



Effect of Short Term Exposure to Cyperdicot on Behavioural and Haematological Responses in African Catfish *Clarias Gariepinus*

GE Odo^{1,*}, JE Agwu¹, N Ivoke¹, VC Ejere¹, Atama C.I¹, CO Ezea², Aguoru GC¹, Anya BC³

¹ Department of Zoology and Environmental Biology, University of Nigeria.

² Federal University of Technology, Owerri.

³ Gregory University Uturu, Okigwe, Abia State.

* Corresponding Author: Tel.: 07031705640;
E-mail: gregory.odo@unn.edu.ng

Received 29 June 2016
Accepted 26 July 2016

Abstract

The effects of short term exposure to cyperdicot on behavioural and haematological responses in 300 *Clarias gariepinus* were investigated. The fish were randomly divided into three groups of 30 fish. Fish in first treatment group were exposed to tap water and served as control, while those in second and third groups were treated with 0.04 and 0.08 mg l⁻¹ of Cyperdicot, respectively. The 24, 48, 72 and 96 h LC₅₀ values were 1.462, 1.094, 1.030 and 0.800 mg l⁻¹, respectively. The safe level for the insecticide varied from 8 x 10⁻³ to 8 x 10⁻⁴ mg l⁻¹. Fish exposed to sub-lethal concentrations of insecticide exhibited alterations in various blood parameters including significant reductions in RBC count, Hb and PCV. A cyperdicot-induced dose- and time-dependent significant increase in WBC count from day 10 onward was observed, while values of blood indices such as MCV, MCH and MCHC in treated fish were not significantly different from those of the control group (P>0.05). This study revealed that the short term exposure to cyperdicot on behavioural and haematological responses in *Clarias gariepinus* elicited reduction of RBC, Hb and PCV values while MCV and MCH caused both macrocytic and microcytic anemia in the fish.

Keywords: Cyperdicot, *Clarias gariepinus*, haematology, behaviour parameters.

Introduction

Cyperdicot, a commercially formulated agrochemical insecticide, is known for its action against a wide range of insects. It is a synthetic pesticide composed of cypermethrin (50 mg l⁻¹) and dimethoate (250 mg l⁻¹) a synthetic pyrethroid and organophosphate derivatives (Agwu *et al.*, 2016). It is a contact insecticide which kills target organisms by altering normal neurotransmission within the nervous system of the organisms by inhibiting the enzyme acetyl cholinesterase (ACHE), which hydrolyses the neurotransmitter acetylcholine (ACH) in cholinergic synapses and neuromuscular junctions. Non-target organisms can be exposed to Cyperdicot by inhalation, ingestion and/or dermal exposure (Glen *et al.*, 2014). It enters the aquatic environment because of its proximity to the agricultural activities along water bodies and has been detected in many rivers in both urban and agricultural regions (Ayoola and Ajani, 2007). Furthermore, the indiscriminate or misuse of the insecticide or discharge of untreated effluents into natural water ways, have harmful effects on the fish populations and other aquatic organisms and may contribute to long term ecotoxicological effects in

resident aquatic organisms (Leilani *et al.* 2015).

In the water, the molecules of these contaminants may bind to the materials in suspension, accumulate in the sediment or can be absorbed by the aquatic organisms with attendant physiological responses including effect on behavior and haematology (Jordan *et al.*, 2013). As result of high water solubility, low persistence and extensive usage of the insecticide in the environment, exposure to non-target aquatic organisms is a source of concern. Changes in enzyme activity and other biomarkers have been studied as possible tools for aquatic toxicological research (Moore and Simpson, 1992; Abuo *et al.*, 2001).

Sub-lethal effects are biochemical in origin, exerting their effects at basic levels of the organisms by reacting with enzymes or metabolites and other functional components of the cell. Transaminase enzymes play vital roles in carbohydrate-protein metabolism in fish and other organism's tissues (Eze, 1983).

The indigenous African catfish, *C. gariepinus* was selected for the bioassay experiments because it can be found in other tropical countries of the world. It is also an aquaculture candidate that can

narrow the gap between the demand for and supply of animal protein in developing countries. The species is also an attractive model for toxicity studies because of its availability throughout the year, voracious feeding habit, prolific reproduction and general hardness in culture environments. The adverse effects of agrochemicals and their residues on non-target organisms have not been seriously considered in Nigeria (Ayoola and Ajani, 2007). Despite a number of researches carried out on the toxicity effects (Fazio et al., 2014, Naccariet al., 2015; Di Bella et al., 2015) of agrochemical insecticide on the haematology of *Clariasgariiepinus*, little is known about the lethal toxicity and haematological changes that *Clariasgariiepinus* may undergo on exposure to Cyperdicot. The purpose of this study was to investigate the effects of short term exposure to cyperdicot on behavioural and haematological responses in African catfish, *Clariasgariiepinus*.

Materials and Methods

Experimental Fish

Three hundred *C.gariiepinus* juveniles, mean weight 150 ± 5.20 g, length= 35.00 ± 2.50 cm, from Sacen Fish Farm, were treated with 0.05% potassium permanganate to avoid possible dermal infections. They were acclimatised for 20 days in a 1000 l plastic tank, fed 3% body weight (BW) in divided rations twice daily (7.00 am and 7.00 pm) with a laboratory-prepared pelleted diet containing 35% crude protein (Eyoet al., 2013). Feeding was terminated 24 h prior to the range -finding and toxicity test, to reduce ammonia content in the water (Ward and Parrish 1982, Reishand and Oshida, 1987). The ethical guidelines of the Animal Care Committee (UNN-EGACC, protocol no. 0430/2013) of the University of Nigeria, Nsukka were strictly followed.

The pH of water and sediment samples was measured in the laboratory using the Hanna pH meter (Hi-1922 model) according to APHA (1992).

The conductivity of water was determined using the Hanna 911 conductivity meter which was standardized with 0.01N potassium chloride (KCl) solution (APHA, 1992). The readings were taken from the display on the meter and values were recorded in micro Siemens per centimeter ($\mu\text{S}/\text{cm}$) (APHA, 1992).

Alkalinity Mg calcium carbonate

$$(\text{CaCO}_3)/\text{L} = \frac{\text{A} \times \text{N} \times 5000}{\text{Volume of Sample}}$$

Where:

A = Volume of acid used.

N = Normality of standard acid used

Hardness, mg equivalent $\text{CaCO}_3/\text{L} = 2.497 [\text{Ca}, \text{mg}/\text{L}] + 4.118 [\text{Mg}, \text{mg}/\text{L}]$

Pesticide

Cyperdicot is composed of cypermethrin and dimethoate. Cypermethrin is an insecticide in the synthetic pyrethroid family, first marketed in 1977. The primary manufacturers in the U.S. are Zeneca Inc., FMC Corp., and American Cyanamid Co. Common brand names are Demon, Cymbush, Ammo and Cynoff. Dimethoate first marketed in 2001 by FAO, is an organ phosphorus and systemic pesticide with stomach and cholinesterase inhibition actions.

The trade names are Danadim, Rogo, and Roxion. The primary manufacturers in Denmark and Italy are Cheminouta.

Determination of LC₅₀ Concentration

A toxicity assay to determine the 96 h LC₅₀ values of Cyperdicot was conducted with a definitive test in a semi-static system in the laboratory following standard methods (APHA, 2005). A range-finding tests (5, 4, 3.5, 3 and 2.5mg L⁻¹) was carried out to determine the concentrations of the test solution for the definitive test. The experiment was conducted in 60 x 30 x 30 cm glass aquaria containing 40 L of de-chlorinated aerated water. The test solution was changed on every alternate day to counter-balance the decreasing pesticide concentrations. To prevent oxygen depletion, experimental tanks were continuously oxygenated using an air pump. Dead fish were immediately removed to avoid possible deterioration of the water quality. Behavioural changes in fin and opercular movements, equilibrium status, swimming rate, air gulping and skin coloration during the test period were observed.

In the definitive test a set of 10 fish specimens was randomly exposed to Cyperdicot at 5, 4, 3.5, 3 and 2.5 mg L⁻¹ concentrations. Another set of 10 fish specimens was simultaneously maintained in tap water, without test chemical, and considered as control. The experiment was set in triplicate to obtain LC₅₀ values of the test chemical under a photoperiod of 12 hour light and 12 hour dark. The LC₅₀ values (95 % confidence limits) of different concentrations of Cyperdicot in *C. gariepinus* were found to be 1.462^a (1.290-3.289) 1.094^a (1.180-1.328) 1.030^b (0.875-1.100) 0.800^c (0.734-0.980), respectively for 24, 48, 72 and 96 h exposure time. Probit analysis (Finney, 1971) was used to determine the concentration at which 50% mortality (LC₅₀) occurred using SPSS version 17.0.

The 96 h LC₅₀ was calculated to be 0.08 mg l⁻¹. The safe level of the test pesticide was estimated by multiplying the 96 h LC₅₀ with different application factors (AF) as suggested by the international Joint Commission (IJC, 1977). The mean water quality of the test solution determined in the experimental tanks following the standard method (APHA, 2005) were (Mean \pm SE): dissolved oxygen 7.02 ± 0.46 mg/l, temperature $25.70 \pm 0.86^\circ\text{C}$, pH 7.04 ± 0.34 ,

conductivity 275 ± 2.30 Scm⁻¹ and total hardness 202.5 ± 4.45 mg/l as CaCO₃. The experiment was conducted following the OECD 173 guidelines for semi-static test conditions (OECD, 1992).

Determination of Sub-lethal Concentrations

The 96 h LC₅₀ value of Cyperdicot on *C. gariepinus* was found to be 0.80 mg l⁻¹. Based on this value, two sub-lethal concentrations of 0.04 and 0.08 mg l⁻¹ corresponding to 1/20th and 1/10th of the 96 h LC₅₀ of the pesticide, respectively, were prepared by serial dilution of the stock solution with dechlorinated water and used for the *in vivo* exposure. A total of 90 fish from the acclimatised batch were used during the *in vivo* experiment. The fish were randomly divided into three groups of 30 fish, without regard to sex. Fish in the first treatment group were exposed to tap water and served as control, while those in second and third groups were treated with 0.04 and 0.08 mg L⁻¹ of Cyperdicot, respectively. Each treatment group was further randomised into three replicates of 10 fish per replicate in 40 L (60 x 30 x 30 cm) glass aquaria. The exposure lasted for a period of 15 days during which the fish were fed daily small quantity of food approximately 1% of total body weight about an hour before the test solution was renewed, to avoid catabolism and subsequent mortality.

Estimation of Haematological Parameters

The total red cell count /cu.mm of blood (RBC) and the total leukocyte count (WBC) were determined using a Neubauer-type hemocytometer with Toisson's solution as the diluting fluid for RBC, and Turk's solution for WBC (Rusia and Sood, 1992). The haemoglobin level of blood was estimated following the cyanmethemoglobin method (Blaxhall and Daisley, 1973) with some modifications. Each 0.02 ml blood sample was mixed with 4 ml Drabkin's solution and allowed to stand for 10 minutes for proper color development, after which absorbance was read at 540 nm in a Unicam spectrophotometer against a blank. Hematocrit (PCV) was analysed by centrifugation of the blood for five minutes at 14,000 × g in heparinised glass capillaries using a microhaematocrit centrifuge (Hawkesley & sons, Lancing, UK) at room temperature (Nelson and Morris, 1989). The haematocrit was read after centrifugation using the microhaematocrit reader and

the result expressed as the percentage of the whole blood. Haematological indices such as MCHC, MCH and MCV were calculated according to the formula proposed by Dacie and Lewis (2001):

$$\text{MCHC (g dl-1)} = \frac{\text{Hb (g dl-1)} \times 100}{\text{PCV (\%)}}$$

$$\text{MCH (pg cell-1)} = \frac{\text{Hb (g dl-1)} \times 10}{\text{RBC count in millions mm}^{-3}}$$

$$\text{MCV (fl cell-1)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC count in millions mm}^{-3}}$$

Statistical Analysis

The data obtained, expressed as means SE, were analysed using the statistical package SPSS 17.0 (SPSS, Chicago). The data were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range tests to determine the significance difference at the 5% probability level. A p-value less than 0.05 were considered statistically significant.

Results

Physico-Chemical Parameters of the Test Water

During the experimental period the test water pH ranged from 6.89 to 7.16, temperature ranged from 25.10 to 27.0 °C, dissolved oxygen varied from 6.61 to 7.82 mg l⁻¹, conductivity ranged from 68.33-71.00 μM cm⁻¹, and total hardness and alkalinity varied from 5.99 to 6.28 mg l⁻¹ and 136.5 to 180.5 mg l⁻¹ as CaCO₃, respectively (Table 1).

Toxicity bioassay, safe level and behavioural characteristics

In the toxicity bioassay, a concentration-dependent increase and time-dependent decrease were observed in the death rate, to the extent that exposure duration time increased from 24 to 96 h, the concentration of Cyperdicot required to kill the fish was reduced. The LC₅₀ values with 95% confidence limits of different concentrations of Cyperdicot in *C. gariepinus* were 1.462^a (1.290-3.289), 1.094^a (1.180-1.328), 1.030^b (0.875-1.100) and 0.800^c (0.734-0.980) mg l⁻¹ for 24, 48, 72 and 96 h exposure times, respectively (Table 2). The estimated safe levels of Cyperdicot in *C. gariepinus* varied from 8 x 10⁻³ to 8 x 10⁻⁴ mg l⁻¹ (Table 3). Behavioural responses of the

Table 1: Physico-chemical parameters of the test water used for lethal concentrations on *C. gariepinus*

Characteristics	Unit	Mean	Range
pH	-	6.98	6.89-7.16
Temperature	°C	26.90	25.10-27.0
Conductivity	μM cm ⁻¹	69.80	68.33-71.00
Dissolved oxygen	mg l ⁻¹	6.85	6.61-7.82
Alkalinity	mg l ⁻¹	24.16	25-27
Total hardness	mg l ⁻¹	6.04	5.99-6.28

Table 2. Lethal concentrations of Cyperdicot (mg l⁻¹) and 95% confidence intervals (in parentheses) for *C. gariepinus* depending on exposure time (n = 10) in three replicates. Each value is the mean ± SE of 10 identical observations. Values in rows with different superscript letters differ significantly (p<0.05)

Lethal Concentration	Exposure time (h)			
	24	48	72	96
LC10	0.897 ^a (0.960-1.231)	0.635 ^a (0.716-0.10)	0.506 ^b (0.615-0.770)	0.510 ^c (0.389-0.720)
LC20	1.065 ^a (1.142-1.650)	0.871 ^a (0.885-1.046)	0.705 ^b (0.730-0.861)	0.690 ^c (0.492-0.790)
LC30	1.202 ^a (1.044-2.127)	1.080 ^a (1.005-1.174)	0.780 ^b (0.820-0.930)	0.750 ^c (0.571-0.850)
LC40	1.430 ^a (1.220-2.660)	1.083 ^a (1.099-1.340)	0.859 ^b (0.900-1.010)	0.805 ^c (0.659-0.910)
LC50	1.462 ^a (1.290-3.289)	1.094 ^a (1.180-1.328)	1.030 ^b (0.875-1.100)	0.800 ^c (0.734-0.980)
LC60	1.704 ^a (1.274-3.070)	1.312 ^a (1.166-1.641)	1.015 ^b (1.046-1.210)	0.913 ^c (0.805-1.081)
LC70	1.762 ^a (1.459-4.110)	1.549 ^a (1.362-1.010)	1.207 ^b (1.123-1.243)	0.987 ^c (0.872-1.222)
LC80	2.172 ^a (1.564-5.690)	1.620 ^b (1.370-2.287)	1.220 ^b (1.217-1.420)	1.062 ^c (0.943-1.224)
LC90	2.410 ^a (1.720-9.724)	2.105 ^a (1.554-3.027)	1.413 ^b (1.357-1.710)	1.203 ^c (1.036-1.798)

Table 3. Estimates of safe levels of Cyperdicot pesticide at 96 h exposure time

Chemical safe level (mg l ⁻¹) Cyperdicot	96 h LC ₅₀ (mg l ⁻¹)	Method	Application Factor
2.30 x 10 ⁻²	0.800	Hart et al. (1948)*	-
8 x 10 ⁻³		Sprague (1971)	0.1
8 x 10 ⁻⁴		CWQC (1972)	0.01
8 x 10 ⁻³ - 8 x 10 ⁻⁷		NAS/NAE (1973)	0.01-0.00001
4 x 10 ⁻³	0.05	CCREM (1991)	
4 x 10 ⁻³	5% LC ₅₀	IJC (1977)	

*C=48 h LC₅₀ x 0.03S², where C is the presumably harmless concentration and S = 24 h LC₅₀ / 48 h LC₅₀

fish to Cyperdicot were observed in the exposed fish as well as in the control, in both the toxicity and sub-lethal concentrations. Normal swimming behaviour was observed in the control throughout the exposure period. In tanks with the test chemical, the fish swam erratically with jerky movements and hyperactivity. Faster opercular movement, surfacing and swallowing of air were observed. With increase in duration of the exposure, swimming and body movements were retarded and copious mucus was secreted and deposited in the buccal cavity and on the gills. The fish subsequently lost balance, became exhausted owing to respiratory difficulties, and finally settled on the bottom and died. In the sub-lethal concentration similar abnormal behaviour was exhibited, but no mortality was recorded (Table 4). Studies on toxicity with *C. gariepinus* indicate variations in LC₅₀ values depending on the pesticide type, duration of exposure and stage of maturity (Table 5).

Haematological Parameters

The red blood cell count (RBC) and hemoglobin in the experimental group were not significantly different from those of the control (P>0.05) throughout the duration of the experiment except on day 15, when they were significantly reduced (P<0.05) Table 6. There was no significant difference in PCV values between the control and exposed fish on day 1 (P>0.05), but PCV was significantly reduced (P<0.05) from day 5 of exposure. A Cyperdicot-induced dose- and time-dependent significant increase in W B C count from day 10 onward (P<0.05) were

observed, while values of blood indices (MCV, MCH and MCHC) in the experimental fish were not significantly different (P>0.05) from the control group throughout the duration of the experiment.

There were dose- and time-dependent significant decreases (P<0.05) in the levels of neutrophils compared to the control throughout the experimental duration Table 7. The lymphocyte levels were significantly elevated (P<0.05) from day 5 onward, but the values of the monocytes, basophils and eosinophils were not significantly different from the control.

Discussions

The abnormal behavioural alterations in Cyperdicot-exposed fish may indicate disturbance in the internal physiology of the fish, which may be attributed to the neurotoxic property of the drug. Studies by Bull *et al.* (2007) indicated that Cyperdicot interferes with signaling at the neuromuscular junction, thereby disrupting the Ca²⁺ voltage-gated channels. This will result in conformational changes in membrane lipid and membrane fluidity, which is a further indication of a neuro-pharmacological effect of Cyperdicot on the fish (Wilson *et al.*, 2003). This, according to Sarai *et al.* (2013) would result in prolonged neuromuscular depolarisation, culminating in the observed uncoordinated and jerky movement that was noticed in Cyperdicot-exposed fish. Similar behavioural responses have been observed in fish exposed to chemotherapeutic compounds (Obiekezie and Okafor, 2005; Mitchell and Hobbs 2007, Wang *et*

Table 4: Behavioural and dermatological changes of *C.gariepinus* juveniles exposed to various concentrations of Cyperdicot

Exposure time(h)	Toxicity Test																							
	24					48					72					96								
Concentration (mg/l)	0	5.0	4.0	3.5	3.0	2.5	0	5.0	4.0	3.5	3.0	2.5	0	5.0	4.0	3.5	3.0	2.5	0	5.0	4.0	3.5	3.0	2.5
Behavioural changes																								
Loss of reflex	-	+	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Air gulping	-	-	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Erratic swimming	-	-	-	-	-	+	-	-	+	-	-	+	-	+	++	-	+	++	-	++	++	-	++	++
Dermatological changes																								
Discoloration	-	+	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	++	++	-	++	++
Exposure time(h)																								
Behavioural changes																								
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Air gulping	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	++	-	++	+++
Erratic swimming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	++	++
Dermatological changes																								
Discoloration	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	++	+	-	++	++	-	++	+++
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

= no significant, +=low severity, ++, = moderate severity, and +++= high severity

al., 2009; Nwaniet al., 2013). Also, similar observations have been reported in *Clariasalbopunctatus* after exposure to monocrotophos (Mgbenkaet al., 2005). Moreover, hypoxic conditions also contributed to the increase surfacing and gulping in surface water, which could also have been an attempt by the fish to avoid breathing in the poisoned water. Hypoxic conditions arise primarily due to damage of gills of fish exposed to pesticide, which hampers oxygen uptake. The increased mucus secretion by the fish after Cyperdicot exposure is probably an adaptive response to counter the irritating effects of the pesticide on body surface and mucus membrane. The observed abnormal behaviour alterations in cyperdicot-exposed fish are consistent with previous reports on organophosphate-based pesticides (Adhikari et al., 2004; Yaji, 2011) and other pesticides such as malathion, profenofos, praziquantel (Pandey et al. 2011, Nwaniet al., 2014) and atrazine (Nwaniet al., 2012).

In this study, the toxicity level of Cyperdicot on *Clariasgariepinus* was found to be 0.800 mg l⁻¹, based on the 96 hr LC₅₀ value. The LC₅₀ value reported in the present study for commercial formulation of Cyperdicot is lower than the 1.05 mg l⁻¹ and 13.6 mg l⁻¹ reported by Usmani and Knowles (2001) and Das and Murkherjee (2003) when organophosphate derivatives-based pesticide were exposed to *Labeorohitafingerlings* and larvae and adults of *Helicoerpezaa* and *Agrotisipsolon* respectively. The LC₅₀ obtained in our present study for commercial formulation of Cyperdicot is also lower than the 3.74 mg l⁻¹ obtained by Dixon and Dick (1985) when *Cyprinus carpio* were exposed to pyrenoid-based pesticide for 48 h as well as the observations made by Fazio et al. 2014, Arukwe et al. 2014, Wågbø et al. 2012, Cangialosi et al. 2012. Our LC₅₀ value 0.800 mg l⁻¹, however, is higher than the 0.620 mg l⁻¹ and

0.0750 mg l⁻¹ 96 h LC₅₀ reported by Nath and Banerjee (1996) and Yaji et al., (2011) when *Channa punctatus* and *Oreochromis niloticus* were exposed to organophosphate commercial formulation pesticide.

Toxicity tests in fish are useful in assessing possible eco-toxicological risks of contaminants (Prusty et al., 2011). The literature indicated that the toxicity of organophosphate-based herbicides varies from one species to another, and even in strains of the same species. Toxicity of chemicals to aquatic organisms has been reported to be affected by temperature, pH, dissolved oxygen, size and age, type of species, water quality, concentration and formulation of test chemicals (Young, 2000). Toxicity of compounds to organisms has however been known to be dependent on concentration, age, sex and exposure period (Saravanan et al., 2012). The safe level obtained for Cyperdicot in the present study varied from 8 x 10⁻³ to 8 x 10⁻⁴ mg l⁻¹. However, due to large variation in safe levels as determined by different methods, the estimates of safe levels cannot be guaranteed (Buikemet al., 1982). Extrapolation of laboratory data to the field is not always meaningful value, and hence it is difficult to determine acceptable concentration based on laboratory experiments that may be considered 'safe' in the field (Abuo et al., 2001; Pandey et al., 2005). Similar observations were recorded by Sampath et al. (1993) in *Oreochromis mossambicus* exposed to organophosphorous, Omoregie et al., (1994) in *Oreochromis niloticus* exposed to formalin, Svoboda et al. (2001) in *Cyprinus carpio* exposed to diazinon and

Gabriel et al. (2007) in *C. gariepinus* exposed to refined crude oil product kerosene.

Exposure of *C. gariepinus* to sub-lethal concentrations of Cyperdicot elicited changes in some haematological parameters. The RBC, Hb and PCV values were appreciably reduced at higher

Table 5. Results of various toxicity studies of some pesticides on *C. gariepinus*

Pesticide	Assay	Result	Reference
Diazinon	96 h LC50	11.80 mg l ⁻¹ in juvenile	Nwani et al. (2011)
Diazinon	96 h LC50	6.60 mg l ⁻¹ in adult	Adedeji et al. (2008)
Endosulfan	96 h LC50	8.8 ppm in juvenile	Agbohessi et al. (2013)
Gammalin20	96 h LC50	30 ppb in fingerlings	Ezemonye and Ogbomida (2010)
Glyphosate	96 h LC50	211.80 mg l ⁻¹ in juvenile	Nwani et al. (2013)
Lambdacyhalothrin	96 h LC50	0.325 ppm in juvenile	Yekeen, Fawole, and Bakare (2013)
Lindane	96 h LC50	1.29 ppm in juvenile	Lawson et al. (2011)
Cyberdicot	96 h LC50	0.80 mg l ⁻¹ in juveniles	This study

Table 6. Effects of exposure to various sub-lethal levels of Cyberdicot on RBC parameters in *C. gariepinus*

Parameters/(s)	Concentration (mg/l)	Duration (days)			
		1	5	10	15
RBC ($\times 10^6$ cells/mm ³)	Control	7.61 \pm 0.81 ^{a1}	8.22 \pm 0.84 ^{a1}	7.86 \pm 0.06 ^{a1}	89.73 \pm 0.64 ^{a1}
	0.04	7.71 \pm 0.86 ^{a1}	6.90 \pm 0.93 ^{a1}	6.95 \pm 0.63 ^{a1}	4.65 \pm 0.60 ^{b2}
	0.08	7.15 \pm 0.77 ^{a1}	7.14 \pm 0.96 ^{a1}	6.87 \pm 0.71 ^{a1}	4.57 \pm 0.58 ^{b2}
PVC (%)	Control	26.00 \pm 0.77 ^{a1}	29.50 \pm 0.71 ^{a1}	30.50 \pm 0.72 ^{a1}	27.50 \pm 1.83 ^{a1}
	0.04	26.50 \pm 0.81 ^{a1}	23.00 \pm 0.83 ^{b2}	23.50 \pm 0.73 ^{b2}	16.00 \pm 0.68 ^{c2}
	0.08	27.00 \pm 0.62 ^{a1}	25.00 \pm 0.49 ^{a2}	24.00 \pm 0.65 ^{a2}	14.50 \pm 0.59 ^{b2}
WBC ($\times 10^4$ cells/mm ³)	Control	6323 \pm 2.63 ^{a1}	8024 \pm 6.41 ^{a1}	8100 \pm 7.72 ^{a1}	7650 \pm 8.41 ^{a1}
	0.04	6474 \pm 7.82 ^{a1}	8124 \pm 6.01 ^{a1}	8350 \pm 6.44 ^{b1}	7930 \pm 7.91 ^{b2}
	0.08	6451 \pm 5.41 ^{a1}	8201 \pm 5.63 ^{a1}	9000 \pm 6.76 ^{c2}	9050 \pm 6.77 ^{c2}
Hb (g/dL)	Control	7.65 \pm 0.66 ^{a1}	8.80 \pm 0.71 ^{a1}	9.45 \pm 0.66 ^{a1}	8.45 \pm 0.81 ^{a1}
	0.04	6.10 \pm 0.54 ^{a1}	6.95 \pm 0.82 ^{a1}	7.15 \pm 0.71 ^{a1}	5.60 \pm 0.71 ^{b2}
	0.08	6.30 \pm 0.63 ^{a1}	7.65 \pm 0.54 ^{a1}	7.30 \pm 0.46 ^{a1}	5.15 \pm 0.62 ^{b2}
MCH (pg/cell)	Control	8.82 \pm 0.83 ^{a1}	9.52 \pm 0.67 ^{a1}	10.78 \pm 0.92 ^{a1}	9.52 \pm 1.01 ^{a1}
	0.04	9.45 \pm 0.66 ^{a1}	9.06 \pm 0.71 ^{a1}	9.25 \pm 0.94 ^{a1}	9.91 \pm 0.83 ^{a1}
	0.08	10.41 \pm 0.45 ^{a1}	9.63 \pm 0.83 ^{a1}	18.55 \pm 1.16 ^{a1}	9.24 \pm 0.46 ^{a1}
MCHC (g/dL)	Control	32.27 \pm 1.45 ^{a1}	32.22 \pm 1.94 ^{a1}	32.18 \pm 1.09 ^{a1}	32.16 \pm 2.11 ^{a1}
	0.04	32.09 \pm 1.16 ^{a1}	32.13 \pm 1.86 ^{a1}	32.27 \pm 3.04 ^{a1}	31.94 \pm 1.64 ^{a1}
	0.08	32.21 \pm 1.84 ^{a1}	32.27 \pm 2.35 ^{a1}	32.20 \pm 2.06 ^{a1}	32.23 \pm 3.09 ^{a1}
MCV (fl/cell)	Control	28.51 \pm 1.86 ^{a1}	30.65 \pm 1.86 ^{a1}	34.52 \pm 1.86 ^{a1}	27.70 \pm 2.06 ^{a1}
	0.04	30.57 \pm 2.45 ^{a1}	29.38 \pm 2.09 ^{a1}	29.82 \pm 1.54 ^{a1}	29.09 \pm 1.07 ^{a1}
	0.08	33.36 \pm 2.11 ^{a1}	30.94 \pm 1.16 ^{a1}	30.77 \pm 1.11 ^{a1}	26.83 \pm 1.08 ^{a1}

Values with different alphabetic (lowercase) superscripts differ significantly ($p < 0.05$) between different concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean standard error of the mean.

concentrations of Cyberdicot. The reduction in these parameters may be attributed to haemolysis caused by the drugs action on the fish. The decrease may also be attributed to the limit in erythrocyte synthesis due to impaired osmoregulation across the gill epithelium and accumulation of the toxicant in the gill region (Saravanan et al., 2011; Pereira et al., 2013). Furthermore, blood indices are often subjected to variations depending upon stress and environmental factors (Goellet al., 1981; Hlavova, 1993; Marsaleket al. 2014). The observed leukocytosis from day 10 of exposure indicated an immune protective response against the stress imposed by the drug (Davis et al., 2008). Exposure to toxicants such as Cyberdicot stimulated the lymphocyte cells in the lymphomyeloid tissue as defense mechanism against the stressor, hence the observed proliferation of WBC in the peripheral blood (Campbell, 1996). The data on MCV and MCH showed that Cyberdicot caused both macrocytic and microcytic anemia on day 1, and between days 5 and 15, respectively. Microcytic

anemia was reported in *O. mykiss* treated with sulfadiazine and trimethoprin (Lunden and Bylund, 2002) and sulfamerazine (SaglamandYonar, 2009; Šišperová et al., 2015). There was no significant change in the values of MCHC in Cyberdicot-treated fish compared to the control. Li et al. (2011c) obtained similar results for the red blood cell indices of *O. mykiss* exposed to the pharmaceutical drug carbamazepine, (Serckova et al., 2016). Changes in leukocyte differentials have been used as good indicators of stress (Cole et al., 2001). Neutrophils and lymphocytes make up the majority of WBC and proliferate in circulation in response to stress (Jain 1993, Thrall, 2004). The observed significant increase in lymphocytes from day 5, and of neutrophils throughout the duration of exposure, may have been provoked by the stress imposed by Cyberdicot on the fish. Stress-induced lymphopenia and neutrophilia have been shown to be related to elevated glucocorticoids secretion which acts to increase their percentage levels (Davis et al., 2008). Similar results

Table 7. Effects of exposure to various sub-lethal levels of Cyperdicot on differential WBC counts (percentage) in *C. gariepinus*

Parameter(s)	Concentrations (mg/l)	Duration (Days)			
		1	5	10	15
Neutrophils	Control	12.00 ± 1.11 ^{a1}	13.00 ± 1.93 ^{a1}	13.60 ± 1.13 ^{a1}	13.8 ± 1.09 ^{a1}
	0.4	16.50 ± 1.86 ^{a2}	17.00 ± 2.06 ^{a2}	23.50 ± 1.41 ^{b2}	25.00 ± 2.11 ^{b2}
	0.8	20.3 ± 1.14 ^{a3}	19.50 ± 1.81 ^{a2}	24.11 ± 1.63 ^{b2}	27.50 ± 3.06 ^{c2}
Lymphocytes	Control	16.50 ± 1.16 ^{a1}	22.00 ± 1.14 ^{a1}	18.00 ± 1.10 ^{a1}	16.00 ± 1.66 ^{a1}
	0.4	29.50 ± 1.09 ^{a1}	23.00 ± 0.83 ^{b1}	23.50 ± 0.93 ^{b2}	23.00 ± 1.41 ^{b2}
	0.8	33.00 ± 0.93 ^{a2}	25.50 ± 0.71 ^{b2}	26.00 ± 0.71 ^{b3}	30.50 ± 0.89 ^{c3}
Monocytes	Control	0.40 ± 0.06 ^{a1}	0.50 ± 0.01 ^{a1}	0.50 ± 0.01 ^{a1}	1.00 ± 0.02 ^{a1}
	0.4	0.40 ± 0.06 ^{a1}	0.30 ± 0.01 ^{a1}	1.00 ± 0.02 ^{a1}	0.60 ± 0.03 ^{a1}
	0.8	2.00 ± 0.06 ^{a1}	1.00 ± 0.04 ^{a1}	1.50 ± 0.11 ^{a1}	0.50 ± 0.07 ^{a1}
Basophils	Control	0.04 ± 0.00 ^{a1}	0.04 ± 0.01 ^{a1}	0.04 ± 0.00 ^{a1}	0.04 ± 0.00 ^{a1}
	0.4	0.04 ± 0.00 ^{a1}	0.04 ± 0.01 ^{a1}	0.03 ± 0.00 ^{a1}	0.01 ± 0.00 ^{a1}
	0.8	0.03 ± 0.01 ^{a1}	0.03 ± 0.01 ^{a1}	0.04 ± 0.01 ^{a1}	0.05 ± 0.00 ^{a1}
Eosinophils	Control	0.40 ± 0.01 ^{a1}	0.10 ± 0.01 ^{a1}	0.40 ± 0.01 ^{a1}	0.30 ± 0.01 ^{a1}
	0.4	0.40 ± 0.02 ^{a1}	0.50 ± 0.03 ^{a1}	0.10 ± 0.04 ^{a1}	0.30 ± 0.02 ^{a1}
	0.8	0.40 ± 0.01 ^{a1}	0.50 ± 0.02 ^{a1}	0.10 ± 0.02 ^{a1}	0.35 ± 0.03 ^{a1}

Values with different alphabetic (lowercase) superscripts differ significantly ($p < 0.05$) between different concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean standard error of the mean.

have been reported in fishes exposed to different concentrations of pharmaceuticals (Kreutzmann, 1977; Lundenet al., 1999, Li et al., 2011). Other leukocyte differentials such as monocytes, basophils and eosinophil were comparable to the control throughout the experimental period. Similar observations have been also reported in other fishes treated with different toxicants (Veliseket al., 2009, Mohammad et al., 2012).

Conclusion

This study showed that both behavioural and sub-lethal concentrations of Cyperdicot affected both the behavioural responses and the haematological parameters of juveniles of *Clarias gariepinus*. It is shown that long-term exposure to Cyperdicot at sub-lethal concentrations induced haematological alterations in *Clarias gariepinus*, and that the use of haematology is adequate in detecting and monitoring possible effects of sub-lethal dose of pesticide in the environment. From the present study it could be concluded that, when fishes are exposed to fourth generation pesticides such as Cyperdicot, they have various haematotoxic effects on the survival of the fish. This in turn also will affect the fecundity of the fish population and other non-targeted organisms such as man through the food chain. Therefore the levels in the aquatic environment should not be higher than the sub-lethal exposure levels. Action is needed when the levels increase markedly.

References

AbuoE ,Naga, E.H., Moselhy, K.M. and Mohamadein, L.I. 2001. Effect of cadmium and copper on the digestive andenzymes of the limpet (*Patella* sp., Mollusca, Gastropoda). *Egyptian Academy of Society and Environmental Development*, 2:29-36. doi: 200531(2)60-

71/1687-4285

- Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C.T., and Ayyappan, S. 2004. Effects of Cypermethrin and carbofuran on certain haematological parameters and predictions of their recovery in a fresh water teleost, Labeorohita. *Ecotoxicology and Environment*, 2:23-43. Paper ID:15157576
- Agwu J.E; Odo G.E.; Oloto J.C. Uwagbae, M. 2016. Effects of Cypermetrin on the Biochemical Profile of the Hemolymph of the African Pest Grasshopper *Zonocerus variegatus* Linn. *International Journal of Biomaterials Science and Engineering*, 3(2):15-19.
- APHA, (American Public Health Association). 2005. *Standard methods for the examination of water and waste water*. 21st edition. New York: American Public Health Association.
- Arellano, J.M., Blasco, J., Ortiz, J.B., Capeta Da Silva, D., Navarro, A., Sanchez-De Piano, M.J. and Sarasquete, C. 2000. Accumulation and histopathological effects of copper in gills and liver of Senegalese sole, *Solea senegalensis* and toad fish, *Halobatrachus didactylus*. *Ecotoxicology and Environment Restoration*, 3: 22-28. doi.org/10.1006/eesa.1999.1801
- Arukwe, A., Olufsen, M., Cicero, N. Hansen M.D. (2014). Effects on development, growth-hormone, and thyroid-hormone systems in eyed eggs and yolk-sac larvae of atlantic salmon (*Salmo salar*) continuously exposed to 3,3',4,4' tetrachlorobiphenyl (pcb-77). *Journal of Toxicology and Environmental Health, Part A*. 77 1-13. ISSN: 1528-7394 print / 1087-2620 online, DOI: 10.1080/15287394.2014.887422
- Ayoola, S.O. and Ajani, E.K. 2007. Histopathological effects of Cypermethrin on juvenile Nile Tilapia (*Oreochromis niloticus*). *African Journal of Livestock Extension*, 13:231-239. doi:10.5897/AJLE11.003
- Blaxhall, P.C and Daisley, K.W. 1973. Routine haematological methods for use with fish blood *Journal of Fish Biology*, 5: 771-781. doi:10.1111/J.1095.8649.1973.TB04510X
- Bull, K., Cook, A., Hoper, N.A., Harder, A., Holtendy Bull, K., Cook, A., Hoper, N.A., Harder, A.,

- Holten – Dye, L. and Walker, I.J. 2007. Effect of the novel anthelminticemodepside on the locomotion, egg-laying behavior and development of *Caenorhabditiselegans*. *International Journal of Parasitology*, 37: 627–636. doi:10.1016/j.ijpara.2006.10.013
- Buikem, J.R., Naidler-Lehner, A.L., and Cairns, J.R. 1982. Biological monitoring: Part IV. *Toxicology testing Environmental Molecular Mutagen*, 33: 239-262. doi: 10.1002/en.22001
- Campbell, T.W. 1996. Reptile medicine and surgery In: Mader D R. (ed.), *Clinical pathology*. Philadelphia: W B Saunders, pp. 248–257.
- Cangialosi, M.V., Corsi, I., Bonacci, S., Sensini, C., Cicero, N., Focardi, S., Mazzola A. 2012. Screening of ecotoxicological, qualitative and reproductive variables in male European sea bass *Dicentrarchus labrax* (L.) reared in three different fish farms: facility location and typology. *NATURAL PRODUCT RESEARCH*, 27 (7), 670-674. DOI:10.1080/14786419.2012.683000
- Clara N., Nicola C., Vincenzo F., Giuseppe G. Antonio V. Andrea M., Francesco N., Giacomo D. 2015. Toxic metals in pelagic, benthic and demersal fish species from Mediterranean FAO zone 37. *Bulletin of Environmental Contamination and Toxicology*. Volume 95, Issue 5, 567-573. DOI: 10.1007/s00128-015-1585-6”.
- Cole, M.B., Arnold, D.E., Watten, B.J., and Krise, W.F. 2001. Haematological and physiological responses of brook charr to untreated and limestone neutralized acid. *Journal of Fish Biology*, 59: 79–91. doi.org/10.3996/072013.JFB
- Dacie, J.V. and Lewis, S.M. 2001. *Practical haematology*, 6th edn: University Press, 633pp.
- Dixon, D.G. and Dick, P.T. 1985. Changes in circulating blood levels of rainbow trout *Salmo gairdneri* (Richardson) following acute and chronic exposure to copper. *Journal of fish Biology*, 26:475–481. doi/10.1111/j.1095-8649.1985.tb04287.x/full
- Eze, L.C. 1983. Isonoid inhibition of liver glutamate oxaloacetic transaminase from goat (*Carpaeaercus*). *International of Journal of Biochemistry*, 15: 13-16.
- Eyo, J.E., Levi, C.A., Asogwa, C.N., Odii, E.C., Chukwuka, C.O., Ivoke, N., Onoja, U.S. and Onyeke, C.C. 2013. Toxicity and effect of *Carica papaya* seed aqueous extract on liver biomarkers of *Clarias gariepinus*. *International Journal of Indigenous Medicinal Plants*, 46: 1301–1307. <https://www.resgate.net/.../2255859120>
- Fazio, F; Piccione, G; Tribulato, K; Ferrantelli, V; Giangrosso, G; Arfuso, F and Faggio, C. 2014. Bioaccumulation of heavy metals in blood and tissue of striped mullet in two Italian Lakes. *J. Aquat. Anim. Health*, 26(4): 278-284.”
- Finney, D. J. 1971. *Probit analysis*, 3rd edition Cambridge: Cambridge: University Press.
- Hart, W.B., Weston, R.F., and Dermann, J.G. 1948. An apparatus for oxygenating test solution in which fish are used as test animals for evaluating toxicity. *American Fishery Society*, 75:288-301. [www.tandfonline.com/.../1548-8659\(1945\)](http://www.tandfonline.com/.../1548-8659(1945))
- Hesser, O.F. 1960. Methods of routine fish haematology. *Progressive Fish Culturist*, 22: 164 – 171.
- Hlavova, V. 1993. Reference values of the haematological indices in grayling *Thymallus thymallus* Linnaeus). *Comparative Biochemistry Physiology*, 105A: 525–532.
- Giuseppa D., Angela G., Potorti, V., Lo T., Daniel B., Patrizia L., Nicola C., Giacomo D. (2015). Trace Elements in Thunnus Thynnus From Mediterranean Sea: Benefit-Risk Assessment For Consumer. *Food Additives & Contaminants: Part B*, Vol. 8, No. 3, 175–181 doi.org/10.1080/19393210.2015.1030347”.
- Glen, V.D.K. 2014. Effects of Atrazine in Fish, Amphibians, and Reptiles: An Analysis Based on Quantitative Weight of Evidence. *Reviews in Toxicology*, 44(S5):1-66 DOI: 10.3109/10408444.2014.967836
- I. J. C. (International Joint Commission). 1977. New and revised Great Lakes water quality objectives. Great Lakes Basin, Windsor; Ottawa. IJC.
- Jain, N.C. 1993. *Essentials of veterinary haematology*. Philadelphia: Blackwell.
- Leilan, B., Richard, A.B., Alan, J.H., Mark, L. H. 2015. Effects of atrazine on egg masses of the yellow-spotted salamander (*Ambystoma maculatum*) and its endosymbiotic alga (*Oophila amblystomatis*). *Environmental Pollution*, 206:324-331 DOI: 10.1016/j.envpol.2015.07.017
- Kreutzmann, H.L. 1977. The effects of chloramphenicol and oxytetracycline on haematopoiesis in the European eel (*Anguilla anguilla*). *Aquaculture*, 10:323-334. doi:10.16/0044-8486(77)90123-5
- Li, Z.H., Zlabek, V., Velisek, J., Grabic, R., Machova, J., Kolarova, J., Li, P. and Randak, T.B. 2011. Acute toxicity of carbamazepine to juvenile rainbow trout (*Oncorhynchus mykiss*): effects on antioxidant responses, haematological parameters and hepatic EROD. *Ecotoxic Journal of Environmental Safety*, 74:319–327. doi:10.1016/j.ecoenv.2010.09.008. Epub.20100ct23
- Li ZH, Zlabek V, Velisek J, Grabic R, Machova J, Kolarova J. and Randak TC. 2011. Hepatic status and hematological parameters in rainbow trout (*Oncorhynchus mykiss*) after chronic exposure to carbamazepine. *Chemical Biological Interaction*, 183: 98–104. doi:10.1016/j.jicbi.2009.09.009
- Lunden, T. and Bylund, G. 2002. Effect of sulfadiazine and trimethoprim on the immune response of Rainbow trout (*Oncorhynchus mykiss*). *Veterinary Immunology and Immunopathology*, 85: 99–108. www.ncbi.nlm.nih.gov/pubmed/1186717
- Lunden, T., Miettinen, S., Lonnstom, L.G. and Lilius, E.M., Bylund, G. 1999. Effect of flor-fenicol on the immune response of rainbow trout (*Oncorhynchus mykiss*). *Veterinary Immunology and Immunopathology*, 67:317–325. www.ncbi.nlm.nih.gov/pubmed/10206200
- Maršálek, P., Mikulíková, I., Modrá, H., Svobodová, Z. 2014. Effect of prochloraz fungicide on neopterin and biopterin concentrations in blood plasma of common carp. *Acta Veterinaria Brno*, 83(2):101-105 DOI: 10.2754/avb201483020101
- Mgbenka, B.O., Oluah, N.S. and Arumgwá, A.A. 2005. Erythropoietic response and haematological parameters in the catfish *Clarias albopunctatus* exposed to sub-lethal concentrations of acclerlic. *Journal of Ecotoxicology and Environmental Safety*, 62:436 – 440. doi:1016/j.ecoenv.2005.03.031
- Mitchell, A.J. and Hobbs, M.S. 2007. The acute toxicity of

- praziquantel to grass carp and golden shiners. *Northern American Journal of Aquaculture*, 69: 203–206. doi:10.1577/A06-056.1
- Mohammad, N.S.M., Soltani, M., Kamali, A., Imanpoor, M.R., Sharifpour, I. and Khara, H. 2012. Effects of organophosphate, diazinon on some haematological and bio-chemical changes in *Rutilus frisii kutum* (Kamensky 1901) male brood stocks. *Iranian Journal of Fisheries Science*, 11: 105–117. www.jifros.ir/adm-10.1-141-4312883
- Moore, M.N. and Simpson, M.G. 1992. Molecular and cellular pathology in environmental impact assessment. *Aquatic Toxicology*, 22: 313–322. doi:10.1016/0166-445x(92)90047Q
- Nath, R., Banerjee, V. 1996. Effects of pesticide methyl parathion and Cyprmethrin on oxygen consumption and blood of *Channa punctatus*. *Journal of Industrial Zoology*, 16: 95 – 102.
- Nelson, D.A. and Morris, M.W. 1989. Basic methodology. Haematology and coagulation; part IV. In: Nelson D A, Henry J B (eds.), *Clinical diagnosis and management by laboratory methods*, 17th edn. Philadelphia: W B Saunder, pp.578–625.
- Nwani, C.D., Oluah, N.S., Echi, P.C. and Nwamba, I. 2014. Mutagenic and physiological responses in the juveniles of African catfish, *Clarias gariepinus* (Burchell 1822) following short term exposure to praziquantel. *Tissue and Cell*, 46(4), pp 264–273. doi.org/10.3109/01480545.2013.86613
- Nwani, C.D., Okeke, O.C., Onyishi, G., Attama, C., Uzoma, C., and Eneje, L.O. 2012. Toxicity and effects of diazinon on behavior and some haematological parameters of African catfish, *Clarias gariepinus*. *Journal of Environmental Biology*, 1: 246 – 253. doi:10.1080/21658005.2012.733555
- Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B., Kumar, P. and Lakra, W.S. 2013. Induction of micronuclei and nuclear lesions in *Channa punctatus* following exposure to carbosulfan, glyphosate and atrazine. *Drug and Chemical Toxicology*, 31(9):138–148. doi:10.3109/01480545.2013.866138. Epub. 2013 Dec. 11
- Obiekezie, A. and Okafor, N. 2005. Toxicity of four commonly used chemotherapeutic compounds to fry of the African catfish, *Clarias gariepinus* (Burchell). *Aquaculture Research*, 26: 441–445.
- OECD (Organisation for Economic Co-operation and Development). 1992. The Organisation for Economic Co-operation and Development's Guidelines on environment and aid, No. 1, Good practices for environmental impact assessment of development projects, *OECD, Paris: Development Assistance Committee*.
- Omoriegie, E., Eseyin, T.G. and Ofojekwu, P.C. 1994. Effects of formulation on erythrocyte counts and plasma glucose of Nile tilapia *Oreochromis niloticus*. *Asian Fisheries Science*, 7:1–6. www.academia.edu/963346
- Pandey, A.K., Nagpure, N.S., Trivedi, S.P., Kumar, R., Kushwaha, B. and Lakra, W.S. 2011. Investigation on acute toxicity and behavioural changes in *Channa punctatus* (Bloch) due to organophosphate pesticide profenofos. *Drug and Chemical Toxicology*, 34: 424–428. doi:10.3109/0148054.2011.585650. Epub 2011 3y9
- Pandey, S., Kumar, R., Nagpure, N.S., and Srivastava, S.K. 2005. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctatus* (Bloch). *Ecotoxicology*, 61: 114–120. doi:10.1016/j.ecoenv.2004.08.004
- Pereira, L., Fernandes, M.N., and Martinez, C.B.R. 2013. Haematological and biochemical alterations in the Fish *Prochilodus lineatus* caused by the herbicide clomazone. *Environmental Toxicology and Pharmacology*, 36:1–8. doi:10.1016/j.etap.2013.02.019
- Prusty, A.K., Kohli, M.P.S., Sahu, N.P., Pal, A.K., Saharan, N., Mohapatra, S. and Gupta, S.K. 2011. Effect of short-term exposure to fenvalerate on biochemical and haematological responses in *Labeo rohita* (Hamilton) fingerlings. *Pesticide Biochemistry Physiology*, 100: 124–129. doi:10.1016/j.pestbp.2011.02.010
- Sampath, K., Velamnia, S.K. and James, R. 1993. Haematological changes and their recovery in *Oreochromis mossambicus* as a function of exposure period and sub-lethal levels of Elalus. *Acta Hydrobiologica*, 35:73–83.
- Sarai, R.S., Kopp, S.R. and Coleman, G.T. 2013. Acetylcholine receptor subunit and p-glycoprotein transcription patterns in levamisole-susceptible and resistant *Haemonchus contortus*. *International Journal of Parasitology*, 3:51–58. doi:10.1016/j.ijpdr.2013.01.002
- Sprague, J.B. 1971. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water Research*, 3: 793–821. doi:10.1016/0043-1354(69)90050-5
- Svoboda, M., Luskova, V., Drastihova, J. and Zlabek, V. 2001. The effect of diazinon on haematological indices of common carp (*Cyprinus carpio*). *Journal of Veterinary Medicine* 70:457 – 465. doi:10.2754/avb200170040457
- Šišperová E, Modrá H, Zikova A, Svobodová Z 2015. The effect of mycotoxin deoxynivalenol (DON) on the oxidative stress markers in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). *Journal of Applied Ichthyology*, 31(5) DOI: 10.1111/jai.12809
- Reish, D.J. and Oshida, R.S. 1987. Manual of methods in aquatic environment research, Part 10. Short-term static bioassay. *FAO Fishery Technical*. Paper 247, 62 pp.
- Rusia, V. and Sood, S.K. 1992. Routine hematological tests. In: Mukerjee KL. (ed.), *Medical Laboratory Technology*. New Delhi: Tata McGraw Hill, pp.252–258.
- Saglam, N. and Yonar, M.E. 2009. Effects of sulfamerazine on selected haematological and immunological parameters in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, 40: 395–404. doi:10.1111/j.1365-2109.2008.02105X
- Saravanan, M., Karthikas, S., Malarvizhi, A. and Ramesh M. 2011. Ecotoxicological impacts of Clofibric acid and diclofenac in common carp (*Cyprinus carpio*) fingerlings: haematological, biochemical ion regulatory and enzymological responses. *Journal of Hazardous Materials*, 195:188–194. doi:10.1016/j.jhazmat.2011.08.029. Epub. 2011. Aug. 16
- Sevcikova, M., Modrá, H., Svobodova, Z., 2016. Haematological and oxidative stress responses of common carp (*Cyprinus carpio* L.) after sub-chronic exposure to copper *Veterinárni Medicina*, 61(01):35–50. DOI: 10.17221/8681-VETMED

- Velisek, J., Stesjskal, V, KourilJ.andSvobodovo, Z.2009. Comparison of the effectsof four anesthetics on biochemical blood profiles of Perch. *Aquaculture Research* 40:354–361.doi:10.1111/j.1365-2109-2008.02102X
- Thrall, M.A. 2004. Haematology of amphibians. Veterinary haematology and clinical chemistry: Text and clinical case presentations. Philadelphia: Lippincott Williams and Wilkins.
- Usmani, K.A. and Knowles, C.O. 2001. Toxicity of pyrethroids and effects of synergist's tolarva and adult *Helicoverpazea*, *Spodopterafrugiperda* and *Agroisipsilon* (Lepidoptera 117.Noctruidae). *Journal of Entomology*,94:868-73. doi:pdf/10.1603/0022-0493-94.94.4.868
- Yaji, A. J., Acute, J. and Onyiye, S. J. (2011). Effect of cypermethrin on behaviour and biochemical indices of the freshwater fish *Oreochromisniloticus*. *Electronic Journal of Environment, Agriculture and Food Chemistry*,10:1927-1934.
www.cabdirect.org/abs/20113/03/0469.html
- Yaji, A. and Auta, J. 2007. Sub-lethal effects of monochrotophos on some haematological indices of African Catfish *Clariasgariiepinus*. *Journal of Fish Physiology*,2: 115 – 717.doi:j.fish.2007.115.117
- Young, R.A. 2000. Spatial sampling and the environment: some issues and directions. *Journal of Occupational Medicine*,29:601-604
- A.M. Wågbo, M.V. Cangialosi, N. Cicero, R.J. Letcher and A. Arukwe 2012. Perfluorooctane Sulfonamide-Mediated Modulation of Hepatocellular Lipid Homeostasis and Oxidative stress Responses in Atlantic Salmon Hepatocytes. *CHEMICAL RESEARCH IN TOXICOLOGY*. 6, 1253–1264 DOI. org/10.1021/tx300110u
- Wang, N., Nkejabega, N., Hien, N.N., Huynh, T.T., Silvestre, F., Phuong, N.T., Danyi, S.,
- Widart, J.,Douny, C., Scippo, M.L., Kestemont, P.andHuong, D.T. 2009. Adverse effects of enrofloxacin when associated with environmental stress in African catfish (*Pangasianodonhypophthalmus*).*Chemosphere*,17: 1577–1584.
- Ward, G.S. and Parrish, P.R. 1982 .Manual of methods in aquatic environmental research, Part 6. Toxicity tests. *FAO Fish Technical Paper185 FIRI/T185*. Rome: FAO.
- Wilson J., Amilwala, K., Harder, A., Holden-Dye, L.and Walker R. 2003. The effect of antihelminthicemodepside at the neuromuscular junction of the parasitic nematode, *Ascarissuum*. *Parasitology*,126: 79–86