Women' Coll. Ann. Rev.
Vol 17 (1992).

3

TOXICITY OF THE PYRETHROID INSECTICIDE DECAMETHRIN AND FENVALERATE TO FRESH-WATER CLARIAS LAZERA

11- Biochemical responces to induced intoxication. with Decametherin and Fenvalerate.

*Moreed Yanni; **Husseen Badawi and *Rokaya Saeed

*1- Department of Zoology, Girls College for Arts, Science and Education, Ain Shams University

**2- Institute of Oceanography and Fisheries Alexandria

ABSTRACT

The effect of acute concentrations of Decamethrin and Fenvalerate (Pyrethroid insecticide) on the different biochemical constituents of blood - liver and muscles of Clarias lazera were investigated. The sublethal concentrations induced many changes in water, protein and fat contents. Water content in blood and liver showed slight decrease than controls after using high concentration of decamethrin.

The two major components for growth and body composition i.e protein and fat were affected by the two

pyrethroid used. General decrease occured in protein content of the three tissues studied (blood, liver and muscles). Moreover a general increase in fat content was observed after exposure to low and high concentrations of Decamethrin and Fenvalerate. The increase in fat was more clarified in blood and liver, while in muscle fat tended to decrease slightly than in controls.

Glutamic oxaloacetic transaminase (Got) and Glutamic pyruvic transaminase (GPT) were studied in serum, liver and muscles. Marked enzymatic inhibition occured in GOT with decamethrin especially in serum. On the other hand Fenvalerate caused obvious decrease in serum, but in liver and muscles GOT decreased only with low dose and the values returned around the initial values with the high dose.

The second transaminase GPT showed significant reduction in the activity in serum and liver with different concentrations of decamethrin and Fenvalerate. However, in muscle GPT insignificant differences were observed after four days with both insecticides used.

The changes in such parameters during the exposure were recorded and the significance of such changes was discussed.

Women' Coll. Ann. Rev.
Vol 17 (1992).

INTRODUCTION

The chemical nature of our environment has been altered by the addition of several thousand synthetic chemical compounds. Some of these were used as pesticides although their general lack of specificity suggests that the term biocide may be more appropriate (Carson, 1962). The environmental persistence, accumulation, and effects of these chemicals in the biosphere are poorly understood.

All of these contaminants or residues reach the aquatic environment. The persistence and low water solubility of many pesticides contribute to their concentration in fish tissues. Residues analyses may in themselves provide us with indications of the action and effects of pesticides on fish and their ecosystem.

It appeared important to examine the effects of induced intoxication with two pyrethroid insecticides (Decamethrin & Fenvalerate) commonly used in Egypt on metabolism and biochemical components of the Nile fish Clarias lazera. Also to clarify more information about this group of pyrethroids which are considered one of the most principle groups of pesticides.

MATERIALS AND METHODS

The experimental animal used was Clarias lazera. The fish were caught from different localities near Cairo. The fish were of both sexes with average body weight of 326 grams and average lengths of 25 cms.

Collected mature Clarias lazera were transferred to 500 liter holding tanks. The animals were acclimatized for 10 days during which time were fed with worms.

Two formulated synthetic pyrethroid pesticides were used:

2.5% Decamethrin.

20 % Fenvalerate.

Utilizing serial dilution techniques by adding one ml of pesticide solution in acetone to each aquaria to obtain final sublethal concentration 3.6 & 7.3 ppm Decamethrin and also the same concentrations for fenvalerate. Control aquaria received only one ml of acetone.

Ten specimens randomly chosen from the tanks were taken for analysis from each aquaria after exposure for 96 hours to each insecticide. The fish were rapidly dissected and samples were taken from blood, liver and muscles.

Women' Coll. Ann. Rev.
Vol 17 (1992).

Blood Sampling:

The blood samples required for different biochemical analyses were taken by using the heart puncture method. Also excess of blood sample can be obtained by cutting the tail of the fish and collecting the blood which exudes.

The exuding blood was allowed to stand at room temperature until it has clotted. Blood samples were centrifuged at 300 R.P.M for 7 minutes, then clear serum was decanted and stored frozen at - 15°C for the time of use.

Tissue samples:

After collection of the blood samples, the fish were rapidly dissected and 2 more samples of skeletal muscles and liver were taken into tarred and stoppered weighing bottles for chemical determination of protein, fat and water contents.

Similar samples to those taken for chemical analysis were separately stored frozen at - 15°C together with blood serum for further determination of enzymatic activities.

Analytical methods:

Determination of water content:

Water content was determined by recording the wet weight of the sample before drying of the tissues in an electric oven at 103 ± 1°C, until a constant weight was

maintained. The weight of the dry sample was then recorded and the difference between the wet and dry weight gives the amount of moisture content in the sample provided. The moisture content is then expressed as percent (Heller, 1949)

Determination of fat content:

Extraction of fat from the dried tissues was carried out by several washings with ethyl ether and petroleum ether (40 - 60°C). The lipid content is given as percent of fresh weight.

Determination of protein content:

Determinations of total nitrogen and total protein contents of the blood, liver and muscles were performed according to the method of Kock and Mc- Meekin (1924).

Determination of transaminases (GOT & GPT)

Both enzymes were determined in serum and tissues on the basis of the technique reported by Reitman and Frankel (1957).

Women' Coll. Ann. Rev.
Vol 17 (1992).

RESULTS AND DISCUSSION

The data concerned with water content are shown in table (1). From this table one can notice that water content of two tissues only (blood & liver) showed a slight decrease than normal after using the high dose of decamethrin.

On the other hand the two major components for growth i.e protein and fat can be affected by the two pyrethroids used. From tables (2 & 3) one can notice that general decrease occurs in protein content of the three tissues studied (blood, liver and muscle) after exposure to different concentrations used for Decamethrin and Fenvalerate. No relation could be detected between the type of pesticide or its dose.

Moreover, a general increase in fat content was shown after treatment with the two concentrations of the pyrethroids used. Marked increase in fat was observed in blood and liver only while in muscle no variation was detected.

In the present study, the decrease in protein and the increase in fat of the tissues of Clarias lazera after exposure for 96 hours to both insecticide used agrees with Inui (1968) who found that rapid decrease 2 was noted after days of carbon tetrachloride exposure and reaching its

lowest level after 7 days. Morevoer, this author added that the decrease in protein content from the 2nd to the 7th days appears to be due to the loss of intracellular substances, including protein, by the deterioration of parynchymal cells. This may explain the same decrease in protein in Clarias lazera in the present investigation. The same author found also the fat of the liver varied markedly with the progress of time.

Thus, there was a trend for liver fat content to increase gradually, reaching its highest level on the 20th day. Moreover, the accumulation of fat in the animals produced by carbon tetrachloride intoxication, has long been a matter of interest to scientists (Reoknagel & Lomberdi 1961; Heimberg & Weinstein 1962; Seakins & Robinson 1963).

Coworkers & Wilson (1965) and Schatz & Recknagel 1960 clearly demonstrated that the fat accumulation is a result of inhibition of triglyceride secretory system in the liver. It is quite protable that a similar inhibitory mechanism plays an important role in the fat accumulation observed in the present study.

Also, it must not be forgotten that some of the previous authors supposed that this accumulation of fat originated from destructed cells of the liver.

However, phillips and Buhler (1979) gave another view regarding fat and protein after using Dieldrin and studying its effect on body composition of Salmo gairdneri. The authors found that various components which lead to fish growth such as protein and lipid are not necessarily influenced in the same way by pesticide exposure, but by diet composition and ration which have important influences on the growth responses of fish exposed to a pesticide. Dietary stress interaction should be more critically considered by reasrchers conducting laboratory studies using fish.

Enzymatic activity:

a- Glutamic oxaloacetc transaminase (GOT).
b- Glutamic pyruvic transaminase (GPT).

The data concerned with Got and GPT are shown in table (4). The data indicate that pesticides used in the present study have significant effect on activity of transaminases of treated Nile fish Clarias lazera. Marked enzymatic inhibition occurs in Got with all concentrations used especially in serum. In liver tissue the decrease in transaminase activity was clear only after the use of high dose of Decamethrin. In muscle Got, it appears to be slightly affected.

On the other hand, Fenvalerate caused obvious decrease in serum Got. In the liver and muscles GoT was inhibited with low dose, but after using the high dose, the values returned around their initial values.

The second transaminase studied (GPT) showd a general decrease in its activity in both serum and liver with the different doses of decamathrin and Fenvalerate. However, in muscle GpT insignificant differences were observed.

From the previous observation one can conclude that serum Got and GPT are more sensitive to the toxicity of pesticides. This sensitivity was noticed also in liver Got and GPT but not at the same level.

Furhtermore, the inhibition in the activity of serum GOT & GPT in the present study agrees with Inui (1968); Shelly & Kaplawitz (1980) and Verma et al (1981) after exposure of many species of fish to different sublethal concentrations of toxic substances and pesticides.

It is worth mentioning that other authors found increased activity of GOT and GPT with poisned salmon, (Bell, 1968). The same author put the following possibilities for the elevation of transaminases in poisned salmon.

a- Leakage of enzyme from damaged tissue, erythrocytes or bacteria.

b- Impaired mechanism for removal of serum enzyme.

c- Hemolysis of erythrocytes by bacterial hemolysis or exogenous poison.

Rosen et al (1958); Agress (1959) and Ogino (1965), considered that variation in the activity of transaminases was attributed to species, sex, age, residual blood in the tissue, nutritional or physiological conditions or other more subtle variables.

From the previous studies on GOT and GPT, it appears that both are sensitive to toxicity and varied markedly with different physiological and environmental factors effecting it. But on the other hand transamination represents one of the principle metabolic pathways for synthesis and deamination of amino acids. It is a reversible reaction and broad scope (Cohen & Salloch, 1961; Guirard & Snell 1964).

Meister (1955) had previously suggested that the amino acid necessary for protein synthesis is regulated by transamination. McAllan and Chefuske (1961a) found a close relationship between synthesis of protein and transamination activity.

From these observation one can explain the inhibition occurs in transaminases in the present work and its close relation to the decrease in protein content of serum and the tissues of Clarias lazera after exposure to two synthetic pyrethroids used (especially decomethrin).

REFERENCES

Agress, C.M. (1959):
Evaluation of the transaminase test. Am. J. Cardiol, 3: 74-93.

Bell, G.R. (1968):
Distribution of transaminases (amino transferases) in the tissues of pacific salom (Oncarhynchus), with emphasis on the properties and diagnostic use of glutamic oxalacetic transaminase. J. Fish. Res. Bd. Canada 25(6): 1247-1268.

Carson. R. (1962):
Silent spring, 368 pp. Boston. Houghton Mifflin.

Cohen, P.P., and Sallach, H.J. (1961):
Nitrogen metabolism of amino acids. In D.M. Green berg (ed.) metabolic pathways, Vol.2. Academic, Press, Inc., N.Y.P. 1-78.

Coworker, R.A., and Wilson, A.J. (1965):
Kinetics and effects of DPT in a tidal march. ditch. Trans. Amer. Fish. Soc., 94(2) 152.

Guirard, B.M., and Snell, E.E. (1964):
Vitamin B6 function in transamination and decarboxylation reaction. In. M. Florkin and E.H. Slotz (ed.)
Comparhensive biochemistry, Vol.15 Elsevier Publishing. Co. N.Y.P. 138-199.

Heimberg, M. and Weinstein, J. (1962):
A mechanism for the induction by carbon tetrachloride of fatty liver in the rat, Biochem. pharm. II.

Heller, H. (1949):
Effect of dehyration on adult and new born rats. J.physiol. (London.) 108, 303.

Inui, Y. (1968):
Pathological study on the effects of carbon tetrachloride posioning on the liver. Bull. Fresh water fish, Res. Lab. Vol. 18(2), 157-167.

Kock, S.C., and Mc Meekim. T.L. (1924):
A new direct nesslerization micro - Kjeldahl method and modification of Nessler folin reagent for ammonia J. Amer. Chem. Soc. 46P. 2066.

Mcallan, J.W., and Chefurka, W. (1961a):
Some physiological aspects of glutamic aspartate transamination in insects. Comp. Biochem. Physiol, 2:290-299.

Meister, A. (1955):
Transamination in amino acid metabolism. Federation Proc., 14: 683-689.

Ogino, C. (1965):
B. vitamins requirements of Carp, Cyprinus carpio 1. Deficiency symptoms and requirement of vitamin B6. Bull. Japan. Soc. Sci, Fish., 31(7): 546-551.

Phillips, G.R., Buhler, D.R. (1979):
Influences of Dieldrin on the growth and body composition of Fingerling Rainbow trout (Salmo gairdneri) Fed oregon noit pellets or tubificid warms (Tubifex. sp). Journal of the Fisheries Reserach Board of Canada 36(1) 77-80.

Reoknagel, R.O., and Lomardi, B. (1961):
Studies of biochemical changes in subcellular particles of rat liver and their relationship to a new hypothesis regarding the pathogenesis of carbon tetrachloride fat accumulation. J. Biol. Chem. 236(2), 564-569

Women' Coll. Ann. Rev.
Vol 17 (1992).

Reitman, S. and Frankel, S. (1957):
Calorimetric method for the determination of serum glutamic oxalocetic and glutamic pyruvic transaminases. Amer. J. Clin. Path. 28, 56-63.

Rosen, F., Frankel, S. (1957):
An enzymatic basis for the gluconeogenic action of hydrocartisone. Science, 127: 287-288.

Schatz., M.C. and Recknagel, R.O. (1960):
Rapid increase of rat liver triglyceride following carbon tetrachloride poisioning. Biochem. Biophys. Acta. 41, 151-152.

Seakims, A. and Robinson, D.S. (1963):
The effect of the administration of carbon tetrachloride on the formation of plasma lipo-proteins in the rat. biochem. J. 86, 401-407.

Shelly, L. and Kaplowitz, N. (1980):
Effect of pyridoxine deficiency on serum and liver transaminase in experimental liver injury in the rat GAS Traenlerology 79(3): 545-549.

Verma, S.R. Saroj. Rani and Dalela, R.C.(1981):
Responses of serum tranaminase in Notopterus notopterus Chronically exposed to phenolic compounds and their combinations. Environ. Res. 24(1): 218-223.

Table (1): Effect of Decamethrin and Fenvalerate on water conent in blood, liver and muscles of Clarias lazera after exposure for 96 hours.

Fish Group	Water Content in		
	Blood	Liver	Muscles
Control	87.07±0.55	75.36±0.35	97.80±0.35
Exposed to low dose (3.6ppm) Decamethrin	87.29±1.37	76.99±1.16	79.22±0.67
Exposed to high dose (7.3ppm) Decamethrin	87.29±1.37	76.99±1.16	79.22±0.67
Exposed to high dose (3.6ppm) Fenvalerate	85.29*±0.81 88.51±0.57	71.96*±0.81 76.07±0.68	79.88±0.52 79.99±0.54
Exposed to high dose (7.3ppm) Fenvalerate	87.84±0.76	76.70±0.77	80.50±0.18

The data is represented as Mean ± S.E (standard error).
* The value is statistically significant at $P<0.05$.

Women' Coll. Ann. Rev.
Vol 17 (1992).

Table (2): The effect of Decamethrin and Fenvalerate on the protein contents in blood, liver and muscles of Clarias lazera after exposure for 96 hours.

Fish Group	Protein Percentage in		
	Blood	Liver	Muscles
Control	37.86±2.46	30.30±1.75	49.49±2.08
Exposed to low dose (3.6ppm) Decamethrin	28.40*±1.14	29.66±1.16	39.12*±2.86
Exposed to high dose (7.3ppm) Decamethrin	28.51*±1.30	26.17*±1.30	37.30*±1.55
Exposed to high dose (3.6ppm) Fenvalerate	29.41*±1.55	27.44*±1.41	41.31±1.26
Exposed to high dose (7.3ppm) Fenvalerate	32.96*±1.56	27.25*±1.78	34.53*±1.94

- Tha data is represented as Mean ± S.E (standard error)
* The value is statistically significant at $P<0.05$.

Women' Coll. Ann. Rev.
Vol 17 (1992).

Table (3): The effect of Decamethrin and Fenvalerate on Fat contents in blood, liver and muscles of Clarias lazera after exposure 96 hours.

Fish Group	Fat Percentage in		
	Blood	Liver	Muscles
Control	0.16±0.01	4.96±0.23	2.85±0.14
Exposed to low dose (3.6ppm) Decamethrin	0.24±0.02	5.91±0.38	2.25±0.22
Exposed to high dose (7.3ppm) Decamethrin	1.32*±1.30	5.41±0.18	2.82±0.18
Exposed to high dose (3.6ppm) Fenvalerate	0.72*±0.15	5.22±0.35	2.36±0.22
Exposed to high dose (7.3ppm) Fenvalerate	0.50*±1.56	5.63±0.45	2.33±0.14

- The data is represented as Mean ± S.E (standard error).
* The value is statistically significant at $P<0.05$.

Table (4) : Comparison between mean values of Enzymes activities of glutamic oxaloacetictransaminase (GOT) and glutamic pyruvic transaminase (GPT) in serum liver and muscles of Clarias lazera after exposure to Decamethrin and Fenvalerate for 96 hours.

Fish group	Glutamic oxaloacetic transaminase (GOT)			Glutamic Pyruvic transaminase (GPT)		
	Serum	Liver	Mucles	Serum	Liver	Muscles
Control	118.80±5.97	20.86±0.69	12.61±0.55	64.24±7.32	11.10±0.89	5.94±0.23
Exposed to low dose (3.6ppm) Fenvalerate	58.62*±6.48	19.16±0.97	10.78*±0.58	47.21*±5.28	5.71*±0.52	5.05±1.03
Exposed to high dose (7.3ppm) Decamethrin	51.92*±8.89	14.29*±0.74	10.73*±0.83	43.89*±7.43	3.05*±0.46	6.73±0.73
Exposed to low dose (3.6ppm) Decamethrin	87.93*±5.47	18.41±0.81	10.98±0.62	49.29*±2.85	6.64*±1.07	6.04±0.62
Exposed to high dose (7.3ppm) Fenvalerate	49.34*±9.03	21.71±0.56	14.15±0.49	54.47*±7.81	4.62*±0.70	5.38±0.42

- The data is represented as mean ± S.E (standard error).
  The data is expresed as u mole/L or gram.
* The value is statistically significant at P<0.05.

Women' Coll. Ann. Rev.
Vol 17 (1992).