Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2013) 37: 535-540
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doi:10.3906/vet-1212-39

Acute toxicity of diazinon and its effects on hematological parameters in the Indian carp, *Cirrhinus mrigala* (Hamilton)

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Received: 21.12.2012 • **Accepted:** 12.05.2013 • **Published Online:** 26.08.2013 • **Printed:** 20.09.2013

Abstract: Diazinon is a commonly used pesticide and its existence in the fresh water reservoirs of Pakistan has been reported by some authors. A semistatic experiment was conducted to find out the acute toxicity of diazinon as an aquatic pollutant on some hematological parameters of Indian carp (*Cirrhinus mrigala*). The 1, 24, 48, 72, and 96 h LC_{50} values of diazinon for Indian carp were estimated as 17.32 mg/L, 15.39 mg/L, 12.64 mg/L, 10.98 mg/L, and 8.15 mg/L respectively. A significant increase in the number of dead fish was observed as the concentration of diazinon and exposure time increased ($P < 0.05$). The results after acute exposure to 8.15 mg/L of diazinon for 96 h showed that compared to the control fish, the total erythrocytes, leukocytes, hemoglobin, and hematocrit values decreased significantly ($P < 0.05$) in exposed fish. The mean corpuscular volume and mean corpuscular hemoglobin increased significantly ($P < 0.05$), while the mean corpuscular hemoglobin concentration of both groups remained unchanged during the study. In conclusion, acute exposure to LC_{50} values of diazinon induced hematological alterations in Indian carp and offers a tool to evaluate toxicity derived alterations.

Key words: Diazinon, acute toxicity, Indian carp, hematology

1. Introduction

In Pakistan, the use of pesticides to increase crop yield by controlling plant pests has increased considerably during the past few decades. As a result, it has become a major source of pollution of the aquatic environment. Diazinon is an organophosphate pesticide; it is widely used as an insecticide to protect many crops from a wide range of hymenopteran and hemipteran insects (1). After agricultural application, diazinon may easily wash away and reach the surface water reservoirs, thus affecting a wide range of nontarget aquatic animals, including invertebrates and fishes. Like other organophosphate pesticides, diazinon produces its toxic effects by inhibiting a series of enzymes, including acetylcholinesterase (2). Depending upon differences in absorption, detoxification, and acetylcholinesterase inhibition, the 96 h sublethal values of diazinon exposure vary across a variety of fish species. The 96 h LC_{50} values of diazinon for zebrafish (*Brachydanio rerio*) have been reported as 8 mg/L, but for guppies (*Poecilia reticulata*) they were found to be 0.8 mg/L (3). Inhibition of acetylcholinesterase in fish after exposure to sublethal doses of diazinon could drastically affect growth, survival and reproductive and feeding behaviors (2,3). Since hematological parameters reflect the healthy condition of a fish, they are widely used as an indicator of disease or stress caused by aquatic pollutants. Although physiological and biochemical changes induced by different organophosphate pesticides have been investigated in several fish species (malathion in *Cyprinion watsoni* (4), cypermethrin *Heteropneustes fossilis* (5), dichlorvos in *Clarias batrachus* (6), and diazinon in *Oreochromis niloticus* (7), *Silurus glanis* (8), and *Cyprinus carpio* (9)), there is a paucity of data regarding the effects of diazinon on the hematological parameters of Indian carp (*Cirrhinus mrigala*). As diazinon is commonly used in agricultural fields and *C. mrigala* is an important fresh water fish of Pakistan, the present study was carried out to find out the acute toxicity of diazinon and its effects on some hematological parameters in *C. mrigala*. Finally, this study would supplement the current knowledge on pesticide toxicity and also help effective management of fresh water reservoirs with respect to the input of diazinon from agricultural fields.

2. Materials and methods

Live specimens of *C. mrigala* were obtained from the Jokhio fish farm (24°16′5″N, 67°35′55″E) in Thatta, located northeast of Karachi, Pakistan. Live juveniles weighing

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18–20 g and having a total length of 4–6 cm were used for the acute toxicity test; for hematological studies, fish of the same strain of 1–2 years old weighing 200–250 g with a length of 15–17 cm were used. The fish were transported in aerated plastic containers to the laboratory and kept in a 500 L fiber glass tank for 1 week to get acclimatized to the laboratory conditions. The water in the tank was aerated continuously. Water was changed daily and its temperature was maintained at 22 ± 2 °C. During the period of acclimation, the fish were fed with a commercial fish food twice a day, but feeding was stopped 24 h prior to the test and throughout the test.

After 1 week of acclimation, a static acute toxicity test was performed following guideline No. 203 of the Organization for Economic Cooperation and Development (OECD). A commercial diazinon, Basudin 60 EC brand, with the active ingredient diazinon [0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate], with purity of 60% and dissolved in 40% acetone, purchased from Syngenta Ltd., Pakistan, was used to prepare test solutions of diazinon. Juveniles of *C. mrigala* were exposed to 6 different concentrations (5, 10, 15, 20, 25, and 30 mg/L) of diazinon to determine 1, 24, 48, 72, and 96 h lethal concentration values for the test fish. For the acute toxicity test, 20 fish were transferred to 40 L aquaria containing dechlorinated tap water. Five replicates for each concentration of diazinon were used to determined lethal concentrations. Prior to the introduction of fish in the aquaria, diazinon was added and water was aerated for 1 h for homogeneous distribution of diazinon in the water. Two control sets were also run containing the same number of fish in the same volume of water, containing 0.6 mg/L of acetone instead of diazinon that was used for the dilution of diazinon. The water quality parameters were: pH 7.5 to 8, temperature 22 ± 2 °C, dissolved oxygen 6.4 to 7.3 mg/L, free carbon dioxide 4.5 to 6.0 mg/L, salinity 108 mg/L, hardness 110 mg/L, and $CaCO_3$ 52 mg/L. The physicochemical characteristics of the experimental water used were determined according to the procedures described in standard methods (10). The water quality parameters were monitored throughout the test and water in the aquaria was changed every 12 h. Fish were considered dead if their gill opercula movement ceased and they failed to respond to the stimulus provided with a glass rod. Their mortalities were recorded at 1, 24, 48, 72, and 96 h after exposure to different concentrations of diazinon; dead specimens were immediately removed from the aquaria and the Probit Analysis test was used to calculate the LC_{50} (11).

Hematological examinations were carried out on specimens of *C. mrigala* 1–2 years old at the end of a 96 h acute toxicity test, with diazinon at a concentration of 8.15 mg/L (96 h LC_{50}). The test was performed in 4 aquaria of 100 L each containing 20 fish, i.e. 3 aquaria with 8.15 mg/L of diazinon and 1 control aquarium without diazinon. The test was performed semistatically for 96 h and the water was changed every 12 h to ensure the presence of diazinon above 80% of the nominal concentration in each aquarium. The water quality parameters in the aquaria were maintained the same as described above, except oxygen saturation of the water was above 60% (85%–95%). The behavioral changes of the fish were observed at 12 h during the study period. At the end of 96 h exposure to 8.15 mg/L of diazinon, a total of 22 fish died. Twelve surviving experimental (4 fish from each aquarium) and 12 control fish were randomly caught using a small dip net with minimum disturbance in the aquaria for hematological examinations. The fish were wrapped and cleaned with a coarse filter paper. The blood was sampled by cardiac puncture using an 18 G needle attached to a heparinized syringe (50 IU sodium heparin/mL of blood) to determine hematological parameters. The hematological parameters measured included erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), and leukocyte count (WBC), and these were determined according to the unified methods for hematological examination of fish (12). The derived blood indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following formulae: MCV = Hct × 100/RBC; MCH = Hb/RBC; and MCHC = Hb × 100/Hct.

Data of all replicates were used to calculate mean values. The difference in mortality values was analyzed by χ^2 test. The values of hematological parameters were presented as mean ± standard deviation. Data for hematological parameters were tested for normality (Kolmogorov–Smirnov test) and then analyzed by one-way analysis of variance (ANOVA) to test the significant differences among different hematological parameters. All statistical analyses were carried out using SPSS 10.1 (SPSS Inc., Chicago, IL USA) and $P < 0.05$ was considered statistically significant.

3. Results

The number of dead *C. mrigala* exposed to different concentrations of diazinon in respective time intervals is given in Table 1. The highest and most rapid fish mortality was observed in fish exposed to the highest concentration of diazinon. The data clearly showed the relationship among the concentration of pesticide, duration of exposure, and number of dead fish ($P < 0.05$ for each case). The LC_{50} values of diazinon for *C. mrigala* were determined as 17.32 mg/L for 1 h, 15.39 mg/L for 24 h, 12.64 mg/L for 48 h, and 10.98 mg/L for 72 h. The 96 h LC_{50} is the basic value in the

Table 1. Cumulative mortality of juvenile *C. mrigala* exposed to different concentrations of diazinon.

Concentrations (mg/L)	Number of dead fish				
	1 h	24 h	48 h	72 h	96 h
Control	–	–	–	–	–
5	–	–	–	–	22
10	–	16	25	35	49
15	25	30	45	64	80
20	52	63	82	94	100
25	80	100	ND		
30	100	ND			
χ^2	1.76	2.01	2.17	3.02	3.13
P	<0.05	<0.05	<0.05	<0.05	<0.05

ND = no data because of 100% mortality; (–) = no dead fish.

acute toxicity test and it was 8.15 mg/L for *C. mrigala*, while a significant difference was observed in the $LC_{10–90}$ values obtained for different times of exposure ($P < 0.05$, Table 2). Clinical symptoms observed at every 12 h of pesticide exposure included accelerated gill movement and loss of coordinated movement with fish lying on their side, or hanging vertically in the water, followed by a short period of excitation with convulsions and then a short resting and motionless period at the bottom of the aquarium. Results of the hematological profile showed that compared to the control fish, the RBC, Hb, Hct, and WBC count of exposed fish decreased significantly after acute exposure to diazinon ($P < 0.05$). On the other hand, the MCV and MCH values of exposed fish showed a significant increase ($P < 0.05$), while the MCHC values of both groups were not significantly different from each other during the study (Table 3).

4. Discussion

In the present study, the 1 and 96 h LC_{50} values of diazinon for Indian carp (*C. mrigala*) were found to be 17.32 and 8.15 mg/L, respectively. In view of this, diazinon is considered a moderately toxic substance to *C. mrigala*. The acute toxicity of diazinon varies in different fish species and ranges in tenths to several tens of milligrams per liter (13). The US Office of Pesticide Programs (14) reported the 96 h LC_{50} values of diazinon for sheepshead minnow (*Cyprinodon variegatus*) as 1.47 mg/L and for fathead minnow (*Pimephales promelas*) as 7.80 mg/L. Keizer et al. (3) reported that 96 h LC_{50} values of diazinon for zebrafish

Table 2. Lethal concentrations of diazinon depending upon time for juvenile *C. mrigala*.

Time	Lethal concentration values with 95% confidence limits (mg/L)*		
	LC_{10}	LC_{50}	LC_{90}
1 h	9.45^a (8.14–10.21)	17.32^a (15.91–18.32)	36.72^a (35.33–37.19)
24 h	7.12^b (6.42–8.24)	15.39^b (14.32–16.22)	29.18^b (27.80–30.22)
48 h	5.40^c (4.72–6.12)	12.64^c (11.32–13.44)	25.21^c (24.48–26.31)
72 h	3.21^d (–)	10.98^d (–)	21.32^d (–)
96 h	2.16^e (–)	8.15^e (–)	16.40^e (–)

(–) = no data because of $P > 0.05$. * = Lethal concentration values in columns with different letters significantly differ ($P < 0.05$).

Table 3. Hematological changes in *C. mrigala* after 96 h of exposure to 8.15 mg/L of diazinon.

Parameters	Control group (n = 12)	Exposed group (n = 12)
RBC (10^6/mm^3)	1.57 ± 0.02[a]	1.05 ± 0.04[b]
Hb (g/dL)	5.14 ± 0.21[a]	3.52 ± 0.24[b]
Hct (%)	14.11 ± 1.18[a]	9.71 ± 0.21[b]
MCV (fL)	208.14 ± 3.10[a]	218.40 ± 5.15[b]
MCH (pg)	24.31 ± 1.26[a]	28.85 ± 0.96[b]
MCHC (%)	22.26 ±1.22[a]	19.25 ± 0.46[a]
WBC (10^3/mm^3)	35.02 ± 0.13[a]	21.24 ± 0.45[b]

Different superscript letters indicate significant ($P < 0.05$) difference between the groups.

(*Brachydanio rerio*) were 8 mg/L and for guppy (*Poecilia reticulata*), 0.8 mg/L. Ahmed (9) estimated 9.76 mg/L for common carp (*Cyprinus carpio*), whereas Shamoushaki et al. (15) reported the 96 h LC_{50} value for *Rutilus frisii kutum* as 0.4 mg/L. The difference in the toxicity of diazinon may be attributed to differences in susceptibility and tolerance regarding absorption, biotransformation, and excretion of diazinon (16). The age of fish, size, and duration of exposure also affect the toxicity potential of pesticides in various fish species; the degree of sensitivity to diazinon varies even among the fish of the same genus and family (17). The 96 h LC_{50} values recorded in this study are higher and indicate that *C. mrigala* is more resistant to diazinon, as its mortality increases at higher doses and it requires longer durations of exposure compared to other fish species.

Behavioral changes in fish are important indicators of potential toxic effects of pesticide exposure. In the present study, the abnormal swimming pattern of exposed fish was recorded. Banaee et al. (18) found a similar behavioral response in common carp exposed to sublethal concentrations of diazinon. Clinical symptoms observed during acute exposure to diazinon in the present study are consistent with the findings of other authors, indicating diazinon-induced suppressed activity of acetylcholinesterase (19). The principal hematological response of Indian carp (*Cirrhinus mrigala*) to the acute exposure of diazinon in 8.15 mg/L concentrations was a significant decrease in RBC, Hb, Hct, and WBC count as compared to the control fish. These findings are consistent with the findings of some other authors studying the responses of other fishes exposed to organophosphorus pesticides. This regards changes in RBC indices after acute exposure to malathion in *Cyprinion watsoni* (4), formothion in *Heteropneustes fossilis* (5), trichlorfon in *Piaractus mesopotamicus* (20), chlorpyrifos in *Cyprinus carpio* (21), and phosalone in *Oreochromis mossambicus* (22). Similarly, decreased RBC, Hb, and Hct values after acute exposure to diazinon have been reported in fingerling European catfish (*Silurus glanis*) (8), male brood stock (*Rutilus frisii kutum*) (15), common carp (*Cyprinus carpio*) (23), and African catfish (*Clarias gariepinus*) (24). The reduction in RBC count and Hb are often accompanied by a decrease in Hct and demonstrates the physiological dysfunction of the hemopoietic system. In order to overcome hypoxic conditions in stressful media, fish usually respond by increasing the MCV and MCH of erythrocytes. In the present study, a significant increase in these indices was observed in the test fish after acute exposure to diazinon. A similar response in various fish exposed to different pesticides has also been reported in other studies. Shamoushaki et al. (15) reported decreased RBC, Hb, PCV, MCH, MCHC, and WBC counts in male brood stock (*Rutilus frisii kutum*) after long-term exposure to diazinon. Rao (25) reported carbaryl-induced increased MCV and MCH in common carp (*Cyprinus carpio*); a decrease in MCHC after acute exposure to bifenthrin in *Oncorhynchus mykiss* has also been reported (26). Khoshbavar-Rostami et al. (27) reported a decrease in RBC, Hb, and WBC values as well as an increase in MCV and MCH in *Acipenser stellatus* exposed to different concentrations of diazinon. Their findings are identical to those of the present study. The increase in MCV and MCH values after exposure to diazinon indicates that a reduced RBC count may be due to the destruction of erythrocytes or their decreased synthesis in bone marrow (28). Significant decreases in the total WBC count of *C. mrigala* after acute exposure to diazinon in the present study supports the results of previous reports (8,15). Ali and Rani (22) also reported decreased WBC counts in tilapia exposed to phosalone. Similarly, a suppressed nonspecific immunity as a result of decreased WBC counts and changes in differential count after acute exposure to diazinon has also been reported in *C. carpio* and *C. gariepinus*, respectively (23,29). Finally, from the results of this study, it is concluded that diazinon is moderately toxic to Indian carp (*Cirrhinus mrigala*). Diazinon exposure in low concentrations produced significant changes in some hematological parameters of *C. mrigala* and caused stress to the fish. Therefore, it is suggested that preventive measures be taken to avoid the possible contamination of aquatic environments when diazinon is used in agricultural fields near freshwater reservoirs.

Acknowledgment

The authors are thankful to Prof M Ayub Qureshi, Head of Department of Zoology, Govt. Superior Science College, Shah Faisal Colony, Karachi, Pakistan, for providing all the facilities for the successful completion of this study.

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