Acta Veterinaria (Beograd), Vol. 60, No. 2-3, 123-131, 2010.

DOI: 10.2298/AVB1003123D UDK 619:612.015.1

THE EFFECT OF DICHLORVOS ON ACETYLCHOLINESTERASE ACTIVITY IN SOME TISSUES IN RATS

DERE E, ARI FERDA and UGUR S

Uludag University, Faculty of Science and Art, Department of Biology/Molecular Biology, Nilüfer, Bursa, Turkey

(Received 27th October 2009)

In this study, the changes with respect to time in the serum, brain, liver, kidney and small intestine acetylcholinesterase activities were investigated in both male and female rats administered dichlorvos intraperitoneally (i.p.). For this purpose, 4 mg kg^{-1} doses of dichlorvos were injected i.p. in the rats. The control groups, on the other hand, were administered physiological saline via the same route. Rats were killed by decapitation at 0, 2, 4, 8, 16, 32, 64 and 72 hours after administration of dichlorvos and tissues were harvested. Enzyme activities were determined following the necessary treatments.

While a significant decrease in enzyme activities in the kidney and small intestine tissues with respect to time were not observed in either sex, a significant decrease in enzyme activities in the serum, as well as in the brain and liver tissues were observed.

As a result of our study, acetylcholinesterase activity was found to be decreased compared to controls in both male and female rats from 2 and 4 hours. Enzyme inhibition continued for up to 72 hours.

Key words: acetylcholinesterase, DDVP (dichlorvos)

INTRODUCTION

Pesticides are chemical substances that lead to poisoning when they enter the body through inhalation and dermal and gastrointestinal absorption. Especially in exposure to organic phosphorus compounds, the observed clinical symptoms include increased secretions, nausea-vomiting, urinary incontinence, dyspnea, bradycardia, tachycardia, muscarinic effects including hypotension, myasthenia, striated muscle effects including fasciculation and central nerve system effects like confusion and coma. In addition, polyneuropathy is observed 2-4 weeks after poisoning and can lead to apoplexy (Koçak *et al*., 2005). Pesticide poisoning is an important health problem, especially in developing countries. In a study from Sri Lanka, it was declared that 62.8% of 239 acute pesticide poisoning cases occurred in males, and the primary reason for poisoning was suicide in 200 cases (84%). Of the suicides, 60% were seen in males and 62% in the 16-29 age group (Van der Hoek and Konradsen, 2005). Another study in Japan showed that

70% of the poisoning cases used pesticides for suicide (Recena *et al*., 2006; Nagami *et al*., 2005).

In a study from Western Australia, Emerson *et al*. (1999) reported that the origin of most of the organophosphate poisoning cases was accidental and that most of these accidents involved children. The consumption of pesticides in Turkey was determined to be 182 044 kg, which has been predicted to be 8.8% of the total insecticide consumption worldwide (Delen *et al*., 2005). Changes in acetylcholinesterase (AChE) activity is frequently used as a biomarker for organophosphorus pesticide contamination in fish, but pesticides of other classes may also affect AChE activity (Dutta and Arends, 2003; Miron *et al*., 2005; Glusczak *et al*., 2006).

Dichlorvos, also known as DDVP, is a widely used organophosphate insecticide and acaricide that affects the stomach and respiratory system. It is primarily used for the control of internal or external parasites of livestock or pets and in pest control for houses and open areas (ATSDR, 1997). Dichlorvos can be absorbed easily in organisms through the skin and by digestion and respiration, since it quickly evaporates (Parmeggiani 1983). Dichlorvos is metabolized quickly by the liver for removal from the body and transformed into desmethyldichlorvos, dimethylphosphate and dichloroacetaldehyde metabolites.

Acetylcholinesterase is a key enzyme in the nervous system of animals. By rapid hydrolysis of the neurotransmitter acetylcholine (ACh), AChE terminates neurotransmission at cholinergic synapses. AChE inhibitors are among the key drugs approved by the Food and Drug Administration (FDA) for management of Alzheimer's disease (AD). The powerful toxicity of organophosphorus (OP) poisons is attributed primarily to their ability to act as potent AChE inhibitors.

In our study, the changes in AChE activity in the brain, liver, kidney, intestinal tissues and serum of rats were investigated. Changes in the activity of this important enzyme may affect the nervous system function directly and other metabolic pathways indirectly. This research contributes to the understanding the effects of the dichlorvos.

MATERIAL AND METHODS

Wistar rats (*Rattus norvegicus*) weighing 250-300 g were used in this study. Animals were obtained from the laboratory of experimental animals at Uludag University. For each trial period, four rats were used (total of 64 rats). Control groups were treated with physiological serum, while experimental groups were injected intraperitoneally with 4 mg kg^{-1} dichlorvos. In order to establish the metabolic synchronization in both groups the animals were left without food and water for 24 hours before injection. Following injection, food and water were regularly given to the animals until the trial periods were completed. Animals were killed via cervical dislocation at 0, 2, 4, 8, 16, 32, 64 or 72 hours after injection. Serum was collected, and the brain, liver, kidney, and small intestine were quickly removed and perfused in ice-cold 0.15 M KCl. The homogenates were prepared after the addition of ice-cold 0.15 M KCl (1/3, mass/volume) in a glass homogenizer with a Teflon pestle, and tissues were homogenized on ice with four

pulses for the brain, liver and kidney samples and eight pulses for the small intestine at 1500 rpm in a T-line laboratory stirrer-type homogenizer (model No: 136-2). Each homogenate was centrifuged in a Sorval RC-5 super speed refrigerated centrifuge (Dupont Instruments) at 48000 g for 30 minutes. The enzymatic reaction rates were determined with freshly made preparations. Centrifugation and homogenization were done with great care at 0-4^{o}C. Acetylcholinesterase activities were estimated spectrophotometrically using the Boehringer Mannheim (1973) method. Protein concentrations were determined using the Bradford (1976) method and bovine serum albumin was used as the protein standard.

Statistical analysis

Data were analyzed using SPSS 13.0 for Windows. All data displayed a normal distribution. Independent t-test was applied to compare data from the control and experimental periods. The significance was calculated using one-way analysis of variance (ANOVA) and Student's *t*-test. Results are presented as means ± standard deviations, and $p<0.05$ was regarded as statistically significant. Pearson correlation analyses were used to determine the relationships between variables (Jerrold, 1984).

RESULTS

AChE enzyme activities during the time course and statistical evaluations are shown in Table 1 for male rat tissues and in Table 2 for female rat tissues. After treatment with dichlorvos, there were no significant changes in AChE enzyme activity in the kidney and intestinal tissues of both female and male rats when compared with the control group ($p>0.05$). While dichlorvos started to inhibit the serum AChE enzyme by 8 hours in male rats, this inhibition started by hour 4 in female rats. Inhibition continued for 72 hours ($p<0.05$, Tables 1, 2). In the brain and liver tissues, AChE inhibition started at 2 hours in both sexes. While enzyme activity in the brain tissue of both female and male rats remained unaffected until 72 hours ($p<0.05$) and inhibition continued for 64 and 72 hours in the liver tissue of female rats, it was not statistically significant ($p>0.05$).

In male rats, the decrease in the AChE enzyme activity in the liver tissue continued until hour 72 ($p<0.05$).

The highest level of AChE enzyme inhibition was measured in the serum at 8 hours in female rats, with activities of 7.49 ± 0.73 U (mg protein)$^{-1}$ x 10^{-1} in the control group and 3.87 ± 0.59 U (mg protein)$^{-1}$ x 10^{-1} in the dichlorvos group. Although this inhibition continued for 72 hours, it decreased. The activities were 7.72 ± 0.51 U (mg protein)$^{-1}$ x 10^{-1} in the control group and 5.08 ± 0.83 U (mg protein)$^{-1}$ x 10^{-1}. The greatest inhibition was detected at 4 and 8 hours in the brain tissue of male rats and 8 and 16 hours in female rats (Table 1, 2), with approximately a third of the activity of the control group. In the liver tissue, inhibition was detected starting at hour 2 and continued to increase, especially in male rats, and reached a maximum at 32 and 64 hours. After 72 hours, a smaller

Table 1. Changing with respect to time the effect of dichlorvos on acetylcholinesterase activity in some tissues of male rats

Time (hour)		0 Mean±SD	2 Mean±SD	4 Mean±SD	8 Mean±SD	16 Mean±SD	32 Mean±SD	64 Mean±SD	72 Mean±SD
Serum	Control	6.25±0.23	7. 52±0.52	7.52±0.48	7.49±0.73	7.62±0.41	7.59±0.92	7.43±0.43	7.72±0.51
	DDVP	6.02±1.11	8.95±1.28	7.29±0.58	3.87±0.59*	3.96±0.71*	4.83±0.93*	4.19±0.72*	5.08±0.83*
Brain	Control	30.25±2.02	32.33±2.45	34.22±2.65	33.26±3.03	32.52±3.12	31.63±2.54	32.44±2.76	32.59±2.70
	DDVP	29.11±1.93	25.10±1.58*	11.23±1.12*	10.91±1.78*	15.32±1.96*	20.22±1.57*	19.11±1.73*	19.36±1.39*
Liver	Control	1.95±0.11	2.71±0.21	2.72±0.23	2.65±0.28	2.58±0.37	2.61±0.31	2.80±0.38	2.80±0.29
	DDVP	1.69±0.12	1.39±0.19*	1.12±0.15*	1.11±0.14*	1.02±0.17*	0.92±0.08*	0.94±0.07*	1.30±0.09*
Kidney	Control	19.04±2.41	22.29±2.02	22.34±1.98	21.19±2.65	22.31±1.94	22.49±1.55	23.91± 2.01	23.30±2.33
	DDVP	18.17±1.51	22.03±1.88	19.93±1.75	19.08±1.66	20.97±1.82	20.11±1.25	19.91±1.53	22.31±1.36
Small intestine	Control	2.05±0.55	2.19±0.27	2.21±0.71	2.28±0.53	2.21±0.47	2.25±0.86	2.37±0.54	2.32±0.47
	DDVP	1.87±0.44	2.08±0.29	2.12±0.01	1.99±0.08	1.19±0.09	1.95±0.11	2.18±0.05	2.14±0.03

* – Data shown in the vertical column for same tissues and same time are different from control at 0.05 statistical levels ($p<0.05$)
r – All data in the table showed enzyme activities as U.(mg protein) $^{-1}$ x 10^{-1}
SD: Standard Deviation

Table 2. Changing with respect to time the effect of dichlorvos on acetylcholinesterase activity in some tissues of female rats

Time (hour)		0 Mean±SD	2 Mean±SD	4 Mean±SD	8 Mean±SD	16 Mean±SD	32 Mean±SD	64 Mean±SD	72 Mean±SD
Serum	Control	7.93±0.89	8.23±0.45	8.56±0.74	8.42±0.59	8.57±0.26	8.64±0.09	8.37±0.81	8.49±0.52
	DDVP	6.23±0.49	9.82±0.93	6.42±0.71*	2.68±0,09*	3.58±0.11*	3.92±0.13*	4.21±0.98*	4.52±1.25*
Brain	Control	33.61±3.25	36.52±3.14	35.42±2.89	35.23± 2.96	36.81±4.21	35.82± 3.52	35.41±3.78	35.82±2.15
	DDVP	30.98±2.14	20.45±2.7*	19.13±1.19*	15.36±1.86*	15.87±1.78*	16.68±1.63*	20.09±1.56 *	21.38±1.48*
Liver	Control	1.70±0.12	2.51±0.18	2.62±0.25	2.63±0.19	2.58±0.18	2.59±0.29	2.49±0.24	2.48±0.23
	DDVP	1.51±0.11	1.47±0.09*	1.43±0.14*	1.37±0.05*	1.35±0.12*	1.52±0.04*	1.55±0.48	1.61±0.68
Kidney	Control	33.22±2.85	40.20±3.02	38.66±2.44	42.13± 2.81	42.14±3.29	39.54±3.22	36.54± 3.05	42.55±3.18
	DDVP	32.16±2.77	37.21±2.56	34.31±2.59	39.52±3.02	41.59±3.22	31.82±2.67	34.11±2.33	38.31±3.21
Small intestine	Control	2.58±0.09	2.73±0.21	2.75±0.41	2.78±0.71	2.59±0.46	2.73±0.28	2.71±0.49	2.63±0.76
	DDVP	2.52±0.81	2.55±0.77	2.59±0.74	2.43±0.92	2.11±0.33	2.13±0.48	2.61±0.71	2.31±0.44

* – Data shown in the vertical column for same tissues and same time are different from control at 0.05 statistical levels ($p<0.05$)
r – All data in the table showed enzyme activities as U.(mg protein) $^{-1}$ x 10^{-1}
SD: Standard Deviation

decrease in inhibition was detected. In the liver tissue of female rats, the enzyme activity was calculated as 1.9-fold of the control value at 8 and 16 hours.

DISCUSSION

AChE exists in two forms. While the real AChE functions essentially in erythrocytes and in the nerve tissue, serum pseudocholinesterase activity is found in the liver, heart, pancreas and brain. The role of cholinesterase is to hydrolyze choline and acetic acid, which are the inactive components of acetylcholine. Inhibition of cholinesterase leads to accumulation of acetylcholine in the nerve synapses and neuromuscular junctions and results in excessive stimulation of acetylcholine receptors. This situation can cause persistent depolarization of skeletal muscles, myasthenia, hypertension and tachycardia (Robey, 2004; Worek, 1996). It has been proposed that organophosphate pesticides inhibit AChE and pseudocholesterase in target tissues (John *et al.*, 2001; Kalender *et al*., 2006), such as pancreas (Yurumez *et al*., 2007), liver (Kalender *et al*., 2005) and heart (Ögütçü *et al*., 2006).

At the neuromuscular junction region, AChE is responsible for preventing restimulation of the stimulated cells after the first action potential. Since irreversible inhibition of AChE leads to continuous stimulation of the parasympathetic system and muscle tissue, this continuous stimulation can result in cell death. It has been seen that while dichlorvos inhibits AChE in the serum, brain and liver tissue of both female and male rats, AChE activity was almost the same when compared with the control groups. Inhibition was also detected at the last time point, 72 hours after administration, indicating that inhibition of AChE was not dependent on time (Tables 1 and 2).

Cellular changes due to the effect of pesticides cause metabolic changes in the organism. While dichlorvos leads to increases in the activities of enzymes, such as glucose 6-phosphate dehydrogenase, malate dehydrogenase, pyruvate kinase and glutathione S-transferase (Dere *et al*., 2007; Dere *et al*., 2008; Özdikicioglu *et al*., 2008), it has been observed through immunocytochemical studies that dichlorvos significantly decreases perforin, granzyme A and granulocyte levels in NK-92Cl cells (Li *et al*., 2002). Dichlorvos leads to histopathological changes in the lungs, liver, kidney, heart and spleen (Luty *et al*., 1998). Especially in hepatocytes where major histopathological lesions are available, parenchymal and vacuolar degenerations have been observed (Mengi *et al*., 2007). Injuries in the granular endoplasmic reticulum membranes among the organelles and cytological damage, like scattering of ribosomes to the cytoplasm, will directly affect protein synthesis.

One of the mechanisms of inhibition in our study is that dichlorvos itself or its metabolites can oxidize amino acids directly related to AChE activity. The World Health Organization (1989) determined that dichlorvos forms free radicals and causes oxidative stress, which leads to damage.

Thanks to the easy accessibility of information through developing technology, one of the problems many of us complain about is memory loss, which progresses as a disease with increasing age. Millions of people worldwide

live with chronic memory loss due to Alzheimer's disease. This disease not only makes remembering difficult, but also creates mental confusion. Since dichlorvos inhibits AChE enzyme long term (Tables 1 and 2), it is possible that it would improve the fragmented acetylcholine release from cholinergic neurons and facilitate cholinergic nerve transmission. With this approach, it can be considered that dichlorvos, as an organophosphate insecticide, could have a regulatory effect on the cognitive defects associated with Alzheimer's disease, which develops through the cholinergic system. As a matter of fact, Farlow *et al*. (1999) argued that Metrifonate, which is used in the treatment of Alzheimer's, functions by metabolic conversion into dichlorvos in the organism. In other studies of Alzheimer's patients, it was shown that Metrifonate meaningfully decreased psychiatric symptoms through dichlorvos and that it positively affected cognitive functions (Cummings *et al*., 2001; Mega *et al*., 2001). We think that the use of dichlorvos as a drug should be studied further.

ACKNOWLEDGEMENTS:
We would like to thank Uludag University, Faculty of Medicine, Department of Pharmacology. This study was supported by the Research Foundation of Uludag University. We wish to thank American Journal Experts (AJE) for their indispensable assistance in the editing of this paper.

Address for correspondence:
Egeman Dere
Uludag University
Faculty of Science and Art
Department of Biology
16059 Nilüfer, Bursa, Turkey
E-mail: edere@uludag.edu.tr

REFERENCES

1. ATSDR Agency for Toxic Substances and Disease Registry: Toxycologycal Profyle For Dychlorvos. U.S. Goverment Printing Office, 1997.
2. Bohringer Manheim, GmbH, 1973, Acetylcholinesterase; in Biochemica information I, 11.
3. *Bradford MM*, 1976, A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding, *Anal Biochem*, *72,* 248-54.
4. *Cummings JL, Nadel A, Masterman D*, 2001, Efficacy of metrifonate in improving the psychiatric and behavioral disturbances of patients with Alzheimer's Disease, *J Geriatr Psychiatry Neurol*, 14, 2, 101-8.
5. *Delen N, Durmusoglu E, Güncan A, Güngör N, Turgut C, Burçak A*, 2005, Türkiye'de Pestisit Kullanimi, Kalinti Ve Organizmalarda Duyarlilik Azalisi Sorunlari. Türkiye Ziraat Mühendisligi 6. Teknik Kongre Bildirisi. Ankara, 3-7 Ekim 2005, sayfa 629-648. Kongre Kitapçigi, Ankara.
6. *Dere E, Özdikicioglu F, Tosunoglu H*, 2007, The Effect of Intraperitoneal Administration of Dichlorvos on the Activity Glucose 6-Phosphate Dehydrogenase and Malate Dehydrogenase in Some Tissues of Rats (*Rattus norvegicus*), Uludag University, *Review of Medicine Faculty*, 33, 1, 5-10.
7. *Dere E, Ari F, Tosunoglu H*, 2008, Pyruvate Kinase Activity In Various Organs Of Rats Exposed To Dinitro-O-Cresol And Dichlorvos, *Acta Vet (Beograd)*, 58, 5-6, 439-47.
8. *Dutta HM, Arends DA*, 2003, Effects of endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish, *Environ Res*, 91, 157-62.
9. *Emerson GM, Gray NM, Jelinek GA, Mountain D, Mead HJ*, 1999, Organophosphate poisoning in Perth, Western Australia, 1987-1996, *J Emerg Med*, 17, 2, 273-7.

10. *Farlow MR, Cyrus PA, Nadel A*, 1999, Metrifonate treatment of Alzheimer's Disease, Influence of APO E genotype, *Neurology*, 53, 2010-6.
11. *Glusczak L, Miron DS, Crestani M, Fonseca MB, Pedron FA, Duarte MF, Vieira VLP*, 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (Leporinus obtusidens), *Ecotoxicol Environm Saf*, 65, 237-41.
12. *Jerrold HZ*, 1984, Biostatistical Analysis prentice-Hall Inc, Englewood Cliffs, New Jersey, 138-78.
13. *John S, Kale M, Rathore N, Bhatnagar D*, 2001, Protective effect of vitamin E dimethoate and malathion induced oxidative stress in rat erythrocytes, *J Nutr Biochem*, 12, 500-4.
14. *Kalender S, Ögütçü A, Uzunhisarcikli M, Açikgöz F, Durak D, Ulusoy Y, Kalender Y*, 2005, Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes, *Toxicology*, 211, 197-206.
15. *Kalender Y, Uzunhisarcikli M, Ögütçü A, Acikgöz F, Kalender S*, 2006, Effects of diazinon pseudocholinesterase activity and haematological indicates in rats: The protective role of vitamin E, *Environ Toxicol Phar*, 22, 46-51.
16. *Koçak A, Senol E, Kök HO, Aktas Ö*, 2005, Organofosfat (Tamaron) Zehirlenmesi Sonrasinda Gelisen Nöropati, *J Forensic Med*, 2, 109-12.
17. *Li Q, Nagahara N, Takahashi H, Takeda K, Okumura K, Minami M,* 2002, Organophosphorus pesticides markedly inhibit the activities of natural killer, cytotoxic T lymphocyte and lymphokine-activated killer: a proposed inhibiting mechanism via granzyme inhibition, *Toxicology*, 172, 3, 181-90.
18. *Luty S, Latuzynska J, Halliop J, Tochman A, Obuchowska D, Przylepa E, Korczak E, Bychawski E*, 1998, Toxicity of dermally absorbed dichlorvos in rats, *Ann Agr Env Med*, 5, 57-64.
19. *Mega MS, Cummings JL, O'Connor SM ve ark*, 2001, Cognitive and metabolic responses to metrifonate therapy in Alzheimer's Disease. *Neuropsychiatry Neuropsychol Behav Neurol*, 14, 1, 63-8.
20. *Mengi A, Toker NY, Oztabak K, Turkay GH, Yardibi H, Yuzbasioglu G*, 2007, Biochemical and histopathologic changes in acute toxicity of dichlorvos in rats, *Indian Veterinary Journal*, 84, 3.
21. *Miron D, Crestani M, Schetinger MR, Morsch VM, Baldisserotto B, Tierno MA, Moraes G, Vieira VLP*, 2005. Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on Acetylcholinesterase activity in the silver catfish (Rhamdia quelen) (Heptapteridae), *Ecotoxicol Environm Saf*, 61, 398-403.
22. *Nagami H, Nishigaki Y, Matsushima S, Matsushima T, Asanuma S, Yajima N, Usuda M, Hirosawa M*, 2005, Hospitalbased survey of pesticide poisoning in Japan, 1998-2002, *Int J Occup Environ Health*, 11, 2, 180-4.
23. *Ögütçü A, Uzunhisarcikli M, Kalender S, Durak D, Bayrakdar F, Kalender Y*, 2006, The effects of organophosphate insecticide diazinon on malondialdehyde levels and myocardial cells in rat heart tissue and protective role of vitamin E, *Pestic Biochem Physiol*, 86, 2, 93-8.
24. *Özdikicioglu F, Dere E, Tosunoglu H*, 2008, The Effect of Dichlorvos on Glutathione S-Transferase Activity in Some Tissues of Rats, *J Appl Biol Sci*, 2, 1, 35-8.
25. *Recena MCP, Pires DX, Caldas ED*, 2006, Acute poisoning with pesticides in the state of Mato Grosso do Sul, Brazil, *Sci Total Environ*, 357, 88-95.
26. *Robey WC, Meggs WJ*, 2004, Insecticides, Herbicides and Rodenticides. In: Tintinalli JE, Kelen GD, Stapczynski JS, eds. Emergency Medicine: a Comprehensive Study Guide. 6th Edn. McGraw-Hill Co, New York, 1134-43.
27. *Parmeggiani L*, 1983, Encyclopedia of occupational health and safety. Vol. 2. Geneva: International Labour Organization.
28. WHO, 1989, Environmental Health Criteria 79: *Dichlorvos*. World Health Organization, Switzerland: Geneva.
29. *Worek F, Kirchner T, Backer M, Szinicz L*,1996, Reactivation by various oximes of human erythrocyte acetylcholinesterase inhibited by different organophosphorus compounds, *Arch Toxicol*, 70, 497-503.
30. *Van der Hoek W, Konradsen F*, 2005, Risk factors for acute pesticide poisoning in Sri Lanka, *Trop Med Int Health*, 10, 6, 589-96.

31. *Yurumez Y, Yavuz Y, Sahin O, Ciftçi IH, Özkan S, Büyükokuroglu ME*, 2007, Can diphenhydramine prevent organophosphate-induced acute pancreatitis? An experimental study in rats, *Pestic Biochem Physiol*, 87, 271-5.

UTICAJ DIHLORVOSA NA AKTIVNOST ACETILHOLIN-ESTERAZE U NEKIM TKIVIMA PACOVA

DERE E, ARI FERDA i UGUR S

SADRŽAJ

U ovim ispitivanjima je praćena aktivnost acetilholin esteraze u krvnom serumu, mozgu, jetri, bubrezima i tankim crevima pacova oba pola, posle intraperitonealne aplikacije dihlorvosa. Preparat je aplikovan u dozi od 4mg kg^{-1} telesne mase, dok je kontrolna grupa dobijala fiziološki rastvor u istoj zapremini i na isti način. Životinje su žrtvovane dekapitacijom, radi uzimanja uzoraka tkiva u nultom satu i zatim 2, 4, 8,16, 32, 64 i 72 sata posle aplikacije, kada je i određivana aktivnost enzima.

U bubrezima i tankom crevu, nisu zapažene promene u aktivnosti enzima u odnosu na pol i vreme aplikacije. Značajno smanjenje aktivnosti Ach – esteraze dokazano je u krvnom serumu, jetri i moždanom tkivu. U odnosu na kontrolne vrednosti, aktivnost Ach – esteraze je bila smanjena počevši od drugog i četvrtog sata i ta inhibicija je trajala sve do kraja ogleda.