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# Investigation on Acute Toxicity and Behavioral Changes in a Freshwater African Catfish, *Clarias gariepinus* (Burchell, 1822), Exposed to Organophosphorous Pesticide, Termifos®

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**Abstract.-** Chlorpyrifos (CPF) O,O-diethyl 0,3,5,6-trichloropyridin-2yl phosphorothioate is commonly used for pest and insect control in agricultural fields and surrounding freshwater reservoirs. A 96 h semi-static acute toxicity bioassay was carried out to determine the $LC_{50}$ value and behavioral responses of commercial formulation of chlorpyrifos (Termifos) on the freshwater fish *Clarias gariepinus*. The $LC_{50}$ values (with 95 % confidence limits) of different concentrations of CPF (Termifos) in *C. gariepinus* were found to be 1.66 (1.40-3.30), 1.30 (1.18-1.53), 1.03 (0.98-1.11) and 0.86 (0.73-0.99) mg/l for 24, 48, 72 and 96 h exposure time, respectively, thus indicating that the pesticide is highly toxic to the fish. The values of safe level of the pesticide in *C. gariepinus* varied from $8.61\times10^{-2}$ – $8.61\times10^{-6}$ mg/l. Fish exposed to various concentrations of the pesticide showed uncoordinated behavior such as erratic and jerky swimming, attempt to jump out of water, frequent surfacing and gulping of air, decrease opercula movement and secretion of mucus on the body and gills followed by exhaustion and death. Our results indicate that commercial formulation of chlorpyrifos (Termifos) is highly toxic to *C. gariepinus*. The pesticide should thus be applied with caution in our environment especially near water bodies to avoid the possible risk associated with its use.

**Key Words:** *Clarias gariepinus*, termifos, toxicity, $LC_{50}$, behavioral changes, safe level

## INTRODUCTION

Chlorpyrifos (CPF; O,O-diethyl 0,3,5,6-trichloropyridin-2yl phosphorothioate) is a broad-spectrum organophosphorous (OP) insecticide widely used in agriculture for control of insect pest on such crops as citrus, alfalfa, wine grapes, nut orchards, pineapple, tomato, maize, tobacco among others. It has also been used as mosquito larvicide in water bodies (WHO, 2004) and as termiticidal barrier in, around or under buildings. CPF is one of the most widely used OP insecticides but its domestic use was recently restricted due to its toxicity. Despite this CPF remains one of the most widely used OP insecticides in the world (Ambali *et al*., 2011) and one of the few OP insecticides which is still allowed in EU (Kralj *et al*., 2007). OP function by binding with acetylcholinesterase (AChE), an enzyme that breaks down the neurotransmitter acetylcholine so that subsequent impulses can be transmitted across the synapse. Inhibiting the AChE thus results in repeated, uncontrolled firing of neurons leading to death usually by asphyxiation as respiratory control is lost (Sparling and Fellers, 2007).

CPF degrades primarily in the soil through microbial action (WHO, 2012) and can persist for about 60-120 days. CPF is highly toxic to aquatic organisms, mobile in the environment and is among the most detected pesticides in streams, rivers, ponds and reservoirs (Phillips *et al*., 2007; Ensminger *et al*., 2011). CPF concentration up to 0.4µg/l has been detected in drinking water (USEPA, 1998). Due to repeated application for the control of insects, large quantities of the insecticides find their ways into water bodies. The indiscriminate use of this pesticide, careless handling, accidental spillage or discharge of untreated effluents into natural water ways have harmful effects on fish population and other aquatic organisms and may contribute to long term effects in the environment.

Some studies have demonstrated that CPF is toxic to fish and can give rise to morphofunctional changes in these animals (Carr *et al*., 1997; Ramesh

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and Saravanna, 2008). Several authors have also shown that CPF and its formulations can be genotoxic to fish (Ali *et al*., 2008; Yin *et al*., 2009; Yong *et al*., 2011; Sandal and Yilmaz, 2011). Xu *et al*. (2011) reported on the adverse effects of CPF on reproduction, nerve and immune systems in fish. Bernabo *et al*. (2011) demonstrated that CPF affect the survival, growth and gill apparatus of *Rana dalmatina* larva. Ambali *et al*. (2010) showed that exposure of CPF to wistar rats increased the erythrocytes fragility and lipid peroxidation while the effects were reversed with the administration of Vitamin E. Other recent laboratory studies have also shown that CPF could lead to immunocyte reduction, cardiac disorder, growth, reproduction and developmental impairment (Eddins *et al*., 2010; Shittu *et al*. 2012).There is paucity of scientific documentation on CPF toxic effects on most indigenous fish species of Africa. *C. gariepinus* was selected for the study because it is indigenous to Africa and can be found in other tropical countries of the world. It is of commercial importance and an aquaculture candidate that can narrow the gap between demand and supply of animal protein in developing countries. The species is also an attractive model for toxicity because of its availability throughout the season, wide distribution in the environment and easy acclimatization to laboratory conditions. Therefore, the present investigation was designed to determine the acute toxicity of commercial formulation of chlorpyrifos (Termifos) and its effects on the behavior of juveniles of *C. gariepinus* under laboratory exposure.

## MATERIALS AND METHODS

*Experimental fish specimen and chemicals*

The juveniles of freshwater African catfish *Clarias gariepinus* (Burchell 1822) (Siluriformes: Clariidae) were caught from nearby ponds and lakes with the help of local fishermen. The fish specimens had an average (±S.D) wet weight and length of 19.5±1.56 g and 22.7±1.92 cm, respectively. Specimens were subjected to a prophylactic treatment by bathing twice in 0.05% potassium permanganate ($KMnO_4$) for two min to avoid any dermal infections. The specimens were then acclimatized for three weeks under laboratory conditions in semi-static systems. They were fed boiled eggs, minced goat liver and poultry waste materials during acclimatization. The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. For the present study, commercial formulation of Chlorpyrifos (40% EC) with trade name 'Termifos®' manufactured by Anderelm limited, United Kingdom was purchased from the local market and used.

*Acute toxicity bioassay*

Acute toxicity assay to determine the 96 h $LC_{50}$ values of CPF was conducted with definitive test in a semi-static system in the laboratory as per the standard methods (APHA, AWWA, WPCE, 2005). The range finding test was carried out prior to determine the concentrations of the test solution for definitive test. The experiment was conducted in glass aquaria (60cm 30cm 30cm size) containing 40L of de-chlorinated and aerated water. The test solution was changed on every alternate day to counter-balance the decreasing pesticide concentrations. In definitive test, a set of 10 fish specimen were randomly exposed to CPF (0.56, 0.68. 0.80, 0.92, 1.04, 1.16 and 1.28 mg/l) concentrations. Another set of 10 fish were simultaneously maintained in tap water, without test chemical, and considered as control. The experiment was set in triplicate to obtain $LC_{50}$ values of the test chemical under the photoperiod of 12 hour light and 12 hour dark. The $LC_{50}$ values (with 95 % confidence limits) of different concentrations of CPF (Termifos) in *C. gariepinus* were found to 1.66 (1.40-3.30), 1.30 (1.18-1.53), 1.03 (0.98-1.11) and 0.86 (0.73-0.99) mg/l, respectively for 24, 48, 72 and 96 h exposure time. The safe level of the test pesticides was estimated by multiplying the 96 h $LC_{50}$ with different application factors (AF) as suggested by Hart *et al*. (1948), Sprague (1971), Committee on Water Quality Criteria (CWQC, 1972), National academy of Sciences/ National Academy of Engineering (NAS/NAE, 1973), Canadian Council of Resources and Environmental Ministry (CCREM, 1991) and the international Joint Commission (IJC, 1977). The physicochemical properties of test water, namely temperature, total alkalinity, pH, dissolved oxygen, conductivity and total hardness were analyzed using standard methods (APHA, AWWA, WPCE, 2005).

*Statistical analysis*

The data obtained were statistically analyzed by statistical package SPSS 16.0 computer program (SPSS Inc. Chicago, Illinois, USA). The data were subjected to one way analysis of variance (ANOVA) and Duncan's multiple range tests to determine the significance difference at 5% probability level. Results were expressed as means ±standard error.

## RESULTS

*Physico-chemical parameters of the test water*

The physico-chemical parameters of the test water showed that the pH ranged from 7.0 to 7.10 (mean 7.03). The water temperature ranged from 22.80 to 26.10°C (mean 23.55) whereas the dissolved oxygen varied from 6.07 to 6.90 mg/l (mean 6.11). The conductivity value ranged from 250 to 290 µM/cm (mean 251 µM/cm) while total hardness and alkalinity varied from 164 to 182 mg/l (mean 172 mg/l) and 136.50 to 180.50 mg/l as $CaCO_3$, respectively, during the experimental period.

**Table I.- Lethal concentrations of Chlorpyrifos with 95% confidence intervals.**

| LC (values) | Concentrations (mg/l) | 95 % confidence limit | |
|---|---|---|---|
| | | Lower | Upper |
| $LC_{10}$ | 0.616 | 0.389 | 0.725 |
| $LC_{20}$ | 0.691 | 0.492 | 0.792 |
| $LC_{30}$ | 0.75 | 0.579 | 0.85 |
| $LC_{40}$ | 0.805 | 0.659 | 0.912 |
| $LC_{50}$ | 0.861 | 0.734 | 0.987 |
| $LC_{60}$ | 0.919 | 0.805 | 1.085 |
| $LC_{70}$ | 0.987 | 0.872 | 1.222 |
| $LC_{80}$ | 1.072 | 0.943 | 1.427 |
| $LC_{90}$ | 1.203 | 1.036 | 1.798 |

*$LC_{50}$, safe levels and behavioral characteristics*

The percentage mortality of African catfish *C. gariepinus* exposed to CPF-termifos concentrations of 0.56, 0.68, 0.80, 0.92, 1.04, 1.16 and 1.28 mg/l at 24, 48, 72 and 96 h duration was shown in Figure 1. A time and dose-dependent increase in mortality rate was observed; thus, as the exposure time increased from 24 to 96 h, the median lethal concentration required to kill the fish was reduced. There were significant differences ($p < 0.05$) in $LC_{10-90}$ values obtained for different times of exposure. During the experimental period no mortality was recorded in the control group.

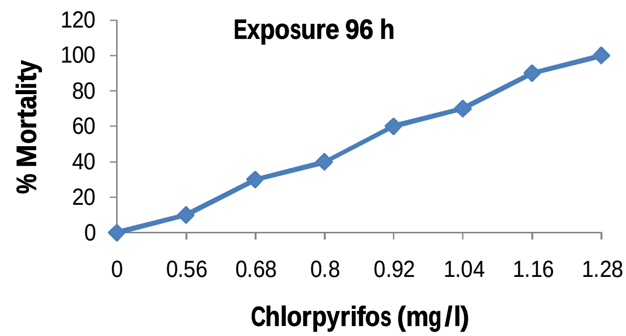


Fig. 1. Mortality of *Clarias gariepinus* during 96 h due to chlorpyrifos (Termifos) exposure.

The highest concentration of 1.28 mg/l showed the highest fish mortality. The 24, 48, 72 and 96 h $LC_{50}$ values (95% confidence limits) of termifos for juveniles African catfish were determined as 1.662, 1.295, 1.034 and 0.861 mg/l, respectively (Table I). The values of safe level of the pesticide in *C. gariepinus* varied from $8.61\times10^{-2}$ – $8.61\times10^{-6}$ mg/l. The least safe level that ranged from $8.61\times10^{-2}$ – $8.61\times10^{-6}$ mg/l[1] was obtained for the pesticide in *C. gariepinus* following the method of NAS/NAE (1973) and using the application factor of $1.0\times10^{-1}$,$1.0\times10^{-5}$. Similar safe levels of $8.61\times10^{-2}$ mg/l and $8.61\times10^{-3}$ mg/l were obtained using the methods of Sprague (1971) and CWQC (1972), respectively. A safe level of $2.36\times10^{-2}$ mg/l was calculated for the pesticide in *C. gariepinus* using the method of Hart *et al* (1948) while the value of $4.305\times10^{-2}$ mg/l was calculated using the methods of CCREM (1971) and IJC (1977). The behavioral responses of the test fish were observed at 24-96 h of exposure (Table II). Normal behavioral was observed in the control fish. Fish exposed to 0.56 and 0.68 mg/l showed normal behavior for the first 48 h but afterwards fish that were alert stopped swimming and remained static in position in response to the sudden changes in the

**Table II.- Effects of Chlorpyrifos on the behavioral characteristics of *Clarias gariepinus* at different duration**

| Concen. (mg/l). | Hyper-activity | Equilibrium status | Swimming rate | Fin move-ment | Jerky move-ment |
|---|---|---|---|---|---|
| **24 h** | | | | | |
| Control | - | +++ | +++ | +++ | - |
| 0.56 | - | +++ | +++ | +++ | - |
| 0.68 | - | +++ | +++ | +++ | - |
| 0.80 | - | +++ | ++ | +++ | + |
| 0.92 | + | ++ | ++ | ++ | + |
| 1.04 | + | + | ++ | + | ++ |
| 1.16 | + | + | + | + | +++ |
| 1.28 | + | + | + | + | +++ |
| **48 h** | | | | | |
| Control | - | +++ | +++ | +++ | - |
| 0.56 | - | +++ | +++ | +++ | - |
| 0.68 | - | +++ | +++ | +++ | - |
| 0.80 | + | ++ | ++ | ++ | - |
| 0.92 | + | ++ | ++ | ++ | + |
| 1.04 | + | + | ++ | ++ | ++ |
| 1.16 | + | + | + | + | +++ |
| 1.28 | + | + | + | + | +++ |
| **72 h** | | | | | |
| Control | - | +++ | +++ | +++ | - |
| 0.56 | - | +++ | +++ | +++ | ++ |
| 0.68 | - | +++ | +++ | ++ | ++ |
| 0.80 | + | ++ | ++ | ++ | +++ |
| 0.92 | + | ++ | ++ | + | +++ |
| 1.04 | + | + | + | + | +++ |
| 1.16 | ++ | + | + | + | +++ |
| 1.28 | ++ | - | - | - | - |
| **96 h** | | | | | |
| Control | - | +++ | +++ | +++ | - |
| 0.56 | - | +++ | +++ | +++ | ++ |
| 0.68 | - | ++ | ++ | ++ | ++ |
| 0.80 | + | ++ | ++ | ++ | +++ |
| 0.92 | + | + | + | + | +++ |
| 1.04 | ++ | + | + | + | +++ |
| 1.16 | ++ | - | - | - | - |
| 1.28 | ++ | - | - | - | - |

None-, mild+, moderate ++, strong +++

surrounding environment. Generally, fish exposed to higher concentrations of the pesticide showed abnormal behavior and tried to avoid the test water by swimming very fast, jumping and displaying erratic with vigorous jerky movements, faster opercula movement, hyperexcitation , surfacing and gulping of air.

## DISCUSSION

Natural pollution of the environment can pose serious hazard to aquatic organisms and to plants which constitute the primary producers in the ecosystem. Asides natural pollution, man influence on the environment poses more serious damage than man has intended. Environmental pollution resulting from industrial effluents and agricultural activities has become a global issue because of the extent of damage caused to the aquatic ecosystems and the disruption in the natural food chain. There are many research works on the extent of damage posed by several agricultural activities such as insecticidal and herbicidal application, industrial activities such as oil spillage etc.

The present study investigates the acute toxicity of commercial formulations of chlorpyrifos (termifos) insecticide on the African catfish *Clarias gariepinus*. The result of 96h $LC_{50}$ value of termifos was found to be 0.861 mg/l, which indicated termifos to be very toxic. The $LC_{50}$ value obtained is lower than 0.92 mg/l and 1.57 mg/l earlier reported by Ogueji *et al*. (2007) and Gül (2005) when chlorpyrifos-ethyl and chlorpyrifos-methyl were exposed to *Clarias gariepinus* and *Oreochromis niloticus* larvae respectively. The $LC_{50}$ obtained in our present study for commercial formulations of chlorpyrifos (Termifos) is also lower than the 3.00 mg/l and 5.17 mg/l obtained by Sparling and Fellers (2007) and Bernabo *et al*. (2011) when *Rana boylii* and *Rana dalmatina* tadpoles were exposed to Chlorpyrifos respectively. In the present study $LC_{50}$ value however is higher than the 0.811mgl$^{-1}$ reported by Ali *et al*. (2008) when freshwater fish *Channa punctatus* were exposed to Chlorpyrifos. Our $LC_{50}$ value is also higher than 0.004mg/l obtained for rainbow trout (*O. mykiss*) (Kikuchi *et al*., (1996), 0.396mg/l for rainbow fish (*M. spendida*) (Humphrey and Klumpp, 2003) and 0.297 mg/l for Chinook salmon (*O. tshawytscha*) (Rao *et al*., 2005). Our results is also higher than 0.098 mg/l obtained for lake trout (*S. namaycush*) (USEPA (1986), 0.0071 mg/l for guppy (*P. reticulata*) (De Silva and Samayawardhena, 2002) and 0.0053 mg/l for *Cyprinus carpio* (Ramesh and Saravanan, 2008). Toxicity of pesticides has been reported to vary depending on species, developmental stages (Bridges and Semlitsch, 2000) and testing protocols (Jones *et al*., 2009). Toxicity of chemicals to aquatic organisms has also been reported to be affected by dissolved oxygen, size, age, water quality and formulations of chemicals (Pandey *et al*., 2012). The safe level of chlorpyrifos formulation (Termifos) in the present study varied from $8.61\times10^{-2}$ to $8.61\times10^{-6}$ mg/l. However, the large variations in the safe levels due to the dependence on application factors for the calculation have resulted in controversy over the acceptability (Buikema *et al*., 1982). Pandey *et al*. (2005) noted that extrapolation of laboratory data to field is not always meaningful value hence it is difficult to decide on acceptable concentration that may be considered "safe" based on laboratory experiments.

There were behavioral changes in the activities of *Clarias garienpinus* treated with different acute concentrations of termifos compared to the control. Among these changes were hyperactivity, decreased equilibrium status, increased erratic swimming, decreased fin movement and an increased jerky movement. The behavioral study gives direct response of the fish to the pesticide and related chemicals. According to Radhaiah *et al*. (1987), the behavioral activity of organisms represents the final integrated result of a diversified biochemical and physiological processes. The observed behavioral alterations in the studied formulations of Chlorpyrifos (Termifos) are consistent with previous reports on chlorpyrifos (Gül, 2005; Ogueji *et al*., 2007; Ramesh and Saravanan, 2008; Ali *et al*., 2008; Sharbidre *et al*., 2011) and in other pesticides such as cypermethrin (Ansari *et al*., 2011) profenofos (Pandey *et al*., 2011), atrazine (Nwani *et al*., 2011), diazinon (Ahmad, 2011), endosulfan (Yekeen and Falowe, 2011; Shao *et al*., 2012), carbosulfan (Altinok *et al*., 2012) and malathion (Ahmad *et al*., 2012). The observed behavioral changes may be attributed to the neurotoxic effect of CPF (Chlorpyrifos) by inhibition of AChE. The inhibition interferes with normal neurotransmission in cholinergic synapses and neuromuscular junctions of the nervous system thus affecting normal functioning of the nerves (Miron *et al*., 2005).

## CONCLUSIONS

From the current study it is concluded that the commercial formulation of chlorpyrifos (Termifos) is very toxic to fish and possible related organisms of the aquatic ecosystems. Termifos has the potential to impair physiological and biochemical activities of the organism leading to observed changes in behavioral pattern, and consequent dose dependent mortality. The use of termifos at the riverside and coastal areas should be strongly monitored and regulated to avoid chlorpyrifos related hazards in aquatic organisms.

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## REFERENCES

AHMAD, Z., 2011. Acute and hematological changes in common carp (*Cyprinus carpio*) caused by diazinon exposure. *Afri. J. Biotech*., **10**: 13852-13859.

AHMAD, Z., 2012. Toxicity bioaasay and effects of sublethal exposure of malathion on biochemical composition and hematological parameters of *Clarias gariepinus*. *Afr. J. Biotech*., 11: 8578-8585.

ALI, D., NAGPURE, N.S., KUMAR, S., KUMAR, R. AND KUSHWAHA, B., 2008. Genotoxicity assessment of acute exposure of chlorpyriphos to fresh water fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. *Chemosphere*, **71**: 1823–1831.

ALTINOK, I., CAPKIN, E. AND BORAN, H., 2012. Mutagenic, genotoxic and inhibitory effects of carbosulfan in rainbow trout *Oncorhynchus mykiss*. *Pestic. Biochem. Physiol*., **102:** 61-67.

AMBALI, S.F., AYO, J.O., ESIEVO, K.A.N. AND OJO, S.A., 2011. Hematoxicity induced by chronic chlorpyrifos exposure in wistar rats: mitigating effect of Vitamin C. *Vet. Med. Int*., DOI: 10.4061/ 2011/945439.

AMBALI, S.F., AYO, J.O., OJO, S.A. AND ESIEVO, K.A.N., 2010. Vitamin E protects wistar rats from chlorpyrifos-induced increase in erythrocyte osmotic fragility. *Fd. Chem. Toxicol.*, **48**: 3477-3480.

ANSARI, R.A., RAHMAN, A., KAUR, M., ANJUM, S. AND RAISUDDIN, S., 2011. *In vivo* cytogenetic and oxidative stress-inducing effects of cypermethrin in freshwater fish, *Channa punctatus*. *Ecotoxicol. environ. Saf.*, **74**: 150-156.

APHA, AWWA, WPCF. 2005. *Standard methods for the examination of water and waste water*, 21st ed. American Public Health Association, Washington, DC.

BERNABO, I., SPERONE, E. AND TRIPEPI, S., 2011. Toxicity of chlorpyrifos to larval *Rana dalmatina*:

Acute and chronic effects on survival, development, growth and gill apparatus. *Arch. environ. Contam.Toxicol*., **61**:704-718.

BRIDGES, C.M. AND SEMLITSCH, R.D., 2000. Variation in pesticide tolerance of tad- poles among and within species of Ranidae and patterns amphibian decline. *Conserv. Biol.,* **14**: 490–1499.

BUIKEMA, J.R., NAIDER-LEHNER, A.L. AND CAIRNS, J.R., 1982. Biological monitoring: Part IV. Toxicology testing. *Environ. Mol. Mutagen*., **33**: 239-262

CARR, R.L., HO, L.L. AND CHAMBERS, J.E., 1997. Selective toxicity of several species of fish during an environmental exposure: Biochemical mechanisms. *Environ. Toxicol. Chem.,* **16**: 2369-2374.

CCREM, 1991. *Canadian water quality guidelines;* Canadian Council of Resources and Environmental Ministry, Inland Waters Directorate, Environment Canada: Ottawa, ON, Canada.

CWQC (COMMITTEE ON WATER QUALITY CRITERIA), 1972. *.A report of the committee on water quality research series,* EPA-R3–73–003, US Environmental Protection Agency Report; CWQC: Cincinnati, OH, USA.

De SILVA, P. M.C.S. AND SAMAYAWARDHENA, L.A., 2002. Low concentrations of lorsban in water result in far reaching behavioral and histological effects in early life stages in guppy. *Ecotoxicol. environ. Saf*., **53**: 248–254.

EDDINS, D., CERUTTI, D., WILLIAMS, P., LINNEY, E. AND LEVIN, E.D., 2010. Zebra-fish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: comparism with nicotine and pilocarpine effects and relation to dopamine deficits. *Neurotox. Teratol*., **32**: 99-108.

ENSMINGER, M., BERGIN, R., SPURLOCK, F. AND GHO, K.S., 2011. Pesticide concentrations in water and sediment and associated invertebrate toxicity in Del Puerto and Orestimba Creeks, California, 2007-2008. *Environ. Monit. Assess.,* **175**: 573-587.

GÜL, A., 2005. Investigation of acute toxicity of chlorpyrifos-methyl on Nile tilapia (*Oreochromis niloticus* L) larvae. *Chemosphere*, **59**: 163-166.

HART, W.B., WESTON, R.F. AND DERMANN, J.G., 1948. An apparatus for oxygenating test solution which fish are used as test animals for evaluating toxicity. *Trans. Am. Fish. Soc*., **75**: 288.

HICKMAN, P. C., LARRY, S. B., SUSAN, S. K., ALLAN, L., HELLEN, I.A. AND DAVID, J. E., 2008. *Integrated priciples of zoology.* McGraw-Hill, New York.

HUMPHREY, C. AND KLUMPP, D.W., 2003. Toxicity of chloropyrifos to early life history stages of eastern rainbowfish Melanotaenia spelndida splendida (Peters 1866) in tropical Australia. *Environ. Toxicol.,* **18**: 418–427.

IJC, 1977. *New and revised great lakes water quality objectives.* Great Lake Basin, IJC: Windsor; Ottawa, ON, Canada.

JONES, D.K., HAMMOND, J.I. AND RELYEA, R.A., 2009. Very highly toxic effects of endosulfan across nine species of tadpole: lag effects and family level selectivity. *Environ. Toxicol. Chem*., **28**:1939–1945

KIKUCHI, M., MIYAGAKI, T. AND WAKABAYASHI, M., 1996. Evaluation of pesticides used in golf links by acute toxicity test on rainbow trout. *Nippon Suisan Gak Bull. Jap. Soc. Sci. Fish,* **62**: 414–419.

KRALJ, M.B., FRANKO, M. AND TREBSE, P., 2007. Applications of bioanalytical techniques in evaluating advanced oxidation processes in pesticide degradation. *Trends Anal. Chem.,* **26**: 1020-1031.

MIRON, D., CRESTANI, M., SCHETINGER, M.R., MORSCH, V.M., BALDISSEROTO, B., TIERNO, M.A., MORALES, G. AND VIEIRA, V.I.P., 2005. Effects of the herbicides clomazone, quinclorac and metsulfuron methyl on acetycholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae). *Ecotoxicol. Environ. Saf .,* **61**: 398-403.

NAS/NAE, 1973. *Water quality criteria*, EPA-R3–033; US Government Printing Office: Washington, DC, USA.

NWANI, C.D., NAGPURE, N.S., KUMAR, RAVINDRA., KUSHWAHA, B., KUMAR, P. AND LAKRA, W.S., 2011. Mutagenic and genotoxic assessment of atrazine-based herbicide to freshwater fish *Channa punctatus* (Bloch) using micronucleus test and single cell gel electrophoresis. *Environ. Toxicol. Pharmacol.,* **31**: 314-322.

OGUEJI, E.O., AUTA, J. AND BALOGUN, J.K., 2007. Effects of acute nominal doses of chlorpyrifos-ethyl on some hematological indices of African catfish *Clarias gariepinus*-Teugels. *.J. Fish. Int*., **2**: 190-194.

OGUEJI, E.O., AUTA, J., BALOGUN, J.K. AND IBRAHIM, N.D.G., 2008. The histopathological effects of sublethal doses of chlorpyrifos-ethyl on the liver and gills of African catfish, *Clarias gariepinus*.*ChemClass J*., **4**: 49-59.

PANDEY, A.K., NAGPURE, N.S., TRIVEDI, S.P., KUMAR, R., KUSHWAHA, B. AND LAKRA, W.S., 2011. Investigation on acute toxicity and behavioral changes in *Channa punctatus* (Bloch) due to organophosphate pesticide profenofos. *Drug Chem. Toxicol*., **34**: 424-428.

PANDEY, S., KUMAR, R., SHARMA S., NAGPURE, N.S., SRIVASTAVA, S.K. AND VERMA, M.S., 2005. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctata* (Bloch). *Ecotoxicol. Environ. Saf.,* **61**:114-1220.

PHILLIPS, P., SCOH, W.A. AND NYSTROM, E.A., 2007. Temporal changes in surface-water insecticide concentrations after phase out of diazinon and chlorpyrifos. *Environ. Sci. Technol.,* **41**: 4246-4251.

RADHAIAH, V., GIRIJA, M. AND RAO, K. J., 1987. Changes in selected biochemical parameters in the kidney and blood of the fish, *Tilapia mossambica* (peters), exposed to heptachlor. *Bull. environ. Contam. Toxicol.*, **39**: 1006-1011.

RAMESH, M. AND SARAVANAN, M., 2008. Hematological and biochemical responses in freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. *Int. J. Integr. Biol.*, **3**: 80-83.

RAO, J. V., BEGUM, G., PALLELA, R., USMAN, P.K. AND RAO, R.N., 2005. Changes in behavior and brain acetylcholinesterase activity in mosquito fish, Gambusia affinis in response to the sub-lethal exposure to chlorpyrifos. *Int. J. environ. Res. Publ. Hlth*, 2: 478–483.

SANDAL, S. AND YILMAZ, B., 2011. Genotoxic effects of chlorpyrifos, cypemethrin, endosulfan and 2,4-D on human peripheral lymphocytes cultured from smokers and non-smokers. *Environ. Toxicol.* **26**: 433-442.

SHAO, B., ZHU, L., DONG, M., WANG, JUN., WANG, J., XIE, H., ZHANG, Q., DU, Z. AND ZHU, S., 2012. DNA damage and oxidative stress induced by endosulfan exposure in Zebra fish *Danio rerio. Ecotoxicology,* **21**: 1533-1540.

SHARBIDRE, A.A., METKARI, V. AND PATODE, P., 2011. Effects of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, *Poecilia yeticulate*. *Pest. Biochem. Physiol.*, **101**: 132-141.

SHITTU, M., AYO, J.O., AMBALI, S.F., KAWU, M.U. AND SALAMI, S.O., 2012. Vitamin E mitigates chronic chlorpyrifos- induced oxidative changes in pituitary glands and testes in wistar rats. *Am. J. appl. Sci*., **9**: 75-82.

SPARLING, D.W. AND FELLERS, G., 2007. Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylii*. *Environ. Pollut.,* **147**: 535-539.

SPRAGUE, J.B., 1971. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water Res.*, **3**: 793–821.

USEPA, 1998. *Chlorpyrifos: Fate and risk assessment*. United States Environmental Protection Agency.

WHO, 2004. *Guidelines for drinking water quality* WHO/SDE/03.04/81.

WHO, 2012. *Chlorpyrifos in drinking-water*. *Background document for development of WHO guidelines for drinking-water quality*. WHO/SDE/WSH/03.04/87.

XING, H., WANG J., LI J., FAN Z., WANG, M AND XU, S., 2010. Effects of atrazine on acetycholinesterase and carboxyesterase in brain and muscle of common carp (*Cyprinus carpio*). *Environ. Toxicol. Pharmacol.,* **30**: 26-30.

XU, W., LI, J., XING, H. AND XU, S., 2011. Review of toxicology of atrazine and chlorpyrifos on fish. *J. Northeast Agric. Univ. China*, **18**: 88-92.

YEKEEN, T.A. AND FAWOLE, O.O., 2011. Toxic effects of endosulfan on hematological and biochemical indices of *Clarias gariepinus*. *Afr. J. Biotech.*, **10**: 14090-14096.

YIN, X., XHU., G., LI, X.B. AND LIU, S., 2009. Genotoxicity evaluation of chlorpyrifos to amphibian Chinese toad (Amphibian: Anura) by comet assay and micronucleus test. *Mutat. Res.,* **680**: 2-6.

YONG, C., GUO, J., XU, B. AND CHEN, Z., 2011. Genotoxicity of chlorpyrifos and cypermethrin to ICR mouse hepatocyte. *Toxic. Mech. Meth.,* **21**: 70-74.

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