A Study on Biochemical Changes in Common carp *Cyprinus carpio* exposed to Lethal and Sublethal concentrations of an Organophosphate Insecticide, Phorate

G.Lakshmaiah

Abstract— Phorate is an organophosphate insecticide (OPI) that is widely used throughout the world and in India and Andhra Pradesh as a broad-spectrum insecticide on numerous crops including paddy and groundnut. It is a soil and systemic insecticide and miticide, used for the control of sucking and chewing insects, mites and soil dwelling pests. Biochemical (BC) alterations were studied in the blood (BL), osmoregulatory organs such as gill (GL) and kidney (KY) and non-osmoregulatory organs such as liver (LR), muscle (MU) and brain (BR) of the fingerlings of *Cyprinus carpio (C. carpio)* on exposure to acute lethal toxicity of Phorate (ALTP) for one day and 4 days and chronic sublethal toxicity of Phorate (CSTP) for 1, 7, 15 and 30 days. Both the lethal and sublethal concentrations of phorate altered the levels of blood glucose (BG) and total carbohydrates (TC) significantly ($P<0.05$) in the all target organs of the experimental fish in acute and chronic toxicity studies (ACTS). The alterations in the levels of BG and TC are significantly dose dependent ($P<0.05$).

Key Words : Acute lethal, Chronic sublethal, *Cyprinus carpio*, Miticide, Organophosphate, Osmoregulatory, Phorate.

1 INTRODUCTION

Carbohydrates (CHs) are a superior short-term fuel for organisms because they are simpler to metabolize than fats or those amino acid portions of proteins that are used for fuel. In animals, the most important carbohydrate (CH) is glucose (GLU), a simple sugar (monosaccharide) that is metabolized by nearly all known organisms. GLU and other CHs are part of a wide variety of metabolic pathways across species. CHs are the first substrates to be utilized in metabolism more so under toxic stress conditions. GLU as the primary fuel is utilized during biological oxidations for energy production. The principal sugar in the blood of fishes and other vertebrates is GLU, serving the tissues as a major metabolic fuel. Blood GLU has been considered the most sensitive parameters in detecting stress responses of fish [1], [2].

The changes in biochemical parameters (BCP) such as CHs are important to indicate the susceptibility of organ systems to pollutants like pesticides (PCs) by altering their function as indicated by Verma et al [3]. PCs are known to alter carbohydrate metabolism (CHM) in fishes. Several investigators reported the influence of various PCs on the CHM in fish and were analyzed at different segments of CHM [4], [5], [6], [7], [8]. Mayes [9] reported that CHs from the energy reserves are affected and depleted when the animals are subjected to the pesticide (PC) pollution.

PCs can cause serious impairment to physiological and health status of fish. Therefore, BC tests are useful in recognizing acute or chronic toxicity of insecticides [10], [11] like Phorate and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish. BL biochemistry test gives indication of the alterations happening in the body of fish exposed to PCs. Due to the severity of the damage to the tissues, particularly liver, synthesis of many biochemical substances may reduce significantly in cells, which can decrease some biochemical factors in BL of fish exposed to PCs. These changes were observed in *Channa punctatus (C. punctatus)* [12], *Oncorhynchus mykiss (O. mykiss)* [13], *Clarias batrachus (C. batrachus)* [14], *C. carpio* [15] and *O. mykiss* [16] which were exposed to monocrotophos, bifenthrin, cypermethrin, diazinon and malathion respectively.

Since fishes are important sources of proteins and lipids in the form of food, health status of these organisms is very important for human beings. Fishes are particularly sensitive to the environmental contamination of water. Hence, pollutants such as PCs may significantly damage certain physiological and biochemical processes (BPs) when they enter into the organs of fishes [17], [18]. Basal or standard metabolism in fish is relatively constant under constant environmental conditions. PCs are known to interfere with the BPs of cellular metabolism at different steps. The present study is aimed to investigate the extent to which non-target animals like fishes are affected, by exposing to the OPI (phorate) through monitoring selected biomarker responses under laboratory conditions in the selected test species *C. carpio*, a representative species from the aquatic environment.

2 MATERIALS AND METHODS

2.1. Material

2.1.1 Test Species

The Indian major carp *C. carpio* (Linnaeus, 1758) has been selected as test species for the present investigation. It is an economically important edible fish, having great commercial value. The animals were starved for 24 hours prior to each

• *Department of Zoology, Sri Krishnadevaraya University, Anantapuramu-515003, A.P, India, PH-+91-9441355066. E-mail: govindulakshmaiah@gmail.com*

estimation to avoid any influence of differential feeding.

2.1.2 Test Chemical

Pesticide selected for this study is phorate (O,O-diethyl S-ethylthiomethyl phosphorodithioate) an OPI which is widely used throughout the world and also in India and Andhra Pradesh as a broad-spectrum insecticide on numerous crops. Commercial names of phorate are Thimet, Rampart, Granutox Agrimet etc and its molecular formula is $C_7 H_{17} O_2 PS_3$.

2.2 Methods

2.2.1 Acute and Chronic toxicity procedures

Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by Probit method of Finney [19]. LC_{50}/96 hours (0.71 ppm/l) of phorate was taken as lethal concentration to study acute toxicity and one-tenth of the LC_{50}/96 hours (0.071 ppm/l) concentration of phorate was taken as the sub-lethal concentration for chronic toxicity study.

2.2.2 Experimental Design

160 fishes were divided into two batches, again batch I was divided into 3 groups and batch II into 5 groups comprising of 20 fishes each. Batch I was exposed for acute toxicity of Phorate (exposed to LC_{50} of Phorate -071 ppm/l) and batch II was exposed for Chronic toxicity of Phorate (exposed to sub lethal concentration = 1/10th of LC_{50} - 0.071 ppm/l). In batch I, group 1 was considered as normal control, group 2 and 3 were experimental groups. The fishes of group 2 were exposed for 1 day and group 3 for 4 days. In batch II, group 1 was considered as normal control group, group 2, 3, 4 and 5 were experimental groups. The fishes of group 2 were exposed for 1 day, group 3 for 7 days, group 4 for 15 days and group 5 for 30 days.

2.2.3 Estimation of Blood glucose

The level of BG was estimated by colorimetric method as described by Nelson and Somogyi [20], in the fish *C. carpio* at 1, 4 days on exposure to lethal concentration of phorate (LCP) in acute toxicity study (ATS) and 1, 7, 15 and 30 days on exposure to sublethal concentration of phorate (SCP) in chronic toxicity study (CTS). 0.1 ml of BL was collected and to it 3.9 ml of deprotenizing solution (5% zinc sulphate and 0.3N sodium hydroxide in 1:1 ratio) was added and the mixture was centrifuged at 3000 rpm, for 10 minutes. The supernatant of 1 ml from this mixture was added with 1 ml of alkaline copper reagent, shaken vigorously and heated in a boiling water bath (BWB) for exactly 20 minutes. Then it was cooled and 1 ml of arsenomolybdate colour reagent (ACR) was added. The entire volume of the solution was raised to 10 ml by adding distilled water and the optical density of the colour developed was measured in a spectrophotometer (SPM) at wavelength of 540 nm. A blank and glucose standards were also run simultaneously. Glucose content was expressed as mg of glucose/100 ml of blood.

2.2.4 Estimation of Total carbohydrates

The TC content was estimated by the method of Caroll et al [21]. A 10% homogenate (w/v) of the tissues such as GL, LR, MU, KY and BR was prepared in 10% TCA. The protein precipitate was removed by centrifuging the homogenates for 15 minutes at 3000 rpm. The clear supernatant was taken for the estimation of TCs. To 0.5 ml of TCA filtrate, 5 ml of anthrone reagent was added and kept in BWB for 15 minutes. Then the contents were cooled and read at 620 nm against a reagent blank in a SPM. The TC levels were expressed as mg/gr wet weight of the tissue.

2.2.4 Statistical Analysis

DMR (Duncan's Multiple Range) test had been employed for the statistical analysis of the BG and TC levels data. P value (level of significance) is significant at < 0.05.

3 RESULTS AND DISCUSSION

3.1 Results

As The data on the levels of BG and TC content in the GL, LR, MU, KY and BR of the fish at 1 and 4 days on exposure to acute toxicity of phorate (ATP) and 1, 7, 15 and 30 days on exposure to chronic toxicity of phorate (CTP), besides controls are presented in the Table- 1 and 2. For comparative assessment, the differences obtained in relation to controls in each parameter of each organ at the above said exposure periods in ACTS of phorate, were converted as mean values and percentages of the corresponding controls and was plotted the graphs of percent changes against exposure periods in Figures 1 and 2.

3.1.1 Blood glucose

From the data presented in the Table-1 and Figure- 1 it is seen that, relative to controls, the BG level is elevated in the fish exposed to ACTS of phorate at all exposure periods. Elevation of BG level in the fish exposed to ATP showed a steady increase from day 1 to day 4 in the order of day 1<4. In the fish exposed CTP the BG level was gradually decreased from initial elevation to reach normalcy. The decrease is greater at day 7 and from day 15 there is reduction in the decrement was observed, which is in the order of day 1>7>15>30.

3.1.2 Total carbohydrates

From the data presented in the Table-2 and Figure-2 a significant decrease was observed in the TC content in all organs, relative to controls, at day 1 and day 4 in the fish exposed to ATP in the order of day 1>4. There was a progressive decrease in the TC levels in all the organs when exposed to ATP. Where as, the TCs decreased and it was gradual at day 1 and day 7 followed by a significant increase at day 15 and day 30 in the fish exposed to CTP in all the organs in the order of day 1>7<15<30.

3.2 Discussion

In the present study the elevation in BG level in the fish *C. carpio* exposed to acute and chronic toxicity of phorate (ACTP) indicates the stepping up of CHM during toxic stress. The hyperglycemic condition (HGC) may be due to stepping up of glycogenolysis or gluconeogenesis or both. HGC indicates a typical stress response in fish, which certainly includes an increased breakdown of LR and MU glycogen (GLY), thereby increasing the blood glucose level (BGL). Such a response can also be caused by a result of hypoxic state caused by PC action

on the GL function. The elevation in the BGLs suggests a physiological response of the fish to meet the critical need of energy under stressed condition.

TABLE 1: BGLs (mg/100ml) in the fish *C. carpio* at different periods of exposure to ACTP.

NAME		EXPOSURE PERIOD IN DAYS							
		ACUTE TOXICITY			CHRONIC TOXICITY				
		CONTROL	1	4	CONTROL	1	7	15	30
BLOOD GLUCOSE mg/100ml	Mean	90.575a	100.355b	140.681c	90.575a	116.821e	100.563d	93.463c	92.666b
	S.D. ±	0.346	0.781	0.679	0.346	0.617	0.435	0.415	0.375
	% change		+10.79	+55.32		+28.97	+11.02	+3.18	+2.30

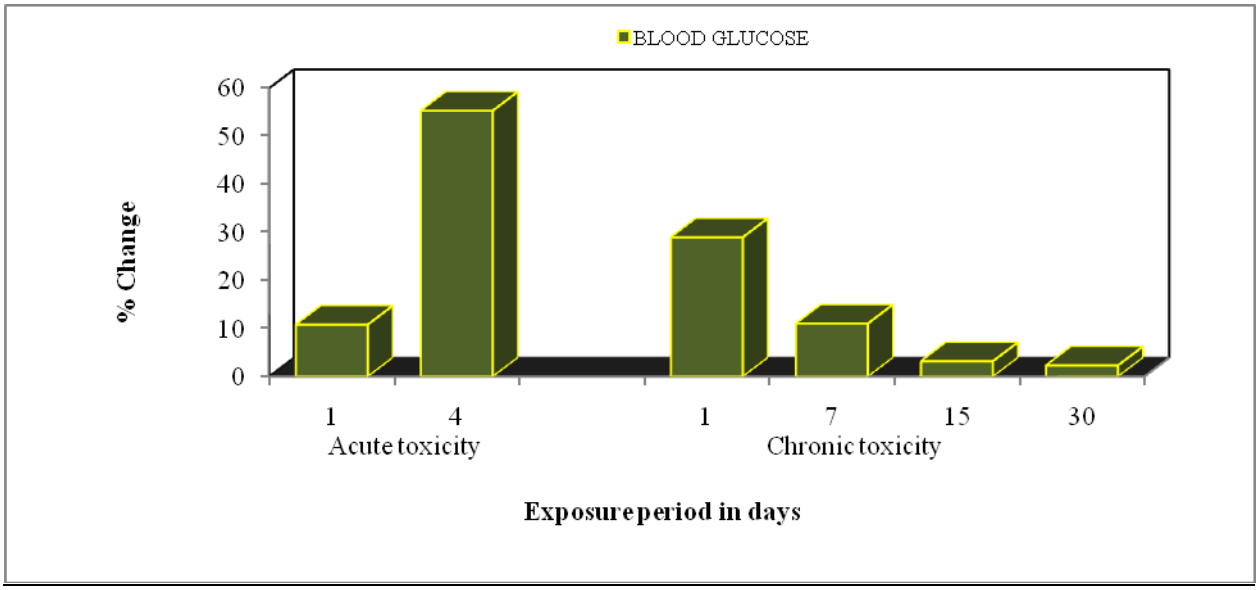
All the values are mean ± SD of six individual observations. Values with different superscripts with in the column are significantly different from each other at P<0.05 according to DMR test.

TABLE 2: TCs (mg/gm wet wt) in different organs of the fish *C. carpio* at different periods of exposure to ACTP

ORGAN		EXPOSURE PERIOD IN DAYS							
		ACUTE TOXICITY			CHRONIC TOXICITY				
		CONTROL	1	4	CONTROL	1	7	15	30
GILL	Mean	19.706c	12.933b	11.435a	19.706e	17.880c	12.785a	14.875b	18.811d
	S.D. ±	0.281	0.165	0.291	0.281	0.449	0.418	0.342	0.319
	% change		-34.37	-41.97		-9.26	-35.12	-24.51	-4.54
LIVER	Mean	58.871c	17.256b	14.378a	58.871e	27.651b	20.165a	28.901c	52.353d
	S.D. ±	0.139	0.329	0.131	0.139	0.423	0.472	0.373	0.349
	% change		-70.68	-75.31		-53.03	-65.74	-50.90	-11.07
MUSCLE	Mean	21.413c	10.293b	8.675a	21.413e	15.443c	11.461a	13.410b	19.951d
	S.D. ±	0.359	0.142	0.068	0.359	0.352	0.417	0.333	0.348
	% change		-51.93	-59.48		-27.87	-46.47	-37.37	-6.82
KIDNEY	Mean	9.265c	5.323b	3.888a	9.265e	7.306c	4.970a	6.730b	8.288d
	S.D. ±	0.064	0.063	0.049	0.064	0.261	0.299	0.034	0.031
	% change		-42.54	-58.03		-21.13	-46.35	-27.36	-10.54
BRAIN	Mean	11.318c	5.871b	4.978a	11.318e	8.088c	6.223a	7.910b	9.851d
	S.D. ±	0.124	0.092	0.099	0.124	0.021	0.029	0.051	0.046
	% change		-48.12	-56.01		-28.53	-45.01	-30.11	-12.95

All the values are mean ± SD of six individual observations. Values with different superscripts with in the column are significantly different from each other at P<0.05 according to DMR test.

Fig. 1: BGL (mg/100ml) in the fish *C. carpio* at different periods of exposure to ACTP.



All the values are mean ± SD of six individual observations.

Fig. 2 TCs (mg/gm wet wt) in different organs of the fish *C. carpio* at different periods of exposure to ACTP.

All the values are mean ± SD of six individual observations.

Similar results were obtained by several authors in support of the present work. Dalela et al [22] observed hyperglycaemia in *Mystus vittatus* exposed to three different PCs thiotox, dichlorvos and carbofuran and their combinations. They suggested that hyperglycaemia indicates the disrupted CHM which might be due to enhanced breakdown of liver GLY, perhaps mediated by adrenocorticortropic hormone (ACTH), glucogon hormone and reduced insulin activity. All types of stress increase the secretion of catecholomines and glucocorticoides from the adrenal tissue of fish which enhanced the glycogenolysis [23]. Mukhopadhyay and Dehadrai [24] observed elevation in the BGLs in *C. batrachus* exposed to malathion and they suggested increased glycogenolysis. Venkateswarlu et al [25] observed similar results in *C. batrachus* with endosulfan and kelthane.

Srivastava and Mishra [26] made a study on fenthion induced haematological and BC changes in the Indian catfish *Heteropneustes fossilis* (*H. fossilis*) and determined the acute toxicity and effect of duration of sublethal exposure to malathion. They reported that fish exposed to the PC for 2, 6, 12, 48 and 96 hours showed MU glycogenolysis with concomitant hyperglycaemia at all time intervals. Logaswamy and Remia [7] reported that sublethal concentration of certain OP PCs caused glycogenolysis which produced hyperglycemia in the African food fish *Tilapia mossambica* (*T. mossambica*) and the Indian catfish, *H. fossilis*. Elevation of CHs might be due to the stress induced by the insecticide as physiology of organisms with the help of corticosteroids [6].

PCs may exert general stress-effects i.e., increases in cortisol and catecholamines in blood plasma. Elevated concentration of these hormones induced increase in BG [27], [28]. The increased levels of glucose in the serum may also be due to the fact that the inhibition in the activity of acetylcholinesterase (AChE) which is accompanied by an increase in acetylcholine levels at neuro-effector sites in adrenal medulla leading to hyper secretion of adrenaline which stimulates the breakdown of GLY to glucose [29], [30].

The stress related hyperglycemia reported in many species of teleosts is mediated mainly by the effects of catecholamines (CAs) on glucose release from the LR, the main CH store in fish, with epinephrine being more potent than noreinephrine [31]. Chan and Woo [32] noted that cortisol has shown to promote catabolism of peripheral tissues via, increased gluconeogenesis, leading to hyperglycemia. In the present study the significant increase of BGL might have resulted from gluconeogenesis to provide energy for the increased metabolic demands imposed by phorate toxicity stress, particularly in osmoregulation which may contribute to the restoration of plasma osmolarity in the face of failing blood levels of Na^+ and Cl^-.

In the fish exposed CHP in the present study, the BG though increased initially declined sharply during later period. Several workers have reported similar HG effect in the freshwater fish like *H. fossilis* and *Anabas testudineus* [33] following PC exposure. This was similar to the findings of Natarajan [34] with regards to hyperglycemia. Hyperglycemia, generally believed to be a response to stressful condition and reaction to stress, is a feature of adaptation.

The TCs in the present study depleted in all the tissues throughout the exposure period in ACTP. The decrease in the amount of TCs is perhaps to keep high GLs in the blood in response to the stressors. Decreased TCs were observed in fresh water rice-field crab *Oziotelpusa senex senex* when exposed to sumithion [35].

The CH reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition. Many workers reported a similar trend of decrease in CHs [5], [8]. Sambasiva Rao et al [36] reported reduced CH levels in the tissues of *C. punctatus* treated with phenthoate. Kannupandi et al [37] observed decreased CH content in different stages of larval development of *Sesarma brockii* at different PC concentrations. A possible explanation for tissue CH depletion during phosphomidon exposure is attributed to the neuroendocrine control of CHM [38].

Changes in the CHM are to meet the changing energy demands which can be expected in animals exposed to stress. Saravana Bhavan and Geraldine [39] reported that the stress induced by carbaryl necessitates the utilization of CH to counteract the required energy demand. Therefore, concentrations of TCs in the tissues of prawn were found to be lower than that of controls. Thus a shift towards anaerobic metabolism, which results in increased glycogenolysis and decrease in oxidative metabolism due to inhibition of AChE and consequent release of catecholamine and glucocorticoids [40]. Operation of such adaptive mechanism has also been reported in prawn *Macrobrachium malcolmsonii* and penaeid shrimp, *Metapenaeus monoceros* due to PCs toxicity [41].Thus the decrease in CH content may result in impairment of CHM due to toxic effect [8].

4 CONCLUSION

The present study yielded important results that improve the understanding on the effects of pesticides on carbohydrate metabolism of aquatic animals. Phorate intoxicication has disturbed the normal functioning of cells with the resultant alterations in the fundamental biochemical mechanisms of carbohydrate metabolism in the fish *Cyprinus carpio.*

REFERENCES

[1] G.A. Wedemeyer, B.A. Barton and A.J. McLeay, "Stress and acclimation" In: *Methods for fish biology* (Eds). Schreck, C.B. and Moyle, P.B.), American Fisheries Society, Bethesda, Maryland, 541 – 589, 1990

[2] S. Fiveslstad J. Schwarz and H. Stromsnes, "Sublethal effects and safe levels of ammonia in sewage water for Atlantic salmon post molts (*Salmo salar* L.)" *Aquacult. Eng.*, **14**: 271-280, 1995.

[3] S.R. Verma, Sarita Rani, I.P. Tonk and R.C. Dalela, "Pesticide-induced dysfunction in carbohydrate metabolism in three freshwater fishes", *Environ. Res*., 32:127-133, 1983.

[4] E.O. Oruc and N. Uner, "Effect of 2,4-Diaminon some parameters of protein and carbohydrate metabolism in the serum, muscle and liver of *Cyprinus carpio",* In: *Environmental Pollution,* 105(2): 267-272, 1999.

[5] G. V. Venkatramana, Sandhya, P. N. Rani and P. S. Moorthy, "Impact of Malathion on the biochemical parameters of gobiid fish", *Glossogobius giuris* (Ham). *J. Environ. Biol*. 27(1):119-122, 2006.

[6] K. M. Remia, S. Logaswamy, K. Logankumar and D. Rajmohan, "Effect of an insecticide (Monocrotophos) on some biochemical constituents of the fish *Tilipia Mossambica", Poll. Res*. 27(3):523-526, 2008.

[7] S. Logaswamy and K. M. Remia, "Impact of Cypermethrin and Ekalux on respiratory and some biochemical activities of a fresh water fish, *Tilapia mossambica", Curr. Biot*. 3(1):65-73, 2009.

[8] C. Thenmozhi, V. Vignesh, R. Thirumurugan and S. Arun, "Impacts of malathion on mortality and biochemical changes of freshwater fish *labeo rohita", Iran. J. Environ. Health. Sci. Eng*. 8(4):189-198, 2010.

[9] P.A. Mayes, In: Review of Physiological Chemistry, 16th edition (Ed) Harper, H.A., Woodwell, V.M. and Mayes, P.A., Longe Medical Publications, California, 1977.

[10] M. Banaee, A.R. Mirvagefei, G.R. Rafei, and B. Majazi Amiri, "Effect of sublethal diazinon concentrations on blood plasma biochemistry", *Intl J of Environ Res,* 2 (2), 189-198, 2008.

[11] M.A. Al-Kahtani, "Effect of an Insecticide Abamectin on Some Biochemical Characteristics of Tilapia Fish (*Oreochromis Niloticus*)", *American Journal of Agricultural and Biological Sciences*, 6 (1): 62-68, 2011.

[12] S. Agrahari, K.C. Pandey and K. Gopal, "Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch)", *P. Biochem and Phys,* 88 (3), 268-272, 2007.

[13] J. Velisek, Z. Svobodova and V. Piackova, "Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*)", *Veterinarni Medicina*, 54, (3): 131–137, 2009.

[14] G. Begum, "In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure", *Pesticide Biochemistry and Physiology*, 82: 185–196, 2005.

[15] M. Banaee, A.R. Mirvaghefi, K. Ahmadi and S. Banaee, "Determination of LC_{50} and investigation of acute toxicity effects of diazinon on hematology and serology indices of common carp (*Cyprinus carpio*)", *Journal of Marine Science and Technology Research*, 3(2): 1-10, 2008.

[16] M. Banaee, A. Sureda, A.R. Mirvaghefi and K. Ahmadi, "Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*)", *Pesticide Biochemistry and Physiology*, 99: 1–6, 2011b.

[17] P. John, "Alteration of certain blood parameters of freshwater teleost *Mystus vittatus* after chronic exposure to Metasystox and Sevin", *Fish Physiol. Biochem*., 33: 15-20, 2007.

[18] M. Banaee, A.R. Mirvaghefi, B. Majazi Amiri, G.R. Rafei and B. Nematdost, "Hematological and Histopathological Study of Experimental Diazinon Poisoning in common carp fish (*Cyprinus carpio*)", *Journal of Fisheries (Iranian Journal of Natural Resources)*, 64(1): 1-14, 2011a.

[19] D.J. Finney, "Probit Analysis", 3rd edition, Cambridge University press, Cambridge, pp.333, 1971.

[20] N. Nelson and N. Somogyi, "A photometric adaptation of Somogyi method for the determination of glucose", 1952.

[21] N.V.R. Carroll, R.W. Longley and J.H. Rae, "The determination of glycogen in liver and muscle by use of Anthrone reagent", *J.Biol. Chem*., 220: 583-598, 1956.

[22] R. C. Dalela, S. Rani, V. Kumar and S. R. Verma, "In vivo haematological alterations in a freshwater fish *Mystus vittatus* following sub acute exposure to pesticides and their combinations", *Journal of Environmental Biology* 2, 79-86, 1981.

[23] N. N. Singh and A. K. Srivastava, "Effects of endosulfan on fish carbohydrate metabolism", *Ecotoxicology and Environmental Safety* 5, 412-417, 1981.

[24] P. K. Mukhopadhyay and P. V. Dehadrai, "Biochemical changes in the air-breathing cat fish *Clarias batrachus* (Linn.) exposed to malathion", *Environmental Pollution (Series A)* 22, 149-158, 1980.

[25] P. V. Venkateshwarlu, Joyce Rani Shobha, C. Janiah and M. S. C. Prasad, "Effect of endosulfan and kelthane on haematology and serum biochemical parameters of the teleost *Clarias batrachus* (Linn.)", *Indian Journal of Comparative Animal Physiology* 8, 8-13, 1990.

[26] A.K. Srivastava and J. Mishra, "Effects of fenthion on the blood tissue chemistry of a teleost fish (*Heteropneustesfossilis*)", *J. Comp. Path*. 93:27-31, 1983.

[27] T. Nakono and N. Tomlinson, "Catecholamines and carbohydrate concentration in rainbow trout (*Salmo gairdneri*) in relation to physiological disturbance", *J. Fish. Res. Bd. Can.,* 24: 1701 – 1715, 1967.

[28] C.B. Schreck and H.W. Lorz, "Stress response of coho salmon (*Oncorhynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress", *J. Fish. Res. Bd. Can.,* 35: 1124: 1129, 1978.

[29] J. Brzezinski and K. Ludwicki, "The interrelationship of the changes of acetylcholineesterase and catecholamines blood and urine levels in rats poisoned with disyston", *Pol. J. pharmacol. Pharm*. 25(3):313-316, 1973.

[30] M.P. Ferrando and E. Andreu-Moliner, "Changes in selected biochemical parameters in the brain of fish, *Anguilla Anguilla* (L.) exposed to lindane", *Bull. Environ. Contam. Toxicol*. 47: 459-464, 1991.

[31] M.T.M. Van Raaji, Van den Thillart GEEJM et al., "Effect of arterially infused catecholamines and insulin on plasma glucose and free fatty acids in carp", Am. J. Physiol. 268:170, 1995.

[32] D.K.O. Chan and N.Y.S. Woo, Effect of cortisol on the metabolism of the eel *Anguilla japonica", Gen. Comp. Endocrinol*.35:205-215, 1978.

[33] K. Kumar and P. Patri, "Variation in biochemical composition of climbing perch *Anabas testudineus* in response to endosulfan toxicity", *J. Environ. Poll*. **7(2)**: 135 – 141, 2000.

[34] G.M. Natarajan, "Effect of sublethal concentration of metasystox on selected oxidative enzymes, tissue respiration and haematology of the freshwater air-breathing fish, *Channa striatus", Pest. Biochem. Physiol.,* 21: 194 – 198, 1984.

[35] A. Bhagyalakshmi, P. Sreenivasulu Reddy and R. Ramamuthy, "Effect of sumithion on some aspects of oxidative metabolism in the fresh water field crab *Oziotelpusa senex senex", Proc. Symp. Phys. Rasp. Anim. Pollutants*, 81-85, 1982.

[36] K.R.S. Sambasiva Rao, Sivaprasad Rao K. and K.V. Ramana Rao, "Impact of technical and commercial grade of phenthoate on some selected parameters of oxidative metabolism in the fish", *Channa punctatus. Pestic. Biochem. Physiol.* 34:1-8, 1984.

[37] T. Kannupandi, G. Vijayakumar and P. Soundarapandian, "Impact of phosphomidon on total protein, carbohydrate and lipid contents during the larval development of *Sesarma brockii* (de man)", *Indian J. Fish*. 48(2): 203-209, 2001.

[38] M. Sreenivasulu Reddy and K.V. Ramana Rao, "Phosphomidon induced changes in the nitrogen metabolic profiles of penaeid prawn *Penaeus indicus* during acute and chronic exposure", *Proc. Indian Acad. Sci.* (Anim. Sci.), 86: 533-536, 1986.

[39] P. Saravana Bhavan and P. Geraldine, "Carbaryl induced alterations in biochemical metabolism of the prawn, *Macrobrachium malcolmsonii", Journal of Environmental Biology,* 23(2), 157-162, 2002.

[40] B.F. Grant and P.M. Mehale, "Endrin toxicosis in rainbow trout, *Salmo gairdneri", J. Fish Res. Boad. Can*., 30:31-40, 1973.

[41] R.A. Uddhav Suryavanshi, Z.A. Sreepada, Ansari, Subhanchi Nigam and Shahin Badesab, "A study on biochemical changes in the penae-

id shrimp, *Metapenaeus monoceros* (Fabricius) following exposure to sublethal doses of organochlorine pesticide (endosulfan)", *Chemosphere*, vol. 77(11) 1540-1550, 2009.