International Journal of Occupational Medicine and Environmental Health, Vol. 12, No 2, 105–117, 1999

ORIGINAL PAPERS

LONG-TERM BEHAVIOURAL EFFECTS OF A REPEATED EXPOSURE TO CHLORPHENVINPHOS IN RATS*

RENATA SOĆKO[1], SŁAWOMIR GRALEWICZ and ROMAN GÓRNY

[1] Department of Scientific Information
The Nofer Institute of Occupational Medicine
Łódź, Poland

Key words: Organophosphorus compounds, Chlorphenvinphos, Rat, Acetylcholinesterase, Open field, One-way active avoidance, Hot-plate

Abstract. The aim of the present study was to investigate persistent neurobehavioural effects of repeated low-level exposure to chlorphenvinphos ((2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate – CVP) in rats. The rats received 10 i.p. injections of CVP at daily doses of 0.5 mg/kg or 1.0 mg/kg (one injection/day, five days/week) which corresponded to 1/20 and 1/10 of LD_{50}, respectively, for this species. In a part of the rats, cholinesterase (ChE) activity in blood (plasma and erythrocytes), and in the selected brain regions was determined at arbitrarily chosen time after the last exposure. The determinations showed that the level of ChE inhibition was dose-related, but the compartments studied differed in the magnitude of this effect. The differences in the level of ChE inhibition between the compartments were particularly evident in rats which had received CVP at the 1.0 mg/kg dose; in these animals 3 h after exposure the ChE activity in erythrocytes, plasma and the brain corresponded to 78%, 48% and 67–70%, respectively, of the control value. Enzyme activity returned to the control level after 14 days in plasma and after 35 days in erythrocytes. In rats receiving CVP at daily doses of 0.5 mg/kg, ChE activity in plasma was decreased by 40.8% and that in erythrocytes by 21.4% 3 h after the last exposure. The activity of ChE in plasma returned to the control level within four days and that in erythrocytes within 14 days. In these rats, in all the brain regions studied except brainstem, ChE activity was not reduced significantly.
In rats selected for behavioural tests, the following behavioural aspects were investigated: response to novelty in an open field, acquisition and extinction of a one-way active avoidance response, and the magnitude and persistence of the footshock-induced analgesia (hot-plate test). Testing in the open field was performed before the exposure and then 1, 3 and 6 weeks after the last exposure. The remaining tests were performed after the exposure. The interval between testing and the last CVP injection was sufficient for recovery of ChE activity. It has been found that in rats of both exposure

* The results of the study were presented at the 6th Meeting of the International Neurotoxicology Society INA-6, June 29–July 4, 1997, Szeged, Hungary and published in the Int J Occup Med Environ Health 4: 375–394, 1997.

Address reprint requests to Dr. R. Soćko, Department of Scientific Information, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Łódź, Poland.

groups the response to novelty in the open field, i.e. the increase in locomotor and exploratory activity in the presence of a new object, was reduced, albeit nonsignificantly, compared to the unexposed animals. In rats which received CVP at the dose of 1.0 mg/kg, acquisition of the one-way active avoidance response was facilitated. No differences between groups were found during extinction of this response. In the hot-plate test, in rats exposed repeatedly to 1.0 mg/kg CVP, the footshock-induced increase in the latency of the paw-lick response to heat (54.5°C) was stronger and more persistent than in the unexposed animals. The above results show that some neurobehavioural effects of exposure to organophosphorous (OP) compounds may be detected after a time sufficient for recovery of ChE activity.

INTRODUCTION

Organophosphorus (OP) compounds are widely used throughout the world as insecticides. The exposed groups include manufacturers, formulators, applicators and the general population. The biocidal properties of OP are due to their ability to disrupt cholinergic neurotransmission by irreversible inhibition of acetylcholinesterase (AChE). There are some experimental data showing that neurobehavioural disturbances resulting from OP exposure are reversible. For example, Costa et al. (7) have shown on mice that memory impairments caused by exposure to disulphoton disappeared when the AChE activity returned to a normal level. However, clinical and epidemiological observations suggest that, in people with a history of acute OP exposure, some neurobehavioural disturbances may be detected long after full restitution of AChE activity (8,12,15,16). Moreover, in humans (9) and in monkeys (4), changes in EEG were observed months or even years after an acute OP exposure. There is no convincing evidence however, that such long-term effects may result from a repeated exposure to OP at doses producing no overt acute symptoms.

In our earlier experiments we have found that even a single exposure of the rat to CVP at a dose of 3.0 mg/kg producing overt acute symptoms (immobility, tremor, salivation, diarrhoea) may result in some neurobehavioural alterations detectable after the restoration of AChE activity in blood and in the brain. The observed changes in behaviour suggested an increased fearfulness and/or increased reactivity to environmental stressors. Some effects (a noticeable reduction of the response to novelty) were also detected in rats receiving CVP at a dose producing no overt symptoms (1.0 mg/kg). The purpose of the present work was to find out whether repeated exposure to CVP at doses producing no overt acute behavioural symptoms (1.0 mg/kg or 0.5 mg/kg) could result in behavioural alterations after the restoration of AChE activity in blood and in the brain.

MATERIALS AND METHODS

Subjects. The experiments were performed on male Wistar rats (Imp:DaK, outbred) 90–120 days old. The animals were housed in groups, six rats per cage, in standard laboratory conditions (temperature: 20–22°C, humidity: 50–60%, L/D cycle 12/12 h with light on at 6 a.m.), and fed with standard rat chow and water *ad libitum*.

Enzyme determinations and behavioural studies were performed on separate groups of rats.

Insecticide. Chlorphenvinphos was obtained from the manufacturer (ORGANIKA-AZOT, Jaworzno, Poland) in the form of a concentrate consisting of 92% CVP (cis- and trans- isomers at 1:8 ratio) and about 8% impurities (enolophosphates). Before use, it was dissolved in warm (about 40°C) sterile olive oil in order to obtain the required concentration.

Determination of cholinesterase activity

Ninety seven animals used in this part of the experiment were divided into three groups: control group and two exposed groups. Each rat was given ten i.p. injections within a period of two weeks (one injection/day, five days a week). The rats were injected with pure olive oil (control group) or oil-diluted CVP at 1.0 mg/kg (group 1.0 mg/kg) or 0.5 mg/kg (group 0.5 mg/kg). This is equivalent to about 1/10 and 1/20 LD_{50} for the rat. The volume of the injected solutions was 1 ml. Samples of animals from each group (consisting of 3–8 animals) were killed by decapitation at a pre-selected time after the exposure (3 h, 4, 7 and 14 days in the 0.5 mg/kg group and 3 h, 4, 7, 14, 21, 35, 42 and 49 days in the 1.0 mg/kg group). The killing was performed at 11–12 a.m. in all cases. For the determination of serum cholinesterase (sChE) and red blood cells acetylcholinesterase (rbcAChE) activities, samples of blood flowing out from the cut vessels were collected. The brain was removed from the skull as quickly as possible and dissected on ice into five parts. Each part was weighted with 0.001 g accuracy and homogenised in a glass Potter-Elvehjen's homogeniser with the addition of cold Sorensen's buffer (0.066 M, pH 8.0). The volume of the buffer as well as the volume of the homogenate samples taken for enzyme determination were adjusted on the basis of earlier determinations of AChE activity in a given part of the brain (19). The activity of enzymes in blood and brain homogenates was determined by spectrophotometry using acetylcholine iodide as substrate and ditiobisnitrobenzoic acid (DTNB) as chromogen (20).

Behavioural investigations

The rats used for the behavioural tests were divided into three groups: two exposed groups and a control group (each consisting of 10 subjects). At the beginning of the experiment the animals were handled 10 min/day for four days. The handling was performed in the same room in which the behavioural tests were performed. This was followed by a two-week injection period. During this period the rats received ten i.p injections (one injection/day except Saturdays and Sundays) of CVP diluted in olive oil in a dose of 1.0 mg/kg/day (1.0 mg/kg group) or 0.5 mg/kg/day (0.5 mg/kg group) or olive oil alone (control group). The behaviour of rats in home cages was observed at least once a day, usually 30 min after the injection, during the whole injection period. Body weight was measured once a week till the end of the experiment.

Open field behaviour. The 'open field' used in the present studies consisted of a white square arena, (100 · 100 cm), surrounded by walls, 20 cm high, and divided into 49 squares (14.3 · 14.3 cm) with black lines painted on the floor. A 25 W bulb, located 100 cm above the centre of the arena, served as the source of light during the testing. The animals were tested in the open field every second day (three times a week with 48 h intervals between sessions) in the week preceding the injection period, and then in weeks 1, 3 and 6 following the last injection. Each

daily session started with placing the rat into middle of the open field. In open arena the rat was observed for five min. During the last three sessions in the open field the rats were presented with a new object (a cylindrical box of 7.0 cm in diameter), placed in the middle of the arena. The measured variables were: the number of squares crossed (locomotor activity), and the number of rearings (exploratory activity).

One-way active avoidance. The testing of one-way active avoidance response was performed in a box composed of two identical compartments (32 · 54 · 24 cm). The floor of each compartment was made of stainless steel rods connected in parallel with the output of an electric shock generator. The compartments were separated by a wall with a small (8 cm · 7 cm) opening secured by a hinged door. The opening was cut out in the middle of the wall at its bottom edge.

The testing of one-way active avoidance response was composed of two phases: the acquisition phase and the extinction phase. Each phase was composed of three daily sessions. During the acquisition phase each trial started from placing the rat in the left compartment. Five seconds later, a 1000 Hz tone (a conditioned stimulus — CS) from a loudspeaker located in the middle of the back wall was switched on. If the rat did not enter the right compartment within 5 sec from the tone onset it received electric footshocks (unconditioned stimulus — US), one per two seconds, via the metal rods of the floor. Each footshock consisted of a series of three rectangular 2.5 mA, 20 msec pulses applied at 3.0 Hz frequency. The CS and US remained on until the rat escaped to the right compartment. Ten seconds later it was transferred to a 'waiting cage' where it remained for 25—40 sec before the next trial. Each day the rat was trained to a criterion which consisted of four avoidances (entries into the right compartment before the US onset) in five consecutive trials. The acquisition rate was assessed on the basis of the number of trials to criterion and the number of footshocks received by the rat in successive daily sessions.

The extinction phase started two days after the end of the acquisition phase. Like during the acquisition phase, each trial during extinction started from placing the rat in the left compartment. The required response, however, consisted in staying in this compartment for 10 sec with CS on. Each entry into the right compartment during CS presentation was punished by electroshock until the rat returned to the left compartment. After a 10-sec staying in the left compartment the CS was turned off and the rat was transferred to the waiting cage for 25—40 sec. On each day of extinction the procedure was continued until the rat reached the criterion: no response, i.e. entering the right compartment, during 10-sec CS presentations in four out of five consecutive trials. The extinction was assessed from the number of trials to criterion and the number of footshocks received in successive daily sessions.

The testing of acquisition and extinction of the active avoidance response was performed on days 47 to 55 after the last injection.

Hot-plate behaviour. The equipment consisted of a hot-plate and a shock chamber. The hot-plate was a flat rectangular copper box (350 · 350 · 35 mm) filled with water. The water temperature was kept constant at 54.5°C (±0.2). A plastic translucent cylinder (280 mm diameter, 250 mm height) with a top cover formed an enclosure preventing the rat from stepping off the plate. The shock chamber was a tight (100 · 200 · 400 mm) equipped box with a metal grid floor which could be electrified.

The test was performed twice, i.e. 59 and 60 days after exposure. It consisted of placing the rat on the hot-plate within the plastic enclosure. After the occurrence of the expected response — licking the foot — or, in the case the licking had not

occurred, after 60 sec of staying on the plate, the animal was transferred into the shock chamber where it received trains of electric footshocks, one every 5 sec for 2 min. The train duration was one second, the frequency of electric pulses in the train – 5 Hz, pulse duration – 6.5 msec, and the current intensity – 3.5 mA. Immediately after shocking, the rat was transferred into the hot plate again. The latency of the paw-lick response before shocking (L1), after shocking (L2) and 24 h after the footshock (L3) was carefully measured with a stop-watch. The stop-watch was switched on at the moment when the rat, being placed into the plastic enclosure, touched the hot plate with four paws. It was switched off immediately after the first lick at the foot of the right or left hind limb had been noticed.

Statistical analysis. In the case of cholinesterase activities (absolute values) the differences between groups were estimated with the use of the nonparametric (blood) or parametric (brain) one-way Kruskal-Wallis, ANOVA and Sheffe's test (17,21). The data obtained from behavioural experiments were analyzed with a parametric two-way ANOVA and the Tukey's test (21).

RESULTS

Biochemical results

The results of ChE (AChE) determinations after repeated i.p. exposure to CVP are presented in Tables 1 and 2.

In both exposed groups the values of successive determinations of sChE differed significantly (in the 1.0 mg/kg group: $X^2 = 35.614$, df = 8, $p < 0.001$, and in the 0.5 mg/kg group: $X^2 = 11.581$, df = 4, $p < 0.05$). Three hours after the last CVP injection, sChE activity was 52% (SE $\pm$ 3.2) and 59.2% (SE $\pm$ 6.2) in the 1.0 mg/kg and 0.5 mg/kg groups, respectively. In the 1.0 mg/kg group, sChE activity returned to the normal level by day 14 after the last exposure, in the 0.5 mg/kg group within 24 h.

Differences between successive determinations were also significant for rbcAChE (the 1.0 mg/kg group: $X^2 = 39.122$, df = 8, $p < 0.001$; the 0.5 mg/kg group: $X^2 = 13.795$, df = 4, $p < 0.05$). Three hours after the last exposure the enzyme activity was 78.6%, SE $\pm$ 7.1 in the 0.5 mg/kg group, and 22.1%, SE $\pm$ 1.8 in the 1.0 mg/kg group. In the 0.5 mg/kg group rbcAChE activity returned to normal level by day 14 after the last injection and that in the 1.0 mg/kg group by day 35.

The analysis of the brain AChE data showed that in the 0.5 mg/kg group the values of successive determinations differed significantly only in the case of the brainstem ($F_{4,13} = 6.543$, $p < 0.001$); 3 h after the last exposure the enzyme activity was 75.7% (SE $\pm$ 6.9) of the control value, and returned to the normal level by day 7. In the remaining brain regions the changes were small and the differences between successive determinations insignificant. In the 1.0 mg/kg group, significant differences between successive determinations were found in all brain regions studied (diencephalon: $F_{8,49} = 21.049$, $p < 0.001$; hippocampus: $F_{8,52} = 31.425$, $p < 0.001$; frontal pole: $F_{8,51} = 21.084$, $p < 0.001$; cerebellum: $F_{8,51} = 13.288$, $p < 0.001$; lower brainstem: $F_{8,52} = 38.496$, $p < 0.001$). Three hours after the last injection the lowest AChE activity was noted in the hippocampus (33.4%, SE $\pm$ 4.3), and in the frontal pole (33.0%, SE $\pm$ 1.5). AChE activity returned to normal level by day 4 in the cerebellum, day 14 in the hippocampus and day 21 or 35 in the remaining brain regions studied.

Table 1. Changes in the blood and brain ChE (AChE) after repeated exposure to CVP. Dose: 0.5 mg/kg

Time after exposure		Blood (U/litre)		Brain (U/gram)				
		plasma	erythrocytes	cerebellum	diencephalon	hippocampus	brainstem	anterior part of the hemisphere
Control	U	186.1 ± 11.2	1136.4 ± 28.0	4.5 ± 0.1	11.7 ± 0.2	11.5 ± 0.3	13.4 ± 0.1	23.2 ± 0.1
n = 21								
3 h	U	110.2 ± 7.1*	892.7 ± 45.4*	3.4 ± 0.1	9.5 ± 0.5	10.5 ± 0.4	10.1 ± 0.3**	17.2 ± 1.2
n = 4	%	59.2 ± 6.2	78.6 ± 7.1	75.4 ± 5.5	81.5 ± 7.1	91.2 ± 7.7	75.7 ± 6.9	74.1 ± 6.7
96 h	U	251.3 ± 8.6	743.3 ± 52.4*	4.3 ± 0.2	9.8 ± 0.2	10.9 ± 0.3	11.0 ± 0.6**	17.3 ± 0.7
n = 4	%	135.0 ± 5.3	65.3 ± 5.3	95.4 ± 11.3	83.4 ± 2.0	95.0 ± 3.2	82.2 ± 5.3	74.5 ± 3.3
168 h	U	179.5 ± 18.1	806.7 ± 93.3*	4.6 ± 0.1	9.6 ± 0.4	9.5 ± 0.5	10.1 ± 0.4**	17.0 ± 1.2
n = 4	%	96.4 ± 11.2	71.0 ± 9.5	90.4 ± 4.4	81.8 ± 4.6	82.3 ± 5.0	75.6 ± 3.6	73.4 ± 5.2
336 h	U	239.7 ± 15.8	1131.0 ± 25.2	4.6 ± 0.1	11.2 ± 0.4	10.7 ± 0.2	12.5 ± 0.2	21.9 ± 0.2
n = 3	%	128.8 ± 8.0	101.7 ± 2.7	101.8 ± 4.0	95.4 ± 4.0	92.9 ± 2.6	90.9 ± 1.3	94.3 ± 7.1

The numbers in the table represent mean values and mean standard errors. U – direct value of ChE activity (international units).

% – relative value of ChE activity (in per cent of the control)

* $p < 0.05$ compared to the control group (one-way ANOVA and Sheffe's test),

** $p < 0.01$ compared to the control group (one-way ANOVA and Sheffe's test).

Table 2. Changes in the blood and brain ChE (AChE) after repeated exposure to CVP. Dose: 1 mg/kg

Time after exposure		Blood (U/litre)		Brain (U/gram)				
		plasma	erythrocytes	cerebellum	diencephalon	hippocampus	anterior part of the hemisphere	brainstem
Control n = 21	U	206.3±11.2	1056.1±32.4	4.4±0.1	12.5±0.3	11.8±0.2	24.4±0.2	13.7±0.2
3 h	U	114.0±10.6*	234.0±19.3*	2.0±0.2*	4.7±0.4*	3.9±0.5*	8.0±0.3*	4.9±0.7*
n = 3	%	52.0± 3.2	22.1± 1.8	44.6±4.9	37.6±3.9	33.4±4.3	33.0±1.5	35.7±5.0
96 h	U	174.0± 5.3*	550.0±48.4*	3.9±0.3	9.1±0.2*	7.9±0.2*	16.7±0.6*	9.7±0.4*
n = 4	%	84.3± 2.5	52.1± 4.6	89.9±6.9	72.5±2.1	65.7±2.2	68.5±2.4	70.6±2.8
168 h	U	137.2± 6.7*	442.2±17.0*	3.8±0.2	8.0±0.1*	7.3±0.4*	15.9±0.3*	9.7±0.2*
n = 4	%	66.7± 3.2	41.9± 1.6	86.4±3.2	64.0±1.2	62.3±4.0	65.2±1.2	70.7±1.5
336 h	U	243.0±13.1	701.0±28.3*	3.7±0.1	9.0±0.5*	9.1±0.5*	17.7±0.8*	11.3±0.5*
n = 5	%	117.8± 6.3	65.3± 2.6	83.4±3.5	72.0±3.8	77.4±4.9	72.5±3.4	82.4±4.0
504 h	U	221.0±18.8	832.0±50.5*	4.1±0.3	9.9±0.6*	9.8±0.4	16.7±1.9*	11.5±0.4*
n = 5	%	107.3± 9.1	78.5± 4.8	93.6±6.1	79.2±5.7	83.0±3.8	68.5±8.0	83.2±3.4
840 h	U	214.0±14.3	1080.0±63.0	4.4±0.1	11.2±0.2	10.7±0.3	24.2±0.6	12.6±0.1
n = 5	%	103.8± 6.9	102.1± 6.0	99.7±1.5	89.5±2.2	90.7±2.6	100.3±2.7	92.0±1.1
1008 h	U	285.0±26.0	1012.0±66.0	4.1±0.2	12.5±0.5	11.4±0.2	23.8±2.1	14.1±0.3
n = 6	%	137.9±12.7	95.8± 6.2	93.6±5.1	100.0±4.4	96.9±1.9	95.4±9.7	103.1±2.4
1176 h	U	235.0±24.0	1003.0±41.0	4.9±0.1	13.0±0.3	13.2±0.3	26.9±1.3	15.9±0.6
n = 8	%	113.7±11.5	95.0± 3.9	116.6±3.9	104.4±3.1	111.6±3.4	110.3±5.1	115.8±4.7

The numbers in the table represent mean values and mean standard errors. *U* – direct value of ChE activity (international units).

% – relative value of ChE activity (in per cent of the control).

* $p < 0.001$ compared to the control group (one-way ANOVA and Sheffe's test).

Behavioural results

Body weight. Differences between groups in the rate of body weight increase during the experiment were not found (data not shown).

Open field behaviour. A two-way ANOVA (groups × sessions) revealed no significant differences between groups or between sessions in the week before the exposure (data not shown). In the first week after the last injection only the effect of the session factor was significant, both in the case of locomotor activity ($F_{1,27} = 13.38$, $p < 0.01$), and exploratory activity ($F_{1,27} = 5.55$, $p < 0.02$); in all groups the exploratory activity in sessions II and III, and the locomotor activity in session III were significantly higher than in session I ($p < 0.05$ in all cases). Results suggesting an effect of exposure were found only in the data from week 6, where a significant group × session interaction was found in the case of exploration ($F_{2,27} = 5.51$, $p < 0.01$) as well as locomotion ($F_{2,27} = 11.91$, $p < 0.001$). In all groups there were some differences between sessions (Figs. 1 a and b). In the control

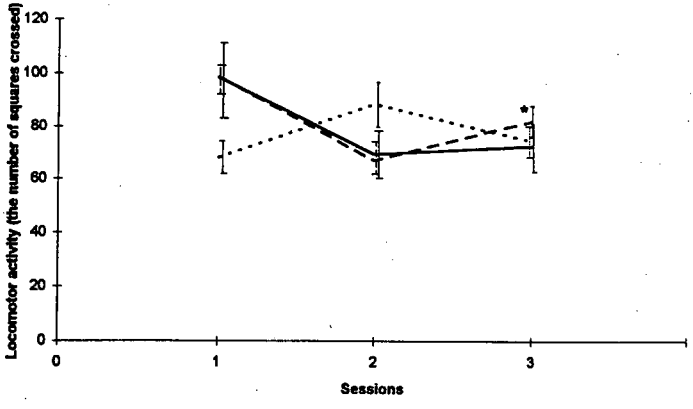

Fig. 1a. Locomotor activity of rats in open field, five weeks after repeated exposure to 0.5 mg/kg or 1 mg/kg chlorphenvinphos.
*$p < 0.05$ compared to session II.

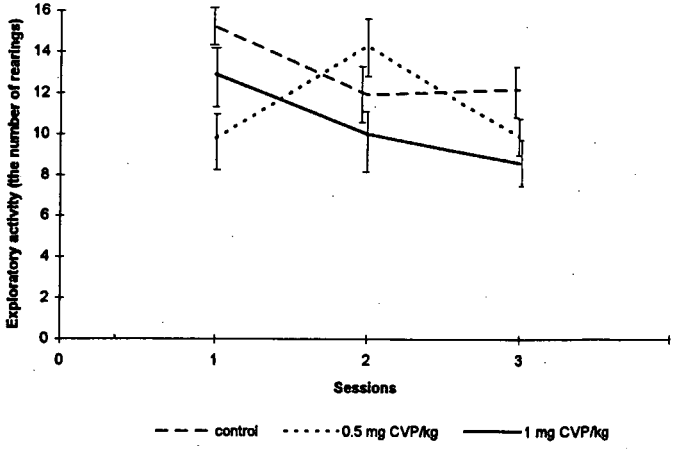

Fig. 1b. Exploratory activity of rats in open field, five weeks after repeated exposure to 0.5 mg/kg or 1 mg/kg chlorphenvinphos.

group the locomotor and exploratory activities in session III (with the new object) were increased. In the case of locomotor activity the increase was significant statistically (compared to session II, $p < 0.05$). On the other hand, in the exposed groups, both the exploratory and locomotor activities were decreased in session III compared to the preceding sessions, but these differences were not significant statistically.

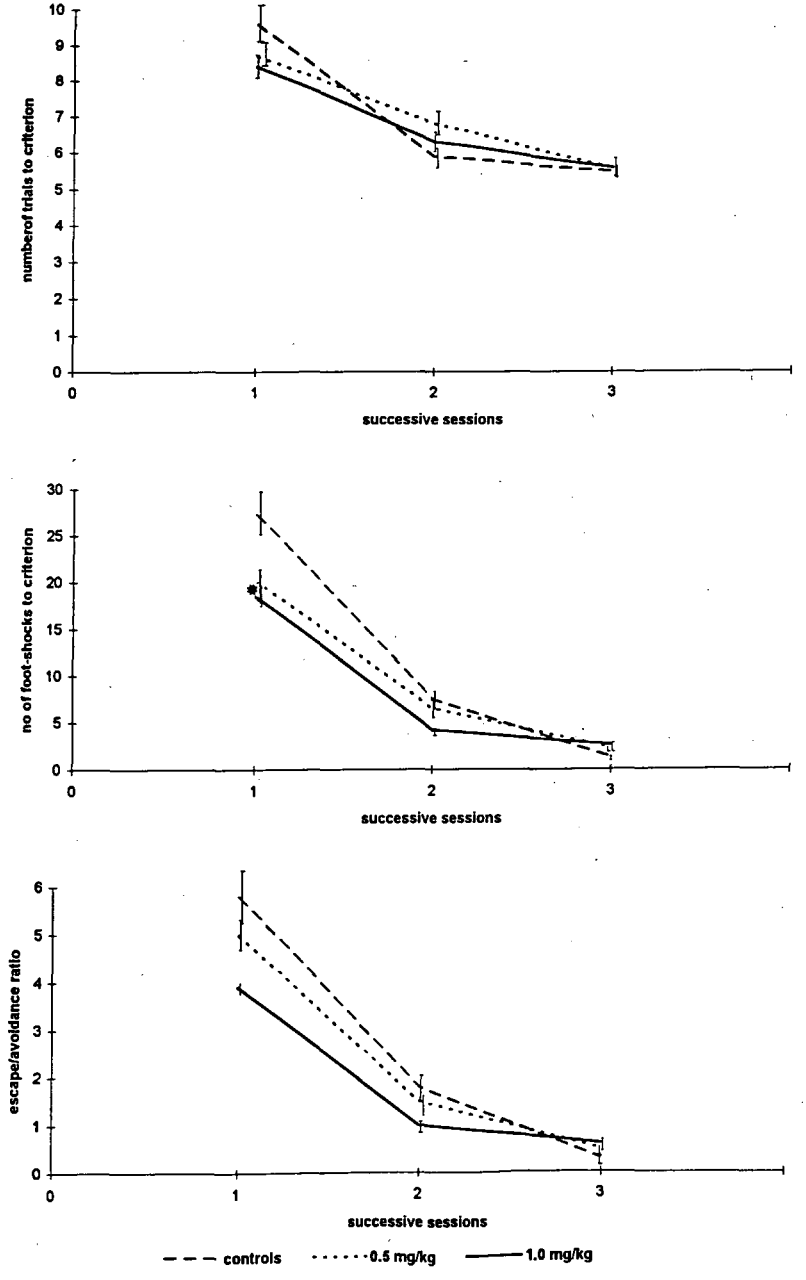

Fig. 2. Acquisition of the active avoidance response in control rats and rats exposed repeatedly to CVP. * $p < 0.05$ compared to the control group

One-way active avoidance: acquisition and extinction. Fig. 2 presents the results of active avoidance acquisition test.

Acquisition. A two-way ANOVA of the number of trials to criterion in successive sessions revealed no effect of the group factor and a significant effect of the session factor ($F_{1,27} = 20.25$, $p < 0.001$); in sessions II and III the number of trials to criterion was significantly smaller than in session I. The groups × sessions interaction was not significant.

For the number of shocks received in successive sessions, the analysis showed a significant effect of the session factor ($F_{1,27} = 70.98$, $p < 0.001$); in session I the number of shocks received by rats was significantly higher compared to sessions II and III. The groups × sessions interaction was also significant ($F_{2,27} = 6.45$, $p < 0.05$). Consequent detailed comparisons showed that in session I the exposed rats received fewer shocks than the control animals but only in the case of the 1.0 mg/kg group this difference was significant statistically ($p < 0.05$). In other words, it was easier to force the exposed rats to escape than the control ones.

The analysis of the escapes/avoidances ratio revealed a significant effect of the session factor ($F_{1,27} = 61.45$, $p < 0.001$) – generally, the value of this ratio in session I was significantly higher than in sessions II and III – and a significant groups × sessions interaction ($F_{2,27} = 4.59$, $p < 0.05$). The consequent comparisons showed that in session I the value of the escape/avoidance ratio in group 1.0 mg/kg was significantly smaller than in the control group.

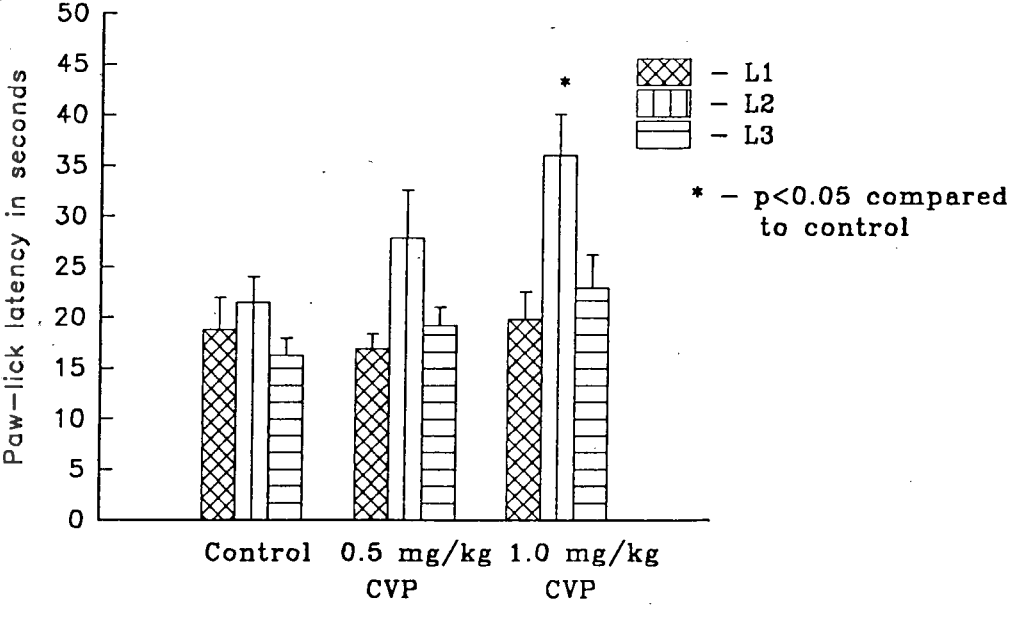

Fig. 3. Diagrams illustrating the effect of a 2-min intermittent footshock on the latency (expressed in seconds) of the paw-lick response to heat in control rats and rats exposed repeatedly (ten times) to CVP at a daily dose of 0.5 mg/kg or 1.0 mg/kg. The bars represent group means and SE. L1, L2 and L3 – paw-lick latencies before footshock, immediately after the footshock and 24 h after the footshock, respectively.

Extinction. The analysis of the results from the extinction part have revealed that in the case of all assessed parameters only the effect of the session factor was statistically significant; in all groups in session I the numbers of trials to criterion and the number of shocks received were significantly higher than in the remaining sessions ($p < 0.001$ in all cases). The effect of the group factor and the groups × sessions interaction were not significant (data not shown).

Hot-plate behaviour. The results of this test are shown in Fig. 3. The analysis of paw-lick latencies before shocking (L1), immediately after shocking (L2) and a day after shocking (L3) revealed a significant effect of time ($F_{1,27} = 14.53$, $p < 0.05$) and significant groups × time interaction ($F_{2,27} = 7.67$, $p < 0.05$). Consequent repeated comparisons showed no differences between groups with respect to L1 and L3. However, L2 appeared to be significantly longer in the 1.0 mg/kg group compared to the control group ($p < 0.05$)

DISCUSSION

In our previous studies we have found that after a single i.p. exposure to CVP at a dose of 3.0 mg/kg producing overt acute behavioural and vegetative symptoms of OP intoxication (immobility, salivation, urination, diarrhoea), or of 1.0 mg/kg not producing such symptoms, subtle changes in the rat behaviour may be detected after a time sufficient for complete or almost complete recovery of AChE activity in blood and in the brain. In the case of the 3.0 mg/kg dose, these changes consisted in a depression of the response to novelty in the open field and, compared to control animals, a stronger and more durable increase in paw-lick latency after footshock in the hot-plate test. In the case of the 1.0 mg/kg dose only a depressed response to novelty was observed (19) . The results of the present studies show, however, that an increased effect of footshock on the hot-plate behaviour may be also detected in rats which received CVP at this dose, i.e 1.0 mg/kg, repeatedly. Moreover, these rats show a facilitated acquisition of the one-way active avoidance, an effect which was not mentioned earlier. It should be stressed again that, judging from the biochemical data, both effects were detected when the level of AChE activity in blood and in the brain was normal. It may be concluded then, that both a single exposure to CVP at a dose producing acute symptoms of OP intoxication, and repeated exposures at doses not producing such symptoms, can lead to behavioural alterations outlasting the depression of AChE activity in blood and in the brain. Nonetheless, the exposure-induced transient depression of AChE activity in the brain continues to be regarded as the most likely cause of these alterations. The level of AChE inhibition seems to be an important factor determining the occurrence of the persistent behavioural effects. In the present experiment they were detected only in the 1.0 mg/kg group where the AChE activity determined 3 h after the last exposure was inhibited by more than 50% in all compartments studied, but not in the 0.5 mg/kg group where in the none of the compartments the AChE was inhibited by more than 35%.

The present study shows that the brain regions differ in the magnitude of the CVP-induced AChE inhibition as well as in the time of AChE recovery. It was particularly evident in the 1.0 mg/kg group. AChE activity in the cerebellum was less inhibited and needed shorter time to recover than AChE activity in the remaining parts of the brain, particularly in the cortex of the frontal pole.

Considering the known role of the frontal cortex in motivational and attentional processes (10), it is likely that the observed behavioural alterations in the 1.0 mg/kg group were related somehow to the consequences of long-lasting depression of AChE activity in this area, possibly at the level of cholinergic receptors. It should be stressed that the level of inhibition and the course of recovery of rbcAChE and brain AChE (except the cerebellum) were similar, which confirms that after OP exposure the rbcAChE activity is a good indicator of AChE activity in the majority of brain areas. (1,11,13).

The results of our present studies, just like those of our earlier works, (19) seem to suggest that rat rbcAChE and brain AChE are more sensitive to CVP than the rat plasma ChE. This is in contrast to humans, where plasma ChE is a more sensitive enzyme (6,14).

The character of the behavioural alterations found in the rat long after exposure to CVP, either repeated (present studies) or acute, suggest an increased fearfulness. This is concordant with persistent emotional disturbances found in OP-exposed humans; common subjective symptoms are: feeling of anxiety and increased fearfulness (2,18). For a long time already, the cholinergic system has been linked with the control of negative emotional states (5), and many studies have shown that cholinergic agonists administered directly to a variety of brain regions induce behavioural and vegetative symptoms characteristic of a defensive behaviour (3). Thus, the behavioural effects noted in our studies may be interpreted as an evidence of an increased activity, or excitability of cholinergic neurons in some parts of the rat brain. It is well known that a single strong or weak but repeated electrical or chemical stimulation of the brain may lead to a permanent decrease in seizure threshold, a phenomenon known as kindling. It has been shown that months after repeated exposure to some pesticides kindling is being facilitated, which suggests that exposure to these compounds leads to a persistent increase in excitability of some brain structures. According to some authors, persistent hyperactivity of the cholinergic system may be responsible for some EEG alterations (9) and psychological disturbances in persons exposed to OP in the past (2,8,12,15,16,18). However, direct experimental proof of this conjecture is not available.

To sum up, the results of our present studies show that in the rat, repeated low level exposure to CVP, an OP pesticide, may result in behavioural alterations detectable after restoration of AChE activity. Further experiments are needed to characterize the basic behavioural deficits produced by the exposure and the changes within the brain responsible for the deficits.

REFERENCES

1. Ambrose AM, Larson PS, Borzelleca JF, Hennigar GR. Toxicologic studies on diethyl-1-/2,4-dichlorophenyl/-2-chlorovinyl phosphate. Toxicol Appl Pharmacol 17: 323–336, 1970.
2. Bazylewicz-Walczak B, Kędzierski A. Neurobehavioural effects of chronic occupational exposure in workers of a chemical factory. Stud Mat Monogr, IMP Łódź 40: 65–75, 1993 (in Polish).
3. Brudzyński SM, Eckersdorf B. Vocalization accompanying emotional-aversive response induced by carbachol in the cat. Reproducibility and dose-response study. Neuropsychopharmacol 1 (4): 311–320, 1988.
4. Burchfiel JL, Duffy FH, Sim VM. Persistent effects of sarin and dieldrin upon the primate electroencephalogram. Toxicol Appl Pharmacol 35: 363–379, 1976.

5. Carlton PL. Cholinergic mechanism in the control of behavior. In: Psychopharmacology: A Review of Progress. DE Efron, ed. Public Health Service Pub 1836: 1968.
6. Chlorphenvinphos. The rationale for the proposed limits of occupational exposure: A document prepared by the Group of Experts for Chemical Factors. Interdepartment Committee for the Actualization of MAC Values, Poland, 1986 (in Polish).
7. Costa LG, Schwab BW, Hand H, Murphy SD. Reduced [H^3] quinuclidynyl benzilate binding to muscarinic receptors in disulfoton – tolerant mice. Toxicol Appl Pharmacol 60: 441–450, 1981.
8. Dilsaver S. Cholinergic mechanism in depression. Brain Res Rev 11: 285–316, 1986.
9. Duffy FH, Burchfiel JL, Bartels PH, Gaon M, Sim VM. Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol Appl Pharmacol 47: 161–176, 1979.
10. Ehlers CL, Somes C, Lopez AL, Robledo P. Long latency event-related potentials in rats: response of amygdala, nucleus accumbens, dorsal hippocampus and frontal cortex to changes in reward characteristics of conditioned stimuli. Brain Res 780 (1): 138–142, 1998.
11. Geller I, Sawa A, Stavinoha WB. Effects of subchronic soman on avoidance-escape behavior and cholinesterase activities. Neurotoxicol Teratol 9: 377–386, 1987.
12. Gershon S, Shaw FH. Psychiatric sequelae of chronic exposure to organophosphorus insecticides. Lancet 1: 1371–1374, 1961.
13. Gralewicz S, Kowalczyk W, Górny R, Soćko R. Brain electrical activity (EEG) after a repetitive exposure to chlorphenvinphos, an organophosphate anticholinesterase. I. Rabbit. Pol J Occup Med 3: 51–68, 1990.
14. Hunter C.G. Dermal toxicity of chlorfenvinphos (CFVP). Industrial Med 38: 25–27, 1969.
15. Metcalf DR, Holmes JH. EEG, psychological and neurological alterations in humans with organophosphorus exposure. Ann NY Acad Sci 160: 375–391, 1969.
16. Savage EP, Keefe TJ, Mounce LM, Heaton RK, Lewis JA, Burcar PJ. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch Environ Health 43: 38–45, 1988.
17. Siegel S. Nonparametric Statistics for the Behavioral Sciences. Mac-Graw-Hill Book Comp., Inc., New York, 1956.
18. Sińczuk-Walczak H. Condition of the nervous system in the employees of an organophosphorous pesticide plant. Stud Mat Monogr, IMP, Łódź 40: 53–64, 1993 (in Polish).
19. Soćko R, Gralewicz S, Górny R. Neurotoxicity of chlorphenvinphos an organophosphorus pesticide: effect on blood and brain cholinesterase activity, open field behaviour and response to change in a "T" maze in rats. Pol J Occup Med 2 (3): 294–308, 1989.
20. Voss G, Sachsse K. Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined by a modified acetyltiocholine/DTNB procedure. Toxicol Appl Pharmacol 16: 764–772, 1970.
21. Winer BJ. Statistical Principles in Experimental Design. McGraw Hill Book Company, New York, 1962.

Received for publication: September 29, 1998
Approved for publication: April 12, 1999