II. REVIEW OF LITERATURE

The toxicological features, systemic effects, safety and efficacy of Amitraz and Cypermethrin have been reviewed and presented in this chapter.

2.1 Amitraz

Formamidines form a class of insecticide-acaricide having the characteristic nitrogen structure -N=CH-N. They are very effective against eggs and very young caterpillars as well as against most stages of mites and ticks. Formamidines such as amitraz, chlordimeform and formetanate are valuable for the control of insects resistant to OPs and carbamates.

Amitraz is a main member of the Formamidines group of insecticide, which acts as octopamine receptor sites in ectoparasites resulting in neuronal hyperexcitability and death (Nathanson, 1985). It is available as a spray or dip for use against mites, lice and ticks in domestic livestock (Taylor, 2000). In cattle, for example amitraz has been widely used in dips, sprays or pour-on formulations for the control of single host and multihost tick species. In dipping baths, it can be stabilized by the addition of calcium hydroxide (Stanford et al., 1981) and maintained by standard replenishment methods for routine tick control. An alternative method has been the use of total replenishment formulations whereby the dip bath is replenished with the full concentration of amitraz at weekly intervals. Amitraz has also been shown to have an expellent action against attached ticks (Baker et al., 1973; Harrison and Palmer, 1981). It has been used against mange in pigs and psoroptic mange in sheep (Curtis, 1985).

In small animals, amitraz is available for topical application for the treatment and control of ticks and for canine demodicosis (Demodex canis) (Farmer and Seawright, 1980) and sarcoptic mange (Sarcoptes scabei) (Curtis, 1999). Amitraz is contraindicated in horses (Auer et al., 1984) and in pregnant or nursing bitches and cats, although it has been used at a reduced concentration to treat feline demodicosis (Cowman and Campbell, 1988). Amitraz is also formulated in collars for tick control in dogs.

Amitraz is a formamidine acaricide and insecticide used on top fruit, cotton and hops, and as a veterinary medicine for the treatment of ectoparasites in pigs, cattle, sheep, goats and dogs. It may be applied topically, as a spray, dip or pour-on.

Amitraz interacts with octopamine receptors in the central nervous system of ectoparasites, inducing increased neuronal activity, abnormal
behaviour, detachment and death. Clinical effects in mammals are due to its $\alpha_2$ adrenoceptor agonist activity.

Amitraz is rapidly and well absorbed after oral administration and eliminated from most of the tissues within few days. Amitraz is rapidly metabolized and excreted, mainly formamidine and 2, 4, dimethyl formanilide. These metabolites still contain the 2, 4 dimethylaniline (2,4DMA) moiety. The end product is 4-amino-3-methylbenzoic acid which is rapidly conjugated and excreted. This metabolic pattern is qualitatively similar in the rat, mouse, cat, dog, boboon, cow and human. In urine of these species 4-amino-3methylbenzoic acid (free and conjugated form) is most predominant (>70%). Other metabolites like N-(2,4dimethylpheny1) N-methyl1 formamidine and 2,4, dimethyl formanilide constitute not more than 10% each.

In an adequate set of in vitro and in vivo mutagenicity tests, covering different genotoxic endpoints, amitraz and its metabolities were regarded as non-genotoxic. In carcinogenicity studies with mice and rats amitraz was regarded as non-carcinogenic.

Besides the acaricidal and insecticidal effects, amitraz has a quite complex pharmacological activity in mammals. In various species of animals, the common signs of acute intoxications are depression of the central nervous system (CNS) and episodes of hyperexcitability, hypotension, bradycardia, hypothermia, hyperglycemia and in some species, alterations in water balance, evidenced by hemoconcentration (Bonsall and Turnbull, 1983).

The signs described above could be related to the capacity of formamidines to inhibit monoamine oxidase (Aziz and Knowles, 1973; Benezet and Knowles, 1976), block the synthesis of prostaglandin E$_2$ (Yim et al., 1978), have a local anesthetic effect (Chinn et al, 1977) and, what appears to be the principal mechanism of action, stimulate $\alpha_2$-adrenergic receptors (Costa and Murphy, 1987).

2.1.1 Experimentation on Rats

2.1.1.1 Acute toxicity
Single doses of amitraz at doses of 50, 100 and 250 mg/kg P.O in or i.p., P.O., i.m. or i.p was non-toxic at 50 mg/kg, toxic at 100 mg/kg and lethal at 250 mg/kg within 24 hours of dosing. Features of toxicity were depression, incoordination of movement, paresis of the limbs, hepatonephrotoxicity, muscular hemorrhage i.p. injection. These changes were accompanied by leucopenia were correlated with alterations in serum AST and amitraz did not inhibit serum Ach activity.

2.1.1.2 Effect on functional behavior

Florio et al. (1989) reported behavioural effects of acute oral administration of amitraz at 60 and 100 mg/kg (N= 10 per group) decreased the rearing frequency of rats in an open field and increased duration of immobility. Acute oral administration of amitraz (20,60 or 100 mg/kg, N=10 per group) increased the convulsive threshold dose of strychnine, picrotoxin and pentyletrazol, in rats.

Moser et al. (1991) used a functional observational battery to assess the effects of 3-day exposure to the pesticide amitraz in different strains of rat at a dose of 50 and 100 mg/kg p.o. Autonomic measures and indicators of general health were affected by amitraz in all rats. The most prominent sign was reddish discharge around the eyes and nose, which dried to a pronounced crusty layer. Moderate lacrimation or chromadacryorrhea, was observed in all strains beginning at 52 hr. inability of pupil to constrict in response to light and ptosis, or drooping eyelids, was recorded in all treated rats. Moderate effects were noted on the measures of neuromuscular function. Gait alterations described as tiptoe walking and a hunched posture was observed. Study reported increased reactivity to manipulation, tenseness and irritability, aggression, depressed arousal and rearing, hypothermia, body weight loss, autonomic effects such as ptosis, chromadacryorrhea, loss of pupil reflex and altered gait. One possible explanation for the increased sensitivity to being handled or touched, irritability, and the self-directed attacks may be due to the presence of paresthesias or sensory irritation.

Moser (1991) investigated amitraz neurotoxicity in adult male Long-Evans rats administered either vehicle or 10, 25, 50, 100 or 200 mg/kg amitraz i.p. and they were tested with Functional observational battery (FOB) immediately before design, at one, four hour, one day, two, four and eight days after dosing. Higher doses (100-200 mg/kg) produced increased reactivity to manipulation, tenseness, and aggression. Most of all doses produced depressed arousal and rearing activity, hypothermia, body weight loss and autonomic changes including ptosis, chromadacryorrhea resulting in facial crustiness, loss of the pupil reflex and decreased defecation. Effects of lower doses (10-50 mg/kg) were reversible by two to four days after treatment.

In investigating the antinociceptive effect of amitraz in rats by comparing it with the effect obtained with xylazine and detomidine, two α2-adrenergic agonists utilized largely in veterinary medicine, Queiroz-Neto et al. (1994) concluded that the effect of amitraz is comparable in potency to that of the traditional α2-adrenergic agonists, being also blocked by idazoxam, an α2-adrenergic blocker.
The effect of prenatal amitraz exposure on physical and behavioral parameters in rats was studied by Palermo Neto et al. (1994). Pregnant rats were orally gavaged with amitraz (20 mg/kg) or with distilled water (1.0 ml/kg) on Day 1, 4, 7, 10, 13, 16 and 19 of pregnancy. Groups prenatally exposed to amitraz showed decreased age of vaginal opening, earlier fur development and a delay in incisor eruption compared to control group. The present findings indicate that prenatal exposure to amitraz caused transient developmental and behavioural changes in the exposed rat offspring.

2.1.1.3 Effect on skin

Effects of amitraz given by different routes on rats was studied by Al-Qarawi et al. (1999). No effects were observed in three month old male Wistar rats sprayed with 250, 500, 1000 and 2000 ppm.

2.1.1.4 Effect on development and behavior

Prenatal exposure to amitraz caused transient developmental and behavioural changes in exposed rat offspring (Palermo Neto et al., 1994) when pregnant rats were orally gavaged with amitraz (20 mg/kg) on Days 1, 4, 7, 10, 13, 16 and 19 of pregnancy. After birth cross fostering was performed thus generating variations in groups. Delay in Vaginal opening, for development, incisor eruption, pinna detachment, eye and ear opening, testes descent, locomoter activity was observed.

Postnatal exposure to amitraz caused transient developmental and behavioral changes in the exposed offspring in a study conducted by Palermo-Neto et al. (1997) in wistar rats, whose lactating dams received the pesticide (10 mg/kg) orally on days 1, 4, 7, 10, 13, 16 and 19 of lactation period. The results showed that the median effective time (FT50) for fur development, eye opening, testis descent ad onset of the startle response were increased in rats postnataaly exposed to amitraz (2.7, 15.1, 21.6 and 15.3 days respectively) compared to those of the control pups (1.8, 14.0, 19.9 and 12.9 days, respectively).

2.1.1.5 Effect on insulin secretion

Smith et al. (1990) reported that the inhibition of insulin release in rats is mediated through the stimulation of α2 adrenergic receptors.

2.1.2 Experimentation on Mice

2.1.2.1 Acute toxicity study
Moser and MacPhail (1985) reported in mice that an i.p. dose of 600 mg/kg of amitraz the cumulative mortality was 80 per cent with in one week no death was observed in the first 48 hr. following amitraz administration.

2.1.2.2 Effect on biochemical parameters

Forty adult male swine mice exposed to tap water containing 0, 40, 80 or 16 ppm amitraz for 12 weeks showed adverse effects on the fertility and reproductive system (Al-Thani et al., 2003). Average body weights gains, fluid consumption, fertility, number of females impregnated, number of viable fetuses, absolute testis weight, weight of epididymis and preputial gland, testicular sperm counts, daily sperm production and epididymal sperm counts were decreased in males received higher doses. The seminal vesicles weight and number of resorption was increased in male mice and females impregnated with the exposed males.

A study was conducted by Filazi et al. (2003) to assess the effect of amitraz on the biochemical parameters in mice. Mice were given 15 or 45 mg/kg body weight of amitraz orally, diluted in dimethylsulphoxide (DMSO). Amitraz caused no effect on serum glucose concentration but increased urea, phosphorous, aspartate transaminase and alanine amino transferase concentrations in the group given 45 mg/kg. A decrease in creatinine and alkaline phosphatase concentration were observed in all groups. No difference was observed in serum calcium and bilirubin concentrations.

2.1.2.3 Effect on functional behavior

Florio et al. (1989) reported behavioral effects in mice administered with amitraz at the doses of 20, 60 and 100 mg/kg i.p. (N=10 animals per group). These was increase in sleeping time in a dose-dependent manner to 96±26, 120±29 and 198±58 min respectively when compared to 45±15 min for control mice indicating that amitraz produces a depressant effects on the central nervous system.

In investigating the antinociceptive effect of amitraz in mice by comparing it with the effect obtained with xylazine and detomidine, two α2-adrenergic agonists utilized largely in veterinary medicine, Queiroz-Neto et al. (1994) concluded that the effect of amitraz is comparable in potency to that of the traditional α2-adrenergic agonists, being also blocked by idazoxan, an α2-adrenergic blocker.

2.1.2.4 Effect on fertility

The effect of amitraz on fertility of male mice was studied by Al-Thani et al. (2002). The fertility was reduced in male mice ingesting 10 or 20 mg/kg/day amitraz. The number of females impregnated by them was reduced. An increase in the total number of resorptions was observed in females impregnated with the exposed males. Absolute testis weight was decreased at 10 mg/kg concentration. Testicular sperm counts and daily sperm production were decreased in males that ingested 10 or 20 mg/kg/day
amitraz. Epididymal sperm counts were decreased in exposed males at 10 or 20 mg/kg amitraz. Exposure to amitraz pesticide caused an adverse effect on the fertility and reproductive system of male mice.

2.1.3 Experimentation on Guinea pigs

2.1.3.1 Effect on hematological and biochemical parameters

The effect of the acaricide amitraz as a 0.1 per cent solution in acetone on the haematology and biochemistry of guinea pigs was investigated by Riberio et al (1999). One group received one ml amitraz solution divided into two doses (0.5 ml in the morning and 0.5 ml in the afternoon) for five days and were examined after 15 days another group received the same treatment every 15 days and were examined after 137 days. Amitraz-treated guinea pigs became anaemic, which was more pronounced after 137 days. Leukocytosis developed and the eosinophil count increased after 15 days and dropped after 137 days. After a slight initial increase the lymphocyte count dropped after 137 days, indicating immunosuppression. Plasma protein and fibrinogen concentration increased, resulting in hyperproteinaemia.

2.1.3.2 Effect on cardiovascular system

Amitraz caused a dose dependent bradycardia and hypotension in pentobarbitone anaesthetized guinea pigs (Pascoe and Reynoldson, 1986). Depression of blood pressure reached a plateau with a dose of 10 mg/kg but heart rate continued to fall in a dose-dependent manner, unto a fall of 90 beats per minute after a total of 160 mg/kg/min.

2.1.3.3 Effect on gastrointestinal tract

The effect of amitraz on in vitro intestinal contractions in the transmurally stimulated guinea pig ileum was studied by Hsu et al. (1987). Amitraz (10^{-7} to 10^{-6} M) produced a dose dependent inhibition of these transmurally-stimulated contractions. This effect of amitraz was blocked and reversed by idazoxan (10^{-6} M), an \( \alpha_2 \) adrenoceptor antagonist, thus amitraz decreased intestinal contraction by activating the \( \alpha_2 \) adrenoceptors in the myentric (Auerbach’s) plexus thus inhibiting the parasympathetic tone.

2.1.4 Experimentation on Dogs

2.1.4.1 Efficacy studies

Amitraz topically applied as an 0.025 or 0.05 per cent aqueous suspension once or twice weekly up to eight weeks cured three cases of generalized squamopapular to particular demodectin mange and effectively controlled the condition in fourth dog (Farmer and Seawright, 1980). A single wash in 0.025 per cent amitraz suspension was found to be sufficient to cure each of five dogs affected with localised demodecosis within two to four weeks. At the dose levels employed the drug was nontoxic and nonirritant to skin and mucous membranes.
Folz et al. (1983) reported that multiple treatments with liquid concentration of 250 ppm amitraz (Mitaban Upjohn) were highly efficacious and safe for treatment of generalized demodicosis without any ocular dermatological or other clinical side effects. When 42 dogs (26 treated and 16 controls) were given three to six treatments topically at 14 day intervals. All (100%) the dogs responded clinically and the mean rate of improvement at four weeks of treatment was 99.1 per cent.

A liquid concentrate formulation of amitraz was diluted and applied as a spongeon therapy as a new treatment for naturally acquired canine scabies in 40 dogs (20 treated and 20 controls) by Folz et al. (1984b). Ninty four per cent of the dogs treated were cleared of mites and returned to normality with a single topical treatment. The miticide was found to be well tolerated by all dogs and there was no evidence of dermal or ocular irritation.

Amitraz liquid concentration of 250 ppm active drug in water applied topically at 14 day intervals in 1721 canines showing localized or generalized demodicosis by 25 veterinary investigators showed approximately safe and efficacious response in 99 per cent of the treated canines thus indicating that amitraz is efficacious and safe for treating canine demodicosis (Folz et al., 1984a).

A liquid concentrate formulation of amitraz (Mitaban Upjohn) was used to topically treat 181 dogs with scabies at an active drug level of 250 ppm of 10.6 ml of concentrate in 2 gallon water by Folz et al. (1984). All treated dogs were clinically improved and 97.8 per cent of dogs got cured, after a single treatment three dogs required two treatments and one dog three treatments. Otodectes cynotis and cheyletiella yasguni in several dogs were also cleared after one treatment. Mild transient sedation occurred in 12.4 per cent of treated dogs with transient vomiting, increased appetite and diarrhea in less than one per cent of treated dogs.

Davis (1985) reported that in 27 clinical cases of canine demodecosis, three of four applications of a wash containing 0.025 per cent amitraz together with antimicrobial and antipruritic therapy were sufficient to effect clinical cure in 25 out of 26 cases mildly to severally affected. One case very severally affected, nine weekly applications together with microbial and antipruritic therapy effected clinical and parasitological cure.

Folz et al. (1985) evaluated the bioactivity and safety of a liquid concentrate formulation of amitraz at a concentration of 250 ppm active drug with or without the addition of a nonionic detergent in 20 dogs naturally acquired generalized demodicosis at 14 days intervals. The liquid concentrate with or without detergent was equally effective and safe as a dermotherapy for demodicosis and the addition of nonionic detergent grossly improved the wetting characteristics of the treatment mixture however it did not alter the biological activity or the safety of the therapy.

Brown dog tick (*Rhipicephalus sanguineus*) and American dog tick (*Dermacentor variabilis*) populations were eliminated and repelled for up to four weeks post treatment with a topical formulation of amitraz applied at 250 ppm active drug as a single treatment to dogs (Folz et al., 1986).
Amitraz for treatment of demodicosis in dogs was studied by Yathiraj et al. (1990). Amitraz liquid concentrate was highly effective and safe in treating 72 cases of both localized and generalized demodicosis in dogs. The drug was applied topically once a week and number of treatments ranged from 3 to 10 for return of cases to clinical normalcy and clearing of mites. The commonly observed side effects were mild sedation and pruritus and these were transient and disappeared without any treatment.

Thirty five dogs infested with *R. sanguineus* ticks and *Ctenocephalides felis orientis* fleas were employed to study the efficacy of amitraz in controlling the ectoparasites by Yathiraj et al. (1992). Ectodex emulsified concentration containing 5.0 amitraz was used in the recommended concentration (250 ppm/litre of water). From this study, it was concluded that single application of amitraz emulsified concentrate was very effective in treating the dogs infested with ticks and fleas. No side effects were observed in the cases or in the personnel’s involved in bathing the dogs.

In a clinical trial conducted by Madleau and Willemse (1995) to assess the efficacy of 0.125 per cent amitraz solution over half the body applied once a day, altering the body half treated in 50 dogs resulted in 25 dogs (61%) success treatment with the median duration of treatment of 6.5 weeks and the median interval from completion of treatment to least post treatment evaluation was 3.4 years. 16 dogs (39%) relapsed were cured after a second course of daily amitraz treatment. Also they reported that in two independent studies, 0.125% amitraz solution applied half the body daily was found 73% effective in curing dogs with demodicosis previously refractory to biweekly or weekly amitraz treatments. Thirteen of the 16 cases that resolved did so after one course of treatment which ranged from four wks to five months in duration. The other three cases initially relapsed but were cured after they were retreated. All cases deemed cured including those relapsed initially were followed for at least one year after their last treatment.

Estrade-Pena and Ascher (1999) did comparison of an amitraz impregnated collar with topical administration of fipronil for the prevention of experimental and natural infestations by brown dog tick (*Rhipicephalus sanguineus*) in dogs. Fipronil and amitraz were acaricidal and inhibited attachment and feeding. Amitraz had greater effect than fipronil on number of live, feeding ticks, egg hatchability and larval viability, indicating partial ability to interrupt the tick life cycle. In field conditions amitraz remained effective over the entire observation period and had stronger and more sustained effects against tick infestation than fipronil.

Amitraz (9%) impregnated collars prevented transmission of *Borrelia burgdorferi* by *Ixodes scapularis* in eight specific pathogen free Beagles dogs (Elfassy et al., 2001) in a laboratory trial. Serum ELISA and Western blot assays confirmed seronegative and absence of antibodies respectively throughout the study in treated dogs compared to control animals thus suggesting amitraz impregnated collars as a useful management tool for the prevention of borreliosis in dogs.
Hugnet et al. (2001) conducted a clinical study to assess the efficacy of 1.25% amitraz solution applied weekly in the treatment of eight dogs with generalized demodicosis and five dogs with sarcoptic mange associated with antidote treatment atipamazole 0.1 mg kg\(^{-1}\) i.m. once and Yohimbine 0.1 mg kg\(^{-1}\) once daily for three days orally. Skin scrapings were used to determine the therapy and the median number of treatments for demodicosis was three (range 2-5) and for sarcoptic mange it was two (range 1-3). No failure or relapses occurred at 6-36 months after treatment.

In a prospective study conducted by Estrada-Pena and Reme (2005) in 72 dogs fitted with test collars impregnated with amitraz (9%), amitraz (9%) and priproxyfen (PPF) or only excipients against experimental tick infestations by Rhipicephalus ranguineus, Ixodes ricinus and Ixodes scapularis revealed collars impregnated with amitraz were efficient in preventing tick infestations in dogs but did not inhibit ovi position in few surviving female ticks. Incorporation of PPF into amitraz impregnated collar resulted in impairment of reproductive ability of ticks.

2.1.4.2 Effect on biochemical parameters

When 3.78 L (containing 2.1g) of amitraz (twice the recommended concentration) was applied to five dogs four hours before glucose (0.6 g/kg of body wt.) administration i.v. The plasma glucose concentration was increased, but the increase in plasma insulin concentration, which usually follows i.v. administered glucose, was suppressed. Amitraz induced hyperglycemia at least partly by inhibiting insulin release (Hsu and Schaffer, 1988).

2.1.4.3 Effect on central nervous system

Seawright (1982) reported that the side effects of amitraz included central nervous system depression, ataxia and vomiting, while more severe toxic signs were hypothermia, bradycardia, hyperventillation and tachycardia. Amitraz is contra indicated in horse, cat an Chihuahua dog due to its undesirable effects. Fatalities have been reported and were more in common in small breeds.

Amitraz produces a characteristic syndrome of hyperactivity, irritability and decreased motor activity as well as numerous other behavioural and physiological effects as observed by Moser et al. (1987).

Andrade and Sakate (2003) conducted a study to compare the efficacy of yohimbine with atipamazole a new \(\alpha_2\) adrenergic antagonist to treat amitraz intoxication in three groups of thirty dogs with Group A receiving 2.5% amitraz iv at 1 mg/kg, Group A receiving 2.5% amitraz iv at 1 mg/kg followed 30 min later by 0.2 mg/kg atipamazole iv. Sedation, loss of reflexes, hypothermia, bradycardia, hypotension, bradypnea and mydriasis were observed in Group A with 3\(^{rd}\) eyelid prolapse, increased diuresis and vomiting in some animals whereas Yohimbine reversed all alterations induced by amitraz but induced cardio respiratory effects. Atipamazole was a useful antagonist for amitraz with
less cardio-respiratory effects suggesting its potential role as an alternative treatment of amitraz intoxication in dogs.

2.1.4.4 Effect on cardiovascular system

Cullen and Reynoldson (1988) reported that the blood pressure and heart rate were recorded continuously in methoxyflurane-anaesthetized dogs. Amitraz (i.v.) caused a dose-dependent rise in blood pressure with a fall in heart rate at lower doses. Pressor responses to amitraz were reduced by phentolamine and slightly enhanced by atropine and hexamethonium, while bradycardia was reduced by phentolamine, atropine and hexamethonium. Amitraz reduced the responses to acetylcholine and enhanced pressor responses to tyramine, while reducing bradycardia. Pressor response to DMPP were reduced, but there was little effect on responses to noradrenaline, isoprenaline or histamine. Amitraz exerted cardiovascular effects by stimulating $\alpha_2$ adrenoceptors and had some similarities to clonidine and xylazine in action.

Amitraz administered through intravenous route in anaesthetized dogs had shown to increase the mean arterial pressure, decreased heart rate and depressed tidal volume, respiratory rate and respiratory minute volume (transiently) immediately after injection (Cullen and Reynoldson, 1987).

Cullen and Reynolds (1990) studied the cardiovascular and respiratory effects of the acaricide, amitraz in thiopentone/ methoxyflurane anaesthetized dogs. Amitraz at dose rates of 1, 2, and 5 mg/kg i.v. dissolved in DMSO increased blood pressure for one hour with decreased heart rate initially and depression of tidal volume, respiratory rate and respiratory minute volume with hyperventilation as a feature. Cumulative doses of amitraz at doses of 0.5, 1.0, 2.0, 5.0 and 10.0 mg/kg i.v. at intervals of five minutes increased blood pressure, decreased heart rate at lower doses and increased at higher doses. Cardiac index was normal while total peripheral resistance showed a dose related increase suggesting an alpha 2-adrenoceptor agonistic action of amitraz.

2.1.4.5 Effect on immune system

The clinical and immunological evaluation of 10 dogs affected with generalized demodicosis was reported by Barboza et al (2000). The animals were teated with amitraz at 250 ppm (0.025%). Before treatment, haematological values were found to be in normal range in most of the patients, some of them were found to have neutrophilia with a regenerative left shift, eosinophilia and monocytosis. With the amitraz treatment, some cases of neutrophilia and monocytosis were observed. The lymphocytes of patients stimulated with mitogens such as phytohaemagglutinin (PHA) and concanavalin A (Con A) with the lymphoblastic transformation test (LTT), showed low proliferation.

2.1.4.6 Effect on insulin secretion
The effect of amitraz on plasma concentrations of glucose and insulin was studied in dogs by Hsu and Schaffer (1988) and observed that, at least in part, the hyperglycemic effect could have been due to its capacity to inhibit insulin release.

Hugnet et al. (1996) reported rapid hyperglycemia and no change in insulin concentration in dogs given 100 mg/kg amitraz. Administration of atipamazole at doses of 50, 100, 200 µg/kg b.wt. i.m. enhanced insulin secretion, resulting in a decrease in blood glucose concentration.

2.1.5 Experimentation on Cats

2.1.5.1 Efficacy study

Amitraz was effective in the treatment of generalized demodicosis in a cat according to a case study by Cowman and Campbell (1988). Generalized demodicosis was diagnosed in a 14 year old castrated male domestic short hair cat with no underlying disease. The cat responded incompletely or poorly to commonly recommended treatment but responded well to total body dipping with 0.0125% amitraz at weekly intervals.

2.1.5.2 Effect on behavior

Gunaratnam et al. (1983) reported that at 0.0125 per cent concentration, cats showed no behavioural changes but food intake was reduced and semi-fluid malodorous faeces were passed within three hours of bathing. At 0.025 per cent concentration, cats appeared sedated, food and fluid intake were reduced for 24 hours but returned to normal at 48 hours. At 0.05 percent concentration, changes were similar but more severe and accompanied by moderate weight loss. No haematological changes were recorded. The mild, transient toxicity produced even at comparatively low dose levels render amitraz unsuitable for use as an acaricidal bath in the cat.

2.1.6 Experimentation on Goats

2.1.6.1 Efficacy studies

Angora goats heavily infested with *Chorioptes bovis* were dipped one time in either 0.05% amitraz, 0.27% coumaphos, 0.05% fenvalerate or 0.03% lindane (Wright et al., 1988). Control of the mites by single dips were evaluated for 21 days. Amitraz had caused 98% mortality of the mites initially. Both coumaphos and lindane had caused greater than 85 per cent mortality at three days, but mite numbers increased rapidly. Only fenvalerate had killed all the mites.

Brugger and Braum (2000) reported that in four year old Toggenburg goat with demodicosis characterized by multifocal nodules containing thick yellow exudates resulted in a marked decrease in the number of skin nodules after the goat was clipped and treated topically every five to seven days for
a total of 12 treatments with a 1:100 dilution of amitraz (Ectodex, Hoechst Roussel Vet). However, new nodules appeared after the treatment was discontinued and complete clinical cure was not achieved.

2.1.7 Experimentation on Cattle

2.1.7.1 Efficacy studies

Trials conducted by Curtis (1985) had indicated about the elimination of mixed infection of chorioptes spp, Psoroptes spp, and sarcopter spp, in cattle using spray applications of amitraz where in some cases organochlorine, organophosphorus and organotin compounds had failed. In a pilot study heavy infestation of chorioptic mange was controlled on a calf using a pour-on formulation of amitraz.

Rapid detachment of all tick instars form animal treated with the application of an aqueous emulsion of amitraz (Bovitraz, Bayer Alt) according to manufacturers recommendations was noticed by Mekonen (2001) when applied on eight cross bred heifer calves aged between six to eight months and heavily infested with ticks. Amitraz was hand sprayed on animals and ticks were counted and identified in sites. 100% tick control was achieved on Day three after acaricide application and this was maintained for a further 18 days and the residual effects protected the animals from re-infestation for 21 days.

2.1.7.2 Effect on central nervous system

Seawright (1982) reported that the treatment with amitraz involves whose body application, the possibility of percutaneous absorption of amitraz may lead to intoxication in calves under hot humid conditions, the main effect being tranquillization which may lead to recumbency.

2.1.8 Experimentation on Buffaloes

2.1.8.1 Effect on haematological parameters

The effect of topical application of amitraz (0.6%) daily once for 14 days on mange affected buffalo calves was studied by Shanth Kumar and Suryanarayana (1995). There was a decrease in TEC, PCV and haemoglobin but there was increased TLC in mange affected calves, while there was gradual increase in TEC, PCV and Hb in treated calves.

2.1.9 Experimentation on Horses

2.1.9.1 Effect on biochemical parameters
Quetroz et al. (2002) reported an increase in BUN value in horses given intravenous injection of amitraz. The mean value for urea was ly more from the mean value obtained for the control group.

2.1.9.2 Effect on cardiovascular system and gastrointestinal tract

Amitraz induced severe stasis of the gastrointestinal tract and major side effects from higher blood levels included CNS depression and stupor in horses (Seawright, 1982).

2.1.9.3 Effect on locomotor activity

Harkins et al. (1997) by studying the sedative effect of the some drugs in a behavior stall, observed that amitraz as well as xylazine and detomidine caused a diminution of the spontaneous locomotor activity of horses, characterizing their sedative effect. Furthermore in this case, the effect of amitraz was also reversed by the administration of an $\alpha_2$-adrenergic blocker yohimbine.

Queiroz-Neto et al. (1998) in considering the potential therapeutic use of amitraz as an $\alpha_2$-adrenergic agonist investigated the sedative and antinociceptive effect of this drug after an i.v. administration at doses of 0.05, 0.10 and 0.15 mg/kg in English Thoroughbred (ETB) horses. The results indicated that amitraz showed a more potent and long-lasting sedative effect in horses when compared to xylazine. The antinociceptive effect, determined by the increase in the skin twitch reflex latency, was observed only at the highest dose of amitraz, indicating that the principal action of this drug is the sedative effect.

Queiroz-Neto et al. (2000) compared the effects of amitraz (0.1mg/kg IV) and xylazine (1 mg/kg IV) on physiological variables in horses. Amitraz caused a reduction in cardiac activity, respiratory frequency and bowel movements, but these effects were not as intense as those caused by xylazine. Amitraz still caused a relaxation of the smooth musculature of the rectum and an apparent increase in sweating and frequency of chewing hay. Rectal temperature was not influenced by amitraz. It was concluded that amitraz, after acute drug administration and in that dose did not cause relevant side effects in horses.

2.1.10 In vitro studies
Maske et al (1994) reported that in vitro studies on efficacy of amitraz against *B. microplus* ticks of cattle showed 100% mortality of adult males in 30 hours after exposure to amitraz at dilution of 0.05% and 75% and 55% mortality at 0.03% and 0.01% concentrations, respectively. Engorged females treated with 0.05%, 0.03% and 0.01% dilutions of amitraz were immobile at 36 h, 48h and 60 h, respectively. Treated females did not lay eggs.

DiFilippo et al. (2000) proposed that amitraz might have an anti-inflammatory effect. These authors examined the possible anti-inflammatory action of this drug in cultured C3H mouse spleen cells stimulated with concanavalin A or lipopolysaccharide.

The role of cytochrome P<sub>450</sub> enzyme induction in hepato toxicity of cypermethrin insecticide was investigated by incubating 100, 200, 400 and 800 ng/ml different concentrations of cypermethrin with a primary culture of rat hepatocytes (El-Tawil and Abdel-Rahman, 2001). The cytotoxic effect measured by decrease in cell viability and leakage of ALT and AST enzymes into culture medium was found at 200, 400 and 800 ng/ml.

### 2.2 Pyrethroids

Pyrethroids are synthetic compounds, the chemical structure of which is patterned after pyrethrins (extracted from the chrysanthemum flower), a mixture of six esters named cinerin I and II, jasmolin I and II and pyrethrin I and II. The synthethic pyrethroids mimick the broad efficacy of the botanical, but, as they contain only one of these esters, insect species tend to develop resistance to them. Compared with the botanical pyrethrum, which contains all six ester properties, no resistance has yet been observed as it becomes very difficult for insects to develop the requisite combination of alternative pathways to these six properties.

Pyrethroids show low mammalian toxicity, but like pyrethrum, they are highly toxic to fish and bees. They are anoxic nerve poisons.

Pyrethrum and the older synthetic pyrethroids such as allethrin, bioallethrin, bioreesmethrin, phenothrin, resmethrin and tetramethrin are very sensitive to sunlight, because their molecules split under ultra-violet light. Therefore, they are not suited for agricultural use, but some of them have good volatility and are very effective against indoor insect pests. For
household use they are formulated as aerosols together with piperonyl butoxide for synergistic action.

From the early 1970s, pyrethroids such as deltamethrin, fenvalerate and permethrin have been produced which have better photo-stability with low volatility.

The latest group of synthetic pyrethroids such as bifenthrin, fenpropathrin, cyfluthrin, flucythrinate, cyhalothrin, fluvalinate, cypermethrin and tetrathrin are photo-stable as well as extremely toxic to insects. These new pyrethroids are not mixed with synergists.

2.2.1 Synthetic Pyrethroids

Ever since the chemical structure of insecticidal components of pyrethrin extracts were elucidated by LaFoge et al. (1936), a number of workers had studied analogous esters of chrysanthemic acid i.e., the synthetic pyrethroids. Gammon et al. (1981) suggested that synthetic pyrethroids were esters of specific acids (e.g., Chrysanthemic acid, halo-substituted chrysanthemic acid, 2-(4-chlorophenyl) 3-methylbutyric acid and alcohol (e.g., allethrolone, 3-phenoxy benzyl alcohol).

Pyrethroids generally have a broad spectrum of activity and high potency when applied topically to insects. They have a low oral and intraperitoneal toxicity to mammals even though some pyrethroids have shown high intrinsic mammalian toxicity after intravenous and intracerebral routes of administration (Gammon et al., 1981).

2.2.1.1 Classification of Pyrethroids

Pyrethroids have been classified into Type-I and Type-II, based on electrophysiological studies with peripheral nerve preparations of frogs. A similar distinction between these two classes of pyrethroids was made on the basis of toxicity signs in mammals and insects (Van Den Bercken et al., 1979, Verschoyle and Aldridge, 1980).

Based on the binding assay on the gamma-aminobutyric acid (GABA) receptor-ionophore complex, synthetic pyrethroids could also be classified into two types the α-cyano-3-phenoxy benzyl pyrethroids and the non-cyano pyrethroids (Gammon et al., 1982; Gammon and Casida, 1983).

2.2.1.1.1 Type-I Pyrethroids
Type-I pyrethroids are the pyrethroids that do not contain an α-cyano group and produce Tremor-syndrome (T-syndrome) in mammals. These pyrethroids gave rise to pronounced repetitive activity in the sensory organs and in sensory nerve fiber (Van Den Brecken et al., 1973). These compounds also induced pronounced repetitive firing of the presynaptic motor terminal in the neuromuscular junction (Van Den Bercken, 1977).

Verschoyl and Aldridge, (1980) reported that the Type-I poisoning syndrome also known as T-syndrome (Tremor syndrome) which was characterized by symptoms such as elevated straitle response, whole body tremors and prostration in the rat. Gammon et al. (1982) reported restlessness, incoordination, prostration and paralysis in insects. Vijveberg and Van Den Bercken, (1982) suggested that Type-I pyrethroids primarily affect the Na⁺ channels in the nerve membrane and caused prolongation of the transient increase in the Na⁺ permeability of the membrane during excitation.

2.2.1.2 Type-II Pyrethroids

Vijveberg and Van Den Bercken, (1979) reported that the Type-II pyrethroids are pyrethroids with an α-cyano group on the 3-phenoxybenzyralcohol and which produced Choreoathetosis salivation syndrome (CS-Syndrome) in mammals. The pyrethorids with an α-cyano group caused an intense repetitive activity on the lateral line organs in the form of long-lasting trains of impulses and also caused frequency dependent depression of the nervous impulse due to depolarization of the nerve membrane as a result of the summation of depolarizing after potentials during train stimulation.

Type-II symptoms produced by Type-II pyrethroids include in-coordination, convulsion and intense hyperactivity. In rats displaying burrowing behaviour, coarse tremors, tonic seizures, sinus writhing and profuse salivation without producing lacrimation and hence termed as Choreoathetosis salivation syndrome (CS-syndrome). Some of the examples of the Type-II pyrethroids are cypermethrin, deltamethrin, fenvalerate, cyhalothrin and lambda cyhalothrin.

2.2.2 Experimentation on Rats

2.2.2.1 Acute oral toxicity

Coombs et al. (1976) and Dewar and Owen (1979) reported that the factors which increased the oral LD₅₀ value of cypermethrin included concentration, vehicle, temperature, age and the strain of animals used. Coombs et al. (1976) also reported the acute oral toxicity of cypermethrin was ranging from 400 mg/kg in aqueous solution in rats.

Iyaniwura and Okonkwo (2004) determined the effects of acute cypermethrin toxicity in rats and its dose response characteristics. The intraperitoneal LD₅₀ of cypermethrin from the study was 44 mg/kg body weight and the symptoms of toxicity were muscular weakness, swaying gait and respiratory distress, prostration occurred at higher doses and convulsions preceded death apparently due to respiratory failure.
2.2.2.2 Effect on haematology parameters

Female rats receiving up to 1600 mg cypermethrin / kg feed for three months reported decreased haemoglobin concentration and RBC count (Hend and Butterworth, 1975).

There was no changes in haematology when cypermethrin was fed in diet up to 1500 mg per kg for 13 weeks (Buckwell and Butterworth, 1977) and (MCausland et al., 1977) also reported that there was no haematological changes in rats fed cypermethrin up to 1000 mg/kg diet for two years.

Clark (1982) found no effects on haematological parameters in rats fed α-cypermethrin up to 27 mg/kg body weight for 13 weeks.

Effects of six months feeding of cypermethrin mixed diet uninterruptedly to male albino on the blood and liver was studied by Shakoori et al. (1998). Rats consumed cypermethrin at a dose of 420 mg active ingredient per kg body weight per day. At the end of the stipulated period hemoglobin content and white blood cell (WBC) count remained unaltered, while red blood cell (RBC) count and packed cell volume (PCV) decreased. Histopathological changes were marked by hypertrophied hepatic cells and nuclei.

Mansee (1998) reported a reduction in RBC count and PCV in rats when cypermethrin and permethrin were administered at a single dose of 10 mg/kg body weight of each pyrethroid.

2.2.2.3 Effect on biochemical parameters

Female rats receiving up to 1600 mg cypermethrin / kg feed for three months reported an increased plasma urea concentration and plasma alkaline phosphatase activity (Hend and Butterworth, 1975).

There was no changes in clinical chemistry reported in rats fed cypermethrin up to 1000 mg/kg diet for two years (MCausland et al., 1977). Clark (1982) found no effects on clinical parameters in rats fed α-cypermethrin up to 27 mg/kg body weight for 13 weeks.

Pretreatment with phenobarbital strongly protected the hepatocytes against cypermethrin induced loss of cell viability percentage and increased enzyme leakage percentage. Coincubation of the hepatocytes with SKF525A, substantially potentiated the effect of cypermethrin on cell viability and enzyme leakage.
Krechniak and Wrzesniowska (1991) reported that cypermethrin (80 mg/kg) administered as a solution in soybean oil orally for 1 to 20 days had resulted in slight microsomal enzymes induction as there was change in the content of cytochrome P-450 and \( \text{b} \), activity of NADPH cytochrome P-450 reductase, P-nitroanisole, O-demethylase in microsomes and concentration of sulfhydryl groups in cytosol. Deltamethrin (15 mg/kg) and permethrin (100 mg/kg) resulted in slight induction of microsomal enzymes.

Effect of six months feeding of cypermethrin mixed diet uninterruptedly to male albino on the blood and liver was studied by Shakoori et al. (1998). Rats consumed cypermethrin at a dose of 420 mg active ingredient per kg body weight per day. At the end of the study, blood serum lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), amylase activities, blood serum protein, free amino acids (FAA) and hepatic GOT was decreased suggesting liver and possibly pancreas malfunction. Glutamate oxaloacetate transaminase (GOT), creatine phosphokinase (CPK) and cholesterol content decreased. Histopathological changes were marked by hypertrophied hepatic cells and nuclei.

### 2.2.2.4 Effect on body weight gain

Hend and Butterworth (1976) reported reduction in body weight gain in rats fed 1600 mg cypermethrin per kg for three months. The growth in rats was reduced at 750 mg of cypermethrin per kg for five weeks.

Clark (1982) reported a decreased growth which correlated with decreased feed intake in both male and female Wistar rats fed 540 mg/kg of \( \alpha \)-cypermethrin.

### 2.2.2.5 Effect on fertility

Elbetieha et al. (2001) had demonstrated the adverse effects of cypermethrin pesticide on fertility and reproduction in male Sprague-Dawley rats exposed to tap water containing 8.571, 17.143 and 34.286 ppm cypermethrin for 12 weeks and based on water consumption per animal per day the rats received 13.15, 18.93 and 39.22 mg cypermethrin in treated groups, respectively. In the rats ingesting 13.15 and 18.93 mg, there was decreased fertility, decreased number of females impregnated, decrease in the perimeter and number of cell layers of the seminiferous tubules. In rats ingesting 39.66 mg, there was decreased implantation sites in females mated with males and decrease in concentrations of testosterone, follicle stimulating hormone and luteinising hormone. They observed reduction in viable fetuses, decreased body weight gain and decreased epididymal and testicular sperm counts in all the cypermethrin exposed male rats.

Cypermethrin at 1/40\(^{th}\) and 1/80\(^{th}\) the LD\(_{50}\) were given for 65 successive days to male rats. These rats showed decreased genital organ weight, spermatozoa. Cell concentration, percentage of live
spermatozoa and spermatozoa motility and increased percentage of total spermatozoa abnormalities with decreased plasma testosterone concentration (Abd el-Khallk et al., 1999).

2.2.2.6 Effect on organ weights

Hend and Butterworth (1976) reported an increase in liver and kidney weights in rats fed cypermethrin up to 1600 mg / kg feed for three months. Increased kidney weights were noted in the group receiving 4000 mg cypermethrin / kg diet.

There was increase in liver, spleen, kidney weights of rats fed 1000 or 3000 mg of cypermethrin per kg diet for five weeks (Hend and Butterworth, 1977a). There was an increase in relative kidney weights of rats fed 300 mg of cypermethrin / kg diet or more and an increase in liver weight at 750 mg cypermethrin / kg diet for five weeks (Hend and Butterworth, 1977b). There were no changes in organ weights despite severe signs of intoxication in beagle hounds fed up to 1500 mg cypermethrin/kg diet for 13 weeks (Buckwell and Butterworth, 1977).

Glaister et al. (1977) reported increased liver microsomal oxidase activity in rats fed up to 1500 mg of cypermethrin / kg diet, but the gross and microscopic examination of tissues and organs did not reveal any changes.

Pickering (1982) reported an increase in weight of liver and kidneys of both male and female rats fed α-cypermethrin up to 540 mg per kg diet for 13 weeks.

2.2.2.7 Effect on central nervous system

Rats fed up to 540 mg α-cypermethrin / kg diet for 13 weeks showed no histopathological abnormalities except for a sparse axonal degeneration in the sciatic nerve (Clark, 1982).

Perinatal effects of two pyrethroid insecticides on brain neurotransmitter function in the neonatal rats was recorded by Malaviya et al. (1993) implied disturbance in dopaminergic and cholinergic pathways which were more pronounced during the ‘growth spurt’ period and may lead to functional delay in brain maturation. Ten mg/kg fenvalerate body weight and 15 mg cypermethrin/kg body weight formulations was given during gestation and lactation periods to pregnant and nursing dams by gavage in corn oil and morphological development and neurochemical indices was studied. Exposed pups showed an increase in concentration of dopamine muscarinic receptors of striatal membrane in both treatment groups and was more marked in lactationally exposed pups with an increase in acetylcholinesterase and decrease in the activity of brain monoamine oxidase and Na⁺, K⁺, ATPase from gestational exposure to fenvalerate. During lactation there was decreased activity of monoamine oxidase and acetylcholinesterase in fenvalerate exposed pups and in acetylcholinesterase and Na⁺, K⁺, ATPase in cypermethrin exposed pups.
Condes et al. (1999) reported that when 300 mg/kg intraperitoneal dose of cypermethrin was administered daily to rats, the frequency of epileptic activity increased throughout the days of exposure which were recorded on electroencephalography in rats.

Effectiveness of anticonvulsants therapy was assessed by alleviation of effects and survival percentage by Salawu et al. (2000). Rat brains implanted with electroencephalographic (EEG) and electromyographic electrodes were given 58 mg cypermethrin/kg body weight orally followed by 5 mg diazepam/kg, 20 mg phenobarbitone/kg or 50 mg diphenylhydantoin/kg body weight. Diazepam at 5 mg or 20 mg phenobarbitone/kg produced 100 per cent survival and alleviated all the signs of poisoning while diphenylhydantoin produced 80 per cent survival at 50 mg/kg suggesting the antidotes antagonised desynchronisation of EEG waves produced in rats exposed to cypermethrin.

Latuszynska et al. (2001) reported an inhibition of cholinesterase and acetylcholinesterase activity and elicited pycnosis of brain neurocytes when a mixture of chlorpyrifos and cypermethrin (Nurelle D550EC) was applied to tail skin of rats based on cognitive function, activity of the blood cholinesterase and brain acetylcholinesterase as well as brain histological examination.

2.2.2.8 Dermal toxicity

The dermal toxic effects of two component preparation Nurelle D550EC (500 g of chlorpyrifos and 50 g cypermethrin per one ml) on female Wistar rats was evaluated by Latuszynska et al. (1999). Two preparations of 200 mg/kg/day of chlorpyrifos plus 20 mg/kg/day of cypermethrin and 1000 mg/kg/day of chlorpyrifos plus, 100 mg/kg/day of cypermethrin was applied dermally on the fall skin of rats for four hours daily for a period of four weeks. There was slight morphological and ultrastructural changes in liver, kidney, lung and heart.

Luty et al. (1998) studied the immunotoxic effects of dermally applied α-cypermethrin in female wistar rats at 50 and 250 mg/kg applied to the tail skin four hours daily for 28 days. Dermal application resulted in slight histological changes in liver, kidney, lung and brain with ultrastructural pathological changes in heart and there was increased phagocytic activity of neutrophils after administration of 50 mg/kg α-cypermethrin.

2.2.2.9 Effect on immune response

The immunotoxicological effects of repeated combined exposure of cypermethrin (CY) at 55.4, 22.2 and 11.1 mg/kg body weight and the heavy metals lead and cadmium was studied by Institoris et al. (1999) in four week old male Wistar albino rats exposed orally for a period of 28 days. The highest dose resulted in an increase of the relative liver weight, and all the three doses resulted in changes in haematocrit and MCV values. The maximum of DTH reaction was decreased in all the three doses. While combination of the highest CY dose with non effective doses of lead or cadmium the immunotoxic effects of the former were modified and alteration of the immunotoxic effects were noticed when immunotoxic doses of cd or pb were combined with the lowest CY dose.
Desi et al. (1985) reported immunotoxicological effects of a daily oral administration of 1/40, 1/20 and 1/10 dose LD$_{50}$ of cypermethrin in rats and rabbits after vaccination with *salmonella typhi*. The two higher doses induced a dose dependent decrease of serum antibody titer and delayed type of hypersensitivity. The skin redness measured in the tuberculin skin test showed dose dependent tendency in rabbits. In sub acute experiments with rats, a dose-dependent decrease in passive hemagglutination test and in the autologous rosette-formation of T-lymphocytes in groups fed with higher doses.

Immunotoxic responses of cypermethrin in male wistar albino rats at dose levels of 0 (control), 5, 10, 20 and 40 mg/kg/day administered once daily orally for 90 days was studied by Varshneya et al. (1992). Leucopenia, decrease in delayed type hypessensitivity reaction and a decrease in spleen weights and increase in adrenal weight were observed in rats receiving 40 mg/kg/day. Humoral response as evidenced by serum haemagglutinin and haemolysis titres did not show any definite change. Total body weights and liver weight did not show any change with any of the doses levels. Results of this study revealed that low doses did not have any adverse effect on the immune-competence of rats.

Tulinska et al. (1995) studied the sub acute exposure of super cypermethrin (SCM) on cellular and humoral immunity in male wistar rats given SCM by gavage for 28 days at 12.5 mg/kg/day, 8.75 mg/kg/day and 4.38 mg/kg/day. The response of splenocytes to the mitogens phytohaemagglutin and concanavalin A and to a T-dependent antigen (Sheep red blood cells) was enhanced at 4.38 mg/kg/day dose and at 12.5 and 8.75 mg/kg/day dose these variable were suppressed. The phagocytic activity of polymorphs was not affected. These SCM at 12.5 mg/kg/day had adverse effect on a number of immunological function in rats but low dose had no effect on these activities.

A study conducted by Madsen et al. (1996) had resulted in increased relative adrenal weight and no severe effect on immune system was found with low effect dose of $\alpha$-cypermethrin at 12 mg/kg body weight in a 28-day study of F344 male rats given $\alpha$cypermetherin of 0, 4, 8 and 12 mg/kg/ day in soy bean oil by gavage.

Varshneya and Sharma (2001), noted immunotoxicity of cypermethrin in rats revealed a reduction in the phagocytic index following exposure at a dose rate of 40 mg/kg over a period of 90 days.

2.2.3 Experimentation on mice

2.2.3.1 Acute oral toxicity

The $\alpha$-cypermethrin was moderate to highly toxic and three to four times more toxic than cypermethrin. The clinical signs of toxicity were typical for a cyano group containing pyrethroid intoxication. The majority of mortalities occurred within the first three hours and surviving mice recovered within seven days (Rose and Dewar, 1978).
Rose (1982) reported clonic convulsions, piloerection and salivation when \( \alpha \)-cypermethrin administered in corn oil. The oral LD\(_{50} \) value was reported to be 798 mg/kg in mice when administered orally. There was a decreased haemoglobin concentration haematocrit value and RBC counts with anaemia when mice were fed cypermethrin up to 1600 mg/kg for 101 weeks.

### 2.2.3.2 Effect on haemotolgy and biochemical parameters

Genotoxic effect of a synthetic cypermethrin insecticide was studied in mice by Bhunya and Pati (1988). Bone marrow chromosome aberrations were not dose, time or route dependent. In the micronucleus test, the occurrence of polychromatic erythrocytes with micronuclei increased slightly with dose but was only a marginal difference in the incidence of sperm abnormalities was noted.

Haratym-Maj (2002) noted hematological alterations in \( \alpha \)-cypermethrin treated male and female Swiss mice there was an increase in the number of leukocytes in peripheral blood. There was no change in haematological parameters in male mice, whereas in female animals the lower dose resulted in an inhibition and a higher dose in mobilisation of hemopoietic system. Haematological changes were noticed in deltamethrin and fenvalerate poisoning.

### 2.2.3.3 Effect on fertility

Adult male Swiss Albino mice were administered i.p suspension solution of cypermethrin in 0.15 per cent. DMSO at doses of 30, 60 and 90 mg/kg body weight daily for five days to demonstrate sperm head shape abnormally (Kumar et al., 2004). The results revealed that body weight gain was considerably reduced in higher dose groups, but the testicular weight did not change in any of the cypermethrin treated groups. However, an elevation in the number of abnormal shape of sperm head was noticed in higher dose groups.

### 2.2.3.4 Effect on immune response

\( \alpha \)-cypermethrin had a suppressive effect on IL-12p70 production in Swiss mice at 25 and 10 mg/kg body weight administered per o.s. daily for a period of 29 days (Luty et al., 2000). Subacute poisoning of mice resulted in decreased bacterial activity of neutrophils in both dose groups with stronger stimulatory effect on phagocytic activity in low dose group. An higher number of monocytes and lymphocytes were noticed in the blood of male mice poisoned with low dose. There was decrease in IL-12p70 serum secretion. Histopathologic and ultrastructural changes were observed in the liver and kidneys.

Tamang et al. (1988) reported that cypermethrin suppressed both cell mediated (CMI) and humoral immune response in mice given cypermethrin 50 mg/kg i.p. for 26 days.
Oral administration of 30, 60 and 120 mg/kg permethrin per day for 14 days to mice did not alter the primary and secondary humoral immune response and protein concentrations. A dose of 120 mg/kg reduced the CMI response and reduced TLC and ALC (Shah and Gupta, 1998).

2.2.3.5 Effect on central nervous system

Pyrethroids with a cyano group on the alcohol moiety such as cyphenothrin, cypermethrin, fenvalerate and fenproparthrin shortened the pentobarbital induced sleeping time while pyrethroids with an ethynyl group on the acid moiety such as prallethrin, furamethrin and empenthrin prolonged sleeping time in mice given pyrethroids orally two hours before the intraperitoneal injection of pentobarbital at a dose of 45 mg/kg (Tsuji et al., 1996).

2.2.4 Experimentation on Rabbits

2.2.4.1 Effect on body weight gain

Cypermethrin of 200 mg per kg when applied topically for six hours a day for 13 weeks reduced feed intake and body weight gain in New Zealand white rabbits (Henderson and Parkinson, 1981).

2.2.4.2 Effect on biochemical parameters

Yousef et al. (2003a) observed changes in haematological and biochemical indices of male New Zealand White rabbits given orally sublethal dose of cypermethrin (24 mg/kg body weight), while isoflavones (2 mg/kg body weight) was given alone or in combination with cypermethrin every other day for 12 weeks. Cypermethrin caused an increase in the concentration of plasma total lipids (TL), cholesterol, triglycerides (TG), glucose, urea, creatinine and total bilirubin, low density lipoprotein (LDL) and very low density lipoprotein (VLDL), while the concentration of high density lipoprotein (HDL) decreased. Isoflavones alone decreased the concentration of TL, cholesterol, TG, LDL and VLDL and increased HDL and alleviated the harmful effect of cypermethrin on lipid profiles. Cypermethrin decreased haemoglobin (Hb), total erythrocyte count (TEC) and packed cell volume (PCV), while total leucocyte count (TLC) increased. Isoflavones alone did not cause any change in these parameters but minimised the toxic effects of cypermethrin.

Isoflavone confers marked protection against cypermethrin induced oxidative stress in rabbit’s plasma, liver, brain and testes as was evident in a study conducted by El-Demerdash et al. (2003) in male New Zealand rabbits given orally sublethal dose of cypermethrin at 24 mg/kg body weight, while isoflavone at 2 mg/kg body weight given every other day for 12 weeks alone or in combination with cypermethrin.
Cypermethrin induced free radicals in plasma, liver, brain and testes. The activities of glutathione S-transferase (GST), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were decreased after cypermethrin treatment and in contradictory the activities of GST, AST, ALT and ALP were increased in plasma. Isoflavone alone decreased the concentration of free radicals in plasma, liver, brain and testes. However, isoflavone did not reverse the changes in enzyme activities due to the effect of cypermethrin.

2.2.4.3 Effect on fertility

Protective role of isoflavones (2 mg/kg body weight) on semen quality and plasma testosterone concentration in male New Zealand White rabbits given sublethal dose (24 mg/kg body weight every other day for 12 weeks) of cypermethrin was studied by Yousef et al. (2003b). Results showed that treatment with cypermethrin caused a decrease in ejaculate volume, sperm concentration, total sperm count, sperm motility (%), total motile sperm per ejaculate (TMS), placed sperm volume (PSV), semen initial fructose and plasma testosterone concentration, live body weight (LBW), dry matter intake (DMI), relative weights of testes and epididymis and there was increased number of abnormal and dead sperms. The presence of isoflavones together with cypermethrin minimised its harmful effect as treatment with isoflavones alone caused increase in libido, PSV, sperm motility and TMS while abnormal and dead sperm were reduced when compared to control animals.

2.2.5 Experimentation on Guinea pigs

2.2.5.1 Effect on central nervous system

A quantitative assessment of pyrethroid induced paraesthesia in the guinea pig flank model was done by Mc killop et al. (1987). The cutaneous effects of classical irritation and vascular responses were characterised by transient facial burning and tingling sensation were related to the ability of pyrethroid to produce trains of nerve impulses in afferent nerves by prolonging the opening of the neuronal sodium channel. Using the guinea pig flank model dose response curves to permethrin, cypermethrin and deltamethrin and to a mixture of veratrum alkaloids (veratrine) were noticed.

2.2.5.2 Effect on gastrointestinal tract

Tonini et al. (1989) suggested that neural Na⁺ channel activation may under lie pyrethroid-induced potentiation of enteric cholinergic transmission and cypermethrin was effective as a non competitive antagonist of GABA-A receptor mediated cholinergic contractions in small intestines from their study in electrically-stimulated longitudinal muscle-myenteric plexus preparations of the guinea pig ileum with 1-100 μl of tetramethrin and 1-100 micro M of cypermethrin.
2.2.6 Experimentation on Dogs

2.2.6.1 Effect on feed intake

Cypermethrin (1500 mg per kg for 13 weeks) caused diminished feed intake in beagle hound dogs. Weight loss, diarrhoea, anorexia, licking and chewing of paws care observed in the treated dogs (Buckwell and Butterworth, 1977).

There was weight loss in beagle dogs fed $\alpha$-cypermethrin at 200 mg per kg for seven days (Green Vough and Goburdun, 1984). $\alpha$-cypermethrin (fed in diet at the rate of 270 mg/kg for 13 weeks to beagle dogs) did not show any pathological changes in organs and tissues.

2.2.7 Experimentation on Cats

2.2.7.1 Effect on immune response

A topical (micro-droplet) bioassay for the cat flea, *Clenocephalides felis* was conducted by Moyses and Gfeller (2001). This method was used to compare the effectiveness of 13 insecticides. LD$_{95}$ values in nanograms per flea for cypermethrin was 5.4. Experiments repeated in a different facility showed only small shifts in potency (0.38 to two fold of the original LD$_{50}$ values).

In a study conducted by Lemke *et al.* (1989) adult cat fleas, *Clenocephalides felis* from Florida and California were exposed to residues of eight pyrethroids to compare their susceptibilities. The Florida strain was more tolerant than the California strain with 6.8, 5.2 and 4.8 fold tolerance to cyfluthrin, cypermethrin and fluvalinate, respectively. The Florida strain showed less than three fold tolerance to the other five insecticides excluding cypermethrin.

2.2.8 Experimentation on cattle

2.2.8.1 Field trials

Populations of southern cattle ticks, *Boophilus microplus* from Mexico had developed resistance to many classes of acaricide including chlorinated hydrocarbons (DDT), pyrethroids, organophosphates and formamidines (amitraz) (Foil *et al.*, 2004). Target site mutations were the most common resistance mechanism observed. In many pyrethroid resistance strains, a single target site mutation on the Na$^+$ channel conferred very high resistance to both DDT and all pyrethroid acaricides. Acetylcholine esterase affinity for OPs was changed in resistant ticks populations. A second mechanism of OP resistance was linked to cytochrome P450 monooxygenase activity. Additionally, a specific metabolic esterase CzEst9 with permethrin hydrolyzing activity was associated with high resistance to permethrin in one Mexican tick population.
Rothwell et al. (1998) determined the efficacy of Zeta-cypermethrin pour-on at 2.5 mg/kg and spray at 62.5 ppm formulations for control of buffalo fly (Haematobia irritans exigua) in groups of 20 cattle each. Buffalo fly counts were conducted three time before treatment and 3, 7, 14, 21, 28 and 35 days after treatment. Zeta-cypermethrin pour-on at 2.5 mg/kg gave good control of buffalo fly for four weeks and the spray at 62.5 ppm gave 14 days control indicating good control of H. irritans exigua for four weeks with 2.5 mg/kg zeta-cypermethrin pour-on.

The addition of piperonyl butoxide (PBO, 5% and 10%) to cypermethrin (5%) did not provide a treatment that would give a long term control of pyrethroid resistant horn flies, Haematobia irritans when synergised formulations used as pour on method in groups of 25 Holstein cows each, naturally infested with H. irritans. The efficacy was higher than 95 per cent for a period of 14 days (Guglielmone et al., 1999).

Zeta-cypermethrin pour-on, given at 2.5 mg/kg, was an effective treatment for cattle lice control (Rothwell et al., 1999). Zeta-cypermethrin and other synthetic pyrethroid pour-on are the treatment of choice to control B. bovis. Zeta-cypermethrin pour-on and deltamethrin pour-on at 2.5 and 0.75 mg/kg were assessed in field trials in south-eastern Australia in groups of 10 cattle each. Lice were counted before treatment and 14, 28, 42 and 56 days after treatment.

Sommer et al. (2001) studied the effect of topical treatment of calves with deltamethrin, flumethrin, cyfluthrin and α-cypermethrin. Larval adult mortality was observed when allowed to feed of dung collected from calves dosed with topical applications. Nulliparous flied fed for six day on dung collected after three days treatment showed little or no ovarian development. The results indicated that the use of synthetic pyrethroids affected the insect dung fauna and such use may reduce dung decomposition.

2.2.8.2 Effect on immune response

Immunosuppressive action of cypermethrin was demonstrated by Tamang et al. (1988) in mice by intraperitoneal injection of the pesticide at 50 mg/kg/body weight per day for 26 days. The results indicated depression of CMI in the cypermethrin treated mice.

Cell mediated immune response and humoral immune response were suppressed in cross bred calves administered 60 mg/kg cypermethrin (Barathrin 25% EC) daily for 30 days (Patel et al., 1996).

2.2.9 Experimentation on Sheep

2.2.9.1 Effect on haematology and biochemical parameters

Yousef et al. (1998) reported decreased RBC, PCV, haemoglobin concentration and increased concentrations of total protein, albumin and globulin and an increased total leucocyte count with decreased AST, ALT and ALP concentration in barki sheep given cypermentrin 6 or 12 mg/kg P.O.
2.2.9.2 Effect on immune response

Mikula et al. (1992) found reduction in the phagocytic index and reduction in the number of rosette forming lymphocytes in 7 month old sheep fed 300 ppm cypermethrin for 4 weeks.

2.2.9.3 Efficacy studies

A synthetic pyrethroid, cypermethrin (NRDC 149) showed activity in killing lice (Damalinia ovis) in the fleece of sheep at concentrations as low as one ppm in a dip (Hall, 1978). In addition, concentrations of 5 and 10 ppm showed a persistent effect and prevented reinfestation from contact challenge sheep for 7 and 19 weeks, respectively.

Cypermethrin pour-on formulation was done by Henderson and McPhee (1983) to control sheep body louse Damalinia ovis. Application rates of 5 mg/kg or more gave 99 to 100 per cent control against D. ovis which caused potential serious problem affecting the value of fleece and reduced weight gains.

Appleyard et al. (1984b) reported that plastic tags containing 8.5% w/w cypermethrin or 10% w/w permethrin reduced the severity of damage caused to sheep by the headfly Hydrotæa irritans. A similar pattern of protection was extended from the tagged ewes to their untagged lambs. Tags containing 8.5% w/w fenvalerate failed to reduce the level of headfly damage in gimmers.

In the evaluation of three synthetic pyrethroids in control of sheep headfly disease, Appleyard et al. (1984a) observed polyvinyl chloride tags containing 8.5 per cent cypermethrin reduced damage in gimmers to a very low level with protection lasting throughout the summer and weight gains of lambs suffering headfly damage were less than unaffected lambs. Deltamethrin (0.01%) and permethrin (0.1%) showed less protection period.

Route of passage of cypermethrin across the surface of sheep skin was studied by Jenkinson et al. (1986). 14C cypermethrin applied topically to the dorsal surface of sheep moved radially across the skin within the stratum corneum of the epidermis at a rate which excluded 11 cm/h, which was accompanied by some dermal infiltration most marked at the site of application.

Henderson and Stevens (1987) conducted trials in 11 farms to assess the effectiveness of 2.5% w/v cypermethrin pour-on for the control of ticks (Ixodes ricinus) in sheep. Treatment at the rate of 5 ml/10 kg body weight gave 92 per cent control of ticks in ewes for up to nine weeks and 88 per cent control on lambs for up to eight weeks.

Three hundred Merino ewe lambs were treated with 300 mg cypermethrin each and run as a separate flock and 100 lambs were used as untreated controls with a further 100 lambs were treated topically with a larval growth regulator as treated control groups by Gruss (1988). The synthetic pyrethroid afforded total protection for three weeks against strike by Luchlia
cuprina, whereas the blowfly challenge was heavy and 26 per cent of the untreated control animals were struck in the four week trial period.

Polymer matrix ear tags containing 13.7% w/w tetrachlorvinphos or 8.5% w/w cypermethrin were applied to Merino withers infested with Damalinia ovis and carrying two months wool. The cypermethrin tags reduced louse numbers by a maximum of 89 per cent in comparison to controls at 16 weeks after treatment and by 85 per cent at the conclusion of the experiment 38 weeks after application compared to tetrachlorvinphos impregnated tags and controls (James et al., 1989).

The application of polymer matrix ear tags impregnated with 8.5% w/w cypermethrin to six wethers following shearing reduced body mice Damalinia ovis lice to non-detectable levels on four of them at 29 weeks after tagging (James et al., 1990). At the conclusion of the study at 45 weeks, the count of lice on tagged wethers was three per sheep compared to 158 on untreated wether.

James et al. (1994) reported that tags impregnated with cypermethrin (8.5% w/w) Lucilia reduced the total number of egg masses of cuprina deposited on the heads of sheep in Hy cage studies over a six week period by 73.3 per cent compared with no treatments in a study conducted to evaluate the efficacy of controlled release insecticide devices for protection of sheep against head strike caused by Lucilia Cuprina.

Insecticide dipping fluid emulsions of diazinon, cyhalothrin and cypermethrin settling rates five cm below the dam water had declined by 72.5, 72.8 and 89.4 per cent, respectively by the addition of one per cent w/v Zinc sulphate. (Morcombe et al., 1995). In two trials, lice were eradicated from sheep and in 12 flock treatments in which 1000 to 2000 sheep were dipped with added zinc sulphate, the concentration of insecticide remained above the minimum lethal concentration for susceptible strains of lice.

A 10% w/w dip formulation of high cis-cypermethrin (cotleece sheep dip) was evaluated as a prophylactic and treatment method against Psoroptes ovis of sheep (O’ Brien et al., 1997). A laboratory trial using groups of healthy sheep under challenge showed that when dipped as directed for one min, they were protected against infection for at least four weeks. 12 sheep infected with psoroptic mange at the laboratory were effectively treated by one dipping cotleece. A field outbreak of psoroptic mange in a flock of 237 cross bred ewer was successfully treated by just one dipping.

Johnson et al., (1997) studied the effect of aqueous formulation of α-cypermethrin on lice in a group of five adult merino sheep treated with a topical application. Number of pyrethroid susceptible lice surviving exposure in vitro for 20 h differed between samples collected at different types after treatment, lice survived for 20 h in wool taken from parts of fleece. The number of lice surviving in sample collected within 28 days after treatment tended to be lower than in those collected from 28 to 98 days.

### 2.2.10 Experimentation on Goats
2.2.10.1 Pathology

The black Bengal goats drenched cypermethrin did not show any variations in thyroid hormone concentration, pathological parameters, indicating that cypermethrin did not have either thyrotoxic or goitrogenic effects on goats (Gupta et al., 1999).

2.2.10.2 Effect on immune response

The immunosuppressive action of cypermethrin was first reported by Tamang et al. (1988). Goats were drenched with cypermethrin at 41.6 mg/kg body weight per day for 30 days. The status of cell mediated immunity (CMI) was assessed by the 24-dinitrofluorobenzene (DNFB) skin sensitivity test which indicated depression of CMI and in addition suppression of the humoral immune reaction via reduction in rate of plaque formation in the lymphocyte suspension.

2.2.11 Experimentation on poultry

2.2.11.1 Effect on immune response

Abdel-Nasser and El-Zanaty (1994) reported that chicken dipped in cypermethrin solution showed decreased number of lymphocytes, thymocytes and splenocytes along with reduced body weight gain and relative weight of the bursa fabricius, thymus and spleen.

Day-old broiler chicks when fed 100 mg/kg cypermethrin in feed for 8 weeks showed reduction in humoral immune responses with a decrease in serum globulin and γ-globulin concentration and antibody titers indicating immunosuppressive effect of cypermethrin (Khurana et al., 1998).

The delayed type of hypersensitivity reaction was depressed in cypermethrin fed birds by dinitrofluorobenzene (DNFB) skin sensitivity test as reflected by reduction in skin thickness and mild inflammatory reaction (Khurana et al., 1999).

A suppression in total leucocyte count, absolute lymphocyte count, and a delayed type hypersensitivity reaction were noted in chicken fed with 100 ppm, a low dose of cypermethrin in feed (Khurana et al., 2000).

2.2.12 In vitro immersion bioassay

Pap et al. (1997) conducted an in vitro immersion bioassay to compare the efficacy of selected pyrethroids against a deltamethrin resistant strain of rabbit ear mite (Psoroptes cuniculai). Deltamethrin proved to be inactive (48 h mortality 21.9% at 1000 mg/kg), each cypermethrin isomer mixture tested, including α-cypermethrin and β-cypermethrin and their mixture at a ratio of 416, β-cypermethrin showed high efficiency (48 h mortality >or=95% at 1000 mg/kg).