International Journal of Occupational Medicine and Environmental Health, Vol. 12, No 4, 331 – 343, 1999

EFFECTS OF ACUTE EXPOSURE TO AROMATIC HYDROCARBONS C 9 ON LOCOMOTOR ACTIVITY IN RATS. TRIMETHYLBENZENE ISOMERS

TADEUSZ TOMAS, RADOSŁAW ŚWIERCZ, DOROTA WIADERNA and SŁAWOMIR GRALEWICZ

Department of Toxicology and Carcinogenesis,
The Nofer Institute of Occupational Medicine
Łódź, Poland

Key words: Toluene, Trimethylbenzene isomers, Locomotor activity, Blood concentration, Rat

Abstract. This study was performed to find out whether in acute exposure to trimethylbenzene (TMB) isomers the dose effect relationship is linear or biphasic. In experiments performed on rats, the effect of four solvents was studied: three TMB isomers: 1,3,5-TMB (mesitylene), 1,2,3-TMB (hemimellitene), and 1,2,4-TMB (pseudocumene), and toluene, known for its biphasic activity, was used as a reference compound. The solvents were dissolved in olive oil and administered to rats orally at the doses of 0.008, 0.016, and 0.032 mol/kg. Spontaneous locomotor activity was assessed with the open-field test. Solvent concentrations in peripheral blood were determined parallelly by gas chromatography on separate groups of animals. Statistics employed a two-way analysis of variance (ANOVA) and Tukey's test. The results showed that oral administration of toluene at a dose of 0.008 mol/kg induced biphasic changes in the animal locomotor activty. It was found that TMB at applied doses increased slightly the animal locomotor activity, but the magnitude of changes did not indicate their stimulating effect. Contrary to toluene, no time-effect relationship was observed after administration of trimethylbenzene isomers. The mean blood concentrations of solvents were dose-related. The highest concentrations were observed after toluene administration.

INTRODUCTION

There seems to be a general agreement that persistent neurobehavioural impairments may develop in workers occupationally exposed to volatile organic solvents like trimethylbenzene isomers (TMB). Due to high volatility of solvents, the lungs are most important route of absorption, due to their lipophilicity, the primary place of action of organic solvents is the central nervous system (3,8,11,12,13). It is known, that exposure to toluene (methylbenzene) and xylene (dimethylbenzene) may, depending on the concentration, induce inverse behavioural changes, stimulation of locomotor activity at low concentration or its decrease and sleepiness at high concentration (1,2,7,18). There is no experimental study concerning long-lasting neurobehavioural effects

Address reprint requests to Dr T. Tomas, Department of Toxicology and Carcinogenesis, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Łódź, Poland.

of exposure to organic solvents. A little is known about spectacular acute effects following exposure to high concentration of solvents.

Our earlier study (9,10) showed, that in conditions of acute inhalation exposure, the irritating effect on respiratory tract and the neurotoxic effect of TMB, estimated on the basis of locomotor activity and sensitivity to pain, are more potent than toluene and xylene. Successive investigations (5,16) revealed that dose-effect relationships following a 28-day inhalation exposure to pseudocumene (1,2,4-TMB) and hemimellitene (1,2,3-TMB) at concentrations of 25, 100, 250 ppm was non-linear. Concentration of 100 ppm appeared to be most effective in inducing long-term behavioural disturbances in rats. It is likely, that the non-linear kind of changes may be determined by the acute effects of exposure to low and high concentrations of TMB. It is possible that the changes which develop during exposure repeated every day are determined by the nature of acute effects. This could partly explain the absence of linearity between the solvent concentration and the magnitude of long-lasting effects. This study was performed to find out whether in exposure to trimethylbenzene (TMB) isomers there is a linear or biphasic relationship between a given dose and acute behavioural effects.

METHODS AND MATERIALS

Animals

Behavioural tests and determinations of solvent concentrations in peripheral blood were performed on WAG/Rij strain of male rats with body weight of 210–270 g. Standard rat food pellets and water were accessible *ad libitum*. The animals were housed in single rat cages at temperature of 21–23°C and humidity of 55–60%, with a light/dark cycle of 12/12 h.

Chemicals

For the experiments toluene (99.9%, Cas 108-88-3) by POCH (Poland) and three TMB isomers: 1,3,5-TMB (mesitylene, 99%, CAS 108-67-8); 1,2,4-TMB (pseudocumene, 97%, CAS 95-63-6); and 1,2,3-TMB (hemimellitene, 90–95%, CAS 526-73-8) by Fluka (Switherland) were used. The solvents were dissolved in olive oil and administered orally at the doses of 0.002, 0.008 and 0.032 mol/kg b.w., volume of 1.5 ml. The control rats were administered with pure olive oil, volume of 1.5 ml.

Behavioural testing

Spontaneous locomotor activity was assessed with the open-field test. The test equipment was a home cage (30 · 60 cm) divided into 8 visible squares of the same size. The rat was placed in the centre of the cage and the number of square borders crossed (locomotor activity) was recorded for 10 min. The animals were divided into five groups: the control group (n = 10) administered with pure olive oil, and four solvent groups (toluene, pseudocumene, hemimellitene and mesitylene), 30 rats each. In addition, the solvent groups were subdivided into three dose-related subgroups, each composed of 10 rats. The rats were given a single dose of the solvent.

Prior to injection, the rats were preliminary tested for 10 min (measurement time point 0). After the injection, the number of square borders crossed was recorded for 70 min at 10 min intervals (measurement time points 1 – 7).

Biochemical assays

Biochemical assays of solvent blood concentrations were performed on 48 rats divided into four solvent groups, 12 animals each. In addition, solvent groups were subdivided into three dose-related subgroups, each composed of 4 rats. Each animal was given a single dose of each compound. After administration, the concentration level was determined in peripheral blood drawn from a tail vein into heparinised glass capillary for 70 min at 10 min intervals (7 assays). The determinations were performed by a Hewlett Packard gas chromatograph with a flame-ionization detector (FID) and HP-1 capillary column.

Statistics

The results of the open-field test were subject to a statistical analysis using a two-way analysis of variance (ANOVA) and Tukey's test (17). The analysis was carried out twice: compound × time interaction for each dose, and dose × time interaction for each compound. The differences were regarded as significant when the null hypothesis probability was less than 5%.

RESULTS

Behavioural investigations. General observations

At time point 0 (control), animals of all groups behaved similarly. After entering the open field, the rat showed evident interest in a new surrounding, demonstrated by an increased locomotor activity, sniffing of new stopping places and alternative rearings. All these activities took place at the edge of the field, that is near the cage walls. The animals urinated and defacated quite often. The high locomotor activity during the first minutes of observation gradually decreased, toilet episodes prolonged, and finally the rat chose one of the open-field corners and stopped there motionless in a sitting position for about 15 min.

After the TMB administration, the animal behaviour was similar in all the three groups (pseudocumene, hemimellitene and mesitylene) at the first and partly at the second time points. After entering the open-field, the rats chose one of its corners where they stayed for 15 min not showing any interest in the sorrounding. Episodes of enhanced locomotor activity occurred in further measurement time points. At these time points the rats behaviour was dose-related. The rats given one of TMB isomers at a dose of 0.008 or 0.016 mol/kg changed their place only rarely but their gait was normal. Besides moderate piloerection, no changes which could evidence the effect of solvents on the animal posture and behaviour were observed. Generally, two hours after the injection the rats behaved normally, only some of them changed the place in their home cage, kicking in sawdust. Neither 24 h following the injection nor during five consecutive days any deaths among rats injected with TMBs at a dose of 0.008 or 0.0016 mol/kg was observed.

The rats given TMB at a dose of 0.032 mol/kg showed an evident increase in mobility 15 min after injection, but their gait was disturbed, and the way of movement (disturbed motor co-ordination, dragging of hind legs) suggested paresis of hind limbs. The rats also showed tachypnea, and tremor which was quite characteristic. Distinct piloerection and frequent blood contaminated secretion from the upper airways, especially in the hemimellitene and pseudocumene groups, were noted. In consecutive measurement time points, rats exhibited motor ataxia with apparent paresis of hind limbs, and were crawling. Nevertheless, they generally showed considerably higher motor excitation than that observed after lower doses. About 90 min after injection, the rats lay motionless in a prone position with stertorous respiration and tachypnea. During 24 h following TMB injection at a dose of 0.032 mol/kg, 9 rats died (in the pseudocumene group 3, hemimellitene 4 and mesitylene 2). During consecutive four days no deaths were recorded, and behaviour of the survivals did not suggest any significant locomotor disorders.

The animals of the toluene group behaved differently from those injected with TMB. Distinct differences between effects of toluene and those described above applied to the doses of 0.008 and 0.016 mol/kg. About 15 min after toluene injection at these two doses a strongly pronounced increase in locomotor activity could be seen. Rats with evident piloerection moved with raised tails (a dose of 0.008 mol/kg) at a long distance diagonally and along the open-field walls, their movements were fast, steady and strong. The rats were excited and this state was pronounced more strongly after a dose of 0.016 than after a dose of 0.008 mol/kg. There was not much difference in the behaviour of rats injected with toluene at a dose of 0.032 mol/kg and those injected with TMB at the same dose.

During 5 days following the injection no deaths were noted in the toluene group of rats.

At measurement time points 1 and 2, the appearance and behaviour of controls which received olive oil were similar to those observed in the solvent groups. After oil administration, the rats took a sitting position in one of the corners of the open field, and stayed motionless for about 15–20 min. At consecutive measurement time points, the locomotor activity of animals was limited to occasional displacements along the open field walls and changes in their position to more comfortable one. No deaths were recorded during five days following the injection.

A quantitative analysis of changes in locomotor activity after solvent administration

The measurement of spontaneous locomotor activity in the open field displayed individual differentiation in the behaviour of rats. In order to diminish the effect of this differentiation on the results of the statistical analysis, the comparisons were based on relative values (%), taking the measurement results (the number of square borders crossed) in time point 0 (10 min prior to solvent injection) as a reference value (100%).

Fig. 1 shows the percentage of changes in locomotor activity among animals after oral administration of olive oil and solvents.

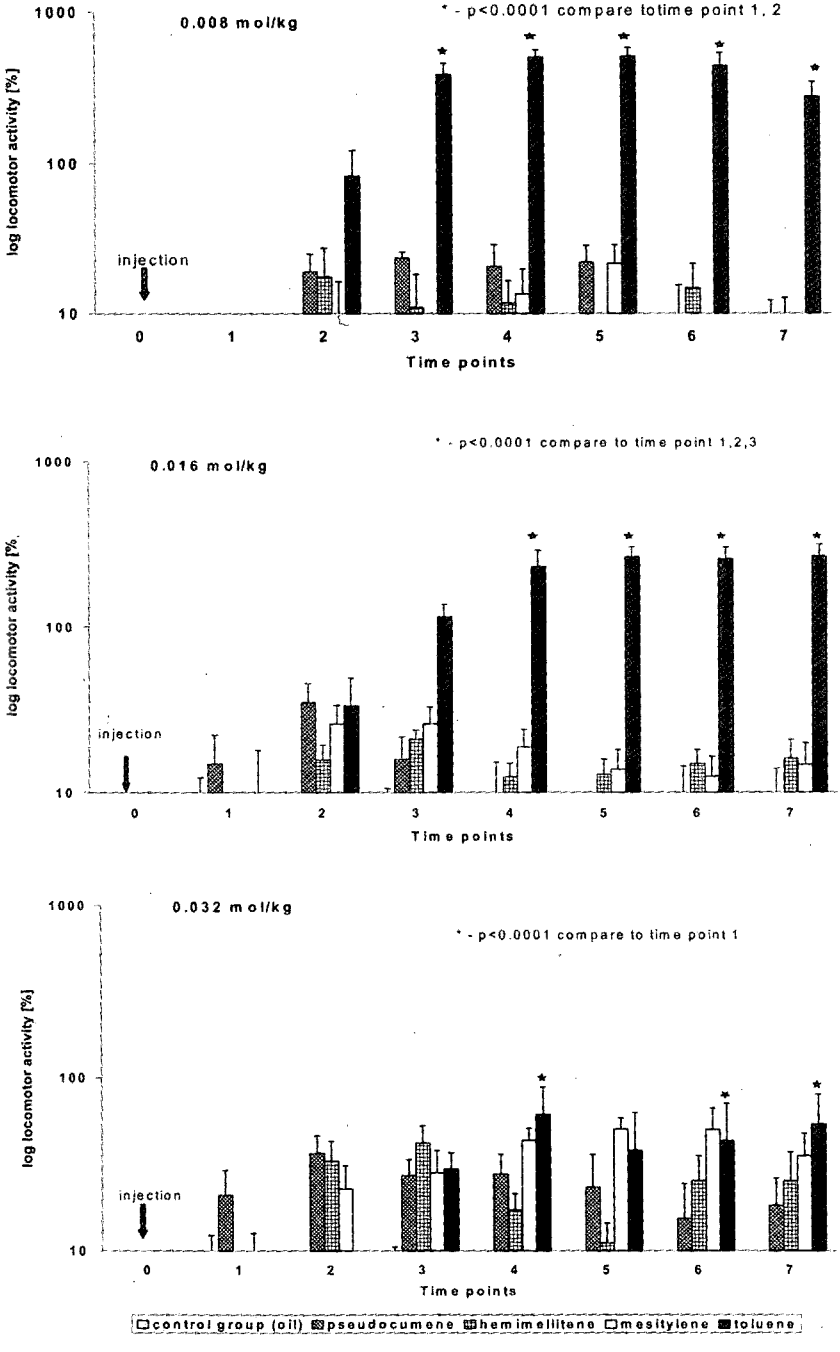


Fig. 1. Locomotor activity following acute exposure to toluene and trimethylbenzene isomers at doses of 0.008 mol/kg, 0.016 mol/kg and 0.032 mol/kg.

Stage 1. An analysis of solvent-related magnitude and dynamics of changes in locomotor activity in the open-field test

A dose of 0.008 mol/kg. Both the kind of the solvent and the post-injection time exerted a significant influence on the parameter studied (solvent effect: $F(4,37) = 29.30$; $p < 0.0001$); time effect: $F(1,37) = 20.86$; $p < 0.0001$). Moreover, the kind of the solvent also determined significantly the dynamics of changes in post-injection locomotor activity (solvent × measurement time point interaction: $F(4,37) = 252.92$; $p < 0.0001$). Between-group comparisons between measurement time points indicated significant differences only in the toluene group ($F(6,222) = 83.89$; $p < 0.00001$). Changes in this group mobility were of typical biphasic character: a gradual increase during 40–50 min after administration (time points 3–7, the number of square borders crossed was significantly larger than at time points 1 and 2), and then a gradual decrease (time point 7, the number of square borders crossed was significantly smaller as compared with time points 4, 5 and 6).

A dose of 0.016 mol/kg. With regard to the 0.016 mol/kg dose, the effect was also significantly related to the solvent and measurement time (solvent effect: $F(4,42) = 38.17$; $p < 0.00001$); time effect: $F(4,42) = 10.42$; $p < 0.005$). The interaction between main factors was also significant ($F(4,42) = 190.96$; $p < 0.00001$). Between-time points comparisons in individual groups indicated significant differences only in the toluene group. In this group, post-injection locomotor activity was increasing gradually for 40–50 min and then was maintained at the attained level until the end of the experiment (the number of square borders crossed at time points 4–7 was significantly larger than in time points 1, 2 and 3).

A dose of 0.032 mol/kg. The effect of both main factors on locomotor activity induced by a dose of 0.032 mol/kg was insignificant but the interaction proved to be significant ($F(4,44) = 31.27$; $p < 0.0001$). Again, between-time points comparisons in individual groups showed differences only in the toluene group ($F(6,264) = 5.97$; $p < 0.0001$); locomotor activity was significantly increased at time points 4, 6 and 7 as compared with time point 1.

The comparisons indicated that of all solvents used only toluene influenced evidently locomotor activity in rats under study. The changes, observed after the administration of a dose of 0.008 mol/kg, were biphasic, which was manifested by the increase in activity followed by its decrease. After doses of 0.016 and 0.032 mol/kg the increase was less pronounced and the decrease was not observed. The changes following the administration of trimethylbenzene isomers were small and irregular.

Stage 2. An analysis of dose-related magnitude and dynamics of changes in locomotor activity

Toluene. Both factors, dose and time, significantly influenced the parameter in question (dose effect: $F(3,36) = 25.16$; $p < 0.0001$; time effect: $F(1,36) = 32.25$, $p < 0.0001$). Also interaction between both factors was significant ($F(3,36) = 106.18$; $p < 0.0001$). The comparison between the effects of individual doses at consecutive time points revealed significant differences at time points 3–7. At those time points locomotor activity of rats which were given toluene in doses of 0.008 and 0.016 mol/kg was significantly increased as compared with the control group and the group given toluene at a dose of 0.032 mol/kg.

Pseudocumene. Only the effect of the group factor (dose) and interaction (dose × time effect:) were significant ($F(3,40) = 3.38$; $p < 0.05$; $F(3,40) = 10.00$; $p < 0.0001$, respectively). In general, locomotor activity in the 0.032 mol/kg group was significantly higher in comparison with the control group. Between-time points comparisons showed differences only at time point 2 ($F(3,280) = 5.6$; $p < 0.0001$); locomotor activity after the administration of the 0.032 mol/kg dose was significantly higher as compared with activity in the control group and in the group given pseudocumene in a dose of 0.008 mol/kg.

Hemimellitene. Like in the case of pseudocumene, an analysis of main effects revealed only dose effect significance ($F(3,31) = 7.91$; $p < 0.001$). Interaction was significant ($F(3,31) = 15.19$; $p < 0.001$), and mobility in the 0.032 mol/kg group was generally significantly higher than that in other groups. Between-group comparisons made at individual time points showed differences only at time point 2 ($F(3,217) = 6.77$; $p < 0.01$), and at time point 3 ($F(3,217) = 8.38$; $p < 0.001$). At both these time points locomotor activity in the 0.032 mol/kg group was significantly higher than that in the control group.

Mesitylene. An analysis of main effects showed a significant influence of the dose on locomotor activity ($F(3,25) = 25.54$; $p < 0.0001$); in general, locomotor activity in rats of the 0.016 mol/kg group was significantly higher than in the control group, and in the 0.032 mol/kg group was significantly increased as compared with other groups. Time effect (measurement time points) appeared insignificant, but the interaction between both main factors was significant ($F(3,25) = 26.38$; $p < 0.0001$). Between-group comparisons at individual time points displayed significant differences at time points 2 and 4–6; at time point 2, locomotor activity in the 0.016 mol/kg group was significantly higher than in the control group, whereas at time points 2 and 4–7, activity in the 0.032 mol/kg group was significantly higher than in the control group.

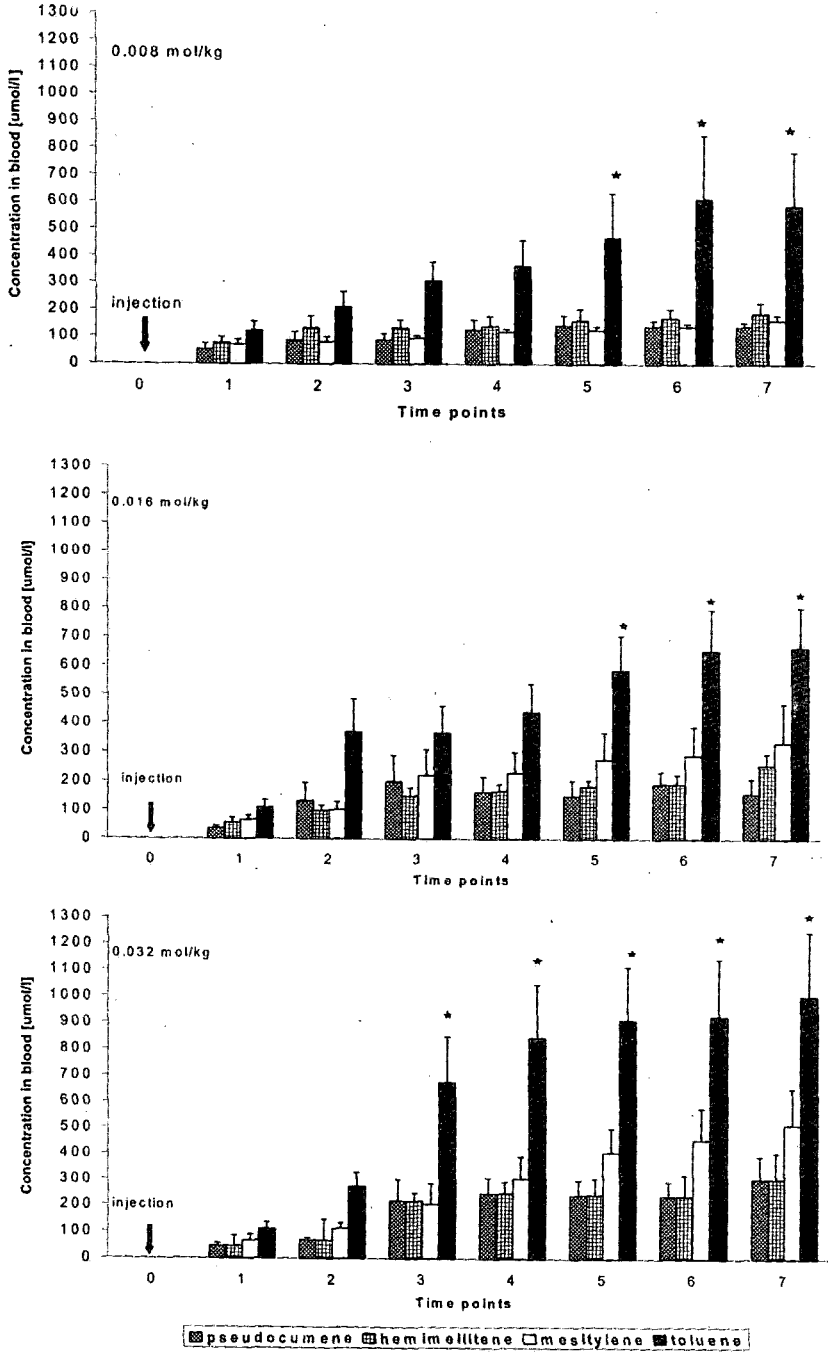
The results of the comparisons, made separately for each solvent, confirmed the following findings disclosed already in stage 1: very strong effect of toluene on locomotor activity, biphasic character of changes observed after its administration and non-linear dose-effect relationship (inverted U). At the same time they indicated that the administration of TMB isomers also stimulated motor behaviour, but this effect, albeit significant, was generally much lower than the effect of toluene, dose-effect relationship was hardly pronounced, and time-effect relationship impossible to define. In addition, the results of stage 1 suggest that TMB isomers do not differ between themselves with regard to the magnitude and character of influence imposed on locomotor activity.

Biochemical assays

The solvent concentrations in peripheral blood (μmol/l) after oral administration are given in Fig. 2. The parameters were analysed for significance by two-way analysis of variance (ANOVA), separately for each dose (solvent × time points) and for each solvent (dose time points).

Stage 1. A comparison of compound-related magnitude and dynamics of changes in blood concentrations

A dose of 0.008 mol/kg. Both the kind of solvent and the measurement time point influenced significantly individual determination values (solvent effect:



* – $p < 0.0001$ compare to trimethylbenzene isomers

Fig. 2. Blood concentration of toluene and trimethylbenzene isomers following acute exposure at doses of 0.008 mol/kg, 0.016 mol/kg and 0.032 mol/kg.

$F(3,12) = 5.34$; $p < 0.02$; time point effect: $F(1,12) = 6.88$; $p < 0.05$). Dynamics of changes in blood concentration of a given solvent was related to the kind of compound administered (solvent × time point interaction: $F(3,12) = 18.51$; $p < 0.0001$). Significant differences occurred at time point 5 ($F(3,84) = 4.27$; $p < 0.005$), time point 6 ($F(3,84) = 9.51$; $p < 0.0001$), and time point 7 ($F(3,84) = 8.01$; $p < 0001$). At these three time points, rats injected with toluene showed significantly increased solvent blood concentration as compared with that of any of TMB groups. Between-time points comparisons in individual groups revealed differences but only in the toluene group in which solvent blood concentration was increasing gradually after the administration reaching its peak (about 600 μmol/l) at time point 6.

A dose of 0.016 mol/kg. Both the main factors and their interaction were significant. In between-group comparisons at individual time points, the toluene group could be easily distinguished. Beginning with time point 5, the values of solvent blood concentration in the toluene group of rats were significantly higher than those in TMB groups. Between-time points comparisons in individual groups demonstrated significant differences in the toluene group ($F(6,72) = 17.02$; $p < 0.0001$), and – as opposed to the effects of the 0.016 mol/kg dose – in the mesitylene group ($F(6,72) = 4.13$; $p < 0.002$). However, the changes (increase) in solvent concentrations at consecutive time points were more dynamic in the toluene group, and in the final time point (70 min after the administration) the concentration was twice as high as in the mesitylene group.

A dose of 0.032 mol/kg. The main factors as well as interaction were significant (group factor effect: $F(3,12) = 5$; $p < 0.02$; time point effect: $F(1,12) = 37.82$, $p < 0.001$; interaction: $F(3,12) = 35.73$, $p < 0.0001$). Beginning with time point 3 the values of solvent blood concentrations were significantly higher in the toluene group than in TMB groups. At the final time point (70 min after the administration) the mean solvent blood concentration accounted for 1001.90 μmol/l in the toluene group; 302.82 μmol/l in the pseudocumene group; 429.18 μmol/l in the hemimellitene group; and 512.85 μmol/l in the mesitylene group. After the solvent administration, its blood concentration was increasing gradually in all groups, but comparisons between consecutive time points revealed significant differences only in the three groups: toluene ($F(6,72) = 33.66$; $p < 0.0001$); hemimellitene ($F(6,72) = 5.27$; $p < 0.001$); and mesitylene ($F(6,72) = 8.19$; $p < 0.00001$).

Stage 2. A comparison of dose-related magnitude and dynamics of changes in solvent blood concentrations

Toluene. The effect of time points ($F(1,9) = 20.53$; $p < 0.002$), and interaction ($F(2,9) = 6,12$; $p < 0.03$) appeared significant. Between-group comparisons revealed significant differences in all groups, but toluene blood concentration was significantly higher at a dose of 0.008 mol/kg at the last two time points; at a dose of 0.016 mol/kg at the last three time points; and at a dose of 0.032 mol/kg at time points 3–7 as compared with time points 1 and 2. This result indicated a significant relationship between solvent concentration dynamics (increase) and the dose.

Pseudocumene. With regard to pseudocumene mean values of solvent blood concentrations at the final measurement time point for doses of 0.008, 0.016 and 0.032 mol/kg accounted for 137.55, 157.18 and 302.8 μmol/l, respectively. Measurement time effect ($F(1,9) = 9.61$; $p < 0.02$) and the dose × time interaction

($F(2,9) = 7.24$; $p < 0.02$) were significant. Between-time points comparisons revealed significant differences for the 0.0016 and 0.032 mol/kg doses: $F(6,54) = 2.82$; $p < 0.02$, and $F(6,54) = 9.22$; $p < 0.0001$, respectively. With regard to the 0.016 mol/kg dose the solvent concentrations were significantly higher at time points 3 and 6 than at time point 1, and in the case of the 0.032 mol/kg these values were increased more significantly at time points 3–7 than in time points 1 and 2.

Hemimellitene. An analysis of main effects did not show any significance of the dose effect, although time effect and interaction were significant (time effect: $F(1,9) = 23.51$; $p < 0.001$; interaction: $F(2,9) = 16.50$; $p < 0.001$). Between-group comparisons indicated significant differences at time point 5 ($F(2,63) = 3.47$; $p < 0.05$), time point 6 ($F(2,63) = 11.66$; $p < 0.001$) and time point 7 ($F(2,63) = 6.22$; $p < 0.01$). At time point 5, in the 0.008 mol/kg group, and at time points 6 and 7 also in the 0.016 mol/kg group the solvent concentration was significantly higher than that in the 0.032 mol/kg group. In the 0.016 and 0.032 mol/kg groups the differences were statistically significant (the former group: $F(6.54) = 5.45$; $p < 0.001$); the latter group: $F(6.54) = 24.67$; $p < 0.0001$). In the 0.016 mol/kg group the solvent blood concentration was significantly higher at time points 6 and 7 than that measured at time point 1. In the 0.032 mol/kg group concentrations measured at time points 5, 6 and 7 were significantly higher than those found at time points 1, 2 and 3.

Mesitylene. Both time effect and interaction were significant (time effect: $F(1,9) = 17.74$; $p < 0.01$); interaction: $F(2,9) = 12.40$; $p < 0.005$). Between-group comparisons revealed significant differences at time point 5 ($F(2,63) = 3.83$; $p < 0.05$); time point 6 ($F(2,63) = 4.86$; $p < 0.02$) and time point 7 ($F(2,63) = 6.00$; $p < 0.005$). In all these groups the solvent blood concentrations in the 0.032 mol/kg rats were significantly higher than those in the remaining ones. Between-time points comparisons disclosed significant differences in the 0.016 mol/kg ($F(6,53) = 5.61$; $p < 0.001$) and 0.032 mol/kg ($F(6,54) = 17.66$; $p < 0.0001$) groups. In the 0.016 mol/kg group, the values determined at time points 5, 6 and 7 were significantly higher than those obtained at time point 1; at time points 6 and 7 they were also higher than at time point 2. In the 0.032 mol/kg group the values determined at time points 4–7 were significantly higher than those measured at time points 1 and 2; and the values determined at time points 5–7 were higher than those obtained at time points 1, 2 and 3; the values of time point 7 were again significantly higher as compared with these measured at time points 1–4.

DISCUSSION

The comparison of changes in general behaviour after oral administration of solvents under study indicated significant differences between acute behavioural effects of toluene and TMB isomers, particularly with regard to the doses of 0.008 and 0.016 mol/kg. The changes in animal behaviour observed after the administration of toluene at these doses – mobility excitation with signs of aggression – recalled the changes observed after the administration of amphetamine what evidenced psychostimulating properties of this solvent (4). Not existing or slight increase in locomotor activity and lack of emotional changes after the administration of any of TMB isomers suggest that these compounds do not act as stimulants,

at least at the doses applied in our experiments. A dose of 0.032 mol/kg did not produce any activation effect. The non-linear dose-effect relationship as well as biphasic character of changes in locomotor activity after a systematic administration or during inhalation exposure are characteristic of many anaesthetic agents and organic solvents, including toluene (1,2). Their occurrence in our experiment confirmed our rightful choice of toluene as a model compound, and indicated that the method applied was sensitive enough to disclose this kind of effects. In this context, the results of comparisons between effects of toluene and those observed after the administration of TMB at the same doses assume even particular importance. As indicated, although TMB isomers exert a stimulating effect on locomotor activity, it is by several times weaker than that of toluene, and noticeable only after the administration of the highest doses of hemimellitene and mesitylene. As opposed to toluene, after TMB administration the time-effect relationship was not clear-cut. It appears that the effect of TMB on locomotor activity differs from that of toluene, at least at doses applied in our investigations. None of TMB isomers at a dose of 0.008 or 0.0016 mol/kg stimulated locomotor activity in the open field. It is also most likely that apparent increase in this activity after the administration of TMB at the highest dose (0.0032 mol/kg) was associated rather with discomfort arising from difficulty in breathing or balance maintaining experienced by animals than with a direct stimulating effect of a given solvent on the systems involved in the movement initiation or performance.

Apart from different effect on locomotor activity, TMB isomers also differ from toluene in respect to post-injection blood concentration, although these differences seem to be only quantitative. At all doses applied, toluene concentration values were usually significantly higher (almost or over twofold) at the final post-injection time points than TMB concentrations in relevant groups of animals. TBM groups did not differ from each other. Similar relations between toluene and TMB concentrations were also observed earlier in rabbits and rats after i.p. injection (10,11). The differences between toluene and TMB blood concentrations after the administration of the same doses may result from considerably higher, compared to toluene, TMB affinity to tissues or lower affinity to blood or from the combination of these two factors. The studies carried out by Zahlsen et al. (19) showed that after a 12-h inhalation exposure to pseudocumene at the concentration of 100 ppm, TMB level in the brain was over two times higher, and in the liver, kidneys and fat tissue almost three times higher than toluene concentration in the same exposure conditions. This may suggest that the transfer of pseudocumene and possibly of other TMB isomers from blood to tissues is more effective than that of toluene.

The fact that there were no significant differences between the mean values of solvent blood concentrations in rats after the administration of different doses of toluene deserves special mention. The comparisons showed that the volume of a dose determined only the dynamics of increase but it did not influence the solvent blood concentration. With regard to pseudocumene this effect was also insignificant and in the mesitylene and hemimellitene groups it was marked only at the final three time points. The results reported suggest that even the lowest dose of each solvent among those applied was high enough to induce its blood concentration at the saturation level.

To sum up, the results indicate that none of orally administered trimethylbenzenes is (at least at doses applied) characterised, at the lowest dose, by a biphasic effect on locomotor activity, i.e. excitation changing into depression. Of the three TMB isomers under study only mesitylene displays some noticeable stimulating effect on locomotor activity in the open field. However, this effect occurs exclusively at the highest dose (among those applied) which induces somatic and autonomous signs of intoxication and causes death in part of animals. Moreover, the effect observed is small in comparison with that noted after the administration of toluene at the lowest dose.

One of possible reasons for the difference between toluene and TMB effects, observed in our investigation, could be the choice of doses applied. Relatively high mortality in groups of rats with the highest doses of TMB and no deaths in the relevant toluene group confirms a considerably higher level of trimethylbenzene toxicity (9,10). As to toluene, the strongest stimulating effect on general behaviour and locomotor activity in the open field test was noted after the lowest dose, while at the highest dose (0.032 mol/kg) a depressant effect predominated. If trimethylbenzenes exert stronger effect than toluene (9,10), it is possible that even the lowest doses of these compounds (among those applied in our experiment) appeared too high to release a stimulating effect. Nevertheless, our studies (the work in preparation for publication) carried out with use of these compounds at doses of 0.002 and 0.004 mol/kg do not confirm these assumptions (lack of stimulating effect). A conclusion which seems to be indisputable is that the kind and magnitude of behavioural changes depends on the solvent concentration in the brain.

REFERENCES

1. Bowen SE, Balster RL. Effects of inhaled 1,1,1-trichloroethane on locomotor activity in mice. Neurotoxicol Teratol 18: 77–81, 1996.
2. Evans EB, Blaster RL. CNS depressant effects of volatile organic solvents. Neurosci Biobehav Rev 15: 233–241, 1987.
3. Fiserova-Bergerova V, Diaz ML. Determination and prediction of tissue-gas partition coefficients. Int Arch Occup Environ Health 58: 75–87, 1986.
4. Glowa JR. Comparisons of some behavioral effects of d-amphetamine and toluene. Neurotoxicol 8: 337–248, 1987.
5. Gralewicz S, Wiaderna D, Tomas T, Rydzyński K. Behavioral changes following 4-week inhalation exposure to pseudocumene (1,2,4-trimethylbenzene) in the rat. Neurotoxicol Teratol 19: 327–333, 1997 (a).
6. Gralewicz S, Wiaderna D, Tomas T. Retardation of the age-related increase in spontaneous cortical spike-wave discharges (SWD) in rats after a 28-day inhalation exposure to an industrial solvent, pseudocumene (1,2,4-trimethylbenzene). Int J Occup Med Environ Health 10: 213–222, 1997 (b).
7. Hinman DJ. Biphasic dose-response relationship for effects of toluene inhalation on locomotor activity. Pharmacol Biochem Behav 26: 65–69, 1987.
8. Järnberg J, Johanson G. Liquid/air partition coefficients of the trimethylbenzenes. Toxicol Ind Health 11: 81–88, 1995.
9. Korsak Z, Rydzyński K. Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats. Int J Occup Med Environ Health 9: 341–349, 1996.
10. Korsak Z. Świercz R. Rydzyński K. Toxic effects of acute inhalation exposure to 1,2,4-trymethylbenzene (pseudocumene) in experiment animals. Int J Occup Med Environ Health 8: 331–337, 1995.

11. Laham S. 1.10 Hemimellitene. Ethel Browning's Toxicity and Metabolism of Industrial Solvents, 2nd edition, vol. 1: 137–142, 1987.
12. Laham S. 1.8 Mesitylene. Ethel Browning's Toxicity and Metabolism of Industrial Solvents, 2nd edition, vol. 1: 121–128, 1987.
13. Laham S. 1.9 Pseudocumene. Ethel Browning's Toxicity and Metabolism of Industrial Solvents, 2nd edition, vol. 1: 129–136, 1987.
14. Tomas T, Świercz R, Wiaderna D. Changes in bioelectric function of rabbit cerebral cortex after intraperitoneal injection of organic solvents. I. Toluene and m-xylene. Med Pr 2: 161–175, 1997 (*in Polish*).
15. Tomas T, Świercz R, Wiaderna D. Changes in bioelectric function of rabbit cerebral cortex after intraperitoneal injection of organic solvents. I. Pseudocumene and 4-ethylotoulene. Med Pr 3: 307–315, 1997 (in Polish).
16. Wiaderna D, Gralewicz S, Tomas T. Behavioural changes following 4-week inhalation exposure to hemimellitene (1,2,3-trimethylbenzene) in rats. Int J Occup Med Environ Health 11: 319–334, 1998.
17. Winer BJ. Statistical principles in experimental design. Ed. McGraw Hill, New York, 1962.
18. Wood RW, Colotla VA. Biphasic changes in mouse motor activity during exposure to toluene. Fundam Appl Toxicol 14: 6–14, 1990.
19. Zalsen K, Eide I. Nilsen AM, Nilsen OG. Inhalation kinetics of C6 to C10 aliphatic, aromatic and naphtenic hydrocarbons in rat after repeated exposure. Pharmacol Toxicol 71: 144–149, 1992.

Received for publication: August 6, 1999
Approved for publication: November 2, 1999