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NEUROTOXICITY ASSESSMENT OF SELECTED ORGANIC SOLVENTS BASED ON SPONTANEOUS AND EVOKED CORTICAL AND HIPPOCAMPAL ACTIVITY IN RATS

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Abstract. In a series of acute experiments on rats the potential of toluene, mesitylene, hemimellitene and pseudocumene to affect the CNS function was assessed following an analysis of spontaneous and evoked hippocampal and cortical activity. The electrophysiological examinations were performed on rats with recording electrodes chronically implanted into selected brain structures. Solvent concentration in peripheral blood was determined by gas chromatography combined with the head space technique in rats with no surgical treatment. The experiments revealed significant quantitative differences between hippocampal and cortical EEG after i.p. injections of equimolar doses of the solvents. A relationship was found between the changes in spontaneous EEG and blood concentration of the solvents. Hemimellitene, with the lowest recorded blood level was found to have the highest potential for inducing the CNS effects.

INTRODUCTION

Organic solvents can be characterised by high volatility and lipid solubility, which enhances their absorption in the tissues and their binding to lipids. As the nervous tissue is composed mostly of lipids it is particularly sensitive to solvent toxicity. At low doses the solvents produce a stimulating effect on the neurons while exposure to high concentrations results in an anaesthetic and narcotic effect due to an impaired information flow between neurons.

The assessment of health effects of occupational exposure to organic solvents has been an essential problem for specialists in both occupational health and experimental toxicology, including neurotoxicology. Chronic exposure to the vapours of organic solvents, which are frequently a mixture of different compounds, may lead to long-term or even permanent functional changes in the CNS, manifested by mental and emotional disorders (7). Unfortunately, universal methods for an effective

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evaluation of solvent-induced CNS impairments have not as yet been available. The psychological and behavioural testing are usually performed to this end. Numerous experimental animal and human studies of exposure to organic solvents which employ the recording of spontaneous and evoked bioelectric activity of the brain have not yielded any universal method of assessment.

The purpose of the present study was to compare, applying an analysis of EEG and evoked potentials, neurotoxicity of trimethylbenzene (TMB) isomers with toluene as the reference compound for which adequate literature data are available. The examined TMB isomers are the components of a commonly used organic solvent under the trade name of Farbasol (Płock Refinery, Poland). The quantitative and qualitative composition of this solvent is similar to that of a few other Polish preparations (Polifarb Cieszyn SA) and some imported products, e.g. Jolasol (J.L.C. Chemie, Austria), Shellsol A (Shell Nederland Chemie B.V.), and Solvesso 100 (Exxon Chemical Belgium). Chemical analysis of Farbasol has revealed that trimethylbenzene isomers constitute 44% of the composition (mesitylene – 11%; pseudocumene – 29.3%; hemimellitene – 3.6%) (12).

The literature on the neurotoxic effects (EEG, evoked potentials) of exposure to organic solvents is very scant. In Poland the maximum admissible concentration (MAC) for trimethylbenzene isomers has been determined to be 100 mg/m^3 which corresponds to the MAC value for tolune and xylene isomers (8). Acute experiments indicate that the irritative effect of TMB vapours on the respiratory tract is 4–8 times as high as that of xylene and toluene (4,5). The proposed MAC value for TMB, based on the acute irritative effect on the respiratory tract in mice, is 50 mg/m^3 (3).

The studies of TMB neurotoxicity, involving motor coordination and pain threshold tests, indicated that TMB isomers produced a neurotoxic effect which was several times more intense compared to that of xylene or toluene. The highest neurotoxic potential was found for hemimellitene (2,6). In the experiments on the bioelectric activity of rabbit cortex after i.p. injection of solvent solutions, the EEG analysis revealed that pseudocumene produced a stronger CNS effect than m-xylene, toluene or 4-ethyltoluene (10,11).

In order to investigate the potential of TMB isomers to induce functional changes in the CNS, we decided to compare the neurotoxic effects of particular compounds and relate the findings to toluene neurotoxicity as the reference, under similar experimental and methodological conditions.

MATERIALS AND METHODS

Chemicals

For the experiments three TMB isomers: 1,3,5-TMB (mesitylene, 99%, CAS 108-67-8); 1,2,4-TMB (pseudocumene, 97%, CAS 95-63-6); 1,2,3-TMB (hemimellitene, 90–95%, CAS 526-73-8) by Fluka (Switzerland) and toluene (99.9% CAS 108-88-3) by POCH (Poland) were used. The solvents were dissolved in olive oil and administered to rats as i.p. injections. That was performed at a two-week intervals, starting on different days for each rat. The rats selected for the determination of blood concentrations of the solvents were divided into 4 groups of 4 rats each and dosed with 6.6 mmol/kg b.w. of a given solvent. The optimum dose was determined in a pilot study.

Experimental animals and treatment

The electrophysiological examinations were performed on 20 white male Wistar outbred rats: Imp: WIST, weighing 400 – 500 g, which had recording electrodes implanted bilaterally in the fronto-parietal cortex and hippocampus (1). Determination of solvent concentration in peripheral blood was carried out in 16 male rats, 455 – 540 g b.w., with no surgical treatment.

Electrophysiological examinations

Subject to assessment was the spontaneous (EEG) and evoked (EP) bioelectric activity of the cortex and hippocampus after acute exposure to organic solvents under study. The examinations started after 30 days of convalescence. Prior to the experiment the animals were tested for the morphology and magnitude of evoked potentials according to the intensity of the stimulus.

Evoked collosal potentials.

Evoked potentials of the cortical and hippocampal derivations were induced by stimulating the contralateral areas of the cortex and hippocampus with 100 – 500 μA rectangular pulses generated every 5 sec and lasting 0.2 ms. The effects of exposure to particular solvents were assessed by analysing averaged EPs from 30 consecutive records. EPs were recorded before administration (reference potential) as well as 30 and 60 min after injection.

Spontaneous bioelectric activity (EEG)

Spontaneous bioelectric activity of the fronto-parietal cortex and hippocampus was recorded at five one-min intervals; hard and soft copies of the records were made at the same time.

Experimental conditions and apparatus

The following stages of the experiment can be distinguished:

1. Control stage:
   (a) recording of pulse-evoked potentials (30 pulses with present intensity, every 5 sec, lasting 0.2 ms; registration time – 250 ms),
   (b) EEG recording and spectral analysis – five one-min sessions.
2. Experimental stage, after solvent injection:
   (a) recording of averaged EPs 30 and 60 min after injection; stimulation and recording parameters as in the control stage,
   (b) EEG recording and on-line spectral analysis at time intervals as in the control stage.

Toxicological examinations

Biochemical testing – determination of blood solvent concentration 30 and 60 min after i.p. injection.

The EEG and EP recordings were performed on the same animal during a single experiment. The animals stayed at their home cages throughout. The recording apparatus was installed in an adjacent room and so was the in-house TV system

which allowed monitoring the animal during the experiment. The bioelectric activity of the brain was recorded by an 8-channel electroencephalograph (ORMED). The electric pulses were generated by a digital pulse stimulator CB-1 (University of Łódź). The outputs of the EEG apparatus amplifiers were connected to the measuring unit of the computerised system for EEG recording and analysis (IMP-NUMERICA). The central unit was 486 IBM PC.

EEG analysis

The applied system for EEG recording and analysis made possible an automatic determination of the power spectrum in the frequency band from 1.0 to 32 Hz. The spectral analysis of signals was carried out using the computer software and the results were saved on hard disk. In the general analysis, the frequency bands making up the highest-percentage components of the total power spectrum, were considered. EEG of the fronto-parietal cortex was analysed in the following frequency bands: 1–3.75 Hz; 4–7.75 Hz; 13–20.75 Hz and of the hippocampus in 1–3.75 Hz; 4–6.75 Hz; 7–9.75 Hz bands, respectively. For the statistical analysis the 13–20.75 Hz bands were selected for the cortical activity, and the 1–3.75 Hz and 7–9.75 Hz bands for the hippocampal activity.

EP analysis

The evoked bioelectric activity of the fronto-parietal cortex was assessed by analysing N1 wave and P1 – N1 wave in averaged commissural potentials (n = 30 averaged records). For the hippocampal activity N1 wave was analysed. Both types of the wave were present in all records from the same site of the brain. Wave latency was the main criterion for their identification.

Determining blood concentration of the solvents

Blood samples were collected from a tail vein into heparinised glass capillary of 100 μl vol. 30 and 60 min after i.p. injection of the solvent. Gas chromatography combined with the head space technique was used for the quantitative determination of toluene or TMB (9). The determinations were performed by a Hewlett Packard 5890 gas chromatograph with a flame-ionization detector (FID) and HP-1 capillary column, 30 m long, 0.53 diam. with stationary phase filter of 2.65 mm. Working parameters: (a) injection chamber temp. 200°C, (b) isoterm temp. 100°C, (c) FID temp. 250°C, (d) carrier gas – argon, (e) gas flow – 10 cm^3/min, (f) injection chamber mode – SPLITLESS

Statistical analysis

The results were subject to a statistical analysis using a two-way ANOVA (13).

RESULTS

Fig. 1 displays sample EP records from the fronto-parietal cortex and hippocampus. After stimulation, two distinct types of waves could be observed – electropositive P1 and electronegative N1. Their latencies approximated 20 ms and 16 ms for the cortical and hippocampal records, respectively.

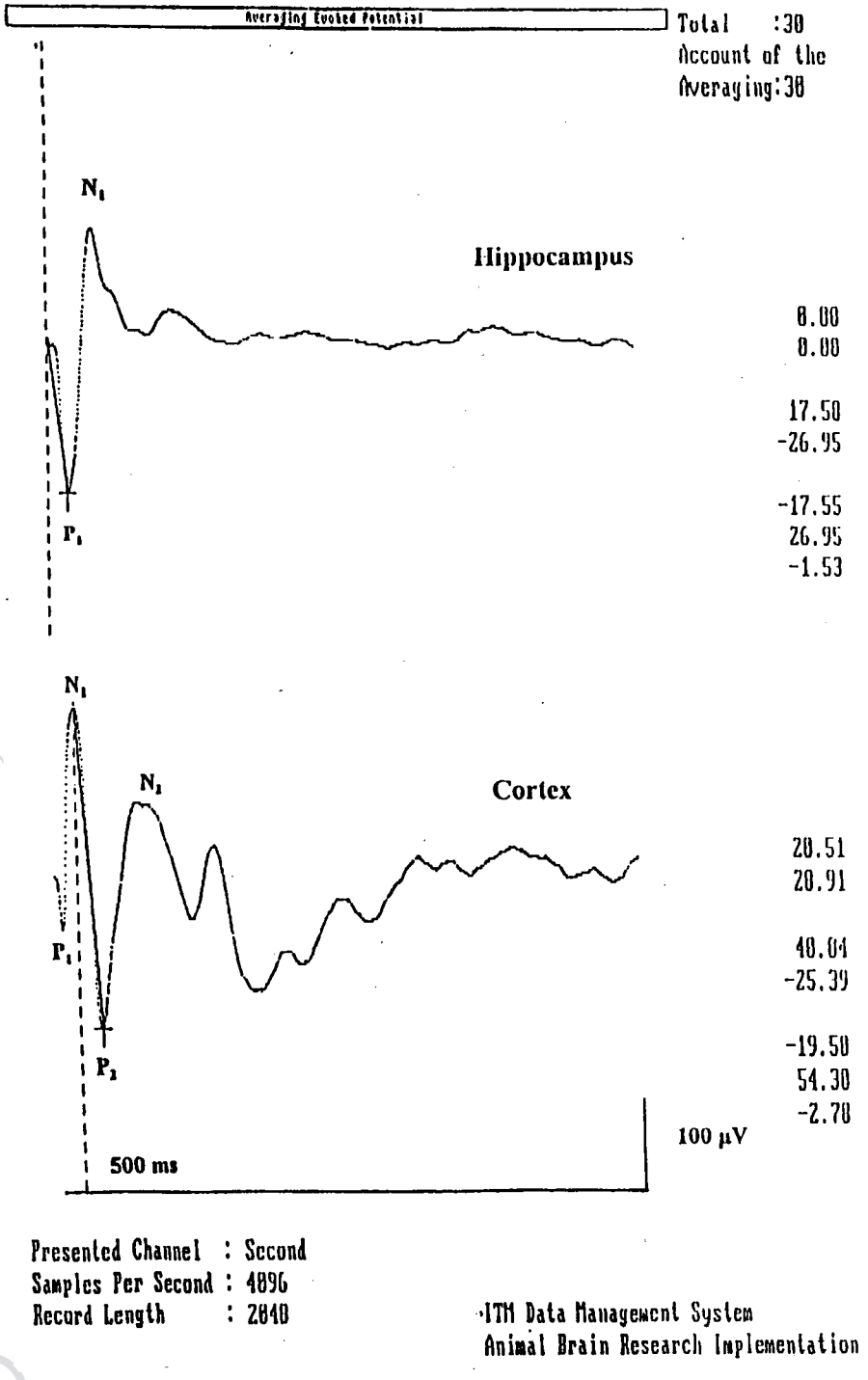

Fig. 1. Sample, averaged (n = 10), evoked potentials from the fronto-parietal cortex and hippocampus.

Amplitude abnormalities in N1 and P1 – N1 waves for cortical activity, and N1 wave for hippocampal activity after i.p. injection of particular solvents were recorded twice 30 and 60 min after injection and compared with the pre-injection values. The results obtained showed that: toluene induced an increase in N1 wave of 7.0% after 30 min and of 12.4% after 60 min on average; mesitylene of 7.0% and of 13.9%, respectively; pseudocumene showed a reverse effect and induced a decrease of 6.5% after 30 min and 2.6% after 60 min; hemimellitene induced a decrease of 8.2% after 30 min and an increase of 4.1% after 60 min (Fig.2).

Toluene induced an increase in cortical P1 – N1 wave of 41.1% after 30 min and 5.9% after 60 min; mesitylene of 2.9% and 11.8%, respectively. The administration of pseudocumene and hemimellitene resulted in decrease of 2.3% and 4.4% after 30 min and a slight increase of 1.6 and 0.8%, respectively after 60 min (Fig.3). Toluene induced an increase in hippocampal N1 wave of 28.5% after 30 min and a decrease of 31.0% after 60 min. For all other solvents a decreased N1 amplitude

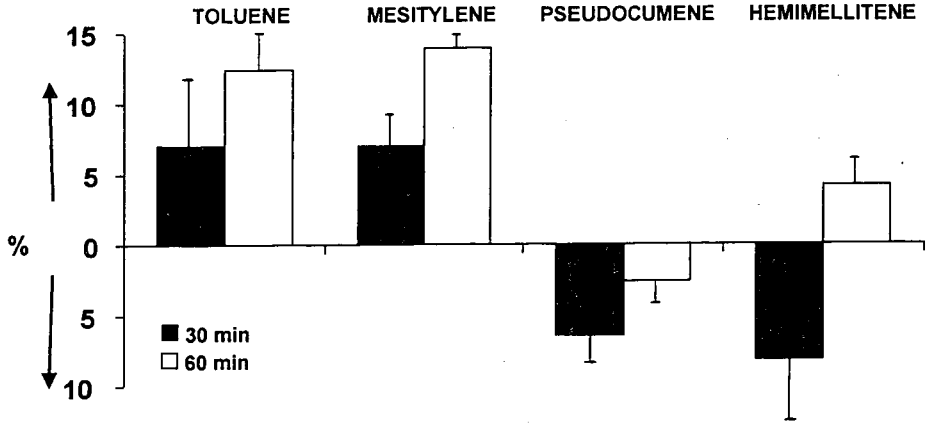

Fig. 2. Amplitude abnormalities of the cortical N1 wave 30 and 60 min after i.p. solvent injection.

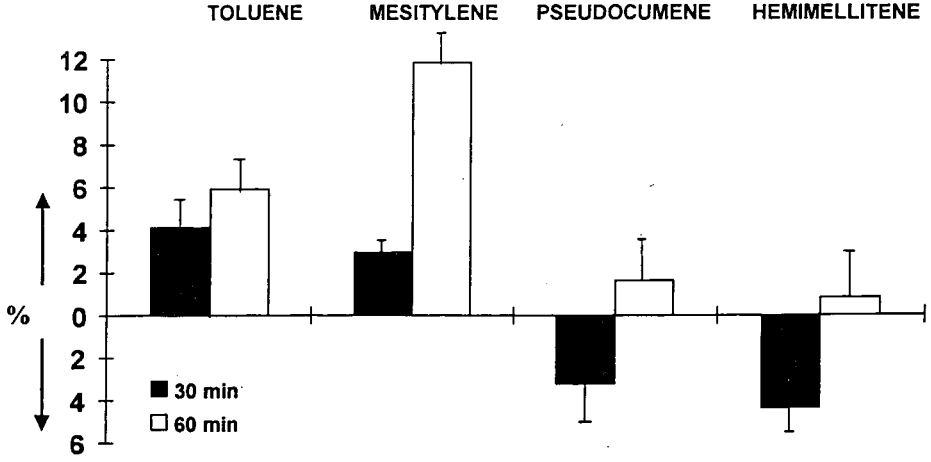

Fig. 3. Amplitude abnormalities of the cortical P1-N1 wave 30 and 60 min after i.p. solvent injection.

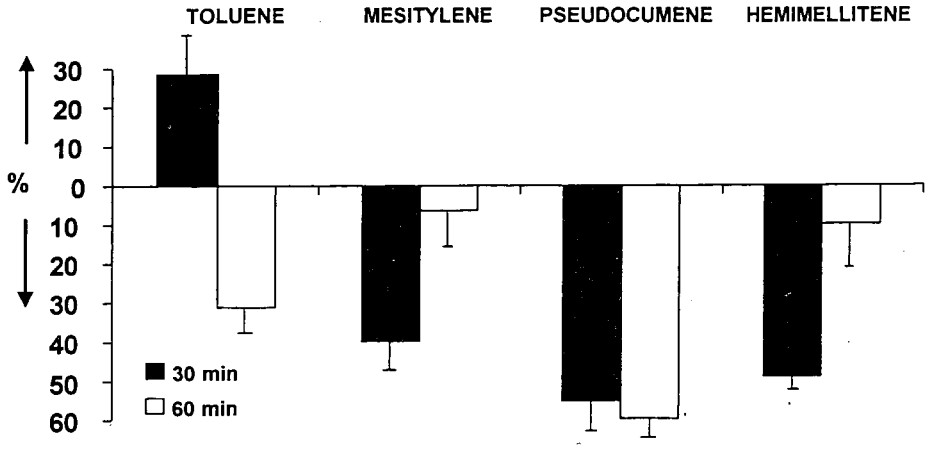

Fig. 4. Amplitude abnormalities of the hippocampal N1 wave 30 and 60 min after i.p. solvent injection.

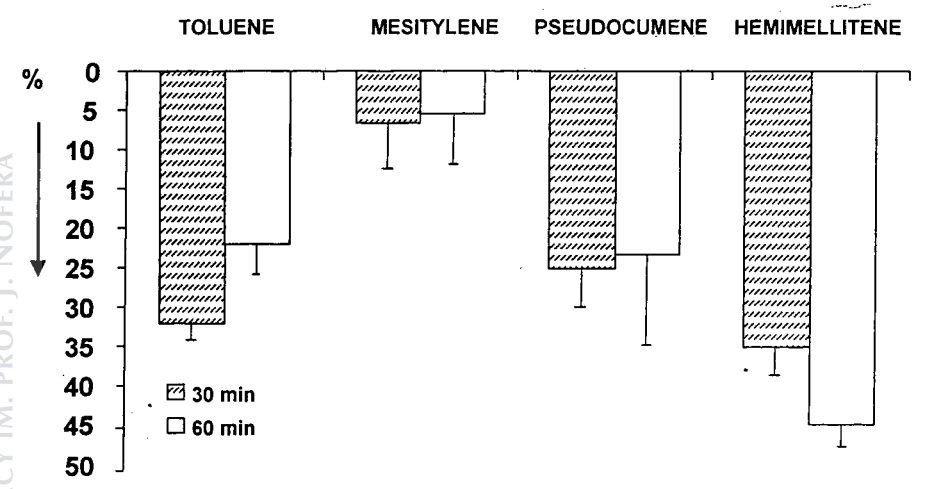

Fig. 5. The effect of i.p. solvent injection on the cortical EEG in the 13–20.75 Hz frequency band.

was found in both records. Mesitylene induced a decrease as high as 38.0% after 30 min and very low of 6.4% after 60 min, whereas the decrease induced by pseudocumene was very high in both records, 55.1% and 59.6%, respectively (Fig. 4).

The effects of administered solvents on the cortical EEG in the 13–20.75 Hz frequency band are presented in Fig. 5. The analysis of major effects revealed a significant influence of the time factor ($F(1.2) = 11.59$, $p < 0.001$) 30 min (TP I) and 60 min (TP II) after exposure. The compound x time interaction appeared to be statistically significant ($F(3.2) = 12.76$, $p < 0.001$). The comparison of bioelectric activity at particular recording periods, carried out separately for each compound, indicated significant differences for pseudocumene ($F(2.4) = 4.49$, $p < 0.01$) and hemimellitene ($F(2.4) = 9.35$, $p < 0.05$). For these two compounds the contribution of the 13–20.75 Hz band to the power spectrum was significantly lower both 30 and 60 min after injection, compared to the pre-exposure records.

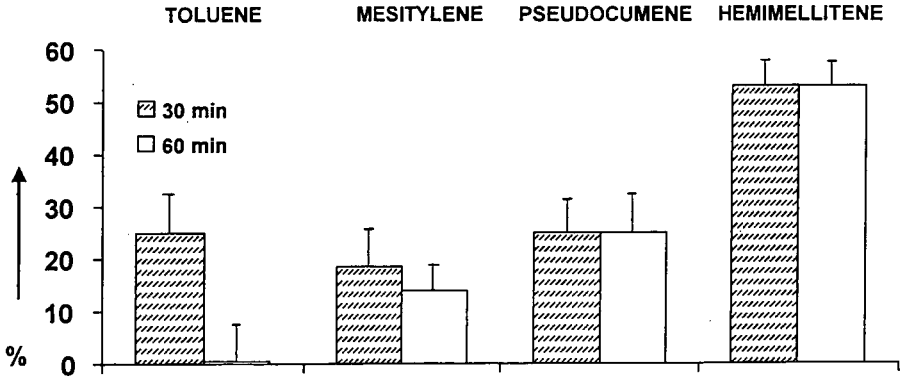

Fig. 6. The effect of i.p. solvent injection on the hippocampal EEG in the 1–3.75 Hz frequency band.

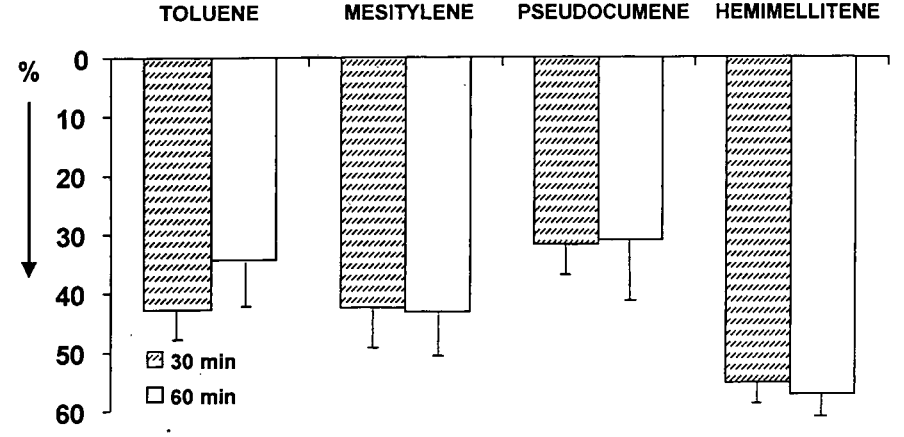

Fig. 7. The effect of i.p. solvent injection on the hippocampal EEG in the 7–9.75 Hz frequency band.

Fig. 6 displays the solvent exposure effects on hippocampal EEG in the 1–3.75 Hz band. The assessment of main effects revealed a significant influence of the time factor on the level of activity in this band ($F(3.20) = 18.05$, $p < 0.001$). When the levels of activity at particular recording periods were compared, a statistically significant increase ($F(2.40) = 12.63$, $p < 0.001$) was found only for hemimellitene.

The effects of the examined solvents on hippocampal EEG in the 7–9.75 Hz band are illustrated in Fig. 7. A statistically significant decrease ($F(1.20) = 75.17$ at $p < 0.01$) in the activity level could be noted for all the solvents both 30 min (TP I) and 60 min after injection. The compound x time interaction was found to be significant ($F(3.20) = 13.02$, $p < 0.001$). The comparison of the activity levels at particular recording periods revealed significant differences between the control stage and the post-exposure records for toluene ($F(2.40) = 15.07$, $p < 0.001$), mesitylene ($F(2.40) = 18.39$, $p < 0.001$), pseudocumene ($F(2.40) = 10.94$, $p < 0.001$) and hemimellitene ($F(2.40) = 35.11$, $p < 0.001$).

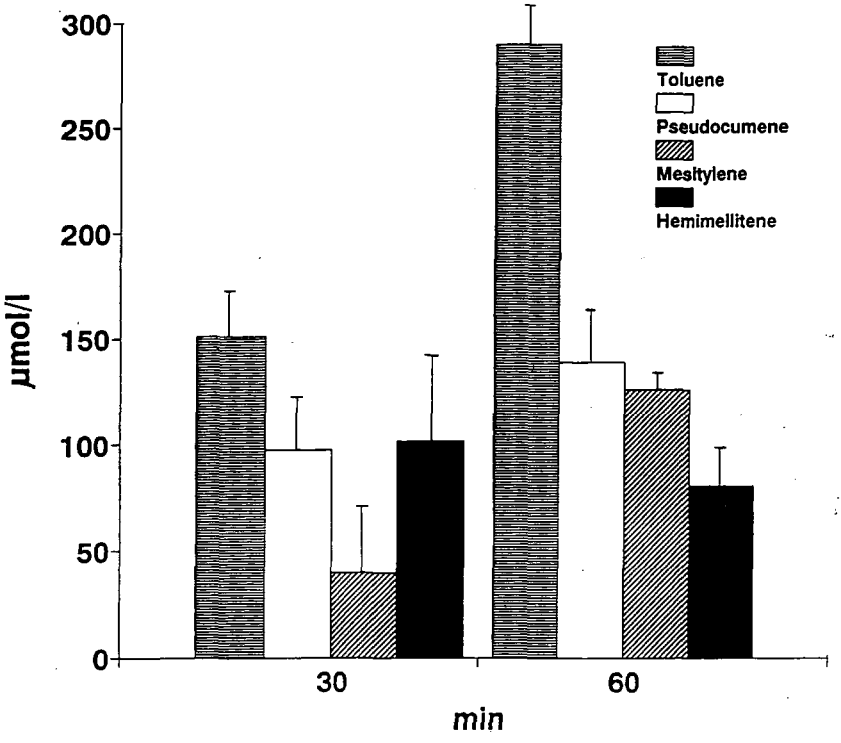

Fig. 8. Blood concentrations of organic solvents after i.p. injection of 6.6 mmol/kg b.w. to rats (n = 4).

The results of the determination of blood solvent concentration after a single i.p. injection are listed in Fig. 8. The mean $\pm$SD blood concentrations of toluene, pseudocumene, mesitylene and hemimellitene accounted for, 151.2 ± 78.3; 97.4 ± 47.3; 39.7 ± 17.7; 101.7 ± 15.9 µmol/l, respectively 30 min, and 289.6 ± 128.3; 138.8 ± 39.5; 125.4 ± 72.6; 80.4 ± 31.1 µmol/l 60 min after injection. Statistical analysis revealed significant differences between the groups of rats exposed to different solvents ($F(3.12) \pm 4.60$, $p < 0.05$). Blood concentrations of mesitylene and hemimellitene turned out to be lower than that of toluene. Significant differences were found between blood levels of the same solvents 60 min and 30 min after exposure ($F(1.12) < 22.97$, $p < 0.01$). The solvent x time interaction appeared to be statistically significant ($F(93.12) = 63.60$, $p < 0.01$). Sixty min after injection a significant decrease in the blood concentration of pseudocumene, mesitylene and hemimellitene, could be noted when compared to the level of toluene ($F(3.24) = 7.96$, $p < 0.01$). For toluene and mesitylene a more significant increase in the blood concentration ($F(1.12 = 29.50$, $p < 0.01$); ($F(1.12) = 11.33$, $p < 0.01$, respectively) was found after 60 min (TP II) than after 30 min following the injection.

DISCUSSION

Considering the morphology of EPs generated by direct electric stimulation of the brain, the dominating effects after solvent injection were the amplitude

abnormalities of N1 and P1-N1 waves and hippocampal N1 wave, however, they were statistically insignificant. The decreasing, albeit insignificant, amplitudinal tendency referring to both the cortical and hippocampal components did not definitely confirm the depressive effects of the solvents on the central nervous system. Their impact on EP morphology was negligible and diverse.

At least two reasons may account for the above. Firstly, the cortical and hippocampal EP could be a very quick monosynaptic response to an external stimulus, in this case to an electric shock. In a statistical analysis these responses are usually affected by pharmacological or physical confounding factors. Secondly, the absence of distinct significant differences may have been associated with individual characteristics resulting from different location of electrodes, their resistance etc.

Among the examined frequency bands in the hippocampal EEG, recorded after the administration of equimolar doses of the solvents, the most evident differences were noted in the 1–3.75 Hz band. The contribution (percentage) of the power spectrum for this band to the total power spectrum was found to reach the highest level after i.p. injection of hemimellitene and then successively, of pseudocumene, mesitylene and toluene. Less explicit differences could be observed in the hippocampal 7–9.75 Hz frequency band. In this case the i.p. injection of the solvents produced a decrease in the power spectrum for this band, being most evident for hemimellitene. The administration of the other three solvents induced similar however, less pronounced effect.

The analysis of cortical EEG in the 13–20.75 Hz frequency band revealed that the highest decrease in the power spectrum could be noted 60 min after hemimellitene injection (45%). As for pseudocumene and mesitylene, the power spectrum for this band at the same moment decreased by 22 and 5, respectively when compared to the pre-injection values.

Generally, the EEG records support a hypothesis that the solvents examined exert a depressive effect on the CNS functions. Both the cortical EEG for 13–20.75 Hz band and the hippocampal EEG for the bands of 1–3.75 Hz and 7–9.75 Hz revealed that the most significant EEG abnormalities followed the administration of hemimellitene. These results, accompanied by similar findings for EP examination, indicate that hemimellitene exhibits the highest potential for affecting the CNS functions. Weaker effects in this respect were found successively for pseudocumene, mesitylene and toluene. Hemimellitene methyl groups configuration at benzene ring is not stable. It causes that the reaction of addition or oxidation of methyl group at position C_2 may take place easier than in the case of pseudocumene or mesitylene. It may be suspected that pseudocumene and mesitylene exert toxic effect similar to that of hemimellitene but at a lower concentration.

The comparison of electrophysiological findings with the results of determining blood concentrations of the solvents made us conclude that the highest impact on the CNS functions is manifested by the solvent with the lowest blood concentration, namely hemimellitene. The weakest neurotoxicity was exhibited by toluene for which the highest blood level was recorded. Further evidence derives from the rabbit study where pseudocumene with the lowest blood concentration appeared to have the highest neurotoxic potential (10,11). A plausible explanation for the lower concentration of hemimellitene, pseudocumene and mesitylene in blood, compared

to that of toluene, is their lower capacity for passing from the peritoneum into the circulatory system or better penetration through blood-brain barrier. Both the possibilities could be verified by comparing EEG records after a direct intravenous injection of each solvent and determination of its concentration in the brain tissue.

The data obtained from the present study made it possible to draw the following conclusions:

1. Intraperitoneal administration of organic solvents: toluene and trimethylbenzene isomers i.e. mesitylene, pseudocumene and hemimellitene, induces abnormal morphology of the evoked potentials in the cortical and hippocampal EEG. The effect on the evoked bioelectric activity is not homogenous or significant. EEG abnormalities provide evidence for the depressive effect of organic solvents on the central nervous system. Among all the examined solvents, hemimellitene exhibits the highest potential to affect the CNS functions.

2. The determination of blood concentrations of the solvents following i.p. injections of their equimolar doses revealed that the strongest effect on the CNS functions is produced by the solvent with the lowest blood concentration (hemimellitene).

3. The method employed in our study enables monitoring of the functional changes in the CNS of the animals exposed to toxic chemicals.

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