GENERAL INTRODUCTION

The advent of modern era of science and technology emanating from the broad spectacular capabilities within the domain of mankind. While miraculous technological success has brought enormous economic gain to man and comfort in his life style. His effort to eradicate the revage caused by the pests and crop diseases in the fields and surroundings date back to the discovery of agriculture. A large number of man-made chemicals of different categories mainly pesticides, fertilizers, food additives etc., are discovered. The increase of gross output of agricultural products is chiefly achieved by the elimination of loss of harvest to pests, plant diseases and weeds. Thus spectacular success of the green revolution is partly due to the application of fertilizers and pesticides during pre- and post-harvest operations. Pesticides are indespensible in modern agriculture to increase the food production by controlling agricultural pests, besides saving the man and his ecological partners from vector borne diseases. The twin objectives of the widespread use of pesticides gives rise to many serious health and environmental problems (Olzna-Marzyset et al., 1973; Nelson et al., 1978).

According to an estimate worked out about ten years ago, nearly 63,000 chemicals were in common use worldwide and it was commonly said that the World's chemical industry was marketing an estimated 200-1000 new synthetic chemicals each year. Many different kinds of pesticides are used in the World today. Kurinnyi and Pilinskaya (1974) estimated 800 pesticidal chemicals. Ridgeway et al., (1978) estimated 50,000 formulations from 1500 basic chemicals which Khilchevskaya (1980) put the former at 1,00,000 and the later at 900. More than 15,000
individual compounds and 35,000 formulations have come into use as pesticides since 1945 (Davies, 1987). India is one of the largest user of agricultural insecticides (Allen et al., 1984). In India, pesticide consumption rose from 2,000 tonnes in 1955 to 43,270 tonnes in 1980-81 and upto 59,000 tonnes in 1988-89. Annual loss of agricultural products due to pests is estimated to be of Rs. 6,000 crore/year (Gahukar, 1992). The food grains production during 1990-91 was 176 million tonnes in India and it is estimated that 220 million tonnes of food grains would be required by 2000 AD to feed its population (Bahl, 1992). This suggests the essential use of pesticides in our daily life. The pesticide act regulates the production, marketing, control and its utilization.

The credit for the discovery of the first modern synthetic pesticides goes to Paul Miller, when he discovered the insecticidal properties of dichloro-diphenyl-trichloro ethane (DDT) in 1939. Since then, there has been a rapid proliferation of both the inorganic and synthetic organic pesticides. Most of the pesticides, used daily are not selective, but in general are toxic to many non-target organisms including human beings. The mammalian endocrine system is very dynamic and undergoes frequent physiological fluctuations due to diurnal variations and cyclical hormonal feedback systems. Both hormonal modulations and chemical/drug perturbations can affect the reproductive systems in females and males. The endocrine system can be disrupted or modulated by many physiological events (e.g. exercise, menstruation, pregnancy etc.), phytoestrogens and xenoestrogens (e.g. chlorinated pesticides) can also affect the dynamics of the endocrine system. Several sites of action can be involved between a drug/chemical and the endocrine system including the central nervous system, specific target organs or sub-pollution of cells, hormone transporting proteins and xenobiotic metabolizing enzymes in the liver (Thomas, 1996).
In the studies conducted in South Asia, suicide is clearly the major cause of pesticides-related deaths (Jayarathnam et al., 1982). Whereas in Latin America occupational exposure is the major cause of pesticide poisonings (Paho, 1986). It is reported that in Sri Lanka 47.3 percent of the 30,490 hospital admissions for poisoning in 1986 were due to pesticides, 33.7 percent being by cholinesterase inhibitors alone (Forget, 1991). A case of gastro-intestinal poisoning by cupermethrin was reported by Poulos et al., (1982) resulting in nausea, prolonged vomiting, tenesnus, diarrhoea, unconsciousness, coma and death. Metcalf (1957) found that in California alone there had been 14,188 cases of accidental organophosphate poisoning. In Japan, there were about 6,000 cases of parathion poisoning during 1953-1959 (Namba and Hiraki, 1958). In the Indian states of Andra Pradesh, Tamil Nadu, Karnataka and Kerala during 1961-1964, 668, 3582, 1091 and 405 cases respectively were reported of organophosphate poisoning (ICAR, 1967).

Toxicological evaluation is aimed towards the human welfare and common laboratory animals have been used as the experimental models which respond to these chemicals. Though man may not form directly an experimental model, but has been subjected to sublethal longterm exposure with increased usage of pesticides and incidences of chronic diseases. Pesticides are by nature toxic to all living things and they not only pollute the environment but also cause harm to humans as well as beneficial organisms (Link et al., 1984). Pesticides are an important class of toxic chemicals to which man is extensively exposed through various means and are known to cause genotoxic effects in the exposed organisms (Jayashree et al., 1994).
The pesticides are classified on the basis of their biological activities into different groups, such as insecticides, herbicides, rodenticides and fungicides etc. On the basis of their chemical nature they are classified into inorganic (arsenols, mercurials, borates etc.), organic (organochlorines, organophosphates and carbamates) and insecticides of vegetative origin (Pyrethrum, rotenone, nicotine etc). Synthetic organic compounds are distinguished by their exceptionally broad spectrum of action and high activity (Gruzdyev et al., 1983). Thus, they predominate the other pesticides in the World market.

The commonly used pesticides are organochlorines, organophosphates and carbamates. The use of these pesticides accumulated, together with the metabolites in the abiotic and biotic environment, finally leads to the pernicious effect on the non-target animal population in the complex ecoweb and thus may be considered to imbalance the eco-populations and eco-energitics (Edwards, 1977) has posed potential health hazards (Matsumura and Ward, 1966; Matsumura et al., 1972). Exposure of human beings to pesticides may occur either accidentally or occupationally, where pesticides are manufactured, laboratories and application to vegetation (Nelson, 1990). In addition to occupational exposures, their entry in the form of residues in or on raw agricultural commodities, dermal exposure etc., are also reported (Hayes, 1961; Hayes and Laws, 1991).

Most of the pesticides used daily are toxic to many mammals. It is not only to invertebrates (Muley and Mane, 1995; Jadhav et al., 1995; Venkateshwarlu and Sunita, 1995), fishes (Bengeri et al., 1984; Jyothirmayee, 1997), amphibians (Mudgal and Patil, 1987), mammals (Saxena and Sarin, 1980; Shivanandappa and Krishnakumari, 1981; Morris
et al., 1996; Faundez et al., 1996; Liu and Pope, 1996; Barlas 1996; Sheets et al., 1997; Kackar et al., 1997), Live stock (Shivanandappa and Krishnakumari, 1983; Sandhu and Malik, 1988; Sitarska et al., 1995), birds (Garrison and Wyttenbach, 1985; Bennett et al., 1990; Elliott et al., 1996; Howe et al., 1996; Roperto and Galati, 1998) and even to human beings (Nelson, 1989; Jong, 1991; Peterson et al., 1992; Minelli and Ribeiro, 1996; Talaska et al., 1996).

Organochlorine pesticides were widely used in agriculture and material control programmes from 1940s to 1960s with dramatic beneficial effects, but have come into disfavour because of their persistence in the environment, wild life and humans. They have become infamous because of their tendency to accumulate in humans, animals, birds and the general environment. It causes derangement of mitochondrial function. Most of the chlorinated non-degradable pesticides tend to leave residues in living organisms for prolonged periods of their life span and are presumably responsible for a variety of known toxic symptoms (Mastumura et al., 1972). Some studies on organochlorine pesticides have been carried out in Brazil (Fernicola and Azevedo, 1982; Lara et al., 1987; Carvalho, 1991).

A number of experimental research work revealed the negative effect of the organochlorine pesticides on female and male reproductive systems (Sircar and Lahiri, 1990; Martinez and Swartz 1991, 1992). Persistent organochlorine compounds such as DDT and its metabolites, hexachloro benzene (HCB) and poly chlorinated biphenyls (PCBS) play an important role in chronic poisoning and take part in number of pathological processes (Lambowicz et al., 1991; Sitarska et al., 1991). Populations of bald eagles have declined during 1950s to 1970s in many parts of North America, largely
due to the reproductive effects of the dichlorodiphenyl trichloroethylene (DDT) metabolite, dichlorodiphenyl dichloroethane (DDE) (Weimeyer et al., 1993) in concern with loss of breeding habitat (Stalmaster, 1987). Spatial and temporal trends in chlorinated hydrocarbon levels and effects on reproduction are reported elsewhere (Elliott et al., 1996).

Organochlorine pesticides are widely recognised as neurotoxic substances, affecting the peripheral and central nervous system, respectively, and causing a hyperexcitability of nerves and muscles (Hassal, 1983). Flemming et al., (1994) found p,p'-DDE, a metabolite of DDT, in the majority of postmortem brain samples from neurological disease cases. Bhatia and Venkatasubramanian (1972) suggested that dieldrin treatment to rats increased liver weight and hepatic microsomes. Proteins and hepatic RNA were affected but DNA was unaffected. Those which are highly stable are shown to cause serious health and environmental hazards and violate the reproductive processes in rats and mice due to their estrogenic activity (Swartz and Mall, 1989; Martinez and Swartz, 1991).

Previous studies have shown that acute exposure of rats or mice or fishes to dieldrin, phenobarbital or endrin produces several changes in liver, including centrilobular hypertrophy, induction of hepatic cytochrome P450 and increased liver weight (Sastry and Sharma, 1978; Kolaja et al., 1996). Treatment with polychlorinated biphenyls (PCB), and DDT produced a broad range of effects on the histological structure of liver, kidney and thyroid (Kimbrough, 1985; Safe, 1994; Chu et al., 1994, 1995, 1996; Lecavalier et al., 1997). It has been reported that the study of testosterone enanthate on rats showed delayed estrous cycle in dose dependent manner (Sawada et al., 1996). The ovarian androgens and
inhibin secretion by follicles may be an important part in the regulation of FSH secretion and follicular dynamics (Evans et al., 1997). Ova viability and implantation are reduced by the chlordecone (Pinkston and Uphouse, 1988). Treatment with DDT, endosulfan, hexachlorohexane (HCH) and carboplatin induced testicular dysfunction in rats with marked testicular atrophy, reduced tubular size and spermatogenic arrest (Burlington and Linderman, 1950; Shivanandappa and Krishnakumari, 1983; Singh and Pandey, 1990; Fuse et al., 1996). When edifenphos, methyl parathion and mancozeb was administered to normal and hemicastrated rats, have shown to inhibits compensatory ovarian hypertrophy, affects estrous cycle and decrease in the number of healthy follicles with concomitant increase in the number of atretic follicles (Nanda, 1995; Math and Kaliwal, 1996; Math et al., 1997; Dhondup and Kaliwal, 1997; Asinathbanu and Kaliwal, 1997; Soratur and Kaliwal, 1999; Mahadevswami, 1999).
DICOFOL

Dicofol (Kelthane) [2, 2, 2-trichloro-1, 1-bis (4-chlorophenyl) ethanol] is a chlorinated hydrocarbon. It was introduced as a commercial chemical in 1955 containing 18.5% (W/W). It is manufactured from United Phosphorus Ltd., Bombay. It was obtained from the local company’s market and dissolved in olive oil. It was used mainly as an effective miticide for citrus fruits, nuts, apples, tea, cotton, beans garden and ornamental crop. Its use still appears to be widespread in many European, South African, Asian and African countries as well as the United States. It is structurally similar to DDT.

Structural formula of dicofol [2, 2, 2-trichloro-1,1-bis (4-chlorophenyl)-ethanol or 4, 4-dichloro-α (trichloromethyl) benzhydrol].

There are little published work on the specific toxic effects of dicofol in experimental animals. At high levels, the animals show a general weakness, coma and death. In rats and rabbits the acute oral LD₅₀ for technical grade dicofol seems to range from 575 to 2000 mg/kg (Smith
et al., 1959; Brown et al., 1969; Ben-Dyke et al., 1970). Rat fed with dicofol for up to 2 years showed no effects on survival at levels below 1000 ppm but growth was impaired (Smith et al., 1959). Females at the level of 250 ppm or higher, and males at 500 ppm and higher showed suppression of growth. The maximum tolerable dose for mice in a subchronic study was 500 ppm (Sato et al., 1987).

Some small adverse effects associated with reproduction in rats and mice have been reported (Brown, 1972; Trifonova and Gladenko, 1980). Some evidence has been obtained for its hepatocarcinogenicity in male B6C3F1 mice but not in rats (NCI, 1978). Histopathological changes are limited to the liver and kidneys and are relatively mild in nature.

Apparently, only one case of possible human poisoning by dicofol has been reported and this was in combination with trichlorfon (Zolotnikova and Somov, 1978). Green house workers suffered frequently from allergic dermatitis. A detailed study of the protection of workers in Florida citrus groves from contamination by dicofol has been reported (Nigg et al., 1986). Dicofol has cyto-kinetic and cytogenetic effects on human lymphoid cells in vitro (Sobti et al., 1983). Decrease in Hb and WBC count was noted by Gvineriya and Sindzharadze (1976) when rats were given kelthane through inhalation route.

Dicofol seems to be metabolized in rats to 4-4-dichlorobenzophenone, which is stored in fat and muscles as well as being excreted in the faeces (Brown et al., 1969). Water soluble metabolites have been detected in the urine of mice given $^3$H- and $^{14}$C-labeled dicofol. Nearly 50% of the administered doses was excreted in the urine.
within 24 hrs. Brown and Casida (1987) showed that in vivo mice convert dicofol to dichlorobenzophenone and dichlorobenzhydrol and that DDE originates from the impurity α-Cl-DDT.

Dicofol was extensively absorbed from the gastrointestinal tract. At near steady state conditions the highest tissue concentrations were found in adipose tissue, followed by the adrenal glands, thyroid and liver. Female rats tend to retain dicofol to a greater extent than males. Dicofol and DDT showed a similar pattern of distribution and elimination. Dicofol is more polar than DDT and therefore less persistent in the body. In rats, dicofol was excreted as polar metabolites, primarily in the faeces, but with lesser amounts in the urine. Dicofol has moderate acute oral toxicity. It produces signs of toxicity consistent with CNS depression. WHO has classified dicofol as slightly hazardous (Pesticide residues in food Report, 1992, 1994). Since, there are no reports on the ovarian follicular growth, estrous cycle, implantation, pregnancy and testes in dicofol treated rats. Therefore, the present investigation was undertaken to elucidate the effect of dicofol on estrous cycle, compensatory ovarian hypertrophy, the pattern of changes in the ovarian follicular population, implantation, pregnancy and testes in albino rats.