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BEHAVIOURAL CHANGES FOLLOWING A FOUR-WEEK INHALATION EXPOSURE TO HEMIMELLITENE (1,2,3-TRIMETHYLBENZENE) IN RATS

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Abstract. Trimethylbenzene isomers (TMBs): 1,2,4-TMB (pseudocumene – PS), 1,2,3-TMB (hemimellitene – HM) and 1,3,5-TMB (mesitylene – MES) are important constituents of solvent mixtures. In the US, the adopted TLV-TWA value for TMBs is 125 mg/m^3 or 25 ppm (ACGIH 1996). Recent experiments at our laboratory have revealed an impaired learning of passive and active avoidance responses and a longer persistence of an effect of footshock (increase in latency of the paw-lick response to heat) in rats tested several weeks after a four-week inhalation exposure (6h/day, five days/week) to PS at a concentration of 100 or 250 ppm (15). The concentration-effect relationship appeared to be nonlinear; the effect of 100 ppm HM was more pronounced than that of 250 ppm. In the present experiment we investigated the effects of a repeated four-week (6h/day, 5 days/week) inhalation exposure to HM at concentrations of 0, 25, 100 or 250 ppm on radial-maze performance, open-field activity, passive and active avoidance learning, and on the shock-induced changes in latency of the paw-lick response to heat (hot-plate test). The tests were performed between days 14 and 61 after the last exposure. No significant effects on radial-maze performance and open-field activity were noted in any of the dose groups. In the remaining tests effects of exposure were noted but, similarly as in the case of PS exposure, the concentration-effect relationship was not linear. In rats exposed to HM at 25 or 100 ppm, but not 250 ppm, learning of the passive avoidance, i.e. refraining from performance of a punished response (stepping off an elevated platform) was significantly impaired. Moreover, in rats exposed to 100, but not 250 ppm of HM, acquisition of the two-way active avoidance in the shuttle-box was slower and the footshock-induced increase in latency of the paw-lick response to heat persisted longer than in the unexposed animals. The results suggest that a low-level inhalation exposure to HM, just like low-level exposure to PS, may lead to long-lasting disturbances in the CNS functions. The nonlinear concentration-effect relationship observed in the case of both TMB-s requires clarification in further studies.

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INTRODUCTION

In spite of years of investigations as well as regulatory and preventive activities, the chronic neurotoxicity of industrial organic solvents remains one of the most important problems of occupational medicine. Generally, epidemiological data seem to confirm that a long-term occupational exposure to organic solvents might result in persistent neurobehavioural impairment in workers (30). It is very likely that it is due to a neglect of hygiene standards at the work place in the past. It cannot be excluded, however, that at least in some cases, the maximum allowable concentration (MAC) values are too high, which means that some compounds may be present in the work environment at concentrations harmful to human health.

Trimethylbenzene isomers (TMBs): hemimellitene (1,2,3-trimethylbenzene – HM), pseudocumene (1,2,4-trimethylbenzene – PS) and mesitylene (1,3,5--trimethylbenzene – MES) are important constituents of some commercial solvent mixtures. In FARBASOL (MZRP, Płock, Poland), for example, their total contribution exceeds 40%, and is likely to be of the same order in other similar petroleum products, e.g. Jolasol, JLC, Chemie, Austria; SHELLSOL A, Shell Nederland, Chemie BV, Holland (36). In the US the TLV-TWA concentration for TMBs has been established at 125 mg/m^3 or 25 ppm (1). The health effects data which served as the basis of this recommendation come from two investigations on the effects of a paint thinner on workers who were exposed to its vapour for a number of years (5,6). The proportion of TMBs in this thinner was about 80%, mainly PS (50%) and MES (30%), with HM in trace quantities only, and the vapour concentration of TMBs in the workplace was found to be between 10 and 60 ppm. Complaints of headache, drowsiness and fatigue occurring during the working hours were common among the workers. These symptoms disappeared after work.

In Poland, the adopted MAC value for TMBs is 100 mg/m^3 (20 ppm) i.e. the same as for toluene and xylene. However, recent animal studies at our laboratory show that under conditions of acute inhalation exposure TMBs affect respiration as well as, motor coordination (rotarod performance) at lower concentrations compared to toluene and xylene (20,21). From those data the authors conclude that the MAC value adopted for TMBs is too high. These experiments have also shown that HM is more potent than PS and MES in inducing effects on respiration and motor coordination. Results of subchronic (12 weeks, 6h/day, 5 days/week) inhalation exposure have revealed that in the case of PS, effects suggestive of neurotoxicity (increased latencies of the paw-lick response to heat suggestive of hypoalgesia, and decreased ability to maintain balance on a rotating rod suggestive of disturbed motor coordination) appeared at concentrations of 100 ppm and higher. For HM, signs of hypoalgesia appeared at a concentration as low as 25 ppm. The above effects were transient; two weeks after the exposure, pain sensitivity was normal and the prevalence of disturbances in rotarod performance was reduced (20). In a recent experiment, however, in which more complex forms of rat behaviour were studied, some alterations were detected several weeks after a four-week exposure to PS at concentration of 100 ppm or 250 ppm but not 25 ppm. These alterations consisted of impaired learning of passive and active avoidance responses and a longer persistence of a footshock-induced increase

in latency of the paw-lick response to heat (15). The results might suggest that inhalation exposure to PS at relatively low concentrations could induce long-lasting disturbances in the CNS functions. However, the concentration-effect relationship seen in these studies was nonlinear; the 100 ppm concentration was apparently more effective than the 250 ppm one. It might give rise to some doubts as to the reliability of the observed effects. The purpose of the present experiment was to find out whether exposure to another TMB isomer, hemimellitene, could produce effects similar to those seen after exposure to PS.

MATERIALS AND METHODS

Animals

Fifty three male outbred Wistar rats from our Institute's breeding farm, 5 mo. old at the beginning of testing, were used. Until the experiment onset they were housed in groups (8 rats/cage), and were not subjected to any experimental procedure or exposed to any chemicals. The rats were divided into four groups not differing with respect to the mean body weight and SD. Littermates were allotted to different groups. During the experiment the rats were housed in single rat cages at 22°C and with a light/dark cycle of 12/12 h (light on at 6:00 AM). In the home cages standard rat food pellets (Murigran) were accessible *ad libitum*. Access to water was limited only during the radial maze test (see below). The groups were exposed to HM at the following concentrations: 0 ppm (HM0 group, n = 13); 25 ppm (HM25 group, n = 13); 100 ppm (HM100 group, n = 14); or 250 ppm (HM250 group, n = 13). The concentrations were the same as those applied in previous studies concerning pseudocumene (15) which enabled us to compare the neurotoxic potential of the two TMB isomers.

Exposure

The rats were exposed to HM vapours (Hemimellitene, Fluka, cat. no. 51500) in 1.3 m^3 dynamic inhalation chambers for 4 weeks (6h/day, from 8:00 AM to 2:00 PM, 5 days/week). In the case of the HM0 group the air flowing through the inhalation chamber contained no HM. Within the inhalation chambers the rats were located in small (25 · 20 · 25 cm) wire-mesh cages, one rat per cage, with no sawdust. They had no access to food and water during the exposure. TMB concentrations in the air samples taken from the exposure chambers were measured every 30 min using a gas Hewlett-Packard chromatograph with a flame-ionization detector. The methodology was described in detail in previous studies from this laboratory (31). Body weight was determined weekly during exposure and fortnightly up to 60 days after the last exposure.

Behavioural testing

In order to assess the effect of exposure on spatial working (short-term) memory, choice accuracy in a radial arm maze was tested (18,24). Effects on spontaneous activity were evaluated by an open-field test. Effects on long-term memory and learning ability were assessed by the conditioned passive and active avoidance tests (23). The hot-plate test (39) was performed in order to compare the groups for the

decrease in responsiveness to a thermal stimulus (54.5°C) after a brief (2 min) intermittent footshock*. According to Bolles and Fanselow (9), exposure to an aversive stimulus conditions fear to the experimental context that inhibits pain. Therefore, the footshock-induced increment in the latency of the unconditioned motor response on the hot-plate (i.e. paw licking), commonly regarded as an index of analgesia, may also be regarded as a robust measure of the emotional fear response.

Previous studies at this laboratory have shown that the sensorimotor disturbances and hypoalgesia induced by a four-week (Korsak et al. in preparation) or 12-week (20) inhalation exposure to TMBs disappear, or are greatly reduced, within two weeks after the end of the exposure. Therefore, like in previous studies concerning PS (15), the behavioural testing was started on day 14 after the exposure cessation. The sequence of the behavioural testing was such that the less stressful tests were carried out first and the more stressful were carried out last. Thus, the sequence was as follows:

1) radial maze – one week before exposure and on days 14–18 after exposure;
2) open-field activity – day 25 after exposure,
3) passive avoidance – days 39-48 after exposure,
4) hot-plate test – days 50 and 51 after exposure,
5) active avoidance – days 54 and 60 after exposure.

The time intervals between successive tests were selected arbitrarily, but in some cases they depended on the accessibility of laboratory facilities. In each case the testing was performed between 8:00 AM and 3:00 PM.

Apparatus and testing procedures

The apparatus used in the present experiment, as well as the procedure of the open-field test, passive avoidance test and the hot-plate test, were the same as those in the previous experiment performed to test the effects of PS (15). Therefore, they are addressed briefly in the present paper. The procedures for the radial-maze test and active avoidance test were slightly modified as described below.

Radial maze. The radial maze consisted of a circular 30 cm dia. arena, from which eight arms, each 60 cm long, 12 cm wide and 4 cm high, radiated at equal angles. Each arm was equipped with a 0.5 ml plastic container fastened to the floor near the back wall. The maze was located in the center of the room, 80 cm above the floor level.

Contrary to the previous studies of the effects of PS exposure, the preexposure testing in the radial maze consisted of one adaptation stage. Five trials (one trial per day) were performed during this stage. The rats were tested individually. During each trial they were allowed to explore the maze for 3 min daily. The rats were not water deprived before the trials and the arms contained no water. After the exposure, the rats were trained in seeking water in the arms of the maze. Two days before the first training trial and during a five-day training

* Unlike analgesia induced by a prolonged (20–30 min) footshock, the analgesia induced by a brief (up to 5 min) footshock is not 'opioid-sensitive'. However, it is sensitive to drugs acting on the central cholinergic system (13).

stage, the rats had access to water in their home cages for 30 min a day only. Before the first trial, they were deprived of water for about 24 h. Before each trial the arms were cleaned and the containers were filled with water. On each trial the rat was allowed to freely enter the arms and empty the containers. The trial ended after eight choices had been made or 5 min had elapsed. The number of arms omitted (omission errors), the number of reentries (perseveration errors), and the trial duration were recorded. The next trial was performed 23.5 h later. The remaining details of the procedure were as described earlier (15).

Open field. The 'open field' was a square (100 · 100 cm) arena surrounded by 20 cm high walls and separated into 49 equal squares. Each rat was placed in the middle of the arena and observed for 5 min. The number of square borders crossed (locomotor activity), the number of rearings (exploratory activity), and the number of grooming episodes were recorded manually by the experimenter. The above testing was performed only once.

Passive avoidance. The passive avoidance situation was a cage (80 · 30 · 30 cm) with transparent roof and front, and a metal grid floor. A cuboidal 22 cm long, 12 cm high and 7 cm wide platform, made of hard cardboard, was fastened to the floor in a central position. The test consisted of two preshock trials (trials 1 and 2), one shock trial (trial 3) and three postshock trials (trials 4, 5 and 6). Trials 1, 2 and 3 were performed at 24 h intervals. Trials 4, 5 and 6 were performed 24h, 3 days and 7 days, respectively, after trial 3. In preshock trials the rat was placed on the top of the platform and the latency to step down onto the floor was measured. After stepping down, the rat was allowed to explore the inside of the box for 1 min. In trial 3, immediately after stepping down, the rat received a series of electric footshocks (100 msec 2.0 mA 1 Hz rectangular pulses) for ten seconds. Returns to the platform during shocking were not prevented but they did not occur in trial 3. In trials 4, 5 and 6, before returning it to the home cage, the rat was allowed to remain on the platform for 3 min or if it stepped down before 3 min had elapsed, to stay on the floor for 1 min.

Hot plate. The apparatus consisted of a flat metal box filled with water (hot plate), a thermostat, and a shock chamber (a 30 · 40 · 30 cm compartment with electrified grid floor). It was described in detail in an earlier report (14). The temperature of the hot plate was kept at 54.5°C (± 0.2°). The testing consisted of three trials, a preshock trial (trial 1) and two postshock trials (trials 2 and 3). Each trial involved placing the rat on the hot-plate within a plastic enclosure where it remained until it responded as expected, i.e. licked the hind foot, or 60 s had elapsed. Then, it was removed from the enclosure which ended the trial. Right after trial 1 the rat was transferred to the shock chamber where it received electric footshocks (100 msec, 2.0 mA), one every 2 s for 2 min. Trial 2 was performed several seconds after the shocking. Trial 3 was performed 24 h after trial 2. The latencies of the paw-lick response in trials 1, 2 and 3 were measured and denoted as L1, L2 and L3, respectively.

Active avoidance. The test enclosure consisted of a box with a metal grid floor. The box was divided into two identical compartments (40.0 · 30.0 · 30.0 cm each) by a partition. A rectangular 8.0 · 8.0 cm opening was provided in the lower middle part of the partition. The conditioned stimulus (CS) was a 500 Hz pulsing tone from

a miniature loud-speaker located at the back wall of each compartment. The unconditioned stimulus (UCS) was a series of electric footshocks (100 ms 2.0 mA 1.0 Hz rectangular pulses) applied through the grid floor. The rats were trained in avoiding the UCS by moving interchangeably from one compartment to another after the presentation of the CS in the compartment occupied by the rat. The CS-UCS interval was 5 s. The CS and UCS terminated immediately after the rat's entry into the second compartment. The inter-trial interval varied randomly from 20–40 s (30 s on average). The testing was comprised of two sessions: an acquisition session and a retention session, separated by a 7-day interval. During each of the two sessions the rats were trained until they reached an arbitrary avoidance criterion which consisted of four avoidance responses during five successive trials.

Statistics. In the case of the open-field data and the active-avoidance data differences between dose groups were estimated with the Kruskal-Wallis non-parametric one-way ANOVA. Multiple comparisons were made with the Sheffe's test (20). Data from the remaining tests were analysed using repeated measures two--factor ANOVA (groups x trials or measurements). In the case of nonhomogeneity of covariances, an approximation procedure which avoids assumption about equal covariances was applied (37). In this procedure the degrees of freedom used in finding the critical values are adjusted. To test the hypothesis that $\delta^2{}_\alpha = 0$, the critical value for the F ratio is: $F_{1-\alpha}$ [1, p(n − 1)] instead of $F_{1-\alpha}$ [(q − 1), p(n − 1)(q − 1)], and to test the hypothesis that $\delta^2{}_{\alpha\beta} = 0$, the critical value is: $F_{1-\alpha}$ [(p − 1), p (n − 1)] instead of $F_{1-\alpha}$ [(p − 1)(q − 1), p(n − 1)(q − 1)]. Whenever insignificant interaction between the main factors was found, Tukey *post hoc* comparisons were made (37).

RESULTS

Body weight. At the onset of the experiment, the mean body weight varied from 401.4 ± 31.6 g (group HM0) to 418.4 ± 25.0 g (group HM250). In all groups body weight increased gradually during the experiment (effect of measurements: $F(1.49) = 61.60$, $p < 0.0001$) but the effect of the group factor, as well as the groups × measurements interaction was not significant (data not shown).

Radial maze performance. Neither before (adaptation) nor after exposure (training) to HM was the effect of the group factor significant for the number of choices made, the speed of making choices (trial duration) or choice accuracy (perseveration errors). During the training stage, the trial duration shortened gradually (effect of trials: $F(1.49) = 57.07$, $p < 0.0001$) and this effect was most evident in group HM0. Although the groups × trials interaction was significant ($F(3.49) = 16.88$, $p < 0.001$), *post hoc* comparisons revealed no significant differences within groups or between groups (Fig.1, upper diagram). The number of perseveration errors showed a tendency to increase across trials (effect of trials: $F(1.49) = 5.07$, $p < 0.03$), which was most pronounced in group HM25. The groups × trials interaction was significant ($F(3.49) = 9.27$, $p < 0.001$). *Post hoc* comparisons revealed no differences between groups at each trial. Comparisons within groups revealed differences only in group HM25; the number of errors in trials 3 and 5 was significantly higher than in trial 1. Such a distribution suggests that the differences might be unrelated to exposure (Fig. 1, lower diagram).

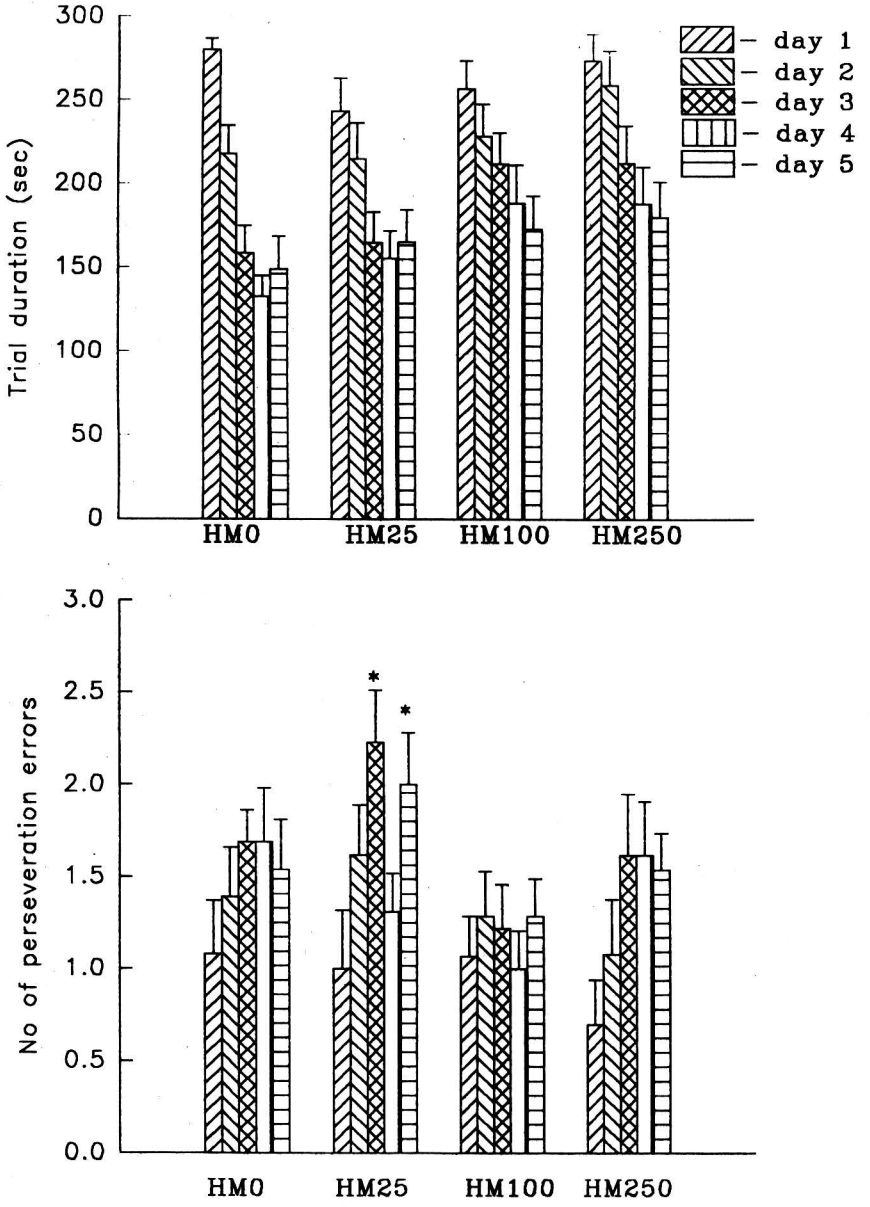

Fig. 1. Radial maze performance of rats exposed for four weeks to hemimellitene vapours. The test (one trial a day) was performed on days 14–18 after exposure. Upper diagram: changes in trial duration, i.e. the time of successive eight arm entries, during successive days of training. Lower diagram: number of perseveration errors in successive daily trials. Denotation of groups: HM0 – sham exposed group (n = 13), HM25, HM100, HM250 – groups exposed to hemimellitene at concentrations of 25 ppm (n = 13), 100 ppm (n = 14) or 250 ppm (n = 13), respectively. The bars represent group means and SE.

* $p < 0.05$ compared to trial 1 in the same group.

Open-field behaviour. Nonparametric one-way ANOVA (Kruskal-Wallis test) revealed no differences between groups in the number of square borders crossed ($X2 = 4.18$, $df = 3$, $p < 0.24$), number of rearings ($X2 = 6.86$, $df = 3$, $p < 0.07$) or the number of grooming episodes ($X2 = 4.88$, $df = 3$, $p < 0.18$) (Fig.2).

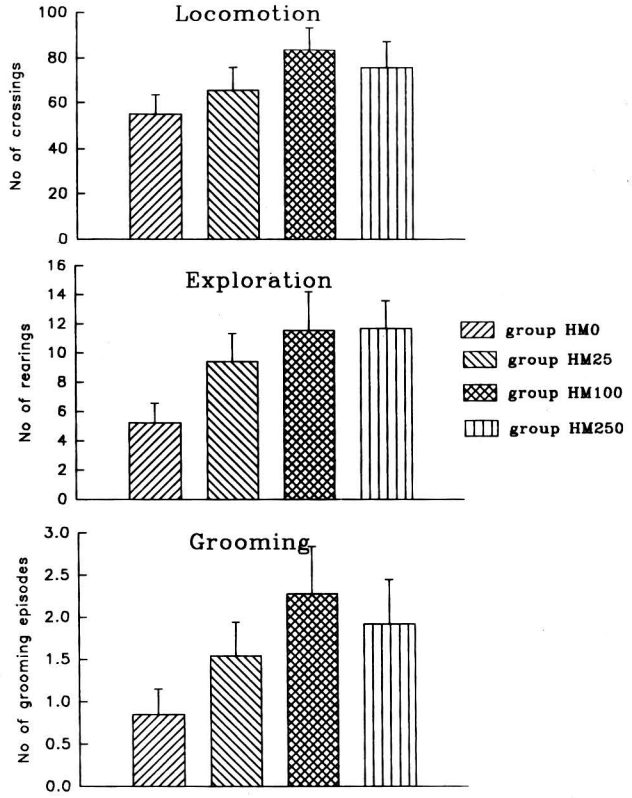

Fig 2. A comparison of spontaneous locomotor (upper diagram), exploratory (middle diagram) and grooming (lower diagram) activity of rats in an open field during a 5-min observation period. The test was performed 25 days after a four-week exposure to HM. Denotation of groups as in Fig. 1. The bars represent group means and SE.

Passive avoidance learning. Changes in the step-down latency during the passive avoidance test are presented in Fig. 3. A two-way ANOVA (groups × trials) revealed that the effect of both the main factors, as well as the groups × trials interaction were significant (effect of groups: $F(3.49) = 2.87$, $p < 0.05$; effect of trials: $F(1.49) = 32.07$, $p < 0.0001$; interaction: $F(3.49) = 16.69$, $p < 0.0001$). *Post hoc* comparisons revealed no significant differences between groups in the step-down latency in preshock trials (trials 1 and 2) and in the shock trial (trial 3). Differences appeared in trial 4 ($F(3.294) = 2.81$, $p < 0.05$), trial 5 ($F(3.294) = 3.49$, $p < 0.0001$), and trial 6 ($F(3.294) = 6.91$, $p < 0.0001$). In all groups the latency of stepping-down increased gradually across postshock trials. However, in group HM25, compared to sham--exposed rats, in all postshock trials the latency of the step-down response was

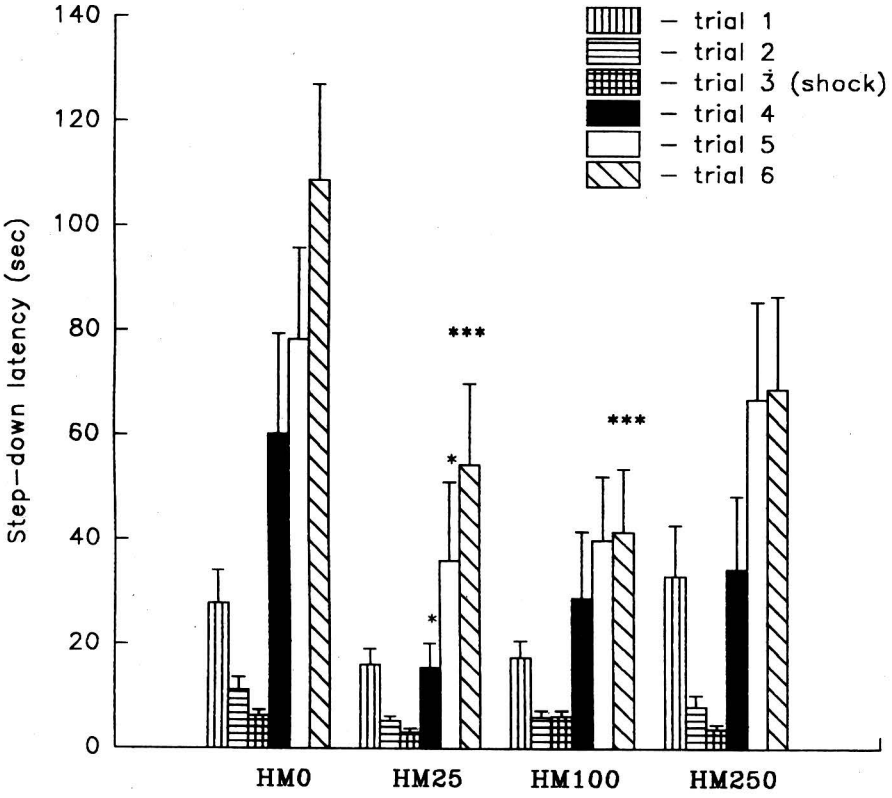

Fig. 3. Diagrams illustrating the effect of a four-week exposure to hemimellitene on the step-down passive avoidance learning in rats. The test was performed on days 39–48 after exposure. Trials 1, 2 and 3 were performed at 24h intervals. The step-down response was punished by a 10 sec-footshock in trial 3 only. Trials 4, 5 and 6 were performed 24h, 3 days and 7 days after trial 3, respectively. The maximum step-down latency was 180 s. Denotations of groups as in Fig 1. The bars represent group means and SE*.

*** $p < 0.05$ and $p < 0.001$, respectively, compared with respective data from group HM0.

significantly shorter. In group HM100, a similar difference was present in trial 6. Rats of the HM250 group did not differ from the sham-exposed rats in respect of the step-down latency in any postshock trial.

Hot-plate behaviour. In the hot-plate test, the expected response to the contact with the hot surface, i.e. licking the hind-paw, was usually preceded by attempts to get out of the plastic enclosure. The more persistent were the attempts, the longer was the paw-lick latency. Statistical comparisons were performed for direct values of the step-down latencies in successive trials, i.e. L1, L2 and L3. In order to assess the effect of footshock more precisely, the L2/L1 and L3/L1 proportions were also compared (Fig. 4). The analysis (a two-way ANOVA, groups × trials) on the direct values showed a significant effect of trials ($F(1.49) = 48.80$, $p < 0.0001$) and insignificant groups × trials interaction ($F(3.49) = 16.30$, $p < 0.0001$). The effect of the group factor was not significant. *Post hoc* comparisons revealed no differences

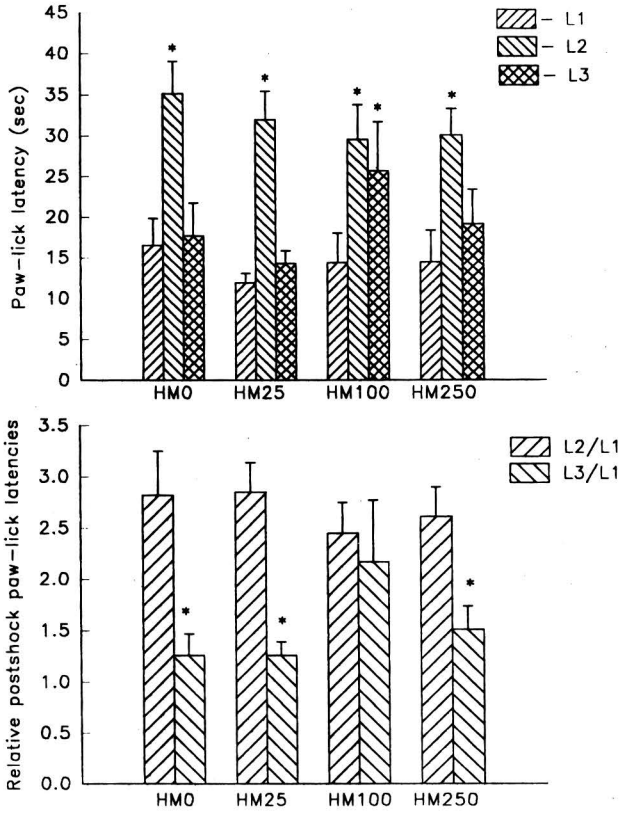

Fig. 4. Hot-plate behaviour tested in rats on day 50 (trials 1 and 2) and day 51 (trial 3) after a four-week exposure to hemimellitene. Denotation of groups as in Fig. 1. The bars represent group means and SE. Upper diagram: A comparison of the latency of the paw-lick response to a thermal stimulus (54.5°C) on day 50. L1 – paw-lick latency in trial 1 performed before a 2 min intermittent footshock. L2 – paw-lick latency in trial 2 performed several sec after the footshock. L3 – paw-lick latency in trial 3 performed 24 h after the footshock.

* $p < 0.5$, compared with L1 in the same group. Lower diagram: A comparison of the change in the paw-lick latency noted immediately (L2/L1) and 24 h (L3/L1) after footshock.

* $p < 0.05$ compared with L2/L1 of the same group.

between groups within successive trials. However, comparison between trials revealed significant differences in group HM0 ($F(2.29) = 16.49$, $p < 0.0001$), HM25 ($F(2.29) = 18.08$, $p < 0.0001$), HM100 ($F(2.29) = 10.02$, $p < 0.001$) and HM250 ($F(2.29) = 9.95$, $p < 0.001$). In groups HM0, HM25 and HM250, the value of L2 (immediately after the shocking) was significantly higher than the L1 and L3 values which did not differ from one another. In group HM100, the L2 and L3 values did not differ from one another and were both significantly higher than the L1 value. It suggests that in group HM100, unlike the remaining groups, 24 h after the shocking, the effect of footshock was still present.

The comparison of the L2/L1 and L3/L1 proportions confirmed this conclusion. In groups HM0, HM25 and HM250, the L3/L1 value was significantly lower than

the L2/L1 value which suggests a dissipation of the effect of shock within 24 hours. In group HM100 however, the values of both proportions did not differ significantly which suggests persistence of the footshock effect.

Active avoidance. A comparison of groups with respect to the number of trials to avoidance criterion (a one-way parametric ANOVA) revealed significant differences in the data from the training session $F(3.49) = 3.39$, $p < 0.03$); the rats of the HM100 group required significantly more trials to attain the avoidance criterion than the sham-exposed animals (Fig. 5, upper diagram). An analysis of the relevant data from the retraining session revealed no differences between groups although the results of the HM100 group were evidently worse (Fig. 5, middle diagram).

In order to assess the avoidance retention, a percentage retention score (%Ret) was calculated for each rat according to the formula: $\%Ret = (1 - Resc/Tesc) \cdot 100$, where Resc and Tesc are numbers of escape responses during retraining and training

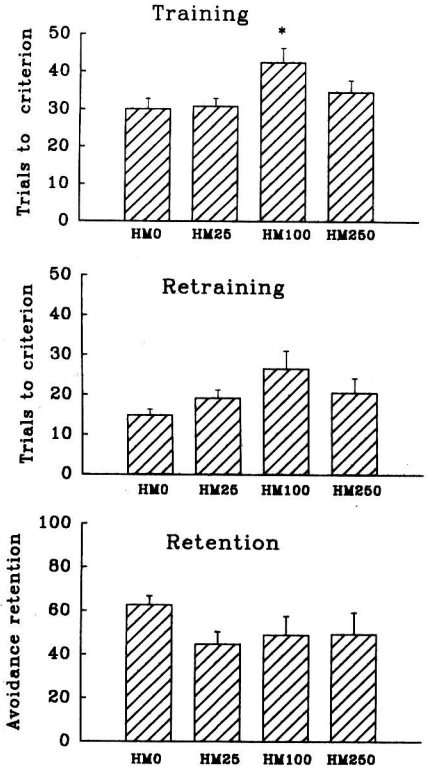

Fig. 5. Active avoidance learning and retention in rats after a four-week inhalation exposure to hemimellitene. Upper and middle diagrams: comparisons of the number of trials to attain an avoidance criterion (four avoidance responses during five successive trials) during the training (upper diagram) and retraining (middle diagram) session. Lower diagram: a retention score calculated according to the formula: $\%Ret = (1 - Resc/Tesc) \cdot 100$, where Resc and Tesc are numbers of escape responses during retraining and training, respectively. Denotation of groups as in Fig. 1. The bars represent group means and SE.

* $p < 0.05$ compared to HM0 group.

session, respectively. As shown in Fig. 5 (lower diagram), in all exposed groups retention was poorer than in the sham-exposed group. Statistical analysis (one-way ANOVA), however, revealed no significant differences between groups.

DISCUSSION

The effects of the four week inhalation exposure to HM described above resemble those resulting from exposure to PS under the same exposure conditions and at the same range of the solvent concentrations (15). As with PS, exposure to HM at any test concentration had no significant effect on body weight gain, suggesting no effect on the general health status of the animals. Choice-making speed and accuracy in the radial maze were similar in the exposed and sham-exposed animals, which suggested no effect of exposure on spatial working memory. At some concentrations however, exposure to HM, just like exposure to PS, resulted in an overt increase in motor activity in the open-field, a reduced ability to withhold a locomotor response (stepping down) in the passive avoidance situation, a persistent foot-shock induced increase in latency of the paw-lick response to heat (hot-plate test), and an impaired acquisition of the two-way active avoidance response. It appears then, that relatively short-lasting and low-level inhalation exposure to HM, just like exposure to PS, may induce long-lasting functional changes within the rat CNS. However, in the case of HM, some of the effects, such as impaired passive avoidance learning, were detected at lower exposure concentrations (25 ppm) than in the case of PS (100 ppm) which suggests that HM may be more potent than PS in inducing long-term neurobehavioural disturbances. The fact that for both of these hydrocarbons, the effective concentrations for producing neurotoxic effects detectable quite a long time after the exposure appeared to be surprisingly low, might raise the question of the validity of the hygiene standards which have been adopted for TMBs.

Apart from the qualitative similarity, the behavioural effects of exposure to HM and PS resemble each other with respect to the type of concentration-effect relationship. In the case of both solvents, the concentration-effect curve for the results of open-field, passive avoidance and hot-plate tests (and active avoidance in the case of HM) resembles an inverted 'U' curve; the highest concentration (250 ppm) being evidently less effective (or not effective at all) than the lower ones. One may argue that this peculiar concentration-effect relationship might have been due to some undetected group differences in pre-exposure behaviour. However, considering the way of alloting rats to different exposure groups (see the method section), the chances that such differences did occur are rather small. Moreover, it is difficult to imagine how the incidental pre-exposure differences (if any) between the test groups in the PS experiment could be replicated in the HM experiment, performed a year later. Therefore, in our opinion, the concentration-effect relationship noted in both these experiments cannot be regarded as an experimental artifact.

Although a non-linear concentration-effect relationship had not been observed following repeated exposure to any solvent, nonlinearity has been frequently reported to result from acute exposures to many solvents, including aromatic hydrocarbons (4,6). For example, a well known effect of acute low-level exposure to toluene is an increase in spontaneous locomotor activity, followed by suppression of such activity at higher exposure levels (17,38).

It is well known that exposure to a drug or neurotoxicant alters the functional equilibrium within the CNS, which is compensated by homeostatic mechanisms. In the case of repeated exposure, these adaptations may have long-term consequences, not necessarily of adaptive value (26,35). It is conceivable that the changes within the CNS resulting from repeated exposure to a drug are determined not only by the character of the drug interaction with structural or molecular components of neural cells, but also by the type of the global CNS response which may be concentration- or dose-dependent. Thus, the lack of linearity in the concentration-effect relationships seen in the present and the previous experiment could be explainable if the acute effects of exposure to TMBs at the concentration of 250 ppm or higher differed qualitatively from those produced by concentrations lower than 250 ppm. Recent data from our laboratory suggest, however, that TMB concentrations of 250 ppm or lower are simply ineffective in producing any overt effect on the rat behaviour tested within an hour after a short (4-hour) inhalation exposure (20).

Another possibility worth considering is that repeated exposure to TMBs results in sensitization. Sensitization is defined as the progressive and enduring enhancement in behavioural and neurochemical measures after repeated intermittent exposure to a variety of stimuli; most commonly psychostimulant drugs, e.g. amphetamine, and environmental stressors (2). Psychostimulants and stress cross-sensitize to produce behavioural sensitization (3,16,25,28,29), which means that a subject sensitized to a psychostimulant will show an augmented behavioural response to stressors, and vice versa. Paramount to our results is that the stimulus initiating sensitization need not be strong; in fact low-level initiating stimuli can induce an increase, whereas high-level initiating stimuli can induce a decrease or reversal in the subsequent sensitized response to another stimulus (4). It is well known that volatile organic solvents may act as psychostimulants. For example, at some concentrations toluene exerts an excitatory effect on EEG (22) and affects behaviour in a similar way as amphetamine does (12). It has also been shown that after an intermittent 28-day inhalation, exposure to toluene at a concentration of 80 ppm, which is far below that producing an overt effect on behaviour (1000 ppm, (12)) or EEG (2000 ppm (32)) rats show an increased locomotor response to apomorphine (34) suggestive of cross-sensitization. The above data allow one to presume that toluene, and possibly other organic solvents including TMBs, may possess sensitizing properties*. Based on the fact that psychostimulants and environmental stressors can cross-sensitize, one may speculate, that the repeated exposure to TMBs produced a sensitization to the behavioural effects of the stress-inducing situational stimuli faced by the rat during the behavioural testing. The fact that significant effects of exposure to HM (and PS) were noted only in the three tests in which the footshock was applied, i.e. passive avoidance, hot plate and active avoidance, makes this conjecture a likely one. On the other hand, the fact that the sensitization effect may be inversely related to the strength of the initiating stimulus (see above) may help understand why in the present and previous experiments the behaviour of rats exposed to TMB at the highest (250 ppm) was less affected than the behaviour of rats exposed to 100 or even 25 ppm TMB.

* According to some authors (7) in humans sensitization participates in initiation and elicitation of susceptibility to low-level environmental chemicals, a symptom complex known as multiple chemical sensititivity (MCS). It is worth emphasizing here that solvents are among the substances MCS patients most frequently report as having induced their conditions (8).

In rats, the sensitized behavioural response to drugs or environmental stressors usually consists of enhanced locomotor activity (19). As discussed in our previous paper (15), the effects of repeated exposure to PS: impaired passive avoidance, persistence of the footshock-induced increase in paw-lick latency on the hot plate, and even the retarded acquisition of the two-way active avoidance response, might also be interpreted in terms of enhanced locomotor activity or a decreased ability to inhibit locomotor response in a fear-inducing experimental context. A similar explanation may be proposed for the effects of HM exposure observed in the present experiment. However, the results of both experiments contain no direct proof in support of the validity of this conjecture. If the sensitization hypothesis of the effects of the TMB exposure comes true, and the proposed explanation of the nature of the behavioural deficits seen after the exposure is valid, one may expect (i) an increasing effect of successive exposures to TMBs on locomotor behaviour, (ii) an augmented locomotor response to footshock (or a psychostimulant) detectable weeks after the last exposure, and (iii) the concentrations of 250 ppm and above should be less effective than concentrations 25 or 100 ppm in producing these effects. Checking the above assumptions is the matter of the next experiment.

In summary, the results of the present and previous (15) experiments demonstrate that a four-week inhalation exposure of rats to TMBs, HM or PS at concentrations producing no overt signs of toxicity may induce changes in the functional state of the rat CNS which are detectable several weeks after the exposure. In the case of HM, the concentration of 25 ppm, which is the TLV value for TMBs adopted in the US, appeared to be effective.

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