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Impact of some chlorinated pesticides on the haematology of the fish *Cyprinus carpio* and *Puntius ticto*

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Abstract: The effects of pesticides on blood characteristics and histological changes in erythrocytes of the fish species *Cyprinus carpio* and *Puntius ticto* were studied. The fishes were exposed to sub lethal concentrations of different chlorinated pesticides namely aldrin, dieldrin, DDT, BHC and chlordane for 10, 20 and 30 d in continuous flow-through test. The LC_{50} values were calculated based on acute toxicity tests and the sublethal doses were arrived at for chronic bioassay studies. Results showed an increase in haemoglobin content of both *Cyprinus carpio* and *Puntius ticto* in case of aldrin and dieldrin. Haemoglobin content reduced from an initial 13 g/100 ml to 8.07 and 10.15 g/100 ml in case of *Cyprinus* at the end of ten days exposure to aldrin and dieldrin respectively, and gradually increased to 8.7 g/100 ml and 10.15 g/100 ml after 20 d of exposure. The haemoglobin content after 30 d exposure to aldrin and dieldrin was 10.15 g/100 ml and 11.6 g/100 ml respectively. In case of *Puntius ticto*, the haemoglobin content in control fishes recorded was 12.8 g/100 ml while in case of fish exposed to aldrin, the haemoglobin content reduced initially on ten days exposure to 10.15 g/100 ml and increased to 11.6 g/100 ml and 13.0 g/100 ml during twenty days and thirty days exposure respectively. This trend was also observed with dieldrin in both the fishes studied. Red blood cells were also counted in case of all the pesticides and exposure periods with respect to *Cyprinus carpio* and *Puntius ticto*. Irrespective of the species and pesticide, the RBC counts uniformly showed decreasing trend with the increase in exposure period, while packed cell volume, PCV(%) showed increasing trend with respect to increase in exposure period in case of aldrin and dieldrin in both the fishes. But DDT, BHC and chlordane showed decreasing trend in PCV(%) values with increasing periods of exposure.

Keywords: histology; aldrin; dieldrin; DDT; BHC; chlordane

Introduction

Chlorinated hydrocarbon pesticides have been of much interest for years because of their persisting nature in the environment including fish, soil and plants. Chlorinated pesticides are very difficult to degrade in nature and hence get accumulated in the flora and fauna.

Fish are in direct contact with the aquatic environment and are susceptible to any environmental changes that may occur. It is expected that some changes would be reflected in the physiology of the fish, particularly in haematological parameters(Blaxhall, 1972). Literature is available on the effect of few chlorinated pesticides like lindane on catfishes (Morgan, 1989; Srivastava, 1985; Rabindranath, 1996; Mishra, 1997), rainbow trout(Betoulle, 2000), zebrafish (Ensenbach, 1995), DDT and dieldrin on the blood of *Channa punctatus* (Lone, 1976), liver of *Parma microlepis* (Tricklebank, 2000) and aldrin on the haematological changes in *Channa punctatus*, *Heteropneustes fossilis* and *Sarotherodon mossambicus* (Mahajan, 1979; Bhargava, 1997; Ghosh, 1986).

Substantial amount of non-biodegradable organochlorine pesticides can get into aquatic ecosystems particularly during monsoon runoff from agricultural fields and reach the body of fish through food chain. Reports are available on the impact of pesticides on haematology of fish. In the Mississippi River fish kills of 1963, the fish were characterized by low red and white blood cell counts and haematocrit values decreased by 50%(Mount, 1966). It is also reported that haemoglobin decreases without change in the erythrocyte count during prolonged exposure of fish to DDT.

Cyprinus carpio exposed to endosulfan showed progressive increase of red blood corpuscle haemoglobin and packed cell volumes and significant alterations of haematological indices were noticed (Naidu, 1987). Literature is available on the effect of certain chlorinated pesticides like chlordane, lindane, endosulfan and also some other pesticides on the haematological alteration in different fishes(Verma, 1979; Madhu, 1984; Rangaswamy, 1984; Agrawal, 1988; Bansal, 1979).

Though little information is available on the toxic effects of different pollutants on fish blood, no detailed studies are available on the toxicity of aldrin, dieldrin, DDT, BHC, etc. on *Cyprinus carpio* and *Puntius ticto*. Haematological studies have become very important parameter in toxicity evaluation studies and hence needs thorough investigation. Therefore an attempt has been made to study the haematological changes induced by aldrin, dieldrin, DDT, BHC and chlordane in *Cyprinus carpio* and *Puntius ticto*.

1 Materials and methods

Fresh water fish *Cyprinus carpio* and *Puntius ticto* were purchased from local fishing contractors. Fish were acclimatized to laboratory conditions in a laboratory scale aquarium of 500 L capacity for about ten days. The test fish were fed regularly with fish food. Fishes of uniform size were selected for the experiments. The aerated and dechlorinated tap water was used as dilution water in all experiments. Dilution water was characterized as per standard methods for the examination of water and wastewater, for few routine

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parameters as indicated in Table 1. After determining LC_{50} values for all the pesticides, sub lethal doses were selected for carrying out chronic toxicity evaluation, for a period of 30 d. Feeding was stopped 24 h before the commencement of the chronic tests. Acute toxicity evaluation was carried out as indicated in the literature.

Table 1 Characteristics of the dilution water

Parameters	Values
Temperature	25—27℃
pH	7.9—8.2 mg/L
Dissolved oxygen	6.6—7.4 mg/L
Total alkalinity as $CaCO_3$	148—180 mg/L
Total hardness	136—160 mg/L
Ca hardness	60—78 mg/L
Mg hardness	76—82 mg/L
Calcium	24—31 mg/L
Magnesium	20—24 mg/L
Sodium	30—32 mg/L
Potassium	2—6 mg/L

An improved continuous dosing unit fabricated in the institute was used for continuous flow through of the test water concentrations and was so regulated as to replace the test solution once in 24 h (Srinivasan, 1970). A total of ten fishes were exposed to different pesticide concentrations. The experiments were normally carried out for a period of ten days and the fish were removed and fed with aquatic crustaceans from the oxidation pond for two days, after which they were again exposed to the pesticide concentration in the experimental aquaria. Each pesticide concentration was investigated for a period extending over 10, 20 and 30 d. Experiments was run in duplicates for all the chlorinated hydrocarbons with both fish species. Separate control was also run simultaneously. After every 24 h, fresh test solution was introduced. Fish were taken out from control and experimental tanks at an interval of 10, 20 and 30 d and examined for residue accumulation, histopathology and haematology.

For haematological studies, blood samples were collected from the caudal vein by cutting a part of the tail transversely. Blood smears were taken on the slides stained with Leishman and dried to see the changes in erythrocytes through microscope. Percent haemoglobin was estimated by Sahlis acid hematin method (Dacie, 1975). Packed cell volume (PCV) was estimated in wintrobes capillary tubes. RBC count or total erythrocyte count was made with Neubaure's double haemtocytometer (Davidson, 1969).

2 Results and discussion

The effects of five chlorinated pesticides on fresh water fishes *Cyprinus carpio* and *Puntius ticto* were studied. The LC_{50} values with safe application rate (SAR) and safe factor (SF) are summarized in Tables 2 and 3. A perusal of LC_{50} values for both fish exposed to 24, 48, 72 and 96 h to five chlorinated pesticides indicated BHC to be least toxic while DDT to be most toxic in major cases. Pesticides are known to alter the blood parameters of fishes. A significant decrease in haemoglobin content and packed cell volume has been reported in fish exposed to different pesticides. This decreasing trend may be attributed to a condition of hypochronic microcytic anaemia.

Table 2 LC_{50} (TLM) values for chlorinated hydrocarbons pesticides

Pesticides	Fish	TLM (LC_{50}), mg/L		
		24	48	96
Aldrin	*Cyprinus*	0.026	0.020	0.0185
	Puntius	0.078	0.048	0.027
Dieldrin	*Cyprinus*	0.016	0.0145	0.0097
	Puntius	0.045	0.031	0.018
DDT	*Cyprinus*	0.027	0.0069	0.0024
	Puntius	0.080	0.070	0.049
BHC	*Cyprinus*	2.8	2.6	2.2
	Puntius	8.20	6.20	4.40
Chlordane	*Cyprinus*	0.074	0.072	0.039
	Puntius	0.069	0.069	0.052

Table 3 Safe factor and safe application rates* of *Cyprinus carpio* and *Puntius ticto*

Pesticides	*Cyprinus carpio*		*Puntius ticto*	
	SF	SAR	SF	SAR
Aldrin	0.500	0.009	0.066	0.002
Dieldrin	0.500	0.005	0.461	0.008
BHC	0.400	0.880	0.125	0.550
DDT	0.133	0.003	0.250	0.012
Chlordane	0.153	0.006	0.375	0.019

Notes: * Based on 96 h values; SF. safe factor; SAR. safe application rate

During the experimentation no mortality of fish was observed in control aquarium while the fish in experimental containers showed characteristics change in behavior, they became restless and also lethargic. Haematological studies showed important changes in RBC count, haemoglobin content and packed cell volume (PCV) in test fishes *Cyprinus carpio* and *Puntius ticto* exposed to sublethal concentrations of aldrin, dieldrin, DDT, BHC and chlordane, as indicated in Tables 4, 5 and 6. The values of RBC count and haemoglobin content obtained in the present study in the control fish *Cyprinus carpio* and *Puntius ticto* are well within the ranges reported in literature.

Table 4 Changes in RBC count in fishes exposed to sublethal concentration of chlorinated pesticides

Pesticides	*Cyprinus carpio*, exposure time days			*Puntius ticto*, exposure time days		
	10*	20	30	10	20	30
Aldrin	2.66 ± 0.22	2.56 ± 0.19	2.38 ± 0.18	2.72 ± 0.36	2.64 ± 0.16	2.60 ± 0.18
Dieldrin	2.44 ± 0.26	2.40 ± 0.18	2.39 ± 0.17	2.49 ± 3.4	2.46 ± 0.18	2.40 ± 0.14
BHC	2.84 ± 0.36	2.80 ± 0.18	2.76 ± 0.20	2.74 ± 0.19	2.70 ± 0.28	2.64 ± 0.18
DDT	2.36 ± 0.18	2.33 ± 0.26	2.32 ± 0.22	2.12 ± 0.22	2.00 ± 0.26	1.98 ± 0.28
Chlordane	2.76 ± 0.45	2.70 ± 0.26	2.64 ± 0.28	2.58 ± 0.19	2.52 ± 0.30	2.47 ± 0.21
Control	2.96 ± 0.12	2.94 ± 0.11	2.94 ± 0.10	2.76	2.76	2.74

Notes: * Readings are average of 5 d; ± *S.D.*

Table 5 Percent haemoglobin content of *Cyprinus carpio* and *Puntius ticto* exposed to different chlorinated pesticides

Pesticides	Dose, mg/L	10 d	20 d	30 d
Cypdrinus carpio				
Aldrin	0.005	8.07 ± 0.12	8.7 ± 0.14	10.15 ± 0.18
Dieldrin	0.002	10.15 ± 0.03	10.15 ± 0.14	11.6 ± 0.20
BHC	1.000	0.15 ± 0.04	8.7 ± 0.30	8.3 ± 0.12
DDT	0.002	8.7 ± 0.12	7.25 ± 0.22	7.0 ± 0.14
Chlordane	0.010	1.8 ± 0.21	8.7 ± 0.20	7.25 ± 0.15
Control	-	13.0 ± 0.12	13.0 ± 0.14	13.0 ± 0.11
Puntius ticto				
Aldrin	0.005	10.15 ± 0.22	11.6 ± 0.22	13.0 ± 0.26
Dieldrin	0.001	7.25 ± 0.14	10.15 ± 0.12	10.55 ± 0.18
BHC	1.00	10.15 ± 0.12	8.7 ± 0.11	7.25 ± 0.14
DDT	0.001	8.70 ± 0.04	7.25 ± 0.16	5.8 ± 0.12
Chlordane	0.002	11.6 ± 0.04	8.7 ± 0.10	8.2 ± 0.11
Control	-	12.8 ± 0.03	12.8 ± 0.04	12.8 ± 0.02

Note: * Average of five numbers of values will *S. D.*

Table 6 Packed cell volume(%) of blood of *Cyprinus carpio* and *Puntius ticto* exposed to different chlorinated pesticides

Pesticides	Dose, mg/L	10 d	20 d	30 d
Cyprinus carpio				
Aldrin	0.005	34.64 ± 1.2	35.86 ± 1.42	39.28 ± 2.6
Dieldrin	0.002	35.05 ± 2.4	37.9 ± 3.6	39.4 ± 4.0
BHC	1.000	33.24 ± 4.0	31.08 ± 3.1	30.62 ± 2.6
DDT	0.002	32.24 ± 1.9	26.72 ± 2.2	24.36 ± 2.0
Chlordane	0.010	32.64 ± 2.9	30.00 ± 2.9	26.46 ± 3.0
Control	-	35.68 ± 2.2	-	-
Puntius ticto				
Aldrin	0.005	33.68 ± 1.82	36.24 ± 2.6	38.82 ± 2.9
Dieldrin	0.001	30.46 ± 2.6	34.26 ± 3.6	36.74 ± 3.2
BHC	1.000	33.72 ± 4.4	30.20 ± 3.2	28.82 ± 1.7
DDT	0.001	31.42 ± 4.4	29.46 ± 2.9	22.32 ± 1.9
Chlordane	0.002	33.88 ± 2.9	28.64 ± 4.0	27.44 ± 3.2
Control	-	34.12 ± 1.1	-	-

It is seen from the results that haemoglobin content of both the fish studied significantly decreased and it was more pronounced in case of *Puntius ticto*. In general, there was more reduction in haemoglobin content after ten days exposure in case of aldrin, dieldrin while DDT, BHC and chlordane showed less reduction and then it slowed down during twenty and thirty days exposure in both the fish. Chlordane showed a minimum decrease in haemoglobin content after ten days exposure and it was around 9.2% and 9.37% in *Cyprinus carpio* and *Puntius ticto* respectively and increased suddenly to 33% and 44.23% during twenty and thirty days exposure in case of *Cyprinus carpio* and 32.03% and 35.93% in case of *Puntius ticto*. Maximum reduction was observed in case of DDT, exposed to 30 d with 44.23% and 54.68% in case of *Cyprinus* and *Puntius* respectively.

Decrease in haemoglobin content may be attributed to the stress induced by the pesticides and reduced respiratory rate which in turn effects oxygen intake and leads to iron deficiency. Similar trend is also reported in case of catfish exposed to aldrin(Yadav, 1994). It can be inferred from the studies that chlordane is least toxic and dieldrin the most toxic of the pesticides with regard to haematology while studies on bioaccumulation indicated DDT to be the most toxic and BHC the least toxic pesticide(Shanta, 1999). With respect to haematology the toxicity in decreasing order was dieldrin > DDT > aldrin > BHC > chlordane. DDT, BHC and chlordane showed almost constant reduction in haemoglobin content in both the fishes(Table 7) i.e. 33% and 32.03%, 21.9% and 20.7% and 9.2% and 9.37% respectively during exposure time of ten day. This same trend was observed during both twenty and thirty day exposure period.

Table 7 Reduction in haemaglobin content in carps exposed to chlorinated pesticides

Pesticides	*Cyprinus carpio*, exposure time days			*Puntius ticto*, exposure time days		
	10	20	30	10	20	30
Aldrin	37.9	33.07	21.92	20.70	9.37	-
Dieldrin	21.92	21.92	10.76	43.33	20.70	17.50
BHC	21.90	33.07	36.15	20.75	32.03	43.35
DDT	33.07	44.23	46.15	32.03	43.33	54.68
Chlordane	9.2	33.0	44.23	9.37	32.03	35.93

While aldrin and dieldrin showed enormous variation in their impacts, aldrin was more toxic in case of *Cyprinus* than in case of *Puntius ticto* as seen from the percent reduction of haemoglobin content(Table 7). Dieldrin showed a toxicity of 21.9% in case of *Cyprinus* on ten days exposure but was around 43.35% in case of *Puntius ticto* under the same exposure time, which already indicated that species specificity plays an important role in evoking response to a particular toxicant. Similar trend in haemoglobin content in fish *Ophiocephalus punctatus* exposed to endosulfan has been reported(Tyagi, 1989).

Initial trend of the impact of aldrin and dieldrin on the haemoglobin content was similar to other pesticides but there appeared a gradual increase in haemoglobin content after twenty and thirty days exposure in case of both *Cyprinus* and *Puntius*. A similar trend has been reported in case of impact of aldrin on *Channa punctatus*(Mahajan, 1979).

Packed cell volume(PCV) also followed the trend like that of haemoglobin in case of aldrin and dieldrin i.e. longer the exposure time, more was the packed cell volume. Whereas in case of DDT, BHC and chlordane, PCV values showed decreasing trend with increasing exposure time as indicated in Table 5. While RBC count showed decreasing trend irrespective of exposure time, type of pesticides used and the fish species. The gradual increase in haemoglobin content and packed cell volume of the *Cyprinus* and *Puntius* might be due to hypoxic condition induced by chlorinated pesticide while RBC count reduced gradually irrespective of increased haemoglobin and PCV in both the fishes exposed to aldrin and dieldrin.

Hypoxic condition leads to more synthesis of haemoglobin to compensate the losses due to aldrin and dieldrin intoxication. This may facilitate transport of more oxygen to the tissues. Similar trend has been reported in literature on *Tilapia massambica* exposed to endosulfan (Rangaswamy, 1984). Increase in haemoglobin and PCV values were also reported in *Labeo rohita* and *Saccobranchus fossilis* during chronic exposure to chlordane(Agrawal, 1988; Verma, 1979).

Fish exposed to chlorinated pesticides even in minute quantities may induce toxic effect and the gills are the first organs to get affected. In the process to cope with the respiratory problems, increase in oxygen intake sets in, which lead to more haemoglobin synthesis. It can be inferred from the studies that the prolonged exposure of fish to even very minute doses of pesticides may pose greater risk for their

survival, on longer time period i.e. chronic toxicity sets in.

Detailed microscopical studies of slides of blood smears showed marked destruction of erythrocytes. In the DDT and BHC exposed fishes, the damage to the red blood cells was maximum, though acute toxicity data and bioaccumulation of pesticides in different tissues showed DDT to be most toxic and BHC the least (Shanta, 1999; 1985). In most cases nucleus was enlarged and extruded, whereas cytoplasm was in the process of degeneration. Erythrocytes in general showed enlargement compared to control cells with respect to all the pesticides. *Cyprinus carpio* exposed to aldrin, dieldrin, BHC and DDT for a period of 30 d showed more cell damage. A vacuole was developed between the cytoplasm and nuclear wall. While *Cyprinus* exposed to chlordane showed serration of cell wall and nucleus was in the verge of extrusion. In BHC exposed fish, cell wall of erythrocytes was completely dissolved with a naked and enlarged nucleus, which was on the verge of disintegration. The granulocytes were observed to be in various stages of destruction. Similar trend was observed in case of *Puntius ticto* also.

3 Conclusions

The studies revealed massive destruction of blood cells in case of both *Cyprinus carpio* and *Puntius ticto* exposed to chlorinated pesticides even at sublethal levels. Irrespective of species and pesticide, the red blood cell counts uniformly shoed decreasing trend with the increase in exposure period, while packed cell volume showed increasing trend with respect to increase in exposure period in case of aldrin and dieldrin in both the fishes. However, BHC, DDT and chlordane showed decreasing trend in PVC values with increasing period of exposure. It indicated that enough care must be taken to prevent discharge of effluent containing even minute quantities of pesticides into water bodies.

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