INTRODUCTION
CHAPTER-I

INTRODUCTION

"Gar Firdaus bar rue Jaminast, Haminast, Haminast, Ham'anast" means "if there is heaven on the earth it is here only", these words said by the mugal emperor Jahangir, in the sense of beauty and purity of Kashmir, the part of India. But, today unfortunately not only the flora and fauna of Kashmir but all parts of India are affected dangerously due to pollution. Truely, nothing can be permanent and things can be changed time to time because about ten thousand years ago when mankind was started to change from hunter gather to today's modern mankind, we began to utilize the environmental resources to fulfill our own requirement. As a result of this, mankind has been depleting the natural resources for his welfare and luxury. He has adopted industrialization, deforestation, urbanization and modern techniques in farming which resulted into today's polluted environment, (Bharucha, E; 2005).

The pollution is referred to any worse change in the natural quality of the environment which may be brought about by physical or chemical or biological factors through the natural or man made processes and these factors are known as pollutants. The pollutant is the most important factor that has harmful effect on living organisms and their ecosystem.
Man as a biological entity which is lived on earth and earth is the only planet that known to sustain a life is largely responsible for environmental pollution (Rachel Carson; 1962). For the industrialization and urbanization, man has plundered the natural resources of the earth without thinking about the long-term consequences of such actions on nature. Hence, it can be aptly said that, of all the biotic factors "Man is the most destructive animal." Man is obviously the benefiter from all these activities but no doubt he is also the culprit behind the generation of pollutants (Chaudhari et. al;1999).

Thus, a day by day, environmental pollution is becoming a major problem on the earth planet and the prevention of pollution and maintenance of nature conservation are truly understood by human being because, the damages to the biosphere has reached such a peak that can not be easily repaired for many decades of years. The adoption of new techniques of crops production have witnessed a tremendous boon in quantitative production of food for incessantly increasing population. But the over enthusiastic use of inputs with a singlemined uppermost goal of increasing agricultural production has led to drastic changes in the environment of soil, underground water quality and the ambient air.

Thus, among the various pollutants, pesticides occupied an unique position as they are deliberately added to the environment by man to suppress or eliminate some harmful forms of life causing damages to
crops. Thus, the main plant protection tool i.e. pesticide developed and used for human welfare, is being considered as to this day as the most disastrous factor in producing the harmful effects in the environment and have become the serious source of pollutant (Rameshwarsing; 2004). It has been universally accepted that insecticides have become indispensable for obtaining high yield of crops (Solanke and Singh; 2000). Application of pesticide has become inevitable to protect crop plants from diseases. A loss in agricultural yield due to pests and diseases was estimated around 50% of total production in developing tropical countries. (Vasumathi, et. al; 2001). Against the pests and harmful disease of crops only the pesticide work as a "Magic bullet", a chemical that affected only the pest and no other living things or aspect of the environment (Botkin and keller; 1982). But according to Khan, Z; (2003) the pesticide can change almost all aspects of both natural and managed ecosystem such as agro-eco system, patterns of energy flow, water and air quality.

In India, pesticides are used for crop protection and public health as extensively as to the extent using nearly 85000 tonnes annually and of these, about 77.8% of pesticides are insecticides and rest are fungicides, herbicides, rodenticides, fumigants and miscellaneous pesticides (Maruthannarayan and Sharmila; 2004). During recent years, three groups of pesticides, viz., Chlorinated hydrocarbons, organo
phosphorous and synthetic prethroids are using to combat the ectoparasites, infection in domestic animals and for protection of the crops from the pests. Unfortunately, the regular utilisation of large quantities of these pesticides into the environment creates a pesticidal pollution which constitute the most dangerous health hazards on the non target organisms, such as fishes (Khare and Singh; 2002). The biochemical changes induced by pesticidal stress led to metabolic disturbances and inhibition of activity of important enzymes (Raje and Mulley; 2006). The cellular damages and tissue abnormality in kidney, muscles and testes were noted in the fresh water fish, Puritius stigma when exposed to endosulfan (Khillare; 1992). The restlessness and irregular, erratic and darting movement with imbalanced swimming activity were seen in fresh water fish, Catla catla when treated with endosulfan (Shivakumar and David; 2006).

The changes in the ultra structure of hepatic cells with decrease in the protein value in brain, gills, muscles, kidney and liver were recorded by Khan, Z; (2003) when Rainbow trout exposed to combine treatment of organochlorine (endosulfan) and organophosphate (disulfoton).

The Pollution due to pesticides and fertilizers is responsible for the decline in amphibian population of aquatic habitats, especially in tropical region of the world. The life of amphibians can affected by the
pesticides because of their semipermeable skin, the development of eggs and larvae in water and the storage of food in webs. Pesticides may also affect the amphibian population by disrupting normal growth and development of the young or by impairing adult reproduction. An extensive research studies carried out in Canada which reflected the fact that hind limb deformities were commonly observed in transformed bullfrogs, greenfrogs and northern leopards (Khan and Law; 2005). The morphological and behavioral changes were also seen due to the endosulfan toxicity in the anuran tadpole (Dey and Gupta; 2002).

Some studies have established that, the reptiles face many impact from human activities including hunting, fisheries interactions loss of nesting habitat due to costal development and pesticidal contamination (Khan and Law; 2005). The environmental contamination such as organochlorine contamination is responsible for modulation of immune function in sea turtles which also interfere in the sex, body conditions and hormone concentrations (Keller; 2005).

The treatment of phytopesticide, a biosal (neem based formulation) and organophosphate, malathion led into the disturbances in the cholinesterase activity in kidney and liver of Calotes versicolor (Khan; 2003). The neurotoxic effects were also recorded due to the intoxication of phosphamidon (Dimecron) in Calotes versicolor (Meenakshi et. al; 1997). Some studies have reported that the lizard,
Gallotia gollotia, is highly sensitive to organophosphorous pesticide and itself served as a bioindicator of organospororous contamination in Canary island due to reduction in its important enzymal activities (Fossi et. al; 1995). A number of disturbing abnormalities were recorded in American alligator such as modification of hormone activities and reproductive abnormalities due to the binding of environmental pesticides such as DDT and organochlorine to the ovarian hormone (Vonier. et. al;1996). Recently, it is proved that the treatment of pesticide, cypermethrin resulted into the decrease in protein level of liver and kidney of Calotes versicolor (Khan et. al;2002).

The birds are also highly affected by the toxicity of organochlorine and organophosphate pesticides. The chronic exposure to dieldrin to mallard ducks caused appreciable depletion in brain amines which then resulted into their behavioral disorders (Sharma; 1973). Similar changes in the chronic reproductive study of mallard such as decline in number of egg laid, number of viable embryos and number of normal hatching due to the effect of some pesticides (PANUS; 1999). The treatment of diazinon and parathion was responsible to develop skeletal deformities and decrease in acetylcholinesterase activity in the embryos of bobwhite quails (Meneely and Wyttenback;1989). The acute and chronic treatment of insecticide, baygon spray to the rats resulted into the significant decline in the learning process which then
returned to normal after 24 hrs. (Mathew et. al; 1989). The long term exposure of various pesticides led to develop disturbances in liver, heme biosynthesis with decrease in serum cholinesterase of sprayers of grape garden (Patil et. al; 2003). A profound hormonal imbalance caused by the exposure of endosulfan was noted in male gonads of rat (Singh and Pandey; 1989). They also showed that the liver metabolism was disturbed due to the action of this pesticide.

Therefore, by referring above reviews, we can conclude that our environment is greatly polluted by the pesticides which are commonly used by the farmers. Thus, it can be established the fact that, pesticides and other related chemicals definitely interfere in the normal activities of all organisms living in the pesticide polluted environment.

It is true, that, indiscriminate use of pesticides without studying their toxicological effects may caused severe damages to our ecosystem and human health. But, at the same time we cannot increase our food production to feed the growing population without the use of pesticides, because it was estimated that 50% of the potential food production in the country was lost due to insect pests, rodents, nematodes, plant pathogens and weeds.

Hence, pesticides are no doubt will remain in the use for our foreseeable future, but man must, therefore learn to live with pesticides. At the same time he must encourage the research works to develop safer
and lesser toxic pesticides and un-harmful natural method to control pest impact.

1) HISTORICAL BACKGROUND OF PESTICIDE -

Organisms that cause economic loss or damage to the physical well being of human being are known as "pests". They may damage crops and cause diseases in them or human being. Pesticides are the substances or a mixture of substances used for destroying, preventing, repelling or mitigating pests. The earliest attempts to control pests chemically included use of naturally occurring toxic substances such as mercury or sulphar or plant extracts such as nicotine, pyrethrum or debris. The era of modern synthetic pesticides largely dates from 1939 when insecticidal properties of DDT (Dichlorodiphenyl trichloroethane) was documented by Paul Muller in 1939. He demonstrated that DDT as a mosquito killer was valuable in the control of diseases typhus and malaria and for that Muller was awarded by the Nobel Prize of Physiology and medicine in 1948. The DDT was the first among the chlorinated hydrocarbons to be developed as pesticide. Pesticides, today include mainly acaricides, algicides, avicides, bactericides, fungicides, herbicides, insecticides, molluscicides, nematocides and rodentocides to control the mites, algae, birds, bacteria, fungi, weeds, insects, molluscs, nematodes and rodents respectively.
Today, there are many categories of chemical pesticides being used throughout the world and the majority of them are synthetic organic compounds which have potential to contaminate the environment. Mackay; (1991) selected about 180 organic compounds, that are hazardous to the environment on the basis of release of large quantities into the environment, persistence, high bioaccumulation, potential toxicity and other characteristics such as odour or ability to influence the physico-chemical balance in the environment.

Although, the pesticides like DDT and BHC were for the first time imported for control of malaria and locusts but their use in agriculture in our country began from 1949 and by 1954 we have started the production of these indigenous pesticides with the installation of DDT and BHC plants.

There are four main classes of insecticides which are as under-

- Organochlorine
- Organophosphate
- Natural and synthetic
- Dinitrophenols

But, out of these, only first two principle classes namely Organochlorine and Organophosphate are discussed here in detail because they are selected for the present studies.
Organochlorine -

Organochlorines are known as chlorinated derivatives of ethane such as, DDT, DDD, Methoxychlor etc. cyclodienes, a chlorinated hydrocarbons which include aldrin, dialdrin, endosulfan, heptachlor, toxphene and hexa-chlorocyclohexane such as lindane. In general, these are very stable chemicals which can withstand the action of various environmental factors like temperature, solar radiations and moisture for long duration of time. In a biological system they are degraded with considerable difficulty. The stability and persistance of these synthetic organics which are responsible for their prolonged toxicity to insects and pests must be of short period. Most of these chemicals dissolve well in organic solvents and fats while their solubility in water is very poor. They have a broad spectrum of action which affect the nervous system of the insect causing disruption of transmission of nerve impulses and dies at last.

These pesticides used extensively during the period of 1940 to 1960 for the control of agricultural pests and malaria. Their properties such as low volatility, chemical stability and environmental persistence are led to their bioaccumulation or biomagnifications in the food chain of fishes, birds and mammals due to their lipophilicity and slow metabolic degradation (Pandey, 2005). Endosulfan, an organochlorine pesticide inhibits the process of spermatogenesis by altering the activities
of responsible hormones in the testis of Druckrey rats (Sinha, et. al; 1995).

➢ **Organophosphorous:**

Among the various group of pesticides that are being used worldwide, the organophosphorous group forms a major and most widely used group accounting for more than 96% of the total world market of pesticides. Organophosphorous was first developed in Germany during second world war in the form of tetraethyl pyrophosphate (TEPP) as a by product of nerve gas development. Among the different categories of pesticides, organophosphates are the choice of today because of their non-persistent nature and rapid biodegradibility. The organophosphorous compounds are normally esters, amides, phosphonic and phosphonothionic (WHO, 1986). They are acutely toxic and act by inhibiting acetylcholine esterase, an important enzyme is unable to work which causing accumulation of acetylcholine, which interfere with the transmission of the nerve impulse at the nerve endings. In human being organophosphorous poisoning develop the symptoms such as general weakness, headache, excessive sweating, salivation, nausea, vomiting, diarrhea, abdominal cramps and tremors. In 1944, Schrader synthesized arathion, a more stable compound, and later in 1946, Mc Combie and Saunders synthesized diisopropyl fluorphosphate (DFP) which is
potentially toxic among organophosphate compounds. This compound is deliberately used into the field for crop protection and disease control.

The organophosphate insecticides are all esters of phosphoric acid and also called organophosphate which include aliphatic phenyl and heterocyclic derivatives and have one of the basic building blocks as a part of their much more complex chemical structure. Organophosphates are used to control the varieties of sucking, chewing and pest attacking crops such as cotton, sugarcane, peanuts, tobacco etc. and they are marketed by many of the worlds major agro-chemical companies. The other some of the main organophosphorous pesticides are parathion, methyl parathion, chlorprifos, malathion, monocrotophus and dimethonate (Kanekar. et. al; 2004).

2) PERSISTANCE OF PESTICIDES IN THE ENVIRONMENT

A large quantity of pesticides are introduced in the environment by the farmers directly by spraying or fumigation, while some quantity of pesticides of industrial waste may be percolated in the soil or carried away with drainage water. The extensive use of pesticides in agricultural, public health and forestry ultimately led to the contamination of soil, and aquatics biotopes which alarming a great threat to the environment. The entry of pesticides into soil, air and aquatic biota resulted into wide range of effects on animal community.
Thus, the soil is an environmental reservoir of these residues of pesticides from which they are taken up by plants which are consumed by the animals. Later, due to decomposition of dead bodies of plants and animals these residual pesticides again added to the soil, water and air.

Due to large scale use of pesticides in the agricultural fields and release of untreated sewage from factories, a huge amount of pesticides enter into the water bodies. Similarly, the aerial environment is also contaminated by spraying and fumigation of pesticides.

Thus, the aerial, aquatic and soil environment are dangerously polluted by the pesticides. The persistence of pesticides in the environment depends upon their chemical and physical properties, dose, and formulation (e.g. liquid, powder or granules etc.), type of the soil, its moisture content, temperature, physical properties of the soil, composition of the soil microflora and the plant species present. It should be noted here that the persistence of these chemicals in the environment depends on a number of factors. Under favourable conditions for decomposition, the degradation may be brought about rather quickly while during adverse conditions it may delay for considerable period of time. For example DDT may be eliminated within two years only in favourable conditions while persistence for periods ranging between 20-25 years also been recorded in unfavourable conditions.
The pesticides are classified on the basis of their persistence in environment and food chain as under-

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Types of pesticides</th>
<th>Example</th>
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<tbody>
<tr>
<td>i)</td>
<td>Non Persistence (Degrade rapidly)</td>
<td>Malathion etc.</td>
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<tr>
<td>ii)</td>
<td>Moderately Persistence (Persist for 1-18 Months)</td>
<td>Atrazine etc.</td>
</tr>
<tr>
<td>iii)</td>
<td>Persistence (Persist for up to 20 years)</td>
<td>Aldrin etc.</td>
</tr>
<tr>
<td>iv)</td>
<td>Permanent Persistence (Never degrade)</td>
<td>Mercuric Chloride</td>
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**3) PESTICIDE TOXICITY HAZARDS**

The use of pesticides undoubtedly have saved the millions of human lives by killing disease causing insects and by producing large scale of food supply. But at the same time, excessive pesticide use is responsible to kill the non target organisms and due to its bad impact can upset the natural balance and ecosystem. The pesticides definitely beneficial to mankind because they play an important role in agriculture and health problems. Besides the beneficial properties of pesticides, they are also hazardous to the environment and human being because they cause-
i) Pesticide hazards due to its production

The pesticide producing factories are the most dangerous pollutant producing media. It is estimated that a double quantity of wastes is produced during the production of total amount DDT which affect the underground water by seepage. Similarly, while producing thousand tones of BHC in our country, its solid and liquid waste at large scale is discharged which then accumulated in soil and underground water. The workers of pesticide factory are liable to be constantly, exposed and affected by the pesticide residues as they have to work in pesticidal environment. Hence, almost all workers of the factory become victims of toxic gases, fumes of liquid material and very fine floating particles of pesticides during their work in factory due to constant exposure. Due to this, factory workers complaining about irritation of eye and skin, headache, diarrhea and stomach disorders.

Similarly, unpleasant toxic gases released during manufacturing of pesticides which disturb the proper oxygen balance in the atmosphere which then producing undesirable effects in a respiratory system of organisms.

One of the best example of chemical pollution hazard was “Bhopal gas tragedy!” on 3rd Dec. 1984 in India in which more than 3300 people lost their lives in the world’s worst environmental disaster caused by leakage of Methyl isocinate (MIC) gas. MIC is an
component of temic pesticide, is a powerful irritant, causing burning and swelling of moist tissues such as eyes, mouth, nasal passages and lungs. (Agrawal;2004).

ii) Pesticide hazards due to its application in agriculture

Pesticide poisoning to human beings and animals is of great loss when used for plant production and health programmes. In our country, numerous cases of pesticide poisoning are reported due to direct and indirect inhalation of pesticide. The harmful effect of pesticide poisoning can be seen in the form of death and physical disorders originating from their use in maize, cotton and rice growing areas where pesticides are in excessive use. Excess of residues of pesticide in cereals, fruits and vegetables may responsible to cause "brainlessness" and the 'still births'.

In one episode, in 1972, a single application of the large dose of insecticide azodrin to combat potato aphides on a farm in Dade country, Florida, killed 10,000 migrating robins in three days. This tragedy was due to the poisoning effect of pesticide on nontarget organisms such as robins.

Similarly, in 1991, in California, a tanker of highly toxic metam sodium herbicide dumped into the Sacramento river and the entire river-ecosystem including aquatic plants, insects, amphibians and at least
1,00,000 trouts were completely died and wiped out for 45 km. down stream.

iii) Pesticidal hazards in aquatic life

The harmful effects on the fishes due to the pesticides have been reported from various parts of world. The mortalities of fishes have also been reported in India due to the same reasons. The route of entry to the aquatic environment such as rivers, ponds, lakes, may be through industrial waste discharge, accident spillage, spray drifts during application, leaching of soil insecticides used for soil pests, air transport or sewage effluents, chlorinated hydrocarbons (DDT and HCH) and certain herbicides are main pollutants for aquatic lives.

iv) Pesticide toxicity hazards in human

Pesticide uptake occurs mainly through the skin and eyes by inhalation or by ingestion. The fat soluble pesticides and to some extent water soluble pesticides are absorbed by the skin while the lungs absorbed the vapours of pesticides and aerosal droplets which are smaller than 5 mm in diameter. The pesticides can be also be ingested through mouth with contaminated hands may also lead to intake of pesticides such as from cigarettes, drinks and other etables etc.
Within the body, the pesticides may be metabolized or may be stored in the fat or excreted. While some fat soluble pesticides which are not-metabolised, are stored in fatty tissues. The organochlorine pesticides such as DDT and HCH accumulated in fatty tissues of body where they remained inactive. But at the time of poor starvation, these stored fats are metabolized and the pesticides are released from these sites into blood stream which may causing toxic effects if their concentration reaches an enough higher level.

The possible harmful effects resulted into human beings due to pesticide toxicity hazards are as under-

a) Skin problem

The pesticide workers are generally suffered from contact dermatitis and allergy sensitization after exposure to several pesticides (Adam; 1983)

b) Bone marrow deformities

The aplastic anaemia and related blood dyscrasias were developed in the pesticide workers associated with the pesticides which may be due to rare idiosyneratic bone marrow caused by the exposure of pesticides. But very rare data is available about it.
c) Cytogenetic effects

The pesticidal effects on cytogenetic damages were also recorded in insecticide sprayers (Wood well et. al; 1967). They found increased chromosomal damages among herbicide and insecticide sprayers during the peak spraying season.

d) Interference in reproduction

Male infertility has been affected in association with exposure to the nematocide, dibromochloropropane (BDCP) as reported by Watson; (1979). Moreover the reproductive effects were related with phenoxy herbicides and their contamination. There have been many investigations but little consistence is found in the results.

v) Pesticide hazards in enzyme activity

In some cases, the induction of liver microsomal enzymes by pesticides was found the animals. Antipyrine was responsible to have shortened half life in workers exposed to variety of pesticides due to their interferance in enzyme activity.

vi) Pesticide hazards in cancer

The international agency for research on cancer (IARC) has evaluated that phenoxy acid, herbicides, chlorophenol and concluded that
there is limited evidence of carcinogenicity for human beings due to the exposure of above pesticides.

Thus, we may say that most of the pesticides have systemic effect and by their attack upon nervous system, inhibit not only different physiological and biochemical activities but also the survival rate of organism.

4) CONTROL OF PESTICIDE HAZARDS

The pesticide hazard can be controlled by

i) Controlled use of pesticides

ii) Avoiding use of banned pesticides

iii) Avoiding use of more toxic pesticides

iv) Following manufacturers instructions while using pesticides

v) Practicing better sanitation practices

vi) Using non-chemical methods of pest control such as mechanical method and biological method.

vii) Following integrated pest management scheme to avoid hazardous effect of agro-chemicals.

5) LOGIC OF THE PRESENT STUDY

Thus, it can be concluded that due to the promiscous and indigious use of pesticides, the whole ecosystem is getting polluted. In
the perspective of the above background, the present study was undertaken in order to evaluate the toxicity of endosulfan and parathion on the Indian garden lizard, *Calotes versicolor* as under-

1) Collection of experimental animal, *Calotes versicolor*.

2) A. Establishment of sublethal and lethal doses for endosulfan and parathion pesticide for *Calotes versicolor*.

   B. Sublethal and lethal doses of pesticides to study their toxicity for *Calotes*.

3) Haematological studies.

   a) Haemoglobin percentage in blood.

   b) Total leucocyte counting in blood (TLC)

   c) Differential leucocyte counting in blood (DLC)

   d) Red Blood cell counting

   e) Blood clotting time

   f) Bleeding time

4) Different tissue biochemicals and blood biochemicals

   C) Biochemical study of tissues

   i) Kidney protein

   ii) Kidney glucose

   iii) Liver protein

   iv) Liver glucose
D) Biochemical study of blood

i) Enzyme activity
   a) Alkaline phosphatase
   b) Acid phosphatase
   c) Serum glutamate oxaloacetate transaminase (SGOT)
   d) Serum glutamate pyruvate transaminase (SGPT)

ii) Blood protein

iii) Blood cholesterol

iv) Blood glucose

v) Blood calcium

vi) Blood phosphate

vii) Blood potassium

viii) Blood sodium