

# Determination of Median Lethal Concentration (LC<sub>50</sub>) and Behavioral Effects of Chlordecone in the Cichlid fish, *Etroplus maculatus*

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**Abstract:** The present study was aimed to investigate the median lethal concentration of chlordecone in the Cichlid fish, *Etroplus maculatus* and also its behavioral changes on the exposure to the environmental contaminant. Behavior is considered as a valid biomarker of pollution and also has high ecological significance, as it influences the fitness of the affected species. Different concentrations of chlordecone were exposed to the fish to evaluate the median lethal concentration at 96 h. The result showed that 35 µg/ L kill 50% of the animal, which is determined by the probit analysis. Behavioral changes such as reddening of eyes and fins, exophthalmia, slow movement and lethargy, hitting on the side walls were observed. Results from this study showed that the change in the behavior pattern due to chlordecone exposure was an ecologically relevant assessment for the effects of chlordecone on the test animal.

**Keywords:** Chlordecone, LC<sub>50</sub>, *Etroplus maculatus*, Behavior, Environmental pollution

## 1. Introduction

Chlordecone was first produced in the United States in the early 1950s and was primarily used as an insecticide. It is mainly used to control the banana root borer, applied on non-fruit-bearing citrus trees to control rust mites, wireworms in tobacco fields, apple scab, powdery mildew, grass mole cricket, and control of slugs, snails, and fire ants (ATSDR, 1995). Its registration was cancelled in 1978; however, it was widely used in India to control several pests. Chlordecone is resistant to degradation in the environment and has a high potential for bioaccumulation in fish and other aquatic organisms (ATSDR, 1995).

Chlordecone has been shown to stay in soil, water, and sediment for years and undergo very slow degradation (Carlson et al., 1976). Fish or other animals that live in waters that contain chlordecone, or that eat other animals contaminated with chlordecone, can build up these substances in their bodies and may be several times greater than the amount in their prey or in the surrounding water (Roberts and Bendl, 1982). Studies have shown that chlordecone has the ability to pass into blood through contaminated food. Once chlordecone is taken up by the body, it is carried by the blood throughout the body and is stored for a long time. Chlordecone has been found mainly in the liver and it is broken down to chlordecone alcohol, which is a less harmful product. Chlordecone and its breakdown product slowly leave the body through the feces and this process takes several weeks to months. It has been reported that a very little chlordecone leaves the body through the urine and it has been also found in human milk as well as in saliva only within the first 24 h after the exposure (Taylor et al., 1978).

The LC<sub>50</sub> value of chlordecone in catfish was reported as 0.24 mg/ L for 96 h by using static acute toxicity bioassay (Srivastava and Srivastava, 1994). In the present study it was aimed to evaluate the median lethal concentration of chlordecone in the Cichlid fish, *Etroplus maculatus* for 96 h

exposure along with the noted behavioral changes due to the toxicity exposure.

## 2. Materials and Methods

### Experimental Animal

The Cichlid fish, *Etroplus maculatus* weighing 7 ± 1 g and length 7 ± 1.5 cm were collected from a fish farm, KKF Nursery, Manjeri, Vaniyambalam. Fishes were acclimatized to the laboratory conditions prior to the experiment and were exposed with constant supply of water and good lighting system. They were maintained in well-aerated tubs (40 L capacity), which was dechlorinated and sustained with fresh water flow and waste water discharge.

### Preliminary Tests

The physico-chemical features of the tap water were estimated as per APHA (1998). Water temperature in the test ranged from 28 ± 2°C during the experiment, oxygen saturation of water ranged between 70 and 100 %, pH is 7.6 which were monitored using a standardized procedures.

### Chemical

Technical grade organophosphate insecticide, chlordecone (Kepone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalen-2-one, 99.9% pure) was obtained from Supelco, USA.

### Evaluation of median lethal concentration (LC<sub>50</sub>)

The LC<sub>50</sub> values in 96 h time interval were determined by probit analysis, with a confident limit of 5 % level (Finney, 1971). The fishes were not fed a day prior to and during the test to reduce fecal and excess food contaminating the test solution. Five specimens were placed in each tub of replicates so that ten fishes were maintained in each test and aerated using tubed motorized pumps. Monofilament netting was used to cover the tanks to prevent the specimens from jumping out of test solutions.

The concentration of the pollutant at which 50 percentage of the test animals dies during a specific period or the

concentration lethal to one half of the test population is referred to as median lethal concentration (LC<sub>50</sub>) or median tolerance limit. For determining LC<sub>50</sub> concentration separate circular plastic tubs of 40 L of water capacity were taken and different concentrations (25, 30, 35, 40, 50 and 80 µg/L) of chlordecone were added. Then, 10 fishes were introduced into each tub. A control tub with 40 L of water and 10 fishes were also maintained (no toxicant). The lethal concentration for 50 % killing (LC<sub>50</sub>) values was computed on the basis of probit analysis (Finney, 1971) for 96 h, which were 35 µg/L. The mortality as well as the behavior of fishes was recorded throughout the study.

**Statistical Analyses**

All experiments were repeated three times and performed in triplicate. Data were analyzed using Probit Analysis as statistical method with SPSS 19.0 statistical analysis software. The LC<sub>50</sub> value (with 95% confidence limits), the correlation between mortality against concentrations and the best-fit line were also obtained.

**3. Results**

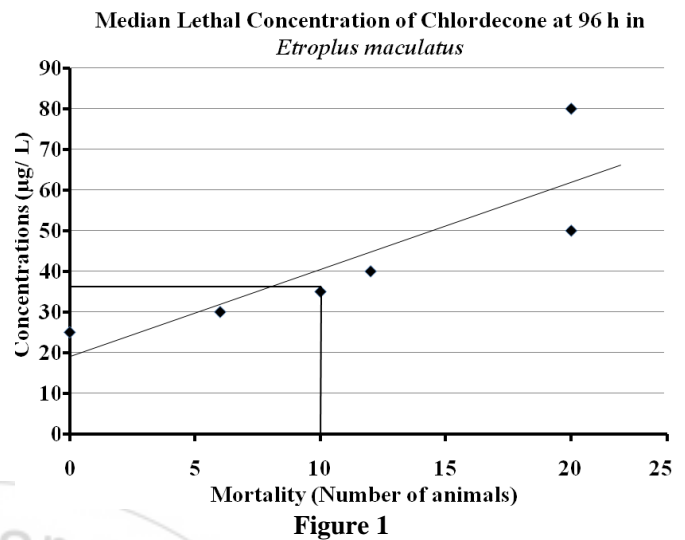
Different concentrations of chlordecone showed different percentage of mortality at different time interval as shown in Table 1. Fishes undergone mortality was immediately removed after death and their numbers registered for calculating percentage of mortality. It was observed only at the concentration of 25 µg/L no mortality was observed at 96 h whereas at 50 and 80 µg/L showed 100% mortality of the animal. Computation of median lethal concentration by probit analysis showed LC<sub>50</sub> value at 35 µg/L. The values are also plotted as graph and the results of correlation analysis showed that mortality (X-variable) against concentrations of chlordecone (Y-variable) was highly positive correlation  $r = +0.8468$  (Figure 1). It was observed that at the concentration above 50 µg/L showed 100% mortality.

The behavior of the animal showed a drastic alteration in chlordecone-treated animal when compared to control group, where at the beginning of the treatment all the fishes showed spontaneous swimming activity and it gradually decreased to become lethargic. All the fishes interact with each other and knock on the walls of the tub frequently finally showed erratic swimming at the time of death. Reddened eyes and fins, especially caudal fin with exophthalmic eyes (Figure 2), high degree of mucus production, increased surfacing-fishes stayed at the water surface with restricted movements, body surfaces became reddened and hemorrhagic, decreased rate of opercular movement, inability to maintain normal posture and balance are the behavioral changes that was observed during the treatment.

**Table 1:** Percentage of fish mortality exposed to different concentrations of chlordecone at 96 h

Concentrations (µg/L)	Mortality (No. of animals)	Mortality (%)	Hour of mortality	Prob
Control	0	0	Nil	0
25	0	0	96 h	0.03622
30	3	30	96 h	0.19580
35	5	50	96 h	0.47518

40	7	70	48 h	0.73432
50	10	100	40 h	0.96213
80	10	100	8 h	0.99999



**4. Discussion**

In the present study the Cichlid fish, *Etroplus maculatus* commonly known as orange chromid was selected as an experimental model as it is one of the endemic fishes of south India found in freshwater, brackish streams, estuaries and lagoons. These fishes are highly sensitive to the environmental pollutants. Chlordecone is a polychlorinated hydrocarbon pesticide having estrogenic effect and has been widely reported to have harmful effects on most vertebrates including human. However, the median lethal concentration (LC<sub>50</sub>) of the Cichlid fish was not yet studied or reported. Therefore, an attempt was made to evaluate the 96 h LC<sub>50</sub> value of chlordecone. Acute toxicity of a pesticide refers to the damage that happens to the test animal when exposed to toxicant from a single exposure, generally of short duration. In fishes, in order to evaluate the acute toxicity or the medial lethal effect usually fishes were exposed for 96 h duration. This toxicity test of pesticides have been commonly performed on fish to acquire rapid estimates of the concentrations that lead to direct, irreversible harm to the tested organisms (Pandey et al., 2005). In the present study the probit analysis clearly states that 35 µg/L as the mean lethal concentration of chlordecone to the Cichlid fish *Etroplus maculatus*.

Studying the behavior of an animal serves as the link between physiological and ecological processes and it is considered as an ideal parameter for studying the effects of environmental pollutants. Fish are an excellent model used to evaluate ecological impact of those pollutants on the aquatic ecosystem. Recently many researchers have proposed several behavioral parameters as indicators for ecologically relevant monitoring of environmental contamination (Atchison et al., 1987). The normal behaviors of fishes are usually triggered by external stimuli acting through neural networks (Weber and Spieler, 1994). Any alterations in their normal behavioral pattern can be affected by numerous physiological and environmental influences.

In the present study the changes in behavioral pattern was also observed in order to see if the toxic effects of aquatic contaminants chlordecone can have severe implications for survival of the fish at short duration of 96 h. During the experiments, the behavior of fishes to the exposed contaminant chlordecone showed an unusual behavior. At the start of exposure, fish were aware that they are exposed to the change in normal environment, which is known by immediate stop in swimming and remained static in position for a while. After some time fishes showed erratic swimming and jumping to avoid from the toxic environment. As they failed, then the fishes moved on the surface with wide opening of gill operculum to engulf air. Finally as a defensive mechanism the fishes secreted mucous all over the body to prevent the entry of chlordecone into the body either through gill, or skin, as a result the rate of opercular movement was decreased. Mucous cells are considered efficient in seizing the toxic agents and thus help in the prevention of the entrance of these agents into the gills (Perry and Laurent, 1993). It was noticed that eyes were red and exophthalmic, bleeding throughout the body of the exposed fishes. Due to inability to maintain normal posture and balance the fishes later a jerky and hyperexcitable movement the fishes followed by settlement at the bottom of the tank, loss of consciousness, lethargic and finally died.

## 5. Conclusions

The median lethal concentration of chlordecone ( $LC_{50}$ ) for *Etrophus maculatus* was determined as 35  $\mu\text{g}/\text{L}$  for 96 h by probit analysis and when the concentration increased above 50  $\mu\text{g}/\text{L}$  showed 100% mortality. The data reported in the study therefore recommend 35  $\mu\text{g}/\text{L}$  as 96 h  $LC_{50}$  of chlordecone for *Etrophus maculatus*.

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