

EFFECT OF SUB-LETHAL DOSES OF CHLORPYRIFOS-ETHYL ON SOME BIOCHEMICAL PARAMETERS OF AFRICAN CATFISH *C. GARIEPINUS*.

E. O. OGUEJI AND J. AUTA

(Received 12 December 2006; Revision Accepted 26 February 2007)

ABSTRACT

The impact of long-term exposure to waterborne Chlorpyrifos-ethyl on *Clarias gariepinus* was evaluated through changes of selected biochemical parameters. Fish was exposed to 0.045mg/l, 0.096mg/l and 0.192mg/l and control with no toxicant for 8 weeks. The parameters measured were serum glucose, protein, cholesterol, triglyceride, glutamic pyruvic acid transaminase (GPT), glutamic oxaloacetic acid transaminase (GOT) and alkaline phosphatase (ALP). There was significant ($P < 0.05$) alterations between the control values and the exposed groups and among groups exposed to different concentrations on all parameters. The alterations in all parameters was significantly ($P < 0.05$) dose and time dependent.

KEYWORDS: Chlorpyrifos-ethyl, serum, biochemical parameters, *Clarias gariepinus*, toxicity.

INTRODUCTION

Chlorpyrifos is an organophosphate insecticide (Cox, 1994). It is very highly toxic to freshwater fish, estuarine and marine organisms (Caroline, 1995). Typically exposures of about 3 ppm are lethal to fish (EPA, 1984). Chlorpyrifos is directly toxic to the nervous system and is also transformed inside animals to chlorpyrifos-oxon and 3, 5, 6-trichloro-2-pyridinol (TCP) both of which are many times more toxic to the nervous system than chlorpyrifos itself (Chambers *et al*, 1989). Aquatic pollution by pesticides results mainly from their widespread use in agriculture and in vector control campaigns. In Nigeria especially in the northern part of the country, there is an upsurge in the application of pesticides in agriculture. A major reason for the increased use of pesticide in the north is the development of dry-season irrigated farming (Mbagwu and Ita, 1994). These poisons are washed into water bodies through surface run-off during the rainy seasons.

The mode of action of chlorpyrifos and their metabolites is inhibition of enzyme acetyl cholinesterase (AChE), and the inhibition of AChE activity caused by chlorpyrifos is more persistent than that caused by other organophosphates and it is believed that this is because chlorpyrifos is lipophilic (Chamber and Carr, 1993). They also inhibit other enzymes e.g ATPase, an enzyme important in cellular respiration (Sarki, 1990). Fish have an important role in the food chain; therefore, investigation of the effects of pesticides on fish has a diagnostic significance in evaluation of adverse effect of pesticides to human health (Begun and Vijayaraghavan, 1996). *Clarias gariepinus* is a fresh water fish and an important food supply for humans.

Biochemical characteristics of blood are among the important indices of the status of the internal environment of the fish organism (Edsall, 1999). Glutamate oxaloacetate (GOT) and glutamate pyruvate (GPT) transaminases are enzymes frequently used in the diagnosis of damage caused by pollutants in various tissues, such as liver, muscle and gills (De la Torre *et al*, 1999). Also alterations in the metabolism of protein and carbohydrate are used for similar purpose. Much work has not been done on the biochemical parameters of *C. gariepinus* in Nigeria. Therefore the aim of this study was to investigate the serum activities of GOT, GPT, ALP, protein and carbohydrate metabolisms after exposure of *Clarias gariepinus* juveniles to nominal sub-lethal concentrations of chlorpyrifos-ethyl (a commonly used insecticide with a view to accessing the possible mechanism of its toxicity).

MATERIALS AND METHODS

Juveniles of *Clarias gariepinus* was purchased from Maigana fish farm in Zaria, Kaduna State Nigeria. The *Clarias* species averaging 14.33 ± 0.50 cm standard length and average body weight of 20.38 ± 1.25 g were used for the study. The fish were conveyed to fisheries laboratory in a portable well-aerated white polythene bag containing water from the Dam. They were held in large water baths of 160L capacity at $24.5-25.5^{\circ}\text{C}$ and acclimatized for two weeks in dechlorinated municipal water. During this period, the fishes were fed with palletized diet containing 35% crude protein twice per day at 3% body weight. Also, the water in the glass aquaria was changed once every two days. The fishes were accepted as well as adapted to laboratory conditions when less than 5% death was recorded for the 14 days period and feeding was discontinued 24 hours before the start of the experimental run (Reish and Oshida, 1987).

SUB-LETHAL BIOASSAY

Based on the result of 96-h LC_{50} , which was estimated to be 0.92mg/l (Auta and Ogueji, 2006) juveniles were exposed to nominal concentrations of Chlorpyrifos-ethyl for 8 weeks. The concentrations used for chronic study of chlorpyrifos-ethyl were 0.045 mg/l, 0.096 mg/l, and 0.192 mg/l. Each treatment was in triplicate and there was a control in each case. With the exception of the control tanks, appropriate volumes of the toxicant were added into each tank. The fishes were randomly assigned to give a loading of 10 fish per tank. Fishes were fed to satiation twice daily. The toxicant and test water were renewed at two days intervals to maintain the toxicants strength and the level of dissolved oxygen as well as minimizing the level of ammonia during the experiment. Four fishes were sampled at the beginning of the experiment, and after every two weeks.

BIOCHEMICAL MEASUREMENTS

For biochemical investigations, The caudal peduncle of fish was cut, blood was collected in non-heparinized tubes. The blood was immediately centrifuged at 1500 rpm for 10min. Serum was then removed and stored at 4°C prior to immediate determination of biochemical parameters, glucose, cholesterol, triglycerides, total protein, glutamic pyruvic acid trasaminase (GPT), glutamic oxaloacetic acid trasaminase (GOT) and alkaline phosphatase (ALP). Blood serum glucose was estimated using the method of Trinder (1969). Serum

cholesterol was measured according to the procedure of Pearson *et al* (1953). Blood serum triglyceride was determined using the method of Rice (1970). The method of Lowry *et al* (1951) was carried out to determine the value of total protein. The activities of blood GPT and GOT were estimated according to the methods of Reitman and Frankel (1957). To determine the activity of blood ALP, Bessey *et al* (1946) method was used.

STATISTICAL ANALYSIS

For the various biochemical parameters, the GenStat statistical analysis software (GenStat, 2006) was used to run analysis of variance (ANOVA) and Duncan multiple range test (DMRT) was used to test for differences between different levels of treatment and to separate means respectively, where applicable (Duncan, 1955). Test of significance was at the 5% level of significance.

RESULTS

The results of the study, shown in Tables 1-2, showed that chlorpyrifos-ethyl had adverse effects on some biochemical parameters of *C. gariepinus* during the exposure

period. Analysis of variance (ANOVA) results of sub-lethal exposure to chlorpyrifos-ethyl indicated a significant ($p < 0.05$) dose dependent elevations in glucose and GPT (Table 1). On the other hand there was a significant ($p < 0.05$) dose dependent inhibitions in protein, cholesterol, triglyceride, GPT and ALP (Table 1). Also the cholesterol, triglyceride, GPT and ALP values of exposed fish were significantly inhibited ($p < 0.05$) in 0.096 mg/l and 0.192 mg/l concentrations (Table 1). The control value of GPT was also significantly higher ($p < 0.05$) when compared with exposed fish in all sub-lethal concentrations. The serum glucose control value was significantly lower ($P < 0.05$) than in all exposed fish (Table 1). The control values of protein, cholesterol and triglyceride were significantly lower ($P < 0.05$) than in 0.045 mg/l exposed fish (Table 1). There were time dependent significant elevations ($p < 0.05$) in the serum values of glucose, protein, triglyceride, GOT and ALP (Table 2). On the other hand, there was time dependent significant ($P < 0.05$) inhibitions in the values of cholesterol and GPT (Table 2).

Table 1: the effect of sub-lethal doses of chlorpyrifos-ethyl on some biochemical parameters of *C. gariepinus*.

Conc (mg/l)	Glucose (mg/dl)	Protein (g/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	GOT (iu/l)	GPT (iu/l)	ALP (iu/l)
0.000(cont.)	55.50+1.29 ^a	3.10+0.08 ^b	127.25+1.70 ^b	143.50+1.29 ^b	50.25+1.70 ^a	58.50+1.29 ^a	17.50+1.29 ^a
0.045	57.00+9.83 ^c	3.98+0.61 ^a	136.50+5.91 ^a	149.50+8.42 ^a	44.00+7.25 ^b	33.00+2.94 ^d	18.00+2.94 ^a
0.096	63.50+3.80 ^b	3.45+0.23 ^b	85.00+9.30 ^c	117.00+31.7 ^c	41.00+7.52 ^c	42.50+6.95 ^c	15.50+2.38 ^b
0.192	77.00+5.22 ^a	3.18+0.20 ^b	77.50+13.32 ^d	101.50+34.68 ^d	35.25+6.13 ^d	51.00+7.23 ^b	12.75+2.0 ^c

Means with the same superscript along columns are not significantly different ($p < 0.05$) (Mean values \pm SD) n=12

Table 2: the time course effect of sub-lethal doses of chlorpyrifos-ethyl on some biochemical parameters of *C. gariepinus*.

Time (Weeks)	Glucose (mg/dl)	Protein (g/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	GOT (iu/l)	GPT (iu/l)	ALP (iu/l)
2	58.50+9.5 ^b	3.39+0.48 ^a	113.00+24.08 ^a	112.00+34.40 ^b	37.75+7.55 ^d	50.25+10.13 ^a	14.25+4.25 ^b
8	68.00+9.4 ⁵	3.81+0.88 ^a	100.12+30.97 ^b	143.75+9.55 ^a	47.50+4.62 ^a	42.25+10.81 ^b	17.62+0.38 ^c

Means with the same superscript along columns are not significantly different ($p < 0.05$) (Mean values \pm SD) n=8

DISCUSSION

Biochemical parameters are a sensitive index to changes due to pesticide toxicity and can constitute an important diagnostic tool in toxicological studies (Rahman and Siddiqui, 2004). The aim of this study was to evaluate some biochemical alterations in *C. gariepinus* after sub chronic administration of chlorpyrifos-ethyl. The study showed that there was significant ($p < 0.05$) increase in glucose which was dose and time dependent. This may be considered to be manifestation of stress induced by Chlorpyrifos-ethyl. Glucose increase is a general response of fish to acute and sub-lethal pollutant effects (Verma *et al*, 1983; Ghazaly, 1994; Ceron *et al*, 1997; Luskova *et al* 2002). Wedemeyer and Mcleay (1981) stated that high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue, resulting in increased level of circulating glucocorticoids (Hontela, *et al*, 1996) and catecholamines (Nakano and Tomlinson, 1967). Both of these groups of hormones produce hyperglycaemia. The observed elevation of blood glucose in this study could be a response to the increased rate of glycogenolysis or gluconeogenesis. These findings and explanation are similar to those considered by Atef (2005) on fish exposed to cadmium.

The increase in serum protein was not significantly time dependent (Table 2). And the observed hyperproteinemia

may possibly be due to (1) water loss in the serum, and (2) the relative changes in the mobilization of blood protein. Our findings are consistent with that of Oruc and Uner (1999). The authors reported increase in liver protein following exposure of *Cyprinus carpio* to 2, 4 -Diamin for 30 days. Salib *et al* (1984) observed that the protein content in all tissues of malathion exposed *Tilapia mossambica* is slightly higher. They suggested that the fish exposed to pesticides may compensate any possible protein loss by increasing its protein synthesis.

Gill *et al* (1991) found an increase in liver proteins following Endosulphan intoxication and noted that protein levels in the liver of *Barbus conchionius* could be due to increased protein turnover. They also concluded that compensatory production of enzymes lost as a result of tissue necrosis or to meet increased demand to detoxify the pesticides might have necessitated enhanced synthesis of enzyme proteins (Gill *et al*, 1990). The quantity of protein is dependent on the rate of protein synthesis, or on rate of its degradation. It may also be affected due to impaired incorporation of amino acids into polypeptide chains (Ram *et al*, 2003). In this investigation there was a significant ($p < 0.05$) serum cholesterol inhibition which was time and dose dependent. The liver is the key organ in the synthesis and excretion of cholesterol, therefore any type of obstruction in the liver either intra or extra hepatic, will cause an increase in total cholesterol levels on the serum. However in chronic conditions such as cirrhosis, that involves considerable destruction of liver cells, the cholesterol levels eventually falls

below normal level since decreased synthesis is taking place (Kamath, 1972).

The observed significant ($p < 0.05$) time dependent triglyceride elevation may be due to impairments in the cell membrane organization and higher energy demands of *C. gariepinus* to get the positive survival value under the imposed sub-lethal concentration stress. Also since decreased thyroid secretion (hypothyroidism) greatly increases triglycerides level in the blood, the hypertriglyceridaemia may also be due to hypothyroidism induced by chlorpyrifos-ethyl and/or liver dysfunction since liver is the principal center of lipid metabolism (Gopal *et al.*, 1997). This observation and explanation was similar to that of Krishna *et al.* (1994) who reported increased levels of phospholipids and cholesterol contents in the tissues of *Tilapia mossambica* subjected to acclimation in sub lethal acidic water (PH 4.0). There was also significant ($p < 0.05$) inhibition as sub-lethal concentration increased, suggesting that more energy was needed at higher sub-lethal concentrations to achieve the positive survival value. Transaminases are intracellular enzymes which exist in only a small amount of the serum. Therefore, damage to the liver cell may result in leakage of the enzymes into the plasma due to large concentration gradient (Wabroblewski and La Due, 1955). The observed significant ($p < 0.05$) dose dependent inhibition and significant ($p < 0.05$) time dependent elevation in serum GOT activities may be due to liver dysfunction. Asztalos *et al.* (1988) had earlier reported elevations in serum GOT activity of *Cyprinus carpio* due to hepatic cellular damage caused by malathion + paraquat. And conversely, Sadhu *et al.* (1985) had decreased GOT activities in the serum of *Channa striatus* following exposure to 0.1 ppm malathion for 10 days. ALP is mainly localized at the cell membrane. Any damage in hepatic cells may result in alteration in ALP activity. The dose and time-dependent inhibition observed in this investigation may also be due to liver dysfunction. This observation is in agreement with the report of many other workers. Sastry and Sharma (1980) reported ALP inhibition after 96h exposure to diazinon, however the normal control values resumed after 96h but later increased due to prolonged exposure time (Sastry and Sharma, 1980). Goel *et al.* (1982), reported serum alkaline and acid phosphatase inhibition by 15% in *Heteropneustes fossilis* resulting from the effect of malathion. Similarly, Das and Mukherjee (2003), reported depletion of ALP due to sub-lethal exposure of *Labeo rohita* fingerlings to cypermethrin. For GPT, there was significant ($p < 0.05$) dose and time dependent inhibition, between control and exposed groups of fishes. This may be due to generalized organ/system failure as the fish approached death due to the effect of chlorpyrifos-ethyl. This finding is in agreement with sadhu *et al.* (1985); Oruc and Uner (1999).

CONCLUSION

Organophosphorus insecticides to which chlorpyrifos-ethyl belongs are widely used in agriculture in Nigeria. They are increasingly being used in veterinary applications on farm and pet animals, for the protection of stored foodstuffs, for the control of endemics and parasites in public health programmes as well as for household applications in kitchens and bedrooms. Chronic exposures of aquatic biota and individuals may result through run-off and adsorption of chlorpyrifos to small dust particles and various other surfaces respectively. Environmental monitoring of pesticides can be for the purpose of generating baseline information, assessing the effects of pesticides to the environment and or continuous measurement of environmental load to ensure that regulatory requirements and standards are being met (EIA Training Manual 2002)

The results of our study suggest that sub lethal exposure of *C. gariepinus* to chlorpyrifos-ethyl could lead to alterations in carbohydrate and lipid metabolism and possible organ damage. In the light of the above observations, it is recommended that chlorpyrifos-ethyl should be used with

caution and in a sustainable manner, as it could be hazardous to aquatic biota, domestic animals and human beings as well.

REFERENCES

- Asztalos, B. and Nemcsok, J., 1985. Effects of pesticides on the LDH activity and isoenzyme pattern of Carp (*Cyprinus carpio*) sera. *Comparative Biochemistry and Physiology* 82: 217-219
- Atef, M. M., 2005. Biochemical Effects of short-term Cadmium Exposure on the fresh water fish, *Oreochromis niloticus*. *Jour. of Bio. Sc.* 5(3): 260-265.
- Auta J, and Ogueji, E. O., 2006. Acute Toxicity and Behavioural effects of chlorpyrifos-ethyl on African catfish *Clarias gariepinus*-Teugels, being a paper presented at the Chemclass National Conference at Ahmadu Bello University Zaria, unpublished.
- Bassey, O.A., Lowery, O. H. and Brock, M.J., 1946. A method for the rapid determination of alkaline phosphatase with 5mm³ of serum. *Jour. Biol. Chem.* 164: 321-329.
- Begum, G., Vijayaraghavan, S., 1996. Alterations in protein metabolism of muscle tissue in the fish *Clarias batrachus* (Linn) by commercial grade dimethoate. *Bull. of Environ. Comtam. And Toxicol.* 57: 223-228.
- Caroline. Cox., 1995. Chlorpyrifos, Part1: Toxicology. *Journal of Pesticide Reform*, 14 (4): 95-20
- Ceron, J. J., Sancho, E., Ferrando, M. D., Gutierrez, C. and Andreu, E., 1997. Changes in carbohydrate metabolism in the eel *Anguilla anguilla*, during short term exposure to diazinon. *Toxicol. Environ. Chem.* 60: 201-210.
- Chambers, J. E. and Carr, R. L., 1993. Inhibition patterns of brain acetylcholinesterase, hepatic and plasma aliesterases following exposures to three phosphorothionate insecticides and their oxons in rats. *Fund Appl. Toxicol.* 21:111-119.
- Chambers, J. E., Forsyth, C. S. and Chamber, H. W., 1989. Bioactivation and detoxification of organophosphorus insecticides in rat brains. In Caldwell, J., Hutson D.H. and Paulson, G.D. *Intermediary xenobiotic metabolism: Methodology, mechanisms and significance*, Basingstoke, U.K: Taylor and Francis. 99-115. pp.
- Cox, C., 1994. Insecticide fact sheet: Chlorpyrifos, Part 1: Toxicology. *Journal of Pesticide Reform*. 14(4): 1-4
- Das, B. K. and Mukherjee, S. C., 2003. Toxicity of Cypermethrin in *Labeo rohita* fingerlings: biochemical enzymatic and haematological consequences. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 134: 109-21.
- De la Torre, F.R., Salibian, A. and Ferrari, L., 1999. Enzyme activities as biomarkers of freshwater pollution: responses of fish branchial (Na⁺ K)-ATPase and liver Transaminases. *Environ. Toxicol.* 14:313-319.
- Duncan, D. B., 1955. Multiple range tests. *Biometric*, No11: 1-42
- Edsall, C. C., 1999. A blood chemistry profile for lake trout. *Journal of Aquatic. Animal Health* 11: 81-86
- EPA, 1984. Pesticide fact sheet: Chlorpyrifos. No. 37. Washington, D.C.

- EIA Training Resource Manual, 2002. Topic 7: Mitigation and Impact Management. Second Edition. GenStat-Release 4.2 2006. Discovery Edition, VSN-International Ltd, Lawes Agricultural Trust. www.worldforestrycenter.org/genstatforafrica
- Ghazaly, K. S., 1995. Carbohydrate metabolism in *Clarias lazara* exposed to three different pesticides. J. Egypt. Ger. Soc. Zool. 16A: 235-251.
- Gill, T.S., Tewari, H. and Pande, J., 1990. Sublethal effects of an organophosphorus insecticide on certain metabolite levels in a freshwater fish, *Puntius conchoni* Hamilton. Pesticide Biochemistry and Physiology 36: 290-299
- Gill, T.S., Tewari, H. and Pande, J., 1991. In vivo and in vitro effects of cadmium on selected enzymes in different organs of the fish *Barbus conchoni* Ham. (Rosy barb). Comp. Biochem. Physiol. 100: 501-505.
- Goel K. A., Tyagi, S. K. and Awasthi, A. K., 1982. Effect of malathion on some haematological values in *Heteropneustes fossilis*. Comp. Physiol. Ecol. 7:259-261.
- Gopal, V. S., 1997. Effect of heavy metals on the blood protein biochemistry of the fish *Cyprinus carpio* and its use as a bio-indicator of pollution stress. Environ. Monitor. And Assess. 48:117-124.
- Hontela, A., Daniel, C. and Ricard, A. C., 1996. Effect of acute and sublethal exposure to Cadmium on the interregional and thyroid function in rainbow trout, *Oncorhynchus mykiss*. Aquat. Toxicol. 35: 171-182.
- Kamarth, 1972. Clinical Biochemistry for medical Technologists. Churchill/Livingstone, London. 89pp.
- Kaneko, J. J., 1989. Clinical Biochemistry of Domestic Animals. 4th Ed, Academic Press, San Diego, 823pp.
- Krishna, M. V., Bhaskar, M., Govindappa, S., 1994. Studies on lipid profiles of fish liver on acclimation to acidic medium. J. Environ. Bio. 15(4): 269-273.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurements with Folin Phenol reagent. J. Biol. Chem. 193: 265 -275.
- Luskova, V., Svoboda, M. and Kolarova, J., 2002. The effects of Diazinon on Blood Plasma Biochemistry in Carp (*Cyprinus carpio* L.) ACTA VET. BRNO 71:117 - 123.
- Mbagwu, I.G. and Ita E.O., 1994. Pesticide use in the sub-humid zones of Nigeria: Implication for conservation of Aquatic Resources. Environmental Conservation 21(3): 214-219.
- Nakano, T. and Tomlinson, N., 1967. Catecholamines and carbohydrate concentration in rainbow trout *Salmo gairdneri* in physical disturbance. J. Fish. Res. Bd. Can 24: 1701-1715.
- Oruc, E.O. and Uner, N., 1999. Effects of 2,4-Diamin on some parameters of protein and carbohydrate metabolisms in the serum, muscle and liver of *Cyprinus carpio* Environmental Pollution 105: 267-272.
- Pearson, S.S., Stern and Mogavack, T.H., 1953. A rapid procedure for the determination of serum cholesterol. J. clin. Endocrinol. 666pp
- Rahman, M. F. and Siddiqui, M. K. J., 2004. Biochemical effects of vepacide (from *Azadirachta indica*) on Wistar rats during subchronic exposure. Ecotoxicology and Environmental Safety. 59: 332-339.
- Ram, P. Y., Digvijay, S., Singh, S. K. and Singh, A., 2003. Metabolic changes in freshwater fish *Channa punctatus* due to stem-bark extract of *Croton tiglium*, Pakistan J. of Biol. Sci. 6(14):1223-1228
- Reish, D. L. and Oshida, P.S., 1987. Manual of methods in aquatic environment research Part 10. Short term static bioassay. FAO. fisheries. Technical paper 247. FAO Rome 1- 62pp.
- Retiman, S. and Frankel, F., 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic acid transaminase. Am. J.Clin Path (2856).
- Rice, E., 1970. Triglycerides (Natural fats) in serum. Stand. Meth. Clin. Chem., Academic Press, New York 6: 215.
- Sadhu, K. A., Chowdhury, D. K. and Mukhopadhyay, P. K. 1985. Relationship between serum enzymes, histological features and enzymes in hepatopancreas after sublethal exposure to malathion and phophamidon in the murrel *Channos striatus* (B.L.). International of Environmental Studies 24: 35-41.
- Sahib, I. K. A., Sambasiva Rao, K. R. S. and Ramana Rao, K. V., 1984. Effect of malathion on protein synthetic potentiality of the tissues of the teleost, *Tilapia mossambica* (Peters), as measured through incorporation of [¹⁴C] amino acids. Toxicology Letters 20: 63-67
- Sarki, R. 1990. Toxicity of organic phosphates and chloronaphthalenes: Part 11. Effect of chlorpyrifos on cholinesterase activity in rats. *Okayama Igakkai Zasshi* 102 (7-8): 997-1006
- Sasty, K. V. and Sharma, K., 1950. Diazinon effect on the activities of brain enzymes from *Opicephalus princatus* (Channa) Bull. Environ. Contam. Toxicol. 24: 326-332.
- Spragne, J. B., 1975. Measurement of pollution toxicity to fish: In Bioassay methods for acute toxicity. Water Resources 3: 346-349.
- Trinder, P., 1969. Determination of blood glucose using 4-aminophenazone. J. Clin. Pathol. 22:246.
- Verma, S. R., Rani, S., Tonk, P. I., and Dalela, R. C., 1983. Pesticide induced dysfunction in carbohydrate metabolism in three freshwater fishes. Environmental Research 32: 127-133.
- Wedemeyer, G., Mcleay, D. J., and Good year, C. P. 1984. Assessing the tolerance of fish and fish population to environmental stress. The problems and methods of monitoring. In Contaminate effects on fisheries Eds. Cairns, W.V. Hodson, P.V. and Nriagu, J.O. 164 - 195pp. John Wiley and son Inc. Newyork.
- Wroblewski, F. and La Due, J. S. 1955. Serum glutamic oxaloacetic transaminase activity as an index of liver cell injury. A preliminary report. Annuals of internal medicine 43:345-360