INTRODUCTION
Ever since the dawn of civilization, man has continuously endeavoured to improve his living conditions. He has been altering his ambience to meet his prodigious needs, his every action affecting the very biological world he inhabits. His interference with the niche of his co-inhabitants has made them to come in conflict with his welfare in the form of pests. Though, pests and diseases are parts of natural processes that are going on since the beginning of the universe and biological evolution, their upsurge is now viewed as man's own creation. The word pest has no defined biological meaning. It implies to any living organism that diminishes the value of resources in which man is interested. It is estimated that over 1,00,000 species of pests destroy food which could be fed to, 135 million people besides acting as vectors for diseases (COINDS, 1986). Man's war against pests is perennial and almost eternal. Pest control has now become the chemistry of human survival. Various techniques and materials were adapted to combat pest menace, pesticides being a major discovery.

Success has it's own cost and this is very true with the use of pesticides in our day to day life as their indiscriminate use is accompanied by a train of disaster and hence has now become a matter of unedifying controversy. Pesticide use is justified and viewed as an inseparable component of agricultural and public health protection. However, it is decried on the grounds that they are pernicious to the environment and jeopardize the personal health of those who come in contact with them directly or indirectly and has become an issue of global concern.
HISTORICAL PERSPECTIVE OF PESTICIDE USE

Pesticides are being used since time immemorable. The earliest known records dates back to classical Greece. Eber's Papyrus written about 1550 B.C. lists preparation to expel fleas from a house. Homer has mentioned the fumigant value of burning sulfur by Odysseus to purge the hall and house of the court (Gallo and Lowryk, 1991). Carvings on stone tablets has revealed use of rat poison before 1000 B.C. By 900 A.D. Chinese were reported to have used arsenals to control garden lizards. During 14th to 18th century mineral oils, arsenals, tobacco extract etc were used. Pyrethrum and soap were used in the early 19th century and the middle of the same century marked the beginning of the first systematic scientific studies into the use of chemicals. By 1900, use of arsenic's and other inorganic heavy metal derivatives were so wide spread that it led to the introduction of what was probably the first pesticide legislation in the world (Hassal, 1982).

It was during the Second World War; the synthetic pesticides were introduced. The discovery of the insecticidal properties of DDT by Paul Miller in 1939 marked the birth of modern insecticide chemistry. These relatively stable, long lasting broad-spectrum organochlorine insecticides appeared as an answer to counteract many undesirable pests. Later, their use declined because of low rate of biodegradability, lipophilicity and bioaccumulation (Edwards, 1973). These persistent chemicals were replaced by less persistent organophosphates and carbamates, which on environmental grounds was considered as a movement in the right direction.
The organophosphorus compounds first appeared in 1944 as the result of success of German industry in finding modification of chemical warfare agents useful for insect control. In addition to being good insecticides and acaricides, chemicals belonging to this group have provided defoliants, fungicides, herbicides and nematicides. Some desirable characters of organophosphorus compounds are:

1. Broad spectrum
2. Highly selective systemic insecticide
3. Short residual action
4. Material with prolonged activity for control of insects of medicinal importance.

Unlike the earlier contact insecticides and stomach poisons they act as neurotoxins inhibiting the cholinesterase enzyme, which is required for the breakdown of acetylcholine at the nerve junctions (O’Brien, 1967).

The indiscriminate use of organophosphorus compounds has increased tremendously during the past decades. The ill effects caused by pesticides are voiced by many. "Silent Spring", written by Rachel Carson in 1962, catalyzed the environmental movement against pesticide use. Agencies like World Health Organization (WHO) are carrying out extensive study on pesticide use and its impact. In view of this, a joint meeting on pesticide residue was organized in 1963 and in 1972 a mathematical model was set up to estimate the deaths caused by pesticide toxicity. United Nations
Environmental Programme (UNEP) is actively involved in health related monitoring of environmental quality since many decades.

Cognizance of pollution hazards associated with pest control chemicals and methods is evident in 6th plan document of Indian Government, which declares that, "while efforts would be made to develop varieties resistant to various pests and diseases, an integrated pest control strategy will be adopted aiming at optimizing the natural controlling factor and ensuring balanced and cheap insecticide which are non-toxic and would fit in with the environment".

Our former prime minister Shri Rajiv Gandhi in his foreword in the report of the world commission on environment and development has stated, "In the name of giving more food and providing more comforts we have polluted the rivers and seas, heated up the globe through the accumulation of carbon dioxide and even depleted the ozone that shielded the biosphere from harmful cosmic radiation's. Ecological degradation affects developing countries more fundamentally then it does to the developed one. Our efforts to provide the minimum needs can be sustained in the long term if we protect our ecology".

This is a testimony to wide spread concern of pesticide pollution and the need to have a systematic scientific study to enunciate the adverse effect caused by them.
NEED FOR PESTICIDE TOXICITY STUDY

Pesticides are used throughout the world, the intensity of use depending on a number of factors, such as dominant crops, stage of development of country, climatic condition and prevalence of pests.

The overall pesticide use in agriculture in terms of amounts applied per hectare has been very much greater in Japan, Europe and USA, than in rest of the world (Edwards, 1986). About 75% of the total world consumption is used by them (Anon, 1985). Though the usage of pesticide in developing countries is comparatively low, the population dependent on agricultural sector is high (63%), thus relatively more people are involved in the handling of pesticides or live in the vicinity of pesticide used (WHO, 1990).

The developed countries export 20% of pesticides to developing nations, sometimes as trade and other times as aid, hiding the actual toxicity data.

In developed countries advances in methods of application of pesticides like ultra low volume spray are used. In contrast many of the application equipment used in developing countries are poorly maintained and supplies of sprays are inadequate. Pesticides are often applied with inefficient hand-sprays, ox-drawn sprayers or dusting equipment or inadequate protective clothing is used. In addition in tropical developing countries the hot
climatic condition, malnutrition and the general lack of technical education and training make pesticide use more dangerous to the operator (WHO, 1990).

Of the pesticide destined for non-agricultural purposes many are used by Governments in public health programs, frequently for the control of vector borne diseases like malaria, filariasis, schistosomiasis and tryptosomiasis (Copplestone, 1985; Edward, 1986). The use of pesticides in public health gives rise to possibility of exposure of spraying staff, the general public and the non-target organisms as well. Pesticides do not know when to stop killing, they harm the non-target organisms in ways often disguised and unknown.

Although, the pesticides are intended for application in fields and orchards they may be wafted by air, washed by rains into surface waters or may be leached into underground basins. Hence, the pesticide problem does not limit itself to the area of application, but emerges from local via regional into global proportions.

Occupational exposure in workers of pesticide manufacturing unit may be a major source. The waste from an insecticide factory may be inadvertently discharged into nearby water bodies, affecting the inhabiting fauna.

The rate of global consumption of organophosphorus compounds is higher. In urban areas organochlorine pesticide use is declined and is replaced by pyrethrins, pyrethroids and organophosphorus insecticides such as chlorpyrifos, dichlorovos, fenitrothion, fenthion, malathion and temephos
Organophosphorus pesticides are likely to continue to be the most important insecticide used in the developing countries (WHO, 1990). 70% of acute occupational poisonings are caused by organophosphorus compounds (Jeyaratnam et al., 1987) and that 5% of these led to persistent neurobehavioural effects (Eskenazi and Maizlish, 1988).

Various regulatory mechanisms are being adapted for pest control but however, pesticide will remain as an important additional tool even within this framework. Hence, a balance should be struck between the benefit and harm caused by pesticides taking humanity and the ecological world into account.

PESTICIDE TOXICITY TO HUMANS

Human populations are exposed to pesticides in different ways and varying degrees. It may be either intentional (suicides and homicides) and some are unintentional which may be either occupational or non-occupational exposure (Davies, 1980). Majorities of deaths in the tropical countries are reported to be due to pesticide poisoning (Ranbird and Oneill, 1994).

Pesticides are reported to cause physiological, pathological and genetical changes in humans, however, the toxic effect depends upon the health status of individual exposed. Malnutrition and dehydration are likely to increase sensitivity to pesticides. Water deprivation make people more susceptible to the effect of anticholinesterase pesticide (Baetjer, 1983). A rise
in ambient temperature often makes the toxic effect of pesticides worst (Kagan, 1985).

Accidental exposure to pesticides accounts for about 4-5% of all accidental poisonings, this proportion is higher in developing countries than in industrialized countries (WHO, 1990). About 60-70% of all cases of unintentional acute pesticide poisoning are due to occupational exposure (Copplestone, 1985).

In a group of 34 workers chronically exposed to organophosphate compounds, the serum pseudocholinesterase activity was depressed and incidences of peripheral neuropathy were observed (Ernest et al., 1995).

Misra et al., (1985), found macular changes in 19% of 79 subjects exposed to organophosphorus pesticide Fenthion, as compared with 3 of 100 controls with an average duration of 7.9 years. Paraquat induced chronic fibrotic change in the survivals of suicide attempts (WHO, 1984).

A report from a Californian chemical company noted azoospermia and oligospermia in their workers (Rengam and Synder, 1991). In utero exposure to organophosphate resulted in birth defects and mental retardation in children. Brain defects in ventricles, corpus callosum, choroid plexus and septum pellucidum were observed (Sherman, 1996).
Chromosomal aberrations have been noted among pesticide workers with symptoms of poisoning (Dulout, 1985).

Wysocki (1985), compared serum concentration of IgA, IgM, IgG and C-3 complement levels among 51 men with occupational exposure to chlorinated pesticide. IgG was increased while IgM and C-3 were lower among the exposed workers.

Subtle behavioural changes have been noted in several cross sectional epidemiological studies among pest control workers, farmers and manufacturing workers and behavioral impairments have also been associated with pesticide exposure in serious accidents among agricultural workers (Maizlish, 1987; Eskenazi and Maizlish, 1988).

The organophosphorus compounds, in addition to producing acute neurological effects and cholinergic symptoms have also been shown to be associated with intermediate and delayed neurological and physiological effects (Savage, 1988). According to NIOSH report, organophosphate insecticides cause impaired psychomotor function of CNS and also cause polyneuropathy (Proctor et al., 1991).

Gershon and Shaw (1961) reported schizophrenic and depressive reactions in individuals exposed to organophosphorus insecticides.
PESTICIDE TOXICITY TO DOMESTIC ANIMALS

Man has been domesticating animals since ages and hence any change brought by him are bound to affect the domestic animals. The use of pesticides in combating both external insects and around barns results in intentional or accidental exposure of large and small animals. These presents the hazard that the environment or the feed may carry residues that may later cause clinical problems in domestic animals or may be found as residue in animal product and thus affect public safety through food chain (Ivie and Dorough, 1977; Shlosberg et al., 1980; Osweiler et al, 1985).

The efficiency of pesticide toxicity in domestic animals varies due to the variety of domestic animals encountered and the interspecific differences occurring in them.

Pesticide exposure in domestic animals is reported to be a common cause of risk and frequent toxicity (Oehme, 1977). Organophosphates and carbamates are commonly used besides organochlorine in swines (Mount et al., 1980), The heaviest exposure of animals to pesticides occur in beef cattle (Oehme,1991). However, organophosphates cause the most common problem in domestic animals.

Watson et al., (1971) reported a case wherein seven cows were killed by disulfoton by chewing bags that were blown into their pasture from an adjacent sprayed potato field. In another case oil – containing triaryl
phosphates was reported to have induced neurotoxicosis in four dairy heifers. The symptoms included dyspnea, dysuria, polyuria, tympanites, incoordination, hind limb weakness and flaccid paralysis (Prantner and Sosalla, 1993).

Errors in formulation, dosage calculation, selecting the correct pesticide, method of application and time may lead to poisoning of large number of animals at times (Oehme, 1977; Dickson et al., 1984). The systemic organophosphorus compounds present some special problem if not applied at appropriate times, if used in over dose or if applied to animals specifically sensitive to their properties (Osweiler et al., 1985).

PESTICIDES TOXICITY TO WILD ANIMALS

The wild life whose food chain is uncontrolled and long, faces a serious threat of pesticidal problem. It is very difficult to predict the type of pesticidal toxicity in wild life. All the factors involved in adsorption, distribution, metabolism and elimination of a pesticide determine the amount of the compound in the wild life (McKim et al., 1985). Pesticides may not induce direct effect on wild animals, but however their effect on habitats or homeostatic mechanisms may alter survival, density, diversity and reproduction (Morrison and Meslow, 1984).

Atmospheric transmigration of pesticides may result in wild life exposure to pesticide (Shukla and Srivastava, 1992). The pesticide burdens
of certain bird species such as the Peregrine falcon, *Falco peregrinus*, can be attributed to overwintering in countries which still use pesticides that are banned in United States (Henny *et al.*, 1982).

Stickel (1975) has reviewed organophosphate pesticide toxicity to wild animals. In a study conducted, azodrin was reported to cause mass mortality of birds of prey (Mendelson, 1977). Inhibition of brain acetylcholinesterase activity in songbirds exposed to fenitrothion was reported during aerial spraying of forests (Busby *et al.*, 1981).

**ORGANOPHOSPHATE INDUCED NEUROTOXICITY**

Organophosphorus insecticides are compounds, which are esters of phosphoric, phosphonic or phosphorothioic or related acids. Phosphates and phosphonates tend to be of higher mammalian toxicity than phosphorothioates (WHO, 1986). Organophosphorus insecticides produce a sequelae of neurological deficit. Their effect as a potent anticholinesterase is a well-documented fact (O’ Brien, 1967 ; Doull *et al.*, 1986 ; Niesink *et al.*, 1996). Acetylcholinesterase depression has been used for years as an index to document exposure to organophosphorus compounds (Saleh *et al.*, 1994). The inhibition of tissue acetylcholinesterase at ganglionic cholinergic synapses in the brain and at the neuromuscular junction is responsible to cause clinical features and the duration of organophosphate intoxication (Besser *et al.*, 1989). The cardinal symptoms observed in cases of organophosphate poisoning in animals are related to deficits of cholinergic
systems. Moser (1995) observed that high dose of organophosphates produced clear autonomic signs of cholinergic over stimulation and lower doses produced a range of effects. There is initial hyperactivity and the subsequent convulsions are followed by tetanic paralysis (Brown, 1978). Pope et al., (1991) investigated that developing mammals are more sensitive to a variety of acetylcholinesterase inhibiting organophosphates. However, not all symptoms manifested can be explicable wholly on acetylcholinesterase inhibition (Abou-Donia, 1978; Chambers et al., 1990).

Some organophosphates are reported to cause an intermediate syndrome in man, which is manifested 24 to 96 hours after poisoning (Senanayake and Karalliedde, 1987; Karademir et al., 1990). It is characterized by acute ventilatory insufficiency due to paralysis of respiratory muscles. Proximal muscles and motor cranial nerves are also affected.

Another distinctive effect of some organophosphorus compounds that is not related to acetylcholinesterase inhibition is a neurodegenerative disorder termed organophosphate induced delayed neuropathy (OPIDN). OPIDN has been characterized as a distal neuropathy, which primarily affects the longest and largest diameter axons in spinal cord and peripheral nervous system (Cavanagh, 1973; Bouldin and Cavanagh, 1974). OPIDN is characterized by a delay period of 6-14 days prior to onset of ataxia and paralysis. Smith et al., (1930) were the first to report a case wherein 20,000 people were paralyzed by drinking "ginger jake" contaminated with TOCP, an organophosphate. Extensive clinical, pathological and experimental

Neurotoxic esterase (NTE) inhibition is deemed to be the target site by several authors, but the current tentative explanation is that promotion should involve a site other than NTE and that NTE inhibitors initiate OPIDN with different efficacy. Impaired axonal flow (Pleasure et al., 1969; James and Austin, 1970) and defective protein metabolism (Patton et al., 1985; Abou-Donia et al., 1988; Abou-Donia, 1995) have been conjectured as putative mechanisms of delayed neuropathy.

Organophosphates are reported to induce neurobehavioral changes in animals (Anitha et al., 1998). Extremely small doses of certain organophosphates are also capable of inducing behavioral changes in experimental animals (Wolthuis and Van Werach, 1984). With few exceptions, behaviour slowed continuously throughout the exposure and returned to normal as exposure continued, though the time course of recovery varied depending on the behaviour (Overstreet, 1984).

Several electrophysiological parameters have been studied during organophosphate intoxication. Post tetanic potentiation was measured in motor neurons innervating slow tonic plantaris muscle in cats treated with diisopropyl phosphorofluoridate (Lowndes et al., 1974) and in hens dosed with
TOCP (Durham and Ecobion, 1984). In another study with repeated dosing of
two potent organophosphorus agents to male rats, the peripheral nerve
conduction increased and reduced refractoriness (Anderson and Dunham,
1985). An electrophysiological study performed in the hens treated with
subneuropathic doses showed that relative refractory period and duration
curve were altered (Robertson et al., 1987). Desi and Nagymajt (1988),
reported dichlorvos induced alterations in EEG recordings of the brain and
peripheral nerves of rats.

Some of the biochemical studies conducted in the nervous system
under organophosphate toxicity include the assessment of changes in
catecholamines and 5-HT in various brain regions (Fiscus and
have also been found to alter the neurotransmitter metabolism in rat brain
(Nag, 1992). Alterations in phosphatases, ATPases, lipid profiles and protein
metabolism in fish and rat brain are also documented (Joshi and Desai, 1983;
Vadhva and Hasan, 1986; Swamy et al., 1992; Karunakaran et al., 1994). The
effect of organophosphorus compounds on the cerebral metabolic effects has
been studied in rats (Miller and Medina, 1986). Repeated exposure to
organophosphate compounds causes decrease in cholinergic muscarinic
receptors in brain and peripheral tissues (Fitzgerald and Costa, 1993).
Ultrastructural studies focusing the effect of organophosphate compounds
have been reported in spinal cord and nerve fibres of rats (Hasan et al.,
1979; Tadokoro et al., 1985), hens (Baron and Johnson, 1964; Husain et al.,
1995).
DDVP TOXICITY

DDVP (O, O – dimethyl-O-2-2- dichlorovinyl phosphate) a derivative of phosphoric acid (Vettorazi, 1976) is a widely used organophosphorus compound. DDVP was first described in the year 1952, but its potential as an insecticide was discovered in 1955 (Mattson et al., 1955). DDVP has been commercially manufactured and used throughout the world since 1961 as a contact and stomach insecticide (WHO, 1988 a). Owing to its high vapour pressure more than any other Organophosphorus compound, it is used to produce insecticidal concentrations in closed places, while it is impractical for use on field crops. It is mainly used for control of insects in tobacco and other warehouses, mushroom houses, green houses, animal shelters, homes restaurants and other food establishments (Gallo and Lowryk, 1991). It is used to spray aircraft to prevent accidental introduction of unwanted pest species (Harte et al., 1993). Its selective action has made it to be used as an antihelmintic in humans and domestic animals (Pyror et al., 1970; Cervoni et al., 1969). It is also impregnated in dog and cat flea collars. It has extremely fast knock effects and residual control of 2-3 weeks may be obtained (Extonet, 1998).

DDVP residues are encountered in the environment not only because of its use directly but it also occurs as a conversion product of another organophosphate pesticide trichlorfon (WHO, 1992) and also as a breakdown product of schistosomiasis drug, metrifonate in in vivo conditions (Nordgren, et al., 1981; Hinz et al., 1998).
The EPA (Environmental Protection Agency, USA) has classified DDVP as highly toxic and it is cited on the hazardous substance list by many authorities, because of its mutagenicity and there is only a small margin of safety for other effects. In India, technical grade DDVP is manufactured at three units. About 1390 MT of indigenous DDVP was consumed in India during 1994-95 (Pestology, 1997). India has placed DDVP on restricted pesticide list (Rengam and Synder, 1991)

Environmental fate and metabolism:

DDVP is volatile and non-persistent in the environment with rapid decomposition in humid air, water and soil both by abiotic and biotic processes. DDVP degrades fairly rapidly with half-life of 2-8 hours in soils ranging from sand to silt. It’s mobility being inversely correlated with the soil organic matter content (EPA, 1987). On land, DDVP is leached into underground water, where it gets hydrolyzed and degraded with half life of 1.5 – 17 days (Howard, 1991).

In water, DDVP hydrolyses with a half-life of approx. 4 days. At pH 4.0 degradation is slow and rapid at pH of 9.0 (Half-life is 4.5 Hrs.). In a study conducted to assess the persistence and fate of DDVP in sea sediments of east coast of India it was observed that DDVP had low stability and was rapidly hydrolysed by the cations present in the sea-sediment (Sarkar and Sen-Gupta, 1986). It hydrolyses yielding dimethylphosphoric acid and dichloroacetaldehyde. DDVP is rapidly lost from the leaf surfaces by
volatilization and hydrolysis. (WHO, 1988a). DDVP is not stored in the body and has not been detected in the milk of cows or rats, even when given in doses that produce severe poisonings (Tracy et al., 1960). When rats and mice inhaled DDVP (90 mg/m³ for 4 hrs) none or very little (upto 0.2 mg/kg) was found in blood, liver, testis, lung and brain. The highest concentrations were found in kidneys and adipose tissue, however, it rapidly disappeared from kidneys with a half-life of approx. 14 min (WHO, 1988a). The rapid degradation is due to the presence of degrading enzymes like esterases in blood and in other tissues (Blair et al., 1975).

**Effects on organisms in environment:**

**Microorganisms:** The effect of DDVP on microorganisms is variable and species dependent. DDVP in concentration of 0.1 – 100 mg/L has little or no toxic affect on microorganisms degrading organic matter in sewage (WHO, 1988a).

**Invertebrates:** Invertebrates are more sensitive to DDVP, levels of 0.05 µg/L is capable of producing deleterious effects. Crustaceans have greater sensitivity to DDVP (McHenery et al., 1991). The LD₅₀ for sand shrimp is 0.004 ppm, grass shrimp 0.015 ppm, hermit crab 0.045 ppm and for *Daphnia pulex* 0.07 µg/L (Extonet, 1998).

**Fishes:** The acute toxicity of DDVP for both freshwater and estuarine species of fish is moderate to high (WHO, 1988a). The LC₅₀ values for stripped mullet
is 0.23 ppm, blue gill (LC$_{50}$ 24) 1mg/L and Rainbow trout 0.1 ppm (Extonet, 1998).

**Birds**: High oral toxicity for birds is reported. The LD$_{50}$ for mallard is 7.8 mg/kg (Extonet, 1998).

**Bees**: DDVP is highly toxic to honey bees (Worthing, 1983).

**Plants**: Among plants cucumber, roses and chrysanthemum are sensitive to DDVP but is non-phytotoxic when used as directed (Harding, 1979).

**Toxicological Effect:**

**Acute toxicity:**

DDVP may cause toxicity due to inhalation, skin absorption or ingestion. The sequence of development of systematic effects varies with the route of entry. Symptoms of acute poisoning develop during exposure or within 12 hours (usually within four hours) of contact. The acute oral LD$_{50}$ for rats is 56 – 80 mg/kg, mice 90 – 175 mg/kg, chick 14.8 mg/kg. Dermal LD 50 for rat is 75 –107 mg/kg. Intraperitoneal mode has an LD$_{50}$ of 18.5 ± 1.3 mg/kg for rats and for mice it is 28-30 mg/kg. By inhalation mode of exposure for 4 hours the LC$_{50}$ is 0.22 mg/Kg for mice and 0.20 mg/kg for rats.

DDVP causes acetylcholinesterase inhibition as typical of all organophosphorus compounds and this has been studied in fishes (Rath and Misra, 1981), rats (Teichert et al., 1976; Bhatnagar et al., 1994) and in dogs (Ward and Glickberg, 1971). Maximum inhibition generally occurs within one hour and is followed by rapid recovery. Primary school children given a single
oral dose of metrifonate induced reduction of plasma cholinesterase levels (Nhachi et al., 1991).

After inhalation of DDVP, respiratory and ocular effects are the first to appear often within few minutes of exposure. Ocular effects include, blurring of distant vision, tearing, rhinorrhea and frontal headache. After ingestion gastro-intestinal effects such as anorexia, nausea, vomiting, abdominal cramps and diarrhea appear within 15 minutes to two hours. After skin absorption, localized sweating and muscular fasciculations in the immediate area occur usually within 15 minutes to four hour, skin absorption is somewhat greater at higher ambient temperatures and is increased by the presence of dermatitis (Taylor, 1985).

With severe intoxication excess of acetylcholine at the neuromuscular junctions of skeletal muscle causes weakness aggravated by exertion, involuntary twitching, fasciculation's and eventually paralysis. Effects on the central nervous system include giddiness, confusion, ataxia, slurred speech, convulsions, coma and loss of reflexes (Menz et al., 1971). Cases of delayed neurotoxicity were reported after consumption of unspecified quantity of DDVP in suicide attempts (Vasilescu and Florescu, 1980; Wadia et al., 1985).

CHRONIC EFFECT:

Daily exposure to concentrations, which are insufficient to produce effect following a single exposure, may result in the onset of symptoms. In a
study of 13 workers exposed for 12 months to an average concentration of 0.7mg/m$^3$ the erythrocyte cholinesterase activity was reduced by approximately 35% and the serum cholinesterase activity was reduced by 60%. Other medical tests conducted at regular intervals were normal (Menz et al., 1971). Other effects reported in workers with chronic exposure to DDVP include impaired memory and concentration, disorientation, severe depression, irritability, confusion, headache, speech difficulties, delayed reaction times, sleepwalking and insomnia. Continuing daily absorption of DDVP may cause influenza like illness characterized by weakness, anorexia and malaise (Morgan, 1982).

A dietary level of 5ppm produced a detectable reduction of blood cholinesterase in only four days (Durham et al, 1957). Continuous exposure for a week to concentrations initially in the range of 1.4 –2.0 mg/m$^3$ produced marked depression of blood cholinesterase in monkeys (Gallo and Lowryk, 1991). Rapid drop in blood cholinesterase but no illness occurred in monkeys exposed for two hours / day for four days to concentrations greater than 7mg/m$^3$ (Extonet, 1998). Cattles fed with DDVP at a dose of two mg/kg in the form of slow release formulations reduced blood cholinesterase (Pitts and Hopkins, 1964). Raine et al., (1989) reported no changes in the activity of SGOT and SGPT in buffalo calves treated with DDVP.

Life time exposure of rats upto 4.0 mg / kg of DDVP affected their behaviour (Schulz et al., 1995).
Cases of dermatitis on DDVP exposure have been documented. Muller (1970) has reported DDVP to cause dermal irritation in domesticated dogs and cats, which had impregnated flea collars around their necks. DDVP was shown to cause a persistent contact dermatitis in one worker and capable of inducing an allergic contact dermatitis (Mathias, 1983).

Carcinogenic effect:

Though DDVP has been classified as a human carcinogen there are very few reports on significant carcinogenic effect of DDVP. In a study conducted by National Cancer Institute (1977), on mice and rats for evaluating carcinogenic effect of DDVP, no statistically significant increase in the incidence of tumors was observed. In another study conducted for two years on rats by administrating DDVP in corn oil by gavage, 5 day / Week showed incidence of carcinogenic effect. Adenomas of the exocrine pancreas were evident. Mononuclear cell leukemia was more frequent in dosed rats than in control. Multiple fibroadenomas and carcinomas occurred in female rats (Extonet, 1998).

Reproductive effects:

To assess the effect of DDVP on reproduction several experiments were carried out but there is no evidence that DDVP affects reproduction. In a conventional reproduction studies male and female rats were fed with a diet containing 100 ppm DDVP prior to mating and throughout gestation and
lactation in female. These studies showed no harmful effect on reproduction (WHO, 1988a). A three generation test in rats with dietary level up to 500 ppm showed a negative result on reproduction (Withetrup et al., 1971). Estrous was delayed for 10 days in female rats exposed to DDVP (Timmons et al., 1975). Marked increase in morphologically abnormal sperms was demonstrated in mice treated with DDVP (Wyrobek and Bruce, 1975).

**Mutagenic effect:**

DDVP is an alkylating agent and binds in *in vitro* to bacterial and mammalian nucleic acids (WHO, 1988a). Direct methylation of DNA has been demonstrated in *in vivo* (Lawley et al., 1974; Saleh et al., 1994). DDVP at doses ranging from 500 μg M and 1000 μg M caused a dose responsive DNA strand breaks in isolated rat hepatocytes (Yamano, 1996).

**Neurotoxic effect:**

Besides its role as an anticholinesterase, DDVP is reported to cause delayed neuropathy in man and test animals (Johnson, 1981) and is also reported to induce biochemical changes. Ali and Hasan (1977) reported diminution of level of some of the free amino acids in brain and spinal cord of rats treated intraperitoneally with DDVP (3 mg/Kg) for 15 days. Levels of dopamine, norepinephrine and 5-hydroxy-tryptamine (5-HT) were significantly altered in different regions of brain in rats while in the spinal cord 5-HT was increased following chronic treatment of DDVP for 10 days (Ali et al., 1979).
DDVP induced increase in the rate of lipid peroxidation in the different regions of rat brain with a chronic exposure for 10 days (Hasan and Ali, 1980). Remarkable depression of locomotory activity and concurrent alterations in brain monoamine levels has been reported by Ali et al., (1980). Exposure of fishes to DDVP daily for 7 days induced dose related differential alterations in lipid levels and increase in lipid peroxidation in various brain regions and spinal cord (Vadhva and Hasan, 1986). DDVP is reported to inhibit glutaminase and glutaminase synthetase activity in rat brain (Nag, 1992).

Some pathological studies reported include structural alterations in rat cerebellum and spinal cord. An abnormal increase in the number of mitochondria in the spinal cord was found. Myelin degeneration was detected in spinal cord and myelin-figures were occasionally noted within oedematous dendritite profile (Hasan et al., 1979). Repeated administration of DDVP (40 mg/Kg body weight) for 10-21 days caused myelin pallor and microvacuolation of the white matter in spinal cord of rats (WHO, 1988 a).

Rats given an acute dose of 88mg/kg of DDVP for a period of 6 weeks, revealed significant changes in central and peripheral nervous system functions as indicated by EEG recordings (Desi and Nagymajt, 1988).
MONOCROTOPHOS TOXICITY

Monocrotophos (O,O — dimethyl — O — (1 — methyl — 3 — oxo — propenyl) phosphate) is a broad spectrum, fast acting organophosphorus insecticide with both systematic and residual contact actions (WHO and IPCS, 1993). It was introduced in 1965 (Gallo and Lowryk, 1991). It is particularly effective against Lepidoptera, Homoptera and certain Coleoptera. The main use of monocrotophos is for foliar application to cotton besides it’s use in cash crops like chillies, sugarcane, pulses, vegetables and fruit orchards (Janardhan and Sisodia, 1990). The EPA (Environmental Protection Agency, USA) has classified monocrotophos has highly toxic (Class I toxicity).

In India, technical grade monocrotophos is produced at 2 units (Ray, 1989). About 4635 MT of monocrotophos was consumed during 1994-95 in India (Pestology, 1997).

Ecological fate and Metabolism:

Monocrotophos is rapidly degraded and is not persistent in the environment. Monocrotophos and its metabolites are rapidly degraded in the soil, biologically to complete mineralisation. It is degraded mainly via hydrolysis and oxidation. Volatilization appears to be the major factor in the rapid loss of residues following application. The breakdown products are of low toxicity (WHO and IPCS, 1993). However, it seems to be persistent in river waters. Under sunlight the original compound was recovered even after
8 weeks in river (Ray, 1989). In plants, N-hydroxy compound is formed in small amounts followed by the nitrogen- dimethylated product and the hydrolyzed fragments. The residual activity of monocrotophos on various parts of vegetable crops was reported to vary from 11.97 to 88.70 ppm (Narkhade et al., 1977) and the residues were reported to last for 9 to 11 days (Puri, 1975). In mammals 45% of monocrotophos injected is excreted in 6 hours and within 24 hours, 58.4 and 5.1% has been recovered from urine and feces (Gallo and Lowryk, 1991).

**Effect on organisms in the environment:**

Monocrotophos has wide variations in toxicity to different species (Janardhan et al., 1986).

**Micro-organisms**: non-toxic to micro-organisms.

**Invertebrates and fishes**: Highly toxic to aquatic invertebrates. LC₅₀ (96 hr) for copepods is 240 µg/L. It is moderately toxic to fish. The LC₅₀ (24 hr) for rainbow trout is 12 mg/L and bluegill 23 mg/L. (Exto net, 1998)

**Birds**: Monocrotophos is highly toxic to birds. The oral LD₅₀ for duck is 3360 µg/L, Quail 4 mg/kg and for pigeons 2.8 mg/kg. (Exto net, 1998)

**Bees**: It is highly toxic to bees. The LD₅₀ ranges between 33 to 84 µg / bee (Anderson and Atkins, 1968).

**Plants**: When applied under cool conditions, monocrotophos has been known to cause phytotoxic effects in apples, cherries, peaches and sorghum (WHO and IPCS, 1993).
TOXICOLOGICAL EFFECT:

Acute toxicity:

Monocrotophos is a potent cholinesterase inhibitor (Gupta et al., 1984; Gallo and Lowryk, 1991). The symptoms of toxicity are similar to those caused by organophosphorus insecticides. In human exposure to monocrotophos causes sweating, salivation, headache, weakness, nausea, abdominal pain, muscle fasciculation, blurred vision, confusion and constricted pupils (Ray, 1989; Gallo and Lowryk, 1991). Monocrotophos has high oral toxicity (Skirpy and Loosi, 1994). In a case of accidental ingestion of monocrotophos, the person was unconscious for four days with bronchopneumonia and thrombophlebitis (Ray, 1989). The acute oral LD$_{50}$ values for mice is 15 mg/kg and for rats 18-20 mg/kg. By intraperitoneal treatment the LD$_{50}$ for mice is 3.8 mg/kg while for rats it is 5 mg/kg. Monocrotophos is reported to inhibit acetylcholinesterase in wistar rats (Ramesh et al., 1996) and in domestic fowls (Sandhu et al., 1991). Blood enzymes and plasma proteins were decreased in buffaloes (Sandhu and Malik, 1988). In an experiment conducted in human volunteers working for 8 hrs in tobacco field, treated with monocrotophos, the plasma and RBC cholinesterase levels were depressed (Guthrie et al., 1976). Intermediate syndrome is documented in many exposed to monocrotophos, the symptoms of which are manifested in 1-4 days after poisoning (Gallo and Lowryk, 1991).
Chronic toxicity:

In a long term feeding study conducted on rat and mouse, the no observable effect level (NOEL) for cholinesterase inhibition was reported to be 0.03 ppm in rats and 1 ppm in mouse (WHO and IPCS, 1993). Daily dosing of monocrotophos to wistar rats for 14 days by oral intubation caused changes in blood biochemistry and haematological parameters. The weight of spleen among the various organs studied, was significantly decreased as compared to control (Kumar et al., 1998).

Reproductive effects:

A multigeneration reproduction study showed that 3 mg/kg of monocrotophos was toxic to pups of F2 generation (WHO and IPCS, 1993). Monocrotophos administered orally at the rate of 3.5 mg/kg for 3 alternative days induced oligospermia and increased pre-implantation losses and hence proved detrimental to male fertility (Rathnasooriya et al., 1992). The toxic effect of monocrotophos on female reproductive performance includes lower body weights and reabsorbtions of foetus in maternal rats. Fertility, parturation indices were reduced in dose dependent fashion, while viability and lactation indices were highly reduced in response to higher doses of monocrotophos (Adilaxmamma et al., 1994).
Teratogenic studies:

Monocrotophos revealed teratogenic potential in a study conducted in rats and rabbits (Janardhan et al., 1984).

Mutagenic Studies:

An invitro study of monocrotophos on human lymphocytes increased chromosomal aberrations (Rupa et al., 1988). In another study conducted in rats, it was concluded that monocrotophos interferes with the integrity of DNA and induces micronuclei (Vijaya Kumar and Janardhan, 1988).

Carcinogenic effect:

In a two-year study on rats at highest dietary concentration of 0.5 mg/kg/day, no evidence of carcinogenecity was seen (WHO and IPCS, 1993).

Neurotoxicity:

Monocrotophos is reported to inhibit brain acetylcholinesterase in rats (Swamy, et al., 1992; Ramesh et al., 1996). Monocrotophos fed orally in sublethal doses to male albino rats for different durations ranging from 1 hour to 16 days elevated the activity of aspartate aminotransferase and alanine aminotransferase while glutamate dehydrogenase activity was inhibited in different brain areas (Swamy and Mohan, 1992). In a study conducted by Nag
(1992), monocrotophos incubated with the mitochondrial and microsomal fractions of rat brain, moderately inhibited $\alpha$-Keto acid activated glutaminase activity. Proteins and phosphatase activity is altered by monocrotophos in the brain of *Tilapia mossambica* (Joshi and Desai, 1983). Chronic daily dosing of monocrotophos (6mg / Kg body weight) by oral intubation to male rats for a period ranging from 1-16 days, induced alterations in protein metabolism in brain. Acidic, neutral and alkaline proteases and acid and alkaline phosphatases were elevated in all brain areas (Swamy et al., 1992). Monocrotophos does not cause delayed neuropathy in hens (WHO and IPCS, 1993). Monocrotophos administered orally to rats and mice in doses of 1,2 and 4 mg/kg caused neurobehavioural effects such as hypothermia and reduced locomotory activity, proving to be potent CNS depressant (Mandhane and Chopde, 1995). No reports are currently available on the electrical activity of nervous system in response to monocrotophos.

**CHOICE OF ANIMAL**

Mice (*Mus musculus*) were preferred for the present investigation considering the following advantages:

1. Easy availability throughout the experimental period.
2. Results obtained can be extrapolated to higher mammals.
3. They get acclimatized quickly to laboratory condition and are easy to handle.
4. The small size of the mice is an added advantage as its spinal cord can be kept viable in isolated media for a longer period.
CHOICE OF PARAMETERS FOR STUDY

The nervous system is the most important and complex organisation, which controls and integrates the various body functions and helps in maintaining the internal stability. The nervous system possesses a number of special features not found in other organ systems. Once damaged it has only a modest capacity for repair and regeneration especially in the central nervous system. This limited ability to regenerate suggests that subtle damages to the nervous system can have serious, long-lasting effects.

The spinal cord is an important part of the central nervous system as a conduit of motor, sensory and sphincter function. It is the primary centre for reflex action for the trunk and limbs and consists of main conducting pathways to and fro from higher centres in the brain to the peripheral nerves.

Any toxicological insult to the spinal cord causes structural alterations with accompanying biochemical and functional changes. Damage to it causes loss of motor functions and if sufficiently extensive promotes paralysis. Previous studies carried out indicate that in case of organophosphate toxicity, the motor functions are disrupted. This implies that the spinal cord may be a probable target for pesticidal action along with the brain, hence the study of the activity of spinal cord in response to such toxicants assumes importance.

DDVP and Monocrotophos were selected as organophosphate toxicants. DDVP is known to induce organophosphate induced delayed
neuropathy (OPIDN) in man as well in test animals (Johnson, 1981), while Monocrotophos does not induce OPIDN (WHO and IPCS, 1993). Hence, a comparative study of these two toxicants will reveal their efficacy in inducing neurotoxicity in the spinal cord.

Neurotoxic insults are not an all-or-non phenomenon, since gradations of effects are induced by neurotoxicants. Neurotoxic compounds have characteristic and individual properties and often cause distinctive morphological and biochemical lesions. However some relatively subtle and non-specific features also constitute part of the overall effect of the neurotoxicant. In the present study an attempt is made to study the efficiency of low acute doses of DDVP and monocrotophos to promote subtle and relatively non-specific effects in the spinal cord of mice other than inhibition of acetylcholinesterases, in contributing to the overall toxicity

The electrical potential generated in the nervous system acts as an index of its functional status. The electrophysiological techniques are utilized as an important research tool by neurotoxicologists to assess the effect of toxicants on the nervous tissue by recording the electrical activities. Further, ionic channels which play a pivotal role in normal functioning of nervous system may also be a putative target for a variety of chemicals, including natural toxins and pesticides alike. The role of ionic channels in neurotoxicity can be well assessed by using specific channel blockers.
The study of rate of conduction of nerve impulses was also undertaken to evaluate the functional status of the spinal cord under the pesticide stress.

Isolated mammalian nervous system preparations have been extensively used for the study of biophysics, physiology and pharmacology of the CNS (Kerkut and Wheal, 1981). The choice of study of isolated spinal cord in the present study was made considering the following advantages it offers:

1. Improved mechanical stability; movements caused by respiration and blood pressure are eliminated.
2. Control over neural activity entering the spinal cord; the supraspinal influences are exterminated.
3. Improved visibility of structures within the preparations allows proper placement of the recording electrodes by visual mean at the precise recording site.
4. The whole spinal cord preparation retains the long tracks allowing the conduction of impulses over the entire cord to be investigated, as in in vivo condition.

All the animals in the present study are sacrificed by decapitation to avoid the depressing actions induced by the use of anesthetics on the synapses (Fox et al., 1982).

Since there is considerable in – animal and between animal variations in electrical activities, and since the population events of the neurons in the
spinal cord are being recorded, a large number of data has to be acquired. Hence, in the present electrophysiological work a series of recordings from a single isolated whole spinal cord were recorded to check the repeatability and the electrophysiological activities of ten such isolated spinal cords were acquired for each dose and time interval effect, to minimize the errors and to facilitate statistical analysis.

Unlike most toxins, organophosphates cause damage first to the motor cells and then to the sensory cells (Niesink et. al., 1996). Hence, the ventral horn region of the spinal cord which constitutes of motor neurons (Chatterjee, 1994b) was chosen for the purpose of electrophysiological recordings.

Considering the complexity of nervous tissue, it is difficult to predict the toxic responses generated by the given pesticide entity solely by electrophysiological recordings. A combination of biophysical or clinical and biochemical studies are recommended to assess pesticide toxicity (Misra et al., 1985; Hayes and Laws, 1991).

Pesticides like most toxicants may disrupt the integrity of membrane structure and its associated enzymes even at relatively low dosages (Hochster et al., 1973). Any kind of disturbance in the activity of animal during chemical toxicosis will be reflected through changes in enzyme activity patterns. Therefore, enzymological studies along with the changes in the
quantitative biochemical constituents would be useful and form meaningful indices of toxicant action.

The mammalian lysosomes are known to be responsive to many type of stressor (Dingle and Fell, 1969). Lysosomal hydrolytic enzyme like acid phosphatase is associated with tissue damage and degenerative diseases (Tietz, 1970). Further, the lysosomal associated enzymes like non-specific esterases may also be activated and playing some role in neuropathy. Hence, it appears reasonable to assume that the lysosomal enzymes are involved sooner or later in DDVP and Monocrotophos induced acute toxicity.

Alkaline phosphatase which occurs in cell membranes (Bretaudiere and Spillman, 1984) is supposedly involved in various secretory and transport processes and is also known to take part in the blood-brain barrier (Shaffi and Habibulla, 1977). Hence, the assay of this phosphatase enzyme was undertaken which may throw some light on membrane transport impairments if any, induced by DDVP and monocrotophos.

Several toxicants are known to manifest their toxicity through mediation of reactive oxygen species. The spinal cord, bearing high lipid contents (about 70%), is more vulnerable to tissue damage by these radicals. Further, the role of free radicals like superoxide anions and defense against them assumes importance in neurotoxicity (Zaleska and Floyd, 1985). Xanthine oxidase is a prime generator of superoxide and may be a significant exacerbating factor in several pathological states, while the antioxidant enzyme superoxide
dismutase is an evidence of an increased oxidant milieu (Bondy, 1994). Hence, the study of these two enzymes was undertaken to see if the generation of reactive oxygen species is involved in the induction of neurodegeneration.

Proteins constitute an important group of macromolecule substance and occupy a pivotal place in both structural and dynamic aspects of living tissue (Frutton and Simmonds, 1975). The quantitative estimation of total proteins serves as a measure of tissue damage as well as altered protein metabolism induced by the toxicants. Similarly assays of mitochondrial proteins were carried out to throw light on metabolic activities.

Tissue creatinine analysis was undertaken as it is an index of endogenous protein metabolism (Chatterjee, 1994a).

The mammalian spinal cord has significantly greater cholesterol content, hence the effect of pesticides on cholesterol level was assessed. Triglyceride content was also estimated.

Carbohydrate occupies an essential role in the energy production of the CNS (O'Neil, 1974). Hence, glucose and glycogen content of the spinal cord was evaluated.
OBJECTIVES OF STUDY

1. To study the acute toxicity of the prototype pesticides DDVP and monocrotophos, on spinal cord and to contemplate the temporal sequential changes induced by it.

2. To assess the spontaneous electroresponsive property of mammalian central nervous system with respect to spinal cord under pesticide induced toxicosis.

3. To elucidate the effect of organophosphate pesticides on the ionic conductance responsible for membrane excitability, which may lead to altered motor co-ordination.

4. To study the action of DDVP and monocrotophos on the conduction of nerve impulses.

5. To study the biochemical alterations induced in spinal cord with respect to lysosomal activity and reactive oxygen species.

PROPOSED PLAN OF RESEARCH

The present investigation was undertaken keeping in view the lacunae existing pertaining to DDVP and Monocrotophos induced neurotoxicity. The literature survey revealed that most workers have concentrated on chronic effect or short-term daily dose studies. There are no reports on single sublethal acute dose effect studies in mammals, particularly in mice.
Secondly, except for a few reports on toxicant effect on spinal cord, most researchers have restricted their studies to different parts of brain; hence an investigation to assess the response of spinal cord to the above mentioned two pesticide toxicant stress in mouse (*Mus musculus*) was necessitated.

Most of the toxicity studies carried out express the LD$_{50}$ dose in terms of mg/kg, and there is absolutely no report in terms of ppm. DDVP, is a volatile organophosphate compound. This volatility coupled with low doses selected for studies creates formulation problems. The formulation based on V / V basis, by use of positive displacement pipettes for dispensing the pesticide, brought the problem under control (Tomaszerowski and Scheer, 1984). Secondly, monocrotophos is also highly soluble in water (WHO and IPCS, 1993). Hence, for the present study, the pesticide suspension was made in physiological saline (VN) and the toxicity evaluation (LD$_{50}$) was performed in terms of ppm of pesticide used.

The route of toxicant administration determines the barriers that the toxicant will encounter and the intensity of the toxicity produced (Loomis and Hayes, 1996). The intraperitoneal mode of drug introduction was selected, as this method has the advantage of bypassing the natural body orifices and secondly, specific amounts can be directly introduced into the test animals.

A set of ten animals, each for respective doses and time intervals was used for both control and experimental doses of 1 ppm, 5 ppm, 10 ppm and
100 ppm and for different time intervals (6, 12, 24, 48, 72, 96, 120 and 240 hours) for DDVP and monocrotophos respectively.

On termination of the time intervals, the spontaneous electrical activities were recorded from the isolated spinal cord to evaluate the responses elicited by the two pesticide toxicants used.

Many research reports in the literature, document the usefulness of biochemical parameters in the discipline of neurotoxicology. Biochemical assays were performed by using standard methods. The enzyme activities viz.: acid phosphatase, alkaline phosphatase, non-specific esterases, xanthine oxidase and superoxide dismutase were carried out. Quantitative biochemical constituents such as total proteins, mitochondrial proteins, creatinine, cholesterol, triglycerides, glucose and glycogen were also evaluated. The pooled data were statistically analysed.