CHAPTER-1

INTRODUCTION
1.1 Brief description on pesticides:

Probably man's struggle against pests of crops is as old as agriculture itself. The numbers of known insect species causing damage to crops are about 1000 out of which 70 species are responsible for maximum loss (Shukla and Upadhyay; 1998). India is loosing annually Rs.5,000 crores worth of agricultural production due to weeds, pests and diseases(Zeheer and Gupta; 1979), according to the National Council of Applied Economic Research. Rodents destroyed 6% of standing crops and 6.8% of stored harvested amounting to a loss of 125 million tones of grain in 1973-1974 (Gunn and Stevans; 1976). Therefore, entomologists and farmers are evolving and using various tools and techniques to control insect pests in order to save the crops in the field as well as under the storage condition.

The control of pests can broadly be divided into natural control and applied control measures. The natural control do not depend upon the activities of human beings and it includes climatic factors viz, rainfall, cold, heat, sunshine and wind; physical factors viz, water reserviours, mountains, streams and presence of predaceous and parasitic insects, fishes, birds and mammals; presence of insect diseases and the conditions which favour the spread of such insect diseases (Shukla and Upadhyay; 1998). But, these pests cause enumerable damage to the crops, which cannot be controlled only by natural control measures. So, the uses of chemicals are one of the most successful means, which includes the use of insecticides, attractants, repellants,
antifeedants, synergists etc. The control of insect pests by insecticidal control is being considered as essence of applied entomology by the farmers. These chemicals are named as pesticides, which is a collective term covering all the chemicals and used to control, destroy or mitigate the crop pests, household pests as well as vermin. It includes insecticides, fungicides, bactericides and weedicides (also called as herbicides or rodenticides). From the definition, it is very confusing to differentiate insecticides from pesticides because most of them are used as pesticides and vice-versa. Some of the pesticides are able to kill wide spectrum of insects and weeds and some of them are totally species specific. The introduction of insecticides has revolutionized the protection technology and with the help of insecticides most of the insect pest population is being managed effectively in a very short period.

1.2 Historical background:
The history of modern insecticides started from 1867 with the use of Paris green for reducing the population of Colorado beetle. Arsenicals were the first insecticide to be used to protect foliage from the attack of insects. Hexachlorocyclohexane (HCH) was first adapted for agricultural use in 1886 (Shukla and Upadhyay; 1998). The era of synthetic organic pesticides began in the 1940s, which brought many benefits. The different pesticides so generated over a period helped farmers to save crops from pests and enabled them to reap harness. They also enabled in curbing insect vector born diseases, which were considered dreaded diseases to human and livestock etc.
Before and during World War II supply of the botanical products became limited. Hence, an effort was made to identify, synthesize and manufacture new insecticides to protect crops from insect pests and from insect born diseases. Scientist of England and France identified the insecticidal properties of Organo-Chlorines and Cyclodienes insecticides and found their low mammalian toxicity and broad spectrum of activity against insecticides used in pest control. But these insecticides were more persistent in the environment and their adverse effect on non target species resulted in the out break and number of secondary pests or the rapid resurgence of the pest through the suppression of beneficial insects. Reports from various resources confirmed the adverse effects to human health and wild life because of their bio­magnification in ecosystem. As a result, most of these products were eventually banned or put under restricted use.

German scientists identified the insecticidal properties of organo-phosphorus compound in 1930s. These compounds were acutely toxic to mammals and had broad spectrum of activity against insect pest and had relatively short environmental persistence than the chlorinated hydrocarbons.

In the late 1940s, methyl carbamate was developed as insecticides. These had the similar properties with organophosphorus insecticides. They had also broad spectrum of activity, and were toxic to non-target species. After that various others insecticides like Pyrethroids, Neonicotinoids, Rotenone etc. were discovered which had the similar toxic effect to the environment and
persistence properties (Reddy et al., 2005). From the literature, it was found that insecticides are of five different families, such as

1. Botanical, eg- Pyrenthrin, Rotenone.
2. Carbamate, eg- Carbaryl.
4. Organochlorine, eg-DDT, BHC, Endosulfan.
5. Organophosphate, eg-Diazion, Malathion.

The use of pesticide in India started only after Independence. The first plant to produce a pesticide on a commercial basis was set up in 1952 and the first public sector plant of DDT came into existence in 1955.

According to formulation, insecticides may be found as dusts, wetable powder, granules, and emulsifiable concentration (E.C). Based upon the mode of physiological action it may be grouped as stomach poison, contact poison, fumigant and systemic poison (Devi; 1994).

1.3. Toxicity of pesticides:

In India the consumption of pesticides has increased from 150 tons in 1953 to 62,6000 tons in 1977 (Nanda Kumar; 1985) due to heavy food loss by the pest. It seems that the consumption of pesticides in India is spectacularly increasing with the phenomenal benefits derived in agriculture. Since 1950s, continuous indiscriminate and universal application of broad- spectrum synthetic organic pesticides brought risks. Their adverse effect on as natural biological control agents, pesticides resistance development in the target pests, health hazards to human beings, aquatic animals, live-stocks and wild fauna, adverse impact on
environment such as soil, water and air etc. The publication of Rachel Carison's famous book, which won the Pulitzer Prize on “Silent Spring” in 1962 brought doubts about the environmental and health risks of pesticides. Developing and implementing alternative to broad-spectrum pesticides have been continued to be a challenge. The ban of certain broad-spectrum insecticides and limitations imposed on the use of certain pesticides had resulted in the development and registration of a number of risk reducing and environmentally safe alternative of pesticides and control strategies. These new pesticides are more pest specific, slower acting and with different mode of action (Reddy et al., 2005).

The nature of some pesticides and insecticides are non-biodegradable, so these pesticides may accumulate in the soil in large quantities. Chlordane and DDT can be found in the soils even after 12-15 yrs of a single application. Pesticides such as Sevin and Lindane are retained in the soil from several months to 2 years (Varonina and Torina; 1967). The toxicity of pesticides may increase due to the influence of some environmental factors, which help to form metabolites. Chlorophos in an alkaline medium converts in to the highly toxic substance dimethyldichlorovinyl phosphate (DDVP). Aldrin converts in to Dieldrin, which is 6-12 times more toxic than aldrin.

Ecologically insecticides have two serious problems, which were not previously anticipated. At the first, many of them have persisted and accumulated in the environment. Later on they have harmed or contaminated
to numerous non-target animals and plants. Secondly, many of them have directly and indirectly affected human health (Verma and Agarwal; 1989). The first available report of toxic effect of insecticides on animal species was described by Kreig; 1925. The first serious fish kill was reported in Lake Balaton in 1967 (Baron et al., 1967) due to high concentration of some agrochemicals. Cottam and Higginis; 1946 reported the effect of DDT on fish and wildlife. Allison et al., 1963 estimated the toxicity of DDT in cutthroat trout. Cope et al., 1947, Hoffman and Sarber; 1949, Harrington and Bildingmayer; 1958, Weise; 1959, Haldon; 1965, Andrews et al., 1966, Anderson; 1968, Buhlar et al., 1969, Fromn and Hunter; 1969, Eaton; 1970, Grant and Mehrle; 1973 also recorded the toxicity of insecticides on fishes. Generally, the pesticide destroys the normal physiological activities of an organism, against which it is applied. Hence, it may cause death or physiological dysfunction of such organism, and reduce its vitality. It may also act as a repellant to keep the pest away. It can promote endless changes in the whole ecosystem. Each of such change is of great significance not only in the successful use of the pesticide itself but also in damaging the natural equilibrium in an ecosystem. Besides these biological ramifications, the use of pesticide is hazardous to many physical components of ecosystem like water, air and soil because more than 99% of the pesticide when applied reaches untargeted spots of the ecosystem. It is estimated that only one percent of applied pesticide is actually used to kill pests (Pimantel and Leistan; 1986).
Pesticide when released in the environment are persistent and harmful, specifically the organochlorine pesticide, not only stored in the tissue causing histological changes but also its bioconcentration occurs in upper trophic level of food chain. (Shashikant; 1987).

Organochlorine insecticides are of different types. These are i) Chlorinated ethane derivatives, such as DDT and Methoxychlor, ii) Cyclodanes derivatives, such as Endrin, Endosulfan, Aldrin, Dieldrin, Chlordane, Haptachlore, Mirex and Hexachlorocyclohexane (HCH). These are highly toxic. They stimulate the nervous system and induce irritability, disturb equilibrium, paresthesia, tremor, and convulsions. Some of these chemicals, such as aldrin, dieldrin, and lindane affect neurotransmitter activity; DDT may exert its toxic effects in the nervous system by adversely effecting ion transport across the axon membrane. (Daughery et al., 1953, Narahashi et al., 1992). Some organochlorine insecticides are hepato-toxic such DDT, BHC, and other cyclodine groups of insects, such as endosulfan, aldrin etc (Devi; 1994).

Organochlorine insecticides are most widely used and include nearly all the hard persistent insecticides that do not break down quickly in the environment. The probable upper limit of persistence of some widely used organochlorine compounds are as Aldrin (14 hours later), Eldrin (14 hours later), Dieldrin (15 hours later), DDT (17 hours later) sited from (Sengupta, 1985). The persistence limit of Endosulfan is 3-6 months (Reddy et al., 2005). In the early 1970's DDT and another organochlorine insecticides used extensively in USA
and this caused biomagnifications. Peregrine falcons and bald eagles consumed fish exposed to DDT and accumulated toxic levels of the pesticide, resulting in reproductive failure (Devi; 1994).

It is ironic that the same properties that made the organochlorine insecticide so successful for insect control (their environment stability, water insolubility and lipoid solubility and their low cost) have resulted in their present position as the most discussed and ubiquitous of the environment micro pollutants and regarded as the prime causes of cancer, mutation, birth defects, spontaneous abortion etc. (Sengupta; 1985).

It is suggested that the insecticides may exert some of their toxic actions by modifying certain mechanisms in the cell membrane on the in vivo metabolism of malathion in rats, Antunes-Madeira and Madeira (1979). The organochlorine insecticides generally accumulate in the tissues of the animals. Tooby and Durbin (1975) examined different tissues of rainbow trout to determine the accumulation and elimination of lindane residue. Verma et al., 1977 noted high accumulation of pesticides in gill, liver, kidney of teleost fishes (L. Rohita, H. fossilis). Hogan and Reclofs; 1971 estimated the concentration of Dieldrin in blood and brain of fish. Dahya and Chauhan; 1982 recorded high percentage of BHC, DDT and Endosulfan in market sample of different fishes.

The pesticide reaching untargeted spots can cause damages to physical and chemical equilibrium when soils are repeatedly exposed to pesticide application. For example, agricultural soils with continuous use of pesticides
in Punjab, Andhra Pradesh and Karnataka as reported to have dangerous levels of HCH (Hydrochloro Hexane), BHC (Benzene Hexa Chloride), and DDE (Dichloro Diphenyl Dichloro Ethylene). Similarly, pesticides such as Atrazine and Aldrin are reported to be found in water in the intensively cultivated areas of the USA. Surveys made in India to estimate the residue of pesticides in the marketed samples of different food items showed that DDT contamination is around 91.7% followed by BHC (Jales; 1980).

Almost all the method of using agro-chemicals widens the spatial and temporal ill-effects on the ecosystem. Any method of application of pesticides cannot avoid the entry of pesticides in to the soil, water, air, crops, micro flora and ecosystem as a pollutant to cause direct and indirect health problems.

In the year 1958, 104 deaths were caused by the organophosphorus pesticides poisoning in Kerela state. Again, fatal poisoning had occurred due to toxicity of ethyl parathion in a formulation and packaging factory in Bombay, in the year 1962 (Shinde; 1979). In Kerela, 100 persons suffered from toxic effect of endrin, of which one died to consumption of contaminated rice in 1964. On consumption of food on banana leaves contaminated by copper sulphate, 160 persons suffered from retching and vomiting in Kerela state (Shinde; 1979).

Mulla (1963) also studied the effect of organochlorine insecticide on fishes and bull frogs. Later on, worker like Macek et al., 1976, Kasymov et al., 1980 studied the effect of insecticides in aquatic animals and fishes. Logier; 1949, Fashingbaurer; 1957, Sanders; 1970 studied the toxicity of different insecticides to amphibia. Hotchkiss and Paugh; 1946, Caburn and Trichler;
1946, George and Mitchell; 1947, Haag et al., 1948, Adam; 1949, Blackmore; 1963, Harrison et al., 1986 recorded the toxic effect of insecticides on birds and mammals.

Mathur (1962) pointed out that fish, subjected to insecticides such as lindane, dieldrin, DDT and BHC die due to pathological disorder. Though the pesticides are selective in their action, they are often highly toxic to human and responsible for most accidental intoxications in agriculture and the pesticide industry (Levine; 1991). Fishes are also affected by different types of heavy metals. Due to urea stress morphological, histopathological, metabolic changes, physiological changes and reproductive changes were observed by Srivastava and Sriwastwa; 1980, Srivastava et al., 1984.

Pesticide hazard on fish mortality and growth and tissue damage have been reported by Shivaji Rao et al., 1967, Edward; 1970, Wildish et al., 1971, Jackson; 1976. Ghosh et al., 1990 observed that due to toxic stress of pesticide reproductive abnormalities with hormonal irregularities occur in fish.

In chronic exposure of organochlorine insecticides, tend to accumulate in the tissues of the organisms. They have the greatest tendency for biological concentration. Fishes are reported to take DDT at 0.265 ppm in water and concentrates it 1,000,000 times in their bodies. In sea urchin DDT can concentrate it 100,000 times in their bodies (Gupta and Gupta; 1976, Pimental and Leistain L; 1986).
There are reports on general toxicity of carbamate to mammals including humans resulting in neurophysiological, behavioural, functional, development, biochemical, bioenergetic and histological changes (Benton; 1976, Risher et al., 1987).

Pesticides can also be retained in plant tissues and can act as a source of human food pollution. Contaminated plant tissue becomes a part of the food chain. As many animals eat such plants or drink pollutant water and the chain is carried forward. They ultimately lead to innumerable health hazards to both human and animal lives. In case of non-vegetarian, the problem of food pollution is more acute because the level of pollution is of higher order due to increase in concentration of pesticide residues in the bodies of the animals, which are taken as food (Joshi; 2005).

Studies of Pandey et al., 1998 has indicated that in Brahmaputra valley, application of pesticides and frequent use of explosive in the hilly areas are adversely affecting the habits of these ornamental and endogenous fishes. Thus existence of fishes is threatened by the use of pesticides.

Fish and other wildlife species, including endangered ones like the peregrine falcon, bald eagle, and osprey, have been victims of pesticides poisoning. Pesticides kill fishes in Canada, particularly in the Maritime Provinces. Pesticides that are not highly toxic can still harmful to wildlife by reducing the abundance of prey species and habitat cover that wildlife need in order to survive. Insecticides reduce food sources, contributing to habitat change or
loss. Repeated exposure to low doses of certain pesticides can result in reduced fish egg production and hatching, nest and brood abandonment, lower resistance to disease, decreased body weight, hormonal changes, and reduced avoidance of predators. (www.wwf.ca).

Now a days, about 67,000 metric tones of pesticides are used annually of which 70% belong to agrochemicals which are banned in several countries and are identified by WHO (1970), as toxic and hazardous. In India, such pesticides are used freely resulting in health hazards to plants and animals of aquatic and terrestrial habitat including human being. In Assam, vast amounts of insecticides are used for protection and increasing the crop and tea production. According to the Assam agricultural report (1982), the insecticides used in Assam in order of priority were i) BHC ii) Sumithion, iii) Acothion, iv) Dimecron, v) Malathion and Cythion, vi) Durspan, vii) Endosulfan, viii) Thimet and ix) Sevin. The organic pesticides such as organophosphate can quickly breakdown and cease to be dangerous. Damage to wild life due to methyl parathion is thus also negligible. The persistence of chemicals such as the chlorinated hydrocarbon is mostly responsible for pollution of the environment. These are desirable in regards to control of insects; but other effects if remain for a long time, becomes undesirable as regards to safety of non-target organisms including man and animals. These are effective against a variety of crop pests and arthropod including insects that cause various human and animal’s diseases. These are also cheap. Thus, large amounts of these are produced and utilized every year (Devi; 1994).
The pesticides get into the soil because of its direct application or by drift during dusting and spraying. From soil, these enter into soil invertebrates or water or by breaking down of microorganisms and physical factors. Because of their large scale and indiscriminate use, the persistence of chemicals in soil gets influenced by various factors. It is also reported that persistence of chemicals gets longer for soils with higher organic matter and clay mineral content. The breakdown of insecticides is more in soil having microorganisms than in sterile soil. The persistence of insecticides in soil does not constitute a great hazard to environment except that of forming a reservoir of insecticides that move in to plants grown on it and into birds upon earthworms and other soil invertebrates having deposits of the chemical in their tissues. When such birds survive, their flesh and egg contain residues of such chemicals through the application by aerial spraying on cultivated crops. Again surface run off from soil treated with the insecticides, disposal and wash off of the insecticide containers etc lead to contamination of water with insecticides. Then the insecticides are absorbed by bottom, sand, mud, plankton, aquatic plants and invertebrates and break down due to hydrolysis. The two main hazards of residues in water are deposition of insecticides in the bodies of the fishes, which are effective filters of suspended particulate matter and in the bodies of the aquatic organisms that form the food for the fish. As a result, the insecticides are deposited in the fishes more than is found free in water. The accumulation of insecticides in fishes is of great importance as fishes are one of the prime food sources for the man and birds (Andal; 2006).
Insecticides carried through the food chains induce harmful effects to reproductive system of aquatic animals due to their lipophilic nature. Thus, it poses a potential danger, by hindering the propagation of all aquatic organisms including ichthio fauna and through residual accumulation to the fish consumers along the food chain, which include man in the highest trophic level.

Benton and Warner; 1976 reported that a pollutant may not kill organisms but have dangerous effect by impairing reproduction. It is demonstrated by the declining population of certain birds (duck, hawks, pelican etc) in U.S.A. These birds have accumulated large amounts of chlorinated hydrocarbon in their bodies. These chemicals affect on these birds by changing their normal breeding behavior, delayed breeding or failure to breed. Again the eggs are so fragile that they break during incubated period causing the embryo to die.

Pesticides have very dangerous effect on the water quality like high acid concentration, high BOD, high COD, and low DO contents (Gupta and Singh;1982, Omkar;1993, Devi; 2001, Joshi,2005). The toxicity of pollutant may be increased or decreased by various water quality factors including pH, temperature, hardness and dissolved oxygen content of the water (Zitko and Carson; 1976, Pascoe et al., 1986). Smith and Heath (1979) showed in several species of fishes that their LC$_{50}$ was decreased with increasing temperature. Insecticides belonging to different classes are usually considered to have positive temperature co-efficient, i.e. their toxicity increases with the increase in the surrounding temperature, (Srivastava;1981). However, the toxicity of
DDT, Lindane and most pyrethroids are negatively correlated with temperature (Kovacs; 1982, Gluth; 1984). Aquatic ecosystem is more sensitive to residual toxicity of chlorinated pesticides than terrestrial ecosystem (Kenegra; 1979). In aquatic environment, organochlorine pesticide residues are of immediate concern to its natural inhabitant, (Edward; 1970, Sackmaucrova et al., 1977). Due to the residuals effect of these pesticides, potential hazards and massive mortality in fishes and wild birds has been reported repeating in the past. (Korswill and Elson; 1955).

Pesticides are very harmful to human being, resulting in neurophysiological, behavioral, functional, developmental and biochemical, bioenergetics and histological changes (Risher et al., 1987, Baron; 1994). These inhibit functioning of brain, red blood cells, plasma, liver, kidney, pancreases, intestine, ovary, adrenal and skeleton muscles of mammals (Baronia and Sahai; 1991).

Pollutant toxicity to fish is generally measured using lethal concentration and determining LC₅₀ values (toxic dose causing 50% mortality of individuals) within 4 days. A toxic concentration is that which cause disruption of the physiological functions of the organisms that may terminate with other pathological changes, morbidity finally leading to lethal effect (Joshi, 2005).

There are various literatures to determine the LC₅₀ dose. Litchfield and Wilcoxon; 1949, Swarup; 1981, Finney; 1971 were generated various methods to determine the sub-lethal concentration. Mathur (1962), Choudhury (1975), determined the toxicity of different insecticides in fishes and aquatic animals.
Santharam et al., 1976 assessed the effect of DDT, endosulfan, fanitrothion to daphnia. Basak and Konar; 1977 suggested a new method for the determination of safe concentration of insecticide to protect fish. Kaundaniya and Rammurthi (1980) conducted bioassay test to determine the LC$_{50}$ value and safe concentration of insecticides (sumithion and sevin) to *Sarotherodon mossambian*. Rao and Rao; 1982 performed the probit analysis in *T. mossambia*.

Haider and Inbraraj (1986) evaluated the relative toxicity of technical and commercial formulation of malathion by determining their LC$_{50}$ value in *Channa punctatus* and found that commercial formulation of malathion was 1.8 times more toxic than the technical materials.

The LC$_{50}$ for acute toxicity of the pesticides depends upon the fishes and duration of exposure. LC$_{50}$ dose of malathion has been found different for different fishes for the same duration of 96 hr. It is 4.0mg/l for *Clarias batrachus* (Dehadrai;1990), 2.5mg/l for *Channa punctatus* (Dubale and Saha;1984), 5.6ppm for *Tilapia mossambica* (Kabeer and Rao;1980), 12.35mg/l for *Notopterus notopterus* (Gupta et al., 1995), 18.36 ppm for fingerlings of Rohu, *Labeo rohita* (Das and Mukherjee;1997),and 11.67 mg/l for *Heteropneustes fossilis* (Dutta et al., 1992).

Pesticides are also responsible for different morphological, behavioral and physiological changes. Srivastava (1981) has reported redness at the base of fins on the belly, in the eyes, heart, liver and kidney under stress of urea above 200 ppm in *Cirrhinus mrigala* fingerlings. He has also reported the lesion on
the jaw region, breaking of pectoral and dorsal fin rays and loosening and shedding of scales was found in *Channa punctatus* under 1000 ppm urea stress.

Broken and wavy concentric lines were observed in *C. mrigala* fingerlings under 500 ppm urea stresses by Srivastava and Srivastava; 1981. Singh and Sahai; 1985 analyzed the effects of Malathion on the mortality and behavior of *Rasbora doniconius* and *Punctius ticto*. Sharma (1990) recorded mercury toxicosis in terms of body weights, change in feeding pattern, behaviour, respiration and morphology of *Channa punctatus*.

Insecticides have produced a numbers of adverse effects on natural ecosystem and damage to fisheries. Insecticides toxicity can also be noted by the changes in the behaviour of the fishes. These insecticides primarily damage the central nervous system resulting in instability, respiratory difficulties and sluggishness. These insecticides also show histopathological alteration in different metabolically important organs like liver, kidney, gills, and intestine. Changes of haematological parameters due to the insecticidal impact is also vulnerable (Chetri; 1990).

**1.4. About Endosulfan:**

Endosulfan is an organochlorine insecticide. It is also widely used as miticide. It is constituted mainly by carbon, hydrogen and chlorine but in some forms certain compounds like oxygen and sulfur are also present. These are cyclodiene insecticides, i.e. these are highly chlorinated cyclic hydrocarbons.
with 'endomythylene- bridged' structure prepared by the diene reaction of Dielder (Andal;2006).

Endosulfan, which has the chemical name 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxa-thiepin-3-oxide is a broad-spectrum insecticide and acaricide first registered for use in the United States in 1954 to control agricultural insect and mite pests on a variety of fields, such as fruits and vegetables crops. U.S. Environmental Protection agency (U.S.E.P.A). 2002. reported that approximately 1.38 million pounds of endosulfan ingredient is used per year. Endosulfan has been found in at least 162 of the 1,569 current National Priorities lists sites by the U.S.E P.A (HazDat 2000). In India, it is widely used against a variety of agricultural pests. During 1999-

![Chemical structure of endosulfan](image)

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Fig No.1.1: Chemical structure of endosulfan 2000, about 81,000 metric tons of endosulfan has manufactured in India and in term of tonnage its production is next only to maneoxezb (103,000 metric tons) and Monocrotophos (95,000 metric tons) (Anonymous;2001).

The chemical nature of endosulfan shows that it has molecular weight of 406.9, with the molecular formula C₉H₆Cl₆O₃S. It is the mixture of a both a chlorinated hydrocarbon and an organic sulphite. The physical state of this insecticide is between crystalline solid. Technical grade endosulfan is
composed of two stereo chemical isomers, \( \alpha \)-endosulfan (endosulfan-1, m.p.109°C) and \( \beta \)-endosulfan (endosulfan-2, m.p.213.3°C), in concentrations of approximately 70% and 30%, respectively. The odor of this insecticide is of sulfur dioxide. The melting point of the technical product is 70-100°C. In market, different trade names of endosulfan are available. These are Thiodon, Tievel, Thimex, Afidan, Cyclodan, Beosit, Devisulfan, Endants, FMC 5462, Hexasulfon, Endocel, Hildan, Hoc 2671, Insectophene, Malix, Phaser, Thimol, Thifor, Thionex etc (EXTOXNET PIP-ENDOSULFAN).

The insecticide properties of endosulfan were first discovered by W. Finkenbrink in 1956. It is highly toxic and restricted pesticide in EPA toxicity class I. It has slight fumigant action. This insecticide is highly cholinergic. Poisoned insects and other animals show violent convulsions and other neuromuscular disturbances due to inhibition of cholinesterase. The toxicity of cyclodiene insecticide is attributed to their lipid solubility.

Endosulfan is principally used to control aphids, thrips, beetles, foliar feeding larvae, mites, borers, cutworms, bugs, whiteflies, leaf-hoppers, and slugs on deciduous, citrus and small fruits, vegetables, forage crops, oil crops, fiber crops, grains as rice, cereals, maize, sorghum, tobacco, coffee, tea, forest. It is also used to control termites and tsetse fly. Sometimes it is used as a wood preservative. This pesticide is used in kharif and rabi and it has high demand in N.E India. This is mostly used in agriculture and form part of the large group of synthetic organic insecticide that has been developed and produced
on a large scale during the last 40-50 years. (Google.com. Endosulfan chemical profile 4/85).

1.5. Toxicity of endosulfan:

Endosulfan is highly toxic to aquatic fauna including fishes. Both vertebrate (fish, amphibians) and invertebrates (mollusca, insects and gastropods) are susceptible with LC₅₀'s in the range of 1 ppb for many species. This is highly toxic to the mammals. The oral LD₅₀ of endosulfan in rats is 18 mg/kg, and for a cat, the LD₅₀ is 2 mg/kg [World Health Organization. (WHO);2002]. Endosulfan has a half life of 60-800 days in soil (ATSDR, 2000). Frank et al., (1982) also has reported its persistence in soil. Endosulfan residues are detected in water samples throughout the year. Several laboratory and green house studies indicate that α and β -endosulfan are strongly adsorbed to soil (Bowman et al., 1965; El Beit et al., 1981 a. 1981b). Endosulfan, a widely used insecticide causes massive mortality to most fishes. Its accumulation has been reported in marine and fresh water animals (Vittozzi et al., 2001). Vajpai and Mathur (1986) have reported alternations in certain components of pituitary of Heteropneustes fossils exposed to a sub lethal concentration of Endosulfan.

Endosulfan is extremely toxic to fish and aquatic invertebrates and is a priority pollutant for international environmental agencies (Awasthi et al., 1999). The solubility of Endosulfan in water is low, but it persists in soil and water environment for 3-6 months or more (Rao and Murty, 1980). Kathpal et al., 1997, Awasthi et al., 2000). Endosulfan contamination is frequently found in
the environment even at a considerable distance from the point of its original 
applications (Sithunathan et al., 2002, Siddique et al., 2003). It has been 
detected in the atmosphere, soils, sediments, estuaries, surface, rainwater, food 
stuff and ground water, which show significant mobility of these chemicals 
(Masse et al., 1994, Jonsson et al., 1995, Miller et al., 1995, Ro et al., 1997, 
Sujatha et al., 1999, Berrakat et al., 2002, Doong et al., 2002, Spark and Suiit, 
2002, Bhattacharya et al., 2003, Cerejeira et al., 2003, Golfinopoulos et al., 
2003).

Endosulfan has hazardous effect to birds and beneficial insects. The biological 
magnification of this insecticide is also high and it is moderately hazardous to 
honeybee. In fact, under greenhouse condition alfalfa, birch and 
chrysanthemum are also affected by this insecticide. This is not recommended 
for use on concord grapes (Endosulfan chemical profile 4/85).

Endosulfan is a neurotoxin, targeting the central nervous system. Stimulation 
of the central nervous system is the major characteristic of endosulfan 
poisoning. Undiluted endosulfan is slowly and incompletely absorbed in the 
body whereas absorption is more rapid in the presence of alcohols, oils and 
emulsifiers. Various symptoms of endosulfan poisonings are recoded such as 
incoordination, loss of ability to stand, gagging, vomiting, diarrhea, agitation, 
convulsion etc. Blindness has been reported in cow where endosulfan was 
Endosulfan is also a reproductive toxicant. Studies have shown adverse effects of endosulfan in the reproductive system of aquatic species and mammals, including reduction of sperm counts, sperm abnormalities and decrease of daily sperm production, as well as decreased testes weight in rats. Human beings are also at risk of exposure of endosulfan. Farmers, pesticide applicators, and individuals living in the vicinity to hazardous waste disposal sites contaminated with endosulfan may receive exposure through skin contact and inhalation. Concentration of endosulfan in breast milk is found higher in woman living in agricultural areas. (www.wwf.ca). Exposure of pregnant rats to endosulfan at 1 mg/kg/day from day 12 through parturition leads to decreased spermatogenesis in offspring (Sinha et al., 2001). Again Dalsenter et al., (2003) has reported similar observations at 3 mg/kg/day and found the strain variation of rats due to toxic effect of this insecticide.

There are reports of testicular toxicity of endosulfan manifested as decreased spermatogenesis and testicular hormone synthesis (steroidogenesis), as evidenced by a decrease in spermatid count in testes and in sperm count in the cauda epididymis and by changes in marker enzymes for testicular steroidogenesis in adult animals (Chitra et al., 1999; Singh and Pandey; 1989, 1990; Sinha et al., 1995). These effects were seen at much lower dosages and shorter durations if exposures occurred during the prenatal or prepubertal periods (Dalsenter et al., 1999; Sinha et al., 1995, 1997, 2001). Singh and Pandey (1990) also reported decreases in the levels of plasma LH, FSH and
testosterone along with decrease in testicular testosterone after the application of endosulfan.

Endosulfan has estrogenic effects (Soto et al., 1994). Synergistic effects with environmentally relevant organochlorine pesticides have also been observed (Arnold et al., 1996). The fate of α and β isomers is dependent upon the physical state of environment. Evidence suggests that exposure to endosulfan may cause mutagenic and teratogenic effects in humans if exposure is great enough. Data from animal studies reveal the organs most likely to be effected include kidneys, liver, blood, and the parathyroid gland.

Endosulfan is a persistent organic pollutant (POP). It persists in environment for extended periods of times, increasing the exposure risk of many non-target animals. It has been detected in wells and surface waters, most likely due to run-off or drifts during application. It volatilizes easily after the application and is transported to areas distant from the application site. It has also been detected in ice and snow samples of the arctic.

Report from a village in Kerela showed that when endosulfan was applied in Kazu plants to control insect-pest, the productions of kazu were increased, but at the same time, a number of handicapped babies were borne in that village. This was taken as the possible cause of exposure to the endosulfan. Since that very incident, endosulfan was banned in Kerela. Again, the same scenario was observed in west Bengal, Bihar and Uttar Pradesh where this insecticide was used in some mango plants without recommended concentration. In that case, birds’ population became less. The layer of egg shell becomes thin due to the
effect of Gamaxine and DDT. As a result no hatching of egg is possible (Prayashi;2005).

Endosulfan is highly toxic via the oral route, with reported oral $LD_{50}$ values ranging from 18 to 160mg/kg in rats, 7.36mg/kg in mice, and 77mg/kg in dogs. It is also highly toxic via the dermal route, with reported dermal $LD_{50}$ values in rats ranging from 78 to 359 mg/kg. Endosulfan may be slightly toxic via inhalation, with a reported inhalation $LC_{50}$ of 21 mg/L for 1 hour, and 8.0mg/L for 4 hours. The alpha-isomer is considered to be more toxic than the beta-isomer. It is also toxic to bird species, with reported oral $LD_{50}$ values in mallard ranging from 31 to 243mg/kg and in pheasants ranging from 80 to greater than 320 mg/kg (EXTOXNET-PIP).

It is reported from EXTOXNET that in an accidental exposure, sheep and pigs grazing on a sprayed field have suffered a lack of muscle coordination and blindness. Several chronic effects have been noted for animal exposed to endosulfan. The dose of endosulfan at 2.5 mg/kg/day on dogs shows vomiting, tremors and convulsions. Higher doses of endosulfan (5.0 mg/kg/day) has caused death in rats, increased resorption and caused skeletal deformities in the rat fetuses. Female mice fed the compound for 78 weeks (0.1 mg/kg/day) has damaged to their reproductive organs. In chronic exposure of endosulfan may result in cellular changes.

The environmental fate of endosulfan is very hazardous. Large amounts of endosulfan can be found in surface water near areas of application. Due to surface run-off through rain and other means, these are contaminated with
different aquatic ecosystem like, ponds, reserviour, wetlands, river etc. Endosulfan is insoluble in water and is usually highly soluble in fat. As the fish represents the highest trophic level in the aquatic ecosystem in food chain therefore these are considered to be more contaminated with the pesticide residues, which affect their population, breeding habitat, behavioral pattern and their normal physiological functions.

Endosulfan is a restricted pesticide in the USA and is banned in Norway and Sweden. In Indonesia, the government is banning 28 pesticides including endosulfan due to their impact on human health. However, there is no restriction in Canada in the use of endosulfan (www.wwf.ca).

1.6. Present study:

In view of the above literature it is clear that endosulfan has got direct impact not only on target animals but also in the general biological system of various animals including fishes.

Among fishes there are many species which are commonly found in the agriculture wetland apart from ponds or lakes. One of these fishes includes *Channa punctatus*, which is common in agricultural wetland and seem to be an easy victim of various pesticides. *Channa punctatus* is a murrel fish, which is considered to be very nutritious and commonly used by people of North east India. The people here have great faith of its medicinal values. It has also got a good market demand owing to their low fat content and less intramuscular spines. *Channa punctatus* are also very important to the paddy field as they increased paddy by tilting soil due to their movement. Again they
Plate No: 1.1. *Channa punctatus* before exposure to endosulfan

Plate No: 1.2. *Channa punctatus* after exposure to endosulfan
feed larvae, worms, nematodes etc in the field, resulting in high yielding of paddy. The excreta of these fishes also serve as additional fertilizers to the crop (Chetri; 1990). These fishes are abundantly found in the beels, marshy land, ponds and paddy fields of Assam, where the insecticide is used continuously.

It is reported that the population of the local fish species are gradually decreasing (Devi; 1994). Various literatures on the impact of pesticides, insecticides, other chemicals and heavy metals on fish species have suggested that indiscriminate use of such chemicals might be one of the reasons for its declining trend. Works on different pollutants like pesticides, insecticides and heavy metals are considered as referential work in this study.

1.7. Aim of the study:

So, keeping in mind the importance of *Channa punctatus*, which is found in wetlands of paddy field and consumed in lunch or dinner by average Assamese community and also it is the direct victim of insecticides exposure, the present work has been undertaken to study the toxicological impact of endosulfan on *Channa punctatus*.

To fulfill the aim of the work following objectives have been taken.

- A general behavioral study to know the effect of the endosulfan.
- To record the histological changes of Liver, Gill and Kidney.
- Haematological study to determine Haemoglobin percentage, WBC Counting, RBC Counting and WBC differential counting.
• Biochemical study to estimate of Serum glucose, Liver glycogen, Muscle glycogen, Serum protein, Tissue protein, Enzymes e.g.-Serum alkaline phosphatase, SGOT (Serum glutamic oxaloacetic transaminase) and SGPT (Serum glutamic transaminase).

• Electron Microscopic Study of Liver, Gill and Kidney to support the histological changes.