CHAPTER V

Summary and Conclusions
In pesticide toxicology, the toxicological studies is earlier always associated with one chemical at a time and the risk associated with exposure to that particular chemical. However, it is common knowledge that we are never exposed to one chemical at a time. Hence in the recent years, a number of combination products consisting of two or three components have been introduced for the effective control of pests, diseases and weeds of crops. Though considerable information is available on individual chemical components of a combination product, toxicological information on it is rather scarce. Therefore, the present investigation was aimed for evaluating the toxicity of a combination fungicide at genotoxic, cellular and morphophysiological levels in adult male rats.

Healthy, adult male albino rats (*Rattus norvegicus*) of Wistar strain was selected and maintained in climatically controlled environment. Dose level of 500 mg/kg body weight in distilled water was opted for daily dosing. This dose level was selected after determining LD$_{50}$ (5150 mg/kg body weight) of M + M and conducting a 28-day oral toxicity study with special emphasis on mortality, gravimetric data and histopathological findings of target organs. During experimental period, control animals were provided only with distilled water. Duration of the
treatment was 30, 60 and 90 days and for studying effects on reproduction, it was restricted to 60 days. Animals of recovery group animals were kept for a further period of 60 days to detect reversibility. The parameters studied were a battery of biochemical tests, blood cell counts, histopathology of tissues, spermiogram, fertility and genotoxic aspects of all experimental groups.

Significant alterations in body weight and kidney, liver and testis weights indicated that this combination fungicide affected general metabolism and growth of animals.

Red blood cell counts, haemoglobin content, haematocrit values and platelet counts were significantly reduced, while WBC count was significantly increased by this fungicide combination revealing its effect on formed elements of blood. Moreover, differential lymphocyte counts were also showed alterations affecting blood defensive functions. However, these effects were found to be reversible after 60 days of post-treatment in these animals. Clinical chemistry parameters were also affected in all experimental animals including withdrawal group manifesting persistent toxicity at milder level except for glucose, protein and cholesterol levels, which showed a recovery in this study, indicating its effect on liver and kidney.

In liver, altered biochemical profiles like protein, phosphatases (ACP and ALP) and glycogen were correlated with histological changes
generated by this combination in our study thus giving an importance, if these tests are done in early diagnosis of liver toxicity. The liver pathological changes exhibited focal sinusoidal distention, marked vacuolar changes and focal hyperaemia. These changes were still persistent partially in withdrawal groups. However, few parameters, enzymes like ALP and ACP showed a recovery but others were persistent revealing its toxicity. In the present report, treated animals also exhibited structural alterations in the kidney including diffused hyperaemia, glomerular retraction and tubular degeneration to certain extent even after withdrawal of M + M treatment like those of biochemical tests in it indicating that in a combination, these fungicides act as nephrotoxicants. These data support the altered biochemical profiles of the serum to demonstrate toxicity of these tissues. This administration of combination fungicide, further induced structural changes due to hormonal imbalances in thyroid gland in our study.

In testis, protein and hydroxysteroid dehydrogenase levels tend to decrease whereas cholesterol level increases thereby inhibiting androgen synthesis in it. These tissue biochemical alterations were consistent with our histological findings. All these effects manifested androgen antagonistic effect of this combination. Further, it is evident by a fall in circulating testosterone levels in the present study. The altered fertility parameters viz., sperm motility, sperm count and viability are probably contributory to a reduction in fertility and implantation loss in mated...
females of these treated rats thus inducing reproductive toxicity of this combination even after withdrawal of the treatment.

The percentage of micronucleated erythrocytes and chromosomal aberration was significantly higher in 90 days combination fungicide treated animals when compared with the control group reflecting on genotoxic potential of this combination due to the persistent nature of the chemical or its delayed effect or through its metabolites formed in the system. This effect is further demonstrated in human blood cultures (Appendix I).

In conclusion, this combination product manifested toxic effects on various parameters probably by binding with cellular components of tissues in treated animals and human blood cultures. Further, it is also obvious that these data would provide a scientific basis for assessing combination fungicide product safety and risk for the intended use in the field of agriculture and other areas of exposure.
Areas of Further Research and Development

Based on the work embodied in the present thesis, the following investigations could be carried out in future.

- Levels of residues in various tissues need to be investigated.
- Teratogenic potential of the compound mixture.
- Electron microscopic studies on various tissues are also to be carried out.
- NOAEL values should be established for data extrapolation to humans and to provide risk assessment information.
- Immunotoxicity will also be carried out to understand its effect on immune system.
- It would be desirable to study the ameliorative and prophylactic effects of both fat soluble and water soluble vitamins along with this fungicide combination.