Summary and Conclusions
INTRODUCTION

The survival of man is dependent upon the existence of animal and plant life around him. From times immemorial, man has been making adjustments with environment, but his unremitting struggle against insect enemies is still continuing. Insect pests are said to be responsible for destruction and damage of over one third of world's crop.

Pesticides are defined as (I) any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (insect, rodent, nematode, fungus, weed and other forms of terrestrial or aquatic, plant or animal life or viruses, bacteria or other microorganisms in living men or other animals) which the administrator declares to be pest and/or (II) any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant.

The declining use of persistent organochlorine pesticides coupled with the increased resistance of insects to organophosphates insecticides have led to the increasing use of synthetic pyrethroids as alternatives. Since as compared to other classes of insecticides, pyrethroids are relatively unstable in the environment and possess low mammalian toxicity, they gained widespread use.

Synthetic pyrethroids have recently been shown to be potentially harmful due to their widespread use. They have now been reported to inhibit the activity of respiratory chain enzymes of mammalian mitochondria.

Fenvalerate is a synthetic pyrethroid. It is an ester of 2-(4-chlorophenyl)-3-methyl butyric acid and α-cyano-3 phenoxy benzyl alcohol. In the present dissertation, an attempt has been made to investigate various toxicity manifestation (particular toxicity to male reproductive system and male
mediated fertility parameters) resulting from inhalation exposure of rats with a formulated Fen preparation (20% E.C) using nose only dynamic inhalation equipment.

**SALIENT FINDINGS OF THE DISSERTATION:**

Clinical toxicity symptoms were noticed in formulated Fen (20% E.C) inhaled rats in a dose dependent manner. The generalised toxicity of Fen exposed rats were evident from significantly decreased body weight gain profile of rats exposed by 1/5th LC$_{50}$ of Fen for 2 and 3 months duration. This decrease in weight gain profile of rats slowly became less prominent with lower doses of Fen exposure and indicated dose dependence.

The male reproductive toxicity manifestations were evaluated by following spermatology and serum testosterone levels in groups of rats exposed to different doses (1/5th, 1/10th and 1/15th LC$_{50}$) of formulated Fen (20% E.C) by nose only inhalation for 70 days (one full spermatogenic cycle in rat). Testicular toxicity resulting from the inhalation exposure of rats with above insecticide was investigated by histopathological examination and concomitant assay of testicular marker enzymes (associated with different cell types of testes) in the group of rats exposed for 2 and 3 months duration with different doses of Fen.

Further, there was significant decrease in epididymal sperm counts and sperm motility alongwith serum testosterone concentration in group of rats exposed to 1/5th LC$_{50}$ of Fen by inhalation for 70 days (one full spermatogenic cycle). Only two testicular marker enzymes viz. 17-β HSD (testicular marker enzyme of leydig cell function) and G6PDH (marker enzyme for interstitial cells) were found to be significantly reduced in group of rats exposed to 1/5th LC$_{50}$ of Fen for 2 and 3 months.

The concomitant histopathological examination of testes from these Fen exposed rats showed good number of tubules and lesser number of
spermatogenic cells and lumens devoid of spermatids. These changes were more pronounced with the presence of marked lesions of interstitial spaces in testicular sections of rats exposed to Fen for 3 months as compared to 2 months exposure duration.

Testicular G6PDH has been reported to be essential for hydroxylation reaction during gonadal testosterone biosynthesis while 17-β HSD is an enzyme involved in the conversion of androstenedione to testosterone during testosterone biosynthesis.

These observations of the present studies hint towards alterations in testicular testosterone bio-synthesis in group of rats exposed to 1/5th LC_{50} of Fen by inhalation for 70 days.

Additionally, testosterone has also been reported to be essential for conversion of stage VII to VIII during spermatogenic cycle of rats. The significant decrease in the serum testosterone levels of rats exposed to 1/5th LC_{50} of Fen for 70 days may be responsible for significantly decreased sperm count and sperm motility in this group of rats.

Similarly, the results of inhalation exposure of rats to different doses of formulated Fen (20% E.C) on male mediated fertility parameters indicate significant decrease in mating index, fertility index and reduced litter size in a dose dependent manner. These observations are being reported for the first time with inhalation exposure of this insecticide in experimental animals and provide mechanistic aspects of male inefficiency resulting by Fen exposure.

Rats exposed to different doses of formulated Fen (20% E.C) by nose only inhalation for 2 and 3 months duration showed pulmonary toxicity also. There was significant increase in absolute and relative weight of lungs of rats exposed to 1/5th LC_{50} of Fen for 2 and 3 months. The biochemical studies and histopathological examination of lungs of rats exposed to different doses of Fen by inhalation were carried out. The results of present studies
indicate significant increase in the activities of enzymes which are considered as indicators of pulmonary inflammation and damage viz. LDH, γ-GT, ACP and ALP in lungs of rats exposed to different doses of Fen for 2 and 3 months in a dose dependent manner.

The histopathological studies of lungs from above Fen exposed rats showed hypertrophied and hyperplastic epithelial lining cells of bronchioles. Some bronchioles also showed desquamated bronchiolar epithelial cells lying loose in lumen. Mostly mononuclear cells admixed with a few polymorphs were evident in alveolar lumen and peribronchiolar and perivascular areas. Some alveolar lumens also showed the presence of eosinophilic edematous fluid. Epithelial lining cells of alveoli were also hypertrophied at places while parenchymatous blood vessels were congested. These histomorphological changes in lungs of Fen exposed rats were more pronounced for 3 months exposure duration as compared to 2 months exposure time period in a dose dependent manner. The above mentioned pulmonary toxic effects in rats exposed to formulated Fen (20% E.C) by inhalation are being reported for the first time in the present dissertation.

Some hepatotoxic changes were also noticed in histopathological examination of liver from rats exposed to different doses of formulated Fen (20% E.C) for 2 and 3 months duration. The histopathological examination of livers showed starting of early degenerative changes of the hepatocytes in centrolobular areas, less marked degenerative changes in midzonal area and periportal area was least affected. Hepatocytes were hypertrophied, showed finely granular cytoplasm and presence of small vacuoles. The nuclei of these cells were enlarged, mostly karyorrhexis and karyolytic nuclei. Blood vessels in the portal triad areas were congested.

These histopathological changes in the liver of rats exposed to Fen (20% E.C) by inhalation for 3 months were more pronounced as compared to the group of rats exposed for 2 months in a dose dependent manner.
During 2 months Fen post exposure period kidney sections showed the presence of a few atrophied glomeruli and hypertrophied tubular epithelial cells along with the presence of casts in tubular lumen. Two places in the interstitial areas had the localised presence of mononuclear cells and necrotic cellular debris.

The kidney lesions during the exposure period of 3 months inhalation of Fen revealed more pronounced degeneration of kidney viz. presence of many atrophied glomeruli along with more atrophied tubular epithelial cells. There were focal collections of mononuclear cells in some interstitial areas. These renal histomorphological changes in Fen exposed rats were found to be dose dependent.

CONCLUSIONS:

The male reproductive toxic effects arising from the inhalation exposure of formulated Fen (20% E.C) along with the role of testosterone in the mechanism of this phenomenon are being reported for the first time. Similarly, the effects of Fen inhalation induced toxic manifestations on male mediated fertility parameters evident as significantly decreased mating index, fertility index and litter size etc., are the novel findings of the present dissertation and were hitherto not known.

The pulmonary toxicity along with biochemical and histopathological evidences in Fen (20% E.C) inhalation exposed rats warrant adequate protective measures by human beings during spraying, handling and formulation of the formulated Fen (20% E.C). The present studies also indicate possibility of some hepato and renal toxic manifestations resulting from the inhalation exposure of formulated Fen (20% E.C) in rats.

The salient findings of present dissertation provide meaningful insight into formulated Fen (20% E.C) induced reproductive, pulmonary,
hepatic and renal toxicity in rats exposed by inhalation. These findings may provide sufficient information to health regulatory agencies in India and abroad to formulate guidelines for safe use of this insecticide by human beings.
References
VIII. REFERENCES


All India Medical Corp. (Undated) Data Sheet: Fenvalerate 20% E.C - Sumitox 20% E.C-Synthetic Pyrethroid Insecticide, Bombay, AIMCO pesticides.


