Polish Journal of Occupational Medicine Vol. 2, No. 3, 1989

NEUROTOXICITY OF CHLORPHENVINPHOS AN ORGANOPHOSPHORUS PESTICIDE: EFFECTS ON BLOOD AND BRAIN CHOLINESTERASE ACTIVITY, OPEN FIELD BEHAVIOR AND RESPONSE-TO-CHANGE IN A "T" MAZE IN RATS

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Key words: Chlorphenvinphos, Cholinesterase, Open field, Response-to-change

Abstract. Cholinesterase (ChE) activity in blood (plasma and erythrocytes) and in different parts of the brain, open field behavior and response-to-change in a "T" maze were investigated in separate groups of rats after a single intraperitoneal exposure to an organophosphorus pesticide — chlorphenvinphos. The doses used were 3.0 mg/kg or 1.0 mg/kg which amounted to approximately, 1/3 and 1/10 of LD_{50} for this species. The exposure resulted in a dose dependent inhibition of ChE in blood, as well as in the brain. No marked differences in the level of ChE inhibition in plasma, erythrocytes, and in selected parts of the brain (cerebellum, brain stem, diencephalon, hippocampus and the anterior part of the hemisphere) were noted after a given dose. In general, 3 hours after the exposure, ChE was inhibited by about 80% in the case of the 3.0 mg/kg dose and by no more than 50% in the case of the 1.0 mg/kg dose. In blood as well as in the brain, the normalization of ChE activity proceeded at a similar rate, being accomplished within 14 days and within 94 hours after the 3.0 mg/kg and 1.0 mg/kg dose, respectively. No changes suggesting an impairment of short-term memory were observed in the "T" maze until the end of testing (i.e. up to the fourteenth day after the exposure). In the open field, a short-term decrease (up to the third day) in locomotor and exploratory activity was observed only in rats exposed to 3.0 mg/kg of the pesticide. At the end of testing (10—14th day after the exposure) the introduction of a new object into the open field resulted in an increase of locomotor and exploratory activity in control animals but not in the rats exposed to chlorphenvinphos in the 3.0 mg/kg as well as in the 1.0 mg/kg. This suggests that exposure to chlorphenvinphos may result in some behavioral disturbances lasting longer than the ChE recovery.

INTRODUCTION

Some clinical and epidemiological data suggest that exposure to organophosphorus compounds (OP) — irreversible inhibitors of cholinesterase — may lead to long-lasting loss of memory (10, 20), emotional

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disturbances (5) and motor impairment (17, 24). Changes in EEG, lasting up to several months after the exposure, have also been observed (6, 18). It has been suggested that at least some of these abnormalities may be due to a persisting functional impairment of the cholinergic system of the brain (6), i.e. system known to be involved in learning and memory and in the control emotional states (1, 2, 5, 13, 16).

In spite of the above data, during the last few years, the interest of toxicologists engaged in OP studies has been concentrated predominantly on delayed neuropathy (24), a peripheral OP effect resulting from the inhibition of an esterase not involved in cholinergic transmission. Less attention has been paid to the functional state of the central nervous system after exposure. What is more, most of the existing experimental data concern the OP-s used as warfare agents (eg. 9, 14, 21), but not those in common use in agriculture and in the home. It is known that OP-s may differ widely with respect to their mammalian toxicity, as well as with respect to their action on cholinergic transmission (7). Thus, conclusions drawn on the basis of studies on a given OP are of limited value.

The present work is part of a broader study concerning 2-chloro-1 (2,4,-dichlorophenyl) vinyl diethyl ester (CVP), an OP insecticide used extensively in agriculture and in the home. To the best of our knowledge, studies on this OP have not extended far beyond routine toxicity testing (3, 15). The mammalian toxicity of CVP differs widely, being highest for the rat. This comes from the ability of the rat brain to accumulate this compound to a concentration markedly exceeding that in blood (15), which makes the rat a useful model for studying the central effects of CVP.

The purpose of the present work was twofold. First, taking into account the data suggesting the accumulation of CVP within the brain, we tried to find out whether brain ChE is inhibited to the same degree as blood ChE after exposure and whether normalization of the enzyme activity proceeds at the same rate in both these areas. Moreover, as the possibility cannot be excluded that not all parts of the brain are equally affected, ChE activity was determined separately in five parts: cerebellum, brain stem, diencephalon, hippocampus and the anterior part of the hemisphere.

The second purpose was to establish whether a single exposure to CVP may influence some more complex forms of behavior, and, if so, what is the relationship between changes in behavior and ChE activity. The behaviors studied were: locomotor and exploratory activity in an open field and response-to-change — a form of alternation — in a „T" maze (4, 19).

MATERIAL AND METHOD

Subjects. The experiments were performed on male Wistar rats (imp-DaK, outbred) 90 — 120 days old. The animals were housed six to a cage in standard laboratory conditions (temperature: 20—22°C, humidity: 50—60%, L/D cycle 12/12 hrs with light on at 6 a.m.), and fed with standard rat chow and water ad libitum. ChE determinations and behavioral 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.

A. Determination of ChE

The animals used in this part of the experiment were separated into two main groups denoted as the High Exposure (H) and the Low Exposure (L) Group, respectively. Each group consisted of control animals and of exposed ones. In H Group, the exposed animals were given CVP in a dose of 3.0 mg/kg i.p. and in L Group, in a dose of 1.0 mg/kg i.p.

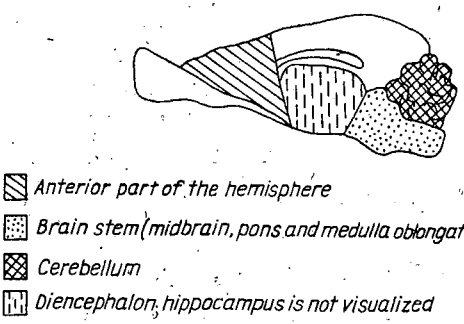

Fig. 1. A schematic drawing showing parts of the rat brain in which ChE was determined.

This amounts to about 1/3 and 1/10 of the LD_{50} for the rat (3, 15). The volume of the injected solutions was about 1 ml in both cases. The control animals of each group were injected with pure oil in a similar volume. Samples of animals from each group (consisting of 3—5 subjects) were killed by decapitation at a pre-selected time after the exposure (3, 24, 48, 94, 168 and 336 hrs). The killing was performed at 11—12 a.m. in all cases. For determination of ChE activity in plasma and in erythrocytes, samples of blood flowing from cut vessels were collected. The brain was removed from the skull as quickly as possible and dis-

sected on ice into five parts (see Fig. 1). The parts were weighed with 0.001 gm accuracy and homogenized in a glass Potter-Elvehjen's homogenizer 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 sample used for ChE determination were adjusted on the basis of earlier determinations of ChE activity in a given part of the brain. The activity of ChE in blood and in brain homogenates was determined spectrophotometrically with the use of acetylcholine iodide as substrate and ditiobisnitrobenzoic acid (DTNB) as chromogen (Voss and Sachsse method (25)).

B. Behavioral investigations

For behavioral studies, just as for ChE determination, two groups of rats, a High Exposure Group (H) and a Low Exposure Group (L), were used. Each of the groups was separated randomly into two subgroups (each consisting of 10—14 subjects): the exposed subgroup and the control. Before the start of the experiment the animals were carried to the experimental room and handled 10 min/day for four days.

Open field behavior

Apparatus. The „open field" used in the present studies consisted of a white, quadratic 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 10×19 cm/whole, cut in one of the walls, led to a small, 20×20×20 cm), darkened start box. The hole was covered by a loosely suspended piece of cotton fabric. A 25 W bulb, located 100 cm above the center of the arena, served as the source of light during the testing.

Procedure. The animals were tested in the open field every second day for two weeks before the injection, on the day of injection (3 hrs after the exposure), and for two weeks after the injection. Each daily session started with placing the rat into the start box. In case the rat had not left the start box within five min it was pushed gently through the hole into open arena where it was observed for five min. During the last three sessions the rats were presented with a new object (a round box of 7.0 cm in diameter), placed in the middle of the arena. The measured variables were: the latency of entry into the open arena, the number of squares crossed (locomotor activity), the number of rearings (exploratory activity) and the number of faecal boluses left in the arena.

Response-to-change in a „T" maze

Apparatus. The „T" maze used for testing the response-to-change was made of plywood. It consisted of a shaft (25×11×40 cm) and three sets of two arms each (40×11×40 cm) which could be changed quickly from trial to trial. One set of arms was painted black, one was white and one was the colour of raw wood.

Procedure. Before the injections, the rats were placed once a day for four days within the shaft of the „T" maze and allowed to explore the maze. The colour of the arms was raw wood. The purpose of this part of the procedure was to establish the directional preference (the most frequently visited arm) of each subject. The first session of the basic test was performed 24 hours after the injection and was repeated every second day (interchangeably with the testing in the open field) four times. Each session consisted of two trials: a presentation trial and a test trial. For the presentation trial, the rat was placed in the shaft of the „T" maze, facing the cross-section, and allowed to explore the shaft and arms for one minute. One of the arms was black and the other white during this trial. Then, the rat was transferred to a plastic box where it remained for another minute. During this time, the arm on the side opposite to the preferred one was changed in such a way that both were of the same colour. During the test trial, the rat was placed again in the shaft. This trial ended immediately after it had made a choice, i.e. entered one of the arms with four paws, or after 3 min of stay in the shaft. The measured variables were: the type of choice (the preferred or the changed arm) and the choice latency.

Statistics. ChE activity in animals exposed to CVP was expressed as a percentage of the values obtained in control animals. Locomotor and exploratory activity in the open field was estimated by the use of the nonparametric Friedman two-way analysis of variances and the Wilcoxon test (23). The data obtained in the „T" maze have been analysed using Fisher's exact probability test.

RESULTS

A. Biochemical

As shown in Figs. 2 and 3, and in Tables 1 and 2, 3 hrs after injection of CVP, in the H group (dose 3.0 mg/kg), ChE activity was strongly inhibited in blood and brain alike. Contrary to our expectations, blood ChE rather than brain ChE was the more inhibited. The differences, however, were not markedly pronounced; in fact, in all cases, the

TABLE 1. ChE Activity in Blood and in the Brain After Exposure to CVP in Dose 3.0 mg/kg (i.p.)

Time after injection		Blood (U/litre)		Brain (U/gram)				
		plasma	erythrocytes	cerebellum	diencephalon	hippocampus	anterior part of the hemisphere	brain stem
Control n = 10	U	187±35.9	761±152.3	4.84±1.0	12.47±1.82	10.01±1.66	24.33±5.01	14.60±2.76
3 h n = 4	U	15±17.6	89±95.1	0.80±0.2	1.95±1.0	1.68±0.9	4.47±1.2	2.42±0.6
	%	8.0±10.87	11.7±14.43	16.5±3.95	15.6±9.73	16.8±10.87	18.4±5.73	16.6±4.63
24 h n = 4	U	65±11.1	131±43.2	1.29±0.4	3.94±0.3	2.62±0.6	5.56±2.1	3.21±0.8
	%	34.8±6.85	17.2±6.54	26.7±9.21	31.6±3.08	26.2±6.82	22.9±9.99	22.0±6.21
48 h n = 4	U	116±11.3	203±46.2	2.10±0.3	5.00±0.4	4.47±0.4	9.79±0.9	6.12±0.4
	%	62.0±6.97	26.7±7.02	43.4±6.05	40.10±3.84	44.7±5.00	40.20±4.36	41.9±3.48
96 h n = 3	U	150±17.4	516±107.7	3.56±0.3	8.68±0.4	7.56±1.3	15.39±1.3	9.53±0.6
	%	80.20±11.38	67.8±17.34	73.6±7.56	69.6±4.35	75.5±16.65	63.3±6.95	65.3±5.20
148 h n = 3	U	176±3.5	520±3.5	4.10±0.2	9.16±0.5	7.35±0.4	16.28±0.8	10.89±0.2
	%	94.1±25.39	68.3±0.64	84.7±5.87	73.5±5.73	73.4±6.51	66.9±4.81	74.6±2.26
336 h n = 6	U	160±41.3	604±91.5	4.15±0.2	10.56±1.4	9.01±0.6	20.17±2.5	13.03±1.5
	%	85.6±24.19	79.4±13.16	85.7±5.37	84.7±12.25	90.0±7.00	82.9±11.5	89.2±11.7

U — direct value of ChE activity (international units)
% — relative value of ChE activity (in percent of the control)

TABLE 2. ChE Activity in Blood and in the Brain After Exposure to CVP in Dose 1.0 mg/kg (i.p.)

Time after injection		Blood (U/litre)		Brain (U/gram)				
		plasma	erythrocytes	cerebellum	diencephalon	hippocampus	anterior part of the hemisphere	brain stem
Control n = 4	U	173±33.5	712±70.8	4.71±0.75	11.55±2.9	11.12±0.28	22.69±0.90	13.03±0.89
3 h	U	96±53.3	457±229.8	3.11±0.93	8.66±3.1	7.68±3.04	9.64±3.35	15.73±4.07
n = 4	%	55.5±30.9	64±32.3	66.0±19.8	75.0±27.1	69.1±27.3	74.0±25.7	69.3±17.9
24 h	U	159±24.8	685±244.8	2.98±0.4	8.60±1.4	7.75±1.7	9.90±2.2	17.86±4.76
n = 4	%	91.9±14.4	96±34.4	63.3±8.7	74.4±12.5	69.7±15.3	76.1±16.6	78.7±21.0
96 h	U	197±31.8	662±75.4	5.1±0.5	12.3±0.7	10.4±0.21	13.1±0.5	20.3±2.3
n = 4	%	113.9±18.4	93±10.6	108.1±11.9	106.9±6.0	93.6±1.9	100.8±4.3	89.3±10.4
336 h	U	208±34.3	691±41.3	4.39±0.3	12.1±0.7	9.82±0.55	12.6±0.5	20.32±0.67
n = 4	%	120.2±19.9	97.1±5.8	93.2±5.5	104.4±6.3	88.3±5.0	97.0±4.1	89.6±2.6

U — direct value of ChE activity (international units)
% — relative value of ChE activity (in percent of the control value)

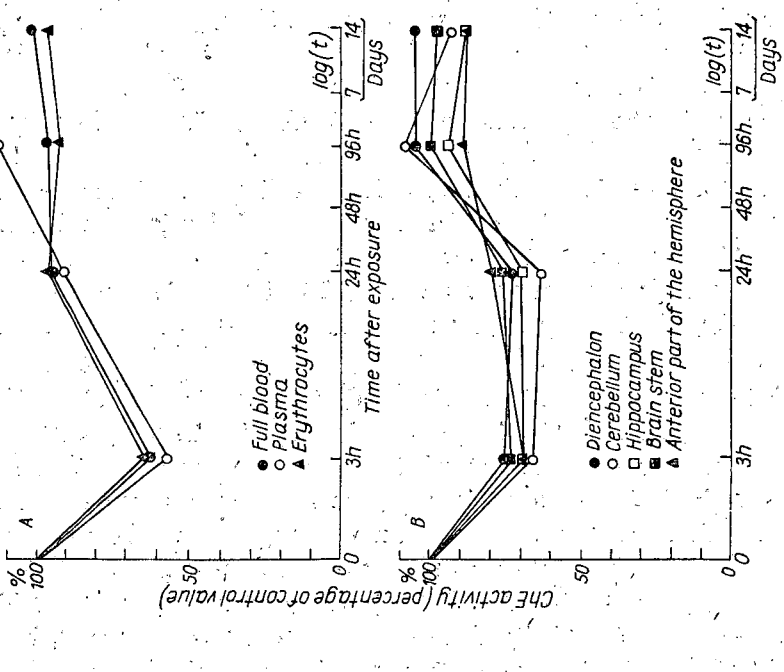

Fig. 3. Time course of changes in ChE activity in blood and in the brain (B) after single exposure to CVP. Dose: 1.0 mg/kg, i.p.

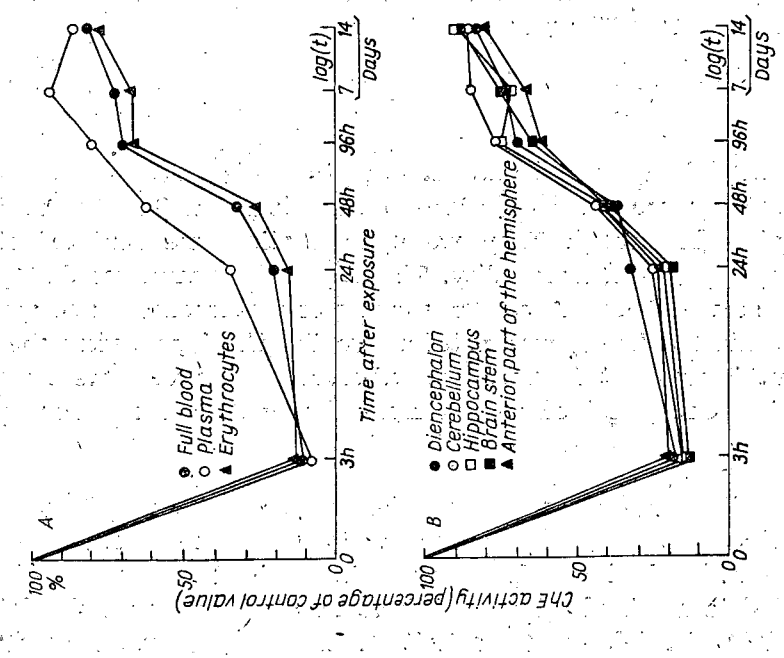

Fig. 2. Time course of changes in ChE activity in blood (A) and in the brain (B) after single exposure to CVP. Dose: 3.0 mg/kg, i.p.

obtained values were very close to each other. The mean ChE activities at this time with respect to control values were: 8.0% (SD 7.0) in plasma, 11.9% (SD 10.9) in erythrocytes, 16.5% (SD 3.9) in the cerebellum, 16.8% (SD 4.6) in the brain stem, 15.6% (SD 9.7) in the diencephalon, 16.8% (SD 10.8) in the hippocampus, and 18.4% (SD 5.7) in the anterior part of the hemisphere. There were no clear-cut differences between the blood and the brain in the rate of normalization of ChE activity after the exposure. One week (168 hrs) after the injection, the level of ChE activity in plasma, erythrocytes and in the analysed parts of the brain approached 70% of the values obtained in the control animals, and after two weeks, the corresponding values in exposed and in control rats were within the same range.

In the L group, marked individual differences in the strength of the effect were observed in the exposed subjects. Three hours after the injection of CVP, in one animal the ChE activity in blood and in the brain was inhibited to a similar degree as in rats given 3.0 mg/kg of the pesticide. In another one the effect was negligible. The mean relative values of ChE activity at this time were as follows: plasma — 55.5% (SD 30.9), erythrocytes — 64.2% (SD 32.3), cerebellum — 66.0% (SD 19.8), brain stem — 74.0% (SD 25.7), diencephalon — 75.0% (SD 27.1), hippocampus — 69.1% (SD 27.3), anterior part of the hemispheres — 69.3% (SD 17.9). In the L Group, just as in the H Group, the normalization of ChE activity in blood and in the brain proceeded at a similar rate. It was completed within 96 hours.

B. Behavioral

a) Changes in general behavior. Symptoms characteristic of acute OP intoxication were noted only in exposed rats given CVP in the dose of 3.0 mg/kg (H Group). They were: lacrimation, frequent urination and defecation, tremor, immobility. The vegetative symptoms disappeared within several hours. A certain slowing of movements (as compared with the control animals) was noted up to the third day after the injection, but it was not assessed quantitatively. Moreover, the exposed animals of H Group gained weight more slowly than the control ones (Fig. 4).

b) Open field behavior. The results of this test are presented in Table 3. The values in columns (blocks) are means of three successive sessions. Thus, blocks I and II contain data obtained before, and blocks III and IV data abtained after the injections. Statistical analysis (Friedman ANOVA) of the locomotor activity (number of squares crossed) re-

vealed significant differences between blocks in the exposed animals of the H group ($x^2=22.9$, $df=3$, $p<0.01$) and in the control animals of the L Group ($x^2=9.2$, $df=3$, $p<0.01$). Detailed comparisons (Wilcoxon test) have shown that the exposed animals of the H Group were less active during the first three sessions after the injection (block III) than during the remaining sessions ($p<0.01$). The control animals of the L Group were more active (crossed more squares) during the last three sessions of the test (block IV) than in the remaining ones ($p < 0.01$ in all cases).

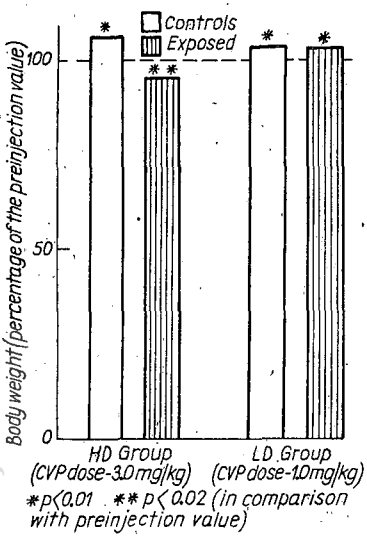

Fig. 4. Body weight of rats two weeks after exposure to CVP.

An analysis of the exploratory activity (number of rearings) has revealed significant differences between blocks in exposed and control animals of the H Group and in the control animals of the L Group (H Group, exposed: $x^2 = 26.8$, $df = 3$, $p < 0.01$; H Group, controls: $x^2 = 12.6$, $df = 3$, $p < 0.01$; L Group, controls: $x^2 = 7.9$, $df = 3$, $p < 0.05$). Detailed comparisons have shown that in the exposed rats of the H Group, the exploratory activity in block III was significantly lower than in the remaining blocks ($p < 0.01$ in all cases). The control animals of both groups (H and L) explored the environment significantly more during the last three sessions of the test (block IV) with the new object in the middle of the arena ($p < 0.01$ in both cases). The exposed animals of both groups did not show such an increase in the exploratory activity in block IV.

The numbers of faecal boluses and the latencies of entry into the arena showed large "within-group" and "between-sessions" variability.

TABLE 3. Locomotor and Exploratory Activity of Rats in Open Field Before (Blocks I, II) and After (Blocks III and IV) Single Exposure to Chlorphenvinphos

Group (CVP dose)	Subgroup	Locomotor activity				Exploratory activity			
		block I	block II	block III	block IV	block I	block II	block III	block IV
HD (3 mg/kg)	Exposed n = 14	241.7 ±108.8	241.4 ±111.0	111.6* ±65.2	228.3 ±84.8	59.0 ±26.9	52.2 ±22.9	18.1* ±12.1	53.1 ±20.9
	Controls n = 10	171.5 ±69.5	207.5 ±87.4	183.0 ±35.3	256.4 ±77.6	45.9 ±16.3	59.0 ±28.7	42.3 ±18.2	74.5* ±18.8
LD (1 mg/kg)	Exposed n = 10	226.9 ±80.1	299.1 ±105.5	295.3 ±118.9	301.2 ±105.4	31.2 ±9.7	46.7 ±18.6	35.2 ±11.9	40.4 ±18.9
	Controls n = 10	190.1 ±148.6	206.1 ±64.8	207.5 ±112.7	324.0* ±73.2	33.0 ±21.9	41.0 ±24.1	35.7 ±22.9	57.7* ±13.4

block I — days 14,12,10 before exposure
block II — days 7,5,3 before exposure
block III — days 0,2,4 after exposure
block IV — days 7,9,11 after exposure
* — $p < 0.05$ in comparison with the remaining blocks within the same group

TABLE 4. Effect of Single i.p. Exposure to Chlorphenvinphos on Response-to-Change in "T" maze

Group (CVP dose)	Subgroup	Time after exposure: 24 hours (session I) type of choice: changed arm	preferred arm	no choice	3 days (session II) type of choice: changed arm	preferred arm	no choice	7 days (session III) type of choice: changed arm	preferred arm	no choice	9 days (session IV) type of choice: changed arm	preferred arm	no choice
HD (3 mg/kg)	exposed n = 14	50*	7.2	42.8	57	14.4	28.6	71	7.1	21.4	64	7.1	28.6
	controls n = 10	90	10.0	0	70	30.0	0	70	20.0	10.0	70	30.0	0
LD (1 mg/kg)	exposed n = 10	80	0	20.0	80	20.0	0	80	0	20.0	60	20.0	20.0
	controls n = 10	90	0	10.0	90	0	10.0	60	10.0	30.0	80	0	20.0

Note: values in columns are percentages of animals from each subgroup
* — $p < 0.05$, Fisher test

As a result, no differences were found between blocks as well as between groups.

c) Response-to-change in the "T" maze. The results of this test are presented in Table 4. During the first two sessions in the "T" maze, the exposed animals of H Group differed significantly from their controls ($p < 0.05$, Fisher exact probability test). However, the source of this difference was not the type of choice (changed vs preferred arm), but rather the animals' eagerness in making the choice: during the test trials, almost half of the exposed animals preferred to stay in the shaft of the "T" maze. During the remaining two sessions, the exposed and control animals of the H group did not differ from each other. The animals of the L Group — exposed and controls — performed equally well from the beginning of this test.

DISCUSSION

The data obtained in the present studies show that in the rat, as a result of a single i.p. exposure to CVP, ChE activity is inhibited to a similar degree and returns to normal at a similar rate in plasma, ery-

throcytes and in different parts of the brain. Such a result is a little surprising: taking into account the ability of the rat brain to accumulate CVP (15), one might expect a stronger inhibition and a slower normalization of the brain ChE, especially when low doses of the pesticide were used. This, however, did not happen, which may suggest that in the rat brain, ChE is not necessarily the preferred site (enzyme) binding CVP. From the practical point of view, the quantitative similarity between blood and brain inhibition of ChE after exposure to CVP is advantageous: determination of ChE in blood of experimental subjects may appear sufficient to assess the level of enzyme activity in the brain. It may be added that a similar parallelism between blood and brain ChE activities were also noted in the case of other ChE inhibitors (8, 11).

The lack of differences in the level of ChE inhibition between different parts of the brain suggest that CVP crosses the brain barriers very efficiently and that the brain cholinergic system as a whole is subjected to its action.

According to literature, 50% decrease of ChE activity may be regarded as the threshold for the appearance of objective and subjective symptoms of OP intoxication (26). Some of our present observations are in agreement with the above: vegetative disturbances and changes in motor behavior appeared only in the rats given CVP in a dose of 3.0 mg/kg, i.e. a dose inhibiting ChE by much more than 50%. The character of the vegetative symptoms noted in our experiments, as well as the inhibitory effect on motor behavior, are in accord with reports from studies concerning other anticholinesterases (12). It is worth emphasizing here that the detectable changes in motor behavior were more durable (days) than the vegatative ones (hours) which was suggested by the lower number of crossed squares and rearings in the open field and by the rats' frequent staying in the shaft, and making no choice in the "T" maze during the first few days after the exposure.

The most interesting fact observed in the exposed rats of both groups, as opposed to controls, is the lack of an increase in exploratory activity in the presence of a new object during the last three sessions (block IV) in the open field. It should be stressed that, according to the biochemical data, the ChE activity at that time was normal in the L Group, and it was very close to normal in the H Group. It is rather unlikely that the above differences in open field behavior between exposed and control animals represented a persisting motor impairment: in the exposed rats of the L Group no changes of locomotor and exploratory activity were observed in block III. Therefore, a better explanation may be that the exposed rats paid no attention to, or were afraid of, the new object in the open field.

The above supposition, however, finds no support in the results obtained in the "T" maze. If the exposed animals suffered an impairment of attentional processes, the percentage of the correct choices should be expected to decrease to chance level or the preferred (unchanged) arm should be chosen more frequently (above chance level). If, on the other hand, the exposed rats feared "novelty", then more frequent refusals to leave the shaft should have occurred (as was observed during the first two sessions in the "T" maze), or the preferred arm should have been the more frequent choice. Nothing like this was observed during the last two sessions in the "T" maze, i.e. at about the same time when the rat response to a new object was tested in the open field.

The "fear" explanation might hold true, however, if the specificity of the two experimental situations, open field and the "T" maze, are taken into account. For the rat, the lighted, white open arena was certainly more stressful than the "T" maze, and the small round box might have been more "new" than an arm of changed color. Owing to this, differences in the emotionality (fearfulness) of the rats might be more reflected in their behavior in the open field than in the "T" maze.

Notwithstanding the possible sources of discrepancies, the fact remains that a single exposure to CVP, not only in a large but also in a relatively low (subsymptomatic) dose, may lead to some subtle abnormalities in rat behavior, which may be detected after a time sufficient for full recovery of ChE activity in blood and in the brain. No matter whether these abnormalities are interpreted in terms of attentional or emotional changes, both interpretations are in accordance with the subjective symptoms noted in humans with a history of OP exposure (10, 22). Thus, the animal data presented above concerning CVP confirm the conclusions which may be drawn from epidemiological observations; namely, that a normal level of ChE after OP exposure does not necessarily mean a full recovery. By the same token, routine testing of ChE in workers involved in manufacturing, distributing and the use of OP-s, may be not sufficient and should be complemented with psychological and neurobehavioral tests, the more so, that accidental, unsymptomatic exposures may pass unnoticed.

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Received for publication: 5.05.1989
Accepted for publication: 5.06.1989