

CHANGE OF ACUTE TOXICITY AND HEMATOLOGICAL PARAMETERS OF PSEUDOBAGRUS FULVIDRACO EXPOSED TO LINDANE

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Abstract : To determine the acute toxicity of pesticide in freshwater ecosystem and its effect on hematological parameters of aquatic organisms, the short-term toxicity screening test and the bioassay experiments were performed using lindane, an organochlorine insecticide and potential toxic pollutant, and bagrid catfish (*Pseudobagrus fulvidraco*). The experiments were repeated in triplicates and the time-LC₅₀ values were determined at 24, 48, 72, and 96 hour, employing the static test method and the water temperature regulated at 23±1°C. The time-LC₅₀ values for bagrid catfish were estimated 38.83, 33.84, 30.30, and 28.43 µg/l for 24, 48, 72, and 96 hour respectively. The hematological parameters, such as red blood cell count, hemoglobin concentration and hematocrit, were decreased with increasing exposure concentrations of lindane, maybe due to the disruptive action of lindane on the erythropoietic tissue.

Key Words : Hematological parameters, Lindane, 96 hr-LC₅₀, *Pseudobagrus fulvidraco*

INTRODUCTION

Contaminants such as organochlorine pesticides in aquatic ecosystem cause various harmful effects on human and wildlife.¹⁾ Organochlorine insecticidal agents, in particular, are stable compounds that persist in the environment and, therefore, are increasingly identified as agents of chronic exposure in aquatic species.^{2~4)}

Presently available lindane contains gamma isomer of 1,2,3,4,5,6 -hexachlorocyclohexane (γ -HCH), whereas the previously produced commercial HCH known as benzene hexachloride (BHC) contained a mixture of α , β ,

γ , δ , and ϵ isomers.⁵⁾ BHC has not been produced since 1978 because of resistance to degradation, but still remains an important environmental contaminant.

Despite the use of lindane (γ -HCH) being banned worldwide, it is still one of the most commonly employed agricultural insecticides and its considerable persistence has made it one of the most common aquatic contaminants. Lindane residues have been detected in continental surface waters^{6,7)} and coastal marine waters.⁸⁾ Although its bioaccumulation is low compared to other organochlorine pesticide (bioconcentration factor for lindane in fish ≤ 800),⁹⁾ relatively high levels of lindane residues have been measured in wild fish caught in fresh and marine waters.¹⁰⁾

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chemicals are commonly extrapolated using an acute toxicity test of short duration.¹¹⁾ Lindane have been reported to be extremely toxic to fish.¹²⁾ The acute toxicity of lindane in fish varies greatly; the 96 hr-LC₅₀ is 9 µg/L for *Menidia menidia*, 77 µg/L for *Lepomis macrochirus*, 138 µg/L for *Lebistes reticulatus*, 1,529 µg/L for *Carassius sp.*, and 670 µg/L for *Anguilla anguilla*.^{13,14)} Also, the 96 hr-LC₅₀ values for fish (*Etroplus maculatus*), prawn (*Macrobrachium idella idella*), clam (*Villorita cyprinoides var Cochinesis*) and crab (*Parathelphusa hydrodromous*) were 700, 12.5, 13,000 and 5,800 µg/L, respectively.¹⁵⁾

Blood parameters are increasingly being used as indicators of the physiological or sublethal stress response to endogenous or exogenous changes in fish. The evaluation depends on the availability of reference values as close as possible to normal values of the various blood components considered as reliable descriptors of healthy fish under natural conditions. Hematological analysis of blood often provides valuable information for diagnosis and surveillance of general health.^{16,17)}

Research on the acute toxicity of pesticide in bagrid catfish has been surprisingly scarce and particularly toxicity endpoints such as haematological effects have rarely been addressed. This study may give helpful information on toxic effects of lindane in bagrid catfish. The aim was to determine the acute toxicity of lindane, a chlorinated insecticide, and to observe its haematological effects on bagrid catfish, using the static test system.

MATERIALS AND METHODS

Bagrid catfish, *pseudobagrus fulvidraco* were obtained from the Inland Fisheries Research Institute, located in Chungcheong buk-do, South Korea. Healthy fish in the weight range of 4.69±0.31 g were selected and acclimated to the laboratory condition where temperature was kept at 23±1°C for two weeks. Fish were fed twice a day with commercial fish feed which contains

Figure 1. Photograph of experimental apparatus.

45% protein, 7% lipid, and 20% carbohydrate (Purina Ltd., Korea) and the feed was withdrawn a day prior to the experimentation to avoid metabolic differences. Test chamber (glass aquaria, 30L capacity each) were filled with 25L of freshwater. All tests were performed semi static and the test solutions were totally renewed at 24 hr intervals. All the determination were repeated in triplicates. Groups of experimental animals, each consisting of 10 individuals, were randomly selected and placed into aerated aquaria. The photograph of experimental apparatus is shown in Figure 1.

Physicochemical characteristics of the experimental water, measured by the Standard Methods,¹⁸⁾ were as follows: pH 7.9±0.5, temperature 23±1°C, dissolved oxygen (DO) 7.2±0.5 mg/L, chemical oxygen demand (COD_{cr}) 1.84±0.25 mg/L, hardness 113±15 mg/L as CaCO₃, and lindane not detectable.

Technical grade lindane (γ-hexachlorocyclohexan, 97%) was from the Aldrich. Dosing solutions were prepared from this stock by diluting with acetone to give the dosing concentrations of 50, 75, 100, 125, and 150 µg/L. After 24 hr of adaptation, the different concentrations of lindane in acetone were added to the experimental aquaria. During the last 24 hr of adaptation and throughout the duration of the experiment, animals were not fed to avoid metabolic differences. Mortality was assessed at 24, 48, 72 and 96 hr after the start of the tests. Dead individuals were removed immediately. The bioassay system was followed according to

the standardized methods.^{18,19)}

At the end of each period, fish were anesthetized in buffered 3-aminobenzoic acid ethyl ester methanesulfonate (Sigma Chemical, St. Louis, MO, USA) and blood samples were taken from caudal vein. The procedure of anesthesia, measurement, and blood withdrawal generally took less than less than 3 min per fish with minimum disturbance. Haematological parameters were evaluated as follows. Hematocrit (Ht) was determined by the microhematocrit technique using capillary tubes with centrifugation at 12,000 g for 5 min. Hemoglobin (Hb) concentration was determined spectrophotometrically at 540 nm using the cyanomethemoglobin method and expressed as g/dL of sample. Red blood cell (RBC) counts were estimated using the improved Neubauer hemocytometer.²⁰⁾ All the procedures used were based on the haematological techniques described by Tvedten²¹⁾ and Campbell.²²⁾

One way analysis of variation (ANOVA) was used to test the hypothesis that the mean LC₅₀ values obtained at different times were equal. Differences between LC₅₀ values of the 24 hr test and the 96 hr acute bioassay were evaluated using a two-tailed t-test.

RESULTS AND DISCUSSION

As shown in Table 1, the calculated time-acute LC₅₀ values along (95% confidence limits also shown) of lindane dissolved in acetone, using a static bioassay system, for bagrid catfish were 38.83 $\mu\text{g/L}$ for 24hr, 33.84 $\mu\text{g/L}$ for 48hr, 30.30 $\mu\text{g/L}$ for 72hr and 28.43 $\mu\text{g/L}$ for 96hr, respectively, and the results show lindane highly toxic to fish. The control mortality was zero.

According to the USDA National Agricultural Pesticide Impact Assessment Program's EXTONET document,¹²⁾ lindane acute toxicity to fish in laboratory tests is reported 96 hr-LC₅₀ values ranging from 1.7 to 90 $\mu\text{g/L}$ in trout (rainbow, brown, and lake), coho salmon, carp, fathead minnow, bluegill, largemouth bass, and yellow perch. Geyer et al.²³⁾ exposed 16 fish species to

Table 1. Acute toxicity value of lindane for bagrid catfish, *Pseudobagrus fulvidraco*

Exposure time (hr)	LC ₅₀ ($\mu\text{g/L}$)	95% Confidence Limit	
		Upper Limit ($\mu\text{g/L}$)	Lower Limit ($\mu\text{g/L}$)
24	38.83	44.58	31.15
48	33.84	40.48	27.50
72	30.30	37.05	23.10
96	28.43	33.20	23.28

various concentrations of lindane. The 48 hr LC₅₀ of the toxicant to these species ranged from 22 to 900 $\mu\text{g/L}$ (mean, 150 $\mu\text{g/L}$). Also, the 96 hr-LC₅₀ ranged from 1.7 to 32 $\mu\text{g/L}$ for trout and salmon and from 44 to 131 $\mu\text{g/L}$ for catfish, perch, and goldfish. These imply the acute toxicity of lindane for cold water fish considerably higher than that for bagrid catfish, which, however, slightly lower than that for warm water fish.

Figure 2, 3, and 4 shows the blood parameters for the fish, such as red blood cell (RBC) count (Fig. 2), hemoglobin (Hb) concentration (Fig. 3), and hematocrit (Ht) value (Fig. 4), decreasing with increasing exposure concentrations of lindane. There were statistically significant changes, relative to the control groups, in these hematological parameters analyzed. The

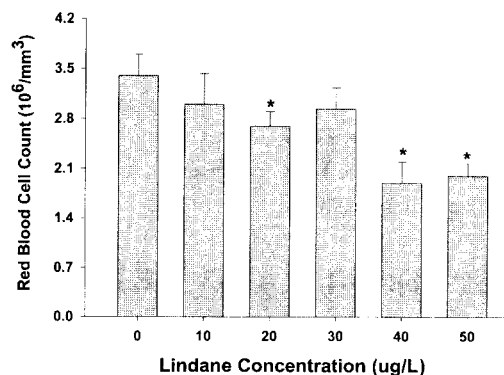


Figure 2. Changes of red blood cell count in *Pseudobagrus fulvidraco* exposed to various concentrations of lindane for 96 hr. Vertical bars indicate standard error (Means \pm S.E., n=10). *; significant difference between control and the others; $p < 0.05$

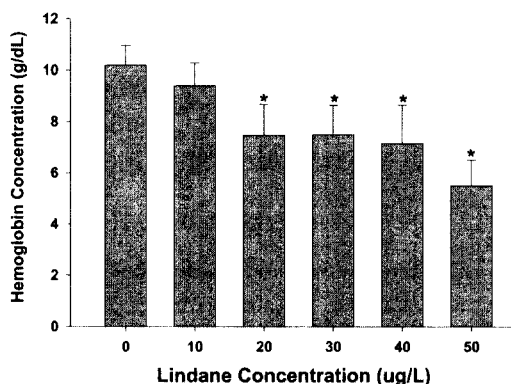


Figure 3. Changes of hemoglobin concentration in *Pseudobagrus fulvidraco* exposed to various concentrations of lindane for 96 hr. Vertical bars indicate standard error (Means±S.E., n=10). *: significant difference between control and the others; p<0.05

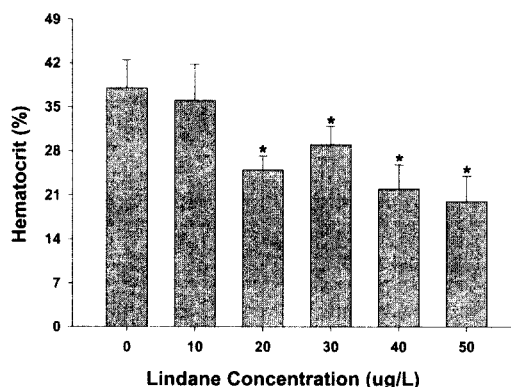


Figure 4. Changes of hematocrit value in *Pseudobagrus fulvidraco* exposed to various concentrations of lindane for 96 hr. Vertical bars indicate standard error (Means±S.E., n=10). *: significant difference between control and the others; p<0.05

predominant hematological finding was a highly significant decrease of RBC count, Hb concentration, and Ht values in fish exposed to 20 µg/L of lindane, thereby indicating a severe anemia (P<0.05). The mean values in the control fish were $3.40 \pm 0.30 \times 10^6 \text{ mm}^3$ for RBC count, $10.20 \pm 0.75 \text{ µg/dL}$ for Hb concentration, and $38.00 \pm 4.50\%$ for Ht value, respectively, during the experiment.

Hematological elements were selected because

blood reflects all the life processes in the body and serves as an indicator of its general condition or metabolic defects. It is widely accepted that the excessive environmental stress causes a variety of detectable, recognizable changes in blood of fish. The change of hematological levels of fish on exposure to organochlorinate pesticides including lindane has been reported by several workers. Bakthavathsalam²⁴⁾ found significant decreases in the Hb concentration of *Anabas testudineus* exposed to lindane, and remarkable differences in the RBC counts between fish treated with lindane and control groups. Nath and Jaipuray²⁵⁾ also reported a reduction of RBC in *Heteropneustes fossilis* under sublethal concentrations of lindane (0.8 µg/L) after 90 days. Ramanujam and Mohanty²⁶⁾ reported the effect of sublethal concentrations of Thiodan® (endosulfan), an organochloride pesticide, on the hematological parameters of a catfish, *H. fossilis*. In both time durations (6 and 10 days) studied, RBC, Hb, MCH (mean corpuscular haemoglobin), and MCHC (Mean corpuscular haemoglobin concentration) decreased significantly, and these changes were relatively higher in fish exposed to the pesticide for 10 days.

The decrease in these levels may be due to the disruptive action of the lindane on the erythropoietic tissue of bagrid catfish, as a result of which the viability of the cells might be affected.

CONCLUSION

Results from this study on changes in time-LC₅₀ values and hematological parameters for bagrid catfish acutely exposed to lindane showed the followings:

1. Time-LC₅₀ values of lindane dissolved in acetone, using a semi static bioassay system, for bagrid catfish were 38.83 µg/L for 24hr, 33.84 µg/L for 48hr, 30.30 µg/L for 72hr and 28.43 µg/L for 96hr, respectively.
2. After 96hr exposure to lindane, RBC counts, Hb concentration, and Ht levels of bagrid

catfish were declined, reflecting erythrocyte hemolysis and/or hemorrhaging from damaged gill filaments. The decreased in these parameters may be due to the disruptive action of the lindane on the erythropoietic tissue, as a result of which the viability of the cells might be affected.

3. It was concluded that bagrid catfish was particularly sensitive to lindane so may be used as a sentinel species in environmental toxicity studies.

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