Summary & Conclusions
SUMMARY AND CONCLUSIONS

1. The present study has been aimed at understanding the toxic effects of a synthetic pyrethroid on a non-target animal. The freshwater field crab, *Oziotelphusa senex senex* which inhabits paddy fields and other freshwater ecosystems where pesticides are frequently used to eradicate insect pests, was selected as the experimental animal. The central nervous system (CNS) and pedipalpal muscle (PM) were selected as the experimental tissues.

2. The pyrethroid compound *Cypermethrin* was chosen as the toxicant, since it is commonly used to control the pests causing damage to crops and public health.

3. The toxicity of cypermethrin to crabs was evaluated in terms of LC$_{50}$/48 h by using probit method of Finney (1964). The mode of exposure was through ambient medium. The LC$_{50}$ value was found to be 2 ppm. The sublethal concentration was taken as the 1/4 of LC$_{50}$. All the experiments on behavioural, physiological and biochemical aspects were conducted at 3, 6, 12, 24 and 48 h of exposure to both sublethal and lethal concentrations of cypermethrin.

4. Behavioural observations following treatment with cypermethrin included restlessness, oozing of dark frothing fluid from the mouth, impaired locomotion leading to paralysis, poor response to mechanical manipulations etc. The severity of these symptoms was more under lethal exposures.
5. Studies on cholinergic system indicated suppression of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity levels in both CNS and PM under cypermethrin intoxication under both in vitro and in vivo exposures. In vivo inhibition of AChE and BuChE increased gradually in both the tissues at different time intervals viz., 3, 6, 12, 24 and 48 h under sublethal and lethal concentrations. Maximum inhibition was observed at 12 h and 24 h under lethal and sublethal concentrations respectively. The extent of inhibition was comparatively more in CNS than PM. Further, inhibition of AChE activity was far higher than that of BuChE activity.

6. In in vitro treatment also both the enzymes were inhibited in the CNS as well as PM in a concentration-dependent manner. Maximum inhibition was recorded at 200 μ moles in both the tissues and the inhibition was more in CNS than in PM, as in in vivo studies. On the contrary, the trend was quite reverse in case of ACh content, i.e., the ACh content was elevated. Maximum elevation was recorded at 12 h and 24 h under lethal and sublethal exposures respectively.

7. During later periods of exposure, there was a gradual recovery of AChE and BuChE activities, and ACh content near control levels under sublethal concentration by 48 h. However, the recovery was negligible under lethal concentration even at 48 h. This indicates that cypermethrin might be acting as a cholinesterase inhibitor like organophosphates and carbamates. Through its effects on the cholinesterase system, cypermethrin may disrupt synaptic transmission. Decreased levels of cholinesterase (AChE and BuChE) activities may lead to the lowering of general metabolism of the animal
during cypermethrin stress. Greater inhibition of cholinesterases in the CNS reiterates the important role of these enzymes in neural function.

8. The total and soluble protein contents also decreased in the CNS and PM of crabs exposed to cypermethrin, suggesting protein degradation or retarded protein synthesis. Maximum decrease was observed at 24 h exposure in both the tissues under sublethal and lethal concentrations of cypermethrin.

9. On the contrary, the free amino acid content increased significantly in both the tissues, indicating hydrolysis of proteins under cypermethrin toxicity. Maximum elevation was recorded at 12 h in both sublethal and lethal concentrations. Protein and FAA contents reverted to near control levels in both the tissues under sublethal concentration, but the recovery was partial under lethal concentration even at 48 h of exposure.

10. Protease (neutral) activity registered elevation in the CNS and PM indicating increased breakdown of proteins resulting in elevation of amino acid pool. This observation further confirmed that cypermethrin caused elevation of FAA levels.

11. Cypermethrin intoxication also caused elevation of aminotransferase (AIAT and AAT) activity in the CNS and PM both in vivo and in vitro, suggesting increased mobilization of alanine and aspartate in the form of pyruvate and oxalacetate respectively into the TCA cycle for energy oxidation. The elevation was maximum at 24 h in both the tissues.
12. Glutamate dehydrogenase (GDH) activity also registered an increase under sublethal and lethal concentrations of cypermethrin, suggesting depletion of glutamate in the CNS and PM.

13. The activities of protease, aminotransferases (AIAT and AAT) and GDH recovered to near control levels in both the tissues under sublethal concentration of cypermethrin by 48 h after exposure. However, the recovery was partial under lethal concentration even at 48 h of exposure.

14. In vitro and in vivo exposure to cypermethrin induced inhibition of ATPase (Na⁺-K⁺ and Mg²⁺) activity levels in the CNS and PM. This inhibition indicates a possible interference in energy metabolism, since ATPases play an important role in the energy transformations in biological systems. Further, it also indicates interference with the synthesis as well as hydrolysis of ATP. Pyrethroids have great affinity for ATPase system and interact with the enzyme molecules thereby inhibiting the ATPase activity (Prasada Rao et al., 1984). The reduction in ATPase activities suggests a general persistent derangement of mitochondrial activities under cypermethrin stress.

15. In contrast to ATPase activities the phosphatase (acidic and alkaline) activities were found to increase upon cypermethrin exposure in the CNS and PM of crabs. Since the phosphatases are lysosomal enzymes, the cellular damage is usually accompanied by an increase in the activity of these enzymes. Elevation of phosphatase activity helps in the liberation of energy by breaking down the phosphate, which may be an adaptive mechanism by the animal to counteract the toxic effects of cypermethrin.
16. In the present study, it was evident that cypermethrin affects the cholinergic system, protein metabolism, ATPase and phosphatase activities both in vivo and in vitro. But the effect was more in vitro than in vivo, and was also more pronounced under lethal concentration than sublethal concentration. So it may be said that the effect of cypermethrin is concentration dependent, whether it is added directly to the tissue homogenate or through ambient exposure. Further, it was also noticed that cypermethrin effect was more on CNS than on PM, suggesting the differential tissue susceptibility to cypermethrin stress.

17. All parameters studied in the present experiment recovered to near control levels in both CNS and PM by 48 h of exposure under sublethal concentration eventhough marked alterations were observed in the cholinergic system, protein metabolism, ATPases and phosphatases. This speaks about the efficiency of detoxification mechanisms operating in the crab to counteract the toxic effects of cypermethrin. This capacity of crabs to recover from the pesticide effects indicates the degradable nature of cypermethrin in living systems. But the detoxification mechanisms seem to be less efficient under lethal concentration.

18. In view of the above observations, it may be concluded that cypermethrin may not cause severe permanent damage to tissues unless it is applied at higher concentrations. Because of its rapid degradation, cypermethrin does not accumulate in biological systems. Hence, if it is prudently employed in agricultural sectors, cypermethrin may not cause irreversible damage to non-target animals like crabs, fish, prawns etc.