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**Full Length Article**

# Acute Toxicity of Endosulfan to the Fish Species *Catla catla*, *Cirrhina mrigala* and *Labeo rohita*

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## Abstract

Acute toxicity (96-h $LC_{50}$ and lethal concentrations) of endosulfan to the fish viz. *Catla catla, Cirrhina mirgala* and *Labeo rohita* of 90-, 120- and 150- day age groups were determined in static water bioassay with three replications for each test dose. Water temperature (29-30˚C), pH (7.5) and total hardness (300 mg $L^{-1}$) were kept constant during acute toxicity trials. Both 96-h $LC_{50}$ and lethal concentrations of endosulfan for all the three fish species varied significantly among all age groups. *C. catla* was more sensitive to endosulfan with the mean $LC_{50}$ concentration of 0.98 µg $L^{-1}$, followed by that of *C. mrigala* and *L. rohita* with the mean concentrations of 1.06 and 2.15 µg $L^{-1}$, respectively. Mean lethal endosulfan concentrations of fish ranged between 1.60 µg $L^{-1}$ (for *C. catla*) and 3.26 µg $L^{-1}$ (for *L. rohita*) with significant differences. Fish age groups exhibited significant differences for their both $LC_{50}$ and lethal concentrations. However, 90-days old fish were significantly more sensitive to endosulfan, while 150-days age fish were least sensitive. Thus, the sensitivity of fish to endosulfan was negatively correlated with age. 

**Keywords:** Fish; Endosulfan; 96-h $LC_{50}$; Lethal concentrations; Age groups

## Introduction

The contamination of natural freshwaters with pesticide residues has become more serious issue all over the world because of their toxic effects on the aquatic animals (Indirabai *et al*., 2010; Muhammad *et al*., 2012; Naz and Javed, 2012). These pollutants are transported from industry, agriculture run off, municipal and man made activities into the aquatic environments. The chemical persistence of these compounds is causing alarmingly high levels of xenobiotic chemicals in the aquatic ecosystems (Brack *et al*., 2002; Diez *et al*., 2002; Naeem *et al*., 2011). Few chemicals are biologically degradable and become harmless, while others are biologically non-degradable and sustain in the aquatic environments for a long period of time. Therefore, there is a growing concern worldwide regarding indiscriminate use of such chemicals that can cause toxicity to the aquatic organisms (Weber *et al*., 2010).

Organochlorines (OCs) are the most widely used class of insecticides in the world and considered to be the most hazardous with respect to environmental pollution. They are persistent and non-biodegradable and their residues buildup in the aquatic food chain and remain there for a long period of time and thus show neurotoxicity to the animals (Adetola *et al*., 2011). Endosulfan, a compound of cyclodiene subgroup of organochlorine pesticides, is used extensively in Pakistan to control insect pests in fruit crops, vegetables, oilseed and cereal crops (Tariq *et al*., 2007). An extensive usage of endosulfan increases its frequency in the aquatic environments even at considerable distances from the point of its original application (Miles and Pfeuffer, 1997). This has also been detected in the soil, sediments, surface and river waters, food stuffs and atmosphere (Tariq *et al*., 2007). The toxicity of endosulfan has created a situation to cause serious damage to the fish fauna in the natural water bodies (Panday *et al*., 2006). Ecological damage caused by anthropogenic factors to the environment, besides presence of other metallic ions, may affect the fish health (Javed and Saeed, 2010). Acute toxicity tests ($LC_{50}$ and lethal) describe the sensitivity of fish towards various chemicals (Abduallah and Javed, 2006). In these tests, mortality data are used to determine the time-specific responses of various fish species to toxicants that could be used for sustainable conservation of indigenous fish species in Pakistan.

Acute toxicity tests are designed to measure the effects of toxic agents on aquatic species during a short period of their life span (Ebrahimpour *et al*., 2010; Javed, 2012a; 2012b) and allow us to assess the effects of various pollutants on the biology of fish (Javed and Saeed, 2010) to find out their abilities to adapt under certain toxicity levels and to forecast possible effects of toxicity on them (Rauf *et al*., 2009; Azmat *et al*., 2012). Although the effect of endosulfan on several fish species has been investigated in several studies but in Pakistan is virtually no scientific documentation on endosulfan's effect on the major carps. Hence, the present investigation, acute toxicity of

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endosulfan, in terms of 96-h $LC_{50}$ and lethal concentrations was determined for major carps viz. *C. catla*, *C. mrigala* and *L. rohita* in order to develop strategies for sustainable conservation of these cyprinids in their natural aquatic habitats.

## Materials and Methods

Acute tests (96-h $LC_{50}$ and lethal concentrations) were conducted with three age groups (90-, 120- and 150-day) of *C. catla*, *C. mrigala* and *L. rohita* to determine their sensitivity against endosulfan. Fingerlings of major carps were obtained from Fish Seed Hatchery, Faisalabad with the following mean average weight, average total and fork lengths (Table 1).

All the fish were acclimatized to laboratory conditions for one week before the start of acute toxicity tests and fed 30% digestible protein with 3 kcal/g digestible energy. However, they were not fed during acute toxicity tests. The technical grade (98.50% purity) endosulfan was dissolved in methanol to prepare stock-1 (1 g/100 mL) solution, while solutions of required concentrations were prepared by further dilutions in deionized water. The exposure concentration of endosulfan in water, for each fish species and age group, was started from zero with an increment of 0.1 µg $L^{-1}$ with three replications for each test dose in 35-L glass aquaria. Three fish species viz. *C. catla*, *C. mrigala* and *L. rohita* of 90-, 120- and 150-day age groups were tested, separately, for different concentrations of endosulfan. These toxicity tests were conducted in the static water bioassay at constant pH (7.5), total hardness (300 mg $L^{-1}$) and water temperature (29-30$^{o}$C). In each aquarium-replicate, ten fish of each species were tested against each test dose. However, the concentration of endosulfan in aquaria was increased gradually up to the test concentration in 5 h. Test media were supplied with constant airflow through air pump. During 96-h exposure, the dead fish were removed from the medium and recorded.

Probit Analysis method was used for the estimation of water-borne 96-h $LC_{50}$ and lethal concentrations with 95% confidence interval values (Siang *et al*., 2007). Data were statistically analyzed using Factorial design (RCBD). Means were compared for statistical differences through Steel *et al*. (1996). Correlations between parameters were also computed by using MSTATC software.

## Results

Both 96-h $LC_{50}$ and lethal concentrations of endosulfan for three fish species varied significantly with 95% confidence interval according to age (Table 2). Among the three age groups, 150-days all the three fish species showed significantly least sensitivity to endosulfan (1.71 µg $L^{-1}$) while 90-days old fish were significantly ($p<0.05$) more sensitive (1.02 µg $L^{-1}$). Among the three fish species *C. catla* was more sensitive ($p<0.05$) in terms of 96-h $LC_{50}$ (0.98 µg $L^{-1}$) to endosulfan, followed by that of *C. mrigala* and *L. rohita* with the mean concentrations of 1.06 and 2.15 µg $L^{-1}$, respectively. During present study, the mean lethal concentrations of endosulfan for three fish species varied between a minimum of 1.60 µg $L^{-1}$ (for *C. catla*) and a maximum mean value of 3.26 µg $L^{-1}$ observed for *L. rohita* (Table 2). The responses of all the three fish age groups, for their sensitivity towards endosulfan varied significantly in terms of 96-h $LC_{50}$ and lethal concentrations of endosulfan. The tolerance limits of three fish species in terms of lethal concentrations (96-h) of endosulfan varied significantly ($p<0.05$) also. *L. rohita* showed significantly least sensitivity to endosulfan (3.26 µg $L^{-1}$) than those of *C. mrigala* (1.67 µg $L^{-1}$) and *C. catla* (1.60 µg $L^{-1}$). The toxicity of endosulfan to three age groups of fish showed significantly negative correlation with their mean acute toxicity measured in terms of $LC_{50}$ and lethal concentrations (Table 3). The present study indicated species specific toxicity of endosulfan for three fish species that varied significantly ($p<0.05$) with fish age.

## Discussion

Endosulfan is highly toxic to various freshwater fish species. The 96-h $LC_{50}$ and lethal concentrations for endosulfan revealed significant differences among three fish species that varied with age also. Among the three age groups, 150-days all the three fish species showed significantly least sensitivity to endosulfan (1.71 µg $L^{-1}$) while 90-days old fish were significantly ($p<0.05$) more sensitive (1.02 µg $L^{-1}$). Capkin *et al*. (2006) reported that toxicity of endosulfan to rainbow trout fish size and age dependent i.e., toxicity of endosulfan decreased with age while fish size showed inverse correlation with endosulfan concentrations. Javed and Abdullah (2006) reported acute toxicity of metals to *C. catla*, *C. mrigala* and *L. rohita* that varied significantly with fish age as well.

Among three fish species *C. catla* was more sensitive ($p<0.05$) in terms of $LC_{50}$ (0.98 µg $L^{-1}$) to endosulfan, followed by that of *C. mrigala* and *L. rohita* with the mean concentrations of 1.06 and 2.15 µg $L^{-1}$, respectively. Adetola *et al*. (2011) reported 96-h $LC_{50}$ of 2.09 µg $L^{-1}$ for African catfish. Kenneth and Willem (2010) reported 96-h $LC_{50}$ value of 10.20 µg $L^{-1}$ for *O. niloticus*, which is higher than that observed during present study. However, Siang *et al.* (2007) reported 96-h $LC_{50}$ (with 95% confidence limits) of 0.42 µg $L^{-1}$ (0.35-0.50) for *M. albus* predicting species specificity to tolerate endosulfan in aquatic media.

During present study, the mean lethal concentrations of endosulfan for three fish species varied between 1.60 µg $L^{-1}$ (for *C. catla*) and 3.26 µg $L^{-1}$ observed for *L. rohita*. The responses of all the three fish age groups, for their sensitivity towards endosulfan varied significantly in terms of 96-h $LC_{50}$ and lethal concentrations of endosulfan. The tolerance limits of three fish species in terms of lethal concentrations (96-h) of endosulfan varied significantly

**Table 1:** Mean average weight, total length and fork length of fish used during acute toxicity tests.

| Age groups | Fish species | Average Weight (g) | Average Total length (mm) | Average Fork length (mm) |
|---|---|---|---|---|
| | *C. catla* | 11.65±1.14 | 81.20±2.07 | 69.54±1.47 |
| 90-day | *C. mrigala* | 11.11±0.89 | 90.62±1.23 | 76.89±1.03 |
| | *L. rohita* | 11.99±1.15 | 84.72±2.14 | 71.26±1.82 |
| | *C. catla* | 13.57±1.14 | 87.90±4.17 | 72.14±1.14 |
| 120-day | *C. mrigala* | 13.37±1.21 | 99.21±3.15 | 84.27±2.04 |
| | *L. rohita* | 14.09±1.09 | 91.89±2.13 | 81.41±1.96 |
| | *C. catla* | 19.91±1.18 | 110.72±3.04 | 91.36±2.57 |
| 150-day | *C. mrigala* | 19.22±2.05 | 129.89±1.17 | 110.28±1.64 |
| | *L. rohita* | 20.06±1.31 | 106.80±1.21 | 91.38±2.19 |

**Table 2:** $LC_{50}$ and lethal concentrations of endosulfan (µg $L^{-1}$) for *C. catla*, *C. mrigala* and *L. rohita*

| 96-h acute toxicity | Age groups | *C. catla* | *C. mrigala* | *L. rohita* |
|---|---|---|---|---|
| | 90-day | 0.67±0.01b (0.56-0.75) | 0.63±0.04b (0.54-0.72) | 1.75±0.02a (1.55-1.92) |
| $LC_{50}$ | 120-day | 1.02±0.01c (0.94-1.11) | 1.20±0.05b (0.92-1.10) | 2.16±0.03a (1.97-2.32) |
| | 150-day | 1.25±0.02c (1.12-1.47) | 1.35±0.02b (1.23-1.45) | 2.53±0.02a (2.30-2.72) |
| | 90-day | 1.23±0.03b (1.10-1.46) | 1.14±0.02c (1.02-1.37) | 2.84±0.02a (2.55-3.36) |
| Lethal concentrations | 120-day | 1.54±0.01c (1.42-1.74) | 1.78±0.01b (1.42-1.74) | 3.11±0.01a (2.87-3.56) |
| | 150-day | 2.04±0.02c (1.86-2.34) | 2.10±0.01b (1.93-2.38) | 3.83±0.10a (3.52-4.40) |
| Age Groups | | | | |
| | 96-h $LC_{50}$ | | Lethal concentrations | |
| 90-day | 1.02±0.63c | | 1.74±0.95c | |
| 120-day | 1.46±0.61b | | 2.14±0.84b | |
| 150-day | 1.71±0.71a | | 2.66±1.02a | |
| Fish Species | | | | |
| *C. catla* | 0.98±0.29c | | 1.60±0.41c | |
| *C. mrigala* | 1.06±0.38b | | 1.67±0.49b | |
| *L. rohita* | 2.15±0.39a | | 3.26±0.51a | |

(The values within brackets are the range values at 95% confidence interval)
The means with similar letters in a single row for a variable are statistically non-significant at p<0.05

**Table 3:** Relationships between 96-h $LC_{50}$ and lethal concentrations for the fish

| Age groups | Regression equation (y= a+bx) | | r | $R^2$ |
|---|---|---|---|---|
| 90-day | Lethal concentrations = 0.21+1.503** ($LC_{50}$) | SE= 0.025 | 0.999 | 0.998 |
| 120-day | Lethal concentrations = 0.14+1.371** ($LC_{50}$) | SE= 0.03 | 0.998 | 0.996 |
| 150-day | Lethal concentrations = 0.22+1.426** ($LC_{50}$) | SE= 0.05 | 0.996 | 0.992 |

**= Highly Significant at p<0.001; SE= Standard error; r=correlation coefficient; $R^2$=coefficient of determination

(p<0.05) also. *L. rohita* showed significantly least sensitivity to endosulfan (3.26 µg $L^{-1}$) than those of *C. mrigala* (1.67 µg $L^{-1}$) and *C. catla* (1.60 µg $L^{-1}$) which is less than 9.0 µg $L^{-1}$ determined for *L. rohita* against melathion by Patil and David (2008). Kamrin (1997) reported 96-h $LC_{50}$ of endosulfan to fathead minnow 1.40 µg $L^{-1}$ while these were 1.50, 1.20 and 1.50 µg $L^{-1}$ for rainbow trout, bluegill sunshine and channel cat fish, respectively. The 96-h $LC_{50}$ of endosulfan for *Anguilla anguilla* was reported as 41.00 µg $L^{-1}$, while these were 1.75, 7.75, 2.40 and 0.10 µg$L^{-1}$ for *C. punctatus*, *O. mykiss*, *B. bidyanus* and the *C. carpio*, respectively (Sunderam *et al*., 1992; Gimeno *et al*., 1994; Capkin *et al*., 2005: Panday *et al*., 2006). The toxicity of endosulfan to three age groups of fish showed significantly negative correlation with their mean acute toxicity measured in terms of $LC_{50}$ and lethal concentrations. Kolankaya (2006) reported the toxicity of organochlorines that showed negative relationship with fish age during acute exposures. The endosulfan was very toxic to the three culture-able fish species. Thus, the use of endosulan should be minimized/ stopped in order to conserve freshwater fish species.

In conclusion, *L. rohita* showed less sensitivity to endosulfan, followed by *C. mrigala* and *C. catla*. The sensitivity of endosulfan to major carps decreased significantly with increase in fish age. Among the three age groups, 90-days old fish showed higher sensitivity to endosulfan, while 150-days fish was least sensitive. Despite such a variable response of the fish species, the use of endosulfan should be minimized/ stopped in order to conserve freshwater fish species.

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