Chapter - I

EVALUATION OF TOXICITY
Toxicity is the degree to which a substance (a toxin or poison) can harm humans or animals. It is the sum of adverse effects or the degree of danger posed by a substance to living organisms. It is expressed generally as a dose response relationship involving the quantity of substance to which the organism is exposed and the route of exposure is skin (absorption), mouth (ingestion), or respiratory tract (inhalation). Toxicity is classified usually as acute, chronic and subchronic toxicity. Acute toxicity involves harmful effects in an organism through a single or short-term exposure. Chronic toxicity is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism (Fauci, Anthony, S. et al., 2008). Subchronic toxicity is the harmful effects produced through repeated or continuous exposure over twelve months or more but less than the normal lifespan of the organism.

The level of toxicity of a toxicant can be measured in terms of its concentration or dose which kills a known number of population of a given species within a definite period of time. Since the evaluation of toxicity of a test chemical is a sensitive phenomenon, which can be influenced by several factors such as size (Heit and Fingerman, 1977; Brazinskii et al., 1979; Jayantha Rao, 1982), weight (Pickering et al., 1962), nutritional status (Pal and Kushwah, 1981; Das and Garg, 1981), its developmental stage (Kamaldeep and Toor, 1977), species specificity (Gouda et al., 1981; Li and Choa, 1981; Jacob et al., 1982; Janardhan et al., 1987; Surendranath et al., 1987a,b.), time of exposure and temperature (Macek et al., 1969), chronobiology of the animal (Uttaman et al., 1979), increase in animal density (Holden, 1973) and sex of the animal (Klein et al., 1983; Chanh et al., 1981). Every pesticide may vary greatly in its toxicity and persistence (Moore, 1969). Thus, various factors influence the LC50 values. These factors are manifold and dependent upon the given set of experimental conditions (Russell and Overstreet, 1987).

Toxicology is a branch of biology, chemistry, and medicine concerned with the study of the adverse effects of chemicals on living organisms. It is the study of symptoms, mechanisms, treatments and detection of poisoning. Extremely high concentrations of pesticides are more toxic to the biological systems. Evaluation of toxicity of a chemical therefore is necessary to know, because it would help us to know its potentiality so that it could be possible to desire more powerful formulations. The toxicity study is essential to find out toxicants limit and safe concentration, so that there will be minimum harm to aquatic
fauna in the near future. The necessity of determining the toxicity of substances to commercially aquatic forms at the lower level of the food chain has been useful and accepted for water quality management.

Unconscious and reckless handling of chemicals resulted in several disastrous incidences of pollution and accidental poisoning. Hence man has recognized the need for better control of the present use and future development of chemicals. In recent years it has become a normal practice to test all new chemicals for the toxicity before they could reach consumers. Major purpose of the toxicological investigations to provide a basis for estimating the maximum dose that may be tolerated by animals throughout their life time without manifesting any adverse effect (Gralla, 1981).

Heavy dependence modern agriculture on agrochemicals such as fertilizers and pesticides is emerging as a threat to the ecological balance of aquatic ecosystems. At present many organic pesticides like organophosphorus, organochlorine and methyl carbamates are currently used for agro-practices. During the last one decade a tremendous progress has been made in the development of new compounds with better toxicity, therefore, a lot of work has been carried out on impact of pesticides on non-target aquatic organisms (Battaglin and Fairchild, 2002; Prasanth et al., 2005).

The pesticides play an important role in the production and preservation of food and other commercial crops by keeping a check on many species of harmful pests. Generally the synthetic organic pesticides are widely used as insecticides. Following decades the over use or misuse or careless use or mishandling of pesticides in agriculture, public health and forestry results in the contamination of soil, water, air, and vegetables posing a great threat to environment and non target organisms. Man has recognized the need for better control of the present use and future development of pesticides. This need has turned in to a demand from the public to have chemicals tested and retested before they reach the market and consumers. So the evaluation of the toxicity of a chemical from time to time is of importance in toxicological research.

The application of various pollutants such as pesticides, in the aquatic environment and their depositions in the biotic system is known to cause several structural and functional changes. Pesticides are unusual among environmental pollutants in that they are used deliberately for the purpose of killing harmful insects and pests. The aquatic ecosystem as a
greater part of the natural environment is also faced with the threat of a disturbing genetic base and biodiversity due to the indiscriminate use of pesticides (Rahman et al., 2002).

Pesticides become easily available in the food chain and subsequent accumulation in both aquatic and terrestrial flora and fauna (Mellamby K, 1967) with possible unquantifiable disastrous consequences are possible on the ecosystem (Terry G, 1987). Due to the residual effects of pesticides, important organ like liver and kidney are damaged in fishes (Rahman et al., 2002). Hence, the role of any pesticide can be well understood by analyzing either tissue or blood of an animal species. The intake of insecticides affects the biochemical composition of fishes (Jebokumar et al., 1990; Prasad et al., 2002). It has been shown by many scientists that insecticide mainly affects liver in fishes (Anthony et al., 1986; Bhushan et al., 2002).

Pesticides used for controlling pests in agriculture are one of the major causes of aquatic pollution. Among synthetic pesticides organophosphates are widely used in agriculture and in health and hygiene programs due to their high effectiveness as insecticides but less persistence in the environment. The shift from organochlorines to organophosphates has resulted into increased occurrence of organophosphates into water bodies causing acute and chronic toxicity to fish fauna (Rao et al., 2005; Velmurugan et al., 2007; Singh et al., 2009). The aquatic environment is currently under threat by the indiscriminate use of synthetic pesticides by the human activities and causing high risk to non-target organisms (Kumar et al., 2010). Pesticides are carried into aquatic ecosystem by surface runoff from sites of application and therefore the health of aquatic ecosystem is being adversely affected because they serve as ultimate sink for these pesticides (Singh et al., 2010). These pesticides are also found to be highly toxic not only to fish but also to other organisms which constitute food of the fish.

The pesticides are varying greatly in their action, toxicity and persistence. Evaluation of toxicity of a chemical could help in knowing its potentiality. The toxicity testing on the non-target organisms would definitely help to understand the hazardous nature of pesticides and to improve health condition of mankind on a long run.

Toxicity assessment is the characterization of the toxicological properties and effects of a substance, specifically the dose response relationship associated with a particular route of exposure. Several studies have been conducted in assessing the toxicity of pesticide to the aquatic biota especially fishes (Verma et al., 1982; Ravikrishnan et al., 1997; Vasit and Patil, 2005; Susan Anita et al., 2010). The wide use of fishes is probably due to their adaptability to
the laboratory conditions as well as their availability and their varying degree of sensitivity to the toxic substance (Verma et al., 1980). So, evaluation of the toxicity of the chemical is an important tool that helps in the field of toxicology.

The popular method of evaluating the toxicity of a toxicant in aquatic organisms is the determination of LC50, which is used in the comparison of toxicities. LC50 is the 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. It is also called as median lethal concentration or population critical concentration 50. LC50 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.

Many workers determined LC50s of different pesticides for a number of fish species (Allison et al., 1963; Burdick et al., 1964; Post and Schroder, 1971; Amminikutty and Rege, 1977; Dubale and Shah, 1979; Dubale and Shah, 1981; Kulshrestha and Arora, 1984; Girija and Jayantha Rao, 1985; Girija et al., 1986; Newsome et al., 1993; Shailaja, M.S. and Singbal, S.Y.S. 1994; Karunakaran et al., 1994 and Rao, 1994).

Organophosphates are highly toxic to fish and non-target aquatic organisms and are powerful nerve poisons, since they inhibit AChE activity (Coppage et al., 1975; Klaverkamp and Hobden, 1980). Several workers investigated the toxicity of organophosphorus pesticides in fish (Lockhart et al., 1973; Koundinya and Ramamurthi, 1979; Johnson and Finley, 1980; Kumar and Gupta, 1997; Santhakumar et al., 2000; Singh et al., 2010; Zhang et al., 2010; Srivastava et al., 2010; Barbieri and Ferreira, 2011; Maniyar et al., 2011).

Perusal of literature reveals paucity of information on acute toxicity of Phorate on freshwater fish, *Cyprinus carpio*. Hence, the present study has been focused to evaluate the acute toxic effects of Phorate on freshwater fish, *C. carpio*, of local importance. It is clear from earlier studies that LC50 of pesticides for a fresh water fish varies from species to species and the same species under the influence of number of factors including size and time of exposure. As these values are highly essential in evaluating the toxicity levels and determining the sublethal concentration, the present study is commenced with the determination of LC50 of phorate to fresh water fish Cyprinus carpio in order to evolve the lethal and safe levels of this toxic compound to fresh water fishes.
RESULTS

The data on the percent and probit mortality of the fish, *Cyprinus carpio* in different concentrations (ppm/l of water) of phorate at 96 hours of exposure are presented in Table-3. It is clear from the data that there was a linear relationship between the percent or probit mortality of the fish and the concentration of phorate. Thus the percent and/or mortality of the fish increased with the increase in phorate concentration. The percent mortality plotted against log concentrations of pesticide gave sigmoid curve (Figure-1), where as the probit mortality plotted against log concentrations gave straight line (Figure-2). The 96 hours LC$_{50}$ of phorate to fish was obtained by taking the means of LC$_{50}$s derived from the percent and probit mortality curves, as well as the value calculated by Dragstedt and Beheren’s method (Carpenter, 1975). The LC$_{50}$s obtained by these three methods along with their mean are presented in Table-4 for comparison. The mean of these three methods was considered in evaluating the level of phorate toxicity in the fish *Cyprinus carpio*.

DISCUSSION

Organophosphates (OPs) have become the most widely used class of insecticides in the world replacing the persistent and problematic organochlorine compounds. Exposure of aquatic ecosystems to these insecticides is difficult to assess because of their short persistence in the water column due to low solubility and rapid degradation. However, monitoring of these insecticides is important, because they are highly toxic to aquatic organisms like freshwater fishes. They are the non-target organisms, which are generally exposed to multiple concentrations of pesticides under field conditions.

Phorate is an organophosphorus insecticide and acaricide used to control sucking and chewing insects, leafhoppers, leafminers, mites, some nematodes, and rootworms (Wagner, 1989; Gallo and Lawryk, 1991). It is used in pine forests and on root and field crops, including corn, cotton, coffee, rice, beetroot, carrots, maize, sorgum, potatoes, tomatoes, soyabean, wheat, chillies, onion, sunflower, groundnut, some ornamental and herbaceous plants and bulbs. Phorate is an important pesticide to which the fresh water fishes are frequently exposed due to the indiscriminate use of this by the farmers. It is highly toxic to, and extremely fast-acting on bird species, freshwater fish, and aquatic invertebrates (Walker, and Keith, 1992). Phorate is very highly toxic to fish. Reported 96-hours LC$_{50}$ values of technical phorate range from 2 to 13 ug/L in cutthroat trout, bluegill sunfish and largemouth...
bass. Other 96-hours LC$_{50}$ values are 110 ug/L in northern pike and 280 ug/L in channel catfish (Johnson and Finley, 1980).

Research in the area of toxicology on the effects of phorate on fish is scarcely done. However, some work was carried out by Saxena and Sarin, (1979, 1980) on desert gerbil Meriones hurrianae; Mohssen Morowati, (1997, 1998, 2001) on the male swiss albino mouse, Mus musculus; Jyothi and Narayan, (1999) on fresh water fish Clarias batrachus and Anand Pratap Singh et al., (2010) on snake headed fish Channa punctatus, about the toxic effects of phorate. Reported 96-hours LC$_{50}$ values of phorate are 0.8 ppm in Clarias batrachus (Jyothi and Narayan, 1999.) and 0.3mg/L in Channa punctatus (Anand Pratap Singh et al.,2010).

In the present study the 96 hours LC$_{50}$ obtained for phorate is 0.71 ppm/L. From the present study it is very clear that phorate is highly toxic to freshwater fish, Cyprinus carpio, as the LC$_{50}$ of this compound to the fish is obtained at very lower concentration. The level of toxicity of pesticides may differ from one type to the another, and from one species to the another (Pickering et al., 1962; Macek and Mc. Allister, 1970; Chambers and Yarbrough, 1974). The differences could be attributed to the chemical nature of the toxicant, interaction of the chemical with biological system, resistance capacity of the animal, detoxification mechanisms involved, assay techniques, purity of the pesticide and the additives or emulsifiers present in the commercial grade formulations.

Different metabolic pathways occurring among fish species may result in different pattern of biotransformation, leading to more or less toxic metabolites (Johnsson and Toledo, 1993). Magnitude of toxic effects of pesticides also depends on length and weight, corporal surface/body weight ratio and breathing rate (Singh and Narain, 1982; Murty, 1986). This is in agreement with Sprague (1969) who observed variation in LC$_{50}$ values for the same species and toxicant depending on size, age and condition of test species along with experimental factors.

In the present study the fish maintained in freshwater without phorate behaved normally i.e., they were very active and movements were well coordinated. They were alert and at any site disturbance they swam faster. But in lethal and near lethal concentrations of phorate they became highly irritable and hyper excitable. Jumping movements were observed, with profuse mucus secretion and loss of equilibrium. Examination of gills revealed significant change in their colour from dark red to brownish black. High mucus films over
surface of gills was also observed. Some of the other behavioral changes observed in the fish exposed to phorate include opercular movement, dullness, loss of equilibrium, stop of food intake, erratic and hysterical swimming, swimming at the water surface, circling movement, and gasping. Prior to death, the fish became less active or generally inactive, remained hanging vertically in the water or lay down on their sides at higher concentrations. Under lethal conditions the fish slowly became sluggish with short jerky movements and erratic opercular activity; finally turned upside down and died. Paralytic movements and suffocation caused by the mucus film over gills could be a few reasons for the death of fish at lethal concentrations.

Behavioral changes observed in the present study in exposed carp, *Cyprinus carpio* appear to be manifestation of phorate toxicity. Upon exposure to this pesticide, increase in surfacing and gulping of surface waters appears to be an attempt by the fish to avoid breathing in poisoned water. Similar observations have been reported in *Anabas testudineus* after exposure to monocrotophos (Santhakumar and Balaji, 2001). Moreover, hypoxic condition also contributes to increase surfacing as reported by Radha et al. (1998). Hypoxic condition arises primarily due to damage of gills of pesticide exposed fish which hampers oxygen uptake (Velmurugan et al., 2007). Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and muscular junctions (Rao et al., 2005).

It may be concluded that the results of this study are highly useful in evaluating the phorate toxicity in fresh water environment. On the whole, with the knowledge of toxicity studies and behavioral observations it could be possible to establish limits of tolerance and susceptibility of the fish to the toxicity of phorate in the fresh water environment.
96 hours percent and probit mortality of the fish, *Cyprinus carpio* in different concentrations of phorate. Each value is a mean of three replicants.

**Table-3**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (ppm)</th>
<th>Log Concentration</th>
<th>Number of Fish exposed</th>
<th>Number of Fish dead</th>
<th>Percent Mortality</th>
<th>Probit Mortality</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>1.6020</td>
<td>30</td>
<td>2</td>
<td>6.7</td>
<td>3.52</td>
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<tr>
<td>2</td>
<td>0.5</td>
<td>1.6989</td>
<td>30</td>
<td>5</td>
<td>16.6</td>
<td>4.05</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>1.7781</td>
<td>30</td>
<td>9</td>
<td>30</td>
<td>4.48</td>
</tr>
<tr>
<td>4</td>
<td>0.7</td>
<td>1.8451</td>
<td>30</td>
<td>15</td>
<td>50</td>
<td>5.0</td>
</tr>
<tr>
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</tr>
<tr>
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<td>27</td>
<td>90</td>
<td>6.28</td>
</tr>
<tr>
<td>7</td>
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<td>2.0414</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>7.33</td>
</tr>
</tbody>
</table>
Table-4

LC$_{50}$ of phorate to the fish, *Cyprinus carpio* after 96 hours exposure

S. D. = Standard Deviation

<table>
<thead>
<tr>
<th>Name of the method</th>
<th>LC$_{50}$/96 hours (ppm/lit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent mortality (sigmoid curve)</td>
<td>0.70</td>
</tr>
<tr>
<td>Probit mortality (linear curve)</td>
<td>0.70</td>
</tr>
<tr>
<td>Dragstedt and Behren’s method</td>
<td>0.75</td>
</tr>
<tr>
<td>Mean</td>
<td>0.71</td>
</tr>
<tr>
<td>S. D. ±</td>
<td>0.029</td>
</tr>
</tbody>
</table>
Toxicity evaluation of phorate to fresh water fish, *Cyprinus carpio*. The graph showing sigmoid curve between percent mortality of the fish against log concentration of phorate.
Toxicity evaluation of phorate to fresh water fish, *Cyprinus carpio*. The graph showing linear curve between probit mortality of the fish against log concentration of phorate.

**Figure-2**

Log Concentration vs Probit Mortality