INTRODUCTION

Pesticides are the compounds that are used to control various pests attacking field crops, farm animals, stored grain and to control insect vector in public health **(Ranvier, *et al.*, 2002).** Pesticides are defined under the Federal Environmental pesticides control act as "any substances or mixture of substances intended for preventing, destroying, repelling and mitigating any pest or use as a plant growth regulator, defoliant or desiccant" **(Hayes, 1975).** Pesticides are first manufactured as technical grade products having high purity. Commercial pesticides are then produced by conversion of the technical products into appropriate formulations, which are tailor-made for field application on disease-insect-pest spectra The technical grade pesticides are transformed into lower concentration, like dusting powder, emulsifiable concentration, water dispensable granules/powder, soluble liquids and granules, etc.

Insecticides may be classified based on·

(i) mode of entry, *viz.* stomach poison, contact poison and fumigants

(ii) mode of action as physical poison, protoplasmic poison, respiratory poison, nerve poison and poison of more general nature

(iii) the chemical nature

Pesticides are also broadly classified as inorganic compounds such as sulphur, zinc, phosphide etc. and as organic compounds, synthetic organic compounds such as organochlorine (DDT, HCH, endosulfan, aldrin endrinate, etc), organophosphates (phorate, chlorpyrifos, methyl parathion, ethion, malathion, etc.) carbamates (carbaryl, carbofuron, aldicarb, etc.) and synthetic pyrethroids (cypermethrin, fenvalerate, bifenthrin, lambda-cyhalothrin, deltamethrin , etc).

The present world population of 6 billions is likely to cross 8 billions mark by 2025 and the provision for adequate food and environmental security would remain the key issues confronting mankind before the turn of the century. The future strategies should therefore be carefully planned to sustain food production with least disruption to fragile agro ecosystem. **(Dhaliwal *et al*,, 1999).**

Pesticides have made great impact on human health, production and protection of food, fiber and other cash crops by controlling disease vectors and keeping a check on the population of many species of unwanted insects and plants. Approximately 70 per cent of the pesticides used in the world are applied in developed countries and remaining 30 per cent in developing countries **(Pimental, 1987).**

Agriculture plays a dominant role in Indian economy as more than 80% of our population is directly dependent on agriculture and our cash crops like tea, coffee, sugarcane, tobacco and cotton are the major foreign exchange earners. The need for pesticides in Indian Agriculture and public health has been well established in protecting the crops from the insect pests and human health from vector borne diseases. India is the largest manufacturer and consumer of pesticides in South Asia, there are over 131 different types of pesticides marketed under 203 different formulations by over 350 companies in the country. India is a bulk consumer of pesticides (73.74%) when compared with other nations (Thailand: 6.61%; Vietnam: 9.59%; Australia: 6.61%; Japan: 2.19% and US: 1.26 %) **(Kannan, 1997).** Today India has made good strides in food production due to new agricultural technology, high yielding varieties, improved seeds, application of fertilizers, increased irrigation and above all proper protection technology by application of pesticides. Pesticides are double-edged weapons. If used injudiciously they may cause very harmful effects not only by contaminating our food and feed but also by affecting ecological balance **(Mukerjee and Tanwar, 1981).**

India is one of the first countries, which started large-scale use of pesticides for control of insects, pests of public health and agricultural importance. India produces over 193 pesticides and hundreds of formulations. It produces 90,000 metric tonnes of pesticides annually; production is the largest in Asia and ranks twelfth in the world. The Indian pesticide industry has a turnover of more than 20 billion rupees, and its average annual growth rate is 2 to 5%. The production of pesticides in India had begun in the mid 50's when the first DDT and BHC plants were set up with the help of World Health Organisation and has now reached a capacity of nearly 140000 metric tonnes.

India is nearly self sufficient in its pesticide requirements – around 95% of the use is met with local production. Further, there are many Indian companies, which export pesticides to other countries. Export of pesticides from India has increased from Rs 882 crores (1998-99) to Rs 1215 crores (2000-01) and imports also have increased from Rs 166 crores (1998-99) to Rs 221 crores (2000-01) as detailed below·

EXPORT & IMPORT OF PESTICIDES (RUPEES IN CRORES)		
Year	Export	Import
1998-99	882	166
1999-00	1005	237
2000-01	1215	221

(Source : Lok Sabha Unstarred Question No. 2477, dated 19.03.2002.,)

The pesticide consumption varies across different states, depending on several factors, including cropping patterns, irrigation facilities, pest resurgence, resistance situations etc. The pesticides consumed by various states in India are provided below:

Consumption of Pesticides (in tones)			
Andhra Pradesh	13000	West Bengal	5800
Uttar Pradesh	11000	Haryana	5200
Tamil Nadu	9500	Gujarat	5100
Maharashtra	6900	Karnataka	4400
Punjab	6400	Rajasthan	2900

(Source.Agricultural Research Data Book, 2001)

Although various methods are used to control pests in different pest management system, pesticides continue to be the major components of most of the pest control programmes and probably will remain so in the near future. In many cases, pesticides are helping in getting highest agricultural production and preventing human/animal diseases.

The bulk of pesticide residues enter the human body through dairy products and also through agriculture products like cereals and vegetables. The chemical pesticides, like most other invention of modern science, constitute a double-edged weapon. The contribution of pesticides to the increased agricultural production and protection of public health has been substantial but the hazards associated with their injudicious use present a serious problem. After natural catastrophies such as floods, drought, and earthquake, India is exposed to another threat of a different nature, i.e., through pesticides which are gradually spreading their tentacles in to the human body.

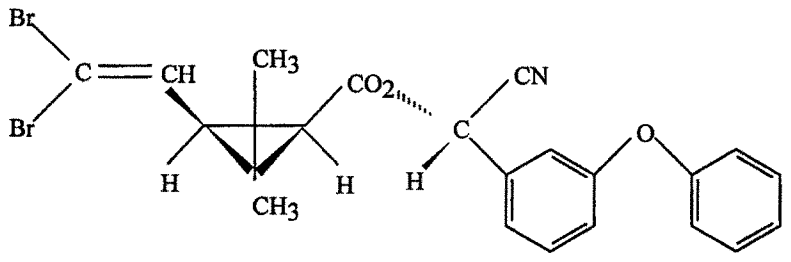
In addition, to the increase in agricultural yield, pesticides have played a vital role in public health. Vectors that may carry various diseases have been the scourge of mankind through recorded history **(Gunther and Jeppson, 1960).** Insecticides were responsible for controlling bubonic plague, malaria, and yellow fever, which were transmitted by insect or avian vectors and resulted in untold misery of millions **(Rao and Schwetz, 1982).** Several chemical pesticides have been tried for the control of the pests **(Singh *et al,* 1993; Shah *et al,* 1994).** Frequent and enormous use of synthetic pyrethroids, organophosphates have posed the resistance problem, resurgence of the pest(s) and health hazards **(Mehrotra, 1990).**

Human Pesticide poisoning is a major concern It has been estimated that annually in the world, there are about 7,50,000 reported pesticide poisoning cases with 13,800 deaths **(Anonymous, 1992).** According to estimates of International Development Research Center, every year around 10,000 people die and another 400,000 suffer from various effects of pesticides in developing countries. Around the world, 3 million people suffer from acute pesticide poisoning every year, of which 200,000 die mostly in countries like India. India accounts for one third of the pesticide poisoning cases in the world **(Dhaliwal *et al.,* 1999).**

Domestic animals are raised and housed in variety of environments and they are often closely associated with the human. The kennels, yards barns and pastures of most animals may be heavily infested with insects, parasites, rodents and other pests. Use of pesticide in domestic animals is economically necessary. Pesticides are required to control insect in livestock, crops and carry disease **(Oehme and Manala, 2001).** The heaviest exposure of animals to pesticides occurs in beef cattle that are raised for rapid weight gain and meat productivity. Pesticide exposure poses a serious risk to all domestic animals and to the environment and public health **(Oehme, 1987).**

Pesticides like synthetic pyrethroids and organophosphates are being used widely across the world. Deltamethrin and triazophos are the example for synthetic pyrethroids and organophosphate, respectively. In the present investigation, the combination insecticide Deltamethrin (Synthetic Pyrethroid) + Triazophos (Organophosphate insecticide) has been selected to study the adverse effects in laboratory animals (Rats – *Rattus norvegicus*) .

Deltamethrin (Synthetic pyrethroid)



[1*R*-[1α(S*),3α]]-cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

Synthetic pyrethroid group of compounds are another class of insecticides widely used in agriculture. These are relatively safe to human being but are innocuous to insect pests because of low persistence in the environment. Deltamethrin, synthetic pyrethroids insecticide is mainly used against agricultural pests. Deltamethrin was synthesized in 1974 and is marketed since 1977 Deltamethrin is commonly used on cotton, and other crops such as coffee, maize, cereals, fruit, soybean and vegetables **(Environmental Health Criteria, 1990).** Deltamethrin is also used for the post-harvest protection of stored cereals, grains, coffee, beans, and dry beans. Major public health applications include, use in the control of Chagas disease and malaria.

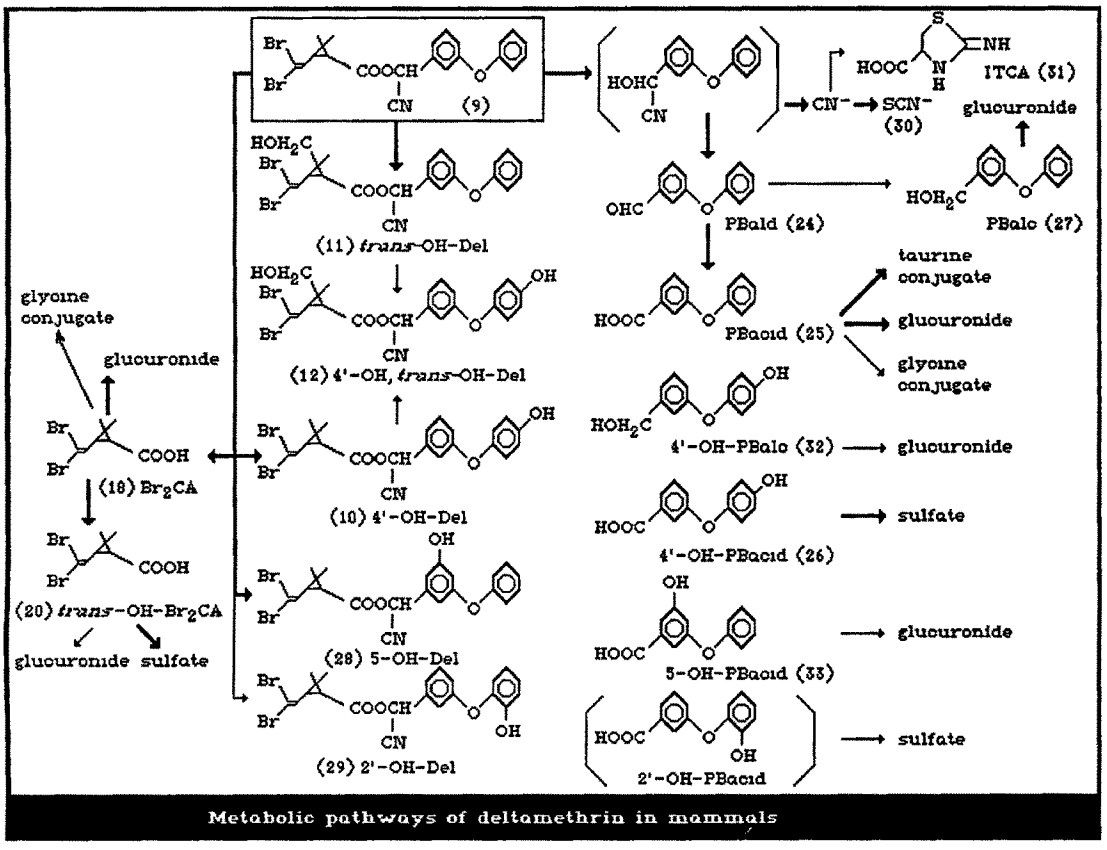
Synthetic pyrethroids are neuropoisons acting on the axons in the peripheral and central nervous system by interacting with sodium channels in mammals and/or insects and cause transient changes in the nervous system such as axonal swelling and breaks and myelin degeneration in the sciatic nerve. Synthetic pyrethroids are known to cause transient itching and burning sensation in humans. They are generally metabolized in mammals through ester hydrolysis, oxidation and conjugation and exhibit no tendency to accumulate in tissues.

The pyrethroids are strongly adsorbed on soil and sediments and are hardly eluted with water. Deltamethrin is readily absorbed by oral route but, less so dermally. Exposure of the compound to the general population is mainly *via* dietary residues, but may occur from its use in public health. Clinical signs observed due to deltamethrin poisoning are tremor, salivation, convulsion, etc. The onset of signs is rapid and they disappear after several days **(Ruzo *et al.*, 1976).**

Deltamethrin has been formulated as solutions, concentrates, granules, powders and aerosols, alone and in combination with other pesticides. Exposure can occur during its production and application and, at much lower levels, from the consumption of food containing its residues. Deltamethrin induce micronucleus formation and chromosomal aberrations in bone marrow and abnormal sperm morphology in mice treated *in vivo* which is an indication of its genotoxic potential **(IARC working group, 1991).**

Deltamethrin is metabolized by liver microsomal esterases and oxidases. Hydrolysis initially cleaves the parent compound into two fragments - cyclopropanecarboxylic acid and 3-phenoxybenzyl alcohols. The latter is then oxidised to 3-phenoxybenzoic acid which is the major excretion compound of this moiety. Deltamethrin is completely eliminated from the body within six to eight days of oral administration.

Metabolic pathways of deltamethrin in mammals



Earlier studies on the metabolism and toxicity of synthetic pyrethroids (fenothrin, furamethrin, proparthrin, resmethrin, tetramethrin and allemethrin) indicate that neither the cis nor trans isomer of eyrysanthemumate is teratogenic in rats, mice or rabbits **(Miyamoto, 1976).** Animals administered with deltamethrin by gavage showed motor in coordination, convulsion, respiratory defects and hypomotolity, shortly after dosing, normal behaviour was observed after 3 days **(Glomot & Chevalier, 1976).**

Oral administration of deltamethrin by oral intubation to pregnant female Sprague-Dawley rats at the dose levels of 0, 0.1, 1 and 10 mg/kg body weight per day on day 6 to 18 of pregnancy revealed slight delayed ossification at the highest dose level **(Glomot & Vannier, 1977).** Decrease in body weight, food consumption and mean pup weights on 21 days of lactation were also observed in a 3-generation study of deltamethrin. Basic reproduction indices were not affected by treatment **(Wrenn *et. al.*, 1980). Vannier and Glomot (1982)** had reported moderate and transient retardation of the development of the foetus in mice treated with deltamethrin on day 6 – 17 of gestation.

Rats treated with deltamethrin at the dose levels of 0, 1.25, 2.5 and 5-mg/kg body weight on day 7-20 of gestation resulted in dose related maternal weight gain during pregnancy. Dams in the high dose group gained only 80% of the control value. Number of implantation sites, fetal mortality, fetal weight, or the number of sternal and caudal ossification centers were not affected **(Kavlock *et. al.*, 1979).**

Husain and Seth (1991) reported adverse effects on morphogenesis, growth, maturation, and functions of the brain. Deltamethrin (7 mg/kg body weight, gestationally) administered to pregnant female rats during 5 to 20 days of gestation and neonates from day 22 to 37 postnatally, showed reduced birth weight and growth rate, also ontogeny of various reflexes and developmental landmarks were delayed Deltamethrin exhibited a significant increase in monoamine oxidase, while polyamine concentration exhibited a differential effect, with an overall increase in several brain areas.

Deltamethrin administered orally to pregnant rats at the dose levels of 1, 2.5 and 5-mg/kg body weight from day 6^{th} to 15^{th} of gestation resulted in higher early embryonic deaths in treated rats. Deltamethrin caused retardation of growth, hypoplasia in lungs, dilation of the renal pelvis and increase in placental weight. No skeletal changes were observed in fetuses **(Abd El-Khalik *et al.*, 1993).**

Increased weight of most genital organs, sperm motility associated with an increase in the percentage of dead and morphologically abnormal spermatozoa, decrease in the plasma testosterone levels and decreased conception rate in non-treated females (mated with treated male) were observed in rats treated with deltamethrin for consecutive 65 days **(Abd el-Aziz *et al.*, 1994).**

The numerous studies on the genotoxic potential of cypermethrin and deltamethrin have demonstrated a wide range of effects, including mitotic/chromosomal abnormalities and the induction of sister chromatid exchanges **(Chauhan *et al.*, 1997).** Significant increase over respective controls was evident for chromosomal aberrations, micronuclei and sperm abnormalities **(Bhunya and Pati, 1990).** The pyrethroid are also capable of producing gross effects on brain maturation and morphology but only, if given at dose levels that cause reduced body weight in the offspring **(Petro *et al.*, 1997).**

In a developmental toxicity study, when deltamethrin was given orally to Charles River Crl.CD VAF/Plus rats/ in corn oil at dose levels of 0, 1.0, 3.3, 7.0 and 11 mg/kg body weight /day from days 6 - 15 of gestation, the treated animals exhibited excessive toxicity at the dose 11 mg/kg body weight/day. Increased mortality, increased salivation and decreased body weight gain were observed. No treatment-related effects on fetal deaths or resorptions, altered growth, or developmental malformations or variations were noted **(EPA, Federal register, 1998).**

A slightly lower daily dose of pyrethroid (4% LD_{50}) over postnatal days 10-16, caused an increase in renal D1 receptor density in rats, which persisted at least until day 90 **(Cantalamessa *et al.*, 1998).** Similarly, low doses of bioalethrin to mice over postnatal days 10-16 caused decrease muscarinic receptor density in adult mouse neocortex and produced lasting changes in adult behaviour **(Talts *et al.*, 1998).** A number of effects of exposure of pyrethroids during early development have been described in rats and mice. Cypermethrin caused an apparent increase in blood brain barrier permeability in 10 days old rat pups after single or repeated doses of about 15% of the LD_{50} but had no effect on adult barrier **(Gupta *et al.*, 1999).** In the studies submitted in support of registration for deltamethrin, no significant developmental toxicity was reported in rats, delayed ossification along with maternal effects was noted in the high dose group in rabbits **(CDPR, 2000).**

Female rats, treated with deltamethrin by oral gavage from day 1 of pregnancy to day 21 of lactation evoked significantly, adverse effects on testicular and epididymal absolute weight and the diameter of seminiferous tubules of male pups at 4 mg/kg body weight **(Andrade *et al.*, 2002).**

Deltamethrin was given to Swiss Albino mice at 1.5, 2.5, or 7.5 mg/kg. Lipid peroxidation was evaluated by determining malondialdehyde (MDA) levels in plasma, and determining glutathione peroxidase (GSH-Px), superoxide dismutase (Cu-Zn SOD) and catalase activities in erythrocytes on days 15, 45, and 60. MDA levels increased in deltamethrin dosed groups, especially for the subchronic and chronic periods. GSH-Px, Cu-Zn SOD and catalase activities in erythrocytes were decreased at high doses of deltamethrin **(Yarsan *et al.*, 2002).**

The effect of deltamethrin pre-treatment on the pharmacokinetics and metabolism of antipyrine was studied in male rats by **Anadon *et al.*, (1991)** and evoked that deltamethrin is capable of inhibiting oxidative metabolism.

Bifenthrin a synthetic pyrethroid was given via gavage to rats in corn oil on days 6 - 15 of gestation exhibited intermittent tremors 2.0 mg/kg between days 10 and 19 of the study. In the second study, bifenthrin was administered in the diet on days 6-20 of gestations evoked treatment-related clinical signs, significant reductions in maternal body weights gains and food consumption at 200 ppm. No significant findings were observed related to developmental and maternal toxicity **(McCarty *et al.*, 2002).**

Cabral *et al.*, (1990) had found that deltamethrin does not appear to be carcinogenic in a carcinogenicity study (2 years) in mice (0, 1, 4 or 8 mg/kg body weight) and rats (0, 3 or 6 mg/kg body weight). An increased incidence of thyroid tumors with a significant increase in the incidence of thyroid adenomas was found at the 3 and 6mg/kg body weight in rats.

Prenatal exposure of deltamethrin to rat pups at 0.08 mg/kg altered latency to float and the activity of striatal dopaminergic system might reflect a persistent effect on animal in motor activity. On the other hand, the decrease in general activity observed in experimental male rats suggests higher levels of emotional lability Data gathered in the present study may be important for the assessment of the safety of pyrethroids insecticides **(Lazarini *et al.*, 2001).**

El-Gohary *et al.*,(1999) found that administration of deltamethrin (1 mg/kg daily for 21 days) to animals resulted in characteristic DNA migration patterns (laddering), thereby providing evidence that apoptosis is the major mechanism of cell death in the testicular tissues In addition, histopathological examination of testicular tissue sections showed that apoptosis was confined to the basal germ cells, primary and secondary spermatocytes. These changes, in addition to the appearance of vacuoles in Sertoli cell in deltamethrin-intoxicated

animals, indicate the suppression of spermatogenesis. Malondialdehyde (MDA) was found to be significantly increased in deltamethrin-treated animals Administration of nitric oxide synthase (NOS) inhibitors such as N (G)-nitro monomethyl L-arginine hydrochloride (L-NMMA, 1 mg/kg) to rats 2 h before exposure to deltamethrin was effective in the reduction of the typically testicular apoptotic DNA fragmentation pattern and the associated histopathological changes which showed that deltamethrin-induced testicular apoptosis is mediated by nitric oxide.

Inhibition of the miotic indices by deltamethrin in a bone marrow study carried out in rat by **Agarwal *et al.*, (1994)** indicated that it is cytotoxic to rat bone marrow, possibly as a result of induction of microtubular and spindle disorders Its ability to induce bone marrow chromosomal disorders and erythrocyte micronuclei indicated that deltamethrin is also a clastogen.

Chauhan *et al.*, (1997) reported that cypermethrin and deltamethrin are genotoxic in the mouse bone marrow sister chromatid exchange through a genotoxic potentail evaluation in mice test and pyrethroid insecticides induced mitotic and chromosomal abnormalities indicate that they present a genotoxic risk to mammals, including humans.

The biological effects of chronic exposure to deltamethrin and dimethoate were studied in rabbits by giving weekly oral doses of 0.1 or 0.01 of LD_{50} for 20 weeks. At 4^{th} week, selected rabbits were sacrificed for analysis. Reduction in body weight and organ weight (adrenal, pituitary, thyroid, and testis) was noted. Dose dependent increases in liver and spleen weights were noted with the significant changes in biochemical parameters. Dimethoate appears to be more hazardous than deltamethrin **(Shaker *et al.*, 1998).**

Forshaw and Bradbury (1983) assessed the cardiovascular actions of 2 pyrethroids, deltamethrin and cismethrin, using the pithed rat. Deltamethrin, increased mean arterial pressure and differential pressure in the pithed rat, and the aortic output and mean systolic aortic pressure in the working heart. In the perfused mesentery, deltamethrin did not modify the action of exogenous noradrenaline (norepinephrine), but increased the response to 10 Hz stimulation. The cardiovascular effects of deltamethrin are evidently due to both increased catecholamine release in peripheral vascular beds, and due to a direct positive effect on the heart.

Akhtar *et al.*, (1992) measured the residues of deltamethrin in milk and tissues of lactating dairy cows fed with deltamethrin (2 or 10 mg kg-1 feed) for 28 consecutive days. Deltamethrin residues were higher relative to dose administered.

Deltamethrin (7.0 mg/kg body weight/day in corn oil for 15 days) exhibited a significant decrease in body weight of rats on day 15 of exposure. Administration of deltamethrin markedly increased the wet weight of the hippocampus and pons medulla region without much effect on the weight of frontal cortex, corpus striatum, hypothalamus, and cerebellum. A significant increase in the activity of monoamine oxidase, acetylcholinesterase and $Na^{+}K^{+}$-ATPase was observed in frontal cortex, hippocampus, and cerebellum due to exposure of deltamethrin **(Husain *et al.*, 1996).**

Studies were conducted on the influence of a synthetic pyrethroid permethrin on cholinesterase activities in six groups of rat. Daily oral administration of permethrin (24-120 mg/kg) for 7 days did not show significant alteration in erythrocytes, plasma or liver cholinesterase activities of rats **(Ayub Shah and Gupta, 2001).** An other repeated dose subacute toxicity studies on permethrin for 30 days resulted in marginal to significant increase in the serum transaminase activities and hyperglycemia at the highest dose (120 mg/kg) without appreciably altering the blood urea, nitrogen and total proteins **(Ayub Shah and Gupta, 2001a).**

Twenty weeks feeding of fenvalerate medicated ration at the rate of 4000 ppm and above resulted in significant decrease in the body weight of cockerels. Diminution in values of TEC, Hb and PCV was recorded in cockerels fed on higher concentration of fenvalerate. A significant ($p < 0.01$) decrease in serum albumin and glucose level and increase in levels of serum AST and ALT was also noted in high dietary groups. It is concluded that prolonged feeding of fenvalerate-medicated ration induces haemotoxic, hepatotoxic and myopathic effects in chicks **(Singh *et al.*, 2001).**

Madsen *et al.*, (1996) evaluated the effects of deltametrin and alpha-cypermetrin on the immune system in male rats through 28-day studies. Increased weight of mesenterial lymph nodes, decreased thymus weight in immunized animals and an increase in numbers of SRBC-PFC, splenic NK cell activity and effect on relative adrenal weight was seen in the 10 mg/kg body wt. The lowest effect level of alpha-cypermetrin was 12 mg/kg body weight/day based on increased relative adrenal weight

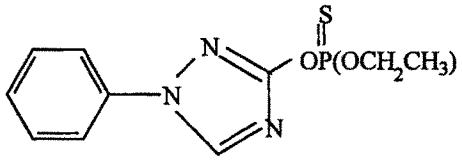
Eriksson *et al.*, (1992) reported persisting changes in neurochemistry and behaviour in mice after administration of very low doses of pesticides such as pyrethroids to the neonate. In another study, **Ray *et al.*, (2002)** examined the reproducibility of these effects during daily

administration of pyrethroids (bioallethrin or deltamethrin at 0.7 or 3.5 mg/kg) from the 10th to 16th postnatal days.

A subchronic biochemical toxicity study on lambda-cyhalothrin 2.5% EC in male wistar rats caused significant elevation of inorganic phosphorus, albumin, creatinine and significant decrease of chloride and alanine aminotranferase suggested a higher dose effect on the blood biochemical profile **(Krishnappa *et al.*, 2000).**

Organophosphates

Triazophos (Organophosphate)



CA Name: *O,O*-diethyl *O*-(1-phenyl- 1*H*-1,2,4-triazol-3-yl)phosphorothioate

Organophosphates (OP,s) were first recognized in 1854, but their general toxicity was not established until 1930s. Tetraethyl pyrophosphate (TEPP) was the first OP insecticide, which was developed in Germany during World War II as a by-product of nerve gas development. Organophosphates are derivatives of phosphoric acid. They are unstable, and therefore break down relatively quickly in the environment. Altogether, over 100,000 OP compounds have been screened for their insecticidal properties, of which over 100 have been developed for commercial use. In humans, poisoning symptoms include: excessive sweating, salivation and lacrimation, nausea, vomiting, diarrhoea, abdominal cramp, general weakness, headache and tremors. In serious cases, respiratory failure and death can occur. OP's are nerve poisons, which kill the target pest (usually insects). In general, organophosphates are ChE inhibitors and because of anticholinesterase activity, organophosphates have most frequently been the offending agents involved in acute poisoning.

The organophosphrous insecticides are not readily degradable in organisms such as insects, plants and animals. The mode of action of organophosphorus insecticides in animals and

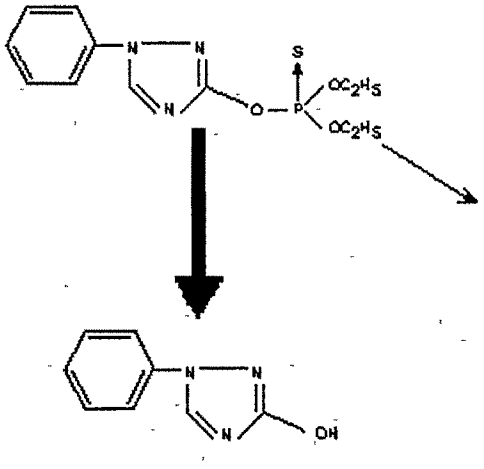
insects is similar and the target for the organophosphorus compound in the organism is also similar. Poisoning by organophosphate is actually toxification by endogenous acetyl choline, which builds up following the inhibition of acetylcholinesterase. The ultimate cause of death in insects is still in dispute but death in mammals is almost certainly due to paralysis of the striated respiratory muscles or paralysis of the respiratory center **(Hutson and Roberts, 1985).**

The biological activity of the organophosphorous compounds is due to the capacity of the central p- atom to phosphorylate the active site of the enzyme cholinesterase which is essential constituent of the nervous system. The phosphorylated enzyme is irreversibly inhibited and therefore is no longer able to carry out its normal function to rapid removal of and destruction of neurochrome (acetyl cholin) from nervous systems **(Shree Ramalu, 1995).**

The organophosphorus compounds (OPs) exert their toxicity mainly by inhibiting cholinesterase. Besides this, there are other metabolic events that are affected by the presence of these compounds. Numerous studies have shown that these compounds have neurotoxic and carcinogenic properties and adversely effect development and reproduction **(Cabello *et al.*, 2001; Mileson *et al.*, 1998 and Ecobichon, 1996).** Several OPs have shown to produce delayed on set neuropathy when administered acutely at a high dose, due to neurotoxic esterase of nerve tissue **(Ehrich *et al.*, 1994).** It is well known that acetylcholinesterase (AchE) and other cholinesterases (ChE) are sensitive targets of OP compounds, which exert their toxic effect primarily by inhibiting these enzymes irreversibily. **(Murphy *et al.*, 1968; Areekul *et al.*, 1981; and Cohen *et al.*, 1985).**

Salim *et al.* (1988) studied the effect of organophosphorus pesticides on semen characteristics of rabbit and demonstrated the deleterious effect on sperm formation together with the decline in testosterone secretion by the pesticide treatment. **Kimbrough and Gaines (1968)** reported deaths, and resorptions were increased in pregnant rats given a single high dose of organophosphorus pesticides on the 11^{th} day of gestation. However, these effects were associated with significant toxic effects on the mothers. Similarly, trichlorfon produced defects in offspring and developed cholinergic symptoms at high dose level given on days 6 – 15 of gestation **(Staples and Goulding, 1979).** A genuine teratogenic effect of moderate doses of trichlorfon has been demonstrated in pregnant pig **(Knox *et al.*, 1978).** Damaged seminiferous tubules were reported in mice treated with organophosphorus pesticides **(Krause and Homola, 1974)** and amiprophos was reported to cause some gonadotropic effects in adult cockerels **(Huang *et al.*, 1979).**

Metabolic Pathway of triazophos



minor amounts (<1%) excreted via feces

five minor metabolites excreted via feces (sum: 7.3%)

excreted via urine (ca. 43% in rats and 18% in dogs, respectively); traces excreted via faeces in dogs (<1%)

1-phenyl-3-hydroxy-(1H)-1,2,4-triazole

conjugation

Glucuronide and Sulfate esters

excreted via urine (ca 43% in rats and 76% in dogs, respectively).

Organophosphorous compounds cause a reduction in brain acetylcholinesterase activity and altered reproductive behavior in a number of species. The reduced acetylcholinesterase activity has been associated with decreased egg production and serum LH and serum progesterone levels **(Rattner *et al.*, 1982).** The standard dominant lethal test in mice was negative for dichlorvos **(Dean and Blair, 1976).** Possible mechanisms of toxicity from studies in trichlorfon and parathion in the rat are thought to be involved in interfering with steroid hormones binding to their receptors in the liver, adrenal, uterii and testes **(Trajkovic *et al.*, 1981).** In a case report, the organophosphate pesticide mercarbam crossed the placental barrier and caused the death of a 5-month foeetus **(Schardein, 1993).** There has been some indication that organophosphate (OPs), in general, may affect the menstrual cycle and cause an early menopause in humans Reproductive effects from exposure of mixture of organophosphates (OPs) have been documented by **Mattison *et al.*, (1983)** and **Nakazawa (1974)** among women in agriculture These effects included abnormal menstruation (e.g. hypermenorrhea, oligomenorrhea, and amenorrhea) and early menopause. On the other hand, **Willis *et al.*, (1993)** found no effects of pesticide exposure (including methyl parathion) on the pregnancy outcome among 535 women enrolled in a Southern California Community clinic perinatal programme

.

The acute interactive toxicity following exposure to two common organophosphorus (OP) insecticides, chlorpyrifos (CPF) and methyl parathion (MPS), was investigated in adult male rats by **Karanth *et al.*, (2004).** Cholinesterase inhibition in plasma, diaphragm, and frontal cortex was generally higher in rats treated sequentially with CPF first than in those treated initially with MPS from 4 to 24 h after dosing. Plasma and liver carboxylesterase inhibition at 4 h was also significantly higher in the CPF first (62-90%) compared with MPS first (22-43%) group. Carboxylesterase (CE) and A-esterase-mediated pathways are markedly less important for methyl paraoxon (MPO) than chlorpyrifos oxon (CPO) detoxification.

A chronic exposure of an organophosphate pesticide (fenthion) to 22 workers induced inhibition in serum acetylcholinesterase and butyrylcholinesterase. Post 3 weeks recovery, serum acetylcholinesterase and butyrylcholinesterase levels were raised significantly **(Misra *et al.*, 1985).** Another experiment on workers engaged in spraying of organophosphorous pesticide for various duration of exposure (3 to 15 years). Estimation of biochemical parameters revealed significant elevation in SGPT and ALP, and significant inhibition in cholinesterase (ChE) activity. Duration of exposure to OPs was significantly correlated with their levels of ChE, SGPT, and ALP but not with serum proteins **(Kamal *et al.*, 1990)**

Misra *et al.*, (1994) studied the cognitive changes to organophosphate (OP) exposure. A clinical and neurophysiological study was performed on workers engaged in spraying fenthion for the 1-14 years. The result suggests a subtle subclinical effect of chronic fenthion exposure on the cognitive functions and event related potentials.

Acute oral median lethal dose (LD_{50}) values of triazophos ranged from approximately 30 mg/kg body weight in mice, 65 mg/kg body weight in rats to about 500 mg/kg body weight in dogs, suggesting a significant species variation. A 3-generation reproduction study in rats suggested 10 ppm as a no-effect level on reproduction. Delayed neurotoxicity study in hens was negative suggesting that it might be too low for its delayed neurotoxic potential. A 2-year feeding study in rats indicated no increase in incidence of any particular type of tumor. A significant individual variation in plasma cholinesterase of human was reported due to effect of triazophos. NOEL for triazophos reported was 1.0 ppm in the diet (equivalent to 0.05 mg/kg body weight) in rats and 0.3 ppm in the diet (equivalent to 0.008 mg/kg body weight) in dogs. Estimation of temporary acceptable daily intake for man was 0-0.0002 mg/kg body weight **(FAO and WHO working and groups, 1982).**

For metabolism and fate study, triazophos was administered orally via stomach tube in Wistar-rats. In, rats receiving a single dose (2.76 mg triazophos), 76.3 % of the radioactivity was identified in the urine, and 21.0 % was identified in the faeces. In rats receiving a repeated dose (0.56 mg triazophos) after 12 days, 69.5 to 83.4 percent of the label was eliminated in urine, and 30.9 to 18.1 percent were eliminated in the feces. Most of orally applied triazophos was excreted via the urine and faeces, and little residue was left in the tissues and organs of rat **(Bock and Thier, 1976).**

Aging and spontaneous reactivation of human plasma cholinesterase (ChE) activity following inhibition by several organophosphate pesticides was studied *in vitro* by **Mason *et al.*, (1993)** in human plasma. Aging and spontaneous reactivation of ChE following inhibition by organophosphates was dependent on the nature of the phosphoryl group bound to the active enzyme site. Spontaneous reactivation occurred to the greatest extent with compounds containing a dimethoxy group. Compounds that contain ethoxy and thiopropyl groups induced the greatest degree of ChE aging.

Single oral administration of 1.0, 2.5 and 5.0 mg/kg body weight of triazophos produced mild to moderate toxic symptoms of anticholinesterase poisoning in buffalo calves. Triazophos inhibited dose dependent erythrocyte cholinesterase (50.3-82.4%) and plasma carboxylesterase (37.4-54.5%). **Sandhu and Bal, (1997)** observed elevation in the plasma levels of total proteins and blood urea nitrogen, following triazophos intoxication. In other

long-term experiment, triazophos in doses of 0.005 and 0.001 mg/kg/day for 150 days had not produced initial inactivation of erythrocyte cholinesterase and plasma cholinesterase **(Sandhu and Bal, 1998).**

The ductus arteriosus (DA) remains patent during the fetal period and is responsible for most of the blood flow in the pulmonary artery (PA) to the aorta. **Shirai et al., (1997)** investigated, whether P=S type organophosphorus insectcides administration late in pregnancy would cause any effect on the fetal ductus arteriosus in the rat. Rats on day 21 of pregnancy were orally administered fenthion (123 mg/kg) or diazinon (143 mg/kg). Fenthion or diazinon-treated groups showed no significant change in the "DA/PA" ratio at any point of measurement, compared to the saline-treated controls.

Oral treatment of gravid female rats with methyl parathion from gestation days 6 to 20, resulted in significantly lower AChE activity in the blood and brain of dams at the 0.30- and 0.60-mg/kg body weight/day. Pups from these dams treated directly from days 11 to 21 postpartum at the same dose levels showed suppression of blood and brain AChE activity at 0.30 and 0.60 mg/kg body weight/day on Day 21 postpartum (**Bevrouty *et al.*, 2001**).

In vitro effects of various organophosphate pesticides were studied on hemolysis, K^+ leakage and lipid peroxidation in rat erythrocytes. **Singh *et al.*, (2004)** found dose and time dependent increase hemolysis and K^+ leakage from erythrocytes. Lipid peroxidation in erythrocyte membrane was decreased.

Kaur *et al.*, (2000) studied blood biochemical and pathomorphological alteration in goat due to subacute exposure chlorpyriphos. Dose and time dependent inhibition of serum ChE with marginal to significant increase in serum total protein, cholesterol, creatinine and transaminase activity with the support of histopathological evaluation evoked in kidney and liver suggest severe toxicity of chlorpyriphos. Another experiment on chlorpyriphos in rats also dropped serum cholinesterase activity 85% in 100mg/kg body weight **(Verma *et al.*, 2002**). Inhibition of ChE activities in RBC and plasma were also reported by **Bhatnagar *et al.*, (1994)** due to exposure of dichlorvos (DDVP) in male mice.

Monocrotophos tested in lactating rats and pups at 0.3, 0.6 and 1.2 mg/kg two weeks premating, during gestation and lactation produced dose dependent and significant elevation in serum cholesterol, urea, nitrogen, GOT, GPT, and ALP was found with significant decrease in blood glucose and cholinesterase **(Adilaxamamma and Reddy, 1995).**

Singh and Sharma (1998) investigated embryotoxicity and teratogenic effect of monocrotophos, administered by oral route at 2 and 4 mg/kg b.wt. The duration of gestation as well as the body weight was affected following the treatment. Skeletal and visceral abnormalities were also higher at high dose group.

Immunotoxic effects of diazinon (DZN), an organophosphate insecticide were investigated by **Neishabouri *et al.*, (2004)** in the C57bl/6 female mice. Exposure produced histopathological changes in thymus and spleen and suppressed both humoral and cellular activities of the immune system at 25-mg/kg b.wt.

The effects of organophosphate insecticide methidathion (MD) on lipid peroxidation and anti-oxidant enzymes and the ameliorating effects of a combination of vitamins E and C against MD toxicity were evaluated in rat erythrocytes. Experimental groups were: control group, MD-treated group (MD), and MD + vitamin E + vitamin C-treated group (MD + Vit). MD and MD + Vit groups were treated orally with a single dose of 8 mg/kg MD body weight at 0 hour. Vitamins E and C were injected at doses of 150 mg/kg body weight, i.m. and 200 mg/kg body weight, i.p., respectively, 30 min after the treatment of MD in the MD + Vit group. Blood samples were taken 24 hours after the MD administration. The level of malondialdehyde (MDA), and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were studied in the erythrocytes. MDA level increased significantly in the MD group compared to the control group ($P < 0.05$) and decreased significantly in the MD + Vit group compared to the MD group ($P < 0.05$). The activities of SOD, GSH-Px, and CAT decreased in the MD group compared to the control group ($P < 0.05$). Only GSH-Px activity increased in the MD + Vit group compared with the MD group These results suggested that treating rats with MD increased LPO and decreased anti-oxidant enzyme activities in erythrocytes. Furthermore, single-dose treatment with a combination of vitamins E and C, 30 min after the administration of MD can reduce LPO caused by MD **(Altuntas *et al.*, 2002).**

The possible genotoxic effects of the organophosphorus insecticides methyl parathion and triazophos were evaluated by their ability to induce gene and chromosome mutations in male germ cells of *Drosophila melanogaster*. Sex-linked recessive lethal (SLRL), total and partial sex-chromosome losses (SCL), and non-disjunction (ND) assays were conducted. The routes of administration included adult feeding, injection, and larval feeding. Methyl parathion was unable to induce point mutations or chromosome mutations, although a small increase in the frequency of non-disjunction was detected after larval treatment. Triazophos induced point mutations when assayed in the SLRL test and induced a weak increase in the non-disjunction frequency, but gave negative results in the SCL test **(Velazquez *et al.*, 1990).**

The effects of early post-natal oral exposures to two organophosphorus insecticides on cholinergic parameters in whole brain and brain regions were investigated. Chlorpyrifos, a diethyl phosphorothionate, and methyl parathion, a dimethyl phosphorothionate, were studied. Incremental daily exposure regimens were used from post-natal day (PND) 1 to 21: chlorpyrifos, low dosage, 1 mg/kg PND 1-21; medium dosage, 1 mg/kg PND1-7, 2 mg/kg PND 8-14, 4 mg/kg, PND 5-21; high dosage, 1.5 mg/kg PND 1-7,3 mg/kg PND 8-14, 6 mg/kg PND 8-14; methyl parathion, low dosage, 0 2 mg/kg PND 1-21; medium dosage, 0.2 mg/kg PND 1-7, 0.4 mg/kg PND 8-14, 0.6mg/kg PND 15-21; high dosage, 0.3 mg/kg PND 1-7, 0.6 mg/kg PND 8-14, 0.9 mg/kg PND 15-21. Cholinesterase (ChE) activity was measured in freshly thawed and ground tissue with a 5-minute continuous assay because of the rapid reactivation of the dimethyl phosphorylated ChE. During the time of treatment, animals from the high dosages displayed about 60% brain ChE inhibition, about 50% for the medium dosage, and about 20% for the low dosage. Muscarinic receptor densities, as monitored with 3H-quinuclidinyl benzilate, were reduced about 20% during the time of the greatest ChE inhibition in the whole brain preparations, with M1/M3 receptor levels (monitored with 3H-4-diphenylacetoxy-N-(2-chloroethyl) piperidine) affected more greatly than M2/M4 receptor levels (monitored with 3H-AF-DX-384); receptor densities were the same as in controls after the cessation of the treatment. Choline acetyltransferase activity, monitored radiometrically, showed a slight decrease after the cessation of treatment at PND 30. The minor reductions in choline acetyltransferase activity observed at PND 30 were not observed in brain regions at PND 40. The changes in receptors and enzyme activities observed during development had not appeared as permanent alterations of brain neurochemistry **(Moore *et al.*, 2003).**

Tamura (2001) showed that fenitrothion is an AR competitive antagonist with a KB value of 2 18 X 10(-8) M using HepG2 human hepatoma cell line transfected with the human AR plus androgen responsive luciferase reporter gene, MMTV-luc. Its value was close to that of flutamide, a known AR antagonist. It was also demonstrated that the AR antagonistic activity decreases as alkyl chain length of derivatives increases and m-methyl substitution on the phenyl ring enhances the AR binding affinity. However, the axon derivative and metabolite of fenitrothion, 3-methyl-4-nitrophenol, had no AR antagonist activity. No electron donating effect of the substituents on the phenyl ring was indicated. Based on the generalization of essential structural requirements on AR binding affinity, we proposed working hypothesis as follows: First, interaction at A-ring binding site is essential for ligand binding. This property mainly depends on the negative atom charge of the functional group as H-bond acceptor. Second, interaction at D-ring binding site determines the character of ligands, whether ligands act as an agonist or an antagonist. This depends on the H-bond strength between the functional group and the amino acid residues at D-ring binding site. As interatomic distance increases as to 10.94 A the character of ligands changes from antagonism to

agonism. This working hypothesis will help to predict the identification of environmental contaminants capable of interacting with AR receptor. Through *In vivo* studies, it was determined whether fenitrothion blocks AR action and quantified the effect on the androgen dependent tissues in the immature male rat by Hershberger assay. Fenitrothion reduced the organ weight of ventral prostate, seminal vesicle plus coagulating glands, and levator ani plus bulbocavermosus muscles. These investigations demonstrated that fenitrothion might cause a disruption of androgen-related sexual differentiation by directly interfering with the androgen signaling pathways resulting in abnormal development of the male reproductive tract.

This study investigated whether increased exposure during pregnancy to organophosphates, restricted fetal growth and/or shortened length of gestation. **Eskenazi *et al.*, (**2003) evaluated their effect in a study population comprising 485 pregnant women receiving prenatal care at six clinics in the Salinas Valley region of California. Significant increase in gestational age and in head circumference and length controlling for gestational age was found with increasing pesticide metabolite levels.

Chlorpyrifos-methyl primarily affects the nervous system through inhibition of cholinesterase. Chlorpyrifos-methyl (1/10th or 1/30th of LD50) was given as a single oral dose to lactating females and their corresponding pups. Chlorpyrifos-methyl was transferred through the lactation route and was able to produce a significant decrease in the activity of brain and serum AChE of both the delivered mothers and their pups. Total glutathione (GSH) content was significantly increased in the livers of suckling pups Lactating mothers showed a significant increase in their liver GST activity, while an adverse effect on serum GST activity was recorded. The present study suggested that lactating females and their corresponding pups might be at risk of significant biochemical alterations, following a single oral dose of chlorpyrifos-methyl **(El-Din Bayoumi *et al.*, 2003).**

Fenitrothion (O,O-dimethyl-O-(4-nitro-m-tolyl) phosphorothioate) is an organophosphate insecticide that is known to antagonize the androgen receptor. To evaluate the ability of fenitrothion to disrupt androgen-dependent sexual differentiation in the male rat, pregnant Crl:CD(SD)BR rats were administered fenitrothion by gavage at 0, 5, 10, 15, 20, or 25 mg/kg/day (n = 6-11/group) from gestation day (GD) 12 to 21. Fenitrothion exposure induced cholinergic stress in the dams and fetal mortality, at the same dose level as found to elicit the transient but significant effects on Anogenital distance (AGD) and nipple retention in the male offsprings **(Turner *et al.*, 2002).**

Nuvacrone, a fast acting organophosphorus pesticide, was tested for its genotoxic effects (chromosomal abnormalities) on adult males, pregnant females of mouse as well as their

embryos. Highly significant and dose dependent increase in structural abnormality and abnormality of germ cell (spermatocytes), inhibition of ChE and gradual increase in gamma glutamyl transferase was induced suggeseting that nuvacrone can be genotoxic and biochemically harmful (**Abd el Aziz *et al*, 2003**). Six organophosphates were tested for developmental toxicity. Specifically, each organophosphate was administered by oral gavage to gravid rats on gestation days 6 to 15. Organophosphates produced biologically significant maternal cholinesterase inhibition without affecting fetal brain cholinesterase activity or any other embryo or fetal parameters and results also suggested that cholinesterase activity inhibition was the most sensitive indicator of maternal organophosphate exposure while conducting a developmental toxicity study (**Astroff *et al.*, 1996**)

Organophosphate pesticides, Dichlorvos and Quinalphos were administered intraperitoneally at the doses 2.5, 5, 10 mg/kg in mice and were found to be significantly mutagenic in both the chromosomal and micronuclear studies The frequency of micronuclei increased significantly both in polychromatic and normochromatic erythrocytes. In foetotoxic study, the pesticides significantly increased the proportion of dead foetuses and resorbtions in uterus. Gross malformations and skeletal abnormalities were also found in the treated mice (**Majeeth *et al.*, 1989**).

Deltamethrin (Synthetic Pyrethroid) + Triazophos (Organophosphate insecticide)

The pesticides combination product (Deltamethrin 10g/L + Triazophos 350g/L) is commercially available in India under the trademark **Spark® 36 EC**. It is easy to handle co-formulation insecticides for effective broad-spectrum pest control in cotton for effective control of sucking and biting insect pests.

The use of multiple pesticides in agriculture is much more effective than for a single pesticide. Therefore use of combination pesticides is more demanding and also economically cost effective to the farmers. Many combinations such as metalaxyl + mancozeb, quinolphos + cypermethrin, Prefenophos +cypermethrin, Chlorpyriphos +cypermethrin, deltamethrin + triazophos etc are available. In assessing the toxicity of combination, it is important to consider chemical and /or physical interaction of the individual chemical, the effect that one chemical may have on absorption metabolism and pharmacokinetic characteristics of another, and the possibility of interaction between parent compound and metabolites (**Sood, 1999**). Effects produced by binary mixture of chemicals are classified as follows:

Independent effect: Substances qualitatively and quantitatively exerts their own toxicity, independent of each other

Additive Effect: Material with similar qualitative toxicity produce a response that is quantitatively equal to the sum of the effect produced by the individual constituents.

Antagonistic Effect: Materials oppose each others toxicity or interferes with the toxicity of another; a particular example is that of antidotal action.

Potentiation Effects: One material, usually of low toxicity enhances the expression of toxicity by another, the result is more severe injury.

Synergistic effect: Two materials, given simultaneously produce toxicity significantly greater than anticipated from that of either material. The effect differs from potentiation in that each substance contributes to toxicity and the net effect is always greater than additive.

The pesticides treatment resulted in decline in body weight, libido ejaculate volume, sperm concentration and semen fructose and an increase in abnormal and dead sperm and methyl blue reduction time **(Qureshi, 1994).** Recently introduced different combination pesticides effectively control both sucking pests as well as internal borers as the combination pesticides have different modes of actions **(Walunj *et al*, 1998).** Organophosphorus and synthetic pyrethroids are widely used in agriculture to control crop-pests and livestock ectoparasites, respectively **(Nolan *et al.*, 1979; Nolan and Edwards, 1982).**

The amount of information available indicates that combination of many pesticides may have additive, potentiative or synergistic effects **(Ball *et al.* 1954; Frawly *et al.*, 1957; Dubois, 1959; Kreitzer and Spann, 1973).** *In vitro* toxicity studies on combination of chemicals indicated the unjustifiability of general application of additive rule for risk assessment of exposure to mixture of chemicals **(Bianichi *et al.*, 1994, Piatti *et al.*, 1994 and Marinovich *et al.*, 1994).**

Triazophos and deltamethrin are the examples of wide spectrum pesticides used in variety of agriculture and non-agriculture application. In recent years, synthetic pyrethroids in combination with organophosphorous (monocrotopos, triazophos profenophos, etc.) are used to control associate sucking pests and foliage feeding larvae. There are indications that potentiation of toxicity may occur when deltamethrin is combined with some organophosphorus compound **(Environmental Health Criteria 97, 1990). Haines *et al.*, (2001)** also reported that organophosphate accentuates the effect of deltamethrin. However,

there is no information available on effect of pesticide combination on reproductive system and hence the present research has been designed to assess the effect of deltamethrin 1% + triazophos 35% on reproductive system of albino rat with the following objectives:

1. **To determine the LD_{50} of the combination and evaluate the dose response relationship.**
2. **To evaluate the potentiation effect of deltamethrin due to triazophos**
3. **To evaluate the effect of deltamethrin1%+tiazophos 35%EC on haematological and serum biochemical alteration.**
4. **To evaluate the effect on reproductive system of the rats by following sperm motility, testicular head count, epididymal sperm count and morphological evaluation.**
5. **To evaluate the maternal toxicity of combination pesticide.**