(A) TOXICITY EVALUATION

INTRODUCTION

Since the World War II the number of pesticides used has dramatically increased. There are today approximately 90 different pesticide ingredients registered for use with in agriculture sector and approximately 1700 tons of these are applied each year accidental spills and dump sites also provide for a part of the environmental pesticide input. In contrast of many other manmade chemicals present in the environment, pesticides are deliberately spread in to the environment. They are manufactured to be harmful to specific target organisms, or groups of organisms and their toxic properties are essential to the pesticides a satisfactory function. Due to pesticides toxic properties there is an obvious risk that non target organisms affected either at the application site, or due to unintentional spreading, at nearby, or even distant, areas.

Chemicals originating from agricultural activity enter the aquatic environment through atmospheric deposition, surface run off or leaching (Kreuger, 1999), and frequently (Lehotay et al., 1998; Kreuger et al., 1999). In all parts of the world pesticides have been found in the aquatic ecosystem and often information of how this pesticides effects inhabiting organisms is missing.

The toxicity of a chemical is totally dependent on the concentration of the chemical in the organism or even the concentration at the target receptor in the organism. Only the bioavailable fraction of the pesticide can be accumulated by aquatic organisms and there by reach the specific target receptors (Hamelink and spacie, 1977). The actual amount of the pesticide that enters the organism, is therefore of critical importance and determines the biological ramifications. In environmental toxicology, environmental concentration is often used as a substitute for knocking the actual amount of a chemical entering an organism. Care must be taken to realize that the entering dose may be only indirectly related to environmental concentration.

Environmental protection has attracted the attention of the wide cross-section of people all over the world which has now become a global issue amongst scientists and
researchers working in this area. Pesticidal pollution constitutes the most dangerous health hazard apart from creating adverse effects on fish production. As the fishes are economically important non-target organisms, they are quite sensitive to a wide variety of toxicants and are used as pollution indicator in the water-quality management.

A toxicant is an agent that can produce an adverse response in a biological system, seriously damaging its structure or function or culminating in death. That adverse response may be defined in terms of a measurement as acute toxicity. A toxicant may be introduced deliberately or accidentally into the aquatic ecosystem, impairing the quality of the water and making it unfavorable for aquatic life. Toxicants enter aquatic ecosystems from:

1. Non-point sources such as agricultural runoff from land, contaminated ground water and bottom sediments, urban runoff, dredged sediment disposal and atmospheric fall out.

2. Point sources such as discharges (effluents) from manufacturing plants, hazardous waste disposal sites and municipal wastewater treatment plants.

The most innocuous chemical substances can have detrimental effects when taken up by an organism in sufficient amounts. In contrast, the uptake of minute quantities of toxic chemicals may result in no apparent adverse effects. Toxicity is a relative property of a chemical, which refers to its potential to have harmful effects on a living organism. It is a function of the concentration of the chemical and the duration of exposure. Toxicity data are commonly used in comparing different chemical substances. Information about the biological mechanisms affected in living system and the conditions under which the toxicant is harmful is also important for this comparison. Toxicity tests are used to evaluate the adverse effects of a chemical on living organisms under standardized, reproducible conditions, which permit a comparison with other chemicals tested.

Different types of toxicity tests serve different purposes. The short-term or acute toxicity test is conducted for a period of 24, 48, 72 or 96 h and is one of the most commonly employed tests in the evaluation of toxicity. The above tests may also be
conducted for long duration, over a period of 30 days to several months (chronic toxicity).

The modern aquatic toxicity protocols in use are the results of a series of attempts at the standardization of the test methodology. The earliest and one of the most useful of these test methods is that of Doudoroff et al. (1951) which forms the basis for all other attempts. In the standard methods of the American Public Health Association (APHA, 1985) bioassay and toxicity test procedures are described in detail. The United States Environmental Protection Agency (USEPA, 1973) and the American Society for Testing and Materials (ASTM, 1992) published a comprehensive review on the methods for conducting acute toxicity tests. Hence, the mortality test is made mandatory in the tired systems of testing followed by the European Economic Community (EEC) and Organization of Economic Cooperation and Development (OECD) committee for any chemical produced on an industrial scale.

It is defined as the concentration of test chemical (Pesticide) that kills all the experimental animals. The toxicity evaluation of the test solution is derived through probit method (Finney 1964) and from this method $LC_{25}$, $LC_{50}$, $LC_{75}$, $LC_{100}$ confidential limits and the slope values can be determined. $LC$ means the “median lethal concentration” and $LC_{25}$, $LC_{50}$, $LC_{75}$ and $LC_{100}$ are the mean concentrations that kills 25%, 50%, 75% and 100% of test animals respectively. When a test chemical is administered either orally or dermally or through injection to the terrestrial animals, it is called “median lethal dose” (LD). The different LD concentrations ($LC_{25}$, $LC_{50}$, $LC_{75}$ and $LC_{100}$) and their corresponding percent mortality of test animals can be derived on the same lines as explained under the lethal concentration (LC).

The concentration of test chemical is expressed in ppm or ppb in the case of aquatic animals and in micrograms or in milligrams in the case of terrestrial animals. The ppm (parts per million), is the number of parts of test chemical per million parts of the substance in question and ppb (parts per billion) is the number of parts of test chemical per billion parts of the substance in question. But in the case of terrestrial animals it is expressed as micrograms/kg or milligram/kg body weight.
Depending on the duration of exposure of animals to test chemical, toxicity studies are broadly classified as follows:

1. **Acute toxicity**: The toxicity of a test chemical is determined at the end of a few hours.

2. **Chronic toxicity**: The toxicity of a test chemical is determined in days or months. For toxicity evaluation, the test chemical can be administered by one of the following methods,

   1. **Oral toxicity**: A test chemical is given through mouth, either by stomach tube or mixed with food or water.

   2. **Dermal and Cutaneous toxicity**: Painting the test chemical on the skin.

   3. **Inhalation toxicity**: Exposure through vapour or dust.

   4. **Subcutaneous toxicity**: Injecting the test chemical just below the skin.

   5. **Intramuscular toxicity**: Injecting the test chemical into the muscle.

   6. **Intravenous toxicity**: Injecting the test chemical into the vein.

   7. **Intraperitoneal toxicity**: Injecting the test chemical into the viscera.

The toxicity of the test chemicals (pesticides) can be influenced by the environmental and biofactors like temperature (Chada et al., 1964; Herz Berg et al., 1980), hardness of water (Pickering et al., 1962), nutrition (Pal and Kushwah 1980; Das and Garg 1983), size (Pickering et al., 1962; Braginskii et al., 1979; Jayantha Rao 1982, Rao, 1981), species specificity (Mayhew, 1955; Gouda et al., 1981; Lee et al., 1981; Santa satyanarayana, 1981; Jacob et al., 1982), salinity (Harzberg, 1980), chronobiology (Uthaman et al., 1979), aeration (Herzel, 1976), duration (Pickering et al., 1962; Abidi, 1980; Gouda et al., 1981; Jacob and Nair 1982), physiological factors (Gupta, 1976), combination of pesticides (Lowerenz et al., 1980; Sing and Srivastava, 1982).
The aquatic environment has become a storehouse for chemical pollutants which infiltrate into aquatic environment by several ways (Verma et al., 1979; Twardowska and Kozlowska, 1980; Barquet and Pfaffenberger, 1981; Bauman, 1981), thus imbalancing the aquatic biota.

The LC₅₀ values will be highly useful in the final evaluation of designing “safe level” or “tolerable level” (TL) of pollution to the aquatic biosphere and thus will pave the way in establishing “limits and levels of acceptability” by the biotic components. It also helps in “programming preferential aquaculture programmes depending on the quality of environment and quantity of pollutants in the same”.

The pesticides have accumulative residual effect on the organisms. The design of toxicity test essentially incorporates the following base components.

1. Selection of test organism.
2. Selection of a response to measure exposure period.
3. Test duration and
4. Series of doses to treat.

Doses are described as Lethal doses (LD) or Lethal concentration (LC) where the response measured is mortality. Construction of the cumulative dose response curve enables us to identify doses that effect a specific percent of the exposed population. It means that LD₅₀ (or) LC₅₀ is the dosage lethal to 50% of the test organisms or one way choose to identify a less hazardous dose such as LD₁₀ (or) LD₀₁. The route of exposure also determines how much of the chemical substance enters or absorbs in to the test animal and which organs are initially exposed.

In a general sense, the toxicity of a substance could be defined as the capacity to cause injury to a living organism (Sanockij, 1970). A highly toxic substance will damage an organism if administered in very small amounts; a substance of low toxicity will not produce an effect unless the amount is very large. Thus, toxicity cannot be defined without reference to the quantity of a substance administered or absorbed (dose), the way
in which this quantity is administered (e.g. Inhalation, ingestion, injection) and
distributed in time (e.g. single dose, repeated doses), the type and severity of injury, and
the time needed to produce that injury.

There is no generally agreed definition of “hazard” associated with a chemical,
but the term is used to indicate the likelihood that a chemical will cause an adverse health
effect (injury) under the conditions in which it is produced or used (Goldwater, 1968;

Risk is a statistical concept and has been defined by the Preparatory Committee of
the United Nations Conference on the Human Environment, as the expected frequency of
undesirable effects arising from exposure to a pollutant. Estimates of risk may be
expressed in absolute terms or in relative terms. The absolute risk is the excess risk due to
exposure. The relative risk is the ratio between the risk in the exposed population and the
risk in the unexposed population (BEIR, 1972).

Most commonly, the term “dose” is used to specify the amount of chemical
administered, usually expressed per unit body weight. If the dose is administered into the
stomach, on the skin, or into the respiratory tract, transport across the membranes may be
incomplete and the absorbed dose will not be identical with the dose administered. In
environmental exposures, an estimate of the dose can be made from the measurement of
environmental and food concentrations as a function of time, and involves the assessment
of food intake, inhalation rate, and the appropriate deposition and retention factors.

The doses in the organs and tissues of interest may be estimated from:

a) Administered dose or intake;

b) Measurement of the concentrations in tissues and organ samples;

c) Measurement of concentrations in excreta or exhaled air.
“Effect” and “response” are often used interchangeably to denote a biological change, either in an individual or in a population, associated with an exposure or dose. Some toxicologists have, however, found it useful to differentiate between an effect and a response by applying the term “effect” to a biological change, and the term “response” to the proportion of a population that demonstrates a defined effect (Pfitzer, 1976).

In this terminology, response means the incidence rate of an effect. For example LD₅₀ value may be described as the dose expected to cause a 50% response in a population tested for the lethal effect of a chemical. This distinction will be made in the present monograph, although it should be recognized that this terminology is not generally accepted.

An effect can usually be measured on a graded scale of intensity or severity and its magnitude related directly to the dose. Certain effects, however, permit no gradation and can be expressed only as “occurring” or “not occurring”. Such effects are usually called “quantal” (Finney, 1971). Typical examples of quantal effects are death or occurrence of a tumour.

The toxic action of chemicals usually affects the whole organism but the primary damage may be localized in a specific target organ or organs in which the toxic injury may manifest itself in terms of dysfunction or overt disease (NIEHS, 1977). According to Sanockij (1975a), the specificity of acute toxic action can be expressed in terms of a “zone of specific action” (Zsp) which is the ratio between the threshold dose of an acute effect at the level of the total organism and the threshold dose for an acute effect at a specific organ or system. If Zsp > 1, the toxic action is specific; if Zsp < 1, it is non-specific.

Acute effects are those that occur or develop rapidly after a single administration (Casarett, 1975) but acute effects may appear after repeated or prolonged exposure as well. Chronic effects may also result from a single exposure but more often they are a consequence of repeated or prolonged exposures. Chronic effects are characterized not only by their duration but also by certain pathological features. They may arise from the accumulation of a toxic substance or its metabolites in the body, or from a summation of
acute effects. The latent period (or the “time-to-occurrence” of observable effects) may sometimes be very long, particularly if the dose or exposure is low.

Considerable literature is available on the toxic potentialities of selected organochlorine insecticides with reference to aquatic biota like fish (Hart et al., 1945; Bhatia et al., 1971; Muirhead-Thomson, 1971; Spargue, 1971; Korn and Earnest, 1974; Toor and Kaur, 1974; Macek and Macellister, 1970; Marking and Mauck, 1975; Basak and konar, 1976; Pillai et al., 1977; Sastry and Sharma, 1978; Basha, 1982), shrimps (Mc Leese and Metcalfe, 1980).

With this background, a preliminary attempt was made to evaluate the toxic potentiality of Lihocin to freshwater teleost fish *Catla catla* under static conditions.

The committee on methods for toxicity tests with aquatic organisms recommended four methods for conducting toxicity tests. They are:

1) **Static technique**: In a static test, the organisms are exposed in still water. The test material is added to the dilution water to produce the desired test concentrations. The control and test organisms are then placed in test chambers and there is no change of water for the duration of the test.

2) **Recirculation technique**: A recirculation test is similar to a static test except that the test solutions and control water are pumped through an apparatus, such as a filter, to maintain water quality but not to reduce the concentration of test material. The water is retained to the test chamber. This type of test is not routinely used as it is expensive to set up and maintain and also due to uncertainty about the effect of the apparatus (aerator / filter / sterilizer) on the test material.

3) **Static renewal technique**: A static renewal test is similar to a static test because it is conducted in still water, but the test solutions and control water are renewed periodically (usually at 24 h intervals) by transferring the test organisms to chambers with freshly prepared material or by removing and replacing the material in the fish containers.
4) **Continuous flow-through technique:** In this method, the test solutions and control water flow into and out of the chambers in which the test organisms are maintained. Two procedures are recommended. In the first procedure large volume of the test solutions are prepared before the beginning of the test and this flow through the test chambers. In the second and most common procedure, fresh test solutions are prepared continuously for every few hours in a toxicant delivery system.

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as *acute toxicity*, the adverse effects should occur within 14 days of the administration of the substance.

Assessment of toxicity on particular organism exposed to a particular toxicant will reveal facts regarding the health of given ecosystem and would eventually help us to propose policies to protect the ecosystem. Toxicity tests will reveal the organism’s sensitivity to a particular toxicant that would help us to determine the permissible limit of a toxicant in an ecosystem.

The decision whether a certain xenobiotic is dangerous for the aquatic system and the food chain, can only be made after the,

a. Mammalian acute toxicity

b. Bacteria acute toxicity

c. Fish acute toxicity and

d. Biological dissociation tests have been carried out in detail (Ardali, 1990).

The fact that increasing use of contaminating chemicals in many industrialised parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity (Brandao et al., 1992). The first step is the acute toxicity test on algae, fish, etc. in order to show the potential risks of these chemicals (OECD, 1993). Although the initial aquatic toxicity tests were carried out using bacteria, invertebrates like
Cladocera and Rotifera and other groups, they can in no way replace the actual test performed on fish, which is the last chain in the aquatic food cycle (Castano et al., 1996).

Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years).

It is widely considered unethical to use humans as test subjects for acute (or chronic) toxicity research. However, some information can be gained from investigating accidental human exposures (e.g. factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, invitro testing methods and inference from data on similar substances.

The assessment of exposure can be performed by measuring the concentration of a substance administered to a particular organism (Oga, 2003). The study of concentration-response or concentration-effect in toxicology is essential and is used to determine the median lethal concentration ($LC_{50}$) of drugs and other chemicals (Goodman and Gilman, 2007). The concentration-response curve is represented by the Gaussian theory, rarely found in practice. This curve is calculated statistically from observations of mortality after exposure related to concentrations of the substance to be tested, and it is widely used to calculate the 50% lethal concentration ($LC_{50}$). The $LC_{50}$ is thus a statistical index which indicates the concentration of a chemical agent capable of causing death in 50% of organisms in a population with defined experimental conditions.

To know the effects of toxic substances and classify them according to their potential lethality or toxicity and concentration-response curve, one needs to perform toxicological tests (Oga, 2003).

**Median Lethal Concentration ($LC_{50}$):** Statistically derived concentration of a substance in an environmental medium expected to kill 50% of organisms in a given population under a defined set of conditions.

Standard measure of the toxicity of the surrounding medium that will kill half of the sample population of a specific test-animal in a specified period through exposure via
inhalation (respiration). LC$_{50}$ is measured in micrograms (or milligrams) of the material per liter, or parts per million (ppm), of air or water; lower the amount, more toxic the material. Used in the comparison of toxicities, LC$_{50}$ values cannot be directly extrapolated from one species to the other or to humans also called Median Lethal Concentration or Population Critical Concentration 50.

Acute toxicity caused by different toxicant on freshwater fish can evaluate by quantitative parameters like survival and mortality of test animals and sensitivity of different fish species against metal’s toxicity (Kausar and Javed, 2012, Azmat et al., 2012, Ebrahipoure et al., 2010). Toxicity testing with a single chemical composition is inadequate identification of pollutant selective toxicity on aquatic biota and does not allow to evaluation pollutant hazard to the environment. Apart from detecting a threshold above which fish are likely to be killed, data obtained on the concentration of selected individual pollutants which are lethal to fish can also provide very necessary information. Toxicity in fish is the culmination of a series of events involving various physical, chemical and biological processes.

**LC$_{50}$:** Estimation of median lethal concentration or dosage (LC$_{50}$ and LD$_{50}$ respectively) is very valuable. LC$_{50}$ or LD$_{50}$ is indicator to the level of resistance of population response to metals (Claude, 2005; Reda et al., 2010). The knowledge obtained from dose response studies in animals is used to set standards for human exposure and the amount of chemical residue that is allowed in the environment. In order to obtain information on pollutant toxicity range, species with generally different susceptibilities or metabolic activities should be used, including those easily available and common in the area where the toxicant may occur. Therefore, comparative toxicity studies should be developed to identify species that produce results suitable to the evaluation of eco toxicity of the pollutant under study (Svecevicius, 2010).
MEASURES OF ACUTE TOXICITY

Regulatory values

Limits for short-term exposure, such as STEL's or CV's are defined only if there is a particular acute toxicity associated with a substance. These limits are set by the American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA), based on experimental data. The values set by these organizations do not always coincide exactly, and in the chemical industry it is general practice to choose the most conservative value in order to ensure the safety of employees. The values can typically be found in a material safety data sheet. There are also different values based on the method of entry of the compound (oral, dermal, or inhalation).

Threshold limit value-time-weighted-average

The maximum concentration to which a worker can be exposed every work day (8 hours) and experience no adverse health effects.

Short-Term Exposure Limit, STEL or Threshold limit value-short-term exposure limit, TLV-STEL: The concentration which no person should be exposed to for more than 15 minutes during an 8 hour work day.

Experimental values

- Observed- adverse-effect level, NOAEL
- Lowest- observed- adverse-effect level, LOAEL
- Maximum tolerable concentration, MTC, LC$_9$; Maximum tolerable dose, MTD, LD$_9$
- Minimum lethal concentration, LC min; Minimum lethal dose, LD Minimum
- Median lethal concentration, LC$_{50}$; Median lethal dose, LD$_{50}$; Median lethal time, LT$_{50}$
MEASURES OF ACUTE TOXICITY

Regulatory values

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Experimental values

- Observed- adverse- effect level, NOAEL
- Lowest- observed- adverse- effect level, LOAEL
- Maximum tolerable concentration, MTC, LC0 ; Maximum tolerable dose, MTD, LD0
- Minimum lethal concentration, LC min; Minimum lethal dose, LD Minimum
- Median lethal concentration, LC50; Median lethal dose, LD50; Median lethal time, LT50
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- Absolute lethal concentration, $LC_{100}$; Absolute lethal dose, $LD_{100}$

- The most referenced value in the chemical industry is the median lethal dose, or $LD_{50}$.

- This is the concentration of substance which resulted in the death of 50% of test subjects (typically mice or rats) in the laboratory.

- The most referenced value in the Aquaculture industry is the median lethal concentration or $LC_{50}$. This is the concentration of substance which resulted in the death of 50% of the test subjects (typically fish, Crabs, Prawns, Snails etc) in the laboratory.

Responses and treatments

When a person has been exposed to an acutely toxic dose of a substance, they can be treated in a number of ways in order to minimize the harmful effects. Obviously, the severity of the response is related to the severity of the toxic response exhibited. These treatment methods include (but are not limited to):

- Emergency showers used for removing irritating or hazardous chemicals from the skin.

- Emergency eye washes used for removing any irritating or hazardous chemicals from the eyes.

- Activated charcoal used to bind and remove harmful substances consumed orally. This is used as an alternative to conventional stomach pumping.

It is customary to express the concentration which kills a certain percentage of test species say 50% as $LC_{50}$ ($X$ mgs) / $y$ hours. Often most of these investigations which refer to the evaluation of toxicity are made for 24, 48, 72 or 96 hours.
The factors which are responsible for variation in toxicological investigation.

1. **Flow of water**: Burke and Ferguson (1968) reported difference in the toxicity of pesticides between static and flowing water to fish.

2. **Temperature**: The pesticide toxicity increases with a raise in temperature of the medium.

3. **Density**: Increase in fish density enhances the toxicity of pesticides (Holden, 1973). Small individuals are more sensitive to toxicants than to large ones.

4. **Water**: The chemistry of water influences the toxicity of chemicals (Pickering et al., 1962).

5. **Size of the fish**: Small individuals are more sensitive to toxicants than the large ones.

As the present study is also eco-physiological in nature an attempt is made in to determine the LC$_{50}$ of Lihcin to *Catla catla* evolve the sub-lethal and safe levels of this toxic compound to both of the fishes.
RESULTS

In the present study the fresh water edible Indian Major Carp fish, *Catla catla* (Hamilton, 1822), commonly known as “Botcha” or “Krishna Botcha” was selected as an experimental model as it is one of the edible fishes of south India found in freshwater streams and it is highly commercially cultured in fresh water ponds. These fishes are highly sensitive to the environmental pollutants.

*Catla (Catla catla)*, also known as the major (Indian) carp, is an economically important South Asian freshwater fish in the carp family Cyprinidae. It is commonly found in rivers and lakes in China, northern India, Nepal, Myanmar, Bangladesh and Pakistan. *Catla* is a fish with large and broad head, a large protruding lower jaw and upturned mouth. It has large, greyish scales on dorsal side and whitish on belly. *Catla* is a surface feeder and mid water feeder also. Adults feed on zooplankton but young ones on both zooplankton and phytoplankton.

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 0.447 mg/ L as the mean lethal concentration of Lihocin to the fresh water edible Indian Major Carp fish *Catla catla*.

Probit and percent mortality data of *Catla catla* in different concentrations (ppm) of Lihocin 96 hours of exposure are presented in Table - 1.1. From the data it is understood that at any period of exposure to the Lihocin showed a linear relationship between the percent or probit mortality of the fish and the Lihocin concentration.
Table 1.1: Effect of different concentrations of Lihocin on the mortality of the freshwater fish, *Catla catla*

<table>
<thead>
<tr>
<th>Conc. of Lihocin (mg/L)</th>
<th>Log Concentration</th>
<th>No. of animals exposed</th>
<th>No. of animals died</th>
<th>Percent mortality</th>
<th>Probit mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.085</td>
<td>-1.070</td>
<td>20</td>
<td>02</td>
<td>10</td>
<td>3.80</td>
</tr>
<tr>
<td>0.170</td>
<td>-0.769</td>
<td>20</td>
<td>05</td>
<td>15</td>
<td>4.04</td>
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<tr>
<td>0.255</td>
<td>-0.593</td>
<td>20</td>
<td>10</td>
<td>25</td>
<td>4.38</td>
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<tr>
<td>0.34</td>
<td>-0.468</td>
<td>20</td>
<td>14</td>
<td>38</td>
<td>4.82</td>
</tr>
<tr>
<td>0.425</td>
<td>-0.371</td>
<td>20</td>
<td>17</td>
<td>58</td>
<td>5.20</td>
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<tr>
<td>0.510</td>
<td>-0.292</td>
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<td>18</td>
<td>78</td>
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</tr>
<tr>
<td>0.595</td>
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<td>19</td>
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<td>6.28</td>
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<td>20</td>
<td>20</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>0.765</td>
<td>-0.116</td>
<td>20</td>
<td>20</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

The data on the percent and probit mortality of the *Catla catla* in different concentrations (ppm) of Lihocin for 96 hours of exposure are presented in Table - 1.1 and Figures - 1.1 and 1.2. It is clear from the data that at any period of exposure to the pesticide, in fish there was a linear relationship between the percent or probit mortality of the fish and the pesticide concentration.

Thus the percent and probit mortality of the fish increased with the increase in the concentration of pesticide. The percent mortality plotted against log concentration of pesticide gave sigmoid curves where as the probit kill plotted against log concentration showed a straight line (Figures - 1.1 and 1.2). The 96 hours LC50 of the pesticide to the fish was derived by taking the mean LC50 obtained from the percent and probit mortality curves.
The Sigmoid shape of the dose-response relationship is caused by the use of low values at the X-axis.

**Figure-1.1: Representing Percent Mortality**

**Figure-1.2: Representing Probit Mortality**

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DISCUSSION

The fish is a sensitive indicator of the quality of the aquatic environment, since it is highly susceptible to very low concentrations of the pesticides in the medium. These pesticides have profound biochemical and physiological effects on the fish, the magnitude of which varies with concentration and duration of exposure. Fish are also often used as sentinel organisms for ecotoxicological studies, because they play numbers of roles in the tropic web, accumulate toxic substances and respond to lower concentration of mutagens (Cavas and Ergne - Gozukara, 2005), therefore, the use of fish biomarkers as indices of the effects of pollution an of increasing importance, and can permit early delectation of aquatic environmental problems (Van Der oost et al., 2003). Acute toxicity has been used to derive heater quality guidelines for regulatory measures.

The result of the median lethal concentration (LC50) of carp fish, Catla catla for organochlorine pesticide, Lihocin in the present study for 96 hours is 0.447 mg/L (Table - 1.1). It is obviously identified that the toxicity of the chemicals to aquatic organisms has been influenced by age, size, sex and health of the species (Abdul - Farah et al., 2004). It is also identified that various physiological parameters such as quality, temperature, pH, dissolved oxygen and turbidity of water, amount and kind of aquatic vegetation, concentration and chemical formulation and its exposure have also greatly influencing, on median lethal concentration, (Gupta et al., 1984 ; Young, 2001). Warren (1997), and pesticides in to aquatic medium might be caused to decrease the DO2 concentration which impaired respiration lead to asphyxiation. The fresh water fish Catla catla exposed to organochlorine insecticide, Lihocin was imposed stress progressively leading to death. The respiratory impairment due to the toxic effect of Lihocin on the gills of Catla catla, similar to the reports of Abdul Farah et al., (2004), Filak et al., (2002) and Ayvoola (2008). In some times death may also accused either by direct toxicity or indirectly by making the medium un conducive for the fish or even by both. The abnormal movements like incessant jumping, restlessness vigorous jerking movements, surface to bottom movements were also appeared, similar to the observations of Pandey et al., (2005) and Chandra (2008).
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(b) Behavioural Changes
(B) BEHAVIOURAL MANIFESTATIONS

INTRODUCTION

Fish serve as consumers of small organism and food chain continues to higher organisms. They contribute to economy of many nations and provided recreation and psychological value to naturalist, sports, enthusiast and aquarist. They also play important role directly or indirectly in the protection of heritage and life of human beings. The threats and the integrity of the environment and germ plasm resource pose serious concern. The behaviour of fish is an index of its physiological status and any change in behaviour indicates a change in the physiological activities of fish and vice versa. Not much literature is available on the ethology of fish under pesticidal toxicity (Gouda et al., 1981; Das and Konar 1974).

In the laboratory, fish behaviour can be sensitive marker of toxicant induced stress (Atchison et al., 1987; Little et al., 1985; Raind, 1985; Westlake, 1984). Behaviour is an organismal level of all the above mentioned parameters including biochemical, physiological state of the animal under the influence of the environment. Further behavioural study should have objectives that should (1) be easily observed in the laboratory or field; (2) be sensitive to the chemicals of interest; (3) be previously well described (4) be ecologically relevant to species survival and (5) integrated several sensory and / or mechanical modalities. In addition, the method should be routinely available and simple to employ.

Behavioural tests typically have been precluded from the hazard assessment process because they are often labour intensive, subjective and difficult to quantify and field variety. Several reviews, however, have demonstrated the sensitivity of fish behavioural toxicity tests and have suggested that these tests be added to the current hazard evaluation process (Olla et al., 1980; Westlake, 1984, Little et al., 1985). Various behavioural changes that have been associated with OP and OC insecticides in a number of species of fish include loss of locomotor control (Raind, 1977), avoidance (Kynard, 1974), failure to select optimal temperature (Domanik and Zar, 1978), decreased feeding, increased aggression, elevated number of comfort movements and respiratory disruption.
(Bull and McInerney, 1974). Very little information is available on the behavioural toxicology of fish exposed to organochlorine insecticides. The present investigation is so designed to study the behavioural manifestation of fresh water teleostean fish, *Catla catla* to median lethal concentration of OC pesticide Lihocin.
RESULTS

The control fish fed on normal feed and exposed to acetone. The percent mortality of fish after exposure to sub lethal concentration of Lihocin for 24, 48, 72 and 96 hrs was recorded. Lihocin treated fish are exhibited various abnormal behavioural changes and all frequently. They also occasionally tried to jump out of water. The experimental fish exhibited increased opercular movement, increased mucous secretion and progressively became sluggish and lethargic. Prior to death in contaminated medium, the fishes mostly showed abnormal swimming movements including loss of orientation and a tendency of muscular tetany. The Lihocin treated fish body became fade and darkened compared to controls. *Catla catla* showed behavioural changes against Lihocin intoxication. These were increase opercular movement, sluggish, lethargic and abnormal swimming, loss of buoyancy and muscular tetany. The treated fishes also showed fading of their body colour. These behavioural changes can be considered as symptoms of stress on account of the toxicological nature of the environment.

The behavioural changes showed by the fishes after Lihocin intoxication are similar to those observed in other fishes exposed to organophosphate pesticides (Simons, 1997). Altered movement of *Channa gachua* at different concentrations of dimethoate exposure has been reported by Verma et al., 1978; Ghose, 1986b; Sweilum, 2006; Ghose, 1986a). The hyperactivity and erratic movements were observed, especially during the first 48 hrs of exposure with dimethoate to the *Cyprinus carpio* fry (Verma et al., 1978).

The behavioural changes showed by the fishes after Lihocin intoxication are similar to those observed in other fishes exposed to organophosphate pesticides. The hyperactivity and erratic movements were observed during the first 24 hrs of exposure with Lihocin to the *Catla catla* fry. In the present study *Catla catla* showed skin discoloration. Such types of changes were also observed in Zebra fish after toxaphene intoxication (Fulton and Key, 2001).
Table 1.3: Behavioural abnormalities monitored in the fish *Catla catla*, treated with sub-lethal concentration of Lihocin at 24h, 48h, 72h and 96h.

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<th>S.NO</th>
<th>EXPOSURE PERIODS</th>
<th>MONITORED BEHAVIOURAL CHANGES</th>
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| 1.   | After 24 hrs exposure | - restlessness, rapid surfacing,  
                                  - peeling of skin and colour fading |
| 2.   | After 48 hrs exposure | - slightly reduced activity  
                                  - gradual increase in colour fading  
                                  - gill adhesion and a thin film of mucous on gills, operculum and general body surface at this stage.  
                                  - nudge and nip |
| 3.   | After 72 hrs exposure | - fish moving towards surface water and gulping of air is increased,  
                                  - loss of balance and jerky movements during swimming.  
                                  - weakened in test animals as compared to controls |
| 4.   | After 96 hrs exposure | - Prominent ulcerations on the base of caudal fin, pectoral fin and on trunk of fish.  
                                  - haemorrhages were also identified on fins and trunk of some fish  
                                  - a thick mucous film was formed on whole body and gills, in all test fishes  
                                  - the experimental fishes lost their natural colouration and become almost reddish black / dark red in colour.  
                                  - S-jerk movements and burst swimming  
                                  - fin flickering |
The following morphological and behavioural abnormalities were observed as -

A) MORPHOLOGY:

a) Change in colour: The fish becomes black in colour on exposure to near lethal (LC$_{50}$) concentration of Lihocin only, but no change in colour was observed in the fish exposed to sub lethal concentration.

b) Change in gill colour: The normal gill colour is red. But in fishes exposed to sub lethal concentration of Lihocin, the gill colour has changed to pale red.

c) Secretion of mucus: A thin film of mucus is observed all over the body and on gill also. The quantity of mucus secretion increased in lethal concentration compared to sub lethal concentration of Lihocin. The secretion of mucus may have a role in protecting the skin etc, against pesticides.

B) BEHAVIOUR BIOLOGY:

a) Irregular and erratic movements: These were observed in fishes exposed to sub lethal concentration of Lihocin within half an hour while this abnormal behaviour sets in after 6 hrs in fishes exposed to sub lethal concentration of Lihocin.

b) Loss of Balance: The fish exposed to sub lethal concentrations of Lihocin, lost their balance and showed parallel movement. The magnitude of change in balanced movements was more in the fish exposed to sub lethal concentration of Lihocin.

c) The frequency of "Surfacing": This phenomenon was more in fishes exposed to sub lethal concentrations of Lihocin over the controls. The "surfacing" phenomenon was higher in the fishes exposed to sub lethal (about 3 to 4 times per minute) concentration. In the control fishes, the surfacing phenomenon was about 2 to 4 time per 10 min. This phenomenon may indicate the prevalence of hypoxic stress on fish. It may also suggest an imbalance in oxygen uptake at animal level where a tilt in the basal metabolism of the fish observed which might be due to the induction of inhibition of oxygen uptake at the animal level. Since the reasons might be varied, it is not clear why animal feels hypoxia, even though there is ample oxygen in the medium. However, the increase in the
frequency of the surfacing phenomenon may be ascribed to the setting up of an imbalance in the physiology of the fish. Besides, the surfacing phenomenon can also be considered a "simple and sensitive bio detector model" just to qualify the aquatic environment.

d) The rate of magnitude of opercular movement: In normal fish the opercular beats are recorded as 65 ± 3.54/minute. However it is observed more in the fishes exposed to lethal concentration (100 ± 12 beats/minute) than in fishes exposed sub lethal concentration (80 ± 15 beats/minute). The possible reason for the greater incidence of opercular movement in the Lihocin exposed fishes might be to facilitate the passing of higher amount of oxygen uptake. This is a sort of "behaviour physiological adaptation" to overcome the possibility of the induced hypoxic condition.

e) Ethology: It is a neurotropically regulated and an energy dependent phenomenon. The organochlorides are not only found to inhibit acetylcholinesterase (Soliman 1981; Matin and Agarwal 1982), but also to favour the release of acetylcholine, in the neural and neuromotor regions, thereby causing "hyper excitability". ATPase system (Mg$^{2+}$, Ca$^{2+}$ and Na$^{+}$ - K$^{+}$ ATPase) plays an important role in the conduction of nerve impulse. The organochlorides are found to inhibit all types of ATPases, especially the Mg$^{2+}$ ATPase (Matsumura et al., 1971, 1975; Koch et al., 1969; Cutkomp et al., 1982). The double effect of inhibition of AChE, release of ACh and inhibition of ATPase complex might be responsible for the manifestation of the abnormal behaviour, like erratic movements, loss of balance etc., there by leading to neurotoxicity, which is also indicated through convulsions and tremors. The accumulation of Ach during organochloride stress has been earlier reported to be the prime factor for convulsions. But recently this theory was ruled out (IRPTC report 1985). Hence it is still a puzzling problem. However, the possible role of NH$_3$ toxicity in inducing tremors cannot be ruled out (Martin et al., 1989; Martin and Agarwal, 1982). Hence one can presume that more inhibition of ATPase system and accumulation of acetylcholine are not the only prime causes. But the organochlorides seem to act upon other biochemical systems which have an equal and important role to play either in the regulation of behavioural movements or in (indirectly) supporting the functioning of the nervous system. In the case of organophosphates and carbonates, this situation does not arise since they are known to be
neurotoxic, where the reasons for the convulsions and tremors could be ascribed to acetylcholine accumulation and AChE inhibition.

Since the anomalous behaviour is an index of the toxicological effect of the Lihocin model namely “Behaviour Index Detector Model” (BIDM), can be designed with fish as the test species in assessing the quality of the aquatic environment. A systematic approach can be made by correlating the degree of toxicity versus the degree of abnormal behaviour and this becomes the basis for formulating a “Simple and sensitive bio detector model” in analysing the possible level of toxicants in the environment. The validity of this model can be further amplified by correlating the abnormal behaviour with the behaviour regulating biofactors like enzymes, hormones etc, under the toxic stress conditions.
DISCUSSION

The migration of the fish to the bottom of the tank following the addition of Lihocin, clearly indicates the avoidance behaviour of the fish. It is obvious that the studying the behaviour 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. It is well known that the Fish are an excellent model used to evaluate ecological impact of those pollutants on the aquatic ecosystem. Recently many researchers have proposed several behavioural parameters as indicators for ecologically relevant monitoring of environmental contamination (Atchison et al., 1987). The normal behaviours of fishes are usually triggered by external stimuli acting through neural networks (Weber and Spieler, 1994). Any alterations in their normal behavioural pattern can be affected by numerous physiological and environmental influences. In the present study the changes in behavioural pattern was also observed in order to see if the toxic effects of aquatic contaminants Lihocin can have severe implications for survival of the fish at short duration of 96 h. During the experiments, the behaviour of fishes to the exposed contaminant Lihocin showed an unusual behaviour. 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.

The behaviour of the animal showed a drastic alteration in Lihocin 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, 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 behavioural changes that was observed during the treatment.
The erratic swimming of the treated fish indicates loss of equilibrium. It is likely that the region in the brain which is associated with the maintenance of equilibrium should have been affected (Rao et al., 1981; Mehrle and Mayer, 1975; Drummond et al., 1986). Loss of equilibrium and erratic swimming are reported in blue gills exposed to Dursban (Mehrle and Mayer, 1975). Excited and erratic movement was observed by Sulaiman et al. (1989); Radhaiah and Jayantha Rao (1988), Henry and Atchison (1986), Sandheinrich and Atchison (1990) and Henry and Atchison, (1984). Increase in fin "flickers" observed in the treated fish is not uncommon (Drummond et al., 1986). These behavioural changes were seen and confirmed in the present investigation also.

In the present study of test fish, *Catla catla* showed normal behaviour in control group but jerky movements, hyper secretion of mucous, opening mouth for gasping, losing scales, hyperactivity were observed experimental group. Behavioural characteristics are obviously sensitive indicators of toxicant effect. In toxic medium of Lihocin the fish sank to bottom of the test chamber and independency in swimming. Subsequently fish moved to the corners of the test chambers, which can be viewed as avoidance behaviour of the fish to the toxicant. In the toxic environment fish exhibited irregular, erratic, burst swimming movements and loss of equilibrium followed by hanging vertically in water. The above symptoms are due to inhibition of AchE activity leading to accumulation of acetylcholine in cholinergic synapses ensuing hyper stimulation.

Increase in opercular movement was initially observed but later decreased with increase of exposure period. They slowly became lethargic, restless, and secreted excess mucus all over the body. Intermittently some of the fish were hyper excited resulting in erratic movements. An excess secretion of mucous in fish forms a non-specific response against toxicants, thereby probably reducing toxicant contact. It also forms a barrier between the body and the toxic medium, so as to minimize its irritating effect, or to scavenge it through epidermal mucus. Similar observations were made by (Rao, 2006; Tripathi et al., 2006) following RPR-V exposure to euryhaline fish, *Oreochromis mossambicus*. Gulping air and swimming at the water surface (surfacing phenomenon) were observed also with mucus secretion on the body in both the lethal and sub lethal
exposure periods (Shivakumar et al., 2005), reported that fish in sub lethal concentration were found under stress but that was not fatal (David et al., 2005).

Reported that the abnormal changes in the fish exposed to lethal concentration cypermethrin are time dependent (David et al., 2005). Observed that the fish is exposed to cypermethrin, erratic swimming, hyper and hypoactive, imbalance in posture, increase in surfacing activity, opercular movement, gradual loss in equilibrium, spreading of excess of mucus all over the surface of the body.

Fishes exhibited a number of behavioural changes when they were exposed to different concentrations of Lihocin. The opercular movement of fishes initially increases and then gradually decreases. Decreased opercular movement probably helps in reducing absorption of pesticide through gills. Edwards (1973); Rao et al. (2005) and Prasanth et al. (2005) have reported that the abnormal swimming and loss of balance was caused by the deficiency in nervous and muscular coordination which may be due accumulation of acetylcholine in synaptic and neuromuscular junctions. It is necessary, to select behavioural indices for monitoring that relates to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural systems.

Finally as a defensive mechanism the fishes secreted mucous all over the body to prevent the entry of Lihocin 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). In the present study the sub lethal concentrations of Lihocin induced that the 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 hyper excitable movement the fishes followed by settlement at the bottom of the tank, loss of consciousness, lethargic and finally died.