CHAPTER VI
SUMMARY AND CONCLUSIONS
6.1 Summary

The present investigation was conducted on female albino mice *Mus musculus*. Inbred, healthy, swiss albino mice were reared in the laboratory. Two months old female mice of about 25-30 gms were used for the study. Animals were divided into four groups and each group was housed with four mice. They were fed with mice feed from Hindustan Lever and water was given *ad libitum*. The first group was considered as control. The second, third and fourth group of animals were exposed to endosulphan subcutaneously for a period of twenty one, sixty and ninety days. After expose they were sacrificed by decapitation method, that is on 22\textsuperscript{nd} day, 61\textsuperscript{st} day and 91\textsuperscript{st} day respectively. The tissues like brain, liver and kidney were dissected out and processed for various biochemical, histopathological and gas chromatographic studies. Daily live body weight, food intake and any observable symptoms were recorded.

Studies like estimation of total protein (Lowry et al., 1951), glycogen (Siefier et al., 1950), total lipid (Folch et al., 1957) acid and alkaline phosphate (Kind and King, 1959) total adenosine tri phosphate (Quinn and White, 1968) Succinic dehydrogenase (Beatty et al., 1966) lactic dehydrogenase (King, 1959) and acetyl cholinesterase (Ellmann, et al., 1961) were done on brain, liver and kidney of mice. Histopathological changes in the vital tissues(Varley,1980) and residue analysis by gas chromatographic (Abraronson, 1978) method were also done.

Daily body weight was taken to find out the effect of endosulphan on growth rate. Glycogen, total protein and total lipid were assayed to see the
effect of endosulphan on carbohydrate, protein and fat metabolism respectively. Acid and alkaline phosphates were estimated to cellular damage or depletion in liver brain and kidney. Total ATPase, succinic dehydrogenase and lactic dehydrogenase were estimated to see effect of endosulphan on energy metabolism. Acetyl cholinesterase activity were measured to understand the effect of insecticide in the control and coordination system if any. Histopathological changes and residue analysis studied to evaluate the extent of organ toxicity caused by endosulphan at the administered doses.

A progressive increase and decrease of body weight and food intake are noticed in control group and treated group respectively. Endosulphan caused pronounced dose and time dependent decrease in glycogen, lipid, protein, total adenoid tri phosphate and acetyl cholinesterase. But there is sharp increase in acid phosphatase and alkaline phosphatase. In the case of succinic dehydrogenase and lactic dehydrogenase there was an increase of enzyme up to 60 days and after that there was decreased.

The histopathological alterations were characterized as oedema, cell swelling, necrosis, proliferation and infiltration of inflammatory cell. Some of these are pre symptoms of inflammatory reactions and cancerous conditions.

Morphological changes like enlargement of cephalic region, loss of whiskers, red patches on the ear, expansion of extreme ends of the tail are noticed in the chronic conditions of exposure.

Residue analysis showed that maximum accumulation of endosulphan were on brain tissue followed by kidney and liver after chronic exposure. It can be concluded that the action of the toxicant brought overall changes in the
physical, chemical and histological architecture of the body of organisms. Extreme exposure leads to death.

6.1 Conclusion

Following conclusions could be drawn from the present observations/investigations.

1. In the present study it seems to be toxic for multiple systems in the mice *Mus muscules* at the doses administered.

2. At the levels used in this study, the Endosulphan caused decrease in body weight and food intake as compared to control group. Decrease in body weight and food intake in Endosulphan treated mice could be due to the effect of pesticide on gastrointestinal tract resulting in decreased appetite and absorption or might be an indication of direct toxicity or stressogenic activity of Endosulphan. The reduction in body weight and food intake also could be attributed to anaerobic properties of insecticide.

3. It is widely accepted fact that glycogen is an important macromolecule which comes first rescue to mice by providing energy from endowing stress caused by endosulphan. Since stress imposes an increased energy requirement. This is achieved through the break down of reserve source of glycogen as its easy availability for energy production. Decrease in glycogen content in various tissues of Mice *Mus musculus* might be an adaptation for the survival of animal in response to stress induced by endosulphan. It can also be suggested that the stress primary affect on endocrine glands stimulating them to releasing large amounts of hormones, some of which are active in carbohydrate metabolism. Break down of
glycogen pool also supplied the energy requirements for detoxification process as well as for the synthesis of conjugates like glucuronic acid which further help in the elimination of pesticide and its metabolites.

4. In light of observation it would be seem that endosulphan toxicity disrupts the protein metabolism also. To over come stress condition animals require high energy and this energy demand may have led to the utilization of these energy yielding compounds. It might also be due to mechanism of lipoprotein formation which will be used to repair damaged cells and tissue organelles. Increased gluconeogenesis is another reasons for the depletion of protein. The endosulphan toxicity stimulate proteolysis in tissues by activating protease enzyme. Protein depletion in tissues may constitute a physiological mechanism and play a role of compensatory mechanism under the stress induced by endosulphan to provide intermediates to Kreb cycle or to enhance osmolality of the body fluids during the pesticidal stress. Structural variations in aminoacids or disruption of active sites by endosulphan and its metabolites may be another reason for depletion of protein.

5. Results of lipid changes in various tissues of mice exposed to endosulphan exhibited a general decline in lipid content in response to increase in time. Increased gluconeogenesis, combination of endosulphan with fatty acids and toxicity induced by endosulphan to lipase binding enymes are chief reasons for the lipid depletion. The decline might be related to impaired food intake, increased energy cost of homestasis or detoxification during the stress.
The increase in ACP and ALP revealed that energy metabolism is greatly disrupted by endosulphan and energy metabolism is enhanced by substrate phosphorylation than oxidative phosphorylation. This may be the reason for increase in ACP and ALP. Along with this inflammatory and necrotic changes are also observed may also induce the increased production of ACP and ALP. It may also be due to cellular damage and non specific tissue irritation induced by endosulphan.

Dose and duration depended decrease in total ATPase have been found in the present investigation. It could be that endosulphan acting as an inhibitor of oxidative phosphorylation, prevents ATP forming mechanism, lowers significantly all respiratory indices in mitochondria and inhibition of electron transfer. Thus, inhibition of ATPase disrupts the coupling of oxidative phosphorylation resulting in reduced energy production.

The results of present study indicated that endosulphan possess the duel properties of an uncoupler of oxidative phosphorylation and an inhibitor of electron transport chain. Thus, inhibition of mitochondrial respiration was found to occur in parallel to the inhibition of enzyme activities of the respiratory electron transport chain of mitochondria. Suppression of both aerobic and anaerobic pathways causes the variation in LDH and SDH activities.

Present study revealed significant reduction in AchE activity in brain, liver and kidney. AchE is extremely important for many physiological tasks variation showed the relation with duration of exposure and concentration of endosulphan. The declination of overall biological activity is due to the effect of endosulphan on controlling system. AchE is an enzyme plays an important
role in neural and muscular activities. Any process which eliminates this
enzyme or inhibits its activity would cause declination in neural and muscular
control. So that decreased movement and paralysis occurs.

Extensive microscopic changes observed in brain, liver and kidney
indicated the cellular toxicity caused due to endosulphan on multiple systems
of animals. Some of the characters are pre symptoms of cancerous conditions.

Residue analysis showed maximum accumulation of endosulphan in
brain tissues. This shows high lipophilic affinity of endosulphan. It can be
concluded that the action of the toxicant endosulphan brought over all changes
in the physical, chemical and histological architecture of the body of
organisms.

Exposure to toxins is prevalent in our age of industrialization and the
entry of chemicals into the body produces deleterious effects in man and other
living beings. The injurious agents however they are introduced into the body,
will target the respective cells and damage them by preference. Thus in order
to meet basal energy requirement and for the adaptation to the extreme
situations under the pesticide stress probably might increase the metabolic rate
resulting in total disruption of physical chemical and biological architecture of
the body. Extreme exposure led to death.

6.2 Recommendations

1. Explicitly acknowledge the health effects/threats possed by current pattern
   of endosulphane use especially in Northern most part of Kerala.

2. Raise awareness of these problems and how they can be avoided through
   well funded, extensive education programmes.
3. Conduct research to further document the extent of Endosulphan related health problems. Further research is required to investigate carcinogenicity, mutagenicity, teratogenicity, reproductive, embryological and neurological disruptions of endosulphan.

4. Conduct coordinated research into current extent of Endosulphan poisoning in other regions of the world.