GENERAL CONSIDERATIONS

Pesticides have made great impact on human health, production and protection of food, fiber and other cash crop by controlling disease vector and keeping check on many species of unwanted insects and plants Agriculture plays a dominant role in Indian economy and more than 80% of our population is directly dependent on agriculture. India is one of the first countries, which started large-scale use of pesticides for controlling insect and pests According to estimation of International Development Research Center, every year around 10,000 people die and another 400,000 suffer from various effects of pesticides in developing countries India accounts for one third of the poisoning cases in the world **(Dhaliwal *et al.*, 1999).**

It has been reported that frequent and enormous use of synthetic pyrethroids/ organophosphate have posed resistance problem, resurgence of the pests and health hazards **(Mehrotra, 1990).** Workers involved in organophosphate spraying for a long period of 3-15 years showed significant pathophysiological symptoms **(Kamal *et al.*, 1990).**

The toxic effect of chemicals on the human health has become a major concern due to several factors. Firstly the number of hazardous chemicals are on the rise in the environment, secondly a relatively large number of individuals get exposed more directly because of occupational exposure in work place, through manufacturing, packaging, or handling. **Quershi (1994)** reported decrease in body weight, sperm concentration and semen fructose and increase in abnormal and dead sperms in intoxicated rats.

The use of multiple pesticides in agriculture is much more effective than using a single pesticide Therefore, use of combination pesticides is more beneficial and also economically cost effective to the farmers. Many combinations such as metalaxyl + mancozeb, quinalphos + cypermethrin, chlorpyriphos + cypermethrin, deltamethrin + triazophos etc. are available. In assessing the toxicity of combination, it is important to consider chemical and /or physical interaction of the individual chemical, the effect that one chemical may have on absorption metabolism and pharmacokinetic characteristics of another, and the possibility of interaction between parent compound and metabolites **(Sood, 1999).** Triazophos and deltamethrin are the examples of wide spectrum pesticides used in variety of agricultural and non-agricultural

application. In recent years, synthetic pyrethroids in combination with organophosphorous (monocrotopos, triazophos, profenophos, etc.) are used to control associate-sucking pests. These combination products are broad-spectrum insecticides and due to the improved biological performance and decreased costs of application, the uses of pesticide mixtures are common and their application is ever increasing. Application of more than one pesticide in a mixture simultaneously saves time, fuel costs and labour. However, Increased and injudicious use of insecticide posses a great risk to the environment and mankind.

The literature available suggest that the combination of organophosphate and synthetic pyrethroid may produce potentiation and synergistic effects. There are indications that potentiation of toxicity may occur when deltamethrin is combined with some organophosphorus compound **(Environmental Health criteria 97, 1990).** It has been reported that organophosphate accentuates the effect of deltamethrin **(Haines *et al.*, 2001).** Hence, the present investigation of the combination insecticide deltamethrin1% + triazophos 35% EC has been selected to study the adverse effects on reproductive system in laboratory animals (Rats – *Rattus norvegicus*).

With a new compound, the customary starting point is toxicological evaluation utilizing lethality as an index. The oral median lethal dose evaluated following a single acute exposure was 128.32 mg/kg body weight **(Chapter I).** From the available literature, LD_{50} values of deltamethrin technical reported is 139 to >5000 mg/kg body weights depending on vehicle/solvent **(Environmental Health criteria 97, 1990). Tomlin (1997)** reported LD_{50} value of deltamethrin at various concentrations (1080 mg/kg b.wt. for 15g/L EC; 535 mg/kg b.wt. for 25g/L EC). In the present study, LD_{50} value of deltamethrin (10g/L EC) found to be 128.32-mg/kg body weight is much lesser than above value suggests the increased toxicity of deltamethrin due triazophos an organophosphate insecticide. Severe toxic symptoms like lethargy, abdominal breathing, tremors, lacrimation, exophthalmos, excessive salivation, diarrhea and convulsion, were observed in the rats. Such manifestations are primarily due to organophosphorous poisoning as the signs and symptoms of poisoning are typically cholinergic.

Based on the results of the acute oral LD_{50} study four groups were selected. One group was treated as control, while other three groups were subjected to deltamethrin 1% + triazophos 35 % EC at the dose levels of 10, 20 and 30 mg/kg body weight. The dose volume administered was maintained within 10 ml/kg body weight. Male animals were dosed 70 days (spermatogenesis period) prior to mating, during mating and thereafter till sacrifice. Female animals were dosed 14 days prior to treatment (at least two cycles), during mating, throughout gestation and lactation period. Treated male and female rats from the same dose

group were allowed for cohabitation (1:1). The pregnant females were isolated and housed individually and observed further during gestation and lactation period.

In the present study, significant increase in cholesterol value was observed in both the male and female rats which might be due to chemical induced stress and increase in both the cortical and medullary activity of the adrenal as described by **Kaur *et al.*, (2000)** who observed similar findings in a sub-acute study in goat following chlorpyrifos exposure. Findings are also in agreement with significant elevation in cholesterol, which was reported, in lactating rat due to exposure of monocrotophos by **Adilaxamamma and Reddy (1995).** The Nephritic syndrome, which is the result of glomerular injury, is characterized by hypercholesteremia, and perhaps hyperadrenocorticism may also be associated with increased cholesterol concentration **(Hall, 1992; Godkar, 1994).** It was reported that the exposure of organophosphate results in increased serum cholesterol in rats **(Hanafy *et al.*, 1991).** Hypercholesteremia due to pesticides may play a role in the pathogenesis of arteriosclerosis **(Patelski, 1976).**

Deltamethrin 1% + triazophos 35% EC induced increase in blood urea nitrogen level in male and female rats. Hepatic dysfuction, parenchymatous damage to kidney and/or increased catabolism of protein are mainly responsible for elevation of blood urea nitrogen **(Finco, 1989).** Increased BUN is an indication of nephrotoxicty as reported with the exposure of organophosphate by **Adilaxamamma and Reddy, (1995).** Findings of present study are also in agreement with that of **Kumari *et al.*, (2002)** who observed significant increase in blood urea nitrogen following synthetic pyrethroid treatment in buffalo calves.

Significant elevation in serum alkaline phosphatase was observed in rats treated with deltamethrin 1% + triazophos 35% EC at doses of 20 and 30 mg/kg body weight. Alteration noted in the present study might be attributed to the hepatic and renal dysfunction. **Adilaxamamma and Reddy (1995)** observed elevated alkaline phosphatase activity in rats subjected to organophosphate insecticide. Increase of ALP is reported in buffalo calves treated with cypermethrin and deltamethrin **Kaur and Sandhu (2001).**

Rats exposed to deltamethrin 1% + triazophos 35% EC revealed significant increase in ALT and AST activity. The rise in serum levels of transaminases is related to the rate and extent of liver cell necrosis and commonly associated with liver disease. The elevation in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in blood has been used as indicator of hepatocellular damage **(Cornelius, 1987).** Significant increase in serum AST and ALT activity in present study could be possibly due to chemical injury to hepatic parenchyma and/or to other vital organs and reported due to treatment of deltamethrin and

other synthetic pyrethroids **(Mohhamad and Adam, 1990; Kaur and Sandhu, 2001; Kumari *et al.*, 2002).** Significant elevation in transaminases activity was reported with the exposure of organophosphate insecticide **(Adilaxamamma and Reddy, 1995; Kaur *et al.*, 2000).**

Significant increase in serum glucose concentration was observed following deltamethrin 1% + triazophos 35% EC in the current study could be related to the increase in secretion of catecholamines from adrenal medulla and/or increase in circulatory glucocorticoids as reported by **Kaur and Sandhu (2001).** Other investigations with synthetic pyrethroids are also supporting the present findings **(Ayub Shah and Gupta, 2001; Kumari *et al.*, 2002).** However, the results of present findings were not in agreement of **Adilaxamamma and Reddy, (1995),** who reported decrease in blood glucose in rats due to organophosphate (monocrotophos) intoxication. Which prompted one to speculate that the hyperglycemia observed in the rats subjected to the current combination pesticide could be a result of pyrethroid component.

The test article induced increase in total serum protein might be due to multiple myeloma as opined by **Kaur *et al.*, (2000).** In the present study, deltamethrin 1% + triazophos 35% EC at high dose caused significant increase in albumin level which was suggestive of toxic hepatitis or hepatic necrosis and thus the findings are in agreement with **Krishnappa *et al.*, (2000)** following the treatment of lambda-cyhalothrin.

Electrolytes are the most important substances, which influence the distribution and retention of body water. Sodium and potassium are the most important osmotically effective electrolytes. The combination pesticide administration leads to significant increase in sodium and potassium level at 30 mg/kg body weight and significant decrease in level of chloride in serum which affects osmotic pressure regulation and acid balance. Hypochloremia observed in the treated rats might be associated with gastrointestinal HCl losses as reported by **Godkar (1994). Krisnappa *et al.*, (2000)** also reported decreased chloride concentration along with no change in Na^+ and K^+ in rats treated with lambda-cyhalothrin.

A significant inhibition in serum cholinesterase activity was observed in the test article treated rats. Anticholinesterase agents act by inhibiting the activity of the cholinesterase enzyme **(Taylor, 1996)** and therefore cholinesterase enzyme is considered an important marker to assess the exposure of an individual to chemicals, especially those which are neurotoxicants. Numerous studies reported inhibition of cholinesterase due to triazophos and other organophosphate insecticides **(Sandhu and Bal, 1998; Kaur *et al.*, 1998; Kaur *et al.*, 2000; Verma *et al.*, 2002).** Inhibition of cholinesterase due to deltamethrin and

cypermethrin intoxication was also reported **(Vanketeshwarlu *et al.*, 1997; Kaur and Sandhu, 2001; Kumari *et al.* 2002).**

Evaluation of haematological changes in the laboratory animals exposed to environmental chemicals is important in the overall assessment of the risks and hazards of potential to human or animal exposure. Repeated dose of deltamethrin 1% + triazophos 35 % EC caused decrease in total erythrocyte count, hemoglobin content and haematocrit value in high dose group. Decrease in haemoglobin (Hb) and haematocrit (HCT) value might be correlated with a decrease in RBC count, which in turn might be due to the effect of the pesticides on blood forming organs suggesting anemia **(Rahman *et al.*, 1990).** Anemia may occur on the primary clinical feature or secondary toxicity (i.e. nephrotoxicity or hepatotoxicity). All causes of anemia including hemolysis, haemorrrhages, blood loss, and decreased red blood cells production have been associated with the chemical exposure. The test material causing lower body weight will also affect the erythrocyte mass **(Hall, 1992). Bhelonde and Ghosh (2004)** reported significant decrease in Hb with the exposure to synthetic pyrethroid fenpropathrin in rats. Decrease in RBC and Hb was also reported by **(Dhembare and Pondhe, 2000)** in fish following treatment of phosphamidon, monocrotophos (organoposphate) and fenevalerate (synthetic pyrethroid).

Rats exposed with deltamethrin 1% + triazophos 35 % EC, in current investigation, revealed dose related decrease in the values of MCH and marked decreased MCHC at high dose group Decrease in MCH and MCHC can be correlated with the decrease in the erythrocyte count and haemoglobin as reported by **Choudhary and Joshi (2002)** in endosulfan toxicity in rats. Similarly, decrease in MCH in rat was reported by **Bhelonde and Ghosh (2004)** following fenpropathrin intoxication.

Dose dependent increase in total leukocyte count (WBC) was observed in female rats treated with deltamethrin 1% + triazophos 35% EC suggesting induction of some pathology as reported in lindane treated birds **(Mandal *et al.*, 1986)** and/or also may be due to toxic effect on bone narrow **(Rahman, 1996).** Total leukocytes count increased during pregnancy. It rises soon after delivery and then gradually returns to normal condition.

Determination of platelet count and clotting time are important tests for the investigation of bleeding disorder. A dose dependent decrease in platelet count and increase in clotting time was observed in rats due to exposure of deltamethrin 1% + triazophos 35% EC. Decreased platelet production is most frequently associated with generelised suppression of hemopoiesis i.e. an aplastic anemia **(Weiss, 2000).** Decrease observed in platelet count might be due to poor clot retraction as suggested by **Godkar (1994)** whereas dose

dependent significant increase in clotting time observed in present study can correlated with the decrease in platelet count.

The treated animals revealed changes in liver such as esionophilic foci, focal necrosis, hypertrophic foci and haemorrhagic spots/area. Haemorrhagic sopts/area, were observed in present study might be due to rupture of blood vessels caused by test substance. **Bhelonde and Ghosh (2004)** had reported fatty changes and hydrophilic degeneration, sinusoids dilation and congestion and necrosis of hepatocytes in liver of rats due to treatment of fenpropathrin. Histopathological examination of mice treated with organophosphorous insecticide revealed congestion and degenerative changes of liver **(Revathi and Sunitha, 2000).**

Kidney showed nephropathic lesions such as dilation of renal pelvis, atrophy of renal papilla, transitional epithelial cell hyperplasia, and nephritis hyperplasia of pelvis. Treatment related hyperplacia in the transitional epithelium has been related to papillary necrosis, mineralisation or calculi formation and chemically exacerbated nephropathy **(Montgomery and Seely, 1990).** Focus of mononuclear cells (MNC) aggregate by the side of pelvis with normal transitional epithelial cell lining of pelvis, was recorded in the present study. Interstitial mononuclear cell infiltrates are also usually associated with nephropathy. Hypercholesterolemia and increase in urea nitrogen observed in present investigation may be correlated with the kidney damage **Bhelonde and Ghosh (2004)** reported tubular degeneration and necrosis in rat kidney following single exposure of fenpropathrin. Examination of mice treated with chlorpyriphos revealed hyperemia in kidney **(Revathi and Sunitha, 2000).**

In order to understand the effect of deltamethrin 1% + Triazophos 35% EC on the reproductive system, a single generation toxicity study was also envisaged**(Chapter 3)** wherein the treated male rats from high dose group exhibited clinical signs of salivation, tremor, lacrimation and nasal discharge. One male rat from the high dose group i.e. 30mg/kg body weight was found dead during 5th week of the treatment. The symptoms were cholinergic and were indicative of CNS involvement and form part of choreoathetosis salivation (CS) syndrome as described for alpha-cyano pyrethroid compound by **Shiva Kumar *et al.*,** (2002).

The exposure of deltamethrin 1% + triazophos 35% EC caused dose dependent reduction in body weight and reduced feed intake in rats. The reduction in the body weight could be attributed to the toxicant induced anorexia and the attendant reduction in feed consumption. Deltamethrin resulted in body weight reduction at high dose in a chronic study **(Hunter *et al.*,**

1977; Shaker *et al.* 1998). A three-generation study of deltamethrin in Charles River rats revealed decrease in body weight **(Wrenn *et al.*, 1980). Singh *et al.*, (2001)** suggested that malabsorption and hepatic dysfunction might be responsible for retardation in body weight gain in animals subjected to synthetic pyrethroids. Since pyrethroids was one of the combination toxicants used in the present study and the hepatic dysfunction, as evidenced by the altered transaminase activity (Chapter 2), was observed in the intoxicated rats a similar reason as opined by **Singh and coworkers (2001)** can not be ruled out.

Many xenobiotics are known to inhibit spermatogenic activity in testis of the animals including man **(Zenick *et al.*, 1994).** In the present experiment, rats treated with deltamethrin and triazophos 35 % EC revealed reduction in sperm motility, testicular sperm head count and epididymal sperm count at 30 mg/kg body weight. Impairment of sperm motility was reported due to retention of the cytoplasmic droplet during their transit from carpus to cauda epididymus **(Akbarsha *et al.*, 2000).** Generally, spermatozoa lose their cytoplasmic droplet during their transit from carpus to cauda epididymus. Loss of cytoplasmic droplets is considered as an index of spermatozoa maturation in mammals **(Bedford, 1975).** Decrease in sperm motility in Wistar rats treated with organophosphorus insecticide (dichlorvos) reported by **Okamura *et al.*, (2004).** The decrease in motility is suggestive of direct effect on developing spermatocytes/spermatids and epididymis or both **(Buttar *et al.*, *1997*). Knobil *et al.*, (1994)** suggested that the decline in sperm motility could be due to altered epididymal physiology.

Testicular homogenization resistant spermatids data are particularly useful for confirming impairment in sperm production. **Lanning, *et al.*, (2002)** suggested that a reduction in testicular homogenization resistant spermatids coincides with a decrease in epididymal sperm count. Results of the current study therefore suggest that the pesticide combination of deltamethrin1% + triazophos 35% EC is inhibiting the sperm production and motility either directly or indirectly. However, the exact mechanism through which this is achieved needs to be further evaluated.

Epididymal sperm morphology is useful to assess the effects of spermatotoxicants. Exposure of deltamethrin 1% + triazophos 35% EC revealed a progressive increase in various morphological abnormalities of sperm. These morphological changes might be considered due to the effect of mutagenic property of the xenobiotic as suggested by **Ron Filler, (1993).**

In the current study, histopathological observation in epididymis revealed lesions such as epithelial vacuolation, complete absence of spermatozoa, luman devoid of spermatozoa etc.

Microvacuolation of the epididymal epithelium can be treated as a specific chemically induced toxic manifestation **(Creasy, 2001).** Microvacuolation and cribriform changes is often seen accompanied by concentration of the atrophic aspermic epididymis. This may represent a normal mechanism of surface area reduction but has also been reported as a toxicological change **(Foley, 2001).** Fluid absorption and secretion are both major functions of the epididymal epithelium; vacuolation is a likely sequel to disturbance of either function **(Lanning *et al.*, 2002).**

Histopathological changes found in testis were focal and diffuse degenerative changes in seminiferous tubules with interstitial cell hyperplasia The most common degenerative change in rats' testis is loss of germinal epithelium with seminiferous tubules. These giant cells, immature germ cells, and cell debris are present in the duct of epididymis when there is testicular degeneration with marked generation and loss of spermatogonial epithelium, diameter of the tubules is often decreased, the wall is slightly thickened, at this stage when testis is grossly reduced in size and germ cells are rarely present and the term atrophy is often used to characterize these lesion **(Boorman *et al.*, 1990).** Hyperplasia is characterized by aggregates of interstitial cells between the seminiferous tubules Some imbalance or lack of negative feedback inhibition may allow pituitary stimulation of the interstitial cells which eventually results in incidences of these proliferative lesion **(Boorman *et al.*, 1990),**

On the other hand when it comes to the female reproductive system, successful pregnancy depends on two sets of physiological events. Transport of gametes through the reproductive tract (so that fertilization can be effected) and establishment of an appropriate hormonal environment (i.e. progesterone state) through cervical stimulation, so that the fertilized egg can implant in the uterus and be maintained during subsequent gestations **Chapin and Heindel (1993).**

Compound that disrupts ovarian cycle and thus the fertility potential could induce a pattern of constant vaginal estrus, repetitive pseudo-pregnancies, or anestrus condition. In the present investigation, during mating period regular vaginal smear examination for the presence of cervical plug or sperm, revealed disturbance in the estrus cycle. Disturbed estrous cycle might be due to disturbed hypothalamic pituitary - ovarian axis whereby both estrogen and progestrogen in combination interfere with pituitary gonadotropic function through reduction of FSH and suppression of LH peak as suggested by **Singh *et al.*, (1998).** Prolonged vaginal diestrus, is indication of interference of follicular development or depletion in the pool of primordial follicles. Irregular cycles may reflect impaired ovulation, as delayed ovulation may extend the period of vaginal cornification (**Cooper *et al.*, 1993).**

The treatment of deltamethrin and triazophos at 30 mg/kg bodyweight body weight revealed reduction in body weight and feed consumption in dam during gestation and lactation and has caused significant diminution in litter size, total litter weight, live birth index at high dose group

Significant reduction in average dam's weight and feed consumption may be probably due to malnutrition arising from indigestibility and malabsorption, while litter size, average pup weight and live birth indices may be reduced because of direct maternal toxicity. Treatment related reduction in the total litter weights was found to be significant at high dose group and was a result of reduced litter size.

Concomitant result obtained by **Singh and Sharma (1998)** in a segment two reproduction toxicity study using monocrotophos support the present observation of embryotoxicity due to combination pesticide. A three-generation reproduction toxicity study using deltamethrin in rats evoked slight but significant decrease in mean pups weight **(Wrenn *et. al.*, 1980). Chapin and Heindel (1993)** had suggested that the most satisfying demonstration of reproductive toxicity is decrease in pup number which supports the data observed in high dose group in the present study, while reduction in the litter size in treated female indicate a female reproductive toxicity probably induced due to altered estrous cyclicity and/or altered mating behavior.

The histopathological evaluation revealed alterations in ovary (angiectasis), uterus (endometrial glandular hyperplasia/luminal dilation) and Mammary gland (undeveloped). The changes observed in ovary and uterus was found to be inconsistent and hence, may not be treatment related. However, undeveloped mammary gland might be correlated with the disturbance observed in the estrous cycle of one female each from mid dose and high dose. It can thus be deduced that toxicant induced disturbed estrous cycle may lead to infertility in female rats.

The present investigation using one of the very successful combination pesticides against insect pests, the pyrethroids and organophosphorus, on rats revealed definite signs of potentiation of toxicity as evident from low median lethal dose value. Clinical symptoms shown by the acutely poisoned rats were a combination of exaggerated cholinergic stimulation (OP induced) and choreoathetosis salivation (Pyrethroid induced). Further the test article evoked alterations in the biochemical and haematological front. The overt among them were the alterations in transaminase and alkaline phosphatases activities, a certain indication of liver and kidney damage a fact that is further reaffirmed by the deranged histoarchitecture. The test article is also found to be affecting the maternal as well as

paternal reproductive system. The embryo toxic effect of the test substance was evident from the reduce litter size and mean litter weight. From the study it is apparent that these toxic signs and symptoms are a product of either synthetic pyrethroids or organophosphorus intoxication; however, what was striking was the severity of toxicity at such a low dose. Nevertheless, the finer mechanisms of actions of the combination pesticide on the biochemical and reproductive toxicological front are still at large. Studies on these lines are in progress as follow up