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ASSESSMENT OF LONG-TERM NEUROTOXIC EFFECTS OF EXPOSURE TO MESITYLENE (1,3,5-TRIMETHYLBENZENE) BASED ON THE ANALYSIS OF SELECTED BEHAVIORAL RESPONSES

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Abstract. Trimetylbenzene isomers: pseudocumene, hemimellitene and mesitylene, are major components of numerous commercial solvents and high-grade fuels. In our earlier research on rats we have proved that inhalation exposure to pseudocumene or hemimellitene at concentrations close to the MAC value results in behavioral changes detectable many weeks after cessation of the exposure. The aim of our present study is to determine whether exposure to mesitylene causes effects similar to those observed for pseudocumene and hemimellitene. Male rats were used in the experiment. The animals were exposed in the inhalation chambers to mesitylene vapors at the following concentrations: 0 ppm – group MES0; 25 ppm (125 mg/m3) – group MES25; 100 ppm (500 mg/m3) – group MES100 and 250 ppm (1250 mg/m3) – group MES250 for 4 weeks (6 h/day, 5 days/week). The following behaviors were tested: 1) ability to find water in a radial maze (14–19 days after the exposure); 2) open field locomotor activity (25 days after the exposure); 3) acquiring the conditioned reaction of active avoidance (35–45 days after the exposure); 4) sensitivity to pain and stress-induced changes of pain sensitivity (50–51 days after the exposure); and 5) acquiring the conditioned reaction of two-way active avoidance (54–60 days after the exposure). Significant between-group differences were noted in passive and active avoidance tests and sensitivity to pain. In the MES25, MES100 and MES250 rats, the persistence of the passive avoidance reaction was shorter, and more trials were required to produce the active avoidance reaction than in controls (group MES0), the MES100 group appeared to be more fearful on the second day of testing on the hot plate. The exposed groups did not differ in the magnitudes of the detected changes (no concentration-effect relationship). These results indicate that inhalation exposure to mesitylene, like that to pseudocumene and hemimellitene, at concentrations close to the current hygiene standard value for trimethylbenzene, may produce long-term functional changes in the rat central nervous system.

Key words:
Mesitylene, Inhalation exposure, Behavior, Rat

INTRODUCTION

Occupational exposure to organic solvent vapors may result in long-term, sometimes even irreversible, neurobehavioral disturbances known as the solvent syndrome [1]. Despite various preventive measures the health effects of exposure to organic solvents continue to be a major problem of occupational medicine. This is probably due to insufficient monitoring, thereby the admissible occupational exposure (or maximum admissible concentration – MAC) values are exceeded. It is also likely that, in some instances, the adopted MAC values are too high. The reliability of MAC values depends on the volume and scope of the toxicological databases for a specified chemical. Until now, however, only few organic solvents have been subjected to thorough toxicological testing.

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Trimethylbenzene isomers: pseudocumene (1,2,4-trimethylbenzene), hemimellitene (1,2,3-trimethylbenzene) and mesitylene (1,3,5-trimethylbenzene), are the major components of numerous commercial solvents used for paint and lacquer production. [2]. They are also used in high-quality fuel grades [3].

Literature data on the neurotoxic effects of trimethylbenzene exposure are scanty. The current Polish MAC value for trimethylbenzene (100 mg/m^3 or 20 ppm) [4] is based mainly on the results of several epidemiological studies. In 1994, the Nofer Institute of Occupational Medicine, Łódź, Poland, started experiments on rats to expand the toxicological data on trimethylbenzene exposure. The data obtained heretofore [5,6] indicate that, under conditions of acute inhalation exposure, both the airway irritating and the neurotoxic (assessed by motoric coordination test) activity of trimethylbenzene isomers are significantly higher than those of toluene and xylene. The current Polish MAC values for toluene and xylene are the same as for trimethylbenzene.

The risk of long-term neurobehavioral impairment is the main problem associated with employment under conditions of exposure to organic solvents. Thus, the aim of a series of studies, including this report, has been to test whether or not repeated exposure to trimethylbenzene isomers may produce long-term impairment of complex behavioral forms for which efficient exploratory and motivation processes are essential. During our earlier research intended to assess the effects of a 4-week (6h/day, 5 days/week, 0, 25, 100, or 250 ppm) exposure to pseudocumene [7] and hemimellitene [8], we have found that the rats tested 4–8 weeks after the exposure score lower on conditioned avoidance reaction tests and their pain-related emotional reaction persists for longer time than in the non-exposed animals. The relationship between the observed effects and solvent concentration was non-linear; for pseudocumene, the effects of exposure to 100 ppm were stronger than those of exposure to 250 ppm, while for hemimellitene the exposure to even 25 ppm appeared to be more effective than that to 100 or 250 ppm. The aim of the work performed in the current stage of the study has been to check whether the behavioral effects of the exposure to mesitylene are similar to those associated with the exposure to pseudocumene or hemimellitene.

MATERIALS AND METHODS

Animals

Male outbred LOD:WIST rats, n = 48, 5 months old at the onset of the experiments, were used in our study. The animals were subjected to a preliminary motor activity test and classified into four groups (12 animals each): control group (MES0) and three groups exposed to mesitylene at 25 ppm (125 mg/m^3), 100 ppm (500 mg/m^3) and 250 ppm (1250 mg/m^3) - groups MES25, MES100, and MES250, respectively.

Exposure

In groups MES25, MES100 and MES250, the rats were exposed to mesitylene, (FLUKA grade) in 1.3m^3 dynamic inhalation chambers 6h/day (from 8:00 to 14:00) five days/week for one month. The air supplied to the chamber housing group MES0 (control group) was free of solvent vapors. Details of the exposure are available in earlier works from the same laboratory [9].

Behavioral tests

Our assessment of the behavioral effects of the exposure was based on the results of the following tests: radial maze test (assay of short-term spatial memory), open field test (assay of spontaneous motor activity), step-down passive avoidance test (assay of long-term memory), hot plate test (assay of sensitivity to pain and pain-related stress level) and conditioned active avoidance reaction test (assay of the ability to learn and memorize).

A pre-training in the radial maze was performed before the exposure, while the basic test was performed between days 14 and 19 after the exposure. The open field test was performed on day 25 after the exposure, the passive avoidance test between days 35 and 45 after the exposure, the hot plate test on day 50 and 51 after the exposure, while

the active avoidance test was effected on day 53 (training) and 60 (retraining) after the exposure.

Radial maze test

The radial maze used in this experiment was made of grey vinidur (polyvinyl chloride); it consisted of a circular 30 cm dia. platform and eight 60 cm long and 12 cm wide arms extending radially from its centre. Side walls of the radial maze were 4.0 cm high. Ca. 0.5 ml vinidur container was placed at the end of each arm. The maze was placed 80 cm above the floor on a metal stand. The experiment was performed in a room with white-painted walls, using artificial light (four 30W neon lamps spaced symmetrically under the ceiling). The maze was placed in the centre of the room, in which other objects (racks with animal cages, auxiliaries) were located at the walls.

Pre-exposure test in the radial maze (adaptation) comprised five trials during five consecutive days (one trial daily). During that phase of the experiment, each rat was allowed to explore the maze 3 min daily during five consecutive days. The basic test (training) was performed between days 14 and 19 after exposure termination. During two days preceding the test and during the test days, water was accessible to the rats only for 30 min daily. Before each daily trial in the radial maze, the containers at each arm end were filled with water. Each trial was started by placing the animal on the central platform and it was finished after 3.0 min had elapsed. Time required for eight selections, the number of omitted arms (omission errors) and the number of reentries to the arms already visited (perseveration errors) were recorded.

Open-field motor activity

A white square 100 • 100 cm arena surrounded by 20 cm walls and provided with black lines painted on its surface, resulted in 49 squares with a 14.3 cm side each. The rat was placed centrally in the arena (open field) and the animal's behavior was observed for 10 min. The following were recorded: the number of crossed square borders (locomotor activity); the number of rearings (exploratory activity); and the number of grooming episodes. This test was performed only once.

Passive avoidance

Passive avoidance was tested in a 80 • 30 • 30 cm cage provided with a transparent roof and a floor made of metal bars connected in parallel to a square-wave direct current (DC) pulse generator. A 22 • 7 • 12 cm cuboidal platform made of hard cardboard was provided at the centre of the floor. The testing comprised six trials. Trials 1, 2, 3 and 4 were performed at daily intervals. Trial 5 was performed after three, and trial 6 after 7 days since trial 3. In trials 1 and 2 the rat was placed on the platform and the time after which the animal stepped down on the floor was recorded. After it had stepped down, the animal was allowed to explore the cage for 1 min and then it was transferred to the breeding cage. In trial 3, directly after stepping down on the floor, the animal received a series of electric footshocks (100 ms 4.0 mA pulses at 1.0 Hz) for 10 s, an then it was immediately transferred to its breeding cage. In trials 4, 5 and 6, the procedure was similar to that in trials 1 and 2, except for the case that the rat did not step down to the floor within 180 s and was removed from the platform and placed in the breeding cage.

Hot plate test

The hot plate test unit comprised a 350 • 350 • 35 mm flat copper container with forced water circulation inside the container, a thermostat and a 80 • 30 • 30 cm cage with electrified floor. A 280 mm diameter and 250 mm high open-ended transparent cylinder with removable cover was placed on the copper container. The temperature of water flowing through the container (hot plate) was 54.5^{o}C. Latency of the unconditioned paw-lick reaction of the rat placed on the hot plate was the studied variable. Immediately after it had licked its paw, the rat was removed from the hot plate to end the trial. The whole test comprised three trials denoted as P1, P2 and P3. Immediately after trial P1, the rat received a series of 4.0 mA, 100 ms electric shocks at 0.5 Hz for 2 min. Trial P2 was performed after 2 to 3 s since the termination of the electric shocks, while trial P3 took place on the next day, after approximately 24 h. Paw lick latencies determined during trials P1, P2 and P3 were denoted as L1, L2 and L3, respectively.

Active avoidance

The active avoidance test unit comprised a 80 • 30 • 30 cm box with metal grid floor, and a controller with electric and acoustic pulse generator. The box was divided into two identical 40 • 30 • 30 cm compartments by a 5 cm high barrier. A miniature loudspeaker connected to the acoustic signal generator was placed at the back wall of each compartment. The metal grid floors of each compartment were connected in parallel to a DC pulse generator. The rats were trained to shift from one compartment to the other to avoid electric footshock (BB). Presentation of the footshock (square wave 100 ms, 4.0 mA, 1.0 Hz pulses) was preceded by a 500 Hz pulse tone presented at 3.0 Hz (BW). The testing consisted of two daily sessions: an acquisition session and a retention session, with a 7-day interval between them. During each session, the interval between two consecutive trials was 30 s (an average of 20 to 40 s). The trial commenced with BW presentation in the compartment occupied at the moment by the rat. If, within 5 s since BW start, the rat did not shift to the other compartment, the electric shock voltage from the generator was applied to the metal grid floor of the compartment occupied at the moment by the rat. BW and BB ceased immediately after the rat had shifted to the other compartment, and the trial was ended. During both sessions, the rats were trained to an arbitrarily selected criterion of at least four shock avoidances in five trials. The maximum number of trials in one session was 60.

Other determinations

The rats were weighed once a week for the whole duration of the experiment.

Statistical analysis

To reveal and to assess the significance of the differences, the numeric data obtained in the experiments were subjected to a variance analysis. Scheffe test for one-way ANOVA or Tukey test for the two-way analysis were used in the detailed comparisons [10].

RESULTS

Body mass and behavioral effects of the exposure

Body mass

The statistical analysis (two-way ANOVA groups x measurements) revealed that only the effect of time factor (measurements) was significant ($F(1,4) = 75.40$, $p < 0.0001$). Throughout the experiment, all rats gradually gained in weight, and the increase was similar in all groups (effect of the group factor and the groups x measurements interaction was statistically insignificant). Data not shown.

Radial maze performance

Neither before (adaptation) nor after exposure did the groups differ with respect to the time for eight selections, the number of omission or perseveration errors (data not shown).

Open-field behavior

Locomotor activity (number of square borders crossed), exploratory activity (number of rearings) and the number of grooming episodes during animal's stay in the open field were assessed by one-way, non-parametrical variance analysis (Kruskal-Wallis test). The groups did not differ in the number of the borders crossed, the number of rearings and grooming episodes (data not shown).

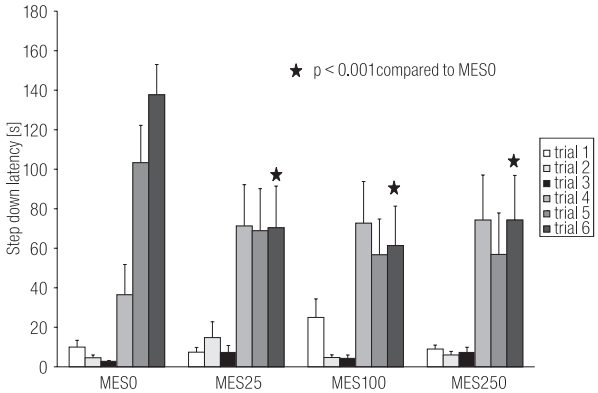

Fig. 1. Passive avoidance. The comparison of the time of staying on the platform in the consecutive test trials. The test was performed between days 35 and 45 after the exposure to mesitylene. Leaving the platform in trial 3 was punished by an electric shock. Trials 1, 2, 3 and 4 were performed at 24-h intervals, while trials 5 and 6 were effected 3 and 7 days after trial 3, respectively.

Passive avoidance

Our analysis (two-way ANOVA, groups x trials) revealed a significant effect of the "trial" factor ($F(1,44) = 37.48$, $p. < 0.0001$) and a significant groups x trials interaction ($F(3,44) = 25.91$, $p < 0.0001$). Between-group comparison showed differences only in trial 6 ($F(3,276) = 6.81$, $p < 0.001$); in this trial, groups MES25, MES100 and MES250 of rats remained on the platform significantly shorter than the MES0 group rats. The differences between the trials were significant for all groups. In all groups, the time for which the animals stayed on the platform in the trials after the shock (trials 4, 5 and 6) were longer than in the trials before the shock (Fig. 1).

Hot plate

The statistical assessment of the paw lick latency in the consecutive trials was based on the absolute values of the consecutive determinations (L1, L2 and L3), and on the values of the proportions L2/L1 and L3/L1. These proportions may be considered to represent stress level (emotional anxiety reaction) caused by 2 min pain stimulation of the paws immediately (L2/L1) and after 24 h since the stimulation (L3/L1).

The analysis (two-way ANOVA, groups x trials) of the absolute latency values showed a significant effect of the "trial" factor ($F(1,44) = 38.39$, $p. < 0.0001$) and a significant groups x trials interaction ($F(3,44) = 10.15$, $p < 0.0001$). The effect of the "group" factor was statistically insignificant. Between-groups comparisons in the individual trials showed differences in trial 3 ($F(3,138) = 3.58$, $p < 0.02$); the latency of the reaction to the thermal stimulus in group MES100 was significantly longer than in groups MES0 and MES25, but did not differ significantly from that observed in group MES250. Within-group comparisons revealed significant differences between trials in all groups: in all trial 2 (immediately after the shock) groups, the latency of the reaction to the thermal stimulus in that trial was significantly longer than that determined for trials 1 and 3. In none of the groups did the reaction latencies in trial 3 differ significantly from those determined in trial 1. No significant differences were detected between the groups in the values of the proportions L2/L1 and L3/L1 (Fig. 2).

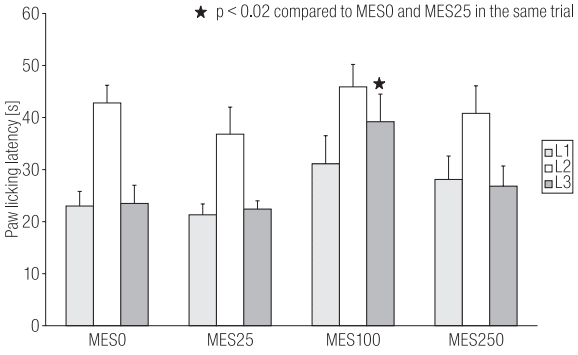

Fig. 2. Hot plate. The comparison of latency of the reaction (paw lick) to the thermal stimulus before (L1), immediately after (L2) and 24 h after (L3) intermittent 2 min electric shock in rats exposed to mesitylene. The test was performed on days 50 and 51 after the exposure.

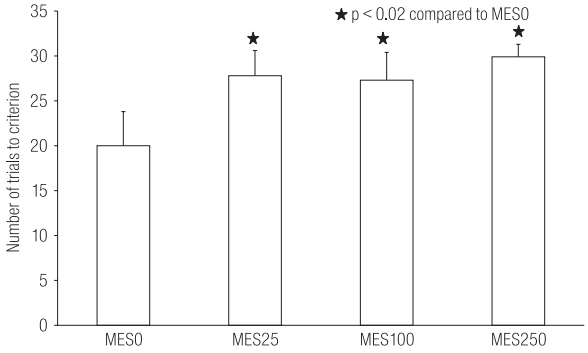

Fig. 3. Active avoidance. The comparison of the rat groups exposed to mesitylene for the number of trials (attempts) required to reach the avoidance criterion (4 shock avoidances) in 5 consecutive trials (attempts) during the training session. The test was performed on day 54 (training) and day 60 (retraining) after the exposure.

Active avoidance

The analysis (one-way non-parametric ANOVA) of the number of attempts to the criterion in the training session revealed significant differences between the groups ($X^2 = 9.882$, $p < 0.02$). In the exposed groups, the number of attempts to the criterion was from 27.25 in group MES100 to 29.9 in group MES250, and in all test groups it was significantly higher than in controls. In the retraining session, the number of trials to the criterion was lower in all groups, but the relations between the groups were similar to those observed in the training session. However, in the latter instance, the differences between the groups were statistically insignificant (Fig. 3).

DISCUSSION

The results of a four week inhalation exposure to mesitylene show that the applied range of concentrations had no significant effect on body weight, suggesting no effect on the general health status of the animals. The absence of differences between groups in the radial maze indicates no long-term effect on short-term spatial memory. At some concentrations, the exposure to mesitylene affects sensitivity to the thermal stimulus (increase in paw-lick response on the second day of testing), impairs animal's ability to acquire the passive avoidance reaction (shorter latencies of staying on the platform after the shock) and the activates avoidance reaction (higher number of attempts required to reach the criterion).

This short summary shows that exposure to mesitylene, like that to pseudocumene or hemimellitene, may cause long-term functional changes in the rat CNS. Our current results show that mesitylene ability to induce those changes is lower than that of the two remaining trimethylbenzene isomers. However in a comparative study [11], behavioral changes caused by a four-week exposure to 100 ppm mesitylene did not differ in their magnitude or scope from those caused by the exposure to 100 ppm pseudocumene, hemimellitene or m-xylene [11]. Earlier studies by Korsak and Rydzyński [5], and Korsak et al. [6] showed that, under conditions of acute exposure, mesitylene did not differ from hemimellitene in its airways irritating activity and its effect on the sensomotor coordination.

The main difference between the effects of mesitylene exposure, observed in the present study and the effects of pseudocumene or hemimellitene exposures observed earlier, applies to the concentration/effect relationship. Both for pseudocumene and hemimellitene, solvent concentration significantly affected the magnitude of the behavioral changes, although the concentration/effect relationship was non-linear: the highest test concentration (250 ppm) was less effective than 100 ppm and, for hemimellitene, even 25 ppm. The results of the present experiment suggest that the observed changes were associated solely with the presence of mesitylene in the inhaled air, while the quantitative factor (solvent concentration) did not play any role.

No data are currently available that would render it possible to explain clearly this fact. It may be guessed (although it seems rather unlikely) that the failure to note the concentration/effect relationship was due to the use of insufficient range or too few values of the concentration. It is more likely that the absence of concentration/effect relationship was due to a considerable variation in the sensitivity of individual animals to mesitylene, particularly in the MES25 and MES100 groups.

The behavioral effects of pseudocumene and hemimellitene have been described by us generally as the reduced ability to inhibit motor reaction in a stressful situation or lower motor reaction threshold in a stressful situation. The effects of mesitylene may be described in a similar way. The increased functional tonus of the dopaminergic system may be one of the causes of such effects [12]. It is generally recognized that exposure to some aromatic hydrocarbons (xylene, toluene) significantly affects the metabolic turnover of the biogenic amines (noradrenaline and dopamine) in the hypothalamus [13,14]. Von Euler et al. [15,16] indicate that a 28-day inhalation exposure to toluene at 80 ppm makes the rat sensitive to apomorphine (a direct agonist of dopaminergic receptors) and induces increased dopaminergic receptor (D2) densities in the striatum. The above effects were detected 14 days after the exposure, which according to the authors, points to their stability. It would not be reasonable to exclude that the exposure to trimethylbenzene isomers can produce similar effects.

The exposure-related functional changes in the dopaminergic system do not necessarily result from the direct solvent interactions with CNS. Trimethylbenzenes, like all organic solvents, have a characteristic smell. Human smell threshold, e.g. for xylene, is 1 ppm. In the rat, the threshold is certainly much lower [17]. Thus, it is quite likely that at least during the first phase of the inhalation exposure, the smell produces a very strong stress reaction, which is additionally increased by animal's limited ability to move (during the exposure, the rats were kept in small single wire mesh cages). Antelman [18–20], and also some other

authors [21] have proved that even a single exposure of the rat to a physical or chemical stressor causes significant changes in the intensity of the behavioral reaction to the dopaminergic-system-affecting drugs. It is extremely important for a correct interpretation of our current and earlier results that the development of the change is gradual, and manifested to the full only after several weeks since the application of the stressor, and that the direction of the reaction may depend on the intensity of the stressogenic agents; weak stressors increase, while the strong ones decrease the magnitude of response to drugs [20]. It is quite likely that exposure to trimethylbenzene (or other solvents) may trigger the same mechanism. This would explain not only the high efficiency of low concentrations in inducing the behavioral changes, but also the fact that the changes could be observed weeks after the exposure, and the non-linear character of the concentration/effect relationship for the effects of pseudocumene or hemimellitene exposure. Experimental verification of the above conjecture would be difficult but feasible. The ability of those chemicals to produce human solvent syndrome cannot be clarified before it has been conclusively determined to what extent the exposure effects, observed in our experiments, represent a long-term effect of nonspecific stress reaction and to what extent they are caused by the direct solvent-CNS element interaction.

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