ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2003, 10, 197–201

NEUROTOXIC EFFECT OF DERMALLY APPLIED CHLORPYRIFOS AND CYPERMETHRIN. REVERSIBILITY OF CHANGES

Jadwiga Latuszyńska, Sabina Luty, Grzegorz Raszewski, Daniela Przebirowska, Małgorzata Tokarska-Rodak

Department of Pathomorphology, Institute of Agricultural Medicine, Lublin, Poland

Latuszyńska J, Luty S, Raszewski G, Przebirowska D, Tokarska-Rodak M: Neurotoxic effect of dermally applied chlorpyrifos and cypermethrin. Reversibility of changes. *Ann Agric Environ Med* 2003, **10**, 197–201.

Abstract: Nurelle D 550 EC preparation (Dow Elanco, USA) was applied in the study. The contents of biologically active substances in 1l of the preparation was: 500 g chlorpyrifos and 50 g of cypermethrin. The experimental studies were conducted on 4 groups of rats (2 control - 10 animals in each group, and 2 experimental - 40 animals in each group). The experimental groups were dermally administered a mixture of 27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin for a period of 1 week and 4 weeks (4 h daily). The control groups did not participate in the experiment. Blood was taken from the heart after 1 day, 1 week, 2 and 3 weeks after the administration of the preparation in order to determine cholinesterase activity in serum, and the brain was taken to evaluate brain cholinesterase activity. The brain for histologic studies was taken from rats 3 weeks after the experiment. The cholinesterase levels in the serum and brain initially decrease and then return to normal at 2 and 3 weeks post-exposure, respectively. Slight histopathological changes in various areas of the brain as well as increased density of the cytoplasm in neurocytes in both experimental groups were observed 3 weeks post-exposure.

Address for correspondence: Dr Jadwiga Latuszyńska, Department of Pathomorphology, Institute of Agricultural Medicine, 20-950 Lublin, P.O. Box 185.

Key words: dermal toxicity, chlorpyrifos, cypermethrin, cholinesterase activity, reversibility of changes.

INTRODUCTION

Previous studies showed that chlorpyrifos and cypermethrin applied in the form of a mixture on rat's tail skin for 4 weeks in the doses of 1,000 mg/kg b.m. and 100 mg/kg respectively, and 200 mg/kg b.m. and 20 mg/kg b.m., resulted in slight histopathological changes in the brain. The changes concerned the density of the cytoplasm of single neurocytes or their foci in the molecular layer of the dentate gyrus, in stratum hippocampi CA 1, in piramidal cells of the cerebral cortex in corpus striatum, as well as in Purkinje cells in the cerebellum [8].

The mixture examined inhibited serum cholinesterase (ChE) and acetylcholinesterase (AChE) activity, after 1-week, as well as 4-weeks application, after the application of both the higher and the lower dose of the preparation [9].

In literature, reports are available concerning the effect of chlorpyrifos and cypermethrin administered separately on the activity of serum cholinesterase (ChE) and brain acetylcholinesterase (AChE), as well as on the reversibility of changes observed in various species of animals [1, 2, 3, 4, 6, 10, 11, 12]. However, no reports have been found dealing with the effect of the mixture of the 2 preparations examined on the reversibility of changes in the above-mentioned enzymes.

Received: 10 July 2003
Accepted: 30 October 2003

Table 1. Analysis of reversibility of changes in cholinesterase (ChE) after 1-week and a 4-week exposure to the mixture of chlorpyrifos 27.8 mg/cm^2 and cypermethrin 2.7 mg/cm^2.

		Time after exposure				
		1 day	1 week	2 weeks	3 weeks	Control group
1-week exposure	x ± SD	305.61 ± 39.52*	614.54 ± 165.08*	1405.00 ± 247.34	1499.45 ± 223.64	1472.86 ± 396.11
	n	10	10	7	10	7
	inhibition (%)	79.3	58.3	4.6	0	0
4-week exposure	x ± SD	149.00 ±15.44*	779.37 ± 104.34*	1711.11 ± 228.11	1929.62 ± 536.24	1844.14 ± 360.55
	n	10	10	10	10	10
	inhibition (%)	92	57.7	0.7	0	0

* $p < 0.0001$ compared to control.

Table 2. Analysis of reversibility of changes in cholinesterase (AChE) after 1-week and 4-week exposure to the mixture of chlorpyrifos 27.8 mg/cm^2 and cypermethrin 2.7 mg/cm^2.

		Time after exposure				
		1 day	1 week	2 weeks	3 weeks	Control group
1-week exposure	x ± SD	3.36 ± 0.326*	4.61 ± 5.08*	5.39 ± 0.250*	6.42 ± 0.229	6.44 ± 0.434
	n	13	17	9	12	8
	inhibition (%)	47.8	28.4	16.3	0	0
4-week exposure	x ± SD	1.23 ± 0.179*	3.96 ± 0.367*	5.21 ± 0.134*	6.47 ± 0.121	6.49 ± 0.289
	n	10	10	10	10	10
	inhibition (%)	81.1	39	19.7	0	0

* $p < 0.0001$ compared to control.

The aim of the study was to determine whether biochemical changes (serum cholinesterase and brain acetylcholinesterase), and histologic changes in the brain after dermal application of the mixture of chlorpyrifos and cypermethrin in acute (1-week) and sub-acute (4-weeks) experiment are reversible, and after what period they return to the initial state.

MATERIAL AND METHODS

The preparation Nurelle D 550 EC (Dow Elanco, USA) was applied in the study. The contents of biologically active substances in 1l of the preparation is: 500 g of chlorpyrifos and 50 g of cypermethrin. The preparation was administered in the form of a water suspension. The mixture used in the study was applied in the same proportion as in the preparation. The study was conducted on 3-month-old female Wistar rats, in good condition, with no macroscopic changes observed on tail skin. The animals were fed with standard fodder LSM and watered *ad libitum* [7]. At the beginning of the experiment, the body mass of rats ranged from 190–230 g.

The study was conducted on 4 groups of rats (2 control groups, 10 animals in each group; and 2 experimental groups - 40 animals in each group). The experimental groups received a mixture of 27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin dermally for a period of 1 and 4 weeks (4 h daily). The preparation was applied on the tail skin (9 cm^2) with the use of an absorptive fabric FPP-15, and covered with aluminium foil to isolate it from the environment [13]. Control groups did not participate in the experiment. After 1 day, 1 week, 2 and 3 weeks of the experiment, blood was taken from the heart to evaluate cholinesterase activity in serum, and the brain was also taken to determine brain acetylcholinesterase activity. The activity of the enzymes was evaluated by the Ellman (1961) colorimetric method using propionylotiocholine iodide and eserine salicylate as an inhibitor of the reaction [5].

The brain for histologic studies was taken 3 weeks after the experiment, and perfused with a solution of methanol, formalin and glacial acetic acid (8:1:1), then cut into sections which were stained by the Nissel method [14].

The results of biochemical tests obtained were subject to statistical analysis by means of a Student's *t* parametric test.

RESULTS

Biochemical studies. The results of the study show that chlorpyrifos and cypermethrin administered to the tail skin of rats in a mixture (27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin) for a period of 1 and 4 weeks, resulted within 1 day after the experiment in a considerable decrease in serum cholinesterase activity ($p < 0.0001$), which in consequence led to 79% inhibition of the enzyme in the 1-week experiment, and 92% inhibition in the 4-week experiment. One week after the exposure, a considerable inhibition of the enzyme in the study was still observed (58% in 1-week, as well as in 4-week experiments), the inibition being highly significant statistically ($p < 0.0001$) (Tab. 1). Two weeks after the

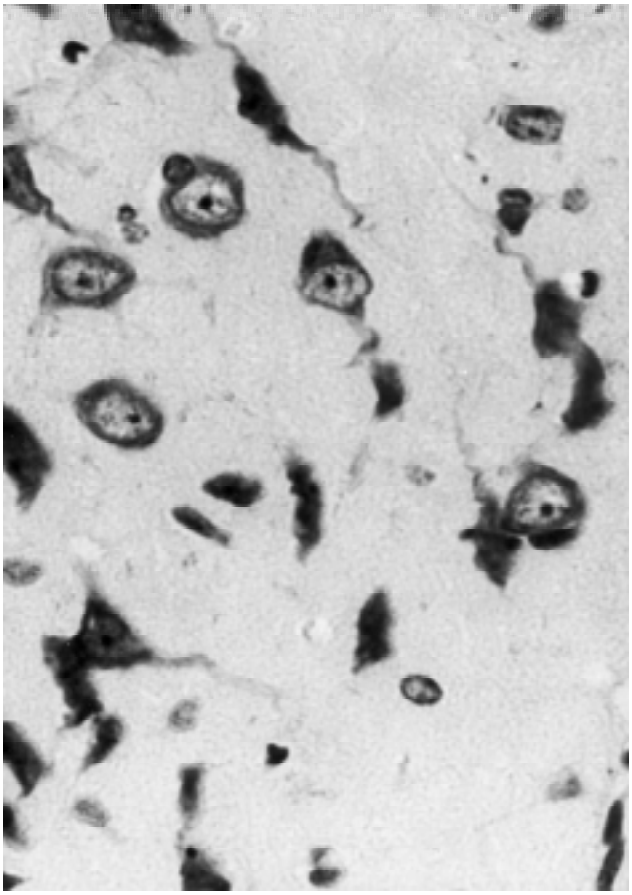

Figure 1. Brain of rat exposed for 1 week to dermal absorption of a mixture of 27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin, 3 weeks after exposure. Pycnosis of the cytoplasm in the cells of the stratum polymorphica of cortex cerebri. Stained according to the Nissel method, × 320.

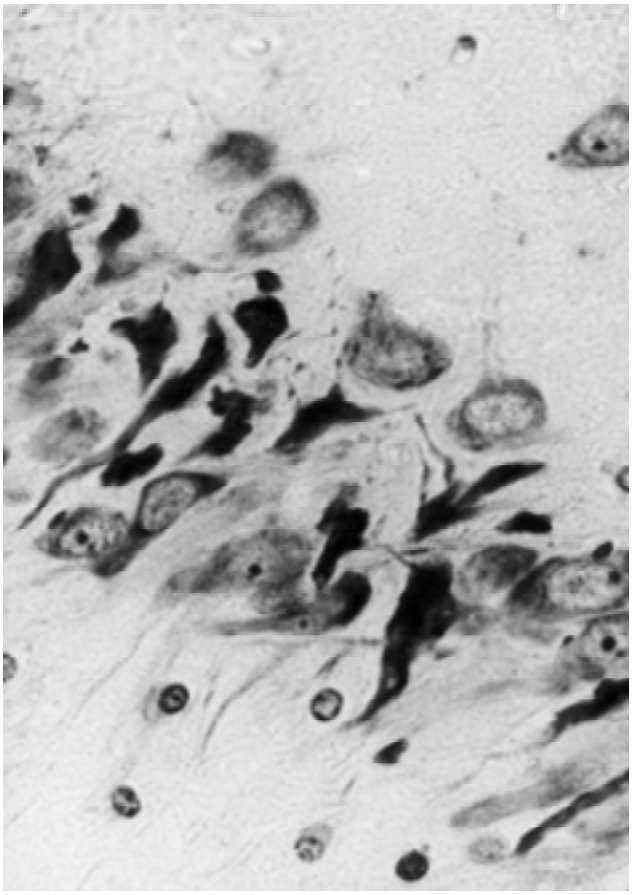

Figure 2. Brain of rat exposed for 4 weeks to dermal absorption of a mixture of 27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin, 3 weeks after exposure. Pycnosis of the cytoplasm in the cells of the hilus fasciae dentatae. Stained according to the Nissel method, × 320.

experiment, the level of the enzyme examined was close to the control values in both experimental groups (5% and 1% inhibition). Three weeks after the experiment, no inhibition of enzyme in the study was observed, neither after 1 nor 4 weeks administration of the preparation.

At 1 day, 1 week and 2 weeks post-exposure, the activity of brain acetylcholinesterase was significantly lower statistically in the two experimental groups, compared to the control group ($p < 0.0001$). After 1-week of exposure, the percentage of inhibition of AChE activity in the experimental group compared to the control was 48%, 28% and 16% respectively (Tab. 2), and after 4-weeks of administration of the substance it was higher - 81%, 39% and 20% respectively. At 3 weeks post-exposure, no difference was noted in the activity of the enzyme examined between the experimental and control groups.

Histologic studies. Three weeks post-exposure of chlorpyrifos and cypermethrin administered in a mixture for periods of 1 and 4 weeks, histological changes in the brain of rats involved increased density of the cytoplasm in cells of the cortex cerebri (Fig. 1), stratum hippocampi CA 1, in the hilus area dentatae (Fig. 2), in the neurocytes of the thalamus nuclei and in Purkinije cells of the cerebellum (Fig. 3).

DISCUSSION

The data from litertature indicate that after the administration of a single dose of cypermethrin, the activity of serum acetylcholinesterase in the erythrocytes of rats initially declines, but shows a tendency towards recovery at 2 weeks post-exposure [6].

Reddy *et al.* [12], after administration of sublethal concentrations of cypermethrin, observed significant changes in acetylcholinesterase (AChE) content in the brain tissue of both juvenile and adult-fish. Maximum inhibition of AChE activity was noticed at 6 and 12 hours after exposure to cypermethrin in juvenile and adult fish respectively. During subsequent periods, the rate of recovery in AChE activity level was variable in both groups.

Chlorpyrifos administered at a dose of 13 mg/kg to quail led to a strong inhibition of brain acetylcholinesterase (AChE) (76.6%) and plasma cholinesterase (ChE) (80.1%) at 8 hours post-administration. Plasma ChE recovered rapidly; however, even after a 4 day recovery, ChE values of quail that had received chlorpyrifos were slightly higher than those of controls. The recovery of brain AChE activity was slower than that of plasma. It reached 59% of controls 2 days after chlorpyriphos

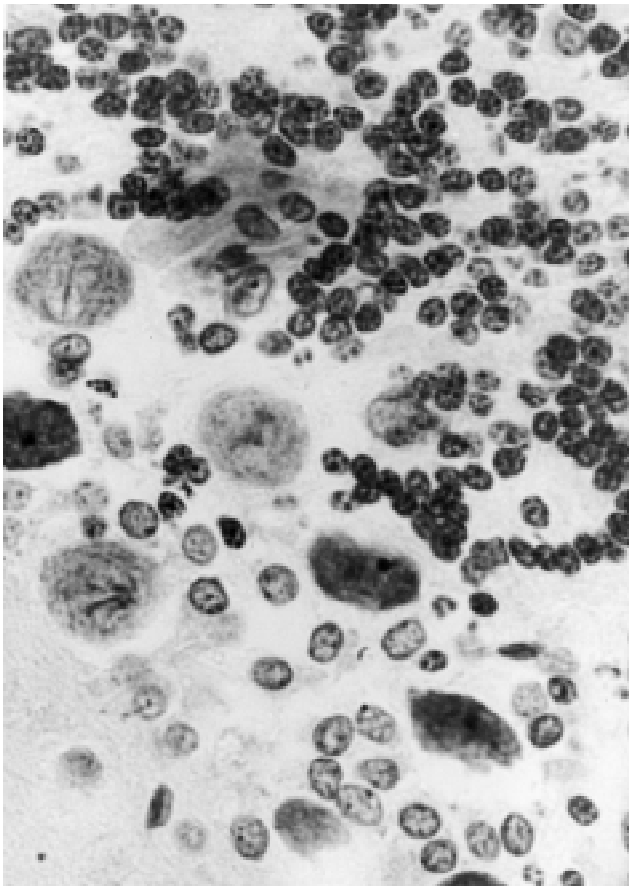

Figure 3. Brain of rat exposed for 4 weeks to dermal absorption of a mixture of 27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin, 3 weeks after exposure. Pycnosis of the cytoplasm of the Purkinje cells in cerebellum. Stained according to the Nissel method, × 320.

administration, then recovered slowly, reaching the control level after 11 days of recovery [4].

Chlorpyrifos administered orally to pregnant female rats (14–18 day of pregnancy) at a dose of 50 mg/kg body mass (61% of oral LD_{50}) resulted in a considerable inhibition of the spinal cord AChE as early as 1 hour after poisoning [2]. Similar results were obtained by Abu-Qare *et al.* [1], where chlorpyrifos was applied dermally to pregnant female rats in a dose of 30 mg/kg body mass (15% of dermal LD_{50}). Subcutaneous administration of a single dose of 279 mg/kg chlorpyrifos resulted in the inhibition of cholinesterase activity in plasma and erythrocytes, as well as high acetylcholinesterase inhibition in the whole brain (over 90%), which persisted for up to 7 days after administration of the preparation [10]. Repeated subcutaneous injections of chlorpyrifos (40 mg/kg for 4 days) caused an extensive inhibition of brain cholinesterase activity in the cortex, hippocampus and striatum among adult rats, 4 and 14 days after the last injection (by 92–98% and 71–78%, respectively) [3].

The present study shows that chlorpyrifos and cypermethrin administered in combination to the tail skin of rats (27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin) for 1 and 4 weeks led, within 1 day after the experiment, to a considerable decrease in cholinesterase activity in serum ($p < 0.0001$), which resulted in 79% inhibition of the enzyme in the 1-week exposure and 92% inhibition in the 4-week exposure. At 1 week post-exposure, considerable inhibition of the enzyme examined was still observed (58% in both 1 week and 4-week experiments), this inhibition being highly significant statistically ($p < 0.0001$). Two weeks after the exposure, the level of the enzyme in the study was close to the control values in both experimental groups (5% and 1% inhibition). Three weeks after the exposure, no inhibition of the enzyme's activity was noted, either after the 1-week or 4-week administration of the preparation.

At 1 day, 1 week, and 2 weeks post-exposure the activity of brain acetylcholinesterase was significantly lower statistically, compared to the control group, in both experimental groups ($p < 0.0001$) - 48%, 28% and 16%, respectively, after the 1-week exposure and 81%, 39% and 20%, respectively, after the 4-week administration of the mixture. Three weeks post-exposure, no differences were observed in the activity of the enzyme examined between the experimental and control groups.

Garcia-Cambero *et al.* [4] observed no clinical signs or mortality after administration of 13 mg/kg chlorpyrifos to quail. Histopathological changes in the central nervous system concerned an encephalytic reaction and necrotic Purkinje cells. In our previous studies, chlorpyrifos (27.8 mg/cm^2) and cypermethrin (2.7 mg/cm^2) after 1 week and 4-weeks application on the tail skin, lead to changes manifested by pycnosis of neurocytes in various areas of the brain. Most often, the changes were clear in the cells of the stratum granulosum in areae dentate, stratum pyramidale hippocampi (CA 1) and pycnosis of the cytoplasm in single cells of layer pyramidale of the cortex cerebri, area pyriformis of the cortex cerebri, Purkinje cells and nucleus medialis in the cerebellum. The brain for histologic studies was taken after the end experiment [9]. In our present study, at 3 weeks post-exposure of the mixture of chlorpyrifos and cypermethrin (27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin), for periods of 1 and 4 weeks, histological changes in the brain of rats involved increased density of the cytoplasm in cells of the cortex cerebri, stratum hippocampi CA 1, in the hilus area dentatae, in the neurocytes of the thalamus nuclei and in Purkinije cells of the cerebellum.

The present study shows that changes concerning the activity of serum cholinesterase, as well as brain acetyl-choliesterase, are reversible (2 and 3 weeks after the end of the experiment, respectively), whereas histopathological changes persist longer.

CONCLUSIONS

The results of the study showed that:

- the level of cholinesterase (ChE) in serum decreases initially after dermal exposure to a mixture of chlorpyrifos and cypermethrin and recovers to control values 2 weeks after the end of the experiment in both 1-week and 4-week exposure groups;

- the level of acetylcholinesterase (AChE) in brain decreases initially after dermal exposure to a mixture of chlorpyrifos and cypermethrin and recovers to the control level 3 weeks after the end of the administration of the preparation in the 1-week and 4-week exposure;
- slight histopathological changes in various areas of the brain so as increased density of the cytoplasm in neurocytes in both experimental groups 3 weeks post-exposure were observed.

REFERENCES

1. Abu-Qare AW, Abdel-Rahman A, Brownie C, Kishk AM, Abou-Donia MB: Inhibition of cholinesterase enzymes following a single dermal dose of chlorpyrifos and methyl parathion, alone and in combination, in pregnant rats. *J Toxicol Environ Health* 2001, **63**, 173-189.
2. Ashry KM, Abu-Qare AW, Saleem FR, Hussein YA, Hamza SM, Kis AM, Abu-Donia MB: Inhibition and recovery of maternal and fetal cholinesterase enzyme following a single oral dose of chlorpyrifos in rats. *Arch Toxicol* 2002, **1**, 30-39.
3. Chakraborti TK, Farrar JD, Pope CN: Comparative neurochemical and neurobehavioral effects of repeated chlorpyrifos exposures in young and adult rats. *Pharmacol Biochem Behav* 1993, **46**, 219-224.
4. Garcia-Cabero JP, Miguez-Santiyan MP, Reja-Sanchez A, Roncero-Cordero V, Soler-rodriguez F: Recovery of brain acetylcholinesterase and plasma cholinesterase activies in quail (Coturnix coturnix) after chlorpyriphos administration and effect of pralidoxime treatment. *Environ Toxicol Chem* 1998, **17**, 1835-1844.
5. Ellman GL, Courtney KD, Anders V, Feathrstone RM: A new and rapid colorimetric determination of acetylcholinoesterase activity. *Biochem Pharmacol* 1961, **7**, 88-95.
6. Kale M, Rathore N, John S, Bhatnagar D: Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol Lett* 1999, **3**, 197-205.
7. Królikowska-Prasał I, Kifer-Wysocka E, Matysiak W: *Gegenbaurus Morphol Jahrb*. Leipzig 1990, **136**, 565-574
8. Latuszyńska J, Luty S, Halliop J, Przylepa E, Tochman A, Obuchowska D, Korczak E: Studies of toxicity of dermally-absorbed Nurelle D 550 EC preparations. *Ann Agric Environ Med* 1999, **6**, 151-159.
9. Latuszyńska J, Luty S, Raszewski G, Tokarska-Rodak M, Przebirowska D, Przylepa E, Haratym-Maj A: Neurotoxic effect of dermally applied chlorpyrifos and cypermethrin in Wistar rats. *Ann Agric Environ Med* 2001, **8**, 1-8.
10. Pope CN, Chakraborti TK, Chapman ML, Farrar JD,Arthun D: Comparision of in vivo cholieserase inhibition in neonatal and adult rats by three organophosphorothioate insecticides. *Toxicol* 1991, **68**, 51-61.
11. Pope CN, Chakraborti TK, Chapman ML, Farrar JD: Long-term neurochemical and behavioral effects induced by acute chlorpyrifos treatment. *Pharmacol Biochem Behav* 1992, **42**, 251-256.
12. Reddy AT, Ayyanna K, Yellamma K: Sensitivity of brain cholinesterase to cypermethrin toxicity in freshwater teleost Tilapia mossambica. *Biochem Int* 1991, **23**, 959-962.
13. Tośl-Luty S, Latuszyńska J, Halliop J, Tochman A, Przylepa E, Bychawski E, Przebirowska D: Skin penetration of selected pesticides. *Ann Agric Environ Med* 1994, **1**, 57-14.
14. Zawistowski S: *Histological Technics*. PZWL, Warsaw 1965 (in Polish).