The advent of man on the earth drastically altered the face of Mother Nature. His distinguished extraordinary cranial capacity introduced scientific logics in every field of life with his own pronounced priorities. This approach towards life sowed the seeds of danger. “In the minds of man the useful has succeeded to the beautiful and is now followed by exploitation”.

Man started the use of pesticides with the growing awareness about their utility in agricultural production, animal husbandry, post harvest technology and welfare of mankind. The synthetic chemicals such as insecticides, weedicides and pesticides which were knights in armor for crops were actually demons in disguise for the members of aquatic communities.

The toxicants exerted pressure on every natural resource but most evident crack has been on the water ecology. The pesticides even when applied in restricted areas are washed and carried away by rains and floods to water bodies which further influence reproductive physiology of fishes adversely. To address the issue of reproductive toxicity of organophosphorus pesticides especially their effects on oocyte quality, the reproductive ability and to study the magnitude and scope of these effects, the present study was undertaken.

The organophosphorus pesticide selected for this study is monocrotophos, which is widely used insecticide in the paddy fields of northern regions of India. Though this pesticide is banned in many parts of the world, unfortunately this pesticide is still widely used and is documented to have wide range of hazardous
effects on the body tissues of the aquatic animals when present in the aquatic environment.

The fish chosen for the present study was *Cyprinus carpio communis*, a pond dweller and found in the vicinity of agricultural lands where it is constantly threatened by the pesticide exposures mainly as runoff during monsoons. This fish is relished by all and is a good source of protective protein. Moreover, this fish is excellent test fish for bioassay studies because of its hardy nature and can be easily maintained in the aquarium. Further, it accepts the artificial feed and exhibit good growth response. Moreover this fish of desired size was available throughout the year. All these considerations are undoubtedly in favour of this as test animals for the present studies. Besides, this biomolecular transformation mechanism of the fish in general is very close to the human system.

The summary of the investigation relating to reproductive toxicological effects on *Cyprinus carpio communis* are as follows:

- The fish was brought from the farm periodically, acclimatized in the glass aquarium for fifteen days before the onset of experiment.
- Acute toxicity test in the form of LC$_{50}$ was conducted to ascertain the effect of toxicant on the fish which appeared within 96 hrs of exposure and LC$_{50}$ of monocrotophos on *Cyprinus carpio communis* came out to be 0.37 ppm by conducting probit analysis with SPSS version 10 programme. This dose response relationship showed linear relationship thus showing that the probit kill increased with increase in the concentration of pesticide.
- The chronic toxicity was further studied by taking three sub-lethal concentrations i.e. $1/7^{th}$ LC$_{50}$ (0.052 ppm), $1/5^{th}$ LC$_{50}$ (0.074 ppm) and $1/3^{rd}$ LC$_{50}$ (0.123 ppm). The Chronic toxicity is the delayed poisonous effect from exposure which was carried for one month in continuum. The behavioral, ultrastructural, morphological and biochemical studies were conducted to ascertain the extent of damage caused by this commonly used organophosphate pesticide in this part of the country.
- The doses of a pesticide determined different degree of effect on its physical and behavioral characteristics. The immediate fish response was studied by studying the following parameters i.e. air gulps, opercular movements, fish movements, etc. The behavioral pattern of fish changed with increase in pesticide concentration i.e. decrease in fish movement, opercular movement.
and increase in air gulps. There was significant decrease in weight at the highest and lowest concentrations of monocrotophos after 30, 45 and 60 days of chronic exposure. The above described erratic behaviour of the fish was due to allergic reactions that bring changes in the immune system i.e. they are not really toxic response. The difference between allergies and toxic reactions is that toxic effect is directly the result of the toxic chemical acting on cells. Allergic response is the result of a chemical stimulating the body to release natural chemicals which are in turn directly responsibly for the effects observed. Thus, it was observed that low concentration of pesticide triggered immune system of fish to secrete maximum mucus that is why it became very slimy and hyperactive in allergic phase. Whereas, the toxic effect at highest concentration showed maximum damage with long resting period, sluggish movement and finally fish became very lethargic and inactive due to toxic phase.

➢ The histological studies following method proposed by Bradbury (1975) was performed to study the characteristic features of various developmental stages of oocyte of Cyprinus carpio as well as the pesticide induced artifacts in the oocytes.

- The process of oogenesis has been classified according to size, appearance of nucleus and nucleoli and distribution of cytoplasmic inclusions in oocytes of fish. The stages identified in Cyprinus carpio communis are oogonia stage, chromatin nucleolus stage, perinucleolar stage, cortical alveoli stage, vitellogenic stage, germinal vesicle migration stage and germinal breakdown stage.

- Monocrotophos induced several deleterious effects on the developmental stages. The reduced size of oocyte with wrinkles on the surface along with presence of more atretic oocyte is very apparent in the study. The development of vacuolization in cytoplasm, damaged peritoneal lining, reduced nucleoli at perinucleolar stage; decline and dissolution of lipid yolk globules (cortical alveoli) in cortical alveoli stage, increase in vacuolization and decline in protein yolk (vitellogenin) in oocytes at vitellogenic stage; deformed oocytes at
germinal vesicle migration stage are the major artifacts observed in the present study.

- The normal as well as treated fishes were insensitized by cervical dislocation and ovaries were processed for scanning electron microscopic studies to view the damage caused by pesticide on the surface of oocytes of the fish.
  - The appearance of the wrinkles on surface of oocytes of treated group is very prominent. The wrinkles became more intense on the surface at the higher concentrations of monocrotophos as well as when the period of exposure of the pesticide increased. The shrinkage around the micropyle is also very clear. The damage to the granulosa layer and decreased compactness of the zona radiata layers on oocyte surface is also apparent in the oocytes of treated fish as compared to that of control fishes group.

- In order to view the damaged caused by pesticide on ultrastructure of oocyte at subcellular level, the tissue (ovary) was processed by the technique of transmission electron microscopy.
  - The monocrotophos effected oocytes revealed atretic oocytes at oogonia stage, distorted nucleus and decreased nucleoli at perinucleolar stage, decreased cortical alveoli at cortical alveoli stage, decreased vitellogenic yolk at vitellogenic stage and wide perivitteline spaces at periphery in nuclear migration stage. All these artifacts followed ascending trend with increase in monocrotophos concentration as well as increase in period of exposure to monocrotophos.
  - Heavy vacuolization in ooplasm of oocyte is observed at all the days of exposure but is maximum on 60th day at 0.123 ppm of monocrotophos concentration. The dissolution of wall separating oocytes, enlarged follicular cells at periphery, wavy superficial layer of oocyte are other artifacts observed at almost all concentrations at exposure periods.
  - The enlarged follicular cell and thecal cell along with the shrinking of ooplasm at periphery developing wide space are prominent at all exposure periods but could not be traced at all concentrations of monocrotophos.
• The intracellular organelles like deformed nuclear membrane and distorted nucleus is observed in oocytes of treated fishes. The scattered endoplasmic reticulum and golgi bodies, increased number of mitochondria in thecal cells, reduced size of mitochondria of oocyte, exposure of microvilli by follicular cell layer are the other subcellular artifacts observed in the oocytes of the treated fishes.

➤ As the pesticide monocrotophos produces the morphological and ultrastructural damage to the fish oocyte, it would definitely pose serious risks to biochemical parameters and enzyme activities in fishes. Thus in order to correlate the structural changes with the biochemical changes, the following biochemical parameters i.e. acetylcholinesterase activity (Ellman et al., 1961), protein content (Lowry et al., 1951), glycogen content (Seifert et al., 1950), lactate dehydrogenase (Anon, 1984), cytochrome oxidase (Sottocosa et al., 1967), alkaline phosphatase (Bergmeyer, 1963), lipid peroxidation (Wills, 1965) and cholesterol (Zlatkis et al., 1953) were analysed.

• The acetylcholinesterase activity shows drastic decrease by 91%, 90% w.r.t controls in ovary on 60th day at 0.052 ppm and 0.074 ppm of monocrotophos concentrations respectively. The activity has been found to be comparatively better on 30th and 45th days of exposure but is quite low than the control. It has been observed that this activity of enzymes decreased in liver. At 0.074 ppm, the enzymatic activity increased on 60th day exposure as compared to 45th and 30th days exposure. At 30th, 45th, 60th days of exposure, acetyl cholinesterase activity showed declining trend.

• The total proteins (mg/ml of homogenate) in the liver and ovaries of treated fish showed a declining trend. These biochemical parameters in ovaries of this fish on 30th day showed a decline by 7%, 12% and 34% w.r.t control at 0.052 ppm, 0.074 ppm and 0.123 ppm of monocrotophos respectively. On the 45th day the value showed 34%, 35% and 35% decrease w.r.t control at all the three concentrations and on the 60th day, the decrease was found to be 69%, 71% and 79% w.r.t control. The liver showed 8%, 30% and 30% decrease on 30th day, 13%, 16% and 18% on 45th day and 11% and 13% decrease on 60th day.
w.r.t their respective controls at 0.052 ppm, 0.074 ppm and 0.123 ppm of monocrotophos treatment respectively.

- Glycogen level in liver and ovary of *Cyprinus carpio communis* exhibited a decreasing trend at 0.052 ppm, 0.074 ppm and 0.123 ppm of monocrotophos exposure. The glycogen content in liver decreased by 7%, 8% and 5% w.r.t control on 30th day, 7%, 7% and 6% w.r.t control on 45th day and 7%, 7% and 10% w.r.t control on 60th day of experiment. In the ovary the decreasing trend has been found to be by 9%, 11%, and 6% w.r.t control on 30th day, 17%, 19% and 20% w.r.t control on 45th day and by 14%, 16% and 26% w.r.t control on 60th day.

- A decrease in cytochrome oxidase activity under the effect of monocrotophos has been observed in liver and ovaries of *Cyprinus carpio communis*. The enzymatic activity decrease by 38%, 29% and 22% w.r.t control on 30th day, 39%, 30% and 28% w.r.t control on 45th day and 38%, 29% and 41% w.r.t control on 60th day in ovaries of fish. Similarly in liver the enzyme activity decreased by 38%, 33% and 29% w.r.t control on 30th day, by 53%, 29% and 30% w.r.t control on 45th day and 53%, 31% and 36% w.r.t control on 60th day of exposure at 0.052 ppm, 0.074 ppm and 0.123 ppm of monocrotophos treatment.

- The alkaline phosphate enzyme analysis in liver showed a decrease by 1%, 5% and 18% w.r.t control on 30th day, 60%, 60% and 23% w.r.t control on 45th day and 92%, 77% and 48% w.r.t control on 60th day. In ovaries, the enzymatic activity has been found to decrease by 14%, 6% and 38% w.r.t control on 30th day 20%, 19% and 39% w.r.t control on 45th day and by 74%, 71% and 89% w.r.t control on 60th day on treatment with 0.052 ppm, 0.074 ppm and 0.123 ppm of monocrotophos exposure.

- The acetylcholinesterase activity, protein content, glycogen, cytochrome oxidase, alkaline phosphate exhibited descending trend in ovary as well in liver with increase in exposure period and increase in concentration of monocrotophos.
• There has been increase in the lipid peroxidation in both tissues e.g. liver and ovary. It means that there is a decrease in total lipids. The increase in lipid peroxidation in liver has been found to be by 64%, 50% and 93% w.r.t control on 30th day, 125%, 100% and 250% w.r.t control on 45th day and by 70%, 60% and 85% w.r.t control on 60th day of monocrotophos exposure. The increase in lipid peroxidation in ovary has been found to be by 110%, 42% and 42% w.r.t control on 30th day, 150%, 105% and 109% w.r.t control on 45th day and 160%, 160% and 120% w.r.t control on 60th day of monocrotophos exposure.

• The effect of administration of monocrotophos on lactate dehydrogenase activity in ovary and liver exhibit ascending trend. The LDH activity in ovary increased by 63%, 50% and 25% w.r.t control on 30th day, 84%, 97% and 57% w.r.t control on 45th day and 70%, 42% and 60% w.r.t control on 60th day. Similar ascending trend was observed in case of liver. The LDH activity in liver increased by 30%, 30% and 27% w.r.t control on 30th day, 33%, 25% and 8% w.r.t control on 45th day and 64%, 38% and 21% w.r.t control on 60th day of exposure at 0.052 ppm, 0.074 ppm and 0.123 ppm of monocrotophos concentration.

• The cholesterol level of the ovary in response to pesticide treatment has been found to the higher than those of their respective control. The increase in ovary has been observed to be by 31%, 32% and 38% w.r.t control on 30th day, 17%, 29%, and 31% w.r.t. control on 45th day and 18%, 20% and 18% w.r.t control on 60th day of treatment at 0.052 ppm, 0.074 ppm and 0.123 ppm of monocrotophos. The cholesterol in the liver increased 38%, 20%and 18% w.r.t control on 30th day, 35%, 20%and 15% w.r.t control on 45th day and 37%, 20%and 16% w.r.t on 60th day of monocrotophos exposure.

• The lactate dehydrogenase, lipid peroxidation and cholesterol level followed ascending trend on monocrotophos exposure in ovary as well in liver of treated fishes as compared to that of the control fishes.

➢ In seasonally breeding fishes, each spawning episode is preceded by a relatively long period of vitellogenesis during which oocytes increase in size
due to acquisition of yolk. The vitellogenin level in the blood plasma of the control and treated fishes is determined by the alkali labile phosphorus method which also confirmed the minimized yolk content in oocytes of fish as was depicted in histological and electron microscopic studies.

- The decrease in vitellogenin level is maximum on 60th day at 0.052 ppm of monocrotophos concentration. The decrease is also evident at other doses and exposure periods. The vitellogenin level in the fish decreased by 37%, 51% and 59% on 30th day, by 35%, 65%, 70% on 45th day and by 58%, 87% and 90% on 60th day of exposure at 0.123 ppm, 0.074 ppm and 0.052 ppm of monocrotophos concentrations respectively. This decreasing trend can be attributed to the lowered level of estradiol in the blood which stimulates hepatocytes to synthesize vitellogenin precursors.

> The functioning of the reproductive activity of the fish i.e. activity of gonads is regulated by gonadotropic hormones via production of steroid hormones. The effect at endocrine level cannot be ignored when pesticide is producing apparent aberrations at morphological, ultrasturctural, biochemical and vitellogenin level in the fish oocyte, Hence, the most important hormone related to the reproductive system fish i.e. estradiol is selected for the study and blood plasma of fish is processed by RIA.

- The 17β estradiol level decreased by large amount on 45th and 60th (3 pg/ml and 1 pg/ml) days of exposure as compared to the control fish. This can be due to the impairment in nerve conduction by monocrotophos as the information or stimulus from brain is required to synthesize the aromatize enzyme in ovary that converts testosterone to estradiol.

Hence it can be concluded that the reduction in enzymatic parameters, proteins, vitellogenin as described earlier and even hormones might be responsible for observed histological and electron microscopic atrophies. Gonadal changes are regulated by varied gonadotrophic hormones and atresia of follicles is due to lack of sufficient endogenous gonadotrophis as growth of follicle is principally dependent on level of gonadotrophin. There is strong evidence that pesticide effects the
functioning of steroid enzyme system in gonads of *Cyprinus carpio communis* (Kapur et al., 1978).

Interference with endocrine hormones affects reproduction, immune function, development and neurological functions in several species of animals. In fish, endocrine disruption interrupts normal reproduction to such an extent that it can cause male fish to have female characteristics. Endocrine disruption causes reduced fertility, lower hatching rate, lowered quality of oocytes and lesser viability of offspring. The present study showed direct relationship between concentrations of pesticides i.e. monocrotophos and depressed estradiol concentrations. This causes decreased production of vitellogenin as well as hampers the development of oocytes in ovary of *Cyprinus carpio communis*. Hence, the check has to be applied on the optimum usage of the pesticide in the agricultural fields especially in the vicinity of water bodies or the safe dose should be calculated so that the rest of the aquatic life is not adversely affected.