GENERAL

INTRODUCTION
The term ‘pesticide’ refers to any substance which can poison or otherwise eliminate an organism (plant or animal) which is considered by man to be a pest.

Pesticides may be defined as the chemicals that can destroy pests such as insects, fungi, nematodes etc. They have the power not only to kill the pests but also to inhibit their further growth. The chemical used to control all kinds of pests are known as pesticides.

Pests are the creatures that attack and destroy food, clothes, furniture and buildings, farm animals etc. The word pest is an all embracing general term which includes insects, fungi, bacteria, viruses, nematodes that damage crops or incite diseases on crops, weeds in cultivators plots that rob the nutrients from soils, snails, birds and rodents that destroy seedlings at sowing time and grains at harvest time, and finally insects, micro organisms and rodents that consume stored grains and fruits in the post harvest stage.

The word ‘pesticides’ includes insecticides, fungicides, nematocides, rodenticides, herbicides and algicides. Pesticides include one or more elements such as arsenic, boron, bromine, cadmium, lead, nitrogen, oxygen, tin, zinc, sodium etc.

**Polluted Rivers in India:**

Many important rivers which pass through or near industrial cities are highly polluted. Most of the Indian rivers are being polluted by some industrial wastes (Chakravarthy *et al.*, 1965; Saxena *et al.*, 1966) and sewage. Yamuna, which passes thorugh Delhi, is highly polluted by sewage and industrial waste. Hoogly, which passes through Calcutta is polluted by various industries such
as jute mills, power plants, chemical industries, detergent, sewage etc. Ganga near Kanpur is highly polluted by the effluent discharged from various industries particularly jute, chemical, metal industries and tanneries (Chakravarthy 1965). Damodar gets pollution from fertilizer industries, steel plants, and thermal power station. Gomti near Lucknow is polluted by paper mills and sewage. Cauvery and Godavari receive pollutants from tanneries, distillaries, paper mills etc. Jamuna river is being polluted between Agra and Delhi by the wastes from the DDT plants near Delhi (Science Today, 1970). Tungabhadra river which is near Kurnool is being polluted by the Rayalaseema paper mills discharges (Manikya Reddy and Venkateswarulu, 1987). In Hyderabad, Hussain Sagar is being polluted by industrial wastes, heavy algal blooms etc. In 1973, in Hyderabad, large variety of fish were killed due to pesticides (Hingorani, 1973). According to the National Environment Engineering Research Institute (NEERI) 70 percent of Indian inland water is not fit for human consumption.

Environmental Protection Agency (South Wick, 1976), is reporting every year that mortality of fishes is due to this polluted water in aquatic ecosystems. For example in Sandiego harbour (USA) in 1962 as per the estimations 378,000,000 fishes were killed by pollution, producing one raft of dead fish 1000 feet long 10 feet wide and 3 feet deep.

Under the U.S. Federal Environmental Pesticide Control Act, the term 'Pesticide' has been defined as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (Gupta and Satankhe, 1985). Due to this pesticide pollution, death in animals and human
beings has been recorded. (Gupta 1978; Schimmel et al., 1980). Sometimes even low concentrations of pesticides are found to be toxic to non-target organisms. (Gralla et al., 1977 and Kilikis et al., 1981)

**Pesticides as pollutants of environment and their adverse effects:**

Sahai and Chauhan (1977) defined the pesticidal pollution as the change in one or more components of the ecosystems by pesticides and their degradative products. These pesticides have posed potential health hazard to livestock, wild life and even to human population (Matsumura, 1975). Uncontrolled usage of pesticides leach into water system. They concentrate slowly in the livers and fatty tissues of non-target organisms like fish, crustanceans and other aquatic organisms. When these are eaten by man as food, they enter the human being and cause drastic effects. Pesticides sprayed on vegetation can also get concentrated by herbivorous animals such as cows, buffaloes etc. By eating flesh and drinking water, human beings concentrate pesticides in their tissues. Pesticides cause pulmonary disorders, dermatitis, allergy, vomittings, cardiac failure and cancerous conditions.

**Water Pollution due to Pesticides:**

Pesticides, inorganic fertilizers and herbicides used in crop field are washed down by rain water or irrigation water to low lying areas, rivers or lakes causing water pollution. The contamination of natural waters with chemical had caused serious problems to the aquatic biota (Arillo et al., 1981; Reber and Borkott, 1983). Oil leaked or spilled from oil tanker and offshore oil rigs float over the sea water polluting the water and thereby causing damage to marine ecosystem. The industrial countries use upto 80 % of the world's
agrochemicals and they are estimated to suffer 1 % of all deaths due to acute poisoning (Jayaratnam, 1985). There are about 45,000 registered pesticides in the market and the annual production of pesticides in India is about 77,840 tonnes (Berry et al., 1974). Application of Bursban in ponds recommended for mosquito control has resulted in mass mortality of blue grills, bass, ducklings and other invertebrates (Hurlbert et al., 1970; Macek et al., 1972). It is estimated that annually, about 5,00,000 accidents take place due to pesticides and 10,000 deaths are reported (Joshi, 1992).

**Pesticide Residue:**

Residues of pesticides persist in soil, water and food and have posed problems all over the world, especially in the U.S.A. where in 1988, global sales of pesticides produced by some top 10 companies of the world amounted to 20 million U.S. dollars, of which herbicides alone constituted 5 billion dollars. Enrichment of pesticides and organic pollutants in the top 100 to 150 cm layer of the sea in Narragansett bay in U.S. (Duce et al., 1972) high levels of organophosphate (OP) residues in the water of Holland Marsh in Southern Ontario (Miles and Harris, 1978), high level of malathion (as high as 500 µg/l) in the waters in Texas (Coppage et al., 1975) are few examples. These provide evidence of indiscriminate use of these chemicals. DDT has been in use since World War II and has permeated our environment and contaminated human beings and wild life of the earth including such remote areas like the Antarctic. The highest quantity of DDT (1.3 mg/kg) has been recorded in fish and Atlantic croakers in the estuary near Pensacola (Hansen et al., 1970), which led to the
banning of DDT by the Environmental Protection Agency in the U.S. (Annon, 1972).

BHC levels in some vegetables in Mysore varied from 10.5 to 20 ppm and BHC levels in Hasan varied from 3.68 to 15.5 ppm (Majumder and Krishna Murthi, 1963). The indiscriminate use of pesticides is posing a serious threat to human health. International Development Centre (Ottawa, Canada) has claimed that every year 10,000 people die and nearly 4,00,000 suffer from effects of pesticide poisoning in the developing countries (Niraj Kulshrestha, 1992). This literature shows that pesticides are toxic to different non-target organisms. Since organochloride and organophosphate (OP) pesticides are more toxic, less toxic pyrethroids have been used widely for control of different agricultural pests and household pests.

**Pyrethroids / Pyrethrins:**

Pesticide products containing synthetic pyrethroids are often described by pest control operations and community mosquito management bureaus as "safe as Chrysanthemum flowers". While pyrethroids are a synthetic version of an extract from the Chrysanthemum plant, they were chemically engineered to be more toxic with longer breakdown times and are often formulated with synergists, increasing potency and compromising the human body's ability to detoxify the pesticide.

In the early 18th century, the Caucasian tribes have used pyrethrum flowers to control body lice. The pyrethrum represents the dried flowers of
Chrysanthemum cinerariaefolium, a member of Asteraceae. Since ancient times, the powder has been used as an insecticide. The original home of the pyrethrum flowers is said to have been the middle and near east.

Gnadinger (1945) and Shepard (1951) discussed thoroughly about the discovery of pyrethrum as an insecticide. Pyrethrins have been considered harmless to plants and mammals but toxic to insects.

**Types of Pyrethroids:**

Pyrethroids are of two types. They are

1. Natural Pyrethroids
2. Synthetic Pyrethroids

**Natural Pyrethroids:**

Natural Pyrethroids are also called as Pyrethrum or "Pyrethrum extract". The pyrethroids contain Pyrethrin I and Pyrethrin II (Head, 1973, Elliott and Janes, 1978). These pyrethroids are used for domestic purposes for the protection of stored food. They are used for the control of pest in agriculture. They are applied in combination with metabolic synergists due to their high biodegradability. Type I Pyrethroids are allethrin, cismethrin, bioresmethrin and 1R, cis-phenothrin. Type II Pyrethroids are deltamethrin, 1R, cis-cypermethrin and 2S, alpha S-fenvalerate.

**Synthetic Pyrethroids:**

Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried chrysanthemum flowers (the term "pyrethrum" is often used as a generic term to describe either natural pyrethrins or synthetic
pyrethroids). The insecticidal properties of pyrethrins are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids. These acids are strongly lipophilic and rapidly penetrate many insects and paralyze their nervous system. Both pyrethrins and synthetic pyrethroids are sold as commercial pesticides used to control pest insects in agriculture, homes, communities, restaurants, hospitals, schools, and as a topical head lice treatment. Various formulations of these pesticides are often combined with other chemicals, known as synergists, to increase potency and persistence in the environment.

While chemically and toxicologically similar, pyrethrins are extremely sensitive to light, heat and moisture. In direct sunlight, half-lives that can be measured in hours. However, the pyrethroids, the synthetic analogues of naturally occurring pesticides, were developed to capture the effective insecticidal activity of this botanical insecticide, with increased stability in light, yielding longer residence times.

The eminent pioneers of Pyrethrum chemistry are Yamamoto (1991) and Yamamoto and Sumi (1923). Chemically, synthetic pyrethroids are esters of specific acids (e.g., chrysanthemic acid, halo-substituted chrysanthemic acid, 2-(4-chlorophenyl)-3-methylbutyric acid) and alcohols (e.g., allethrolone, 3-phenoxybenzyl alcohol). For certain pyrethroids, the asymmetric centre(s) exist in the acid and/or alcohol moiety, and the commercial products sometimes consist of a mixture of both optical (1R/1S or d/l) and geometric (cis/trans) isomers.
**Action of Pyrethroids:**

The action of pyrethroids closely resembles that of the insecticide DDT in the peripheral nervous system of the frog. DDT also causes pronounced repetitive activity in sense organs, in sensory nerve fibres, and in motor nerve terminals, due to a prolongation of the transient increase in sodium permeability of the nerve membrane during excitation (Van den Bercken *et al.*, 1982; Vijverberg *et al.*, 1982).

In the electrophysiological experiments using giant axons of crayfish, the type I pyrethroids and DDT analogues retain sodium channels in a modified open state only intermittantly, cause large depolarizing afterpotentials, and evoke repetitive firing with minimal effect on the resting potential (Narahashi, 1985).

These results strongly suggest that permethrin and cismethrin, like allethrin, primarily affect the sodium channels in the nerve membrane and cause a prolongation of the transient increase in sodium permeability of the membrane during excitation.

Type I pyrethroids (allethrin, cismethrin, bioresmethrin, and 1R, cis-phenothrin) caused moderate presynaptic repetitive activity, resulting in the occurrence of multiple end-plate potentials.

In the electrophysiological experiments using giant axons of crayfish, the Type II pyrethroids retain sodium channels in a modified continuous open state persistently, depolarize the membrane, and block the action potential without causing repetitive firing (Narahashi, 1985).
The most toxic pyrethroids of Type II were the most potent inhibitors of $[^3H] \text{-Ro 5-4864}$ specific binding to rat brain membranes. The $[^3H]$-dihydropicrotoxin and $[^35S]$-TBPS binding studies with pyrethroids strongly indicated that Type II effects of pyrethroids are mediated, at least in part, through an interaction with a GABA-regulated chloride ionophore-associated binding site.

The Type II pyrethroids (deltamethrin, 1R, cis-cypermethrin and [2S, alpha S]-fenvalerate) increased the input resistance of crayfish claw opener muscle fibres bathed in GABA.

Pyrethroids also cause pronounced repetitive activity and a prolongation of the transient increase in sodium permeability of the nerve membrane in insects and other invertebrates.

Although it has been established that sense organs and nerve endings are the most vulnerable to the action of pyrethroids, the ultimate lesion that causes death will depend on the animal species, environmental conditions, and on the chemical structure and physical characteristics of the pyrethroids molecule (Vijverberg and Van den Bercken, 1982).

**Pyrethroids and Health effects:**

Pyrethroids have irritant and/or sensitizing properties. They are not easily absorbed through the skin, but are absorbed through the gut and pulmonary membrane. Tests of some pyrethroids on laboratory animals reveal striking neurotoxicity when administered by injection or orally. Systemic toxicity by inhalation and dermal absorption is low. The acute
toxicity, calculated by LD50's, ranges from low to high, depending on the specific formulation. Low toxicity is attributed to two factors: limited absorption of some pyrethroids, and rapid biodegradation by mammalian liver enzymes (ester hydrolysis and oxidation). Insects, without this liver function, exhibit greater susceptibility to the chemicals.

Pyrethroids interfere with the ionic conductance of nerve membranes by prolonging the sodium current. This stimulates nerves to discharge repeatedly causing hyper-excitability in poisoned animals. The World Health Organisation explains that synthetic pyrethroids are neurotoxins acting on the axons in the peripheral and central nervous systems by interacting with sodium channels in mammals and/or insects. The main systems for metabolism include breakage of the ester bond by esterase action and oxidation at various parts of the molecule. Induction of liver microsomal enzymes has also been observed (WHO, 1999).

**Symptoms of poisoning with Pyrethroid compounds:**

Signs and symptoms of poisoning by pyrethroids may take several forms. Because of the similarities to crude pyrethrum, pyrethroids may act as dermal and respiratory allergens. Severe anaphylactic (allergic) reactions with peripheral vascular collapse and respiratory difficulty are rare. Other symptoms of acute toxicity due to inhalation include sneezing, nasal stuffiness, headache, nausea, incoordination, tremors, convulsions, facial flushing and swelling, and burning and itching sensations. The most severe poisonings have been reported in infants, who are not able to efficiently break down pyrethroids. With orally ingested doses, nervous
symptoms may occur, which include excitation and convulsions leading to paralysis, accompanied by muscular fibrillation and diarrhoea. Death in these cases is due to respiratory failure. Symptoms of acute exposure last about 2 days.

**Pyrethroids and the Environment:**

Roberts (1981) has reviewed the environmental degradation of pyrethroid insecticides. The rate of degradation in soil depends on the type of soil, aerobic or anaerobic conditions etc. Permethrin has a half life of 1 to 3 weeks in various silt and clay soils and up to 15 weeks in organic soils (Belenger and Hamilton, 1979; Williams and Brown, 1979; Chapman et al., 1981). Cypermethrin has a half-life of two to four weeks in mineral and organic soils.

While the development of the synthetic pyrethroids was heralded with claims of selective toxicity to insects, both pyrethroids and pyrethrins are extremely toxic to aquatic organisms, including fish such as the bluegill and lake trout, with LC$_{50}$ values less than 1.0 parts per billion. These levels are similar to those for mosquito, blackfly and tsetse fly larvae, often the actual target of the pyrethroid application. Lobster, shrimp, mayfly nymphs and zooplankton are the most susceptible non-target aquatic organisms. The nonlethal effects of pyrethroids on fish include damage to the gills and behavioral changes.
**Pyrethroids Residues / Persistence:**

As mentioned before, pyrethroids are designed to breakdown more slowly than the naturally occurring pyrethrins. While pyrethrins, extremely sensitive to light, heat and moisture, break down in a few hours, the synthetic pyrethroids are stable and persist and in the environment much longer. With a few exceptions, pyrethroids break down most quickly in direct sunlight, usually just a few days after application, with a few exceptions. However, in areas with limited sunlight, such as grain soils and subway tunnels, pyrethroids can persist for months.

**Synergists:**

Both pyrethroids and pyrethrins are often formulated with oils or petroleum distillates and packaged in combination with synergists, such as piperonyl butoxide (PBO) and n-octyl bicycloheptene dicarboximide. Synergists are added to increase the potency of the pesticide. A range of products from repellants to foggers to pediculicides (lice killers) to garden sprays contain synergists. Many formulations of synthetic pyrethroids, including Scourge™ and Anvil™, used along the East Coast for mosquito control to combat the West Nile Virus, contain the synergist Piperonyl butoxide (PBO).

Piperonyl butoxide (PBO) inhibits important liver enzymes responsible for breakdown of some toxins, including the active ingredients of pesticides. Specifically, it has been shown to inhibit hepatic microsomal oxidase enzymes in laboratory rodents and interfere in humans. Because
these enzymes act to detoxify many drugs and other chemicals, a heavy exposure to an insecticidal synergist may make a person temporarily vulnerable to a variety of toxic insults that would normally be easily tolerated. Symptoms of Piperonyl butoxide (PBO) poisoning include anorexia, vomiting, diarrhoea, intestinal inflammation, pulmonary hemorrhage and perhaps mild central nervous system depression. Repeated contact may cause slight skin irritation. Chronic toxicity studies have shown increased liver weights, even at the lowest doses, 30 mg/kg/day. While not considered a carcinogen by EPA, animal studies have shown hepatocellular carcinomas, even treatments as low as 1.2%.

**Toxicity of Pyrethroids in Fish:**

Natural pyrethrins are highly toxic to aquatic organisms (Mauck et al., 1976). Substances, which are similar to pyrethrins, have been synthesized (Casida, 1973) and these pyrethroids are more toxic to fish (Mauck et al., 1976). Holcombe et al., (1982) concluded that synthetic pyrethroids are more toxic to fish than organochlorides and organophosphates when they conducted toxicity tests with pydrin and permethrin on fish such as fathead minnows and rainbow trout.

**Significance of the Investigation:**

The pesticides produce not only morphological or pathological changes but also cause biochemical alterations in the living system (Edwards 1973; O'Brien, 1977). Both morphological and biochemical
parameters should be established (Shakoori et al., 1976) in order to know the type of damage caused by the insecticides.

The freshwater fish *Labeo rohita* is consumed in and around Kurnool District. The cypermethrin is a commonly used pesticide on crops in this region and the information is not sufficient to understand the impact of this pyrethroid on *Labeo rohita*. The present investigation has been undertaken to assess the effect of cypermethrin on some physiological and biochemical aspects in the freshwater fish *Labeo rohita*. 