EVALUATION OF CYPERMETHRIN TOXICITY
INTRODUCTION:

Water pollution by toxic chemicals comes from run-off urban streets or of agricultural chemicals from cultivated fields, from sewage, or from a specific industrial sources such as refineries, smelters or chemical plants. It is safe to say that significant quantities of every persistent pesticide applied to terrestrial crop will reach surface waters. Non-persistent substances, on the other hand, contaminate only those water bodies near enough to cultivated lands to receive direct fallout.

The imbalance between the number of ecological studies and the number of pesticides in use precludes authoritative assessment of the impact each of these chemicals will have on various ecosystems to which they are applied.

The detection of so many different organic chemicals in the environment alone would be a mammoth undertaking. The amount of ecological research already completed in miniscule compared to that skill required to elucidate the numerous aspects of the pesticide impact on biotic system and the reaction of these system re-establish on equilibrium.

The toxicity of pesticides or any other toxicant to a particular organism of the aquatic environment is usually expressed in terms of LC50 (Lethal Concentration). This represents the amount of poison per unit weight, required to kill 50% of a particular population of the animal species employed for the tests and this designates the median tolerate limit (TLM) measurement (Finney, 1964). Usually the poison used in water
is expressed as parts per million (ppm) or mgs/litre. In this respect, the 
LC$_{50}$ figures for organochlorides are commonly expressed in parts per 
billon, where as synthetic pyrethroids are less toxic to fish that they are 
expressed in parts per million. Majority of investigations on the toxic 
effects of pesticides on fish exposure is usually 24, 48, 72 (or) 96 hours.

Cypermethrin at 96 hr LC$_{50}$ (0.4 – 2.2 µg/l) proves to the acutely 
toxic to some fish species like Salmo, Tilapia, Cyprinus and Scardimin 
(Stephenson, 1982; Goel 1989) observed toxicity of mentanial yellow and 
Derma orange (Textile dyes) to a freshwater teleost Channa punctatus. The 
medium tolerance limit values for 96 hours were obtained after the 
exposure of these two dyes separately. The 96 hrs LC$_{50}$ values from Derma 
orange and mentanial yellow were found out to be 0.252 g/l and 0.155 g/l 
respectively.

Thus the foreside account on the toxicity of insecticides in fishes 
and other animals indicates the significance of the study of immediate and 
chronic effects of pesticides on fishes. Also, the data provided on toxicity in 
fish with different insecticides will be highly useful in the final evaluation 
of the extent of pollution or aquatic environment by agricultural 
chemicals.

METHOD:

The toxicity can be defined as the inherent capacity of a toxicant to 
affect adversely biological activity of an organism. The best method of
evaluation of the toxicity of a toxicant in aquatic organisms is the determination of LC$_{50}$ values (Finney, 1971)

The levels of toxicity of pesticides may differ from one type of pesticide to another and one group of animal to another (Pickering et al., 1962; Macek and Mc Allister, 1970). A good number of reports are available on the effect of pyrethroids on aquatic organisms. (Elliott et al., 1978; Anderson, 1982). The toxicity studies with reference to osmotic stress, temperature, species, age, sex and nutrition of test animal were also carried out to a certain extent (Mc Kenny and Hamarker, 1984; Desi et al., 1986).

These differences could be attributed to the differences in the nature of the chemical, the interaction of the chemical with the biological system, resistance capacity of the animal, detoxification mechanisms involved, assay techniques, purity of the pesticide and the additives and emulsifiers present in the commercial grade consumption.

The period of exposure of an animal to a toxicant is also considerably important in evaluating the levels of toxicity. Depending upon the nature of the toxicant, LC$_{50}$ values will be generally assessed at 24 or 48 or 72 or 96 hours exposure (Spehar et al., 1982).

The LC$_{50}$ studies are useful not only to establish tolerance limits and safe levels of toxic agents for the biota of aquatic environment but also to observe various symptoms of poisoning. There exist a considerably body of
information on the effects of pesticides on behavioural biology of fishes (Hansen et al., 1970).

The pesticides produce behavioural pathology, which differs at different concentrations (Bhagyalakshmi, 1981).

For the evaluation of the cypermethrin toxicity, the static bioassay is followed, where the biological responses of the animal were recorded in static water (Doudroff et al., 1951), so that any change due to extraneous influences can be easily nullified by maintaining suitable control fishes in static water. For $LC_{50}$ determination (Finney, 1964) 10 different concentrations of cypermethrin like (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ppm) were used. A stock solution of cypermethrin prepared in acetone (each gram of cypermethrin is dissolved in acetone). From this stock solution, required dilutions as given below were prepared with tap water. Acetone used in the quantity was found to be non-toxic to fish (Pickering et al., 1962). The Experimental fishes were separated into 7 batches of 10 each. One batch was exposed to freshwater without fenvalerate (control) while the other 6 batches were exposed to media containing 10 different cypermethrin concentrations and the mortality of the fishes in these concentrations within 96 hours exposure was noted (the fish were starved during this exposure period). Each experiment in these selected concentrations of cypermethrin were repeated for 3 times, $LC_{50}$ was calculated by graphical plots of probit mortality versus cypermethrin concentration (Finney, 1964). For subsequent verification of the $LC_{50}$ values obtained by graphical method Dragsted-Behrens as given
by Carpenter (1975) was employed separately. Where the fishes were exposed to concentrations of cypermethrin (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ppm) for 96 hours and the present mortality is calculated from the cumulative mortality. The LC$_{50}$ is calculated by adopting the following formula.

\[
\text{Log LC}_{50} = \log \frac{\text{A} - \text{a}}{\text{b} - \text{a}} \times \log 2
\]

Where

\begin{align*}
\text{A} & = \text{Concentration of cypermethrin which kills 50% of the animals} \\
& = 0.4 \text{ ppm} \\
\text{a} & = \% \text{mortality observed immediately below 50% mortality} \\
& = 40 \\
\text{b} & = \% \text{mortality observed immediately above 50% mortality} \\
& = 70
\end{align*}

Thus for small fish

\[
\text{Log LC}_{50} = \log \frac{0.4 - 40}{70 - 40} \times \log 2
\]

\[
\text{A} = 0.4 \text{ ppm} \\
\text{a} = 40\% \\
\text{b} = 70\%
\]

Therefore for small, LC$_{50}$ value = 0.5003 ppm

Similarly for large fish

\[
\text{Log LC}_{50} = \log \frac{0.4 - \text{a}}{\text{b} - \text{a}} \times \log 2
\]
A = 0.6 ppm
a = 40%
b = 70%
LC50 = 0.7003 ppm

The mean and standard deviation of the LC50 values (ppm) obtained from the three sources was found to be 0.4216 (± 0.185 SD) ppm for small fish and 0.6302 (± 0.298 SD) ppm for large fish (Tables 1 to 3).

**PREPARATION OF SUBLETHAL CONCENTRATION OF CYPERMETHRIN FOR FURTHER STUDIES OF THIS INVESTIGATION:**

There is an increasing realization that the effect of pesticides on the response to the fish other than the easily observable mortality effects, must be taken into account in evaluating the complete ecological impact of the contaminatory substance. This is particularly important, because distinct changes involving the sequence of events in the responses of the fish to the pesticides is likely to occur at sublethal exposure (Anderson 1982; Bashamohideen, 1984). Hence, the sublethal concentration of cypermethrin was selected from the LC50 values obtained.

In this investigation, the LC50 values of cypermethrin obtained for small fish and large fish of *Labeo rohita* is 0.4216 ppm and 0.6302 ppm respectively. Since the sublethal concentration was reported to be taken approximately one-fifth of the LC50 value (Reed and Muenchi, 1938; Konar, 1969) which happens to be nearly 0.0843 ppm and 0.1260 ppm. For the sake of convenience 0.08-ppm cypermethrin is selected to represent the ideal sublethal concentration with an exposure period of 30 days for further studies in this investigation.
RESULTS:

The data for the mortality of 10 individuals of *Labeo rohita* sizes namely, the small individuals and the large individuals in different concentrations of cypermethrin (ppm) at 96 hours is presented in (Fig. 1 and 2, Tables 1 to 3). It is clear from the data that there is a linear relationship between the % mortality of the fish and cypermethrin concentration in that the % mortality increase with an increase in the cypermethrin concentration. Thus in the case of small fishes at 96 hours, the cypermethrin toxicity shown the lowest mortality in 0.1 ppm cypermethrin at normal temperature (28°C). In case of adult fish at 96 hours, the cypermethrin toxicity studies shown lowest mortality in 0.2-ppm cypermethrin, the maximum mortality (100%) in 0.9-ppm cypermethrin at normal temperature (28°C). In both the cases, the % mortality of this teleost fish *Labeo rohita* at different concentrations of cypermethrin at 96 hours was plotted against log concentrations, which has shown a typical sigmoid curve (Fig.1). But when probit mortalities are plotted against log concentrations of cypermethrin, shown a straight line (Fig. 2). The calculated methods are almost the same [Table 1 to 3]. Thus the mean LC$_{50}$ values (0.4216 and 0.6302 ppm) respectively for small and adult fish obtained through the three sources was taken as LC$_{50}$ of cypermethrin for two stages of *Labeo rohita*.

DISCUSSION:

Though the pesticides are essential tools in agriculture as well as forestry, the gradual degradation of ecosystem and also the consequent
disaster by dint of their excessive and discriminative need to be taken into on account (Konar, 1977). As they drain into water the pesticides hamper the metabolism, growth and reproduction of fishes that represent most typical and massive population of the aquatic environment (Johnson, 1967; Burdic et al., 1972). Therefore, an urgent need exist for judicious use of pesticide, which could be done by the determination of the safe levels of the pesticides, and further due to these pollutional hazards in fishes it is highly necessary and significant to conduct studies on the sublethal effects of these pesticides.

Infact such studies are conspicuous by their absence especially in commercially important food fishes like *Labeo rohita* therefore, in this investigation sublethal effects in the form of physiological and biochemical responses of the teleost fish, *Labeo rohita* subjected a synthetic pyrethroid cypermethrin exposure is investigated.

Toxicity can be defined as the inherent capacity of a toxicant to affect adversely any biological organism. In general, the extent of biological effect can be seen at the molecular collar tissue, organism, family and population level through behavioural, physiological and pathological means (Green, 1984). The best accepted method of evaluating the toxicity of pesticide is the determination of values i.e. the toxicity of pesticides to fish commonly assessed by the concentrations of the toxic compound in water that kills half of the animals exposed for a specified period of time.

In the present study, the percent mortality in different concentrations of cypermethrin shown a straight line, when log
concentrations of cypermethrin plotted against probit mortality in both small and large fish of *Labeo rohita*, thus agreeing with probit analysis (Finney, 1964). No mortality was observed up to 0.2ppm in small individuals. Hence cypermethrin is not toxic up to 0.3ppm in large individuals. The LC$_{50}$ values obtain by the graphical and calculated methods are almost the same. Thus, the Mean LC$_{50}$ values in both the small and large individuals of *Labeo rohita* is 0.4216ppm for small and 0.6302 ppm for large fish.

Malla Reddy (1988) found the LC$_{50}$ value as 0.030ppm when *Cyprinus carpio* exposed to cypermethrin for 48 hrs exposure periods.

Studies involving the determination of LC$_{50}$ values at the present study are highly useful in determining the sublethal concentration of pesticides, which are to be approximately one third of the LC$_{50}$ value (Read and Muenchi, 1938; Konar, 1969). Further the period for which the LC$_{50}$ value determined is of considerable importance, because the LC$_{50}$ values are much higher at 24hrs than at 96hrs exposure (Pickering *et al.*, 1962; Kabeer Ahamed, 1979; Koundinya and Ramamurthy, 1980; Basha Mohideen and Subba Rao, 1982; Basha Mohideen and Dhanunjaya, 1986; Basha Mohideen, 1986, 1987a).

In this respect most of the investigation including the present study on the toxic effects of pesticides on fish have preferred 96hrs exposure because of its relatively longer period than 24 hrs when the effects may not be consistent.
Table -1

Mortality of the small fish of *Labeo rohita* in different concentrations (ppm) of cypermethrin at 96 hrs of exposure.

Mortality expressed in both percent and probit kill.

Each value represents an average of three replications.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of Pesticide (ppm)</th>
<th>Log concentration</th>
<th>Number of fish exposed</th>
<th>No. of fish dead</th>
<th>% Kill</th>
<th>Probit kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>20</td>
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<td>50</td>
<td>5.25</td>
</tr>
<tr>
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<td>1.6990</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td>5.52</td>
</tr>
<tr>
<td>6</td>
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<td>1.7782</td>
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<td>8</td>
<td>80</td>
<td>5.84</td>
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<td>10</td>
<td>10</td>
<td>100</td>
<td>8.09</td>
</tr>
</tbody>
</table>
Table - 2

Mortality of the large fish of *Labeo rohita* in different concentrations (ppm) of cypermethrin at 96 hrs of exposure.

Mortality expressed in both percent and probit kill.

Each value represents an average of three replications.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of Pesticide (ppm)</th>
<th>Log concentration</th>
<th>Number of fish exposed</th>
<th>No. of fish dead</th>
<th>% Kill</th>
<th>Probit kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>2</td>
<td>20</td>
<td>4.15</td>
</tr>
<tr>
<td>5</td>
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<td>1.6990</td>
<td>10</td>
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<td>100</td>
<td>8.09</td>
</tr>
<tr>
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<td>1.0</td>
<td>0.0000</td>
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<td>10</td>
<td>100</td>
<td>8.09</td>
</tr>
</tbody>
</table>
**Table - 3**

LC₅₀ values in ppm (96 hours) of small and large individuals of *Labeo rohita* exposed to cypermethrin.

Mean LC₅₀ values calculated from three different sources.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Size of the fish</th>
<th>LC₅₀ values through</th>
<th>Mean LC₅₀ value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Kill</td>
<td>Probit Kill</td>
</tr>
<tr>
<td>1</td>
<td>Small SD±</td>
<td>0.4000</td>
<td>0.4000</td>
</tr>
<tr>
<td>2</td>
<td>Large SD±</td>
<td>0.6000</td>
<td>0.6000</td>
</tr>
</tbody>
</table>

**Small Vs Large**

<table>
<thead>
<tr>
<th></th>
<th>Small fish</th>
<th>Large fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC₅₀</td>
<td>0.4216</td>
<td>0.6302</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.185</td>
<td>0.298</td>
</tr>
<tr>
<td>'t' test</td>
<td>----</td>
<td>N.S.</td>
</tr>
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</table>
Percent mortality of small and large individuals of *Labeo rohita* at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ppm) of cypermethrin (represented in log concentrations). Each point represents an average of three replicates.
Probit mortality of small and large individuals of *Labeo rohita* at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ppm) of cypermethrin (represented in log concentrations). Mortality expressed in probit mortality (percent mortality was transformed into probit mortality from Table-1, Finney 1964).