IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 3 Issue 6, June 2016
ISSN (Online) 2348 – 7968 | Impact Factor (2015) - 4.332
www.ijiset.com

Integration of Cholinesterase, cortisol and blood glucose in the fish, Labeo rohita as biomarkers of pollution

[a] S.N.C Ray & [b*] R.C. Sinha

[a & b] CENC, Deptt. of Zoology, Patna University, Patna-800005, India
[*]Chairman, Bihar State Environment Impact Assessment Authority, Govt. of India
&
Corresponding Author

1. Abstract:

Signal transduction by xenobiotics in fish has gained much attention in the recent decade. Organophosphates and carbamates bind specifically to the membrane bound enzyme acetylcholinesterase affecting neural transmission. Since nervous system is one of the important physiological systems in animals, hypofunction of nervous system leads to secondary hypothalamic functions. Even low levels of xenobiotics are sufficient enough to bring about remarkable changes in the functional physiology of fish. In the present study, sub-lethal acute toxicity of methyl parathion in fish, *Labeo rohita* was assessed using an integration of biomarkers namely acetylcholinesterase, butyrylcholinesterase, cortisol and glucose. These biomarkers were selected as indicators of key physiological functions in the fish *Labeo rohita* as a function of methyl parathion. These biochemical end- points could therefore be recommended to the policy makers in India for using these biomarkers of pollution to be used as complementary parameters in the assessment of water quality.

Key words: Cholinesterases, Labeo rohita, Biomarker, Methyl parathion

2. Introduction:

India is an agricultural country, pesticide usage has increased to a great extent and as such environmental monitoring of the pesticides has become imperative. Contamination by pesticides is an important public health problem mainly in developing countries. The intensity and the amount of pesticide use for the pest control for the improvement of crops in intensive agriculture are posing serious hazards to terrestrial as well as fragile ecosystems and biota including fish [1, 2, 3, 4, 5].

The physiological stress response is commonly activated in experiencing potentially harmful toxicants in aquatic environment. The response to stress in fish is characterized by the stimulation of the hypothalamus which results in the activation of the neuroendocrine system and subsequently metabolic and physiological changes [6, 7].

Fish being strictly aquatic are directly exposed to these pesticides by absorption through the skin, breathing in and oral intake of pesticide contaminated prey [8]. Among various biomarkars of pesticide exposure the family of cholinesterases (acetylcholinesterase, AchE) and butyrylcholinesterase (BchE) have been widely used as biomarker to evaluate the noxious effects of pesticides especially organophosphates and carbamates. BchE is synthesized in the liver and present in the plasma, smooth muscles, skin, brain and heart. Although, its physiological function is not very clear, it is present as one of the most effective detoxifying enzymes and scavenges a broad range of xenobiotics compounds. Moreover, AchE and BchE are different concerning several aspects, while AchE has *in- vivo* half life of 120 days, BchE lasts for 7-12 days. AchE is inhibited by substrate excess but BchE is activated by substrate excess.

Fishes give the nippy signal of pesticide intoxication long before their death and therefore, they are taken as model organisms [9, 10, 11, 12]. AchE plays an important role in neurotransmission at cholinergic synapses and neuromuscular junction by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate. Butyrylcholinesterase is crucial for different parts of immune system. BchE is considered as one of the core detoxifying enzymes [13, 14, 15, 16, 17]. Some of the investigations hyposethize that BchE protect AchE against xenobiotics like pesticides [18].

Inhibition of AchE induces alteration in swimming behaviour, shaking palsy and other undesirable effects. Behavioural changes are the most sensitive indicators of the potential toxic effects. The behavioural and swimming patterns of the fish exposed to methyl parathion are because of the interference of the nervous system and sensory receptors [19]. The effect of certain insecticides on the activity acetylcholinesterase may lead to decreased mobility in fish [20]. In this way, the results may provide insight into the integrity of the ecosystem as a whole. Measurements of toxicity in sentinel species (*Labeo rohita*) can be used as an early warning of population decline and as an ecological relevant end point. Ecological risk assessment must aim at the preservation of the integrity of the ecosystem.

Plasma cortisol is widely used as a general indicator of stressful conditions in fish [21]. Under the condition of stress, the body of the fish emits immediate responses recognized as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system (CNS) and the release of stress hormones, cortisol and catecholamines into the blood stream. Secondary responses occur as a consequence of the released stress hormone causing changes in the blood and tissue chemistry e.g. an increase in plasma glucose [22, 23]. This entire metabolic pathway produces a burst of energy to prepare the fish for an emergency situation [24, 25]. Since stress has been reported to increase plasma cortisol and glucose levels many researchers consider as a “rule of thumb” that fishes undergoing stressful situation exhibit plasmatic increase of cortisol and glucose [25, 26, 27]. Cortisol activates glycolysis and gluconeogenesis and modulates cardiovascular and respiratory functions [28]. The whole process increases the substrate level (glucose) to produce enough energy according to the demand of the animal [25].

The aim of the present study is to estimate the acute toxicity of the pesticidal stress of methyl parathion and integrate an array of biomarkers: acetylcholinesterase, cortisol and glucose in the most common edible and commercial fish, *Labeo rohita*.

3. Material and methods:

3.1. Ethical Statement:

Presently, we do not have any Ethical Committee in our University. But however, we have followed the ethical norms, which are being followed elsewhere which is evident in the Materials & Methods Section.

3.2. Maintenance of animals:

Labeo rohita, a common carp was obtained from the local hatchery. Fishes were acclimated to laboratory conditions for about 5-7 days. They were kept in aquarium tank (250 L) and water was constantly aerated by a static system. During the acclimation period, they were given artificial (commercial) feed composed of ground shrimps available in the local market to avoid the possible effects of starvation. The feeding and maintenance of the fishes and physico-chemical characteristics of the aquaria water were measured (Tab. 1). Short-term test of acute toxicity over a period of 96 h were performed on the fishes following the renewal of bioassay. The fishes were exposed intra-coelomatically

with 1/3rd of LC_{50} value of the pesticide methyl parathion. After 24, 48, 72 and 96 h of exposure fishes were processed for further investigations.

3.3. Determination of LC_{50}: The experiments were repeated several times and only arithmetic mean of the experiments at each concentration was taken to express the results. LC_{50} values were determined by EPA Probit analysis program [29]. The LC_{50} of methyl parathion for the fish *Labeo rohita* was 16.8 ppm.

3.4. Blood collection: The fishes were taken out of the aquarium water individually through fish net with a minimum possible disturbance. After preliminary investigations, the blood samples were collected from caudal fin as described by many authors. In the present study, the blood collection from caudal fin had to be abandoned because there was an unusual elevation in Lactate dehydrogenase (LDH) and Creatine phosphokinase (CPK) activities which were recorded due to leakage of these enzymes from the surrounding muscle tissues. Thus, cardiac sampling was the only suitable method available as an alternative to obtain blood under the present study. After the blood collection, the serum were separated and processed for enzymatic and hormonal assays.

3.5. Assay of cholinesterase: Estimation of acetylcholinesterase was done by the spectrophotometric method of Ellman *et al* (1961) [30] using DTNB (dithionitrobenzoic acid) as a chromogen and acetylcholine iodide as the substrate. The reaction was rapid and the assay was also sensitive (i.e. 10µl sample of blood was adequate). For brain cholinesterase activity, 100 mg of brain was homogenized in 5 ml of phosphate buffer (pH-7.4) and assayed for acetylcholinesterase activity.

3.6. Behavioural: The behaviour and condition of fishes in both control and treated were noted every 24h upto 96h. Behavioural manifestations of acute toxicity of methyl parathion were observed.

3.7. Assay of Cortisol: The concentration of serum cortisol was estimated by Chemiluminescence Immuno Assay Reader Neo-Lumax Model-4901 because it has measuring ranges over many other Immunoassay methods and excellent for detection and quantification.

3.8. Assay of blood glucose: Serum glucose was determined spectrophotometrically by GoD-PoD method [31]'

4. Results:

Table1: Physico-chemical characteristics of aquaria water

Sl no.	Parameter	Value
1.	Temperature	(24±2) ^{0}C
2.	pH	7.1± 0.2 at 24^{0}C
3.	Dissolved Oxygen	8.5 ±0.5 mg/L
4.	Total Hardness	23.4± 3.4mg $CaCO_3$/L
5.	Conductivity	<10 µs/cm

Figure 1: Butyrylcholinesterase Activity (BchE) in Plasma and Acetylcholinesterase activity (AchE) in Brain

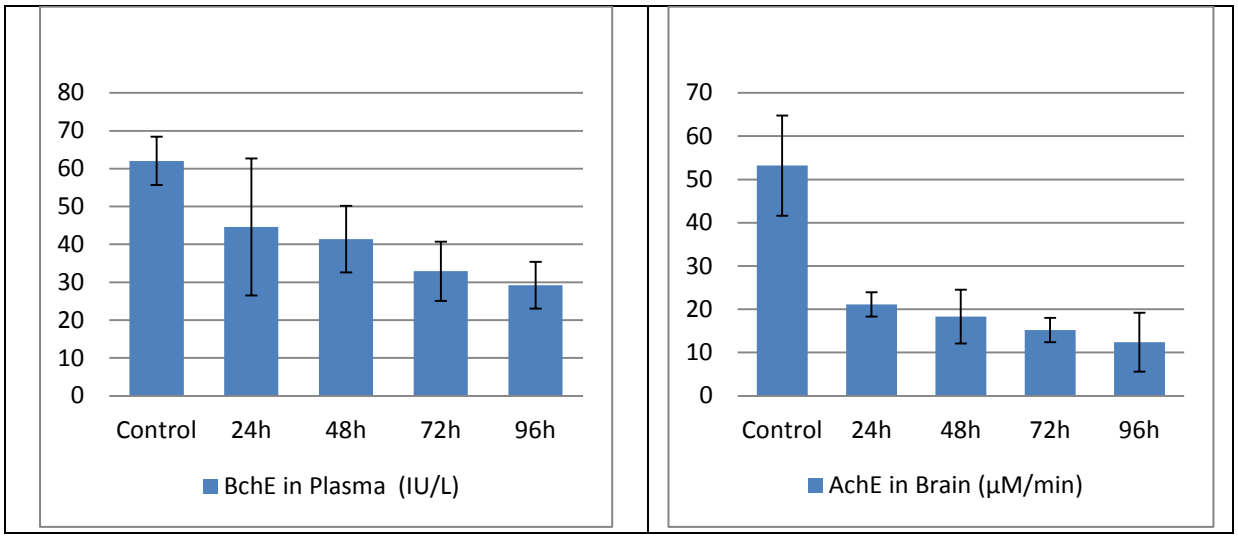
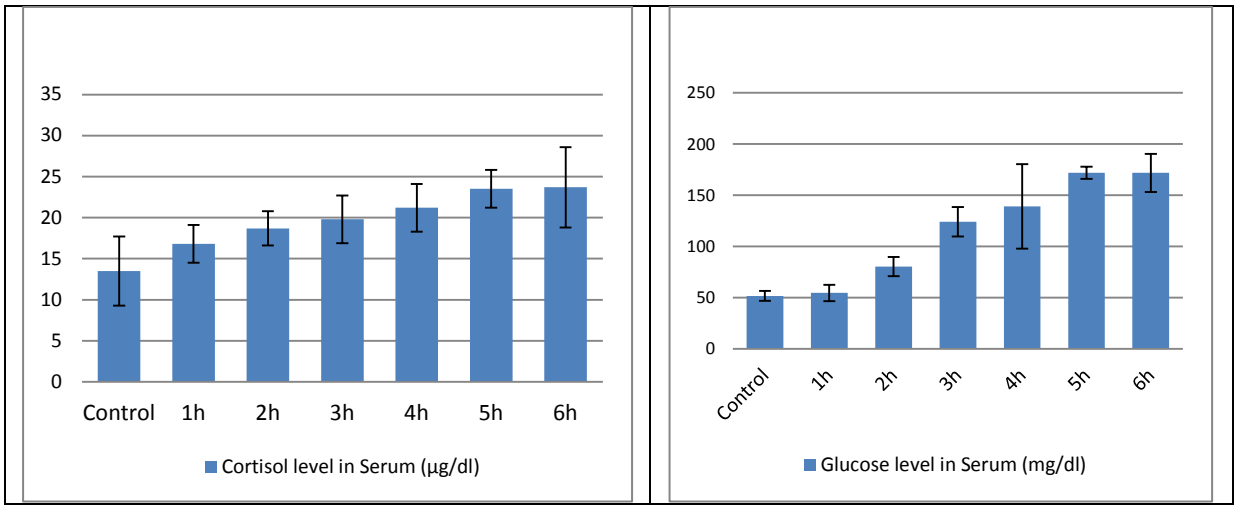


Figure 2: Cortisol and glucose levels in Serum



5. Discussion:

Cholinergic biomarkers are generally divided into two main classes: acetylcholinesterase and butyrylcholinesterase in most of the organisms which are extensively used as a diagnostic tool for ecotoxicological assessment of pesticides as well as xenobiotics in aquatic environment [10]. Inhibition of cholinesterase has been reported in several tissues and organs with a focus on brain tissues [32, 33]. In the present study, sensitivity of the fish, *Labeo rohita* to the exposure of methyl parathion has shown that acetylcholinesterase activity in the brain is inhibited significantly $p<0.14$, 0.002, 0.001 and 0.009 in 24, 48, 72 and 96h respectively. When, AchE activity decreases (Fig. 1) Ach is not hydrolysed and accumulates within the synapses which therefore cannot function in a normal way [34, 35]. Similarly, butyrylcholinesterase activity decreased significantly $p<0.07$, 0.01, 0.001 and 0.004 in 24, 48, 72 and 96h respectively. Following the exposure of methyl parathion, the inhibition of AchE in the brain has been observed in the present study which reflected in the behavioural pattern of the fish. Fish behaviour

provides a link between physiology and ecology and its environment [36]. It is an attempt to adjust to external and internal stimuli to meet the challenges of surviving in an altered surrounding. Behaviour represents an integrated response of the fish to the toxicant (methyl parathion) induced stress [37]. It was observed in the present study that there was a fast and abrupt movement of the fish in the experimental tank had a tendency to escape from toxic water. Thus, behaviour is a selective response that is constantly adapting through direct interaction with physical, chemical, social and physiological aspects of the environment. In the present study, the control fish behaved normally that is, they were active with well coordinated movements. They were alert to the slightest disturbances but in the toxic environment the fish exhibited irregular, erratic swimming movements and loss of equilibrium due to the inhibition of AchE activity leading to the accumulation of acetylcholine at the cholinergic synapses resulting in hyperstimulation and finally lethargic condition. Similar reports have been made by Munshigeri and David, 2005; Kumari *et al*, 2011 [38, 39]. Since inhibition of AchE is a typical characteristic of organophosphate [40, 41, 42, 43, 44] behavioural pattern changes. The fish secreted excess mucous all over their bodies which formed a barrier between the body and the toxic media thereby reduced contact with the toxicant to minimize their irritating effect and eliminating the toxicant through epidermal mucus. Similar observations were made by Kumari and Sinha, 2011; Rao *et al* 2003 [39, 45]. Gulping of air at the surface was observed. Surface phenomena and gulping of air may help to take in more air to meet the challenge of hypoxic condition. The behavioural responses can be used as a tool in biomonitoring programme to monitor ecotoxicity risk of methyl parathion to the test species.

It is clear from Fig. 1 that the percentage of inhibition of butyrylcholinesterase was 52.9%, whereas AchE in the brain was 76.7% after 96h of exposure as compared to the Control. Evidently, the inhibition in the plasma butyrylcholinesterase by methyl parathion was much less (Fig. 1) which could be due to the irreversible binding of BchE to the molecule of the pesticide and inactivated the methyl parathion molecule before it escaped from the circulation and reached the physiological target, the brain. Similar observation has been reported by Ravesh *et al*, 1997 [46]. It is known that loss of AchE function leads to muscle paralysis and may cause death by hypoxia. BchE can be considered as an endogenous scavenger of anti-cholinesterase compounds like methyl parathion because BchE detoxifies them before they reach AchE at physiologically important target sites. Thus, BchE acts as prophylactic agent against methyl parathion. As such, the methyl parathion is mediated by the inhibition of neurotransmission since nervous system has integrative and coordinative functions to maintain normal hormonal homeostasis dysfunction of the nervous system leading to cascade of inhibitory events in endocrine system.

Cortisol is the principal glucocorticoid secreted by the interrenal tissue located in the head of the teleost fish [47]. This hormone is released by the activation of hypothalamus-pituitary-interrenal axis (HPI axis) [48]. When the fish undergoes stressed condition, the hypothalamus releases corticotropin-releasing factor (CRF) towards blood circulation. This polypeptide further stimulates the secretion of adrenocorticotropin hormone (ACTH) from the anterior pituitary [49] which finally activates the release of cortisol by the inter-renal tissue.

Serum cortisol has been widely used to assess the state of health of the fish exposed to stress [50, 51]. In the present study, since there is a significant increase in the cortisol concentration ($p<0.003$, $p<0.66$, $p<0.26$, $p<0.002$, $p<0.006$, $p<0.16$ in 1^{st} , 2^{nd} , 3^{rd} , 4^{th} , 5^{th} and 6^{th} h respectively) immediately after exposure (Fig. 2), thus it could be concluded that fishes exposed to the sub-lethal concentration of methyl parathion undergo an immediate stress and continue to be under stress till 6^{th} h with a parallel increase of glucose concentration (Fig. 2). Many authors have reported that it is catecholamines which are known as “fight and flight” hormone which increase glucose initially and thereafter the cortisol further increases the blood glucose [25]. Glucose increase is a general response of fish to acute pollutant effects, including organophosphates [52]. Hyperglycemic effect of cortisol was due to glycogenolysis to

meet the energy demand during the pesticidal stress and secretion of cortisol was slower than catecholamines but its effects were more prolonged for the restoration of homeostasis [25]. An adverse role of cortisol was suggested to be linked to mobilization of energy reserves through catabolic functions. After stress, the cortisol level showed no increase (Fig. 2) to avoid tissue damage because it is well known that high level of cortisol could cause death in fishes by tissue degeneration and aberration in homeostatic mechanism. Thus cholinesterases, cortisol and glucose could be treated as biomarkers of pollution for acute stress.

6. Conclusion :

In conclusion, the importance of integrating biomarkers to assess the effect of methyl parathion in the fish, *Labeo rohita* has been confirmed by the results showing significant inhibition of cholinesterase activity and increase in the stress hormone, cortisol resulting in the increase in blood glucose level to meet the energy demand during stress. This showed the significance of integrating biochemical parameters in assessing methyl parathion toxicity in fishes. It is therefore recommended to the policy makers in India that these biochemical end points to be used as biomarkers as complementary parameters in the assessment of water quality.

7. Acknowledgement:

The authors are grateful to DBT, Govt. of India for financial assistance for the project (Sanction Letter no. BT/PR/4762/BCE/8/903/2012; Dated: 11/3/2013). The authors also thank the Head of the Department of Zoology for extending all logistic support.

8. References:

[1] Mora P, Michel X and Naarbonne JF. Cholinesterase activity as a potential biomarker in two bivalves. Environ Toxicol Pharmacol 1999; 7: 253-260.

[2] Yaqin K and Hensen PD. He use of cholinergic biomarker cholinensterase activity of the blue mussel, *Mytilus edulis* to detect the effects of organophosphate pesticides. Afr J Biochem Res 2010; 4: 265-272.

[3] Mehboob S, Ghazala S, Sultana AS, Al- Akil AB Al- Kahem, Al- Misned F and Zubair M. Pesticide residues in flesh of *Cirrhinus mrigala* collected from a commercial farm and river Chenab at Trimu head, Jhang. Pak J Zoo 2011; 43: 97-101.

[4] Ahmed L, Khan A and Khan MZ. Pyrethroid induced reproductive toxicopathology in non-target species. Pak Vet J 2012, 32: 1-9.

[5] Muhammed F, Javed I, Akhtar M, Rahman ZU, Awais MM, Saleem MK and Anwar MI. Quantitative structure activity relationship and risk analysis of some pesticides in the cattle milk. Pak Vet J 2012; 32: 589-592.

[6] Wedemeyer GA, Barton BA ad Mcleay DJ. Stress and acclimation. In: Schreck, CB and Moyle PB. (eds) Methods for Fish Biology, American Fisheries Society, Bethesda, 1990, pp. 491-527.

[7] Lowe CJ and Davidson W. Plasma osmolarity, glucose concentration and erythrocyte concentration and erythrocyte responses of two Antarctic notothendid fishes to acute and chronic thermal change. J Fish Biol 2005; 67: 752-766

[8] Mathur SM and Singh JK. Chemical spraying in Agriculture and its environmental impact. All India Seminar on Environmental Impact Assessment, The Institution of Engineers, Udaipur 2006.

[9] Rodriguez- Fuentes G and Bouchet G. Environmental monitoring using acetylcholinesterase inhibition *in- vitro*: A case study in two Mexican lagoons. Marine Environ Res 2000; 50: 357-360.

[10] Torre FR, Ferrari D and Salibian A. Freshwater pollution biomarker: response to brain acetylcholinesterase activity in two fish species. Comp Biochem Physiol 2002; 131: 271-280.
[11] Ferreiro NGC, Fernanda R, Domingues I, Carla F, Amaden MPV and Susana L. Acetylcholinesterase characterization in the terrestrial isopod, *Porcellionides pruinosus*. Interdisciplinary Studies on Environmental Chemistry, Biological Responses to Contaminants 2010; 227-236.
[12] Adedejio B. Responses of acetylcholinesterase activity in the brain of *Clarias gariepinus* in sub-lethal concentration of diazinon. J Appl Sci Environ Sanit 2011; 6: 137-141.
[13] Shea TB and Berry ES. Supression of interferon synthesis by pesticide carbaryl as a mechanism for enhancement of goldfish virus-2 replication. Appl Environ Microbial 1984; 47: 250-252.
[14] Soraq H and Zakut H. Human acetylcholinesterase and anticholinesterase. Academic Press, San Diago, California, USA, 1993.
[15] Behera M, Cousin X, Bertrand C, Vonesch JL, Biellman D, Chattonnet and Strahle UD. Acetylcholinesterase is required for neuronal and muscular development in the Zebrafish embryo. Nat Neurosci 2002; 5: 11-118.
[16] Assis CR, Castro PF, Amral IP, Carvalho EV and Bezerra RS. Concentration of acetylcholinesterase from brain of Amazonian Tambaquil (*Colossoma macropomium*) and *in vitro* of organophosphate and carbamate pesticides. Environ Toxicol Chem 2010; 29: 2243-2248.
[17] Gandahi JA, Gandahi P, Yang XG, Bian, Shah MG, Malhi M, Zhang, LL, Zhang, Q and Chen Q. Disribution of acetylcholinesterase positive neurons in the oviduct of laying hen. Pak Vet J 2013, 33: 213-217.
[18] Whitaker M. Cholinesterase. Karger, Basel, New York 1986; pp. 1-126.
[19] Pan G and Dutta HM. The inhibition of brain acetylcholinesterase activity of juvenile largemouth bass, *icropterus salmoides* by sub- lethal concentrations of diazinon. Env Res Sec 1998, 79: 133-137.
[20] Bretaud S, Toutant JP and Sagilo P. Effects of carbofuran, diuron and nicosulfuron on acetylcholinesterase activity in gold fish (*Carassius auratus*). Ecotoxicol Environ Saf 2000, 47: 117-124.
[21] Pickering AD, Pottiger GG and Christie P. Recover of brown trout, *Salmo turtica* from acute handling stress: A time- course study. J Fish Biol 1982; 20: 229-244.
[22] Barton BA, Schreck CB and Flower LG. Fasting and diet content affect stress- induced changes in plasma glucose and cortisol level in juvenile chinook salmon. The Progressive Fish Culturist 1988; 50: 16-22.
[23] Begg K and Pankhurst NW. Endocrine and metabolic responses to stress in a laboratory population of tropical damselfish *Acanthochromis polyacanthus*. J Fish Biol 2004; 64: 133-145.
[24] Rottman RW, Francis- Floyed R and Durborow R. The role of stress in fish diseases. SARC Publications no. 474; 1992. p. 4.
[25] Ray SNC and Sinha RC. Serum cortisol and glucose: Reliable bioindicators of stress in the fish, *Labeo rohita*. Int J Innovative Sci, Engg and Technol 2014, 1: 6-17.
[26] Hattingh J. Blood sugar as an indicator of stress in the fresh water fish, *Labeo capinsis*. J Fish Biol 1997; 10: 191-195.
[27] Barcellos LJG, Nicolaiwsky S deSouza and Luthier F. Plasmatic levels of cortisol in response to acute stress in Nile Tilapia previously exposed to chronic stress. Aqucul Res 1999; 30: 437-444.
[28] Reid SG, Vijayan MM and Perry SF. Modulation of catecholamine storage and release by pituitary- integral axis in the rainbow trout, *Oncorhynchus mykiss*. J Comp Physiol B 1996, 165: 665-676.
[29] Finney DJ. Probit analysis 3rd edition, Cambridge University Press, London, 1971, pp. 330.

[30] Ellman GL, Courtney D, Andres V and Featherstone R. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961, 7: 88-95.

[31] Kirschner KN and Woods RJ. Solvent interactions determine carbohydrate confirmation. Proc Natl Acad Sci USA 2001; 98(19): 10541-10545.

[32] Fernandez- Vega C, Sancho E, Ferrando MD and Andrew E. Thiobencarb induced changes in acetylcholinesterase activity of the fish *Anguila anguila*. Pestici Biochem Physiol 2002, 72: 55-63.

[33] Pena-Lilopis S, Ferrando MD and Penna JB. Fish tolerance to organophosphate- induced oxidative stress is dependent on glutathione metabolism and enhanced by N- acetylcystine. Aqua Toxicol 2013, 65: 337-360.

[34] Dutta HM and Meizer HJM. Sublethal effects of diazinon on the structure of the testis of blue gill, *Leopomis macrochirus*, a microscopic analysis. Environ Pollu 2003, 125: 355-360.

[35] Kumari K and Sinha RC. Cholinesterase activity as a potential biomarker in the frog, *Rana tigrina*. J Ecophysiol Occup Hlth 2006, 6: 153-157.

[36] Little EE and Brewer SK. Neurobehavioural toxicity in fish. In: Schlenk D and Benson WH (ed.) Target organ toxicity in marine and fresh water teleosts, New perspective: Toxicology and the Environment, Taylor and Francis, London and New York, 2001, 2: 139-174.

[37] Kane AS, Salierno JD and Brower SK. Fish models in the behavioural toxicology: Automated techniques, updates and perspectives. In: Ostrander GK. (ed) Meyhods in aquatic toxicology, 2nd edition, Lewis Publishers, Boca Raton, 1998, pp. 559-590.

[38] Munshigeri SB and David M. Fenvalerate induced changes in the AchE and associated AchE activity in different tissues of fish, *Cirrhinus mrigala* (Hamilton) under lethal and sublethal exposure period. Enviro Toxicol and Pharmacol 2005, 20: 65-72.

[39] Kumari K, Ranjan N and Sinha RC. Multiple biomarker responses in the fish *Labeo rohia* due to hexvalent chromium. In: 2nd International conference on biotechnology and food sciences, 2011, 155-158.

[40] Holmsterd B. Structure activity relationship of the organophosphorus anticholinesterase agents. In: Koelle GB (ed.) Cholinesterase and anticholinesterase agents, Haudbuch der Experinentellen Pharmakologie Springer- Verlag-Berlin, 1963, 428-485.

[41] Habig C and DiGiulio RD. Biochemical charecteristics of cholinesterase in aquatic organism. In: Mineau P (ed.) Cholinesterase inhibiting insecticides: their impact on wildlife and environment-chemicals in agriculture, Elsevier, New York, 1991, 2: 19-34.

[42] Padila S. Regulatory and research issues related to cholinesterase inhibition. Toxicology 1995, 102: 215.

[43] Timchalk C, Nlan RJ, Mendrala AL, Dittenber DA, Brazak RN and Mattsm JL. A physiologically based pharmacokinetic and pharmacodynamic model for the organophosphate insecticide chlorpyrofos in rats and humans. Toxicological Sciences 2002, 66: 34-53.

[44] Kumari K and Sinha RC. Acetylcholinesterase activity in the plasma and brain of frog, *Rana tigrina* and the toad *Bufo melanostictus* following the treatment with carbaryl and methyl parathion. J of VN Karazin Kharkiv National University USSR 2010, 117-120.

[45] Rao JV, Rani CHS, Kavitha P, Rao RN and Madhavendra SS. Toxicity of chloropyrifos to the fish, *Oreochromis mosambicus*Bull of Env Contam & Toxicol 2003, 70: 985-992.

[46] Ravesh L Grunwald J Marcus D, Papier Y, Cohen E and Ashani Y. Human butyrylcholinesterase as a general prophylactic antidote for nerve agent toxicity: *In- vitro* and *in- vivo* quantitative charecterization. Biochem Pharmacol 1993; 45: 2465-2474.

[47] Iwamma GK, Monga JD and Barton BA. Simple field methods for monitoring stress and general conditions of fish. Aqucul Res 1995; 26: 273-282.

[48] Mommsen TP, Vijayan MM and Moon TW. Cortisol in teleosts: dynamics mechanisms of action and metabolic regulation. Rev Fish Biol and Fisheries 1999, 2: 211-268.
[49] Fryer JL and Lederis K. Control of corticotrophin section in teleost fishes. Am Zoolists 1986; 26: 1017-1026.
[50] Mazeaud MM and Mazeaud F. Adrenergic responses to stress in fish. In: Pickering AD. (ed) Stress & Fish, Academic Press, London, New York, 1981, pp. 49-75.
[51] Pickering AD. Growth and stress in fish production. Aquaculture 1993, 111: 51-63.
[52] Sancho E, Ferrando ND and Andrev E. Sublethal effects of an organophosphate insecticide on the Eurepoen ell, *Anguilla Anguilla*. Ecotoxicol Environ Saf 1997, 36: 57-65.

First Author

R. C. Sinha is currently working as Chief Executive, CENC, Department of Zoology, Patna University, Patna and Chairman, Bihar State Environment Impact Assessment Authority, Government of India. He has worked as Professor of Zoology in Patna University and also as Chairman, Bihar State Pollution Control Board from 1991- 2001. He had worked on pollution load of river Ganges and Damodar sponsored by World Bank through CPCB, Delhi and also on new technique for water quality assessment sponsored by CPCB, MoEF, Government of India. Currently, he is working on cytopathological and biochemical markers of pollution sponsored by Department of Biotechnology, Government of India, N. Delhi. He has published more than 100 research papers in international journals.

Second Author

S.N.C. Ray is currently working as Senior Research Fellow in a research project sponsored by Department of Biotechnology, Government of India in Department of Zoology, Patna University, Patna under Prof. R.C. Sinha. He has an experience of 10 years in the field of Environment after his M. Sc. In Biochemistry from Patna University, Patna in association with Prof. Sinha. He has published more than 15 research publications in both National and International peer reviewed journals.