Chapter 2

Review of Literature

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The chapter, review of literature presents the investigations made in different parts of the world, on aquatic organisms after exposure to pesticides, especially organophosphorus compounds. The scientific papers are mainly be categorized under those relating to the solubility of the selected pesticide, biochemical aspects, haematological findings, histological observations and pesticide residue-studies.

2.1: Solubility of chlorpyrifos

The rapid dissipation of chlorpyrifos from aquatic ecosystems has important implications for aquatic risk assessment. Toxicity profiles observed during prolonged, constant concentration exposure in the laboratory may not accurately reflect toxicological responses to pulsed and rapidly declining concentrations in water under field conditions.

The major chlorpyrifos derivative, 3,5,6-trichloro pyridinol (TCP), does not cause cholinesterase inhibition and is of low to moderate toxicity to aquatic and terrestrial biota. Evaluation of LD$_{50}$ values (mg/kg) indicated that aquatic insects (Siegfried, 1993) might be more sensitive than terrestrial insects. Chlorpyrifos is primarily used to kill mosquitoes in the immature larval stages of development. It is generally considered to be non-persistent in the environment (Sharom et al, 1980).

Many organic substances are much more soluble in lipids than in water. These compounds enter animals because they are lipid soluble and then accumulate in the body fat of the animal. This bioaccumulation of substances in animals due to the high fat solubility of compounds has long been recognized (Randall et al, 1996).

According to Mace and Woodburnt (1995) the predominant determinant of chlorpyrifos toxicity to fish appears to be the test species, but toxicity may be influenced by exposure conditions, formulation, source and size of fish and water quality. Water hardness and pH do not appear to influence toxicity in laboratory tests because chlorpyrifos is nonpolar and non-ionizable. However, pH and temperature can affect the dissipation rate in water, which may influence
environmental exposures (Racke, 1993). Size of fish has been reported to influence toxicity in static tests (eg., El-Refai et al, 1976), possibly because absorption by the fish decreases the exposure concentration (Barron et al, 1993).

Technical grade chlorpyrifos generally appears to be of similar or greater toxicity than controlled release or emulsifiable concentrate formulations with lower LC₅₀ values (Jarvinen and Tanner, 1982).

In many of the pesticide toxicity studies, acetone has been used as the vehicle. Acetone was used by Bakhthavathsalam and Reddy (1983) to prepare the test solution of lindane and Miny and Sastry (1989) in the preparation of Monocrotophos solution. David (2005) used analytical grade acetone to prepare fenvalerate test solution and Shivakumar and David (2004) to prepare the solution of endosulfan. Acetone was found to be non-toxic to fish (Pickering et al, 1962). When embarking upon a series of toxicity studies, whenever possible, the test article to be investigated should be "technical grade" material of similar composition to what humans are expected to be exposed to and the vehicle used in formulating the test article is also appropriate for use as the control (Keller and Banks, 2006). According to Mallinckrodt Chemicals, acetone is expected to readily biodegrade and quickly evaporates, when released into water. This material has a log octanol-water partition coefficient of less than 3.0. This material is not expected to significantly bioaccumulate.

2.2: Biochemical studies

A number of studies have been made on the toxicity of different pesticides on aquatic and terrestrial organisms. Most of the studies dealing with the effects of pesticides on fish primarily focus on the short-term investigations involving whole animal responses such as gross abnormalities, behavioral changes in growth rate and mortality. Recently, more research is being conducted on physiological and biochemical responses of the agricultural pesticides on fish. In general, the pesticides increase the activities of some enzymes and decrease the activities of others, while the activities of a few enzymes remain unchanged in various tissues of
Signs of acute toxicity in fish include increased cough frequency and ventilation volume, and decreased ventilation frequency (Bradbury et al., 1991).

The chronic toxicity of chlorpyrifos to fish has been evaluated in early life stage studies (embryo or larval stage through juvenile life stages) and full life cycle studies. In general, growth was the most sensitive measure of toxicity in the majority of chronic toxicity tests with chlorpyrifos (Cripe et al., 1986; Goodman et al., 1985a; Jarvinen and Tanner, 1982). In addition to effects on growth and survival, reported sublethal effects of chlorpyrifos include behavioural avoidance, changes in temperature preference and biochemical alterations (Mace et al., 1995).

Alterations in the chemical composition of the natural aquatic environment usually affect behavioural and physiological systems of the inhabitants, particularly those of the fish (Radhaiah et al., 1987). The fish show restlessness, rapid body movement, convulsions, difficulty in respiration, excess in mucus secretion, changes in colour and loss of balance when exposed to pesticides. Similar changes in behaviour are also observed in fishes exposed to different pesticides (Haider and Inbaraj, 1986). Liver, kidney, brain and gills are the most vulnerable organs of a fish exposed to the medium containing any type of toxicant (Jana and Bandyopadhyaya, 1987).

Fish species are sensitive to enzymatic and hormone disruptors. Chronic exposure to low levels of pesticides may have a more significant effect on fish populations than acute poisoning. Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behaviour and physiology that impair both survival and reproduction (Kegley et al., 1999). Experimental exposure of fish to pesticides has been shown to depress protein values in brain, gills, muscle, kidney and liver. In the kidney and liver, stress-induced significant decrease in the protein content was observed by Tilak et al., 1991.

A significant decline was observed in the globulin content in the blood of chlorpyrifos-treated mice compared to the control mice. A significant decrease in acetylcholinesterase was evident in chlorpyrifos treated mice. Hegazi (1989)
reported that sublethal concentrations of chlorpyrifos reduced brain Acetylcholinesterase (AChE) of the catfish (Clarias lazera). He also reported that sublethal concentrations of chlorpyrifos reduced muscle and liver glycogen and blood glucose levels of C. lazera. The inhibition of acetylcholinesterase by organophosphate compounds has become an index of organophosphate pollution in the aquatic environment (Williams and Sova, 1966). Organophosphates effectively poison the enzyme by phosphorylation and thus block the hydrolysis of acetylcholine. This group of pesticides interferes with the process of synaptic transmission by inhibiting the activity of acetylcholinesterase. This enzyme is important for the neurological functioning of the sensory, integrative and neuromuscular systems in fish. The inhibition of this enzyme alters respiration (Klaverkamp et al, 1977), swimming (Post and Leisure, 1974) and social interaction (Symons, 1973) in salmonides. Chlorpyrifos causes many damages to human and animal health. Its effects on nervous system are well known through the inhibition of the acetylcholinesterase enzyme, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine into choline and acetate (Garcia et al., 2005). Several reports suggest that various organophosphorus pesticides at concentrations close to their LC\textsubscript{50} values can induce a decrease in the enzyme level to 60-20% of their normal physiological activity in fish. Similar changes were found and used by various authors to evaluate the effects of these pesticides on fish (Salte et al, 1987).

The exposure of fish to organophosphates inhibits the activities of several enzymes such as glucose-6-phosphatase, acid phosphatase, pyruvate dehydrogenase, succinate dehydrogenase and acetylcholinesterase in brain. Inhibition in Cytochrome oxidase activity in brain, kidney, gill, liver and muscle have reported by Sastry and Sharma, 1980; Natarajan, 1984. Sastry and Sharma showed that the activity of alkaline phosphatase, ATPase and lactate dehydrogenase remained unchanged in the brain of Ophiocephalus punctatus after 15 days of exposure to an organophosphate pesticide. The exposure of methyl parathion to the freshwater fish, Tilapia mossambica, for 48hr decreased the activity of succinate and lactate dehydrogenase in the gill, liver and muscle tissues (Rao and Rao, 1979).
Reddy et al (1986) reported an increase in the activity of acid phosphatase in the hepatopancreas of the crab Oziotelphusa senex after Methyl parathion exposure. From the study it is inferred that the exposure attributed the stimulation of acid phosphatase activity, alteration in osteoblasts which resulted in more production and liberation of the enzyme, proliferation of smooth endoplasmic reticulum in the parenchymatous cells, that leads to increased production and release of microsomal enzymes. The pesticide may also induce peroxidation of lysosomal membrane leading to breakdown or increased permeability. Both can be responsible for the liberation of acid phosphatase thereby resulting in degeneration and necrosis in tissues (Gopalakrishnan, 1990).

Subburaju and Selvarajan (1989), reported changes in free sugar and amino acids, protein metabolism and lipid content in various regions of the brain of Tilapia mossambica exposed for 4 days to 0.7 μg/L chlorpyrifos. Reported behavioral effects from acute exposures have included immobility and erratic swimming (Subburaju and Selvarajan, 1988; Thirugnanam and Forgash, 1977).

A marked inhibition of DNA, RNA and protein contents was observed in the liver of the fish Brachydanio reieo by malathion and carbaryl. The effect of these pesticides on the in vitro protein synthesis by liver of the freshwater teleost Channa punctatus was studied by Saxena et al. (1988). Exposure period dependent depletion in protein content in endosulfan treated fish Oreochromis mossambicus was reported by Ganesan et al. (1989). Dose-dependent depletion of protein content in Barsillus bendelisis under toxicity to thiodon was reported by Deoray and Wagh (1991).

The organochlorine compounds have been shown to behave as antithyroid materials in fish. They reduce the metabolic activity and oxygen consumption in various tissues (Brown, 1957). The adverse effects of endosulfan and its isomers on the tissue protein, glycogen and lipid content of the freshwater fish, Channa punctatus, has been reported (Murty and Devi, 1982). The organochlorine pesticides have been shown to inhibit the activity of Na⁺K⁺-ATPase in several tissues of fish (Davis et al, 1971; Desaijah and Koch, 1975a). Dalela et al. (1988) have reported
inhibition in the activity of ATPase in several tissues of a freshwater teleost, *Channa gachua*.

Tripathi and Shukla (1990) have shown that an exposure for 7 days of an organophosphate pesticide, methyl parathion and an organochlorine pesticide, endosulfan caused a decline in the efficiency of TCA cycle and the anaerobic glycolytic pathway as reflected by the reduced activities of cytoplasmic malate dehydrogenase, mitochondrial malate dehydrogenase and lactate dehydrogenase in the liver and the skeletal muscle of the freshwater catfish, *Clarias batrachus*.

Since mature chloride cells are in permanent contact with the surrounding water they form an obvious target for aquatic pollutants (Mallatt, 1985; Evans, 1987).

### 2.3: Histological studies

The pesticides in aquatic ecosystems affect nontarget organisms such as fishes and prawns (Gupta, 2007). A number of pathological changes have been reported in fishes exposed to different organochlorine, organophosphate, carbamate and synthetic pyrethroid pesticides (Vijayalakshmi and Tilak, 1996; Tilak et al, 2001 a, b). Pesticide hazard on fish mortality, growth and tissue damage have been amply reported by Wildish et al. (1971) and Jackson (1976). Sudha and Mehrotra (1999) observed severe damage in the outermost serosa of muscle layers, necrosis in intestinal villi and increase in the number and size of muscle cells under sublethal exposure of carbaryl for a period of one month. Ramachandra (2000) observed that *Channa punctatus* on sublethal exposure of malathion caused significant reduction in the ovarian weight and diameter of developing oocytes and also degeneration of growing oocytes.

Braunbeck (1994) reports that the typical reaction of a rainbow trout hepatocyte exposed to organic toxicants is likely to include (1) disturbance of the highly cytoplasmic compartmentation; (2) augmentation of nucleoli and nuclear deformation in conjunction with a stimulation of karyokinesis, but not of cytokinesis; (3) reduction and gradual disintegration of rough endoplasmic reticulum; (4)
proliferation of smooth endoplasmic reticulum, peroxisomes and lysosomal elements; (5) increased heterogeneity of mitochondria and peroxisomes; (6) glycogen depletion; and (7) an immigration of macrophages. Other unique alterations include induction of peroxisomal cores after endosulfan exposure, granulocyte invasion with atrazine or condensation of tremendous amounts of glycogen in multinucleate hepatocytes of 4-chloroaniline-contaminated rainbow trout.

Mallatt (1985) extensively reviewed the morphological changes taking place in fish gills in the presence of environmental pollutants. The most common changes, applicable to a wide range of pollutants, are: lifting of the epithelium covering the secondary lamellae, increased number of lymphatic spaces, changed blood flow patterns and appearance of granulocytes in the epithelium. Furthermore, hypertrophy and hyperplasia of epithelial cells including mucous and chloride cells are often observed. Svobodova et al., 1994 reported that considerable circulatory disorders are the dominant histopathological changes on chronic exposure to copper in all the organs (particularly gills) of the rainbow trout fingerling. These disorders contribute to the respiratory epithelium and liver tubule epithelium vacuolisation and disintegration, in hepatocyte vacuolisation, and in damage to the brain nerve cells (wrinkling and hyperchromasia).

2.4: Haematological studies

Haematological characteristics are tools for screening pathological status. The haematological parameters constitute a good indicator of physiological responses (Blaxhall, 1972). Many reports have been published on the toxic effects of pesticides on haematology of fishes (Koundinya and Ramamurthi, 1979; Sharma and Gupta, 1984; Thakur and Pandey, 1990). A significant decrease in red blood cell (RBC) count, Haemoglobin (Hb) content and packed cell volume (PCV) has been observed earlier in fishes exposed to different pesticides (Koundinya and Ramamurthy, 1979; Sambasiva Rao et al., 1955) and such decreasing effect has been primarily attributed to a condition of hypochromic microcytic anaemia (Bhai et al., 1971; Raja Rishi, 1986). Shankar (1975) has reported a significant increasing trend, observed in the number of white blood cell (WBC), in catfish exposed to sublethal concentration of
phosphamidon. Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin (MCH) along with Mean corpuscular haemoglobin concentration (MCHC) showed appreciable decrease in exposed fishes. Similar observations were reported by Thomas et al. (2007).

But Subramanian and Ramalingam (2003) reported that Hb content showed an increase in fishes exposed to 0.001ppm of DDT, 0.95ppm of malathion and 0.09ppm of mercury chloride. RBC count showed an increase when exposed to DDT and mercury when compared to control. Packed cell volume also showed an increase in DDT and mercury. Erythrocyte sedimentation rate showed no significant change in both control and test groups.

According to the reports by Subburaju and Selvarajan (1988), reduction in acetylcholinesterase in plasma, red blood cell and brain in a variety of fish species acutely exposed to chlorpyrifos. Effects of exposure on the blood chemistry of fish include decreased arterial oxygen, CO₂, pH, haematocrit and haemoglobin levels (Bradbury et al., 1991).

Reports on variations of qualitative tissue proteins are very few and are much wanting, especially with reference to pesticide toxicity (Arockia et al, 2007). A comparative electrophoretic study on the tissue proteins of some catfishes was carried out by Hussain and Siddiqui (1974).

Svobodova et al (1994) reported that significant increases in the erythrocyte count and haematocrit was found in carp after toxic exposure to organophosphorus pesticides. The changes like an increased volume of erythrocytes, mean corpuscular volume (MCV) and a decreased level of mean corpuscular haemoglobin concentration (MCHC) have also been reported after acute exposure to organophosphorus pesticide.

### 2.5: Studies on pesticide residues

Bioconcentration factors (BCF) for chlorpyrifos in invertebrates and fish range from 42 to 5100mL/g, depending on the species, exposure concentration, and
exposure conditions. Neely and Blau (1977) modelled the disposition of chlorpyrifos in a pond environment; the model estimated the BCF in fish to be 700mL/g and predicted the maximum concentration would occur in 37.5 days under conditions of a declining water concentration (Mace, 1995). Environmental monitoring programs have been conducted to determine the occurrence of chlorpyrifos and other pesticides in surface waters as a result of agricultural inputs. In general, levels of chlorpyrifos in surface water have ranged from nondetectable to aqueous concentrations of <0.04-0.134μg/L (Natale et al., 1988) and 0.2-1.6μg/L (Braun and Frank, 1980). The effects of chlorpyrifos in aquatic ecosystems have been assessed in a variety of field studies by monitoring changes in population densities and functional parameters in estuarine and agricultural environments, following accidental introductions into aquatic systems, and in lotic and lentic systems. Mace et al (1995) reviewed the report by Ludwig et al (1968) that an application of 0.028kg/ha resulted in no apparent effects on caged shrimp, minnows, blue crabs or fish (multiple species). A second application of 0.056kg/ha resulted in reduction in brown shrimp abundance and mortality of small fish (species not specified); no mortality of larger fish or blue crabs was observed. Maximum chlorpyrifos concentrations measured in oysters were 0.042, 0.006 and less than 0.005mg/kg at 1, 2, and 3 days following application respectively. The ecological effects of chlorpyrifos on aquatic agricultural environments have been evaluated in rice fields. Linn (1968) made general observations of the toxicity of aerially applied chlorpyrifos on caged and released fish (green sunfish (Lepomis cyanellus), black bullheads) in rice fields (California, U.S). Linn concluded that application rates of 0.028kg/ha had negligible effects on fish survival, whereas 0.056kg/ha appeared to cause mortality of sensitive fish species.

Forgash (1976) and Thirugnanam and Forgash (1977) evaluated the toxicity of four sequential applications of granular chlorpyrifos (0.028kg/ha) on a salt marsh environment (New Jersey, U.S) during a 10-week period. There were no significant effects on marsh grasses (growth of roots or shoots, total productivity), aquatic invertebrates (eg. Isopods, amphipods, snails, shrimp), terrestrial invertebrates or birds (eg. Seaside sparrow, sharp-tailed sparrow). Caged killifish (Fundulus

Biochemical effects of the pesticide Chlorpyrifos on the fish Oreochromis mossambicus (Peters)
heteroclitus) exhibited abnormal behaviour within 24hr of the first application. Fish mortality was 18% following the first application and 36% following the second application; 59% of fish exhibited abnormal behaviour eg. Immobility, loss of equilibrium, reduced feeding. Growth of young fish was reduced for at least 2 weeks following the final chlorpyrifos application. Live fish exhibited a 96% depression of brain AChE activity. Average inhibition of brain AChE activity was 62% at 69 days after the final application of chlorpyrifos. A recent report indicated that 10.5% of farm-raised channel catfish (Ictalurus punctatus) sampled fillets contained detectable chlorpyrifos residues (Santerre et al, 1999). Data from the National Contaminate Biomonitoring Program (EPA, 1992) showed that chlorpyrifos was found, in nine out of sixteen wild channel catfish samples with concentrations above the detection limit (2.5ppb).

Currently chlorpyrifos is used for different purposes. A number of studies in various aspects are in running stage to investigate the toxicity of this insecticide on terrestrial and aquatic organisms.