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NEUROTOXIC EFFECTS OF ACUTE AND SUBCHRONIC INHALATION EXPOSURE TO TRIMETHYLBENZENE ISOMERS (PSEUDOCUMENE, MESITYLENE, HEMIMELLITENE) IN RATS

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Abstract: Neurotoxic effects of trimethylbenzene isomers (pseudocumene, mesitylene and hemimellitene) in male rats were investigated in conditions of acute and subchronic inhalation exposure.

Rotarod performance and pain sensitivity behaviour were tested in rats exposed to trimethylbenzenes at concentrations of 250 – 2000 ppm immediately after termination of a 4-hour exposure. Exposure to each of trimethylbenzene isomers resulted in concentration-dependent disturbances in rotarod performance, and decrease in pain sensitivity in rats.

Pseudocumene, mesitylene and hemimellitene EC_{50} values for rotarod performance behaviour disturbances were 954, 963, 768 ppm and for decreases in pain sensitivity EC_{50} were 1155, 1212, 848, ppm, respectively.

In conditions of subchronic inhalation exposure, pseudocumene and hemimellitene at concentrations of 25, 100 and 250 ppm caused concentration-dependent disturbances in rotarod performance behaviour and decrease in pain sensitivity. Neurotoxic effect of hemimellitene was more pronounced than that of pseudocumene and mesitylene.

Two weeks after cessation of inhalation exposure to pseudocumene or hemimellitene no recovery in rotarod performance behaviour was observed.

INTRODUCTION

Trimethylbenzene isomers are produced mostly during catalytic reforming of petroleum and they enter into the composition of many commonly used commercial solvent mixtures like Solvesso 100 (Exxon Chemical Belgium), Shellsol A (Shell Netherland Chemie B.V.), Jolasol (J.L.C Chemie, Austria) and Farbasol (Polifarb-Cieszyn S.A., Poland).

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Among other trimethylbenzenes, isomers are one of the substantial constituents of these solvent mixtures. A large number of studies on the neurotoxic effects of exposure to organic solvents have been reported over the last three decades (5,8,10).

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).

The ACGIH TLV-TWA concentration of 125 mg/m^3 (25 ppm) and the Polish MAC value (100 mg/m^3) for single trimethylbenzene isomers (pseudocumene, mesitylene and hemimellitene) have been established on the grounds of incomplete, uncertain and no longer valid data (1,2).

Symptoms and signs of impairment of the respiratory, hematopoietic (probably due to benzene contaminations) and nervous systems were observed in 27 industrial workers, exposed for a number of years to a paint thinner containing various alkylbenzenes (pseudocumene – 50%, mesitylene – 30%; hemimellitene, 1-methyl--2-ethylbenzene and 1-methyl-4-ethylbenzene: percentages not mentioned). The levels of hydrocarbon vapours in the atmosphere ranged from 49–295 mg/m^3 (10–60 ppm) (1,2).

Bearing in mind three very active methyl groups in trimethylbenzene isomer molecules and the broad use of trimethylbenzenes, the present study was aimed at evaluating neurotoxic effects in the condition of acute and subchronic inhalation.

MATERIALS AND METHODS

Chemicals

Mesitylene (1,3,5-trimethylbenzene) purris (GC), pseudocumene (1,2,4-trimethylbenzene) purum > 97% (GC), hemimellitene (1,2,3-trimethylbenzene) pract 90–95% (GC) were supplied by Fluka.

Conversion factors for trimethylbenzene isomers:

1 ppm $\approx$ 4.92 mg/m^3

1 mg/m^3 $\approx$ 0.20 ppm

Animals

Male wistar rats of IMP:DAK stock outbred body weight 250–300 g were used. Animals were housed in wire mesh stainless steel cages. LSK lab chow and water were provided *ad libitum*. Animal rooms were maintained at 22–25°C with a 12-hr light-dark cycle (lights on 6:00 AM). Animals were acclimatised for 1 week prior to the use and, in the case of acute exposure, were used within 4 weeks upon arrival.

Inhalation exposure

Animals were exposed to vapours of trimethylbenzene isomers in a dynamic inhalation chamber (volume of 1.3 m^3, 12 to 15 air changes per hour). Vapours were generated by heating of liquid solvent in washers. The desired concentrations of vapours were obtained by diluting them in the air.

Concentration of solvent vapours in the exposure chamber were measured every 30 min with a gas chromatograph Hewlett-Packard with a flame-ionization detector using a 5 m metal column with 10% OV-17 on chromosorb WHD (80/100 mesh) as a stationary phase at a column temperature of 150°C.

In the acute experiment, rats were exposed to particular trimethylebenzene isomers at tested concentrations of 250–2000 ppm for 4 hours. In the subchronic experiment, 10 rat groups were exposed (6 hours/day, 5 days/week for 3 months) to pseudocumene and to hemimellitene at concentrations of 25 ppm, 100 ppm, 250 ppm, and were accompanied by sham-exposed control groups.

Since in the condition of acute exposure the neurotoxic effect of pseudocumene and mesitylene was similar, and the effect of hemimellitene was more pronounced, pseudocumene and hemimellitene were chosen for subchronic exposure.

Rotarod performance was tested according to the principle described by Kaplan and Murphy (12). The used rotarod apparatus consisted of a 8-cm diameter wooden rod rotating 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 both, acute and subchronic experiments, 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 experiments. In the acute experiment, rotarod performance was tested before exposure and immediately after termination of exposure to particular concentrations of trimethylbenzene isomers, and in sham exposed control animals for four hours. Each group consisted of 10 rats.

In subchronic experiments, rotarod performance was tested prior to the start of the study, weekly during the experiment and two weeks after the termination of exposure.

Hot plate behaviour was tested immediately after termination of 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 of the foot, or after 60 sec, the 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. For calculation of ED_{50} value the observed latency elongation over the control was used. Latency of 60 sec was considered as 100% inhibition of pain sensitivity.

STATISTICS

Probit analysis was applied to determine the medial effective concentration EC_{50} value in the rotarod performance test (6). The concentration which increases the latency of the paw-lick response (decrease of sensitivity to the pain) to 50% (EC_{50}) was calculated from the least squares regression lines of concentration – effect relationship (11). The Fisher exact test (17) was applied for evaluation of motor coordination disturbances and Kruskall-Wallis test (16) for changes in pain sensitivity.

RESULTS

All rats exposed for 4 hours to trimethylbenzene isomers at all applied concentrations survived the exposure. Each one of trimethylbenzene isomers caused concentration-dependent disturbance in the rotarod performance of rats (Fig. 1). The rotarod performance behaviour disturbance EC_{50} value, determined with its 95% confidence intervals, amounted for: pseudocumene to 4693 mg/m^3 (3891–5493 mg/m^3) –

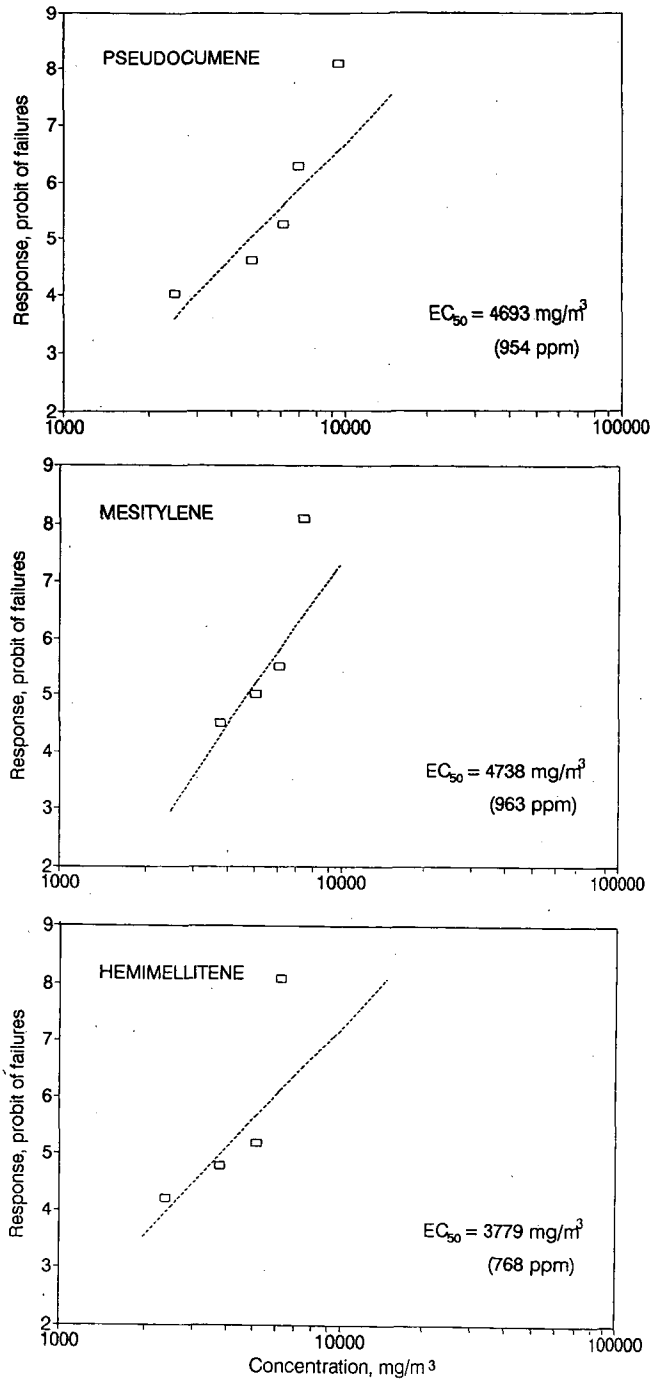

Fig. 1. Rotarod performance of rats exposed to trimethylbenzene isomers. Rats were exposed to solvent vapours for 4 hours. Rotarod performance was tested immediately after termination of exposure. Each point represents probit of failures on rotarod in a group of 10 rats.

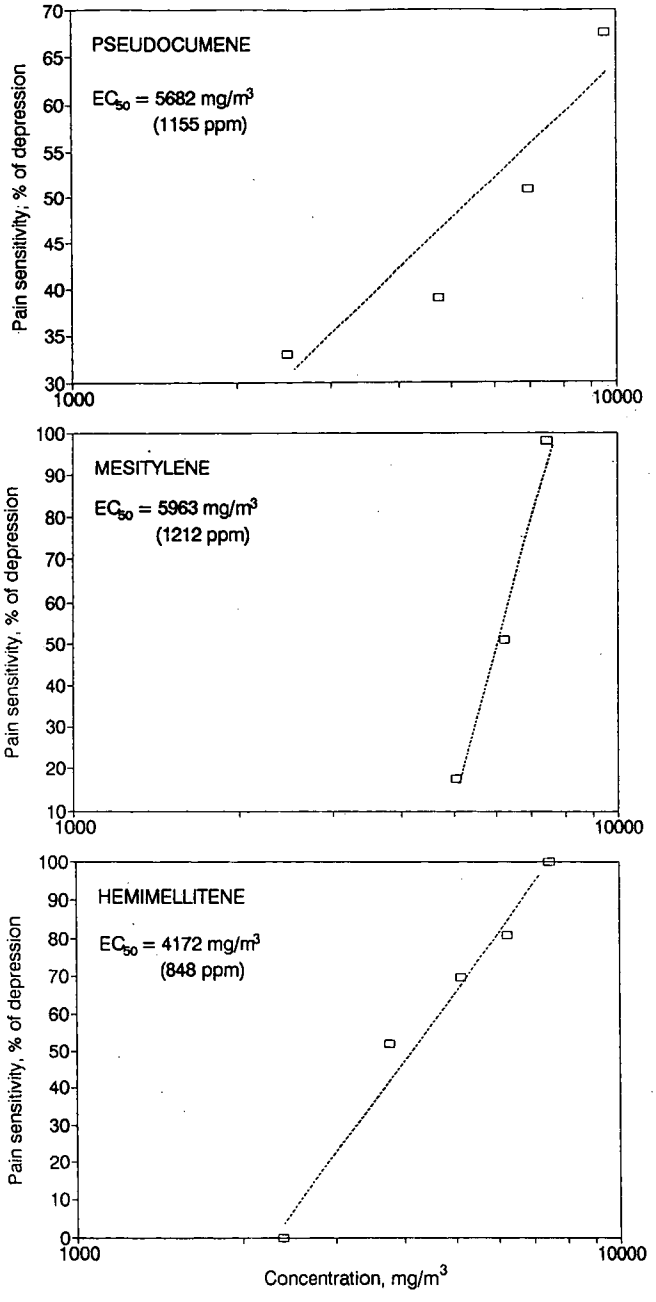

Fig. 2. Hot-plate behavious in rats exposed to trimethylbenzene isomers. Rats were exposed to vapours of each isomer for 4 hours. Hot-plate behaviour was tested immediately after termination of exposure. Each point represents the mean value of separate measurments of latency in 10 rats. Latency of 60 sec was considered as 100% inhibition of pain sesitivity.

954 ppm (791–1113 ppm); mesitylene to 4738 mg/m^3 (3675–5453 mg/m^3) – 963 ppm (750–1113 ppm), and hemimellitene to 3779 mg/m^3 (2832–4615 mg/m^3) – 768 ppm (578–942 ppm).

Trimethylbenzene isomers EC_{50} values determined for observed disturbances in the rotarod performance test indicate a more pronounced neurotoxic effect of hemimellitene.

The pain sensitivity measured as latency of the paw-lick response changed in rats exposed to all trimethylbenzenes. The observed decrease in sensitivity to pain was concentration-dependent (Fig 2). Pain sensitivity decrease EC_{50} value with its 95% confidence intervals amounted to 5682 mg/m^3 (2715–7596 mg/m^3) – 1155 ppm (552–1544 ppm) for pseudocumene; to 5938 mg/m^3 (5194–6512 mg/m^3)

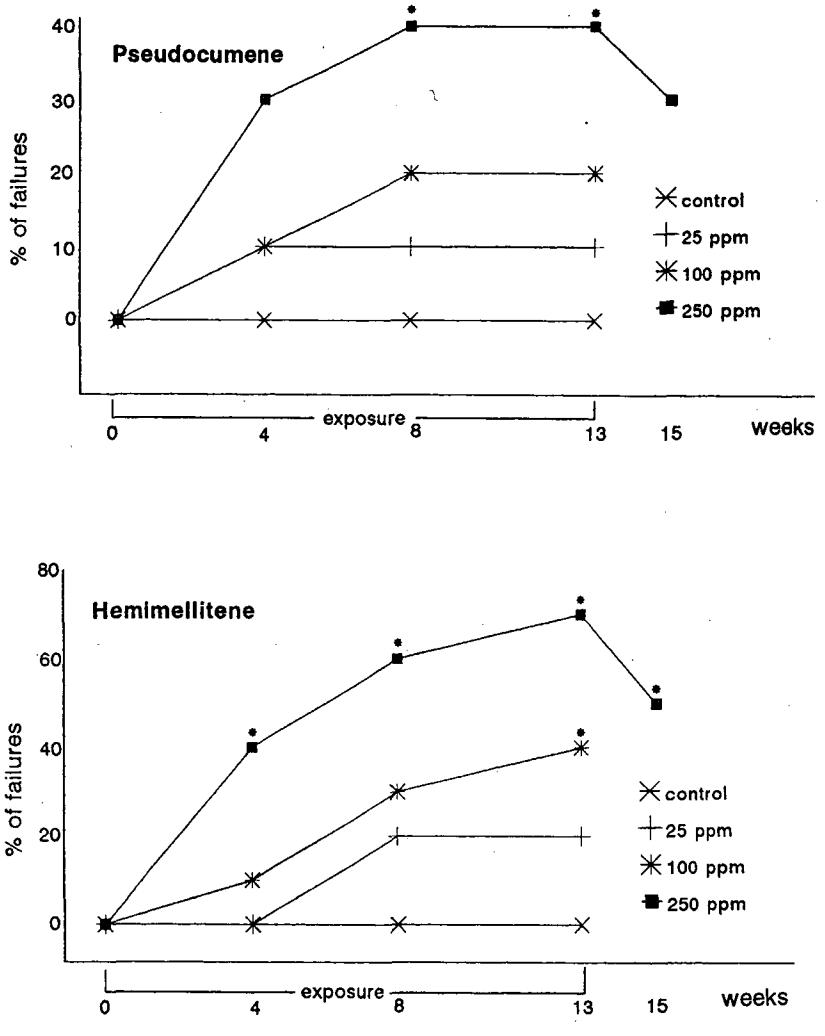

Fig. 3. Rotarod performance of rats exposed to pseudocumene and hemimellitene at concentrations of 25, 100 and 250 ppm. Rats were exposed to vapours of solvents for 6 h/day, 5 days/week, 3 months. Statistical significance marked by asterisks $p < 0.005$.

– 1212 ppm (1086–1329 ppm) for mesitylene; and to 4155 mg/m^3 (3400–4811 mg/m^3) – 848 ppm (694–982 ppm) for hemimellitene. As in the case of motor coordination disturbances, the EC_{50} values for pain sensitivity of trimethylbenzenes indicate a more pronounced analgesic effect of hemimellitene.

All rats exposed for 3 months to pseudocumene and hemimellitene survived the experiment. Clinical observations were unremarkable. Nor were significant differences in final body weights observed in rats exposed to both trimethylbenzene isomers when compared with controls.

In animals exposed for 3 months to pseudocumene and hemimellitene, the disturbances in rotarod performance were observed and changes were dose-dependent (Fig. 3). All control animals performed correctly in the test throughout the experiment. In the case of hemimellitene, the observed disturbances in rotarod performance were more pronounced and statistically significant at concentrations of 100 ppm and 250 ppm, whereas pseudocumene-caused disturbances were significant at the concentration of 250 ppm.

The recovery in the rotarod performance was not observed two weeks after cessation of inhalation exposure to pseudocumene and hemimellitene at the concentration of 250 ppm (Fig. 3).

The changes in pain sensitivity measured as latency of the paw-lick response were observed after a three-month exposure to both trimethylbenzene isomers (Table 1). The decrease in sensitivity to pain was concentration-dependent and statistically significant at 100 ppm and 250 ppm of pseudocumene and at all applied concentrations of hemimellitene. The recovery in hot-plate behaviour was observed two weeks after cessation of inhalation exposure to pseudocumene as well as to hemimellitene (Table 1).

Table 1. Effect of a three-month exposure to pseudocumene and hemimellitene on the latency of the paw-lick response (hot-plate behaviour) in rats

Group	Latency of the paw-lick response, sec	
	Pseudocumene	Hemimellitene
Control	(n = 9) 15.4±5.8	(n = 30) 9.7±2.1
25 ppm	(n = 10) 18.2±5.7	(n = 20) 11.8±3.8*
100 ppm	(n = 9) 27.6±3.2**	(n = 10) 16.3±6.3
250 ppm	(n = 10) 30.1±7.9**	(n = 10) 17.3±3.4**
250 ppm (two weeks after termination of exposure)	(n = 10) 17.3±(3.9)	(n = 10) 11.0±2.4

Statistically significant difference as compared to controls *$p \leq 0.05$, **$p \leq 0.01$.

DISCUSSION AND CONCLUSIONS

Results of rotarod performance and hot plate behaviour tests in rats exposed for 4 hours to vapours of trimethylbenzene isomers at studied concentrations provide an evidence of their neurotoxic effects. Disturbances in rotarod performance and decrease in pain sensitivity observed in acute experiment, were concentration-dependent and indicated a more pronounced neurotoxic effect of hemimellitene than of pseudocumene or mesitylen. The EC_{50} value for rotarod performance

behaviour proved more pronounced toxic effect of trimethylbenzene isomers than that of toluene (ED_{50} – 4050 ppm) and xylene (ED_{50} – 1982 ppm), established in similar experimental conditions (13,14). In the condition of a three-month inhalation exposure, pseudocumene and hemimellitene also exerted neurotoxic effects, and observed effects of hemimellitene were more pronounced, in both applied tests, than those of pseudocumene.

The remaining of rotarod behaviour disturbances, two weeks after cessation of a three-month exposure may indicate long-lasting neurotoxic effects of trimethylbenzene isomers. Similar effects were observed after four-week inhalation exposure to pseudocumene at the same concentrations (7).

It is known that addition of methyl groups to the benzene ring, which raises boiling points and decreases volatility, brings about a change in the equilibrium between the gaseous phase and tissue absorption, leading to the higher effective doses absorbed (4). Thus, stronger neurotoxic effects of trimethylbenzene isomers than those of toluene and xylene may depend on the number of methyl groups in the benzene ring. The more pronounced neurotoxic effects of hemimellitene may be due to 1,2,3-position of methyl groups in the benzene ring.

Very little is known about the cellular solvent mechanism of action in the nervous system. On the basis of the similarity between solvent and classical CNS depressant drugs, the action of solvents is attributed to alterations in membrane structure and function and/or their ability to alter aminergic transmission (5). To explain this hypothesis further research on cellular mechanisms of solvent action is necessary.

Occupational Exposure Limits for Solvents are mostly based on prevention of their neurotoxic effects. Taking into consideration the results of present experiments the MAC value for trimethylbenzene isomers should be considerably lower than the ACGH TLV value (125 mg/m^3 – 25 ppm) and the Polish MAC value (100 mg/m^3 – 20 ppm) (15).

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