Introduction
1. **INTRODUCTION**

Ever since the dawn of civilization, man has been struggling to improve his living conditions. Securing relief from hunger, one of the basic human needs, is a major concern for humans. Today India is engaged in the gigantic task of feeding over 1000 million people and a huge cattle population on which the poor farmer is dependent for his livelihood. The Green Revolution of 1960, which has given reasonable hope for the country being not only self sufficient in the production of adequate food and fodder for feeding its teeming human and animal population but has become the largest producer of some important commodities. The role played by the magic chemicals, called "pesticides" are quite significant but have given rise to serious other health problems (Gupta, 1989). With a shuddering chill in the spine we recall the horror of Bhopal. The catastrophe, resulting from the leakage of methyl isocyanate (MIC) gas from the pesticide factory of Union Carbide Limited at Bhopal in the morning hours of 3 December, 1984, in which thousands of human beings and animals died, will never be forgotten. Most of the chlorinated non-degradable pesticides leave residues in various living systems for prolonged periods of their life span and are presumably responsible for a variety of known and unknown toxic symptoms (Gupta, 1985). Even when present in minute quantities, they show adverse effect on ecological systems to which human welfare is closely associated (Gupta, 1986).

Pesticide is a substance, or mixture of substance, that are used to control pests such as plant parasitic viruses, bacteria, nematodes, fungi, insects,
weeds, rodents, and birds. Pesticides include all materials that are used to prevent, destroy, repel, attract, or reduce pest organism. Therefore, pesticides find application in different area viz., forestry, landscaping, agriculture and domestic use.

Pesticides have been in use for over thousands of year. Some of the early demonstrations include burning of sulphur to control insects by Romans and the use of arsenic and pyrethrum by the Chinese. By the early 20th century, two classes of pesticides were primarily used, viz., botanicals, natural chemicals derived from plant material, and preparation of inorganic salts, which were widely used as fungicides, herbicides and insecticides. The era of synthetic pesticides started with the discovery of DDT (Dichloro diphenyl trichloroethane) in 1934, which possessed insecticidal properties. Since then, innumerable preparations have been in use in the name of insecticides, fungicides, herbicides, etc. Development of resistant pests led to the banning of DDT in the 1960s. Globally, about 2.5 million tons of pesticides are targeted on agricultural crops. Even though the use of pesticides had a very positive impact in the overall increase in food production, the risks associated with this include deterioration of human health, water contamination, livestock animal poisoning and death of beneficial insects, wildlife endangerment and pesticide tolerance (Gupta, 2004).

1.1. Role of pesticides in public health

In the year 1948, DDT was imported for the control of diseases such as malaria, filariasis, dengue, Japanese encephalitis, cholera, and louse-borne typhus. Pesticides played a significant role in the control of vector-borne
diseases. In 1947, when India became independent, there were 75 million cases of malaria with about 800,000 deaths. During this period the National Malaria Eradication Program was launched, where in DDT played a crucial role and in the first 7 years of its operation, the malaria cases were drastically reduced. Therefore DDT is still among the magic chemicals as far as public hygiene is concerned. However, recent reports indicate that a large number of cases of dengue fever in different part of India are emerging (Anonymous, 2003), this is a matter of serious concern.

1.2. Trends in pesticide use

The three commonly used pesticides, BHC (only gamma-BHC is allowed), DDT and malathion account for 70% of the total pesticide consumption (Gupta, 2004). These pesticides are still preferred by the small farmers because they are cost effective, easily available, and display a wide spectrum of bioactivity. Out of the total consumption of pesticides in India, 80% are in the form of insecticides, 15% are herbicides, 1.46% is fungicide and less than 3% are others. In comparison, the worldwide consumption of herbicides is 47.5%; insecticides contribute 29.5%, fungicides, 17.5% and others account for 5.5% only (Gupta, 2004). The consumption of herbicides in India is probably low, because weed control is mainly done by hand weeding. In addition to public health and agricultural use, pesticides also find their use in other sectors (Gupta, 2004).

Pesticides can be classified according to their chemical structure. Inorganic pesticides are poisons made from common natural highly toxic and indestructible chemicals like arsenic, copper, lead and mercury; hence can
accumulate in the environment. Natural or organic pesticides are generally compounds extracted from plants. Many plants like tobacco, chrysanthemum, and conifers, have evolved the ability to produce substances that are used for this purpose. Another class of chemicals are fumigants, used to fumigate the insects but due to the danger associated it, finds only limited application.

1.3. Ecological effects of pesticides

All the pollutants released on land will eventually find their way to the rivers and sea as the final repository. Since estuaries are the links to the freshwater and marine systems, they contain a variety of anthropogenic chemicals referred to as xenobiotics, viz. oil derivatives, heavy metals, pesticides, fertilizers, polychlorinated biphenyls, etc. All the organic xenobiotics have the potential to affect normal physiology of aquatic animals.

Pesticides are included in a broad range of organic micro-pollutants that have tremendous ecological impacts. Different categories of pesticides have different effects on living organisms and hence generalization is difficult. Although terrestrial impacts by pesticides do occur, the principal pathway that causes ecological impacts is that of water contaminated by pesticides runoff. Fish and aquatic animals are exposed to pesticides in three ways namely (i) direct absorption through the skin by swimming in pesticide contaminated waters, (ii) direct uptake of pesticides through the gills during respiration, and (iii) drinking of pesticides contaminated water or feeding on pesticide contaminated prey. Exposure of fish and other aquatic animals to a pesticide depends on its biological availability, bio concentration, biomagnifications and its persistence in the environment.
The organophosphorus pesticide (OP) Folidol 600, which contains methyl parathion as the active substance, is extensively applied as a pesticide in agriculture, food storage shelters, pest control programs, and fish culture tanks to kill the aquatic larval stages of predator insects that threaten fish larvae. The kinds and amounts of pesticides applied to environments are almost out of control, and about one-third of organophosphorus pesticides (OPs) are selectively toxic to fishes (Aguiar et al., 1994). In tropical countries several OPs are applied to soil and are washed into nearby water bodies where they affect, in particular, the aquatic organisms.

1.4. Mechanisms of pesticide action

Pesticides can be classified according to their mechanisms of action. For example, insecticides like organochlorines, organophosphates, and carbamates act primarily by disrupting the functioning of nervous system, while herbicides often target photosynthetic pathways (Ecobichon, 1991; DeLorenzo et al., 2001). Mechanism of action of some of the pesticides is given below:

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Chemical mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphates and carbamates</td>
<td>Acetylcholinesterase inhibitors</td>
</tr>
<tr>
<td></td>
<td>Receptor or Agonists</td>
</tr>
<tr>
<td>Chlorinate hydrocarbon/pyrethroid</td>
<td>Na⁺ Channel,</td>
</tr>
<tr>
<td>Cyclodienes, Avermectines</td>
<td>Cl⁻ Channel</td>
</tr>
<tr>
<td>Nitro and Chlorophenols, Bipyridyls</td>
<td>Cytotoxins</td>
</tr>
<tr>
<td>Pyrethrin/ pyrethroids</td>
<td>Allergines</td>
</tr>
<tr>
<td>Thiocarbamates</td>
<td>Mitosis blockers</td>
</tr>
<tr>
<td>Several groups of fungicides</td>
<td>Sterol inhibitors</td>
</tr>
<tr>
<td>Organophosphate fungicides</td>
<td>Chitin inhibitors</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>Amino acid synthesis blockers</td>
</tr>
<tr>
<td>Rodenticides (varies chemistries)</td>
<td>Anticoagulants</td>
</tr>
</tbody>
</table>
1.4.1. Insecticides

Organochlorines are insecticides, affecting the nervous system. Chemically they are relatively unreactive stable compounds and are characterized by their long-lasting effects. Recently, Younis et al., (2002) identified that the DDT target protein in insects. The target protein was shown to be a subunit of the adenosine triphosphatase synthase (ATPase), which is involved in the neuronal repolarization and thus important for normal nerve function. At the level of neuronal membrane, DDT affects the permeability to potassium ions, reducing potassium transport across the membrane and alters the channels, through which sodium ions pass, thereby interfering with the active transport of sodium out of the nerve axon during repolarization. Finally, DDT inhibits the ability of calmodulin, a calcium mediator in the nerve, to transport calcium ions essential for the release of neurotransmitters. Two other organochlorine insecticides, endrin and lindane, in addition to the DDT-resembling effects are also inhibitor of the γ-aminobutyric acid (GABA) receptors in both insect and vertebrate central nervous system (Wafford et al., 1989). The GABA receptor is an ion channel glycoprotein that traverses the cell membrane and functions as a receptor for the neurotransmitter GABA.

Fig-1. Potential sites of action of classes of insecticides on the axon and the terminal portions of the nerve (Ecobichon 1991).
The organophosphorus group of insecticides is also neurotoxic and this group acts on the nervous system by inhibiting the enzyme acetylcholinesterase (AChE). The reaction between an organophosphorus insecticide and the active site in the AChE protein results in the formation of a transient, intermediate complex that partially hydrolyzes, leaving a stable, phosphorylated, and largely unreactive, inhibited enzyme that, under normal circumstances, can be reactivated only at very slow rate (Fig 2). With many organophosphorus insecticides, an irreversibly inhibited enzyme is formed and the signs and symptoms of intoxication are prolonged and persistent. However, some organophosphorus insecticides are thought to be toxic only after metabolism by the cytochrome P-450 monooxygenase enzyme systems. This bioactivation step creates a metabolite that is a much stronger inhibitor of the AChE than the parent compound (Belden and Lydy, 2000).

Organophosphorus insecticides, like malathion and parathion, replaced the organochlorines in malaria eradication and other global programs for control of disease vectors when some the organochlorines, including DDT, were banned and withdrawn from the market in many countries (Mulla and Mian, 1981).

![Fig-2](image)

*Fig-2. The interaction between an organophosphorus insecticide with the hydroxyl group in the active site of the enzyme acetylcholinesterase (E-OH). The intermediate, unstable complex formed before the release of the "leaving" group (ZH) is not shown. The dephosphorylation of the inhibited enzyme is the rate-limiting step to forming free enzyme. (Ecobichon, 1991)*
Carbamates are also AChE inhibitors, attaching to the reactive site of this enzyme (Ecobichon, 1991). However, in contrast to organophosphorus insecticides, carbamates are poor substrates for the cholinesterase-type enzymes, resulting in a short and reversible inhibition of AChE.

Synthetic pyrethroids belong to the newest of the major classes of insecticides. The pyrethroids show two different characteristic acidic portions, chrysanthemic or pyrethric acids resulting in type I and type II syndrome. Type II syndrome involves primarily an action in the central nervous system, whereas with the type I syndrome, peripheral nerves are also involved (Ecobichon, 1991). Both type I and II pyrethroid insecticides affect the sodium channels in the nerve membranes, causing repetitive neuronal discharge, with effects being quite similar to those produced by DDT. There appears to be a prolongation of sodium influx with a delay in the closing of the sodium activation gate, resulting in an increased and prolonged sodium tail current (Narahashi, 1986; Bradbury and Coats, 1989; Ecobichon, 1991). Type II pyrethroids prolongs the sodium channel open-time much more drastically than type I pyrethroids (Narahashi, 1986). Also other sites of action have been noted for the pyrethroid insecticides.

Pyrethroid insecticides inhibit Ca\(^{2+}\), Mg\(^{2+}\)-ATPase, thereby interfering with calcium removal from the nerve endings, resulting in increased neurotransmitter release in the postsynaptic gap. In addition, the protein calmodulin, responsible for the intracellular binding of calcium ions to reduce spontaneous neurotransmitter release, also get inhibited. Type II pyrethroids have also been shown to bind to the GABA-receptor chloride channel
complex, blocking chloride ion transport into the nerve cell. To summarize, there are many similarities between the mechanism of action of pyrethriod insecticides and organochlorines, consequently there is a risk for additive or even synergistic effects.

1.4.2. Herbicides

Herbicides are produced to kill or injure plants and therefore affect various mechanisms associated e.g. photosynthesis, respiration, growth, cell and nucleus division, or synthesis of proteins, carotenoids or lipids (Ecobichon, 1991).

The herbicide glyphosate, which is the active substance in the commonly used preparation Roundup™, controls weeds by inhibiting a single plant enzyme, 5-enolpyruvoylshikimate 3-phosphate synthase (EPSPS) (Sikorski and Gruys, 1997; Baylis, 2000). EPSPS is a key enzyme in the aromatic amino acid biosynthetic pathway and a blockage of this enzyme, adversely affects the protein synthesis. Although EPSPS is the only known enzyme target of glyphosate, the herbicide affects many physicochemical and physiological processes (Cole, 1985). Among these are the reduction in photosynthesis and the degradation of chlorophyll, as well as inhibited transport of the plant growth hormone auxin and enhancement of auxin oxidation.

The commonly used chlorophenoxy herbicides, including 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and MCPA (4-chloro-o-toloyacetic acid) mimic the action of the growth hormone auxin in plants (Ecobichon, 1991; Grossmann, 2000). When the herbicides
are present in low concentrations at the cellular site of action, growth by cell division and elongation is usually stimulated. However, with increasing concentrations, a variety of growth abnormalities are induced within 24 h of treatment (Grossmann, 2000).

1.4.3. Fungicides

The breakdown of organic molecules provides energy for the survival of living systems. In fungi, as well as in other eukaryotes, a part of this catabolic process takes place in the mitochondria and lead to the synthesis of the high energy intermediate ATP. Several groups of fungicides disturb the energy supply in fungi and all such compounds are powerful inhibitors of spore germination (Leroux, 1992). Among them are the dithiocarbamates (e.g. maneb and thiram) and the R-S-CCl₃ compounds (e.g. captan and dichlofluanid). These fungicides have a multisite action by inhibiting several enzymes involved in the respiratory processes.

Another group of fungicides, the phenylpyrroles, including fenpiclonil and iprodionel are known to inhibit spore germination and induce morphological alterations of germ tubes, i.e. inhibit germ-tube elongation (Leroux et al., 1992). In addition these fungicides are able to uncouple oxidative phosphorylation and inhibit electron transport in respiration processes. However, more recent studies indicate that the main effect induced by fenpiclonil is due to the inhibition of wall glycan biosynthesis and to the accumulation of natural sugars. These effects indicate that the mechanism of action may be related to glucose metabolism (Jespers and de Waard, 1995). The effects of phenylpyrroles in fungi are thereby very close to the effect of
the herbicide dichlobenil in plants. According to Delmer et al., (1987),
dichlobenil may interfere with a membrane-bound protein involved with the
regulation of the β-glucan synthesis. Consequently there is a risk for additive
or even synergistic effects when the phenylpyrroles fungicides and the
herbicide dichlobenil are present at the same time in the environment.

1.5. Indian Scenario

Use of pesticides in India began in 1948 when DDT was imported for malaria
control and BHC for locust control. India started pesticide production with
manufacturing plant for DDT and benzene hexachloride (BHC) (HCH) in the
year 1952. In 1958, India was producing over 5000 metric tonnes of
pesticides. Currently, there are approximately 145 pesticides registered for
use, and production has increased to approximately 85,000 metric tonnes.
Rampant use of these chemicals has given rise to several short-term and
long-term adverse effects of these chemicals. Despite the fact that the
consumption of pesticides in India is still very low, about 0.5 kg/ha of
pesticides against 6.60 and 12.0 kg/ha in Korea and Japan respectively, there
has been a widespread contamination of food commodities with pesticide
residues, basically due to non-judicious usage. In India, 51% of food
commodities are contaminated with pesticide residues and out of these, 20%
have pesticides residues above the maximum residue level values on a
worldwide basis (Gupta, 2004; Agnihotri, 1999). It has been observed that
their long-term, low-dose exposure are increasingly linked to human health
effects such as immune-suppression, hormone disruption, diminished
intelligence, reproductive abnormalities, and cancer. In this light, problems of
pesticide safety, regulation of pesticide use, use of biotechnology, and biopesticides are some of the possible future strategies for minimizing human exposure to pesticides. The worldwide consumption of pesticides is about two million tonnes per year, of which 24% is consumed in the USA alone, 45% in Europe and 25% in the rest of the world (Gupta, 2004)  

1.6. Poisoning from pesticides

The rampant uses of pesticides have played havoc with human and other life forms. There is a serious hurdle in documentation because of lack of systematic and authentic data on poisonings. Pesticides account for a small but significant fraction of acute human poisonings. There has been a number of outbreaks of accidental poisoning by pesticides that deserve special mention. In India, the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion Folidol E 605 (Karunakaran, 1958).

Subsequently, several cases of human and animal poisonings, besides deaths of birds and fishes, have been reported (Sethuraman, 1977; Banerjee, 1979). In Indore, out of the 35 cases of malathion (diazole) poisoning reported during 1967-1968. In another report from Madhya Pradesh, 12 humans who consumed wheat for 6-12 months contaminated with aldrin dust and y BHC developed symptoms of poisoning which consisted of myoclonic jerks, generalized clonic convulsions, and weakness in the extremities (Gupta, 1975). There are a number of similar cases of pesticides poising elsewhere in the country (Nag et al., 1977, Anonymous, 1981, Gupta, 1986).
In general, it has been observed that organophosphorus pesticides are responsible for death in more than 70% cases of intentional poisonings (mainly attempted or successful suicides) make up a large proportion of the poisonings by pesticides of high toxicity in certain developing countries (Anonymous, 1990). In Indonesia, Malaysia, and Thailand, for example, the proportion of acute pesticide poisonings that are due to suicide attempts has been reported to be 62.6, 67.9, and 61.4%, respectively (Jeyaratnam, 1987). In India such suicide poisoning may even go up to 70% because such compounds are easily available in many households. However, no systematic population-based data on the role of pesticides on homicides are available.

The Bhopal gas tragedy is a catastrophe that has no parallel in industry history. In the early morning of 3 December, 1984 a rolling wind carried a poisonous gray cloud past the wall of the Union Carbide plant in Bhopal, Madhya Pradesh, India. An estimated 8000 or more people died. More than 4000 animals died within minutes of exposure to the gas and almost 15,000 animals suffered the toxic gas effect while surviving (over three times the officially announced total. Adverse effects included pulmonary edema which was the cause of death in most cases, with many deaths resulting from secondary respiratory infections such as bronchitis and bronchial pneumonia (Pandey, 1986; Jain and Dave, 1986).

1.7. Health hazards of occupational exposure

In addition to intentional exposure (suicides and homicides), workers are exposed to occupational hazards in industrial settings and operational hazards during distribution and use in the field. Pesticides are toxic chemicals
and as such they represent risks to users. In developing countries where users are often illiterate, ill-trained, and lacking appropriate protective devices, the risks are magnified. The Poison Information Centre in National Institute of Occupational Health in Ahmedabad reported that organophosphorus pesticides were responsible for the maximum number of poisonings (73%) among all agricultural chemicals (Dewan and Sayed, 1998). In a study on 190 patients of acute organophosphorus pesticide poisoning muscarinic manifestations were recorded (Bhatnagar, 2001). Studies on 356 workers in four units manufacturing HCH revealed neurological symptoms (21%) with significant increase in liver related enzymes which were related to the intensity of exposure (Nigam et al., 1993). Observations confined to health surveillance in male formulators engaged in production of dust and liquid formulations of various pesticides (malathion, methyl parathion, DDT, and lindane) revealed several types of adverse effects and reproductive problems (Gupta et al., 1984; Gupta, 2004). The thyroid function of formulators exposed to a combination of pesticides in the organized sector demonstrated a significant suppression of T3 while a marginal decrease (7%) was noticed in T4 level. TSH levels were also elevated by 28% but the rise was statistically insignificant (Zaidi et al., 2000).

A cytogenic study revealed a significant increase in chromatid breaks and gaps in chromosomes in the peripheral blood in grape garden workers exposed to pesticides (Rita et al., 1987). Separate studies conducted in malaria spray men spraying HCH, DDT, malathion, and cyfluthrin, showed increased levels of serum IgG (malathion exposure) and serum IgA (cyfluthrin exposure) (Karnik et al., 1993). Observations on malaria spray men exposed
to HCH indicated changes in serum A/G ratio, glucose levels, and HCH residues (Kashyap et al., 1980).

1.8. Assessment of human exposure

Pesticides have been primarily criticized for the presence of their residues in various samples of human blood, human fat, human milk, and fat samples, and food commodities. There is no denial of the fact that there is some element of risk involved in the use of pesticides like in any other product or service. Therefore, the pesticide-residue level is an indicator of the accidental exposure and/or average measure of body burden to persistent pesticides. This could either be due to direct exposure or indirectly through the food chain. In India, National Occupational Health Centre (NIOH) and Indian Council of Agricultural Research Institute (ICAR) are carrying out monitoring of health status and pesticide-residues in various samples. Analysis of samples of human blood, human fat, human milk, and fat samples was carried out by the National Institute of Occupational Health, Ahmedabad. Residues of organochlorine insecticides, especially DDT and HCH have been detected in man and his environment the world over. However, by comparison very high levels of these have been reported in human blood, fat, and milk samples in India (Bhatnagar, 2001). Higher level of these chemicals in human samples and in mother’s milk is a reflection of their increased burden and their translactation passage. The toxicological implications of these findings could not be assessed precisely, however, preventive measures are warranted to reduce their body burden to avoid any potential health effect. Residues of pesticides from food commodities were monitored by All India Co-coordinated
Research Project on Pesticide Residues under the Indian Council of Agricultural Research, New Delhi, through their centers located in different parts of the country. Recently, analysis of bottled water, colas and other soft drinks carried out by the Centre for Science and Environment, New Delhi revealed very high content of pesticide residues (Anonymous, 2003a, b, c).

1.9. Consumption of pesticides in India

India is presently the second largest manufacturer of basic pesticides in Asia and ranks 12th globally. Currently, the consumption of pesticide is showing a slight declining trend, probably due to shift of farmers toward biopesticides, natural plant sources and other alternative methods (Das et al., 2002; Gupta, 2003). Despite such a large consumption of pesticides, it is estimated that crop losses vary between 10-30% due to pests alone. In monetary terms, these losses amount to Rs.290,000 million per year (Agnihotri, 1999).

1.10. Objective of the study

Fish have been widely used as models to evaluate the health of aquatic ecosystems and in toxicological pathology studies (Law, 2003). Research in fish has demonstrated that mammalian and piscine systems exhibit similar toxicological and adaptive responses to oxidative stress. This suggests that piscine models, in addition to traditional mammalian models, may be useful for further understanding the mechanisms underlying the oxidative stress response.

The fresh water aquaculture system constitutes one third of the total fish production of India and the major carps (Catla catla, Labeo rohita and
Cirrhinius mrigala) being the dominant species. Aquaculture areas are not exclusive areas and usually get exposed to influx from surrounding water bodies particularly during rainy seasons. Therefore the contamination likely to be present in the water bodies may affect the aquaculture produce irrespective of its size. Most of the toxicological studies in fish are confined to the effects of pesticides on fingerlings and therefore a study on the growing/grown out fish is envisaged in this study. The effect of methylparathion on the biochemical enzymatic changes in rohu (Labeo rohita) an important candidate species in a polycultural system is the theme of this work. Therefore the objectives of the study include

1. Determination of lethal concentration (LC₅₀) of methylparathion in grown up L.rohita (75 ± 6g size).

2. Effect of lethal/sub-lethal concentration of methylparathion on tissue metabolism by evaluating

   a) Acetylcholinesterase activity in brain tissues.

   b) Detoxifying enzyme systems in liver

   c) Alteration in liver diseases diagnostic marker enzymes

   d) Alteration in lipid metabolism in major organs

   e) Changes in tissue muscle protein metabolism

   f) Histopathological alteration in fish organ during exposure to methylparathion