VI. SUMMARY

Acute oral toxicity, Repeated dose 28-day oral toxicity, Dermal toxicity and Immunotoxicity studies were conducted in Wistar albino rats. Acute eye irritation and Skin sensitization studies were conducted in New Zealand White rabbits and guinea pigs, respectively. *In vitro* pharmacological efficacy studies were conducted against different stage of *Rhipicephalus sanguineus* ticks by Immersion test method. *In vivo* acaricidal efficacy studies were conducted in dogs, cattle and sheep. All the studies were conducted according to standard guidelines and methods.

Acute oral toxicity study was conducted in both male and female rats separately. The LD₅₀ values of technical grade cypermethrin and amitraz pesticide combination in male and female rats were found to be 224.13 and 218.73 mg/kg body weight respectively.

Animals in Acute oral toxicity study showed the clinical symptoms of depression, profuse salivation, tremors, hyperexcitability, burrowing behaviours, hunched back, startle response, muscular incoordination, weakness, altered gait and convulsions, writhing movement of neck and tail, violent twisting movements sometimes lifted the body from floor in severely affected animals. Choreathetosis became continuous and the righting reflex was gradually lost. At the terminal stage animals showed laboured breathing, gasping and mortality. This was indicative of CNS involvement particularly affecting the motor activities.

Repeated dose 28-day oral toxicity study was conducted in five groups each consisting of 10 male and another five groups each consisting of 10 female Wistar albino rats. The doses administered orally were saline (control), vehicle, 30+30, 60+60 and 120+120 mg/kg body weight of cypermethrin and amitraz pesticide combination at equal concentrations for a period of 28 days.

There was no significant change in feed consumption observed in both the sexes, but there was significant decrease in body weight gain in high dose
and mid dose group in both male and female rats during treatment period, but
the satellite high dose group animals showed recovery in body weight gain
after the administration of pesticide combination was stopped. There was
significant decrease in TEC, Hb and Hct in high dose group during the
treatment period indicating adverse effect on hemopoietic system.

Significant increase in AST, ALT, ALP, BUN, creatinine and glucose
was noticed in mid dose and high dose groups of both male and female rats
during the study period indicating mild hepatotoxicity and nephrotoxicity.

Satellite high dose groups of both male and female animals showed
normal recovery in both haematological and biochemical parameters.

There was a significant decrease in liver, spleen and kidney weights in
mid dose and high dose groups of both male and female animals. There was
also a significant decrease in organ to body weight ratio of liver and kidneys in
mid dose and high dose groups of both the sexes.

Histopathologically liver, kidney, lungs, heart, spleen and brain did not
show any significant change. Satellite high dose group animals did not show
any histopathological changes when compared to satellite control group
animals.

Dermal toxicity study was conducted in five groups of male and five
groups of female Wistar albino rats consisting of 10 animals each by applying
the pesticide combination at a concentration of 1, 2 and 4 ml/ltr of water
along with saline and vehicle control as per EPA guidelines. Satellite high
dose group for both the sexes were maintained. Haematological parameters
such as TEC, Hb, Hct, MCV, MCH, MCHC, TLC and DLC and biochemical
parameters such as AST, ALT, ALP, BUN, creatinine, glucose, TSP and
albumin did not show any significant changes. Histopathology of organs such
as liver, kidney, spleen, heart, lungs and brain showed normal appearance.
Satellite high dose group did not show any significant changes in
haematological, biochemical and histopathological parameters compared
satellite control group animals.
Immunotoxicity study was conducted in 10 groups of 10 male and 10 female Wister albino rats separately. One group was maintained as control in both sexes and other groups were administered vehicle, 30+30, 60+60 and 120+120 mg/kg body weight of cypermethrin and amitraz pesticide combination orally daily for a period of 28 days. Sheep RBC antigen was administered intraperitonially to all the group of both sexes on Day 14 and 20. There was a significant decrease in body weight, TSP, albumin concentrations and haemagglutination titre values indicating humoral immune supression.

Acute eye irritation study was conducted in four groups of New Zealand White rabbits consisting of four rabbits in each by applying 0.1 ml of the doses. The pesticide combination containing amitraz (1%) and cypermethrin (1%) at doses employed in the study are zero (control), 1, 2 and 4 ml/ltr of distilled water with one control group. The ocular grading was done as per standard grading system. Acute eye irritation study in rabbits indicated lacrimation and congested conjunctiva as adverse effects on mammalian eyes at mid and high dose.

Skin sensitization study was carried out in guinea pigs following Buehler test method. Four groups of guinea pigs consisting of four animals in each were maintained in the study. The doses considered for the study were 1, 2 and 4 ml/ltr of containing cypermethrin (1%) and amitraz (1%) combination pesticide with saline and vehicle control groups. The skin and systemic reaction were observed and recorded according to Magnusson / Kiligman grading system. In the Group IV, at four ml/ltr of water of the pesticide combination concentration there was slight patchy erythema which was transient and disappeared in three guinea pigs.

In vitro pharmacological acaricidal efficacy was conducted against different stages of *Rhipicephalus sanguineus* ticks by Immersion test method. The concentrations of the pesticide combination containing cypermethrin (1%) and amitraz (1%) employed to assess the efficacy was 0, 0.25, 0.50, 1.0 and 2.0 ml/ltr of water for both the test methods. 2.0 ml/litre of water pesticide combination was found to be highly effective in all the stages such as eggs,
larvae, adult male, unengorged female and engorged female. *R. sanguineus* ticks. The combination pesticide at 1.0 ml/ltr concentration was also found to be effective to some extent.

The pesticide combination containing cypermethrin (1%) and amitraz (1%) was assessed for the pharmacological acaricidal efficacy of 20 naturally infected dogs, cattle and sheep. For dogs, cattle and sheep the pesticide combination at 3 ml/ ltr of water was used as a single application.

The pesticide combination was found to be highly efficacious against *R. sanguineus* and *Ctenocephalides canis* within 24 hours after spraying. In cattle and sheep also The pesticide combination was highly efficacious against cattle ticks *Bhooophilus microplus* and sheep ticks *Hyalomma anatolicum anatolicum*.

There were no adverse reactions or clinical symptoms in pesticide combination treated animals. No side effects were observed on the treated animals. There were no alterations in haematology and serum biochemical parameters of dogs, cattle and sheep indicating pesticide combination was safe and efficacious against ticks of domestic and pet animals.