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STUDY THE EFFECT OF CHLORPYRIFOS ON ACETYLCHOLINESTERASE AND HEMATOLOGICAL RESPONSE IN FRESHWATER FISH CHANNA PUNCTATUS (BLOCH)

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ABSTRACT

The toxic effect of chlorpyrifos (CPF) on biochemical and hematology in different concentrations and exposure periods was investigated in the fish, Channa punctatus (C. punctatus). The LC50 - 96h of technical-grade CPF was evaluated 811.98 µg/l for C. punctatus in a semi-static system and on the basis of LC50 value two sublethal concentrations viz. 203.0 and 68.0 µg/l were determined. C. punctatus were exposed to sublethal concentrations of CPF for 1, 3, 7, 14 and 21 days. The AChE activity, erythrocyte, hemoglobin percentage, haematocrit and leucocyte were decreased significantly (p<0.01) as concentrations and exposure periods increased. The highest reduction in AChE activity was recorded in brain followed by gill and blood plasma in both sublethal concentrations. Thus our results suggested that AChE activity and blood parameters could be used as potential biomarkers for environmental contaminants in aquatic system.

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KEY WORDS

Chlorpyrifos; Biochemical parameters; Hematology; Channa punctatus

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[I] INTRODUCTION

Chlorpyrifos [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl) phosphorothionate] is a broad spectrum organophosphate pesticide widely used in agriculture and residential pest control throughout the world. As a consequence of chlorpyrifos (CPF) widespread use in crop fields, it easily washed into surface water, enters the ground water and aquatic environment in large quantities [1]. On the other hand, the toxicological and epidemiological studies of pesticides in human health concerns are now at forefront due to litany of pesticide residues found in human blood samples, drinking water and foods [2, 3]. Ricceri et al. [4] reported that exposure to CPF at certain gestational or neonatal times of development are associated with neurobehavioral changes. CPF alters synaptic neurotransmission, inhibits neural cell replication, neurite outgrowth, evokes oxidative stress; interfere with signaling cascades and transcriptional events involved in neural cell differentiation [5].

CPF may also regarded as hazardous, since it persists long time in sediments. In India, CPF is classified as an extremely hazardous pesticide [6]; its residue has been found in scented roses and their products [7]. Its maximum concentration has been reported to be 88.6 µg/g in tissues of fishes *Channa striata* and *Catla catla* from Kolleru Lake, India [8] and 198.5 µg/g in sediment, prawn and water samples from prawn ponds near Kolleru Lake wetland [9]. Surprisingly, the soft drinks also contain CPF in a concentration of 4.8 µg/l, which is 47 times higher than permissible limit [10]. Its genotoxicity was reported in *C. punctatus*, mice leukocytes and root meristem cells of *Crepis capillaries* [11, 12, 13, 14].

The measurement of the AChE activities in fish has been suggested as a diagnostic biomarker, with decreased activities indicating water contamination by organophosphorus pesticide [15, 16]. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fisheries management and disease investigation [17].The hematological abnormalities under toxic stress may also be reflected in other physiological activities like oxygen consumption and metabolism, which result in death [18].

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Oxygen transport in blood depends upon the hemoglobin content of erythrocytes in blood of fishes [19].

Keeping these facts in view, we have envisaged this study for in-vivo toxic effect of CPF on the activity of AChE in different tissues as well as their tissue specific effects in order to establish the target tissue and their relative efficacy and blood parameters of *C. punctatus*. Finally, our findings would be a useful tool for the control of regional reservoirs and their effective management with respect to the input of CPF from agricultural areas.

[II] MATERIALS AND METHODS

2.1. Chemicals

Technical-grade CPF (20% EC) with trade name Tricel (manufactured by Excel crop care Ltd. Mumbai) was purchased from local market. Trichloroacetic acid (TCA), zinc sulfate ($ZnSO_4.7H_2O$), acetylthiocholine iodide and other chemicals were purchased from Merck. Bovine serum albumin (BSA) for protein assay was purchased from Sigma (U.S.A.).

2.2. Experimental animal

Freshwater fish *C. punctatus* (Bloch) were procured from the local outlets. The fish specimens were an average wet weight and length of 30±2.0 g and 14±3.0 cm, respectively. The fishes were given prophylactic treatment by bathing them twice in 0.05% potassium permanganate ($KMnO_4$) solution for two min to avoid any dermal infections. The fishes were then acclimatized for one month under laboratory condition before CPF exposure. The fishes were fed boiled eggs, goat liver and poultry waste material. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Every effort as suggested by Bennett and Dooley [20] was made to maintain optimal conditions during acclimatization.

2.3. Determination of sub lethal concentrations

The acute toxicity bioassay to determine the LC50-96h value of CPF in fish was conducted in the semi-static system. A facility for oxygenation of the test solution was provided with the help of showers fixed above the test chambers. The acute bioassay procedure was based on standard methods [21].The stock solution of CPF was prepared by dissolving it in acetone.

A set of ten acclimatized fish specimens was randomly exposed to each of the six CPF target concentrations (0.3, 0.6, 0.8, 1.0, 1.2 and 1.5 mg/l) and the experiment was repeated five times to obtain the LC50-96 h value of the test chemical for C. punctauts.

The LC50-96 h value of CPF was determined as 811.98 µgl-1 for C. punctauts following the probit analysis method as described by Finney [22]. Based on the LC50-96 h value, the two sublethal concentrations of CPF viz., sublethal 1 (1/4th of LC50 = ~203.0 µg/l) and sublethal 2 (1/12th of LC50 = ~ 68.0 µg/l) were estimated.

2.4. In vivo exposure experiment

The fish *C. punctauts* were exposed to the two aforementioned test concentrations of CPF in a semi-static system with the change of test water on every 96 h. The exposure was continued up to 21 days and tissue sampling was done at intervals of 1, 3, 7, 14 and 21 days at the rate of five fishes per duration. The fishes maintained in tap water were considered as negative control. The concentration of acetone was 0.1% in all test solutions and solvent control.

On each sampling day, the blood, gills and brain tissues were collected and immediately processed for estimation AChE and hematological parameters. The blood samples were collected from the fish by caudal vein puncture technique using heparinized syringe. The physicochemical properties of test water, namely temperature, pH, conductivity, dissolved oxygen, chloride, total hardness and total alkalinity were analyzed by standard methods [21].

2.5. AChE and blood parameters analysis

Blood was collected from caudal vein by heparinized syringes from fish C. punctauts and then fishes were sacrificed and tissue like brain and gills were quickly excised in ringer solution. The excess blood was washed with 0.15 M KCl (cold) then weighed. These were homogenized (10% w/v) in 0.1M, pH 8 Tris HCl buffer using homogenizer fitted with teflon pestle. The homogenate were centrifuged at 5000 rpm for 10 min.

AChE activity was determined using the colorimetric technique described by Ellman et al. [23]. The reaction performed at 37ºC was initiated by adding small aliquots of varying concentrations of the substrate (acetylthiocholine iodide) to yield a final volume of 3 ml. The absorbance at 412 nm was recorded continuously for 5 min. The corresponding blanks lacking AChE were substrate to yield the enzymatic activity rate. The typical runs for all experiments used were 2.7 ml buffer, 0.1 M phosphate buffer, pH 8, 50 µl (0.16 mM) DTNB, 100µl (1 mg/ ml protein and 100 µl of substrate. The protein concentrations were measured at 595 nm by Lowry et al. [24], using bovine serum albumin as standard.

Calculation

$$V = \triangle A/\min \times \frac{3}{\text{Protein}} \times \frac{1}{14.3} \ \mu M/\min/mg \text{ protein}$$

Δ A/min is change in optical density.
3 is ml of solution in cuvette.
14.3 molar extinction coefficient of DTNB M/min/mg protein

The blood parameters viz. total erythrocyte count (TEC), total leukocyte count (TLC) were calculated by Neubauer Haemocytometr and Hb%, hematocrit (PCV) by Sahli's hemoglobin meter from collected blood. One aliquot of the sample was used for total erythrocyte count, total leucocyte count, hemoglobin and hematocrit calculations. Chemicals used were of the highest analytical grade and measurements were determined in triplicate.

2.6. Determination of total protein

Protein contents in fish-tissue were determined according to the method of Lowry et al. [24] using bovine serum albumin (BSA) as standard.

2.7. Statistical analysis

The one-way analysis of variance (ANOVA) was applied to compare the mean differences in the AChE levels between tissues within concentration, between durations within concentration and tissue. The different blood parameters were compared between durations within concentration and between concentrations within duration using Mann-Whitney test. P values less than 0.01 was considered statistically significant.

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[III] RESULTS

3.1. Physicochemical properties of the test water

The test water temperature varied from 26.7 to 28.4 ^{o}C and the pH ranged from 7.2 to 8.1. The dissolved oxygen concentration was normal, varied from 6.0 to 8.05 mg/l, during experimental period. The conductivity of the water ranged from 248 to 296 µM/cm and the chloride, total hardness and total alkalinity ranged from 45-54 mg/l, 160-180 mg/land 260-290 mg/l, as $CaCO_3$, respectively.

3.2. Acetylcholinesterase activity and hematological parameters

AChE activity did not differ significantly ($p<0.01$) in solvent and solvent-free control fish. Therefore, the data of AChE activity of solvent and solvent-free controls were combined for statistical analysis. The AChE activity was decreased 51.83% in brain, 44.63% in gill and 39.44% in blood plasma at 203.0 µg/l of CPF **[Figure–1A, Table1]**, while at 68.0 µg/l CPF it reduced 34.86% in brain, 28.52% in gill and 21.60% in blood plasma **[Figure–1B, Table 1]**. Brain AChE level was decreased about half in exposed fish than control.

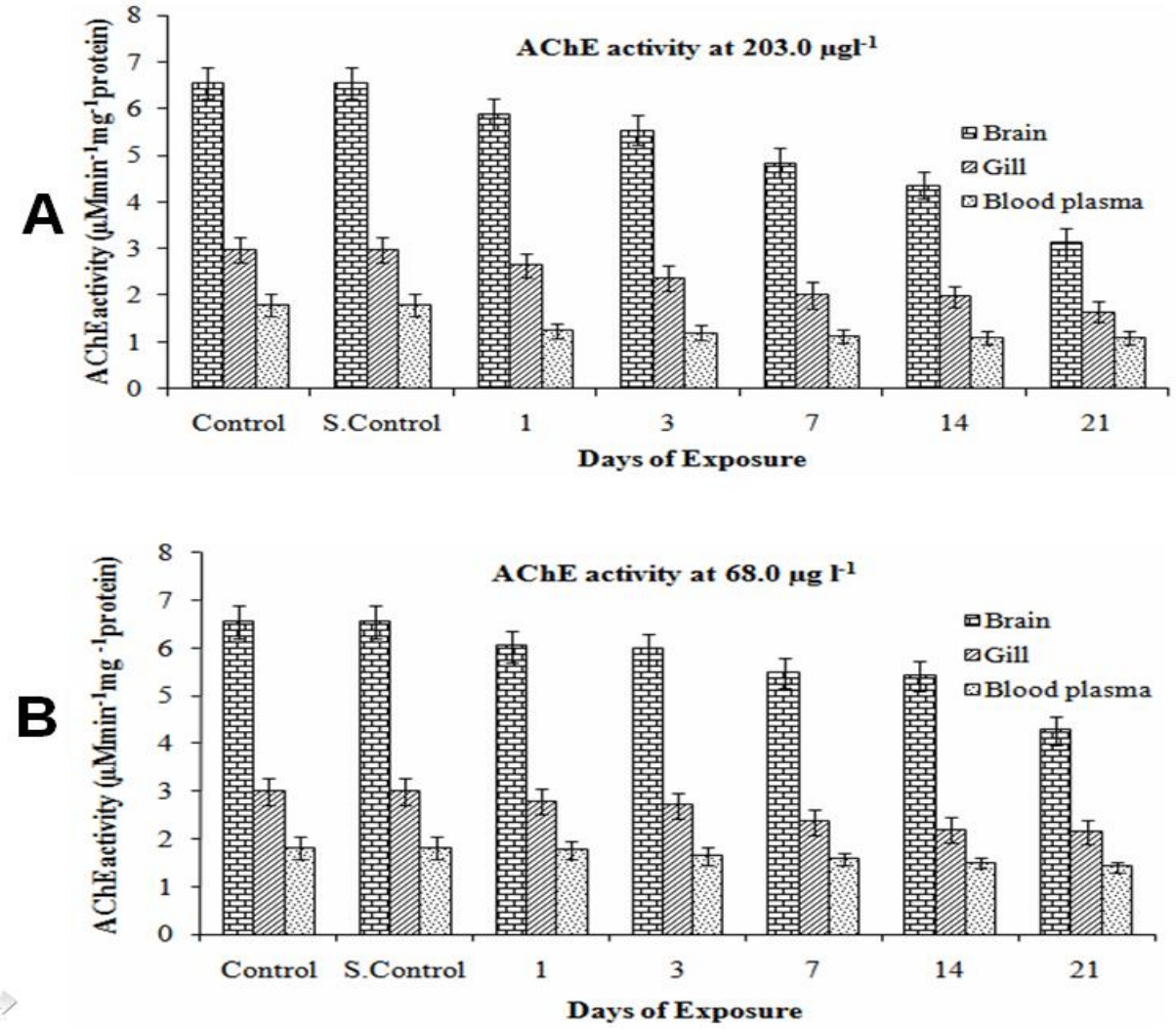

Fig:1. Changes in acetyl cholinesterase activity in brain, gills and blood plasma by **A.** 203.0 µg/l. **B.** 68.0 µg/l of chlorpyrifos.

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Table: 1. AChE activity (µM min/mg/ protein) in different tissues of *C. punctatus* exposed to different concentrations of chorpyrifos at various time intervals

Expo.times	Brain		Gill		Blood plasma	
	203.0 µg/l	68.0 µg/l	203.0 µg/l	68.0 µg/l	203.0 µg/l	68.0 µg/l
Control	6.54 ± 0.34^{a1}	6.54 ± 0.34^{a1}	2.98 ± 0.28^{a1}	2.98 ± 0.28^{a1}	1.80 ± 0.23^{a1}	1.80 ± 0.23^{a1}
S.Control	6.54 ± 0.34^{a1}	6.54 ± 0.34^{a1}	2.98 ± 0.28^{a1}	2.98 ± 0.28^{a1}	1.80 ± 0.23^{a1}	1.80 ± 0.23^{a1}
1 day	5.88 ± 0.33^{a2}	6.03 ± 0.33^{b2}	2.64 ± 0.27^{a1}	2.78 ± 0.28^{a2}	1.24 ± 0.17^{a2}	1.76 ± 0.19^{b1}
3 day	5.53 ± 0.33^{a2}	5.97 ± 0.33^{b2}	2.38 ± 0.26^{a2}	2.70 ± 0.27^{b2}	1.21 ± 0.17^{a2}	1.64 ± 0.18^{b2}
7 day	4.83 ± 0.31^{a3}	5.46 ± 0.32^{b3}	2.01 ± 0.28^{a3}	2.35 ± 0.27^{b3}	1.12 ± 0.14^{a3}	1.57 ± 0.12^{b2}
14 day	4.36 ± 0.32^{a4}	5.40 ± 0.31^{b3}	1.97 ± 0.23^{a3}	2.18 ± 0.27^{b4}	1.10 ± 0.14^{a3}	1.49 ± 0.11^{b3}
21 day	3.15 ± 0.30^{a5}	4.26 ± 0.31^{b4}	1.65 ± 0.23^{a4}	2.13 ± 0.25^{b4}	1.09 ± 0.12^{a3}	1.41 ± 0.11^{b3}

Values with different alphabet superscripts differ significantly ($P<0.01$) between concentrations within tissue. Values with different numeric superscripts differ significantly ($P<0.01$) between durations within concentration and tissue.

Blood parameters namely erythrocyte, leukocyte, hemoglobin and hematocrit mean levels decreased significantly ($p<0.01$) with increase in exposure time and concentration of CPF to the fish **[Figure–2A-D, Table 2]**.

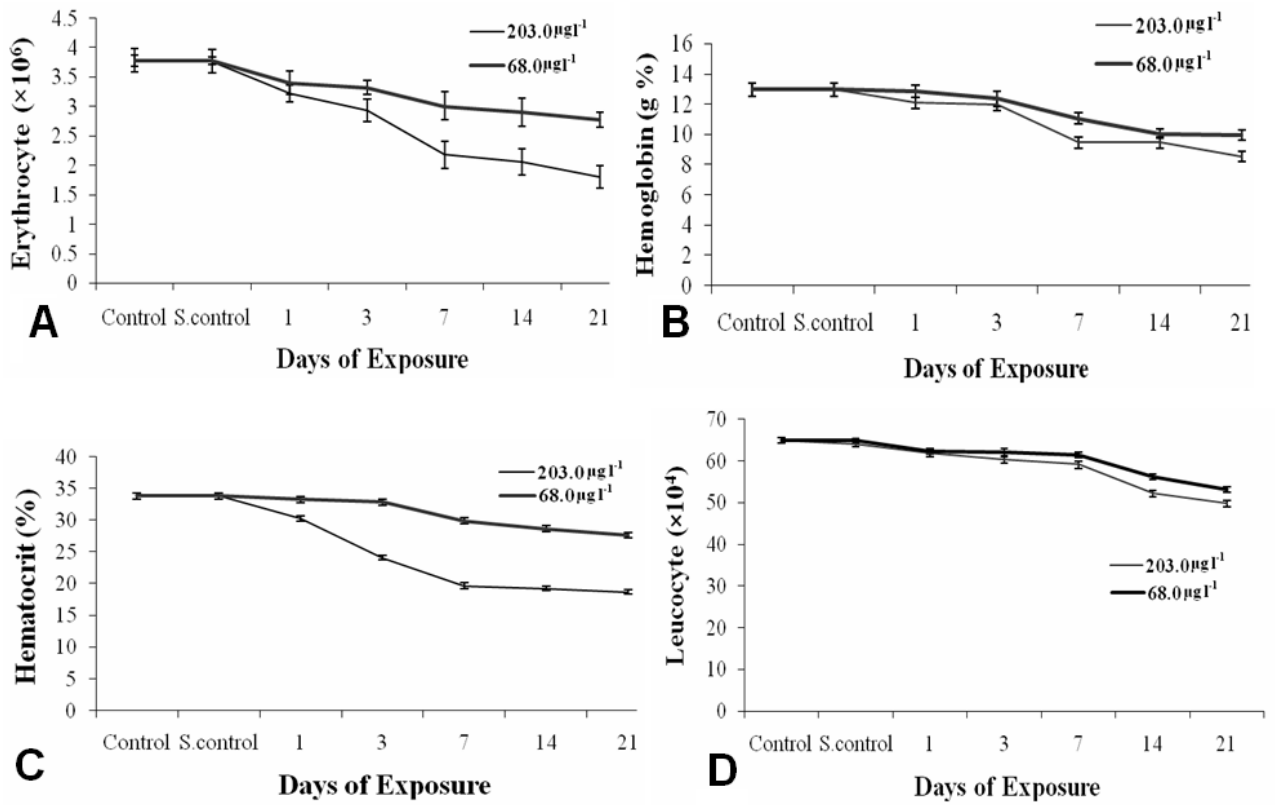

Fig: 2. Changes in blood parameters A. Erythrocyte **B**. Hemoglobin **C**. Hematocrit **D**. Leucocyte after exposure of 203.0 µg/l and 68.0 µg/l of chlorpyrifos for 21days

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Table: 2. Effect of different concentrations of chorpyrifos on hematological parameters of *C. punctatus* at various time intervals.

Expo. times	TEC (×10^5)		TLC (×10^4)		Hb (gm %)		Hematocrit (%)	
	203.0 µg/l	68.0 µg/l	203.0 µg/l	68.0 µg/l	203.0 µg/l	68.0 µg/l	203.0 µg/l	68.0 µg/l
Control	3.78±0.26^{a1}	3.78±0.26^{a1}	64.87±0.63^{a1}	64.87±0.63^{a1}	12.96±0.44^{a1}	12.96±0.44^{a1}	33.80±0.53^{a1}	33.80±0.53^{a1}
S.Control	3.78±0.26^{a1}	3.78±0.26^{a1}	64.82±0.63^{a1}	64.86±0.63^{a1}	12.96±0.44^{a1}	12.96±0.44^{a1}	33.80±0.53^{a1}	33.80±0.53^{a1}
1 day	3.22±0.24^{a1}	3.40±0.25^{b2}	61.87±0.80^{a2}	62.26±0.65^{a2}	12.08±0.39^{a1}	12.86±0.42^{a2}	30.26±0.46^{a2}	33.27±0.51^{b1}
3 day	2.94±0.24^{a2}	3.32±0.24^{b2}	60.20±0.81^{a2}	62.04±0.65^{a2}	11.96±0.38^{a2}	12.34±0.41^{b2}	24.10±0.37^{a3}	32.78±0.49^{b1}
7 day	2.18±0.23^{a3}	3.01±0.24^{b3}	59.10 0.82^{a2}	61.50±0.65^{a2}	9.46±0.36^{a3}	11.04±0.37^{b2}	19.65±0.36^{a4}	29.88±0.49^{b2}
14 day	2.06±0.23^{a3}	2.90±0.24^{b3}	52.23±0.81^{a3}	56.17±0.68^{b3}	9.45±0.36^{a3}	10.06±0.33^{b3}	19.24±0.36^{a4}	28.66±0.48^{b2}
21 day	1.80±0.19^{a4}	2.76±0.22^{b4}	49.76±0.84^{a3}	53.32±0.68^{b4}	8.54±0.34^{a4}	9.90±0.36^{b3}	18.70±0.34^{a4}	27.64±0.41^{b3}

Values with different alphabet superscripts differ significantly (P<0.01) between concentrations within tissue. Values with different numeric superscripts differ significantly (P<0.01) between durations within concentration and tissue.

[IV] DISCUSSION

AChE inhibition was quite high in brain in comparison to other tissues. Natoff [25] reported that increase of acetylcholine at cholinergic synapse resulting from the inhibition of AChE in brain. AChE inhibition process was in two steps due to organophosphate pesticides with the active site of AChE followed by covalent bonding (phosphorylation) of phosphorous of organophosphate pesticide to the oxygen of hydroxyl group of serine [26].

Furthermore, these tissues showed variations in the degree of AChE inhibition for separate treatment and exposure period of CPF. This may be due to differences in the type of interaction between CPF and its metabolites with AChE in various organs, as well as the relative coherence between AChE inhibition and the degree of nerve innervations in these organs. In Tilapia, the highest level of AChE inhibition was noticed in brain followed by kidney, gill [27]. The behavioral changes have been noticed in the form of loss of equilibrium, rapid rate of swimming, convulsions, in the present study, which is in accordance with the findings of Kumar et al. [28] due to neurotoxicity caused by cypermethrin and k-cyhalothrin in *C. punctatus*.

The changes in blood parameters were observed due to long term exposure to CPF in fish. The reduction of erythrocyte count and hemoglobin content in Cyprinus carpio after acute exposure to diazinon were also reported by Svoboda et al. [29].Organophosphate pesticides induce changes in blood parameters, which give evidence for decreased hemotopoiesis followed by anemia induction in fish. Changes in erythrocyte profile induced by acute effect of dichlorvos in Clarias batrachus [30], formothion in *Heteropneustes fossilis* [31], malathion in *Cyprinion watsoni* [32] and trichlorphon in *Piaractus mesopotamicus* [33]. The decrease in erythrocyte number and hemoglobin content observed in this study may be due to the disruptive action of the pesticides on the erythropoietic tissue as a result of which the viability of the red blood cells might be affected. Morgan et al. [34] reported that changes in the hematological parameters were brought about by diazinon as an anemic condition due to decreased synthesis of red blood cells and erythrocyte in bone marrow. Monocrotophos reduced the ventilatory movements and decreased the oxygen intake by impairing neuromuscular transmission through AChE inhibition [35].

The total leukocyte counts (TLC) profile in fish due to CPF exposure showed significant variations. CPF exposure resulted in a significant decrease in the total leukocyte counts that may be due to increased neutrophil leucocytosis. Jain [36] reported that neutrophils are the first line of defense against infections, tissue injury, and parasite attack and in inflammatory response against foreign materials. Svoboda et al. [29] reported a decrease of non-specific immunity in *Cyprinus carpio* after acute exposure to organophosphate pesticides due to decreased leukocyte count. These changes in differential leukocyte count also give evidence for decreased level of non-specific immunity in fish after acute exposure to toxic substances. The adaptation to long term toxicity in relation to lymphocytes could be because lymphocytes are known to play significant role in antigen antibody reaction.

Thus the present study suggest that CPF contaminated water has the potential adverse effect in aquatic organism like fish and further demonstrate the usefulness of the biomarker AChE and blood parameters in an exposure scenario, where it is difficult to get a representative picture of contamination with conventional chemical analysis.

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[IV] CONCLUSION

The technical-grade CPF was found to be neurotoxic to fishes even at lower concentration (i.e.1/12th of LC50 = ~ 68.0 µg/l), which indicates apprehension about the potential hazards of CPF to aquatic organisms. The present investigations indicated that the AChE and blood parameters are sensitive tools for demonstration of toxic effects of CPF in different fish tissues. The results of this investigation may help in guarding against the toxic hazard to human population and the environment through judicious and careful use of this pesticide in agricultural and non-agricultural areas.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

FINANCIAL DISCLOSURE

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REFERENCES

[1] Singh PB ,Singh V, Nayak PK. [2008] Pesticide residues and reproductive dysfunction in different vertebrates from north India, *Food and Chem.Toxicol*. 46:2533–2539.

[2] Fenske RA, Kedan G, Lu C. [2002] Assessment of organophosphorous pesticide exposures in the diets of preschool children in Washington State, *J. Expo. Anal. Environ. Epidemiol* 12: 21–28.

[3] Fenske RA, Lu C, Barr D. [2002] Children's exposure to chlorpyrifos and parathion in an agricultural community in central Washington State. Environ. *Health Perspect 110*: 549–553.

[4] Ricceri L, Venerosi A, Capone F, Cometa MF, Lorenzini S, Fortuna G. [2006] Calamandrei, Developmental neurotoxicity of organophosphorous pesticides: Fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice, *Toxicol.Sci*.93:105–113.

[5] Qiao D, Seidler FJ, Slotkin TA. [2001] Developmental neurotoxicity of chlorpyrifos modeled in vitro: comparative effects of metabolites and other cholinesterase inhibitors on DNA synthesis in PC12 and C 6 cells. Environ. *Health Perspect* 109:909–913.

[6] Industrial Toxicology Research Centre (ITRC) [1989] Toxicity Data Handbook: Pesticide-B TDS. 192: 458– 463.

[7] Kumar A, Nadda G, Shanker A. [2004] Determination of chlorpyrifos 20% EC (Dursban 20% EC) in scented rose and its products. *J.Chromat* 1050:193–502.

[8] Amaraneni SR, Pillai RR. [2001] Concentrations of pesticide residues in tissues of fish from Kolleru Lake in India. Environ. *Toxicol* 16:550–556.

[9] Amaraneni SR. [2006] Distribution of pesticides, PAHs and heavy metals in prawn ponds near Kolleru Lake wetland. *Environ. Interna* 32:294–302.

[10] Centre for Science and Environment, Issue in Depth. http://www.cseindia.org/misc/cola-indepth/cola2006/cola_press2006.htm, 2006.

[11] Porichha SK, Sarangi PK, Prasad R. [1998] Genotoxic effect ofchlorpyrifos in Channa punctatus. *Pres. Cytol. Genet*. 9:631–638.

[12] Rahman MF, Mahboob M, Danadevi K, Banu BS, Grover P, [2002] Assessment of genotoxic effects of chlorpyriphos and acephate by the comet assay in mice leucocytes. Mutat. Res. 516 139–147.

[13] Ali D, Nagpure NS, Kumar S, Kumar R, Kushwaha B. [2008] Genotoxicity assessment of acute exposure of chlorpyrifos to freshwater fish Channa punctauts (Bloch) using micronucleus assay and alkaline single cell gel electrophoresis, *Chemosphere* 71:1823–1831.

[14] Dimitrov B, Gadeva P. [1997] Studies on the insecticide dursban in root meristem cells of Crepis capillaris L., Environ. Exp. Bot.37: 199–209.

[15] Coppage DL, Braidech TE. [1976] River pollution by anticholinesterase agents, *Water Res* 10:19–24.

[16] Zinkl JG, Shea PJ, Nakamoto RJ, Callman J. [1987] Technical and biological considerations for the analysis of brain acetylcholinesterase of rainbow trout. *The American Fisheries Society116*:570–573.

[17] Mulcahy MF. [1975] Fish blood changes associated with disease: a hematological study of pike lymphoma and salmon ulcerative dermal necrosis. In: W.E. Ribelin and C. Migaki Madison, Editors, 1975. The Pathology of Fishes, University of Wisconsin, Wisconsin, USA, pp. 925–944.

[18] Aruna D, Gopal V. [1987] Toxic effects of sublethal level of mercury on hematological parameters, I.J. *Environ. Health* 30:52--56.

[19] Randall DJ, Hoar WS. [1975] General comparative physiology, Eds.2, 875p.

[20] Bennett JK, Dooley RO. [1982] Copper uptake by two sympatric species of killifish F. heteroclitus (L.) and L. majalis (Walkbaum). *J Fish Biol* 21: 381–398.

[21] APHA, AWWA, WPCF. [2005] Standard methods for the examination of water and wastewater. 21st Ed. Washington, DC: A.P.H.A.

[22] Finney DJ. [1971] Probit Analysis. University Press, Cambridge.333 p.

[23] Ellman G, Courtney KD, Andres VJ, Featherstone RM. [1961] A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol.7:88–95.

[24] Lowry OH. [1951] Rosenbrough NJ, Farr AL, Randall RJ, Protein measurement with folin phenol reagent, The J Biol. Chem. 193 :265–275.

[25] Natoff IL. [1971] Organophosphorus pesticides: Pharmacology, *Prog. Med. Chem* 8:1–37.

[26] Korp MR. [1951] Reviews of environmental contamination and toxicology sixth Eds. springer edit. Ware GW 163:57p.

[27] Kabeer AS, Rao KV. [l981] Effect of malathion exposure on some physical parameters of whole body and on tissue cations of teleost. Tilapia mossambica (Peters), *J. Biosci*. 3: 17–28.
[28] Kumar A, Sharma B, Pandey RS. [2007] Preliminary evaluation of the toxicity of cypermethrin and k-cyhalothrin to Channa punctatus. Bull. *Environ. Contam. Toxicol.*79:613–616.
[29] Svoboda M, Luskova V, Drastichova J, Zlabek V. [2001] The effect of diazinon on hematological indices of common carp (Cyprinus carpio L.). Acta Veterianaria Brunensis 70 457–465.
[30] Benarji G, Rajendranath T. [1990] Hematological changes induced by an organophosphorous insecticide in a freshwater fish Clarias batrachus (Linn). *Trop. Freshwater Biol* 21:97–202.
[31] Singh NN, Srivastava AK. [1994] Formothion induced hematological changes in the freshwater Indian catfish, Heteropneustes fossilis. *J Ecotox. Environ. Monit.* 4 137–140.
[32] Khattak IUD, Hafeez MA. [1996] Effect of malathion on blood parameters of the fish, Cyprinion watsoni. Pak. *J Zoology* 28:45–49.
[33] Tavares DM, Martins ML, Nascimento KS. [1999] Evalution of the hematological parameters in *Piaractus mesopotamicus* Holmberg (Osteichthyes, Characidae) with Argulus sp. (Crustacea, Brachiura) infestation and treatment with organophosphate, Revista *Brasileira de Zoologia* 16:553–555.
[34] Morgan DP, Stockdale EM, Roberts RJ, Walter HW, [1980] Anemia associated with exposure to lindane, Arch.Environ. *Health* 35: 307–310.
[35] Murphy SD. [1980] Pesticides In: J. Doull, C.D. Klassen, M.D. Amdur (Eds.) Toxicology: The basic science of poisons, Mac Millan Pub. Co., Inc., NewYork, p357–408.
[36] Jain SK. [1988] Evidence for membrane lipid peroxidation during the in vivo ageing of human erythrocytes, Biochim. Biophys. *Acta* 937:205–210.