J.Pest Cont. & Environ. Sci. 8(3):45-58(2000).

BIOCHEMICAL EFFECTS OF THE FUNGICIDE, PYRAZOPHOS ON COMMON CARP (*CYPRINUS CARPIO L.*)

By

Nagat Mohamed Aly

Pesticide Central Lab.

Agriculture Research Center, Alexandria.

Received18/7/2000, Accepted 22/8/2000.

ABSTRACT

The effects of three concentrations (4, 12 and 18 mg / L) of the organophosphorus fungicide; pyrazophos on the activity of certain enzymes; acetylcholinesterase (AChE) and buterylcholinesterase (BuChE) in brain, some liver detoxifying enzymes (glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), acid phosphatase (AcP), glutathione-S-transferase (GST) and catalase (C-ase) in liver and lipid peroxidation of common carp in different organs (liver, kidney, gill and muscle) after 24 hours of the treatment were investigated.

The data showed a significant inhibition of the AChE and BuChE activities in a concentration dependent manner. Activities of GOT and GPT were significantly decreased with all tested concentrations, with the exception of a significant induction which was found in the fish exposed to the lowest concentration of 4 mg / L.

No significant changes on AcP and GST activities were noted. On the other hand, the lowest concentration of pyrazophos caused a significant reduction in catalase activity. Also, the data illustrated that lipid peroxidation was significantly increased in liver and gill with all concentrations when compared with the control.

INTRODUCTION

The environmental contamination of air, land and water by pesticides, and their deleterious effects on non-target organisms, is probably the greatest public environmental problem since the 1960's. However, the adverse impact of pollutants, including all pesticides groups, is much greater on the aquatic organisms than the terrestrial organisms (Holden, 1973). Widespread contamination of aquatic ecosystems by a whole range of pesticidal chemicals has been responsible for the hulking fish kills and recognized as a potential human health hazard as well (Sekine *et al.*, 1996 and Ismail *et al.*, 1997). The spray drift or run off of many pesticides may affect aquatic life including fish (Saunders, 1969 and Grande *et al.*, 1994). Fish has long been used as a biomarker of aquatic pollution caused by insecticides (Tejada, 1996, El-Gendy *et al.*, 1996 and El-Gougary *et al.*, 1999).

Pesticides poisoning is an important cause of mortality and a serious adverse health problem particularly in developing countries. OP's rank high among pesticides causing human hazard. Pharmacologically, the OP's may be classified as anticholinesterase (O'Brien, 1969) referring to their ability to bind with acetylcholinesterase (AChE). However, it is known that OP's compounds are not only anti-cholinergic agents, but also they cause a variety of other pharmacological and toxicological effects (Eto, 1974).

Many pesticides affect the activity of some enzymes; acid phosphatase and alkaline phosphatase (Abo-Donia, 1986 and El-Gendy *et al.*, 1990a), glutathione-S-transferase (Saffi, 1996), transaminases (Bogusz,1986 and El-Gourgary *et al.*, 1999) and catalase (El-Gendy *et al.*, 1990b and 1996) in living organisms.

The present study was carried out to explore the effect of three levels of the organophosphorus fungicide, pyrazophos on the activity of

some enzymes of common carp fish in different organs after 24 hours of the treatment.

MATERIALS AND METHODS

Tested Compound:

The fungicide, pyrazophos (30 % EC), O, O diethyl, O- (5-methyl-6-ethoxy carbonyl-pyrazolo- (1,5a)-pyrimid-2yl) thionophosphate was provided from Hoechst Company, West Germany. The concentrations administrated in this study were 4, 12 and 18 mg / L. These concentrations responded the 2/3, 2 and 3 fold LC_{50} (6 mg / L) of pyrazophos according to Worthing and Hance (1991).

All other chemicals of highest purity were obtained from Sigma, BDH and Aldrich Chemical Companies.

Experimental fish:

Common carp (*Cyprinus carpio l.*) was obtained from Saft Kaleid Farm in Etay Al-Barood, Behera Governorate. The fish weighing 124 ± 23 gm was kept in large tanks with aerated tap water and left to acclimate to the laboratory conditions for about two weeks before the experiment began. During rearing, standard fish diet was introduced.

Animals treatment:

The healthy fish each approximately 124 ± 23 gm weight were exposed to the tested concentrations (4, 12 and 18 mg / L) of the organophosphorus fungicide; pyrazophos for 24 h. Nine replicates were used for each treatment. The fourth group used as control (0 mg / L) and prepared under identical conditions with the same number of fish. The treated and untreated fish were killed, then brain, liver, kidney, gill and muscle were removed rapidly, washed with physiological saline solution and used for the determination of AChE, BuChE, GOT, GPT, AcP, GST, c-ase and lipid peroxidation.

Enzymes Assay:

Tissues from control and treated groups were homogenized in cold suitable buffer (1:10 w/v). The homogenates were centrifuged at 8,000 xg for 30 min. at 4 °C and supernatants were used as the enzymes source. The activities of AChE and BuChE were assayed in brain by the method of Elman *et al.*,(1961). Activities of the transaminases GOT and GPT were determined spectrophotmetrically using the method of Reitman and Frankel, (1957). Activity of AcP was measured according to Bessey *et al.*, (1946). GST activity was determined by the procedure of Vessey and Boyer (1984), with 1,chloro-2, 4 dinitrobenzene as substrate. C-ase activity was estimated by the method involving measurement of H_2O_2 consumption rate at 240 nm and 25 °C (Beers and Sizer, 1952) and expressed in Bergmeyer Units (BU). The amount of catalase, which decomposes gram of H_2O_2, equals one BU.

Lipid peroxidation was determined according to Placer *et al.*, (1966) and Nair and Tuner (1984) using thiobarbituric acid and expressed as n moles malondialdhyde (MDA). The protein was determined by the method described by Lowry *et al.*, (1951) using bovine serum albumin as a standard.

The data were calculated as mean ± SD and analyzed using analysis of variance technique (ANOVA). The p value of < 0.5 was considered significant.

RESULTS AND DISCUSSION

AChE and BuChE activities:

Table (1) illustrated the *in vivo* effects of the tested concentrations of pyrazophos on brain AChE and BuChE activities of common carp. The results showed that the activities of both enzymes were significantly reduced, and the reduction was in a concentration dependent manner. There were no significant differences between the three concentrations in both enzymes, except for AChE at the sublethal concentration (4 mg / L). Our data are in agreement with those of Safi, (1996) who found that a single oral dose of pyrazophos (242 mg / kg) caused significant reduction

in AChE and BuChE activities of hen liver after 24 h. Some investigators as El-Gendy *et al.*,(1990b) and (1996) reported that AChE inhibition is considered the principle target of many OP's compounds in different organs of fish. The results are parallel to those of Nemcsok *et al.*, (1984) who noticed that paraquate inhibited ChE activities in serum, brain, heart, and muscle of *(Cyprinus carpio L)*.

The inhibition of these enzymes disturbs the normal nervous function, leading finally to the death of animals.

Table (1): Effects of pyrazophos concentrations on AChE and BuChE activities of brain common carp *(Cyprinus carpio L.)* after 24 h of the treatment.

Concentrations (mg / L)	Mean ± SD (μ moles of analyzed substrate / mg protein / min.)	
	AchE	BuChE
0	35.79 ± 3.67^{c}	$0.66 \pm .006^{b}$
4 mg / l	17.36 ± 0.673^{b}	0.374 ± 0.06^{a}
12 mg / l	10.42 ± 0.72^{a}	0.349 ± 0.9^{a}
18 mg / l	9.0 ± 0.30^{a}	0.228 ± 0.04^{a}

Means have the same letter is considered non-significant different, $p < 0.05$.

Detoxifying enzymes in liver :

Transaminases and phosphatases are important in the biological processes. They are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference with these activities out the normal range

should lead to biochemical impairment and lesions of the tissues and cellular functions (Latner, 1978).

The obtained data of GOT and GPT activities (Table 2), illustrated that the lowest concentration (4 mg / L) of pyrazophos caused a significant increase in the activities of liver GOT and GPT. Then the activities were significantly depressed by accessing the concentrations when compared with control. The percentage of inhibition of liver GOT and GPT was in a concentration dependent manner. The present results are in agreement with those of El-Gougary *et al.*, (1999) who found different effects on the serum GOT and GPT of bolti fish (*Orechromus niloticus*) after 24 h of exposure to the sublethal concentrations, 0.32, 1.28, 2.26 and 3.2 mg / L of the organophosphorus insecticide, pirimiphos-methyl. In contrast, El-Gendy *et al.*, (1990b) reported that pyrazophos at LC_{50} and half LC_{50} caused stimulation in the liver GOT and GPT activities of common carp *(Cyprinus carpio L.)*.

Table (2): Effects of pyrazophos concentrations on liver some detoxifying enzymes activity of common carp *(Cyprinus carpio L.)* after 24 h of the treatment.

Enzymes	Concentrations (mg / L)			
	0	4	12	18
GOT	28.5 ± 1.2^{b}	38.9 ± 5.5^{c}	19.85 ± 2.4^{a}	16.3 ± 0.72^{a}
GPT	15.9 ± 1.5^{b}	21.98 ± 0.6^{c}	14.99 ± 1.9^{b}	8.9 ± 0.7^{a}
AcP	3.1 ± 0.2^{a}	4.1 ± 0.73^{a}	3.6 ± 0.4^{a}	3.51 ± 0.54^{a}
GST	0.0254 ± 0.001^{a}	0.024 ± 0.003^{a}	0.0253 ± 0.003^{a}	0.026 ± 0.001^{a}
C-ase	1.1 ± 0.11^{b}	0.83 ± 0.09^{a}	1.38 ± 0.01^{b}	1.22 ± 0.27^{b}

GOT and GPT activities expressed as Units / mg protein
AcP activity expressed as (μ moles of p-nitrophenol / mg protein / min.)
GST activity expressed as OD / mg protein / min.
C-ase activity expressed as Units / gm wet tissue
Means have the same letter is considered non-significant different, $p < 0.05$.

Liver was used to evaluate the *in vivo* effects of pyrasophos tested concentrations on acid phosphatase of common carp after 24 h of exposure (Table 2). The data showed that the tested concentrations caused no significant stimulation in the liver AcP activity of treated fish. This data is parallel to Safi, (1996) who found that a single oral dose of pyrazophos showed inductive effect on the liver AcP activity after 24 h of hen treatment. The inductive effect of liver AcP after OP's treatment in this study agree with other investigators (El-Sebae *et al.*, 1987 and El-Gendy *et al.*, 1996). It can be suggested that changes in the AcP activity may be related to the biotransformation and elimination of the tested pesticide (Hans, *et al.*, 1993).

GST is thought to play a physiological role in the detoxification and elimination of toxic compounds (Chasseaud, 1973). The effect of the different concentrations of pyrasophos on liver GST activity of common carp is summarized in Table (2). It is quite clear from the data, a slight change in GST activity occurred. Some investigators found that GST activity decreased or did not change by treatment with some pesticides (El-Sebae *et al.*, 1987, El-Gendy *et al.*, 1990b and Jensen *et al.*, 1991). These changes of GST activity might be due to partial damage of tissues or it may suggest that pyrazophos have a poor affinity for the enzyme.

C-ase, which eliminates H_2O_2 from environment, is a part of the cellular defense system against " active oxygen form". A significant decrease in liver C-ase activity was produced by the lowest concentration (4 mg / L). While, no significant increase was found in the enzyme activity in treated animals with the two other concentrations, 12 and 18 mg / L. These observations are in agreement with those obtained by Matkovics *et al.*, (1980) who noticed that the liver catalase activity of the rat was increased by trichlorfan, while sumithion had no effect at all. On the other hands, El- Gendy *et al.*, (1990a) illustrated that the three pesticides; edifenphos, glyohosate and niclosamide at 1/1000 field recommended rate caused significant induction in liver C-ase of *Tilapia nilotica*.

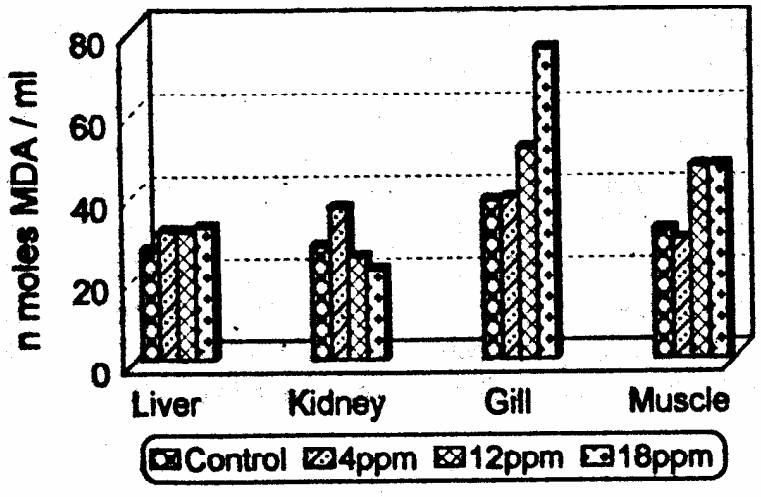
Changes in the type of effect of the pesticide on the enzyme from an increase to the opposite by changing the concentration may suggest two distinct binding sites, one of which may be the active site on the enzyme (Vessey and Boyer, 1988).

Lipid peroxidation levels:

The relatively high content of fat in fish tissues especially of polyunsaturated fatty acids (PUFA) is a well-known fact. PUFA are particularly abundant in biological membranes and their oxidation constitutes an obvious threat to the integrity of these structures. It was reported that relative rate of peroxidation was increasing with increasing number of double bonds (Dirks *et al.*, 1982).

Effects of the tested concentrations of pyrazophos on common carp lipid peroxidation in the different organs were illustrated in Figure (1). The data showed that all concentrations caused a significant activation in the level of liver lipid peroxidation, when compared with the control. There were no significant differences between the three concentrations.

Fig. (1): Effect of pyrazophos concentrations on Lipid peroxidation in different organs of Common carp (*Cyprinus carpio L.*)



The value of lipid peroxidation in kidney was also, significantly increased with the lowest concentration (4 mg / L), but the level was decreased by increasing the concentration. In contrast, the level in muscle was significantly increased with the concentrations, 12 and 18 mg / L to 49.8 % and 50.2 % respectively, when compared with the control.
Lipid peroxidation level in gill was increased in a concentration dependent manner. Our data was in agreement with El-Gendy *et al.*, (1990a) who reported that lipid peroxidation level increased in liver of carp by increasing the concentrations of glyphosate and fenvalerate.
Increase of the lipid peroxidation level and catalase activity pointed to the phenomenon connected with degeneration processes of the dystrophic muscle, entailing increase in the free radicals (Witas et., al.1984). The present study showed that the sublethal concentration, 2/3 LC_{50} (4 mg / L) and the higher concentrations, 2 and 3 fold LC_{50} (12 and 18 mg / L) cased adverse effect on the tested biochemical parameters of the common carp fish.

In general, it can be concluded that the exposure to pesticides either under low or high concentrations may cause changes (increase or decrease) in the activities of many enzymes in different organs. Also, we can postulate that non-target organisms are frequently exposed to pesticides. If these animals did not killed out right, their ability to reproduce or survive may be compromised. So, it is recommended to reduce and be extremely careful in application of such pesticides in the environment.

REFERENCES

Abou-Donia, M. B., K. M. Abdo , P. R. Timmons and J. E. Roctor (1986). Brain acetylcholinesterase, acid phosphatase and 2,3 cyclic nucleotide 3-phosphothydrolase and plasma butyryl –. cholinesterase activities in hens treated with a single dermal neurotoxic dose of S,S,S–tri-n-butyl phosphorotrithioate. Toxicol. Appl. Pharmacol, 83 :461-473.

Beers, R.F. Jr. and I. W. Sizer. (1952). Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, J. Biol. Chem.,195:133-140.

Bessey, D.A., O.H. Lowry and M.I. Brock (1946). Determination in serum with p-nitrophenylphosphate. J. Biol. Chem., 164: 321-329.

Bogusz, M. (1986). Studies on the activity of certain enzymatic systems in agriculture workers exposed to the action of organophosphorurs insecticides. P. I. Lek. Wiadomosci. Lik.,32: 786.

Chasseaud, L.F. (1973). The nature and distribution of enzymes catalyzing the conjugation of glutathione with foreign compounds. Drug Metab. Rev., 2: 185-220.

Dirks, R. C., M. D. Faiman and E. S. Huyser (1982). The role of lipid free radical initiator and oxygen on the kinetics of lipid peroxidation. Toxicol. Appl. Pharmacol., 63: 21-28.

El-Gendy, K. S., N. S. Ahmed, N. M. Aly, N. Saber and A. H. El-Sebae (1990a) Effect of some pesticides on the antioxidant enzymes and lipid peroxidation in carp tissues. J. Pest. Control. Environ. Sci., 2: 21-27.

El-Gendy, K. S., N. M. Aly, N. S. Ahmed and A. H. El-Sebae (1990b). Comparative toxicity of some pesticides to common carp and their effects on biochemical targets in living tissues. J. Pest. Control. Environ. Sci., 2: 29-41.

El-Gendy, K. S., N. M. Aly, N. Saber and A. A. El Sebae (1996). Toxicological effects of some pesticides on *Tilapia nilotica*. Alex. Sci. Exch., 17 (3): 243-251.

El-Gougary, O. A., M. Saad, M. Ismail and Y. M. Ahmed (1999). Protein and transaminases activities modulation by sublethal concentrations of pirimiphos-methyl in blood serum of bolti fish *Orechromus niloticus*. J. Pest. Cont. Environ. Sci., 7 (1): 71-84.

Ellman, G.L., K. D. Courtney, V. Jr. Andress and R. M. Featherstone (1961). A new and rapid calorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88-95.

El-Sebae, H.A., N. S. Ahmed, K. S. El-Gendy, A.S. El-Bakary and S. A. Soliman (1987). Methamidophos (Tamaron) a delayed neuropathic compound to man, but negative to chickens. Proceedings of Second National Conference of Pests and Diseases of Vegetable and Fruit Crops in Egypt. Suez Canal Univ. Fac. Agric. Ismailia. Egypt. pp: 474-485.

Eto, M. (1974). Organophosphorus pesticides: Organic and biological Chemistry. CRC Press, Inc.: Cleveland, Ohio, pp. 387.

Grande, M., S. Andersen and D. Berge (1994). Effects of pesticides on fish Experimental and field studies. Norges land brukshoegskole Fagtjenesten., 1: 195-209.

Hans, R.K., M. A. Khan, M. Faroog and M. U. Beg. (1993). Glutathione-S-transferase activity in an earthworm (*Phcretima posthuma*) exposed to three insecticides. Soil Biol. Biochem., 25(4): 509-511.

Holden, A.V. (1973). Effects of pesticides on fish. In Environmental pollution by pesticides (ed. C. A. Edwards) Plenum, London, pp. 213-253.

Ismail, S.M, Y., M. Ahmed, Y.Y. Moselh and M. T. Ahmed (1997). The activities of some proteins and protein related enzymes of earthworms as biomarkers for atrazine exposure. Toxicol. and Environ. Chem.,63: 141-148.

Jensen, E. G., J. U. Shaare, E. Egaas and A. Goksyr (1991). Response of xenobiotic metabolizing enzymes in rainbow trout (*Oncorhynchus mykiss*) to endosulfan detected by enzyme activities and immunochemical method. Aq. Toxicol., 21(2-1): 81-92.

Latner, L. A.(1978). " Aminotransferases " Clinical Biochemistry Seventh Ed. W. B. Saunders Comp. Philadelphia, London, Toronto, pp.553-568.

Lowry, O. H., N. J. Rosebrough, A. L. Farrand and R. J. Randall (1951). Protein measurement with the folin phenol reagent. J. Biol.Chem.,193: 265-275.

Vessey, D. A. and T. D. Boyer (1988). Characterization of the activation of rat liver glutathione-S-transferase by nonsubestrate ligands. Toxicol. Appl. Pharmacol., 93:275-280.

Warthing, R. and R. J. Hance (1991). The Pesticide manual 4th Ed published by the British Crop Protection Couincil.

Witas, H., T. Gabryelak and B. Matcovics (1984). Comparative studies on superoxide dismutase and catalase activities in liver of fish and other Antarctic vertebrates. Comp. Biochem. Physiol., 77c (2): 409-411.

الملخص العربى

التأثيرات البيوكيميائية لمبيد البيرازوفوس على سمك البلطى

نجاة محمد على
مركز البحوث الزراعية – الصبحية – الاسكندرية

يهدف البحث دراسة تأثير ثلاث تركيزات وهى ٤ ، ١٢ ، ١٨ ملجم / لتر (التى تمثل ٣/٢ ، ٢ ، ٣ أمثال التركيز المميت لـ ٥٠ % من الأفراد المعاملة بعد ٩٦ ساعة) من المبيد الفطرى ، البيرازوفوس على إنزيمى الأسيتيل كولين إستيريز و البيوتيريل كولين إستيريز فى المخ و بعض إنزيمات إزالة السمية فى الكبد (الجلوتاميك اوكسالو أسيتيك ترانس أمينيز ، الجلوتاميك بيروفيك ترانس أمينيز ، الفوسفاتيز الحامضى ، الجلوتاثيون – إس– ترانسفيريز و الكتاليز) و على عملية الأكسدة للدهن فى الكبد– الكلية– الخياشيم– العضلات للسمك البلطى بعد ٢٤ ساعة من المعاملة.

أوضحت النتائج انه حدث تثبيط لنشاط كل من الأسيتيل كولين إستيريز و البيوتيريل كولين إستيريز بعلاقة طردية مع التركيز . وحدث إنخفاض معنوى فى نشاط كل من الجلوتاميك اوكسالو أسيتيك ترانس أمينيز و الجلوتاميك بيروفيك ترانس أمينيز مع التركيزات المرتفعة(١٢ و ١٨ ملجم / لتر) بينما حدثت زيادة معنوية عند التركيز المنخفض (٤ ملجم / لتر) . أحدثت التركيزات الثلاثة للمبيد تغيرات معنوية سواء بالزيادة أو الإنخفاض فى نشاط كل من الفوسفاتيز الحامضى و الجلوتاثيون– إس– ترانس فيريز فى كبد السمك.

أيضا أوضحت النتائج انه حدث إنخفاض معنوى لنشاط إنزيم الكتاليز فى الكبد مع التركيزات المرتفعة (١٢ و ١٨ ملجم / لتر) بينما حدثت زيادة معنوية مع التركيز المنخفض (٤ ملجم / لتر) . أما بالنسبة لمعدل الأكسدة للدهن فحدثت زيادة معنوية مع كل التركيزات فى كل من الكبد والخياشيم بالمقارنة بالكنترول.