

**ELECTROPHYSIOLOGICAL
AND BIOCHEMICAL STUDIES OF
SPINAL CORD OF MOUSE
(*MUS MUSCULUS*) UNDER THE EFFECT
OF DDVP AND MONOCROTOPHOS**

Thesis

Submitted to the Goa University for the degree of

**DOCTOR OF PHILOSOPHY
IN ZOOLOGY**

By

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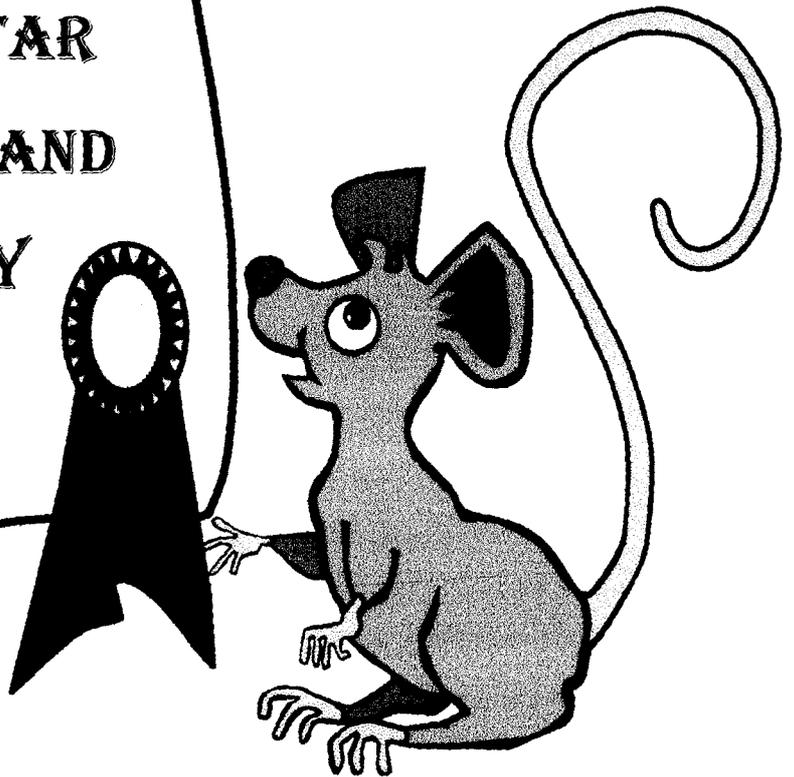
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The literature conceiving the problem investigated has been cited. Due acknowledgements have been made wherever facilities have been availed of.

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CERTIFICATE

This is to certify that
the thesis entitled

**" Electrophysiological and biochemical
studies of spinal cord of mouse (*Mus
musculus*) under the effect of DDVP and
monocrotophos"**

submitted by

Miss. Renuka Ramachandra for the award of
degree of Doctor of Philosophy in Zoology is
based on the result of laboratory
experiments carried out by her under my
supervision. The thesis or any part thereof
has not previously been submitted for any
other degree or diploma.



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PREFACE

The global challenge for production of more food for the ever-increasing population and the management of public health, necessitated continued use of pesticides as a desirable tool in the hands of agriculturists and hygienists. Pesticide usage though vital, poses a tremendous threat to humans and other non-target organisms alike. Besides the harm caused at the site of application, the transmigration of pesticides to remote areas affects the habitat or homeostatic mechanisms, which further alters the very survival of organisms and aggravates the problem globally. Even with the foregoing caveat, regarding the environmental sensitivity of pesticidal compounds, their use is not declined as much as expected. Hence there is an imperative need to gain and disseminate knowledge concerning the effects of the pesticides on living systems. Research as a prevention component serves to identify and evaluate pesticides that may produce such deleterious effects.

Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate and toxicology of the harmful pesticides loosened upon the earth. Among the sequelae of this broad emphasis is a need for a study of the biochemical and physiological changes promoted by these pesticides in particular organ system of mammals to throw light on the mechanism of pesticide action.

Various forms of pesticides are available ranging from heavy metals, organochlorine, organophosphates and carbamates to synthetic pyrethroids.

Organochlorine use increased with industrialization, however, due to large-scale ill effects soon their use declined and there was a concomitant rise in the use of organophosphorus compounds.

Today, organophosphorus pesticides are not only employed in controlling the pestilent organisms, while improving agrarian economy but also are extensively used in public health sector and as medicine in animal husbandry, thus affecting the environment in toto.

The present investigation comprising electrophysiological and biochemical studies is an attempt to elucidate the neurotoxic effect of two organophosphate pesticides, DDVP and monocrotophos. DDVP is widely used for agricultural and domestic practices for its pesticidal and schistosomicidal effect. Monocrotophos is a broad – spectrum insecticide, widely used for foliar application in cotton fields.

The Thesis is presented in four chapters:

The first introductory chapter gives a brief historical account of pesticide use, review of literature pertaining to organophosphate neurotoxicity and toxicity induced by DDVP and monocrotophos. It also focuses on the need for pesticide study, objectives, choice of parameters and experimental animal. It also includes an outline of the proposed research plan.

The second chapter deals with materials and methods. It gives the physical and chemical properties of DDVP and monocrotophos. The toxicity

evaluation method, electrophysiological data acquisition and analysis and biochemical assay methods are presented.

The observations and results are compiled and presented in the third chapter. The first half deals with electrophysiological recordings while the second half deals with the alterations in biochemical parameters.

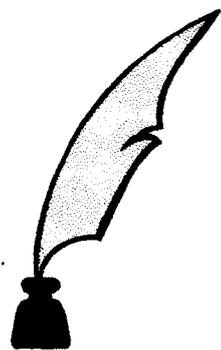
The findings are discussed in the fourth chapter. This is followed by summary, recommendations for future research and bibliography.

The present investigation is an effort to add further information into the stream of current knowledge on the mechanisms of pesticide induced neurotoxicity.

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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 its 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 been 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 (Coppelstone, 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

(WHO, 1988 b). 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 (Coppelstone, 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 Dorrough, 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 over wintering 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

description of OPIDN are summarized and reviewed by Davies (1963), Cavanagh (1973), Johnson (1980), Davies and Richardson (1980), Abou-Donia (1981), Zech and Chemnitius (1987), Carrington (1989), Lotti (1992) and Johnson and Glynn (1995), Gayathri *et al.*, (1998).

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 phosphoroflouridate (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 VanMeeter, 1977; Ali *et al.*, 1979; Gupta *et al.*, 1984). Organophosphates 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 (McHenry *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 (LC₅₀ 24) 1mg / L and Rainbow trout 0.1 ppm (Extonet, 1998).

Birds: High oral toxicity for birds is reported. The LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 LC₅₀ 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 $0.7\text{mg}/\text{m}^3$ 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 $1.4 - 2.0\text{ mg}/\text{m}^3$ 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 $7\text{mg}/\text{m}^3$ (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 administering 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 upto 500 ppm showed a negative result on reproduction (Withettrup *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 *invitro* 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 μM and 1000 μM 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 as 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 LD₅₀ values for mice is 15 mg/kg and for rats 18-20 mg/kg. By intraperitoneal treatment the LD₅₀ 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 1ppm 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 3mg/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 reabsorptions 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 carcinogenicity 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 α -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 LD₅₀ 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 (Tomaszeroski 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 (V/V) and the toxicity evaluation (LD₅₀) 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.

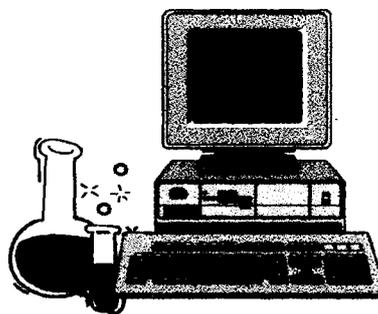
A set of ten animals, each for respective doses and time intervals was used for both control and experimental doses of 1 ppm , 5 ppm, 10 ppm and

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.

MATERIALS AND METHODS



Swiss albino mice (*Mus musculus*) were procured from vaccine institute, Belgaum, karnataka. Healthy, 10-week-old male mice weighing 20-22 gms were chosen for the present study. The mice were housed in clean polypropylene cages (Tarsons, 290 x 220 x 140mm) with paddy husk for bedding. Prior to the experiments, animals were acclimated for a minimum period of 10 days to laboratory conditions with temperature 30^o C, humidity 75 – 80 % and 14 / 10 hours of light and dark regime. They were fed with standard laboratory mice feed *ad libitum* and were given clean tap water for drinking.

PESTICIDES

Technical grade DDVP (90% pure) and Monocrotophos (95% pure), procured from Ciba Geigy Ltd, Goa were used as pesticide toxicants.

DDVP

SYNONYMES AND TRADE NAMES

Dichlorvos, Chlorvinphos, Nogos, Nuvan, Vapona, Dede vap, Atgard, canogard, Task, Equigard, Aparap, Divipan, Herkal, Krecalvin, Lindan, Mafu, marvex, Nerkol, Oko, Phosvit, Vaponite, No-pest strip.

CHEMICAL NAME :

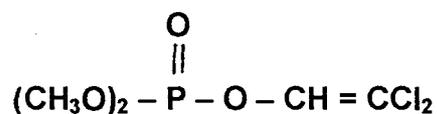
O, O – dimethyl – O –2, 2 – dichlorovinyl phosphate

NIOSH: TC 0350000

CAS Registry number : 62 – 73 – 7

Molecular formula: $C_4H_7Cl_2O_4P$

Structure:



Molecular Weight: 220.93

PHYSICAL PROPERTIES :

Colourless to amber liquid with a mild aromatic odor. Density at 25^o C is 1.415. The boiling point is 35^o C at 0.05 mm Hg and 120^o C at 14 mm Hg. The vapour pressure is 1.2 x10⁻⁴ mm Hg at 20^o C. It's solubility is about 1% in water and 3 % in kerosene and mineral oils.

CHEMICAL PROPERTIES:

DDVP is miscible with alcohol and in aromatic and chlorinated hydrocarbon solvents except glycerol and mineral oils. Concentrates are readily decomposed by strong acids and bases. Dilute DDVP is hydrolyzed rapidly in the presence of moisture.

FORMULATIONS:

Available as E.C., granules, oil miscible liquids, aerosol generator, bait.

MONOCROTOPHOS

SYNONYMES AND TRADE NAMES :

Azodrin, nuvacron, Bilobran, apodrin, Monocron.

Chemical Name:

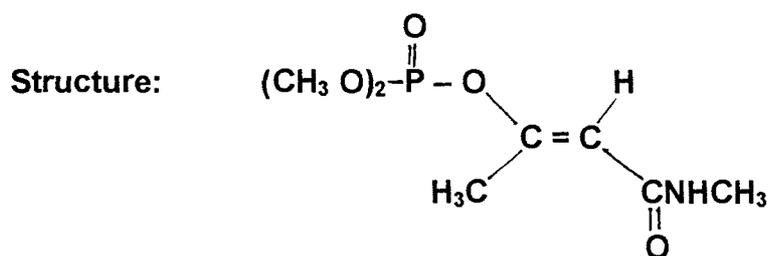
Dimethyl (E)- 1- methyl - methylcarbamolvinyl – phosphate.

NIOSH: TC 4375000

CAS Registry number:

E isomer: 6923 – 22 – 4

Molecular formula: $C_7H_{11}NO_5P$



Molecular weight: 223.2

PHYSICAL PROPERTIES:

Pure monocrotophos is in the form of colourless crystals with a melting point of 54 – 55⁰ C. Technical material is a reddish brown solid with a melting point of 25 – 30⁰ C. It has a mild ester odor. Boiling point is 125⁰ C at 6.65 x 10⁻⁴ mm Hg. Vapor pressure is 7x10⁻⁵ mm Hg at 20⁰ C. It is soluble in water, acetone and alcohol but only slightly soluble in mineral oils.

CHEMICAL PROPERTIES:

Monocrotophos is unstable in low molecular weight alcohols, glycols but stable in ketones. It decomposes on some inert materials. It is corrosive to iron and brass. It decomposes slowly in water. The half-life in a 2 ppm solution at pH 7 and 38⁰ C is 23 days; at pH 4.6 and 100⁰ C, the half life is 80 min.

FORMULATIONS:

Available as S.C., ULV liquid, granules.

TOXICITY EVALUATION

The lethal dose (LD_{50}) effect for both the pesticide toxicants were ascertained by probit analysis method described by Finney (1971), simplified by Busvine (1971). Suspensions of the toxicants were made in physiological saline (V/V). An initial rough dose experiment was performed to select suspected doses based on prior information gathered on DDVP and Monocrotophos toxicity. A second series of analysis was carried out to narrow the range of toxic doses using 4 animals in each experimental group. The final toxicity evaluation test involved randomly assigned sets of 10 animals of same age, sex and weight. The test animals were administered different doses of formulations, which previously had been estimated to induce mortality between 10 % – 90%. The animals were kept under observation and their mortality was noted from the end of 6 hours to the end of 240 hours from the period of toxicant administration. The LD_{50} was statistically determined for different time interval. A regression line was plotted to detect the LD_{50} by the following method:

Different doses of pesticides administered, number of animals used per set, observed mortality rate in percent and their corrected mortalities are represented in column I – IV respectively of Table 1.01 to 1.16. The empirical probit values corresponding to the percent mortality were determined from the probit transformation Table 7 of Busvine, (1971) and placed in column VI of Table 1.01 to 1.16.

A graph was plotted with empirical probit values (Y-axis) against log doses (x-axis). Expected probit (from Y-axis) were read from the provisional line for corresponding log doses on x-intercept and were tabulated in column VII of Table 1.01 to 1.16. Working probits (Y) were then calculated using the formula $V = Y_0 + Kp$; where p= percentage killed and Y_0 and K were read from Table 8 of Busvine (1971) for the appropriate value of expected probit and values were then placed in column VIII of Table 1.01 to 1.16. Weighing coefficient (w) for each point were read from Table 8 of Busvine (1971) and were included in column IX of table 1.01 to 1.16.

Each weighing coefficient was multiplied by the number of experimental animals and then probits (W) were listed in column X of Table 1.01 to 1.16. Now for each row of table 1.01 to 1.16 the value of W and X, W and Y were multiplied and the products WX and WY were listed in column XI and XII of table 1.01 to 1.16. The products of column X, XI and XII for table 1.1 to 1.16 were summed up at the foot of each respective column giving abbreviations as ΣW , ΣWX and ΣWY respectively. Then values for X and Y were determined using following equations.

$$X = \frac{\Sigma WX}{\Sigma W} \quad \text{and} \quad Y = \frac{\Sigma WY}{\Sigma W}$$

Where X and Y are the arithmetic means with weighing coefficient as frequency distribution.

For each row of table 1.01 to 1.16, the values of WX was multiplied by the values of X and the product of WX² at the foot of column XIII. Similarly the value of WY was multiplied by the values of Y and product WY² summed up as $\sum WXY^2$ at the foot of column XIV. For each row of table 1.01 to 1.16 again the value of WX was multiplied by value of Y and products WXY were summed up as $\sum WXY$ at the foot of column XV.

Now the values of the estimated regression coefficient b was calculated by the following equation.

$$b = \frac{\sum WXY - X \cdot \sum WY}{\sum WX^2 - X \cdot \sum WX}$$

The regression equation may now be written as follows:

$$Y' = (Y - bX) + bX$$

or

$$Y' = Y + b(X - X)$$

From the above equation improved expected probit Y' were calculated and tabulated in column XVI. This value of improved expected probit Y' did not differ by more than 0.2 as compared to working probit Y in column VII of all tabled which marks significance of the values (Y'). Similarly X', were calculated using the formula

$$X' = \frac{Y - (Y - bX)}{B}$$

And the average was calculated and placed at the foot of column XVII using the abbreviation m.

LD₅₀ (column XXII) for each exposure period was then calculated using following formulae.

For monocrotophos (in ppm)

$$LD_{50} = \text{Antilog} \left[\frac{5 - (Y - bX)}{b} \right] \times 100$$

For DDVP (in ppm)

$$LD_{50} = \text{Antilog} \left[\frac{5 - (Y - bX)}{b} \right] \times 1000$$

Value $\frac{5 - (Y - bX)}{b}$ was substituted by abbreviation m and were tabulated in column XVIII of table 1.01 to 1.16. The variance of m was calculated by expression

$$V = \frac{1}{\sum W} \left[\frac{1}{\sum W} + \frac{(m - x)^2}{\frac{\sum WX^2 (\sum WX)^2}{\sum W}} \right]$$

Where V is variance which is statistical device to measure the scatterance of calculated value. The fiducial limits also called an interval estimate, is a parameter given by two numbers between which calculated

value (m) may be considered to lie between m_1 the lower confidence level m_2 (the upper confidence limit) with 95% confidence were calculated from variance (V) by using the following formulae

$$m_1 = m - 1.96 \sqrt{V}$$

$$m_2 = m + 1.96 \sqrt{V}$$

From the above calculated LD_{50} values for 6, 12, 24, 48, 72, 96, 120 and 240 hours time intervals; 1 ppm, 5 ppm, 10 ppm and 100 ppm were chosen as biologically significant doses for the present study.

The doses were administered intraperitoneally in a volume of 1ml as a single acute dose per animal. Concurrent sets of animals administered identical dose of physiological saline served as controls.

The animals were exposed to varying time intervals (6, 12, 24, 48, 72, 96, 120 and 240 hours) to assess the temporal alteration produced by the pesticide toxicants in the spinal cord. At the end of the set time interval, the animals were sacrificed by decapitation. Both the control and treated animals were sacrificed by 9.00 am to avoid circadian rhythm if any.

Table 1.01 : Probit analysis data of DDVP toxicity for 6 hr time interval

Dose D in ppm	No.of animals	corrected % mortality	X= $\frac{D}{100}$	X=log X_{10}	Emph erical probit	Expect ed probit	Y= $Y_0 + Kp$	weighi ng co- eff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
3570	10	10	3.57	0.5527	4.16	4.00	3.750	0.439	4.390	2.426	16.463	1.341	61.734	9.099	3.656	0.5670	0.7586	0.0008780	0.6999	0.8161	5736.00
4750	10	30	4.75	0.6767	4.75	4.50	4.460	0.581	5.810	3.932	25.913	2.661	115.57	17.535	4.465	0.6759					
5940	10	50	5.94	0.7738	5.00	5.00	5.000	0.637	6.370	4.929	31.850	3.814	159.25	24.646	5.099	0.7586					
7130	10	70	7.13	0.8531	5.25	5.50	5.530	0.581	5.810	4.951	32.129	4.228	117.675	27.410	5.616	0.8398					
8310	10	90	8.31	0.9196	6.28	6.00	6.240	0.439	4.390	4.037	27.394	3.712	170.936	25.191	6.050	0.9487					
Avg(M)									ΣW	ΣWX	ΣWY	ΣWX^2	ΣWY^2	ΣWXY	Avg(m)						
0.7552									26.77	20.281	133.749	15.756	685.165	103.881	0.758						

Table 1.02 : Probit analysis data of DDVP toxicity for 12 hr time interval

Dose D in ppm	No.of animals	corrected % mortality	X= $\frac{D}{100}$	X=log X_{10}	Emph erical probit	Expect ed probit	Y= $Y_0 + Kp$	weighi ng co- eff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
3530	10	10	3.530	0.5478	4.16	4.00	3.750	0.439	4.390	2.405	16.463	1.317	61.734	9.018	3.660	0.5617	0.7536	0.0008806	0.6948	0.8112	5670.00
4700	10	30	4.700	0.6721	4.75	4.50	4.460	0.581	5.810	3.905	25.913	2.624	115.570	17.416	4.469	0.6707					
5880	10	50	5.880	0.7694	5.00	5.00	5.000	0.637	6.370	4.901	31.850	3.771	159.250	24.505	5.103	0.7536					
7050	10	70	7.050	0.8482	5.25	5.50	5.530	0.581	5.810	4.928	32.129	4.180	117.675	27.252	5.616	0.8350					
8220	10	90	8.220	0.9149	6.28	6.00	6.240	0.439	4.390	4.016	27.394	3.675	170.936	25.062	6.057	0.9440					
Avg(M)									ΣW	ΣWX	ΣWY	ΣWX^2	ΣWY^2	ΣWXY	Avg(m)						
0.7505									26.77	20.155	133.749	15.567	685.165	103.253	0.7530						

Table 1.03 : Probit analysis data of DDVP toxicity for 24 hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_0 + Kp$	weighting coeff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
3450	10	10	3.45	0.5378	4.16	4.00	3.75	0.439	4.39	2.361	16.463	1.270	61.734	8.854	3.662	0.5513	0.7436	0.000885	0.6847	0.8013	5342.00
4600	10	30	4.60	0.6628	4.75	4.50	4.46	0.581	5.81	3.851	25.913	2.552	151.570	17.175	4.475	0.6605					
5750	10	50	5.75	0.7597	5.00	5.00	5.00	0.637	6.37	4.839	31.850	3.676	159.250	24.196	5.104	0.7436					
6900	10	70	6.90	0.8388	5.25	5.50	5.53	0.581	5.81	4.873	32.129	4.088	117.675	26.950	5.618	0.8252					
8050	10	90	8.05	0.9058	6.28	6.00	6.24	0.439	4.39	3.976	27.394	3.602	170.940	24.813	6.054	0.9344					
Avg(M)									26.77	19.90	133.749	15.188	685.165	101.988	0.743						

Table 1.04: Probit analysis data of DDVP toxicity for 48 hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_0 + Kp$	weighting coeff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
3300	10	10	3.30	0.5185	4.16	4.00	3.75	0.439	4.39	2.276	16.463	1.180	61.734	8.536	3.661	0.5322	0.7246	0.0008851	0.6657	0.7823	5304.00
4400	10	30	4.40	0.6435	4.75	4.50	4.46	0.581	5.81	3.739	25.913	2.406	115.570	16.675	4.473	0.6415					
5500	10	50	5.50	0.7404	5.00	5.00	5.00	0.637	6.37	4.716	31.850	3.492	159.250	23.582	5.102	0.7246					
6600	10	70	6.60	0.8195	5.25	5.50	5.53	0.581	5.81	4.761	32.129	3.902	117.675	26.330	5.616	0.8062					
7700	10	90	7.70	0.8865	6.28	6.00	6.24	0.439	4.39	3.892	27.394	3.450	170.936	24.284	6.052	0.9155					
Avg(M)									26.77	19.384	133.749	14.43	685.165	99.407	0.724						

Table 1.05 : Probit analysis data of DDVP toxicity for 72 hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_o + Kp$	weighing coeff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	\bar{m}	V	m ₁	m ₂	LD ₅₀ in ppm
3150	10	10	3.15	0.4983	4.16	4.00	3.75	0.439	4.39	2.188	16.463	1.090	61.734	8.203	3.658	0.5124	0.7046	0.0008836	0.6457	0.7623	5065.00
4200	10	30	4.20	0.6232	4.75	4.50	4.46	0.581	5.81	3.621	25.913	2.256	115.570	16.149	4.471	0.6216					
5250	10	50	5.25	0.7207	5.0	5.00	5.00	0.637	6.37	4.588	31.850	3.304	159.250	22.938	5.101	0.7046					
6300	10	70	6.30	0.7993	5.25	5.50	5.53	0.581	5.81	4.644	32.129	3.712	117.675	25.681	5.616	0.7861					
7350	10	90	7.35	0.8663	6.28	6.00	6.24	0.439	4.39	3.803	27.394	3.295	170.936	23.731	6.052	0.8453					
				Avg(M) 0.7015					ΣW 26.77	ΣWX 18.884	ΣWY 133.749	ΣWX^2 13.657	ΣWY^2 685.165	ΣWXY 96.702	Avg(m) 0.704						

Table 1.06 : Probit analysis data of DDVP toxicity for 96 hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_o + Kp$	weighing coeff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	\bar{m}	V	m ₁	m ₂	LD ₅₀ in ppm
3000	10	10	3.00	0.4771	4.16	4.00	3.75	0.439	4.39	2.094	16.463	0.999	61.734	7.854	3.658	0.4913	0.6836	0.000885	0.6247	0.7413	4826.00
4000	10	30	4.00	0.6021	4.75	4.50	4.46	0.581	5.81	3.498	25.913	2.106	115.570	15.602	4.470	0.6005					
5000	10	50	5.00	0.6990	5.00	5.00	5.00	0.637	6.37	4.453	31.850	3.112	159.250	22.263	5.100	0.6836					
6000	10	70	6.00	0.7782	5.25	5.50	5.53	0.581	5.81	4.521	32.129	3.519	117.675	25.003	5.615	0.7652					
7000	10	90	7.00	0.8451	6.28	6.00	6.24	0.439	4.39	3.710	27.394	3.135	170.936	23.150	6.049	0.8744					
				Avg(M) 0.6803					ΣW 26.77	ΣWX 18.276	ΣWY 133.749	ΣWX^2 12.871	ΣWY^2 685.165	ΣWXY 93.872	Avg(m) 0.683						

Table 1.07 : Probit analysis data of DDVP toxicity for 120 hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_o + Kp$	weighing coeff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
2850	10	10	2.85	0.4548	4.16	4.00	3.75	0.439	4.39	1.997	16.463	0.908	61.734	7.487	3.650	0.4701	0.6616	0.0008772	0.6029	0.7191	4587.00
3800	10	30	3.80	0.5798	4.75	4.50	4.46	0.581	5.81	3.369	25.913	1.953	115.570	15.024	4.466	0.5789					
4750	10	50	4.75	0.6767	5.00	5.00	5.00	0.637	6.37	4.311	31.850	2.917	159.250	21.553	5.098	0.6616					
5700	10	70	5.70	0.7559	5.25	5.50	5.53	0.581	5.81	4.392	32.129	3.320	117.675	24.287	5.615	0.7428					
6650	10	90	6.65	0.8228	6.28	6.00	6.24	4.39	4.39	3.614	27.394	2.972	170.936	22.550	6.052	0.8516					
Avg(M)									ΣW	ΣWX	ΣWY	ΣWX ²	ΣWY ²	ΣWXY	Avg(m)						
0.658									26.77	17.683	133.749	12.07	685.165	90.901	0.661						

Table 1.08 : Probit analysis data of DDVP toxicity for 240 hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_o + Kp$	weighing coeff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
2100	10	10	2.10	0.3222	4.16	4.00	3.75	0.439	4.39	1.415	16.463	0.4559	61.734	5.304	3.663	0.3357	0.5286	0.0008892	0.4696	0.5864	3378.00
2800	10	30	2.80	0.4472	4.75	4.50	4.46	0.581	5.81	2.598	25.913	1.1619	115.570	11.588	4.473	0.4453					
3500	10	50	3.50	0.5441	5.00	5.00	5.00	0.637	6.37	3.466	31.850	1.8858	159.250	17.330	5.101	0.5286					
4200	10	70	4.20	0.6232	5.25	5.50	5.53	0.581	5.81	3.621	32.129	2.2565	117.675	20.023	5.613	0.6104					
4900	10	90	4.90	0.6902	6.28	6.00	6.24	4.39	4.39	3.030	27.394	2.0913	170.936	18.906	6.047	0.7200					
Avg(M)									ΣW	ΣWX	ΣWY	ΣWX ²	ΣWY ²	ΣWXY	Avg(m)						
0.5254									26.77	14.129	133.749	7.851	685.165	73.152	0.528						

Table 1.09 : Probit analysis data of Monocrotophos toxicity for 6hr time interval

Dose D in ppm	No. of animals	corrected % mortality	X= D/100	X=log X ₁₀	Empirical probit	Expected probit	Y=Y _o + Kp	weighting coeff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
297	10	10	2.97	0.4728	4.16	4.02	3.75	0.439	4.39	2.056	16.463	0.981	61.73	7.783	3.702	0.408	0.6776	0.0009304	0.6171	0.7367	476.00
395	10	30	3.95	0.5966	4.75	4.75	4.46	0.581	5.81	3.466	25.913	2.068	115.57	15.459	4.486	0.592					
495	10	50	4.95	0.6946	5.00	5.00	5.00	0.637	6.37	4.425	31.85	3.073	159.25	22.123	5.108	0.678					
593	10	70	5.93	0.7731	5.25	5.25	5.53	0.581	5.81	4.492	32.129	3.473	177.68	24.839	5.605	0.761					
692	10	90	6.92	0.8401	6.28	6.28	6.24	0.439	4.39	3.688	27.394	3.098	170.94	23.013	6.030	0.873					
				Avg(M) 0.6754						ΣW 26.77	ΣWX 18.127	ΣWY 133.75	ΣWX ² 12.693	ΣWY ² 685.165	ΣWXY 93.217		Avg(m) 0.6769				

Table 1.10 : Probit analysis data of Monocrotophos toxicity for 12hr time interval

Dose D in ppm	No. of animals	corrected % mortality	X= D/100	X=log X ₁₀	Empirical probit	Expected probit	Y=Y _o + Kp	weighting coeff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
293	10	10	2.93	0.4669	4.16	4.00	3.75	0.439	4.39	2.056	16.463	0.957	61.734	7.686	3.660	0.4809	0.6749	0.000889	0.6144	0.7312	473.10
390	10	30	3.90	0.5911	4.75	4.50	4.46	0.581	5.81	3.434	25.913	2.030	115.570	15.317	4.460	0.5911					
488	10	50	4.88	0.6884	5.00	4.84	4.97	0.637	6.37	4.316	31.162	2.971	154.875	21.452	5.090	0.6703					
585	10	70	5.85	0.7612	5.25	5.30	5.51	0.581	5.81	4.803	34.493	3.685	190.054	26.463	5.595	0.7541					
683	10	90	6.83	0.8344	6.28	6.00	6.24	0.439	4.39	3.663	27.394	3.056	170.946	22.857	6.027	0.8674					
				Avg(M) 0.6696						ΣW 27.12	ΣWX 18.266	ΣWY 135.425	ΣWX ² 12.699	ΣWY ² 693.169	ΣWXY 93.775		Avg(m) 0.6728				

Table 1.11 : Probit analysis data of Monocrotophos toxicity for 24hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_o + K_p$	weighting coeff. w	Weight W	WX	WY	WX^2	WY^2	WXY	Y'	X'	m	V	m_1	m_2	LD ₅₀ in ppm
285	10	10	2.85	0.4548	4.16	4.00	3.750	0.439	4.39	1.997	16.463	0.9080	61.734	7.489	3.654	0.4695	0.6606	0.0007844	0.6051	0.7149	457.70
380	10	30	3.80	0.5798	4.75	4.50	4.46	0.581	5.81	3.369	25.913	1.9531	115.570	15.026	4.471	0.5781					
475	10	50	4.75	0.6767	5.00	5.00	5.00	0.637	6.37	4.311	31.850	2.917	159.250	21.555	5.105	0.6606					
570	10	70	5.70	0.7559	5.25	5.50	5.53	0.581	5.81	4.392	32.129	3.3197	177.675	24.288	5.632	0.7416					
665	10	90	6.65	0.8228	6.28	6.00	6.24	0.439	4.39	3.612	27.394	2.9720	170.936	22.539	6.061	0.8502					
				Avg(M)					ΣW	ΣWX	ΣWY	ΣWX^2	ΣWY^2	ΣWXY	Avg(m)						
				0.6580					26.77	17.681	133.749	12.070	685.16	90.897	0.6600						

Table 1.12 : Probit analysis data of Monocrotophos toxicity for 48hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_o + K_p$	weighting coeff. w	Weight W	WX	WY	WX^2	WY^2	WXY	Y'	X'	m	V	m_1	m_2	LD ₅₀ in ppm
270	10	10	2.70	0.4314	4.16	4.00	3.75	0.439	4.39	1.894	16.463	0.8170	61.734	7.102	3.662	0.4450	0.6375	0.0008874	0.5777	0.6945	434.0
360	10	30	3.60	0.5563	4.75	4.50	4.46	0.581	5.81	3.232	25.913	1.7980	115.57	14.415	4.473	0.5544					
450	10	50	4.50	0.6532	5.00	5.00	5.00	0.637	6.37	4.161	31.850	2.7179	159.25	20.804	5.102	0.6376					
540	10	70	5.40	0.7324	5.25	5.50	5.53	0.581	5.81	4.255	32.129	3.1165	177.68	23.530	5.616	0.7147					
630	10	90	6.30	0.7993	6.28	6.00	6.24	0.439	4.39	3.509	27.394	2.8047	170.94	21.896	6.050	0.8287					
				Avg(M)					ΣW	ΣWX	ΣWY	ΣWX^2	ΣWY^2	ΣWXY	Avg(m)						
				0.6345					26.77	17.051	133.75	11.254	685.16	87.747	0.6361						

Table 1.13 : Probit analysis data of Monocrotophos toxicity for 72hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_o + K_p$	weighing co-eff. w	Weight W	WX	WY	WX^2	WY^2	WXY	Y'	X'	m	V	m_1	m_2	LD ₅₀ in ppm
255	10	10	2.55	0.4065	4.16	4.00	3.75	0.439	4.39	1.784	16.463	0.725	61.734	6.692	3.659	0.4205	0.6126	0.000883	0.5538	0.6702	409.9
340	10	30	3.40	0.5315	4.75	4.50	4.46	0.581	5.81	3.088	25.913	1.641	115.75	13.773	4.472	0.5296					
425	10	50	4.25	0.6284	5.00	5.00	5.00	0.637	6.37	4.003	31.850	2.515	159.25	20.105	5.103	0.6126					
510	10	70	5.10	0.7076	5.25	5.50	5.53	0.581	5.81	4.111	32.129	2.909	177.67	22.735	5.618	0.6141					
595	10	90	5.95	0.7745	6.28	6.00	6.24	0.439	4.39	3.400	27.394	2.633	170.94	21.216	6.053	0.8032					
Avg(M)									ΣW	ΣWX	ΣWY	ΣWX ²	ΣWY ²	ΣWXY	Avg(m)						
0.6696									27.12	18.266	135.42	12.699	693.17	93.775	0.6728						

Table 1.14 : Probit analysis data of Monocrotophos toxicity for 96hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Empirical probit	Expected probit	$Y = Y_o + K_p$	weighing co-eff. w	Weight W	WX	WY	WX^2	WY^2	WXY	Y'	X'	m	V	m_1	m_2	LD ₅₀ in ppm
240	10	10	2.40	0.3802	4.16	4.00	3.75	0.439	4.39	1.669	16.463	0.635	61.734	6.259	3.672	0.3924	0.5858	0.0009026	0.5272	0.6450	385.3
320	10	30	3.20	0.5051	4.75	4.50	4.46	0.581	5.81	2.935	25.913	1.482	115.57	13.088	4.475	0.5027					
400	10	50	4.00	0.6021	5.00	5.00	5.00	0.637	6.37	3.835	31.850	2.309	159.25	19.177	5.099	0.5867					
480	10	70	4.80	0.6812	5.25	5.50	5.53	0.581	5.81	3.958	32.129	2.696	177.68	21.866	4.382	0.6691					
560	10	90	5.60	0.7482	6.28	6.00	6.24	0.439	4.39	3.285	27.394	2.458	176.94	20.496	6.039	0.7794					
Avg(M)									ΣW	ΣWX	ΣWY	ΣWX ²	ΣWY ²	ΣWXY	Avg(m)						
0.5834									26.77	15.682	133.749	9.58	685.165	86.886	0.5861						

Table 1.15 : Probit analysis data of Monocrotophos toxicity for 120 hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Emphirical probit	Expected probit	$Y = Y_0 + Kp$	weighing co-eff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
225	10	10	2.25	0.3522	3.72	4.00	3.75	0.439	4.39	1.546	16.463	0.5446	61.734	5.798	3.666	0.3651	0.559	0.000895	0.4994	0.6166	362.2
300	10	30	3.00	0.4771	4.48	4.50	4.46	0.581	5.81	2.772	25.913	1.323	115.57	12.363	4.473	0.4750					
375	10	50	3.75	0.5740	5.00	5.00	5.00	0.637	6.37	3.656	31.850	2.099	159.25	18.280	5.099	0.5586					
450	10	70	4.50	0.6532	5.54	5.50	5.53	0.581	5.81	3.795	32.129	2.479	177.67	20.986	5.611	0.6407					
525	10	90	5.25	0.7202	6.28	6.00	6.24	0.439	4.39	3.162	27.394	2.277	170.94	19.731	6.043	0.7506					
				Avg(M) 0.5553					ΣW 26.77	ΣWX 14.931	ΣWY 133.749	ΣWX^2 8.722	ΣWY^2 685.165	ΣWXY 77.156	Avg(m) 0.5580						

Table 1.16 : Probit analysis data of Monocrotophos toxicity for 240 hr time interval

Dose D in ppm	No. of animals	corrected % mortality	$X = \frac{D}{100}$	$X = \log X_{10}$	Emphirical probit	Expected probit	$Y = Y_0 + Kp$	weighing co-eff. w	Weight W	WX	WY	WX ²	WY ²	WXY	Y'	X'	m	V	m ₁	m ₂	LD ₅₀ in ppm
150	10	10	1.50	0.1760	3.72	3.92	3.74	0.405	4.05	0.7128	15.147	0.1254	56.650	2.666	3.649	0.1894	0.3831	0.0008896	0.3230	0.4399	241.63
200	10	30	2.00	0.3010	4.48	4.48	4.46	0.581	5.81	1.7488	25.913	0.5463	115.57	7.800	4.464	0.3003					
250	10	50	2.50	0.3979	5.00	5.00	5.00	0.631	6.37	2.5346	31.850	1.6085	159.25	12.673	5.096	0.3831					
300	10	70	3.00	0.4771	5.54	5.54	5.54	0.581	5.81	2.7719	32.071	1.3224	177.03	15.301	5.613	0.4628					
350	10	90	3.50	0.5440	6.28	6.05	6.05	0.439	4.39	2.3881	27.350	1.991	170.38	14.878	6.049	0.5717					
				Avg(M) 0.3792					ΣW 26.43	ΣWX 10.186	ΣWY 132.33	ΣWX^2 4.2817	ΣWY^2 678.891	ΣWXY 53.317	Avg(m) 0.3816						

ELECTROPHYSIOLOGICAL TECHNIQUE

Immediately following decapitation, the entire vertebral column was aseptically dissected out and placed in a shallow dissecting dish containing modified Kreb's solution consisting of (in mM) 118.2 NaCl, 3.0 KCl, 1.2 CaCl₂, 0.6 MgSO₄ · H₂O, 1.2 KH₂PO₄, 11.1 glucose, 25.0 NaHCO₃, pH 7.44. The vertebral column was cut open from the thoracic region to expose the underlying spinal cord. This preparation was kept for 30 mins in the modified Kreb's solution prior to recording to allow it to recover from surgical trauma.

The spinal cord preparation was then transferred into the recording chamber, which was perfused with Kreb's solution. The temperature of the solution was maintained at 23 – 25 °C throughout the experimental period as it enhances the survival of the isolated spinal cord. The recording chamber consisted of a platform for the placement of the isolated tissue. A clamp was attached to the chamber for holding the electrodes in position during recording.

For the purpose of recording two identical platinum wire (0.35 mm diameter and resistance 0.1 Ω) were used. The electrodes were connected to the computerised recording unit, UnkelScope (MIT, USA : version 1984) via a 20 port cord. The recordings consisted of a horizontal scale recording the duration and a vertical scale measuring the voltage of the electrical activity. The source for the horizontal trace was 200 sec full scale and the recording

frequency was set at an interval of 0.2 sec at 5 Hz. The source of vertical trace was analog 0, with a span of 50 mv full scale.

Of the two platinum electrodes used, one was a recording electrode while the other served as differential electrode and was placed outside the spinal cord. The recording electrode was placed on the ventral side (anterior motor horn region) of the spinal cord surface till a slight dimpling was visible at the point of contact. The recordings were taken only when they steadied down. The recordings were scaled down to 10 sec for the purpose of calculations. The average values of the electrical activities are presented in tabular form and segments of recordings are also presented.

For each recording the following parameters were taken into consideration:

1. Electrical properties :

- a. Frequency of spikes and plateau phases for action potential and inward currents.
- b. Frequency of isoelectric phases.
- c. Frequency of hyperpolarisations.

(The frequency was calculated for 10 sec initially and then extrapolated to 60 sec).

2. Duration of electrical potential:

- a. Average time taken for spike and plateau phases of action potential and inward current.

(Duration was calculated in sec from the onset of depolarisation to

the return of the action potential and inward current to the baseline in reference to horizontal trace of the recording).

b. Duration of isoelectric phases

3. Electrical potentials:

a. Change in baseline potential

b. The amplitude of action potential and inward current.

(the amplitude was measured in mv with reference to the vertical trace of the recording)

4. Conduction velocity:

(Velocity was calculated in m / s computed from the inter-electrode distance and the time taken to reach peak depolarisation)

Ionic channels study:

To evaluate the role of ionic channels in pesticide induced toxicity, specific ionic channel blockers were used. 0.2N CdCl₂ was used as calcium channel blocker (Walton and Fulton, 1986; Narahashi, 1989). 0.1N BaCl₂ was used as a potassium channel blocker (Armstrong and Taylor, 1980 ; Hotson and Prince, 1980). The solutions were perfused into the bathing media of the spinal cord and the recordings were taken after 3- 5 min.

BIOCHEMICAL ASSAYS

ACID PHOSPHATASE (E.C. 3.1.3.2)

Orthophosphoric monoesterase phosphohydrolase.

The assays were carried out according to the method described by Lindart and Walter (1965).

p-nitrophenol phosphate was purchased from Merck and all chemicals were of analar grade.

Reagents:

- Substrate buffer solution : 0.41 g citric acid, 1.125 g of sodium citrate and 165 mg Na-p-nitrophenol phosphate dissolved in 100 ml of distilled water (store at 4^o C).
- 0.05 M phosphate buffer (pH 4.8)
- 0.1N NaOH

Preparation of sample:

A tissue extract of 1% w / v was prepared in phosphate buffer (pH 4.8). The homogenisation of the spinal cord tissue was carried out in a glass mortar. The glass mortar and pestle were rinsed in distilled water, dried and kept in deep freezer for nearly 5 hours prior to homogenisation. The homogenisation was carried out by placing the mortar on an ice block. Such a

method is advantageous because no loss of enzyme activity occurs as the temperature rise due to the friction of mortar and pestle is minimised. Appropriate volume of chilled sodium citrate buffer (0.05M, pH 4.8) was added and the homogenisation continued to get a well-suspended tissue homogenate. This was then centrifuged at 2000 rpm for 15 min. The supernatant was used as an enzyme source.

Protocol:

The reaction mixture in each test tube contained 1.0 ml p-nitrophenol substrate buffer solution and 0.2 ml of enzyme source. This mixture was allowed to equilibrate for 5 to 10 minutes. A single blank test tube contained 1.0 ml of substrate buffer solution only. All the tubes were shaken gently to mix aliquotes properly. These were then stoppered with cork stoppers and incubation was carried out in an incubator at 37.5⁰C for 30 minutes.

At the end of the incubation, the reaction was terminated by adding 4ml of 0.1 N NaOH. The OD was read at 405 nm on 305 Systronics UV spectrophotometer using control as a reference.

Calculations: Optical density was converted to μ mols of p-nitrophenol from the formula suggested by Lindart and Walter (1965) with assay volume of 0.2 ml of extract and an incubation period of 30 minutes; a unit of acid phosphatase activity corresponds to an optical density of 0.362 at 400 nm and is equivalent to 2.76. Factor 2.76 x O.D. of the assayed experimental mixture

was phosphatase activity for 0.2 ml extracts which was then calculated for 1g of tissue. The acid phosphatase activity was expressed as μ moles of p-nitrophenol / g of tissue.

Formula :

$$\mu \text{ moles of of p-nitrophenol / g wet wt. of tissue} = \frac{\text{O.D.} \times 2.76}{0.2 \times \text{wt.in g} \times 1000}$$

ALKALINE PHOSPHATASE (E.C.3.1.3.1)

Orthophosphoric monoesterase phosphohydrolase

p-nitrophenol phosphate assay of alkaline phosphatase was carried out according to the method of King and Armstrong, 1934.

Reagents:

- Substrate buffer solution : 375 mg of glycine, 10 mg $MgCl_2 \cdot 6H_2O$ and 165 mg of Na-p-nitrophenol phosphate dissolved in 42 ml of 0.1 N NaOH and diluted to 100 ml with double distilled water (store at 4^o C).
- 0.05 M phosphate buffer (pH 10.5)
- 0.02 N NaOH

The tissue extract was prepared in glycine buffer following the method described previously for acid phosphatase.

Protocol :

Into each test tube, 1.0 ml of substrate buffer solution and 0.1 ml of tissue homogenate were pipetted out. This mixture was allowed to equilibrate for 5 – 10 minutes. A single test tube containing 1.0 ml glycine substrate buffer served as control. All the tubes were shaken gently to mix aliquotes properly and incubation was carried out in an incubator at 37.5^oC for 30 minutes.

At the end of incubation period, the reaction was terminated by adding 10 ml of 0.02 N NaOH and tubes were centrifuged for 5 minutes at 2000 rpm. The optical density was read at 405 nm on 305 systronics spectrophotometer with control as reference.

Calculations : Optical density was converted into μ moles of p-nitrophenol from the calibrated nitrophenol curve. The activity was calculated using the formula suggested by King and Armstrong (1934) and expressed as μ moles of p-nitrophenol / g of tissue.

Formula :

μ moles of p-nitrophenol /g wet wt. of tissue =

μ moles of p-nitrophenol (from std. graph) x 20

NON- SPECIFIC ESTERASES

(E.C.3.1.1.)

p-nitrophenol assay of esterase was carried out according to the method described by Glenner (1963).

Reagents:

0.1M p-nitrophenol acetate: 63 mg p-nitrophenol acetate in 10 ml ethanol (stock solution). The working substrate solution was prepared by diluting 0.1 ml of stock solution to 10ml.

The tissue extract was prepared by using phosphate buffer (0.66 M, pH 7).

Protocol :

In each test tube was added 5ml of ice cold water, 2 ml phosphate buffer (0.66 M, pH 7), 1ml of tissue homogenate and 2ml of working substrate solution. They were then incubated for 20 min at 25⁰C. A test tube without the enzyme source was used as control. At the end of incubation period the readings were taken at 400nm on a spectrophotometer. The OD was converted into micromoles of p-nitrophenol from the calibrated p-nitrophenol standard curve.

Calculations : Optical density was converted into μ moles of p-nitrophenol from the calibrated nitrophenol curve. The non-specific esterase activity was expressed as μ mols of p-nitrophenol / g of tissue.

Formula :

μ moles of p-nitrophenol / g wet wt. of tissue =

μ moles of p-nitrophenol (from std. graph) x dilution

wt. in g

XANTHINE OXIDASE

(EC 1.2.3.2.)

The assay was carried out according to the method described by Worthington, 1988. Xanthine oxidase activity is determined as the rate of formation of urate from hypoxanthine.

Reagents

- The substrate was prepared by dissolving 10mg hypoxanthine in 500ml reagent grade water.
- 1% W/V tissue extract is prepared in phosphate buffer (0.05 M, pH 7.5)

Protocol:

Into each cuvettes were pipetted, 1.9 ml of phosphate buffer, 1ml substrate and 0.1 ml of tissue extract. The control tube contained the same reaction mixture except the enzyme source. Readings were taken at 290 nm on spectrophotometer.

Calculations: The molar absorbancy of uric acid at 290 nm is $1.22 \times 10^4 \text{ cm}^{-1}$ units /mg of protein. The xanthine oxidase activity was expressed as nmols of urate formed / mg of protein.

Formula :

$$\text{nmols of urate/ mg of protein} = \frac{\Delta A/\text{min} \times 1000}{1.22 \times 10^4 \times \text{mg/ml reaction mixture}}$$

SUPEROXIDE DISMUTASE

(E.C.1.15.1.1)

The assay was carried out according to the method of Winterbourn *et al.*, 1975, which is based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium (NBT) by superoxide anion. One unit is defined as that amount of enzyme causing one half the maximum inhibition of NBT reduction.

Reagents:

- 0.067 M Potassium phosphate buffer pH 7.8
- 0.1 M ethylene diamine tetracetic acid (EDTA) containing 0.3 mM sodium cyanide.
- 0.12 mM riboflavin (stored cold in a dark bottle)
- 0.12 nitro blue tetrazolium (stored cold)
- Standard SOD enzyme (Sigma).
- The tissue extract was prepared using potassium phosphate buffer (0.067 M, pH 7.8).

Protocol :

The blank test tube reaction mixture consisted of 0.2 ml of EDTA containing 0.3 mM sodium cyanide. 0.1 ml of NBT and 2.7 ml of phosphate buffer. For the preparation of SOD standard curve a series of concentrations

of enzyme were used. The sample estimation too was carried out using atleast five various concentrations to achieve accuracy. The tubes were placed in a light box providing uniform light intensity (a foil lined box approximately 4" X 8" X 6" with an internally mounted 40 W fluorescent bulb). Incubation was carried out for 5 - 8 minutes to achieve a standard temperature and 0.05 ml of riboflavin was added. All the tubes were incubated in light box for 12 min. The Optical Density was read at 560 nm.

Calculations :

The catalytic activity of the enzyme is given as the amount of enzyme required to inhibit the colour formation by 50%.

The standard SOD reference curve was plotted, using the formula:

$$\text{Units / mg of protein} = \frac{1000}{\mu\text{g of enzyme resulting } \frac{1}{2} \text{ max. inhibition.}}$$

The samples SOD concentration were calculated referring to the standard SOD graph and expressed as units / mg of protein.

TOTAL PROTEINS

Proteins were estimated by Lowry's method (Lowry *et al.*, 1951).

Reagents :

- Standard proteins solution : 13 mg Bovine albumin was dissolved in 250 ml distilled water made alkaline.
- Lowry's A solution : 2.0% sodium carbonate solution in 0.01 NaOH.
- Lowry's B₁ solution : 2.0% sodium citrate solution in distilled water.
- Lowry's B₂ solution : 1.0% copper sulphate solution in distilled water.
- Lowry's C solution : 1.0 ml of Lowry's B₁ + 1.0 ml of Lowry's B₂ +
100 ml of Lowry's A
(used within fifteen minutes of the preparation).
- Folin's phenol reagent : market available form was used.

The tissue extract was prepared by homogenising the tissue in mammalian saline.

Protocol:

Test tube containing a reaction mixture of 1.5 ml of distilled water and 3.0 ml of Lowry's C, served as blank. Into the sample test tubes 1.3

ml of distilled water was added and after 15 min 0.5 ml of folin's phenol reagent was added. After mixing well it was kept aside undisturbed for an hour. At the end of 1hr incubation, the readings were taken on spectrophotometer at 660 nm using control as reference. Bovine albumin was used as standard.

Calculations : The optical density was converted to g of protein / g wet weight of tissue using the following formula.

Formula : g of protein / g wet wt. of tissue =

$$\frac{\text{O.D. of sample}}{\text{O.D. standard}} \times \text{Known conc. of Std.}$$

MITOCHONDRIAL PROTEINS

Free mitochondrial fraction from the spinal cord was purified by differential centrifugation method described by Christian (1963).

Reagents

Sucrose solution : 0.32 M, 0.8 M, 1.0 M, 1.2 M, 1.4M.

Protocol:

The whole frozen spinal cord was homogenized with 9 volumes of 0.32 M sucrose. The nuclei were separated by centrifugation at 1000g for 10 minutes. The pellet was discarded and the supernatant were centrifuged at 10,000 for 30 minutes at 4^o C to obtain crude mitochondrial pellet. The pellet was washed twice and put on top of a discontinuous sucrose gradient (0.8, 1.0, 1.2 and 1.4 M sucrose solution). After centrifugation at 75,000 g for 2 hours, the mitochondrial pellet was obtained at the interface of 1.4M sucrose solution. The pure mitochondrial pellet was further diluted and was used as sample source for the estimation of protein content by Lowry's method as given earlier. The values are expressed as g of mitochondrial proteins / g of mitochondrial pellet.

Formula : g of mitochondrial protein / g of pellet =

$$\frac{\text{O.D. of sample}}{\text{O.D. standard}} \times \text{Known conc. of Std.}$$

CREATININE

Auto span kit was used for creatinine analysis (Bowers, 1980)

Principle:

Creatinine reacts with picric acid in an alkaline medium to form an orange coloured complex. The rate of formation of this complex is measured by noting the change in absorbance at 505 nm in a selected interval of time and is proportional to the concentration of creatinine.

The reaction time and the concentration of picric acid and sodium hydroxide have been optimized to avoid interference from keto acids.

Reagents

Reagent 1. Picrate reagent

Reagent 2. Sodium hydroxide

Reagent 3. Creatinine standard.

Preparation of working reagent

Equal volumes of picrate reagent and sodium hydroxide was combined and mixed by swirling gently for 2 minutes to prepare working creatinine reagent and is stored at 2 – 8⁰ C.

The tissue extract was prepared in chilled distilled water.

Protocol :

1 ml of working creatinine reagent was pipetted out into all the test tubes. In the standard, 50 μ m of creatinine standard solution was added and into the sample test tubes, 50 μ m of tissue extracts were added. The test tube contents were mixed and the readings were taken after 30 sec. on spectrophotometer at 505 nm.

Calculations : The optical density was converted to mg of creatinine by using the following formula.

Formula : mg creatinine / g wet wt. of tissue =

$$\frac{\text{O.D. of sample}}{\text{O.D. standard}} \times \text{Known conc. of creatinine in Std.}$$

CHOLESTEROL

Cholesterol was estimated by one step method of Wybenga and Pileggi (1970) using Span diagnostic kit.

Principle:

Cholesterol reacts with hot solution of Ferric Perchlorate, Ethyl acetate and Sulphuric acid (Cholesterol Reagent) and gives a lavender coloured complex, which is measured spectrophotometrically.

Reagents :

Reagent 1. Cholesterol reagent

Reagent 2. Working cholesterol standard, 200 mg %.

Protocol :

5 ml of cholesterol reagent was pipetted into the test tubes. In standard 0.05 ml of standard solution was added, while in the test 0.05ml of tissue extract was added. All the contents in the tubes were mixed and immediately kept in boiling water bath exactly for 90 seconds. The test tubes were then cooled to room temperature under running tap water. The OD of standard and test were measured against blank at 560 nm.

Calculations : The optical density was converted to mg of cholesterol / g wet wt. of tissue.

Formula : mg of cholesterol / g wet wt. of tissue =

$$\frac{\text{O.D. of sample}}{\text{O.D. standard}} \times \text{Known conc. of cholesterol in Std.}$$

TRIGLYCERIDES

The triglyceride content in the tissue was estimated by GPO-ESPAS method using Autospan diagnostic kit (McGowan, 1983 ; Naegele, 1984).

Principle:

Triglycerides are hydrolysed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerokinase the glycerol is converted to glycerol – 3 – phosphate. The glycerol – 3 – phosphate is then oxidised by glycerol – 3 – phosphate oxidase to yield hydrogen peroxide. This hydrogen peroxide reacts in the presence of peroxidase with ESPAS (N- ethyl- N- sulfopropyl -N- anisidine) and 4- aminoantipyrine to form a coloured complex. The intensity of the colour developed is proportional to triglycerides concentrations and is measured photometrically at 546 nm.

Reagents:

Reagent 1: TG buffer

Reagent 2: TG Reagent

Reagent 3: TG Standard 200 mg / dl

Preparation of Working reagent :

Dissolve the contents of 1 bottle of reagent 2, TG reagent in 1 bottle of reagent 1, TG buffer. Store the reagent in amber glass bottle at 20-25⁰ C.

Protocol :

1 ml of working TG reagent was pipetted out into test tubes. In the standard 10 μ l of TG standard was added. In the others 10 μ l of the tissue extract was added. A blank with only working reagent was used. All the test tubes were incubated at 37⁰ C for 5 mins. The OD was observed at 546 nm on a spectrophotometer.

Calculations : The optical density obtained was converted mg of triglyceride / g wet wt. of tissue.

Formula : mg of triglyceride/ g wet wt. of tissue =

$$\frac{\text{O.D. of sample}}{\text{O.D. standard}} \times \text{Known conc. of triglyceride in Std.}$$

GLUCOSE

Glucose was estimated by the method of Nelson – Somogyi, 1954.

Reagents:

- Anthrone – 0.2 % in Conc. H₂SO₄.
- Glucose – 0.1 g / L
- ZnSO₄ – 10 %

Protocol :

ZnSO₄ was added to the homogenate to precipitate proteins. The test tubes were centrifuged at 3000 rpm for 15 minutes. The clear, protein free supernatant was decanted and used for glucose estimation.

1ml of supernatant was taken and 4 ml of freshly prepared anthrone reagent was added. The test tubes were shaken vigorously and were kept in boiling water bath for 10 minutes. After heating, the tubes were cooled down to room temperature and the readings were taken at 620 nm against blank.

Calculations: The optical density was converted to mg of glucose / g wet wt. of tissue.

$$\text{Formula : } \quad \text{mg of glucose / g wet wt. of tissue} = \frac{\text{O.D. of sample}}{\text{O.D. standard}} \times \text{Known conc. of glucose in Std.}$$

GLYCOGEN

Anthrone method – (Plummer, 1988)

Principle :

The anthrone reaction is a rapid and convenient method for the determination of hexoses, aldopentoses and hexuronic acids, either free or present in polysaccharides. The blue-green solution shows absorption maximum at 620nm, although some carbohydrates may give other colours. The reaction is not suitable when proteins containing a large amount of tryptophan are present, since a red colour is obtained under these conditions. The extinction depends on the compound investigated, but is constant for a particular molecule.

Reagents:

- Anthrone reagent – 2 g / litre in concentrated H_2SO_4
- Glycogen – 0.1 g / litre.

Protocol:

4 ml of anthrone reagent was added to 1ml of protein free carbohydrate solution and mixed rapidly. The test tubes are then placed in boiling water bath for 10 min with a marble on top to protect loss of water by evaporation.

The tubes were then cooled and extinction was read at 620 nm against a reagent blank.

Calculations : Standard graph was prepared and the glycogen concentration of the unknown was found out by referring the standard curve.

Formula : mg of glycogen / g wet wt. of tissue =

$$\frac{\text{O.D. of sample}}{\text{O.D. standard}} \times \text{Known conc. of glycogen in Std.}$$

STATISTICAL ANALYSIS

The pooled data were statistically analysed using the software package systat Version 5.0. One-way Analysis of variance (ANOVA) was carried out and 0.05 level of significance for probability was considered to be statistically significant.

OBSERVATIONS



ELECTROPHYSIOLOGICAL ALTERATIONS OF SPINAL CORD OF MICE EXPOSED TO DDVP AND MONOCROTOPHOS

Mice administered with various doses of DDVP and monocrotophos for different time intervals exhibited notable alterations in frequencies of electrical activities in general, durations of electrical activities, electrical potentials and conduction patterns of the spinal cord. The averages of all the electrical recordings are tabulated and segments of actual electrical recordings are also given.

I (a). EFFECT OF DDVP ON FREQUENCIES OF ELECTRICAL ACTIVITIES

The recordings of the electrical activities including frequencies of action potential (spike and plateau phases), inward currents (spike and plateau phases), isoelectric phases and hyperpolarisation of the spinal cord of mice exposed to DDVP are tabulated in table 2.01 and represented in Fig. 2.1(a) – 2.4(a). The percentage of changes in the frequencies of electrical activities are presented in table 2.02.

Changes in frequency of action potentials

DDVP induced increase in the frequencies of spikes and plateau phases, especially the spike frequency elevated a little more as the concentration of the drug was increased. The control group of animals

exhibited spike frequencies in the range of 15.0 ± 1.5 to 21.0 ± 4.0 spikes / min, while the plateau phase frequencies ranged between 6.0 ± 0 to 18 ± 2.0 PPs / min for a period of 6 to 240 hours.

Under the influence of 1ppm dose of DDVP the spike frequencies of action potential elevated at all the time intervals. The spike frequency were in the range of 19.5 ± 4.2 to 34.0 ± 8.6 / min (Table 2.01). The changes in the spike frequencies in terms of percentage are given in table 2.02. The elevations in the spike frequency ranged from 25.0 % to 100%. The maximum elevation in the spike frequency was observed at the end of 72 hours while a minimum increase of spike frequency was noted at the end of 120 hours (Table 2.02).

The mice treated with 1 ppm dose of DDVP showed elevation in plateau phase frequencies at the end of 6,12,48 and 72 hours, while there was no change in plateau phase frequency at the end of 24 hours. But the plateau phase frequencies declined at the end of 96,120 and 240 hours (Table 2.02). The plateau phase frequencies were in the range of 6.0 ± 1.0 to 18.0 ± 2.0 / min (Table 2.01). The maximum of 53.85% hike in plateau phase frequency was observed at the end of 6 hours, while the maximum decline of 50 % was observed at the end of 96 hours (Table 2.02).

The exposure of mice to 5 ppm dose of DDVP resulted in elevations of spike frequencies of action potential at all the time intervals (Table 2.02). The spike frequencies of action potential ranged between 42.8 ± 5.4 to 29.2 ± 4.7

spike / min. the elevations in spike frequencies ranged between 72.22 to 185.33 %. The maximum elevations of spike potential were observed at the end of 72 hours, while minimum hike of spike potential was noted at the end of 6 hours (Table 2.02).

The exposure of mice to 5 ppm dose of DDVP led to elevations in plateau phase potentials at all the time intervals except at the end of 120 and 240 hours (Table 2.02). The plateau phase frequencies ranged between 9.0 ± 2.4 and 24.0 ± 4.3 / min (Table 2.01). At the end of 120 and 240 hours the plateau phase frequency remained to 18.0 ± 2.0 / 2.5. The maximum of 200% elevation was observed at the end of 72 hours, while the minimum of 10.0 % hike in plateau phase frequency was observed at the end of 12 hours (Table 2.02).

The mice subjected to 10 ppm dose of DDVP showed elevations in spike frequencies of action potential at all the time intervals (Table 2.02). The spike frequencies ranged between 34.8 ± 7.8 / min and 54.0 ± 8.7 / min (Table 2.01). The elevation in the spike frequencies ranged from 93.38% to 260.0%. The maximum elevation in spike frequency was observed at the end of 72 hour, while the minimum hike in spike frequency was noted at the end of 120 hours (Table 2.02).

Under the influence of 10 ppm dose of DDVP, the plateau phase frequencies elevated at all the time intervals except at the end of 240 hours (Table 2.02). The plateau phase frequencies ranged between 12.0 ± 1.2 / min

and 27.6 ± 6.4 /min (Table 2.01). The elevations in plateau phase frequencies ranged between 13.89 % and 330.0 %. The maximum hike in plateau phase frequency was observed at the end of 72 hours while the minimum hike was observed at the end of 120 hours (Table 2.02). However, at the end of 240 hours the plateau phase frequency decreased by 8.88%.

The mice administered 100 ppm dose of DDVP showed elevations in spike frequencies of action potential at all the time intervals (Table 2.02). The spike frequencies ranged between 38.2 ± 6.5 /min and 78.0 ± 4.6 / min (Table 2.01). The elevations in the spike frequencies ranged between 95.24 % and 333.33 %. The maximum increase in spike frequency was observed at the end of 96 hours, while the minimum hike of spike frequency was observed at the end of 24 hours (Table 2.02).

The mice subjected to 100 ppm dose of DDVP showed elevation in plateau phase frequencies at all the time intervals except at the end of 96 hours (Table 2.02). There was no change in plateau phase frequency at the end of 96 hours. The plateau phase frequency ranged between 13.0 ± 2.1 / min and 36.0 ± 6.2 / min (Table 2.01). The increase in plateau phase frequency ranged between 37.22% and 358.33%. The maximum hike in plateau phase frequency was observed at the end of 72 hours, while the minimum elevation of the plateau phase frequency was observed at the end of 240 hours. There was no change in the plateau phase frequency at the end of 96 hours (Table 2.02).

Changes in frequency inward currents

The inward current spike and plateau phase frequencies were increased in response to various doses of DDVP for different time intervals barring a few exceptions (Table 2.01 and 2.02). The control animals exhibited the spike frequency in the range of 14.2 ± 2.5 to 24 ± 2.5 spikes / min and the plateau phase frequency ranged from 6.0 ± 0.0 to 12.0 ± 2.0 PPs / min.

The exposure of mice to 1 ppm dose of DDVP led to an increase in spike frequencies of action potentials at all the time intervals. The spike frequencies ranged between 18.0 ± 4.1 to 28.6 ± 5.7 spikes / min (Table 2.01). The increases of spike frequency of inward current with respect to the control were in the range of 1.67 to 54.59% (Table 2.02). The elevation of spike frequency observed at the end of 6 hours was non-significant while they were highly significant for rest of the time intervals. The maximum elevation of inward current spike frequency was observed at the end of 48 hours (Table 2.02).

Mice subjected to 1 ppm dose of DDVP, revealed elevations in the inward current plateau phase frequencies at the end of 6,24,48,72,96 and 120 hours but it declined at the end of 12 and 240 hours (Table 2.02). The plateau frequencies were in the range of 8.0 ± 0 to 13.5 ± 2.0 PPs / min (Table 2.01). The increases in plateau phase frequency were in the range of 6.67 to 106.67%, while the decline at the end of 120 and 240 hours was by 15.0 and 33.3 % respectively (Table 2.02). The maximum elevation in plateau phase

frequency was observed at the end of 96 hours, while an 100.0 % increase in plateau phase frequency was observed at the end of 24,48 and 120 hours. The maximum decline in plateau phase frequency was noted at the end of 240 hours.

Under the influence of 5 ppm dose of DDVP, the inward current spike frequencies were in the range of 25.8 ± 7.2 to 42.0 ± 8.7 spikes /min (Table 2.01). The spike frequencies elevated at all the time intervals and the increase were in the range of 37.5 to 163.38%. The maximum elevation in inward current spike frequency was observed at the end of 120 hours, while the minimum increase was observed at the end of 6 hours (Table 2.02).

Mice administered with 5 ppm dose of DDVP exhibited plateau phase frequencies in the range of 14.1 ± 4.5 to 18.4 ± 3.6 PPs / min (Table 2.01). The plateau phase frequencies were elevated at all the time intervals and the increase was in the range of 17.50 to 206.67%. The maximum of about 3-fold increase in plateau phase frequency was observed at the end of 48 hours while the minimum elevation was observed at the end of 240 hours.

Under the influence of 10 ppm dose of DDVP, the inward current spike frequencies ranged between 24.7 ± 7.1 to 42.3 ± 9.7 spikes / min (Table 2.01). The spike frequencies were elevated at all the time intervals and the increases were in the range of 28.33 to 135.0%. The maximum elevation of spike frequency was observed at the end of 72 hours, while the minimum hike was observed at the end of 240 hours.

Mice subjected to 10 ppm dose of DDVP showed elevations in the inward current plateau phase frequencies at all the time intervals. The plateau phase frequencies were in the range of 18.0 ± 2.5 to 26.8 ± 3.5 PPs / min (Table 2.01). The increases in plateau phase frequencies ranged from 50.0% to 270.0%. The maximum hike in plateau phase frequency was observed at the end of 48 hours, while the minimum hike was observed at the end of 6 and 240 hours (Table 2.02).

100 ppm dose of DDVP induced elevations in the inward current spike frequencies at all the time intervals. The spike frequencies ranged between 20.2 ± 8.4 to 38.0 ± 9.6 spikes / min (Table 2.01). The increases in the spike frequencies were in range of 49.17 to 135.21 % (Table 2.02). The maximum hike in spike frequency was observed at the end of 120 hours, while the minimum hike was observed at the end of 72 hours.

Under the influence of 100 ppm dose of DDVP the plateau phase frequencies were in the range of 18.0 ± 2.5 to 19.8 ± 4.6 (Table 2.01). The plateau phase frequencies were elevated at all the time intervals and the elevations were in the range of 50.0 to 205.0%. The maximum elevation of more than 3 folds (205.0%) in plateau phase frequency was observed at the end of 96 hours while the minimum elevation was observed at the end of 12 hours.

CHANGES IN FREQUENCIES OF ISOELECTRIC PHASES

The isoelectric phase frequencies declined in response to treatment with various doses of DDVP at all the time intervals. The isoelectric phase frequencies for control animals were in the range of 36.0 ± 4.0 to 48.0 ± 2.0 IPs / min.

Mice treated with 1 ppm dose of DDVP revealed a decline in isoelectric phase frequencies at all the time intervals. The isoelectric phase frequency was in the range of 25.2 ± 4.5 to 40.0 ± 5.0 IPs / min (Table 2.01). The decline in the isoelectric phase frequencies were in the range of 4.76 to 47.5%. The maximum decline of isoelectric phase frequency was observed at the end of 240 hours while the minimum decline was observed at the end of 6 hours (Table 2.02).

Under influence of 5 ppm dose of DDVP, the isoelectric frequency was in the range of 20.8 ± 3.2 to 42.0 ± 6.6 IPs / min (Table 2.01). The isoelectric phase frequencies declined as compared to the control at all the time intervals and the percentages of decline in the isoelectric phase frequencies was in the range of 12.5 to 50.48% (Table 2.02). The maximum decline in isoelectric phase frequency was observed at the end of 72 hours while the minimum reduction was found at the end of 240 hours.

In response to 10 ppm dose of DDVP the isoelectric phase frequencies declined at all the time intervals. The isoelectric frequencies were in the range

of 24.0 ± 3.7 to 37.5 ± 6.4 IPs /min (Table 2.01). The percentages of decline in isoelectric phase frequencies were in the range of 10.71 to 48.75 % (Table 2.02). The minimum decline in isoelectric phase frequency was observed at the end of 96 hours, while the maximum was observed at the end of 24 hours.

Mice treated with 100 ppm dose of DDVP exhibited the isoelectric phase frequencies in the range of 15.3 ± 4.7 to 34.8 ± 5.2 IPs / min. The isoelectric phase frequencies declined at all the time intervals and the declines were in the range of 27.50 to 63.57 % (Table 2.02). The maximum decline was observed at the end of 120 hours, while the minimum decline was observed at the end of 72 hours.

CHANGES IN FREQUENCIES OF HYPERPOLARISATIONS

The hyperpolarisations appeared in varying frequencies in mice treated with DDVP. The control mice did not exhibit any hyperpolarisations. The hyperpolarisation frequencies are tabulated in table 2.01. The percent changes are not calculated, as controls had no hyperpolarisation frequencies.

Under the influence of 1 ppm dose of DDVP, hyperpolarisations were introduced at all the time intervals except at the end of 72 hour. The hyperpolarisation frequency was in the range of 6.6 ± 1.2 to 12.8 ± 1.6 HPs / min. The maximum number of hyperpolarisation frequencies was observed at the end of 12 hours (Table 2.01).

5 ppm dose of DDVP induced the occurrence of hyperpolarisation frequency in the range of 12.0 ± 2.5 to 18.7 ± 3.0 HPs / min. The maximum frequency of hyperpolarisation was observed at the end of 72 hours, and the minimum hyperpolarisation frequency was observed at the end of 12 and 240 hours (Table 2.01).

Mice subjected to 10 ppm dose of DDVP, showed appearance of hyperpolarisations at all the time intervals. The hyperpolarisation frequency was in the range of 14.6 ± 2.8 to 22.8 ± 4.5 HPs / min. The maximum hyperpolarisation frequencies were observed at the end of 48 hours, while the minimum number of hyperpolarisation frequencies were found at the end of 240 hours (Table 2.01).

Under the influence of 100 ppm dose of DDVP, the occurrence of hyperpolarisation frequencies ranged between 18.0 ± 2.1 and 32.4 ± 6.3 HPs / min. The maximum hyperpolarisation frequencies were observed at the end of 96 hours, while the minimum hyperpolarisation frequencies were observed at the end of 6 and 24 hours (Table 2.01).

**Table 2.01: EFFECT OF DDVP ON THE FREQUENCIES OF ELECTRICAL ACTIVITIES OF SPINAL CORD OF MOUSE
(*Mus musculus*)**

Time intervals	CONTROL						1ppm						5 ppm						10 ppm						100 ppm					
	AP/min		IC/min		IP/ min	HP/ min	AP/min		IC/min		IP/ min	HP/ min	AP/min		IC/min		IP/ min	HP/ min	AP/min		IC/min		IP/ min	HP/ min	AP/min		IC/min		IP/ min	HP/ min
	S	P	S	P			S	P	S	P			S	P	S	P			S	P	S	P			S	P	S	P		
6Hr	18.0 ±2.0	6.5 ±1.5	24.0 ±2.5	12.0 ±2.0	42.0 ±5.0	-	25.2 ±3.4	10.0 ±1.2	24.4 ±1.2	12.8 ±3.4	40.0 ±5.0	12.0 ±2.0	31.0 ±5.3	9.6 ±2.4	33.0 ±4.6	16.0 ±2.5	25.8 ±5.2	13.2 ±2.7	36.7 ±7.1	12.0 ±1.2	30.8 ±6.0	18.0 ±2.0	31.5 ±4.8	15.4 ±1.8	39.0 ±8.3	13.0 ±2.1	35.8 ±7.9	19.2 ±3.2	25.3 ±4.5	18.0 ±2.1
12Hr	18.5 ±4.0	12.0 ±2.0	24.0 ±3.0	12.0 ±1.5	36.0 ±4.0	-	27.0 ±4.2	14.0 ±2.0	21.6 ±5.4	10.2 ±2.5	33.6 ±4.5	12.8 ±1.6	32.4 ±5.8	13.2 ±1.6	30.8 ±5.4	16.8 ±3.0	30.5 ±5.8	12.0 ±2.5	39.2 ±6.9	17.0 ±6.0	34.7 ±5.7	18.5 ±2.3	24.7 ±5.0	18.6 ±2.2	38.2 ±6.5	18.0 ±3.0	36.0 ±8.4	18.0 ±2.5	18.7 ±2.4	24.0 ±3.3
24Hr	21.0 ±4.0	12.0 ±2.5	18.0 ±4.0	6.0 ±0	48.0 ±5.0	-	34.0 ±8.6	12.0 ±1.0	27.6 ±4.2	12.0 ±1.8	35.5 ±6.7	9.0 ±2.0	42.0 ±7.3	15.0 ±2.5	38.3 ±7.8	18.0 ±2.8	34.4 ±6.3	18.0 ±4.2	45.4 ±6.2	24.0 ±4.8	37.9 ±7.2	18.0 ±2.8	24.6 ±5.3	18.3 ±2.0	41.0 ±7.0	18.0 ±3.7	36.1 ±7.8	19.8 ±4.6	27.4 ±5.4	18.0 ±3.2
48Hr	18.0 ±2.0	12.0 ±1.2	18.5 ±1.0	6.0 ±0	38.5 ±4.3	-	31.7 ±7.3	18.0 ±2.0	28.6 ±5.7	12.0 ±1.5	25.2 ±2.8	7.2 ±1.8	41.6 ±6.3	18.0 ±3.0	42.0 ±8.7	18.4 ±3.6	25.3 ±5.2	18.0 ±3.4	51.0 ±9.1	27.0 ±4.4	41.5 ±7.8	22.2 ±3.7	34.2 ±4.7	22.8 ±4.5	60.4 ±4.7	24.0 ±3.2	38.0 ±9.6	17.2 ±3.2	24.8 ±4.3	30.8 ±6.6
72Hr	15.0 ±1.5	6.0 ±0	18.0 ±2.5	8.2 ±1.4	42.0 ±3.8	-	30.0 ±4.8	9.0 ±1.5	27.8 ±6.3	13.5 ±2.0	30.0 ±4.0	-	42.8 ±5.4	18.0 ±2.8	42.0 ±10.6	17.7 ±4.8	20.8 ±3.2	18.7 ±3.0	54.0 ±8.7	25.8 ±5.8	48.3 ±9.7	26.8 ±3.5	24.0 ±3.7	20.2 ±4.0	64.0 ±11.3	27.5 ±5.0	20.2 ±8.4	18.0 ±2.4	17.1 ±4.1	31.2 ±4.0
96Hr	18.0 ±4.0	12.0 ±1.5	17.4 ±3.0	6.0 ±1.0	42.0 ±5.2	-	30.0 ±6.5	6.0 ±1.0	21.7 ±5.2	12.4 ±1.0	32.4 ±5.1	8.4 ±1.5	37.2 ±5.0	24.0 ±4.3	36.0 ±8.2	14.4 ±3.8	24.0 ±3.7	16.8 ±2.0	46.0 ±5.7	27.6 ±6.4	40.3 ±11.2	18.0 ±2.0	37.5 ±6.4	20.3 ±3.6	78.0 ±14.6	12.0 ±5.5	37.3 ±9.8	18.3 ±2.0	15.3 ±4.7	32.4 ±6.3
120Hr	18.0 ±2.5	18.0 ±3.0	14.2 ±2.5	6.0 ±0	45.7 ±4.0	-	22.5 ±5.5	12.0 ±2.5	18.0 ±4.1	12.0 ±1.0	36.0 ±5.8	12.0 ±1.0	39.0 ±6.3	18.0 ±2.5	37.4 ±7.8	15.0 ±3.8	33.5 ±4.3	12.4 ±1.8	34.8 ±7.8	20.5 ±5.0	35.4 ±9.5	18.2 ±2.0	32.4 ±5.7	19.4 ±3.0	66.0 ±17.3	36.0 ±6.2	33.4 ±11.4	15.8 ±2.7	19.6 ±2.2	28.0 ±5.5
240Hr	15.2 ±1.5	18.0 ±2.0	15.0 ±0	12.0 ±1.0	48.0 ±2.0	-	19.5 ±4.2	12.0 ±2.0	20.4 ±3.6	8.0 ±0	25.2 ±4.5	6.6 ±1.2	29.2 ±4.7	18.0 ±2.0	25.8 ±7.2	14.1 ±4.5	42.0 ±6.6	12.0 ±2.0	35.3 ±10.4	16.4 ±4.9	24.7 ±7.1	18.0 ±2.5	28.7 ±4.4	14.6 ±2.8	40.0 ±11.5	24.7 ±5.8	24.2 ±10.3	18.8 ±4.6	34.8 ±5.2	21.4 ±4.6

AP – Action Potential
IC – Inward Current
IP – Isoelectric phase

HP - Hyperpolarisation
S – Spike
P – plateau phase

**Table 2.02 : EFFECT OF DDVP ON THE FREQUENCIES OF ELECTRICAL ACTIVITIES OF SPINAL CORD OF
MOUSE (*Mus musculus*) EXPRESSED AS PERCENT CHANGES.**

Time intervals	1 ppm					5 ppm					10 ppm					100 ppm				
	AP/min		IC / min		IP/ min	AP/ min		IC/ min		IP/ min	AP/ min		IC/ min		IP / min	AP/ min		IC / min		IP / min
	S	P	S	P		S	P	S	P		S	P	S	P		S	P			
6 Hr	40.00	53.85	1.67	6.67	-4.76	72.22	47.69	37.50	33.33	-38.57	103.89	84.61	28.33	50.00	-25.0	116.67	100.0	49.17	60.0	-39.76
12 Hr	45.90	16.67	20.0	-15.0	-6.67	75.13	10.0	71.11	40.0	-15.28	111.89	41.67	92.78	54.47	-31.39	106.49	50.00	100.0	50.0	-48.06
24 Hr	61.90	0.0	53.33	100.0	-26.04	100.0	25.0	112.77	200.0	-28.33	116.19	100.0	110.56	200.0	-48.75	95.24	50.00	100.55	230.0	-42.92
48 Hr	76.11	50.0	54.59	100.0	-34.5	131.1	50.0	127.03	206.67	-34.28	183.33	125.0	124.32	270.0	-11.17	235.55	100.0	58.33	186.67	-35.58
72 Hr	100.0	50.0	54.44	64.63	-28.57	185.33	200.0	133.33	115.85	-50.48	260.0	330.0	135.0	226.83	-42.86	326.67	358.33	12.22	119.51	-59.28
96 Hr	66.67	-50.0	24.71	106.6	-22.86	106.67	100.0	106.89	140.0	-42.86	148.65	130.0	131.61	200.0	-10.71	333.33	0.0	114.36	205.0	-63.57
120 Hr	25.0	-33.3	26.76	100.0	-21.23	116.67	0.0	163.38	150.0	-26.68	93.33	13.89	149.29	203.33	-29.10	266.67	100.0	135.21	163.33	-57.11
240 Hr	28.29	-33.3	36.00	-33.3	-47.5	92.10	0.0	72.0	17.50	-12.50	132.23	-8.88	64.66	50.00	-40.21	163.16	37.22	61.33	56.67	-27.50

Percent changes for Hyperpolarisation frequency were not calculated

I(b). EFFECT OF DDVP ON THE DURATION OF ELECTRICAL ACTIVITIES

The effect of DDVP on the durations of electrical activity of spinal cord of mice are tabulated in table 2.03 and the changes in durations expressed as percentage are given in table 2.04. Fig 2.1 (b) – 2.4 (b) represents the changes observed in durations of the electrical activities.

CHANGES IN THE DURATIONS OF ACTION POTENTIALS

The duration of the spike forms of action potentials elevated at a few time intervals under the influence of 1, 5 and 10 ppm doses of DDVP while 100 ppm dose induced elevation at all the time intervals. DDVP induced much alterations in the duration of plateau phases as compared to those observed for spikes. The duration of spikes of the spinal cord of control animals was equivalent to 0.40 ± 0 sec at all the time intervals while the duration of plateau phases was 0.60 ± 0 sec.

1 ppm dose of DDVP did not alter the duration of spikes for almost all the time intervals except at the end of 48 and 96 hours. The duration of spikes observed at the end of 48 and 96 hours was about 0.42 sec (Table 2.03) and was elevated by 5.0% as compared to control (Table 2.03). The duration of spikes was 0.40 sec for rest of the time intervals.

Under the influence of 1 ppm dose of DDVP, the duration of plateau phases remained unaltered as compared to control at all the time intervals

the duration of plateau phase (0.52 ± 0.08 sec) declined below that observed for control and the decrease was by 13.33% (Table 2.04). At the end of 48 and 120 hours the duration of plateau phase was elevated to 0.65 ± 0.05 and 0.63 ± 0.06 sec respectively and the elevations were by 8.33 and 5.0% respectively.

5 ppm dose of DDVP induced slight elevations in the duration of spikes at the end of 12, 72 and 120 hours. The durations of spikes were equivalent to 0.42 ± 0.03 , 0.43 ± 0.05 and 0.41 ± 0.02 sec (Table 2.03) and the percentage of increases were 5.0, 7.5 and 2.5 % respectively. The durations of the spikes for the rest of the time intervals remained unaltered with reference to control and were equivalent to 0.40 sec (Table 2.03 and 2.04).

5 ppm dose of DDVP did not alter the duration of plateau phase at the end of 6 hours and it was similar to control (0.60 ± 0 sec). The plateau phase durations were elevated from the end of 12 hours to the end of 240 hours and they ranged between 0.62 ± 0.03 to 0.70 ± 0.05 sec (Table 2.03). The increases in the durations of plateau phase frequencies were in the range of 3.33 to 16.67 % (Table 2.04). The minimum hike in the duration of plateau phase was observed at the end of 24 hours, while the maximum hike was observed at the end of 72, 96 and 120 hours.

Administration of 10 ppm dose of DDVP resulted in the elevation of the durations of spikes at the end of 6, 24, 48, 72 and 120 hours, while at the rest of the time intervals it remained similar to that observed for control. The

durations for the spike, equivalent to 0.42 sec was observed at the end of 6, 24 and 120 hours while the durations at the end of 48 and 72 hours were equivalent to 0.43 ± 0.04 and 0.55 ± 0.05 sec respectively (Table 2.03). The percentage of increases in spike durations at the end of 6, 24 and 120 hours was 5.0% and at the end of 48 and 72 hours it was 7.5 and 37.5% respectively (Table 2.04).

10 ppm dose of DDVP induced elevations in the duration of plateau phases at all the time intervals. The duration of plateau phase was in the range of 0.62 ± 0.12 to 0.72 ± 0.14 sec (Table 2.03). The increases in the durations of plateau phases ranged between 3.33 to 20.0 %. The maximum hike of plateau phase was observed at the end of 72 hours while the minimum hike was noted at the end of 6 hours (Table 2.04).

Under the influence of 100 ppm dose of DDVP, the durations of spikes were elevated at all the time intervals as compared to control. The durations of spikes were in the range of 0.42 ± 0.05 to 0.60 ± 0.05 sec (Table 2.03). The increases in the durations of spikes ranged between 5.0 and 50 %. The maximum hike of the duration of spikes was observed at the end of 72 hours, while a minimum hike was observed at the end of 12, 48 and 240 hours (Table 2.04).

The mice treated with 100 ppm dose of DDVP, showed elevations in the durations of plateau phases at all the time intervals. The durations of plateau phases were in the range of 0.64 ± 0.06 and 0.82 ± 0.28 sec (Table

2.03). The increases in the durations of the plateau phases ranged between 6.67 to 36.67%. The maximum hike in duration of plateau phase was observed at the end of 120 hours, while the minimum hike was observed at the end of 24 hours.

CHANGES IN DURATIONS OF INWARD CURRENTS

Administration of DDVP to mice did not induce significant increase in the duration of spikes of inward current barring a few exceptions. The durations of plateau phases were significantly elevated under the influence of a few of the doses at some time intervals. The duration of spikes of control animals was 0.40 sec while that of plateau phase was 0.60 ± 0 sec at all the time intervals (Table 2.03).

Under the influence of 1 ppm dose of DDVP the durations of the spikes of inward current were not altered at all the time intervals. The duration of the spikes was found to be 0.40 sec.

Animals subjected to 1 ppm dose of DDVP showed no change in the duration of the plateau phase at the end of 6, 96, 120 and 240 hours while it was elevated at the end of 12, 24, 48 and 72 hours. The duration of plateau phase was equivalent to 0.64 sec at the end of 12 and 72 hours, while at the end of 24 and 48 hours it was 0.70 sec (Table 2.03). The changes in the durations of plateau phase were by 6.67% at the end of 12 and 72 hours and by 16.67 % at the end of 24 and 48 hours (Table 2.04).

5 ppm dose of DDVP promoted increase in the durations of spikes at the end of 24,72,96 and 120 hours while it remained unaltered at the rest of the time intervals. The durations of the spikes were in the range of 0.41 ± 0.02 to 0.43 ± 0.05 sec (Table 2.03). The increases in the durations of the spike ranged between 2.5 to 7.5 %. The maximum hike in the duration of spikes was evident at the end of 72 hours while the minimum hike was observed at the end of 120 hours (Table 2.04). The increases in the duration of plateau phase ranged between 8.33 to 33.33 %. The maximum hike in the duration of plateau phase was observed at the end of 96 hours and the minimum hike was observed at the end of 24 hours (Table 2.04).

10 ppm dose of DDVP elevated the durations of spike at the end of 24, 48, 72, 120 and 240 hours while it remained unaltered at rest of the time intervals. The durations of spikes were in the range of 0.40 ± 0 to 0.48 ± 0.04 sec (Table 2.03). The increases of the durations of the spikes ranged between 5.0 to 20.0 %. The highest hike in the durations of the spike was observed at the end of 72 hours while the lowest hike was observed at the end of 120 and 240 hours (Table 2.04).

Under the influence of 10 ppm dose of DDVP the durations of plateau phase of inward current were elevated at all the time intervals except at the end of 12 hours. The durations of plateau phase were in the range of 0.60 ± 0 to 0.80 ± 0.08 sec (Table 2.03). The increases in the durations of plateau phase ranged between 3.33 to 33.33 %. The maximum increase in duration of

plateau phase was observed at the end of 72 and 96 hours while the minimum hike was evident at the end of 6 hours (Table 2.04).

Mice treated with 100 ppm dose of DDVP did not show alterations in the durations of spikes at the end of 6 and 12 hours but subsequently the durations of spikes were elevated till the end of 240 hours. The durations of spikes were in the range of 0.40 ± 0 to 0.60 ± 0.08 sec (Table 2.03). The increases in the durations of spike were in the range of 5.0 to 50.0 %. The maximum increase in the duration of spike was observed at the end of 72 hours and 120 hours while the minimum hike was noted at the end of 24 hours (Table 2.04).

100 ppm dose of DDVP induced alterations in the durations of plateau phases in the range of 0.50 ± 0.14 to 0.84 ± 0.13 sec (Table 2.3). The duration of plateau phases elevated at the end of 6, 12, 24, 48, 120 and 240 hours while it declined as compared to control at the end of 72 and 96 hours. The elevations were in the range of 13.33 to 40.0 % while the decline was by 16.67 and 13.33 % at the end of 72 and 96 hours respectively. The maximum hike in the durations of plateau phase was observed at the end of 12 hours (Table 2.4).

Changes in durations of isoelectric phases

The durations of isoelectric phases exhibited fluctuations under the influence of DDVP at different time intervals. However, in general the

durations of isoelectric phases declined as compared to control barring a few exceptions. The durations of isoelectric phase in the control mice ranged between 0.44 ± 0.11 to 0.62 ± 0.09 sec.

Animals subjected to 1 ppm dose of DDVP exhibited fluctuations in the durations of isoelectric phases at different time intervals and were in the range of 0.45 ± 0.04 to 0.63 ± 0.12 sec (Table 2.03). The durations of isoelectric phases were elevated as compared to control at the end of 6, 12, 96, 120 and 240 hours. It was similar to control at the end of 24 hours and declined below the control at the end of 48 and 72 hours by 19.64 and 41.93 %. The increases in durations of isoelectric phases ranged between 4.30 to 43.18 %. The maximum hike in durations of isoelectric phases was observed at the end of 96 hours while the maximum decline was noted at the end of 72 hours (Table 2.04).

The administration of 5 ppm dose of DDVP promoted changes in the durations of isoelectric phases in the range of 0.36 ± 0.05 to 0.67 ± 0.21 sec (Table 2.03). The durations of isoelectric phase were elevated at the end of 12, 24, 48, 96 and 240 hours and the elevations were in the range of 8.21 to 41.30 %. At the end of 6, 72 and 120 hours the durations of isoelectric phases were below the control level and the decline ranged between 6.0 to 28.07 % (Table 2.04). The maximum hike in duration of isoelectric phase was observed at the end of 240 hours while the maximum decline was observed at the end of 6 hours.

Under the influence of 10 ppm dose of DDVP the durations of isoelectric phases varied in the range of 0.29 ± 0.05 to 0.69 ± 0.13 sec (Table 2.03). In comparison to control, the durations of isoelectric phase were elevated at the end of 96, 120 and 240 hours while it declined below the control level at rest of the time intervals. The increases in the durations of isoelectric phases were in the range of 14.0 % while the decline was in range of 19.35 to 44.23 % (Table 2.04). The maximum hike in durations of isoelectric phase was observed at the end of 240 hours while the maximum decline was at the end of 12 hours.

100 ppm dose of DDVP altered the durations of isoelectric phase in the range of 0.18 ± 0.05 to 0.60 ± 0.08 sec (Table 2.03). The durations of isoelectric phases was declined below the control at all the time intervals except at the end of 6 hours (Table 2.04). The durations of isoelectric phases were elevated by 5.26 % at the end of 6 hours while the decline in the durations of isoelectric phases ranged between 15.38 to 64.0 %. The maximum decline in the durations of isoelectric phase was observed at the end of 120 hours.

TABLE 2.03: EFFECT OF DDVP ON THE DURATION OF ELECTRICAL ACTIVITIES OF SPINAL CORD OF Mouse (*Mus musculus*)

Time interval	Control					1 ppm					5 ppm					10 ppm					100 ppm				
	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec
	S	P	S	P		S	P	S	P		S	P	S	P		S	P	S	P		S	P			
6Hr	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.57 ±0.14	0.04 ±0	0.52 ±0.08	0.04 ±0	0.60 ±0	0.62 ±0.12	0.04 ±0	0.60 ±0	0.04 ±0	0.75 ±0.07	0.41 ±0.08	0.42 ±0.06	0.62 ±1.2	0.04 ±0	0.62 ±0.05	0.40 ±0.10	0.45 ±0.06	0.70 ±0.10	0.04 ±0	0.80 ±0.15	0.60 ±0.08
12Hr	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.52 ±0.15	0.04 ±0	0.60 ±0	0.04 ±0	0.64 ±0.06	0.55 ±0.08	0.42 ±0.03	0.64 ±0.05	0.04 ±0	0.60 ±0	0.67 ±0.21	0.04 ±0	0.71 ±0.13	0.04 ±0	0.60 ±0	0.29 ±0.05	0.42 ±0.05	0.66 ±0.05	0.04 ±0	0.84 ±0.13	0.44 ±0.10
24Hr	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.60 ±0.21	0.04 ±0	0.60 ±0	0.04 ±0	0.70 ±0.05	0.60 ±0.09	0.04 ±0	0.62 ±0.03	0.42 ±0.02	0.65 ±0.08	0.65 ±0.18	0.42 ±0.02	0.70 ±0.12	0.43 ±0.05	0.63 ±0.04	0.45 ±0.13	0.51 ±0.03	0.64 ±0.06	0.42 ±0.03	0.70 ±0.10	0.45 ±0.12
48Hr	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.56 ±0.14	0.42 ±0.04	0.65 ±0.05	0.04 ±0	0.70 ±0.06	0.45 ±0.04	0.04 ±0	0.67 ±0.04	0.04 ±0	0.66 ±0.10	0.606 ±0.08	0.43 ±0.04	0.70 ±0.10	0.47 ±0.05	0.65 ±0.05	0.36 ±0.11	0.42 ±0.04	0.80 ±0.08	0.43 ±0.05	0.77 ±0.08	0.32 ±0.16
72Hr	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.62 ±0.09	0.04 ±0	0.60 ±0	0.04 ±0	0.64 ±0.02	0.36 ±0.05	0.43 ±0.05	0.70 ±0.05	0.43 ±0.05	0.75 ±0.12	0.46 ±0.05	0.55 ±0.05	0.72 ±0.14	0.48 ±0.04	0.80 ±0.08	0.50 ±0.14	0.53 ±0.05	0.72 ±0.13	0.60 ±0.08	0.50 ±0.14	0.32 ±0.14
96Hr	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.44 ±0.11	0.42 ±0.03	0.60 ±0	0.04 ±0	0.60 ±0	0.63 ±0.12	0.04 ±0	0.70 ±0.05	0.42 ±0.02	0.80 ±0.15	0.36 ±0.05	0.04 ±0	0.66 ±0.13	0.04 ±0	0.80 ±0.12	0.55 ±0.16	0.52 ±0.08	0.67 ±0.12	0.46 ±0.04	0.52 ±0.13	0.21 ±0.15
120Hr	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.50 ±0.08	0.04 ±0	0.63 ±0.06	0.04 ±0	0.60 ±0	0.58 ±0.10	0.41 ±0.02	0.70 ±0.07	0.41 ±0.02	0.70 ±0.10	0.47 ±0.07	0.42 ±0.06	0.68 ±0.11	0.42 ±0.04	0.67 ±0.06	0.57 ±0.15	0.60 ±0.10	0.82 ±0.28	0.60 ±0.08	0.74 ±0.31	0.18 ±0.05
240Hr	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.46 ±0.09	0.04 ±0	0.60 ±0	0.04 ±0	0.60 ±0	0.48 ±0.05	0.04 ±0	0.64 ±0.05	0.04 ±0	0.60 ±0	0.65 ±0.07	0.04 ±0	0.66 ±0.10	0.42 ±0.03	0.64 ±0.05	0.69 ±0.13	0.42 ±0.04	0.72 ±0.13	0.43 ±0.08	0.68 ±0.19	0.25 ±0.22

AP – Action Potential IC – Inward Current
S – Spike IP – Isoelectric phase
P – plateau phase

**Table 2.04 : EFFECT OF DDVP ON THE DURATIONS OF ELECTRICAL ACTIVITIES OF SPINAL CORD OF
MOUSE (*Mus musculus*) EXPRESSED AS PERCENT CHANGES.**

Time intervals	1 ppm					5 ppm					10 ppm					100 ppm				
	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec
	S	P	S	P		S	P	S	P		S	P	S	P		S	P			
6 Hr	0.0	-13.33	0.0	0.0	8.77	0.0	0.0	0.0	25.0	-28.07	5.0	3.33	0.0	3.33	-29.82	12.5	16.67	0.0	33.33	5.26
12 Hr	0.0	0.0	0.0	6.67	5.76	5.0	6.67	0.0	0.0	28.85	0.0	18.33	0.0	0.0	-44.23	5.0	10.0	0.0	40.0	-15.38
24 Hr	0.0	0.0	0.0	16.67	0.0	0.0	3.33	5.0	8.33	8.33	5.0	16.67	7.5	5.0	-25.0	27.5	6.67	5.0	16.66	-25.0
48 Hr	5.0	8.33	0.0	16.67	-19.64	0.0	11.67	0.0	10.0	8.21	7.5	16.67	17.5	8.33	-35.71	5.0	33.33	7.5	28.33	-42.85
72 Hr	0.0	0.0	0.0	6.67	-41.93	7.5	16.67	7.5	25.0	-25.81	37.5	20.0	20.0	33.33	-19.35	32.5	20.0	50.0	-16.67	-48.39
96 Hr	5.0	0.0	0.0	0.0	43.18	0.0	16.67	5.0	33.33	18.18	0.0	10.0	0.0	33.33	25.0	30.0	11.67	15.0	-13.33	-52.27
120 Hr	0.0	5.0	0.0	0.0	16.0	2.5	16.67	2.5	16.67	-6.0	5.0	13.33	5.0	11.67	14.0	50.0	36.67	50.0	23.33	-64.0
240 Hr	0.0	0.0	0.0	0.0	4.3	0.0	6.67	0.0	0.0	41.30	0.0	10.0	5.0	6.67	50.0	5.0	20.0	7.5	13.33	-45.65

I (c). EFFECT OF DDVP ON THE ELECTRICAL POTENTIALS

The electrical potential including baseline potentials, amplitudes of action potentials and amplitudes of inward current of the spinal cord of mice in response to DDVP treatment are tabulated in table 2.05 and the percent changes of these electrical potentials are presented in table 2.06.

Changes in baseline potential

A nearly dose dependent elevation in baseline potential was observed in mice treated with various doses of DDVP for different time intervals (Table 2.05 and 2.06). The baseline potential of the control mice was in the range of 13.7 ± 2.4 to 16.8 ± 3.6 mv.

Under the influence of 1ppm dose of DDVP the baseline potential elevated at all the time intervals. The baseline potentials were in the range of 15.0 ± 4.7 to 27.0 ± 4.1 (Table 2.05). The increases in the baseline potential were in the range of 3.45 to 197.08% (Table 2.06). The maximum hike in baseline potential was observed at the end of 48 hours while the minimum increase was noted at the end of 6 hours. The increases in the baseline potential were statistically significant at 48 and 72 hours ($P < 0.005$).

5 ppm dose of DDVP induced elevations in the baseline potential at all the time intervals. The baseline potentials were in the range of 23.5 ± 3.7 to 35.2 ± 6.2 mv (Table 2.05). The increases in the baseline potentials ranged

from 48.15 to 133.58%. The maximum elevation in baseline potential was observed at the end of 48 hours while the minimum was observed at the end of 12 hours (Table 2.06).

The mice subjected to 10 ppm dose of DDVP, showed elevations in baseline potentials at all the time intervals. The baseline potentials were in the range of 22.6 ± 2.6 to 38.0 ± 4.2 mv (Table 2.05). The increases in the baseline potential in comparison to control were in the range of 54.79 to 158.39 % (Table 2.06). The highest hike was noted at the end of 120 hours. The elevations in baseline potentials were statistically significant from the end of 12 to 96 hours.

Under the influence of 100 ppm dose of DDVP the baseline potential were elevated sharply. The baseline potentials were in the range of 31.6 ± 4.0 to 82.4 ± 7.1 mv (Table 2.05). The increases in the baseline potentials ranged between 106.17 to 405.81 %. The minimum increase in baseline potentials was observed at the end of 12 hours while the maximum elevation was observed at the end of 72 hours. The elevations in the baseline potentials were statistically significant at all the time intervals.

Changes in amplitude of action potentials

Mice treated with various doses of DDVP exhibited an increase in amplitude of action potentials at almost all the time intervals. The amplitude of

action potentials in the control animals remained steady at 2.5 mv at all the time intervals (Table 2.05).

1 ppm dose of DDVP increased the amplitude of action potentials at all the time intervals. The amplitude was in the range of 2.6 ± 0.6 to 3.1 ± 1.0 mv (Table 2.05). The increases in the amplitude of action potentials with reference to control were in the range of 4.0 to 24.0%. The lowest hike in the amplitude of action potentials was equivalent to 4.0% and was observed at the end of 6,48,96 and 240 hours while the maximum hike of 24.0% was observed at the end of 24 hours.

Under the influence of 5 ppm dose of DDVP, amplitude of action potentials were in the range of 2.5 ± 0.1 to 3.8 ± 1.2 mv (Table 2.5). The amplitude of action potentials were elevated from the end of 6 hours to 120 hours while at the end of 240 hours it remained similar to that observed for control. The increases in the amplitude of action potentials were in the range of 12.0 to 52.0% (Table 2.6). The maximum elevation in amplitude of action potentials was observed at the end of 48 hours while the minimum elevation was observed at the end of 120 hours.

The mice treated with 10 ppm dose of DDVP showed elevations in the amplitude of action potentials at all the time intervals. The amplitudes were in the range of 2.6 ± 0.4 to 4.2 ± 1.5 mv (Table 2.05). The increases in the amplitude in action potentials ranged between 4.0 to 68.0%. The maximum

hike in the amplitude in action potentials was observed at the end of 72 hours while the minimum was observed at the end of 240 hours.

The treatment of mice with 100 ppm dose of DDVP resulted in elevations of amplitude of action potentials at all the time intervals. The amplitudes were in the range of 2.8 ± 0.6 to 6.8 ± 1.0 mv (Table 2.05). The maximum elevation in the amplitude of action potentials was observed at the end of 72 hours while the minimum hike was observed at the end of 120 and 240 hours (Table 2.06). The elevations in the amplitude of action potentials were by 12.0 to 124.0 %.

Changes in the amplitude of inward currents

The changes in the amplitude of inward currents recorded in mice treated with various doses of DDVP are tabulated in table 2.5 and the percent changes are presented in table 2.6. The amplitude of inward current in the control mice remained at 2.5 ± 0 mv at all the time intervals.

1 ppm dose of DDVP did not alter the amplitude of inward currents at all the time intervals in comparison to control except for a slight insignificant increase of about 4.0% at the end of 12 hours (Table 2.06). The amplitude was equivalent to 2.6 ± 0.3 mv at the end of 12 hours while it remained at 2.5 mv at the rest of the time intervals.

Under the influence of 5 ppm of DDVP, the amplitudes of inward currents were elevated only at the end of 48, 72 and 240 hours. The amplitude was equivalent to 3.2 ± 0.4 mv at the end of 48 hours and at the end of 72 and 240 hours it was 2.6 ± 0.2 mv (Table 2.05). At the rest of all the time intervals the amplitude of inward currents were similar to control (2.5 mv). The maximum increase in the amplitude of inward currents (28.0%) was noted at the end of 48 hours while the minimum hike of 4.0% was noted at the end of 72 and 240 hours.

10 ppm dose of DDVP, did not alter the amplitude of inward current as compared to control at the end of 6 hours, however, it elevated at the rest of the time intervals. The amplitude of inward current was in the range of 2.5 ± 0 to 3.0 ± 0.5 mv (Table 2.05). The increases in amplitude of inward current ranged between 4.0 to 20.0%. The maximum hike in amplitude of inward current was observed at the end of 72 hours while the minimum hike was observed at the end of 12, 24 and 240 hours.

The treatment of mice with 100 ppm dose of DDVP led to increase in the amplitude of inward current at all the time intervals. The amplitude of inward current ranged between 2.7 ± 0.4 to 5.0 ± 1.3 mv (Table 2.05). The changes in inward current were in the range of 12.0 to 100.0%. The maximum hike in the amplitude of inward current was observed at the end of 96 hours, while the minimum hike was observed at the end of 240 hours.

TABLE 2.05: EFFECT OF DDVP ON THE ELECTRICAL POTENTIAL OF SPINAL CORD OF MOUSE (*Mus musculus*)

Time interval	CONTROL			1ppm			5 ppm			10 ppm			100 ppm		
	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv
6Hr	14.5 ±3.3	2.5 ±0	2.5 ±0	15.0 ±4.7	2.6 ±0.6	2.5 ±0	25.5 ±2.8	3.0 ±0.8	2.5 ±0	27.2 ±3.1	3.0 ±0.5	2.5 ±0	31.6 ^c ±4.0	4.3 ±0.5	2.8 ±0.3
12Hr	16.2 ±4.1	2.5 ±0	2.5 ±0	20.5 ±3.3	2.8 ±0.6	2.6 ±0.3	24.0 ±3.1	3.2 ±1.0	2.5 ±0	33.2 ^b ±3.0	3.1 ±0.5	2.6 ±0.7	33.4 ^b ±3.4	4.0 ±0.6	3.0 ±0.5
24Hr	15.4 ±2.7	2.5 ±0	2.5 ±0	23.8 ±3.6	3.1 ±1.0	2.5 ±0	25.5 ±3.2	2.9 ±0.9	2.5 ±0	31.8 ^c ±3.7	2.9 ±0.6	2.6 ±0.5	36.5 ^b ±3.4	3.8 ±0.9	3.0 ±0.2
48Hr	13.7 ±2.4	2.5 ±0	2.5 ±0	27.0 ^c ±4.1	2.6 ±0.4	2.5 ±0	32.0 ^b ±3.6	3.8 ±1.2	3.2 ±0.4	35.4 ^b ±3.3	3.4 ±1.3	2.9 ±0.4	42.0 ^a ±3.8	5.2 ±1.2	3.3 ±0.9
72Hr	15.5 ±3.1	2.5 ±0	2.5 ±0	26.2 ^c ±3.7	2.8 ±0.5	2.5 ±0	35.2 ^b ±6.2	2.9 ±0.3	2.6 ±0.2	38.0 ^b ±4.2	4.2 ±1.5	3.0 ±0.5	78.4 ^a ±6.3	6.8 ±1.0	4.2 ±1.2
96Hr	16.8 ±3.6	2.5 ±0	2.5 ±0	22.4 ±3.5	2.6 ±0.5	2.5 ±0	34.6 ^b ±4.5	3.0 ±0.5	2.5 ±0	35.7 ^b ±3.7	3.2 ±0.5	2.8 ±0.5	56.4 ^a ±6.4	5.6 ±1.1	5.0 ±1.3
120Hr	14.6 ±2.6	2.5 ±0	2.5 ±0	20.3 ±4.2	2.7 ±0.6	2.5 ±0	27.8 ^c ±3.2	2.8 ±0.6	2.5 ±0	22.6 ±2.6	2.8 ±0.2	2.7 ±0.6	71.4 ^a ±7.7	2.8 ±0.6	3.8 ±1.2
240Hr	14.3 ±3.0	2.5 ±0	2.5 ±0	17.5 ±2.7	2.6 ±0.4	2.5 ±0	23.5 ±3.7	2.5 ±0.1	2.6 ±0.2	24.7 ±1.9	2.6 ±0.4	2.6 ±0.3	62.4 ±7.1	2.8 ±0.3	2.7 ±0.4

BP – Baseline Potential a= p< 0.001
AP – Action Potential b=p< 0.01
IC – Inward Current c = p<0.05

**Table 2.06 : EFFECT OF DDVP ON ELECTRICAL POTENTIALS OF SPINAL CORD OF MOUSE (*Mus musculus*)
EXPRESSED AS PERCENT CHANGES.**

Time Intervals	1 ppm			5 ppm			10 ppm			100 ppm		
	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv
6 Hr	3.45	4.0	0.0	75.86	20.0	0.0	87.58	20.0	0.0	117.93	72.0	12.0
12 Hr	26.54	12.0	4.0	48.15	28.0	0.0	104.94	24.0	4.0	106.17	60.0	20.0
24 Hr	54.54	24.0	0.0	65.58	16.0	0.0	106.49	16.0	4.0	137.01	52.0	20.0
48 Hr	97.08	4.0	0.0	133.58	52.0	28.0	158.39	36.0	16.0	206.57	108.0	32.0
72 Hr	69.03	12.0	0.0	127.10	16.0	4.0	145.16	68.0	20.0	405.81	172.0	68.0
96 Hr	33.33	4.0	0.0	105.95	20.0	0.0	112.5	28.0	12.0	235.71	124.0	100.0
120 Hr	39.0	8.0	0.0	90.41	12.0	0.0	54.79	12.0	8.0	389.04	12.0	52.0
240 Hr	22.38	4.0	0.0	64.33	0.0	4.0	72.73	4.0	4.0	336.36	12.0	8.0

FIG 2.1(a) : Electrical activity of spinal cord of mice in response to

DDVP treatment (1 ppm dose)

(segments of actual recordings for 10 secs)

S - spike

P- plateau phase

AP – action potential

IC- inward current

IP- isoelectric phase

HP- hyperpolarisation



CONTROL



6 hr



12 hr



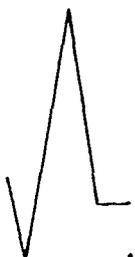
24 hr



48 hr



72 hr



96 hr

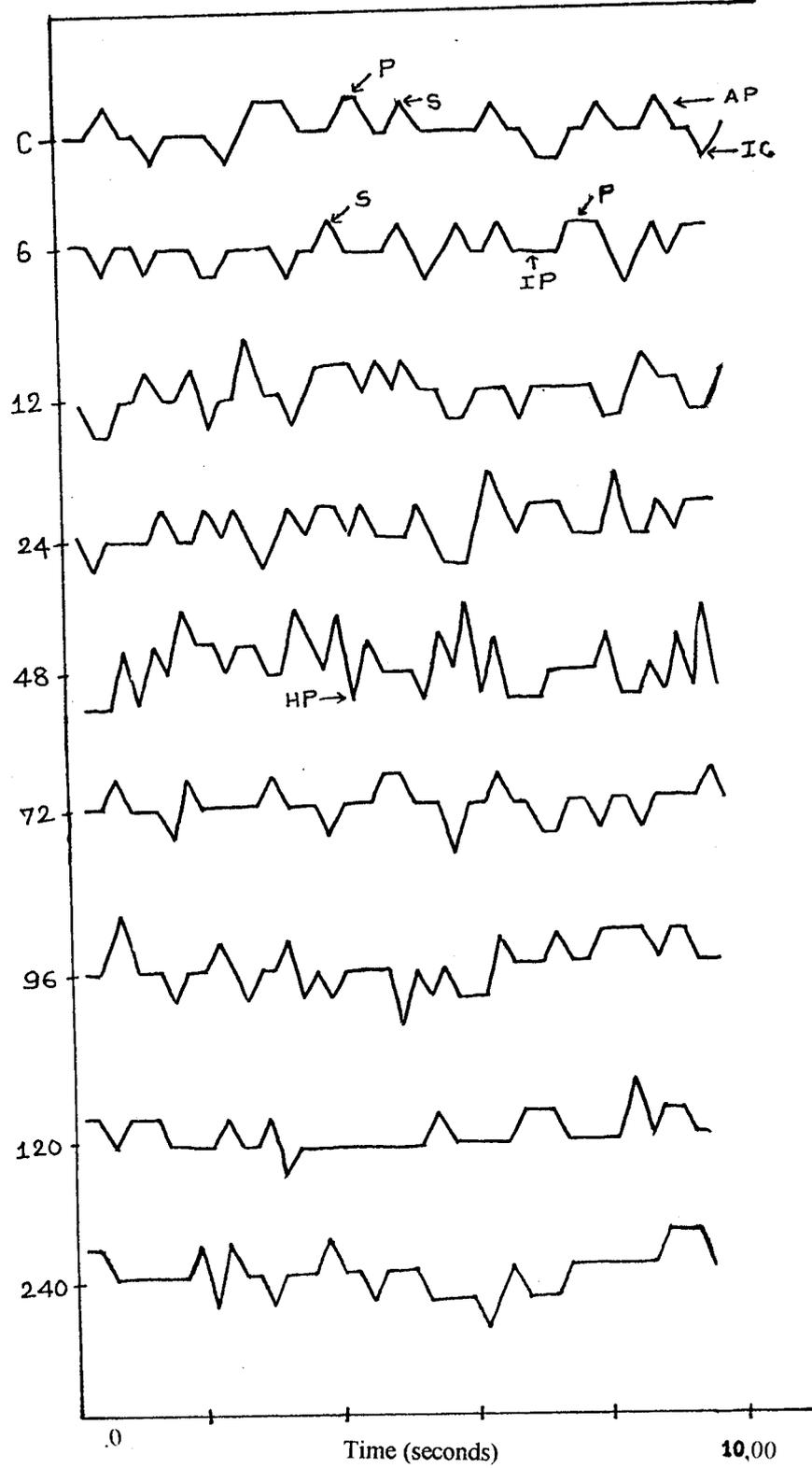


120 hr



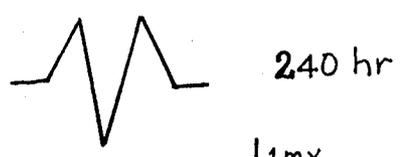
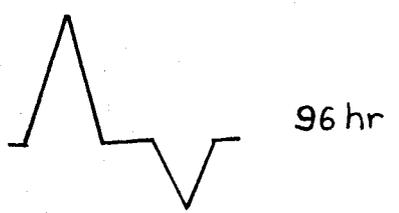
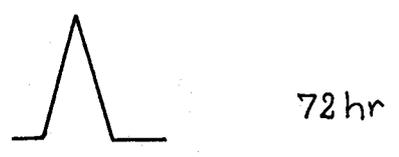
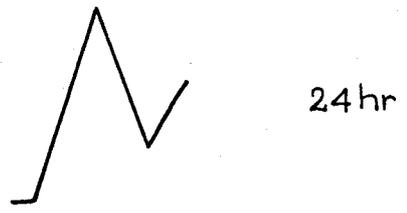
240 hr

1mv
0.2sec



**FIG 2.1 (b): Electrical activity of spinal cord of mice in response to
DDVP treatment (1 ppm dose)**

**Enlarged recordings indicating the changes in the durations
and amplitudes of the action potentials**



1mv
0.2sec

**FIG 2.2(a): Electrical activity of spinal cord of mice in response to
DDVP treatment (5 ppm dose)
(segments of actual recordings for 10 secs)**

S - spike

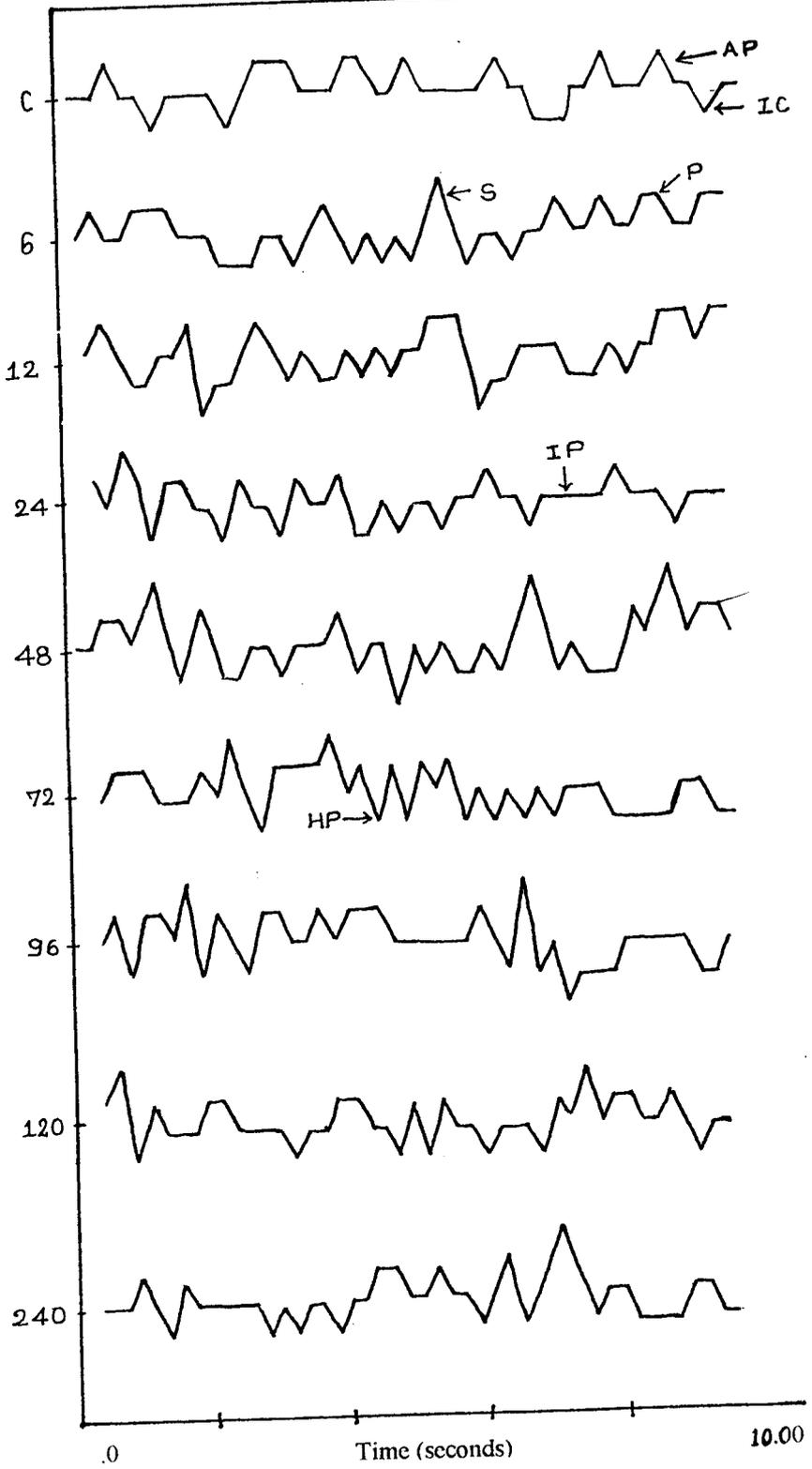
P- plateau phase

AP – action potential

IC- inward current

IP- isoelectric phase

HP- hyperpolarisation



**FIG 2.2 (b): Electrical activity of spinal cord of mice in response to
DDVP treatment (5 ppm dose)**

**Enlarged recordings indicating the changes in the durations
and amplitudes of the action potentials**

FIG 2.3 (a): Electrical activity of spinal cord of mice in response to

DDVP treatment (10 ppm dose)

(segments of actual recordings for 10 secs)

S - spike

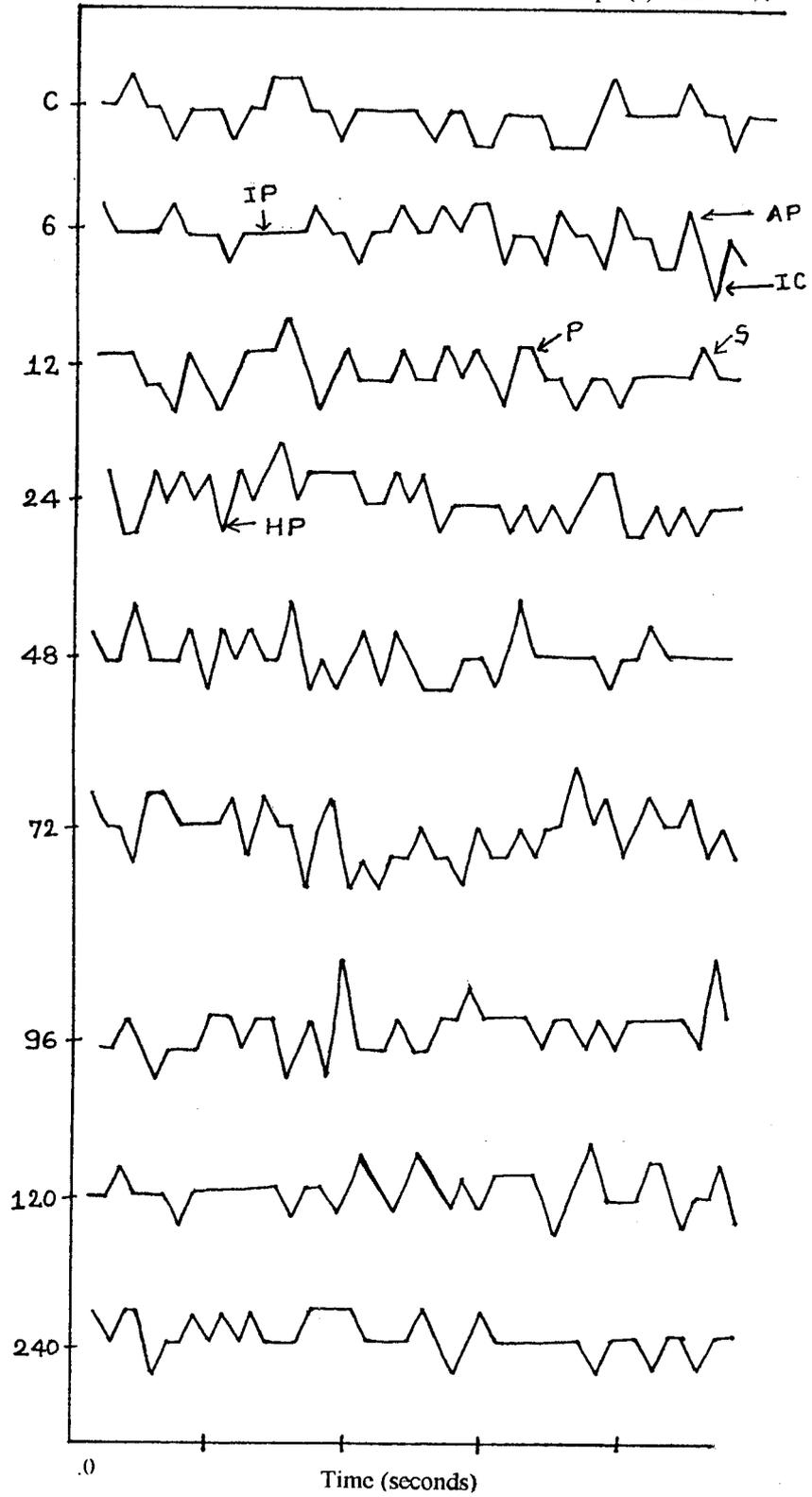
P- plateau phase

AP – action potential

IC- inward current

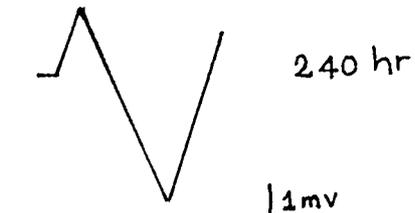
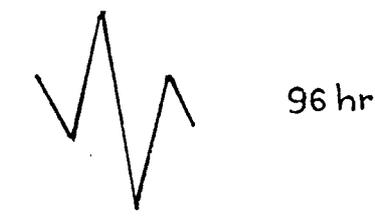
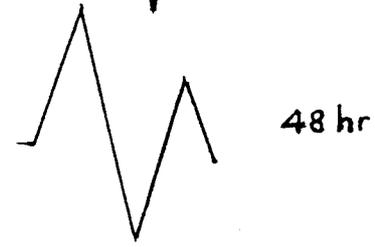
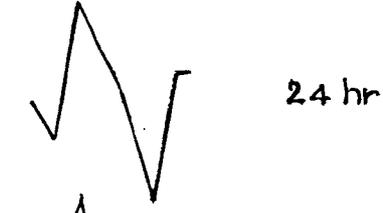
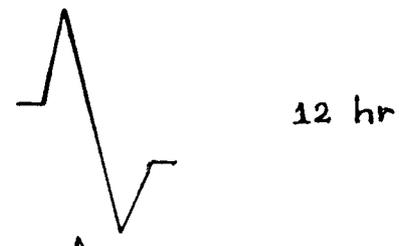
IP- isoelectric phase

HP- hyperpolarisation



**FIG 2.3 (b): Electrical activity of spinal cord of mice in response to
DDVP treatment (10 ppm dose)**

**Enlarged recordings indicating the changes in the durations
and amplitudes of the action potentials**



1mv
0.2sec

**FIG 2.4 (a): Electrical activity of spinal cord of mice in response to
DDVP treatment (100 ppm dose)
(segments of actual recordings for 10 secs)**

S - spike

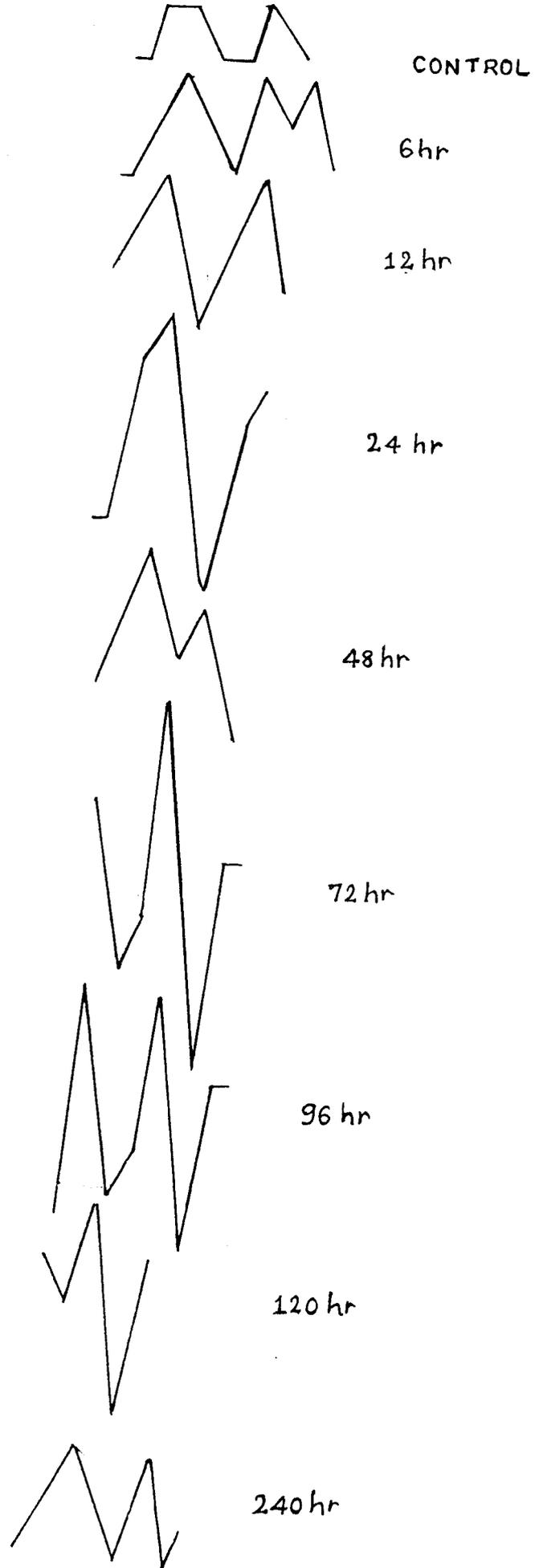
P- plateau phase

AP – action potential

IC- inward current

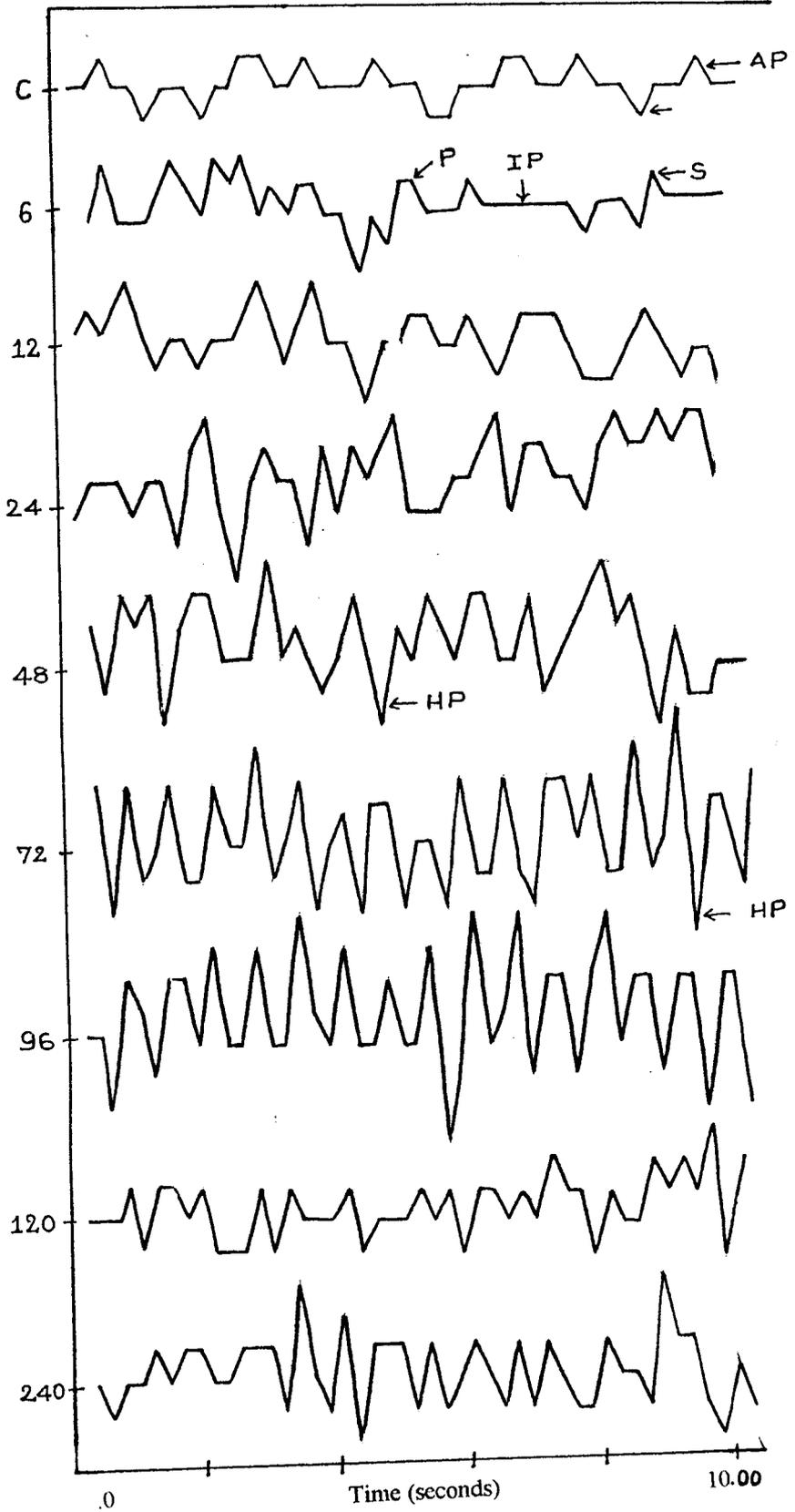
IP- isoelectric phase

HP- hyperpolarisation



**FIG 2.4 (b): Electrical activity of spinal cord of mice in response to
DDVP treatment (100 ppm dose)**

**Enlarged recordings indicating the changes in the durations
and amplitudes of the action potentials**



I (d). EFFECT OF DDVP ON CONDUCTION VELOCITY

The conduction velocity of the spinal cord was altered by DDVP. The conduction velocities are represented in table 2.07 and the percent changes are tabulated in table 2.08. The control mice exhibited conduction velocity in the range of 14.90 ± 2.3 to 16.90 ± 3.1 m / sec.

1 ppm dose of DDVP induced conduction velocity in the range of 13.8 ± 2.7 to 21.40 ± 7.3 m / sec (Table 2.07). The conduction velocity was declined below the control level at the end of 6 and 240 hours and the decline was equivalent to 1.23 and 2.36 % respectively. At the rest of the time intervals the velocity elevated and ranged between 7.23 to 39.86 % (Table 2.08). The maximum elevation in conduction velocity was observed at the end of 48 hours while the maximum decline was observed at the end of 240 hours.

Under the influence of 5 ppm dose of DDVP the conduction velocity was elevated at all the time intervals except at the end of 240 hours. The conduction velocity was in the range of 16.10 ± 3.3 to 22.30 ± 5.8 m / sec (Table 2.07). The increases were in the range of 12.96 to 45.75 %. The maximum increase in conduction velocity was observed at the end of 48 hours while the decline (4.73%) was observed at the end of 240 hours (Table 2.08).

10 ppm dose of DDVP promoted conduction velocity in the range of 16.45 ± 3.5 to 23.10 ± 4.8 m / sec (Table 2.07). In comparison to control the

conduction velocity increased at all the time intervals except at the end of 240 hours. The increases were in the range of 3.70 to 46.31 %. The decline of conductive velocity at the end of 240 hours was equivalent to 2.96 %. The maximum elevation in conduction velocity was observed at the end of 24 hours (Table 2.08).

In response to 100 ppm dose of DDVP the conduction velocity was in the range of 16.20 ± 3.6 to 38.20 ± 6.7 m / sec. The conduction velocity elevated at all the time intervals except for a decline at the end of 240 hours. The increases were in the range of 20.37 to 127.38 %. The maximum increase was observed at the end of 72 hours while a decline of about 4.14 % was observed at the end of 240 hours.

**TABLE 2.07: EFFECT OF DDVP ON THE CONDUCTION VELOCITY
OF SPINAL CORD OF MOUSE (*Mus musculus*)**

Time intervals	Control	1 ppm	5 ppm	10 ppm	100 ppm
6 hours	16.2 ± 2.6	16.0 ±3.1	18.3 ±4.0	16.80 ±3.4	19.5 ±2.4
12 hours	15.3 ±2.7	18.4 ±3.7	20.1 ±5.3	17.6 ±4.3	20.4 ±4.1
24 hours	14.9 ±2.3	18.9 ±3.7	20.4 ±4.9	21.8 ±3.5	22.6 ±3.5
48 hours	15.3 ±4.1	21.4 ±7.3	22.3 ±5.8	22.2 ±3.9	34.3 ±5.2
72 hours	16.8 ±1.7	18.2 ±3.5	21.4 ±5.3	23.10 ±4.8	38.2 ±6.7
96 hours	15.7 ±2.7	19.4 ±2.5	21.3 ±4.5	20.8 ±2.8	30.10 ±5.3
120 hours	15.2 ±3.5	16.3 ±2.5	21.0 ±5.3	20.20 ±3.0	21.20 ±4.9
240 hours	16.90 ±3.1	16.5 ±2.7	16.10 ±3.3	16.45 ±3.5	16.2 ±3.6

TABLE 2.08: EFFECT OF DDVP ON THE CONDUCTION VELOCITY OF SPINAL CORD OF MOUSE (*Mus musculus*) EXPRESSED AS PERCENT CHANGES

Time intervals	1 ppm	5 ppm	10 ppm	100 ppm
6 hours	-1.23	12.96	3.70	20.37
12 hours	20.26	31.37	15.03	33.33
24 hours	26.84	36.91	46.31	51.68
48 hours	39.86	45.75	45.09	124.18
72 hours	8.30	27.38	37.50	127.38
96 hours	23.57	35.67	32.48	91.72
120 hours	7.23	38.15	32.89	39.47
240 hours	-2.36	-4.73	-2.96	-4.14

II (a). EFFECT OF MONOCROTOPHOS ON FREQUENCIES OF ELECTRICAL ACTIVITIES

The recordings of the electrical activities including the frequencies of action potentials, inward current, isoelectric phase and hyperpolarisations of spinal cord of mice exposed to monocrotophos for varying time intervals are tabulated in table 2.09 and the percentage of changes in electrical activities are presented in table 2.10. The segments of actual recordings are represented in fig 2.5 (a) - 2.8 (a).

Changes in frequencies of action potentials

The frequencies of spikes and plateau phases of action potential elevated in spinal cord of mice in response to monocrotophos treatment at all the time intervals. The increments in spike frequencies were more pronounced than those of the plateau phase frequencies. The spike frequencies in the control animals ranged between 18.0 ± 1.5 to 20.0 ± 1.8 spikes / min while the plateau phase frequencies were in the range of 6.0 ± 1.0 to 18.0 ± 1.6 / min.

Under the influence of 1 ppm dose the spike frequencies of action potentials elevated at all the time intervals and were in the range of 21.6 ± 5.4 to 34.0 ± 6.5 spikes / min (Table 2.09). The increases in the spike frequencies ranged between 20.0 to 83.78%. The maximum hike in the spike frequency was observed at the end of 24 hours and the minimum hike was observed at the end of 6 hours (Table 2.10).

Mice treated with 1 ppm dose of monocrotophos revealed plateau phase frequencies in the range of 12.0 ± 3.0 to 21.6 ± 5.4 PPs / min (Table 2.09). The plateau phase frequencies were elevated at the end of 6, 24, 48, 120 and 240 hours. It remained similar to that of controls at the end of 12 and 72 hours and declined below the control level at the end of 96 hours. The increases in the plateau phase frequencies ranged between 25.0 to 220.0% while the decline was equivalent to 13.33%. The maximum elevation in the plateau phase frequency was observed at the end of 24 hours while the minimum elevation was observed at the end of 120 hours (Table 2.10).

5 ppm dose of monocrotophos caused elevations in spike frequencies at all the time intervals and the frequencies ranged between 24.4 ± 6.6 to 39.6 ± 6.0 spikes / min (Table 2.09). The percentages of elevations were in the range of 35.55 to 120.0%. The highest increment in spike frequencies was observed at the end of 48 hours while the lowest hike was observed at the end of 6 hours (Table 2.10).

Under the influence of 5 ppm dose of monocrotophos the plateau phase frequencies were elevated at all the exposure periods and the frequencies were in the range of 13.2 ± 1.5 to 27.6 ± 2.4 PPs /min (Table 2.09). The increases in the plateau phase frequencies ranged between 10.0 to 303.33%. The maximum hike in plateau phase frequency was observed at the end of 24 hours, while the minimum increase was observed at the end of 6 hours (Table 2.10).

The treatment of mice with 10 ppm dose of monocrotophos resulted in elevation of spike frequencies at all the time intervals. The spike frequencies were in the range of 26.4 ± 3.2 to 50.4 ± 7.2 spikes / min (Table 2.09). The increases in the spike frequencies were in the range of 46.67 to 180.0%. The maximum elevation in spike frequency was observed at the end of 48 hours while the minimum elevation was evident at the end of 240 hours (Table 2.10).

In response to administration of 10 ppm dose of monocrotophos the plateau phase frequencies elevated in the range of 12.8 ± 2.5 to 28.6 ± 5.0 PPs / min (Table 2.09). The plateau phases were elevated at all the time intervals except at the end of 96 hours and the plateau phase frequency remained almost similar to that observed for control. The elevations for plateau phase frequencies at the rest of the time intervals ranged between 28.89 to 138.33%. The maximum hike was noted at the end of 48 hours, while the minimum hike was observed at the end of 72 hours (Table 2.10).

Under the influence of 100 ppm dose of monocrotophos the spike frequencies were elevated at all the time intervals and the frequencies ranged between 22.6 ± 4.8 to 57.5 ± 7.8 spikes / min (Table 2.09). The increases in spike frequencies ranged between 68.65 to 287.5%. The maximum increase in spike frequency was observed at the end of 96 hours, while the minimum increase was observed at the end of 24 hours (Table 2.10).

Mice administered with 100 ppm dose of monocrotophos exhibited plateau phase frequencies in the range of 14.5 ± 3.2 to 27.0 ± 8.4 PPs / min

(Table 2.09). In comparison to controls the plateau phase frequencies were elevated at the end of 6, 12, 24, 48, 120 and 240 hours while it declined below the control at the end of 72 and 96 hours. The increases in plateau phase frequencies were in the range of 40.0 to 300.0%. The maximum elevation in plateau phase frequency was observed at the end of 24 hours while the maximum decline was observed at the end of 72 hours (Table 2.10).

Changes in frequencies of inward currents

The spike frequencies of inward current increased in general, barring a few exceptions in response to various doses of monocrotophos for different time intervals. However, the plateau phase frequencies were elevated only from the end of 72 hours to the end 120 hours barring a few exceptions under the influence of all doses of monocrotophos. The control animals exhibited spike frequencies in the range of 18.0 ± 1.2 to 25.3 ± 3.5 spikes / min and the plateau phase frequencies were in the range of 10.2 ± 1.0 to 18.0 ± 2.0 PPs / min.

Mice administered with 1ppm dose of monocrotophos showed spike frequencies in the range of 18.0 ± 2.5 to 36.0 ± 6.4 spikes / min (Table 2.09). In comparison to controls the spike frequencies declined at the end of 6 and 12 hours and subsequently elevated at rest of the time intervals. The increases in the spike frequencies ranged between 13.33 to 50.0%. The maximum decline in spike frequency was observed at the end of 12 hours while the maximum increase in spike frequency was observed at the end of 48 hours (Table 2.10).

Mice treated with 1 ppm dose of monocrotophos had plateau phase frequencies in the range of 9.6 ± 3.0 to 18.0 ± 5.4 PPs / min (Table 2.09). With reference to the controls the plateau phase frequencies declined below the control levels at the end of 6, 12, 24 and 240 hours and the decline was in the range of 16.67 to 46.67%. The plateau phase frequency at the end of 48 hours remained similar to the corresponding control while the plateau phase frequencies elevated at the end of 72, 96 and 120 hours. The increases were in the range of 17.65 and 50.0%. The maximum elevation in the plateau phase frequency was observed at the end of 72 and 120 hours while the maximum decline was observed at the end of 12 hours (Table 2.10).

Under the influence of 5 ppm dose of monocrotophos the spike frequencies of inward current ranged between 18.3 ± 2.0 to 36.4 ± 6.2 spikes / min (Table 2.09). The spike frequency was below the corresponding control level at the end of 6 hours while it elevated at rest of the time intervals. The increases in spike frequencies ranged between 4.44 to 51.67%. The maximum hike in spike frequency was observed at the end of 72 hours while the minimum hike was noted at the end of 120 hours (Table 2.10).

The mice given a dose of 5 ppm of monocrotophos exhibited the plateau phase frequencies in the range of 10.5 ± 2.0 to 15.0 ± 2.3 PPs / min (Table 2.09). With respect to the controls the plateau phase frequencies were declined at the end of 6, 12, 24, 48 and 240 hours and the reductions were in the range of 17.78 to 41.67% (Table 2.10). At the end of 72 and 96 hours the plateau phase frequencies were elevated and the elevations were by 25.0 and

31.37% respectively. At the end of 120 hours the plateau phase frequency was similar to that observed for control (Table 2.10). The maximum hike in plateau phase frequency was observed at the end of 96 hours while the maximum reduction was observed at the end of 12 and 48 hours.

10 ppm dose of monocrotophos elevated spike frequencies at all the exposure periods and they were in the range of 24.2 ± 3.5 to 37.0 ± 4.6 spikes / min (Table 2.09). The percentages of increases in spike frequencies ranged between 2.5 to 100.0%. The maximum elevation in spike frequency was observed at the end of 120 hours while the minimum elevation was noted at the end of 6 hours (Table 2.10).

In response to treatment with 10 ppm dose of monocrotophos the plateau phase frequencies ranged between 10.2 ± 1.5 to 18.2 ± 2.6 PPs / min (Table 2.09). With reference to the controls the plateau phase frequencies were declined at the end of 6, 12, 120 and 240 hours and the decrement was in the range of 4.44 to 30.56%. The plateau phase frequencies elevated at the end of 72 and 96 hours and the increases were by 51.67 and 76.47% respectively. At the end of 24 and 48 hours the plateau phase frequencies remained almost similar to those observed for controls (Table 2.10). The maximum decline in plateau phase frequency was observed at the end of 240 hours while the maximum hike was observed at the end of 96 hours.

Under the influence of 100 ppm dose of monocrotophos the spike frequencies elevated at all the time intervals and were in the range of $26.4 \pm$

7.2 to 34.8 ± 3.0 spikes /min (Table 2.09). The increases in spike frequencies ranged between 10.0 to 59.44%. The maximum elevation was observed at the end of 240 hours while the minimum elevation was observed at the end of 6 hours (Table 2.10).

Mice treated with 100 ppm dose of monocrotophos exhibited plateau phase frequencies in the range of 18.0 ± 3.2 to 20.0 ± 2.5 PPs / min (Table 2.09). At the end of 6, 24, 48 and 240 hours the plateau phase frequencies remained unaltered and were similar to those observed for controls that is 18.0 PPs / min (Table 2.09). At the end of 12 hours the plateau phase frequency declined below the corresponding control and the decline was by 14.44%. The plateau phase frequencies elevated at the end of 72, 96 and 120 hours and the elevations were equivalent to 66.67, 96.08 and 51.67% respectively. The maximum hike in plateau phase frequency was observed at the end of 96 hours, while the maximum decline was at the end of 12 hours (Table 2.10).

Changes in frequencies of isoelectric phases

Mice treated with monocrotophos exhibited wide fluctuations in isoelectric phase frequencies, in response to various doses and time intervals. The isoelectric phase frequencies of the control animals were in the range of 36.0 ± 3.0 to 54.0 ± 4.5 IPs / min. The isoelectric phase frequencies of treated animals were below those observed in controls nearly at all the time intervals and for almost all the doses.

Under the influence of 1 ppm dose of monocrotophos isoelectric phase frequencies were in the range of 24.0 ± 4.0 to 39.0 ± 4.8 IPs / min (Table 2.09). With respect to the controls the isoelectric phase frequencies declined at all the time intervals except at the end of 72 hours, where the isoelectric phase frequency was almost similar to that observed for control. The reduction in the isoelectric phase frequencies ranged between 5.71 to 64.29 %. The maximum decline in isoelectric phase frequency was observed at the end of 240 hours while the minimum increase was noted at the end of 24 hours (Table 2.10).

Under the influence of 5 ppm dose of monocrotophos, the isoelectric phase frequencies fluctuated from the end of 6 hours to the end of 240 hours and were in the range of 26.4 ± 3.0 to 45.6 ± 7.2 / min (Table 2.09). The isoelectric phase frequencies were below the control level at all the time intervals except at the end of 48 and 240 hours. The reductions in isoelectric phase frequencies were in the range of 13.81 to 45.0%. At the end of 48 and 240 hours the isoelectric phase frequencies were elevated and the hikes were equivalent to 6.67 and 8.57% respectively. The maximum decline in isoelectric phase frequency was observed at the end of 6 hours and the maximum increase was observed at the end of 240 hours (Table 2.10).

Mice treated with 10 ppm dose of monocrotophos showed the isoelectric phase frequency in the range of 27.2 ± 6.9 to 39.6 ± 9.0 / min (Table 2.09). The isoelectric phase frequencies declined as compared to controls at all the time intervals and the decline was in the range of 5.71 to

94.35%. The maximum decline in isoelectric phase frequency was observed at the end of 120 hours while the minimum decline was observed at the end of 24 hours (Table 2.10).

100 ppm dose of monocrotophos maintained isoelectric phase frequencies in the range of 27.5 ± 5.8 to 33.6 ± 6.8 IPs / min (Table 2.09). The isoelectric phase frequencies were below the control level at all time intervals and the declines were in the range of -20.0 to 58.81% . The maximum decline in isoelectric phase frequency was observed at the end of 96 hours while the minimum decline was noted at the end of 24 hours (Table 2.10).

Changes in frequencies of hyperpolarisation

The hyperpolarisation frequencies elevated nearly in a dose dependent manner in general barring a few exceptions. Hyperpolarisations were not evident in the control mice. The hyperpolarisation frequencies are tabulated in table 2.09. The percent changes were not calculated, as controls had no hyperpolarisation frequencies.

1 ppm dose of monocrotophos induced hyperpolarisation frequencies only at the end of 6, 24, 72 and 96 hours while hyperpolarisations were not introduced at the end of 12, 48, 120 and 240 hours. The hyperpolarisation frequencies were in the range of 6.0 ± 1.0 to 12.0 ± 1.2 / min. The maximum number of hyperpolarisations of about 12.0 / min were observed at the end of

24, 72 and 96 hours while the minimum number of hyperpolarisation was noted at the end of 6 hours.

Under the influence of 5 ppm dose of monocrotophos hyperpolarisations were evident at all the time intervals and their frequencies ranged between 12.0 ± 3.0 to 24.0 ± 3.0 / min (Table 2.09). The maximum frequency of hyperpolarisation was observed at the end of 48 hours while a minimum of about 12.0 / min were observed at the end of 24, 72, 96 and 12 hours.

Mice treated with 10 ppm dose of monocrotophos showed hyperpolarisation frequencies in the range of 12.0 ± 2.0 to 28.0 ± 2.8 / min (Table 2.09). The maximum frequency of hyperpolarisation was observed at the end of 72 hours while the minimum frequency was observed at the end of 120 and 240 hours.

100 ppm dose of monocrotophos induced hyperpolarisation frequencies at all the time intervals and they were in the range of 18.0 ± 3.0 to 34.0 ± 5.0 / min (Table 2.09). The maximum frequency of hyperpolarisation was observed at the end of 72 hours while the minimum frequency was noted at the end of 240 hours.

Table 2.09: EFFECT OF MONOCROTOPHOS ON THE FREQUENCIES OF ELECTRICAL ACTIVITIES OF SPINAL CORD OF MOUSE (*Mus musculus*)

Time interval	CONTROL						1ppm						5 ppm						10 ppm						100 ppm					
	AP/min		IC/min		IP/ min	HP/ min	AP/min		IC/min		IP/ min	HP/ min	AP/min		IC/min		IP/ min	HP/ min	AP/min		IC/min		IP/ min	HP/ min	AP/min		IC/min		IP/ min	HP/ min
	S	P	S	P			S	P	S	P			S	P	S	P			S	P	S	P			S	P	S	P		
6Hr	18.0 ±1.2	12.0 ±1.0	24.0 ±2.0	18.0 ±1.5	48.0 ±8.0	-	21.6 ±5.4	18.0 ±4.0	19.5 ±3.0	12.0 ±3.0	32.4 ±6.2	6.0 ±1.0	24.4 ±6.6	13.2 ±1.5	18.3 ±2.0	12.0 ±2.0	26.4 ±3.0	18.0 ±3.0	42.8 ±4.3	16.8 ±2.0	24.6 ±2.4	17.2 ±3.0	30.2 ±5.4	24.0 ±4.5	38.8 ±6.0	18.2 ±3.5	26.4 ±7.2	18.0 ±2.0	27.6 ±6.3	21.0 ±3.4
12Hr	18.0 ±1.5	12.0 ±1.2	24.0 ±1.5	18.0 ±1.0	42.0 ±3.0	-	26.4 ±5.2	12.0 ±3.0	18.0 ±2.5	9.6 ±3.0	36.0 ±6.0	-	31.2 ±4.2	19.2 ±3.5	28.8 ±6.4	10.5 ±2.0	36.2 ±3.5	18.0 ±3.0	36.5 ±4.2	27.6 ±4.4	24.8 ±1.5	16.6 ±2.2	33.6 ±3.0	24.0 ±4.0	37.2 ±4.8	24.0 ±5.4	34.8 ±8.4	15.4 ±2.3	32.2 ±5.3	26.5 ±3.2
24Hr	18.5 ±1.2	6.0 ±1.0	22.5 ±1.5	18.0 ±1.5	42.0 ±2.0	-	34.0 ±6.5	19.2 ±4.0	25.5 ±6.0	12.0 ±3.0	39.6 ±4.8	12.0 ±1.5	33.0 ±4.8	24.2 ±3.7	30.0 ±5.5	14.8 ±3.1	28.6 ±2.5	12.0 ±2.0	38.2 ±6.0	12.8 ±2.5	32.4 ±2.8	18.0 ±2.5	39.6 ±9.0	20.4 ±3.0	31.2 ±4.8	24.0 ±2.4	30.8 ±4.5	18.0 ±1.5	33.6 ±6.8	24.0 ±4.0
48Hr	18.0 ±1.5	12.0 ±2.0	24.0 ±2.5	18.0 ±2.0	36.0 ±2.0	-	27.4 ±3.1	21.6 ±5.4	36.0 ±6.4	18.0 ±1.0	26.4 ±6.0	-	39.6 ±6.0	27.6 ±2.4	28.6 ±4.2	10.5 ±1.6	38.4 ±5.0	24.0 ±3.0	50.4 ±7.2	28.6 ±5.0	34.8 ±4.6	18.0 ±2.8	33.2 ±4.6	25.2 ±4.5	56.4 ±9.2	16.8 ±3.0	36.0 ±4.2	18.0 ±2.5	24.1 ±9.0	30.0 ±4.0
72Hr	18.2 ±1.2	18.0 ±1.6	24.0 ±1.8	12.0 ±1.2	36.0 ±3.0	-	28.8 ±6.2	18.0 ±4.0	28.8 ±5.8	18.0 ±3.0	36.0 ±6.6	12.0 ±2.0	36.0 ±7.2	25.2 ±2.7	36.4 ±6.2	15.0 ±2.3	27.0 ±3.6	12.0 ±2.5	48.0 ±4.5	23.2 ±2.1	37.0 ±4.6	18.2 ±2.6	32.0 ±8.0	28.0 ±2.8	54.2 ±7.5	14.5 ±3.2	34.0 ±3.8	20.0 ±2.0	24.0 ±4.5	34.0 ±5.0
96Hr	20.0 ±1.8	18.0 ±1.3	25.3 ±3.5	10.2 ±0.0	42.0 ±2.5	-	27.0 ±4.5	15.6 ±4.8	30.0 ±6.0	12.0 ±3.5	24.0 ±4.0	12.0 ±1.2	32.0 ±9.0	22.5 ±3.6	24.8 ±4.0	13.4 ±1.5	34.0 ±4.5	12.0 ±3.0	36.0 ±5.1	18.0 ±1.5	33.6 ±3.2	18.0 ±4.1	27.2 ±6.9	18.0 ±2.0	57.5 ±7.8	16.0 ±2.0	34.8 ±3.0	20.0 ±2.5	17.3 ±3.0	20.0 ±3.0
120Hr	18.0 ±1.2	12.0 ±0.8	18.0 ±1.0	12.0 ±1.8	54.0 ±4.5	-	24.2 ±6.0	15.0 ±5.0	25.2 ±6.6	18.0 ±5.4	36.0 ±6.0	-	32.0 ±7.1	18.0 ±2.0	18.8 ±3.2	12.0 ±1.6	34.8 ±6.6	12.0 ±3.0	44.2 ±5.3	25.3 ±2.5	36.0 ±5.0	10.2 ±1.5	30.5 ±6.0	12.0 ±2.0	42.7 ±6.8	25.0 ±4.0	28.7 ±3.5	18.2 ±3.2	27.5 ±5.8	24.0 ±4.0
240Hr	18.0 ±1.1	12.0 ±1.2	18.0 ±1.2	18.0 ±2.0	42.0 ±4.0	-	22.8 ±6.0	21.0 ±6.0	21.6 ±4.8	15.0 ±6.0	30.0 ±6.0	-	29.4 ±5.4	18.0 ±1.5	20.4 ±3.2	12.0 ±1.5	45.6 ±7.2	18.0 ±4.0	26.4 ±3.2	27.2 ±4.5	24.2 ±3.5	12.5 ±2.3	31.2 ±4.6	12.0 ±1.5	22.6 ±4.8	27.0 ±8.4	37.2 ±3.5	18.0 ±2.0	32.2 ±5.7	18.0 ±3.0

AP – Action Potential HP - Hyperpolarisation
 IC – Inward Current S – Spike
 IP – Isoelectric phase P – plateau phase

Table 2.10: EFFECT OF MONOCROTOPHOS ON THE FREQUENCIES OF ELECTRICAL PROPERTIES OF SPINAL CORD OF MOUSE (*Mus musculus*) EXPRESSED AS PERCENT CHANGES.

Time Intervals	1 ppm					5 ppm					10 ppm					100 ppm				
	AP / min		IC / min		IP / min	AP / min		IC / min		IP / min	AP / min		IC / min		IP / min	AP / min		IC / min		IP / min
	S	P	S	P		S	P	S	P		S	P	S	P		S	P			
	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P		
6 Hr	20.0	50.0	-18.75	-33.3	-32.5	35.55	10.0	-23.75	-33.35	-45.0	137.78	40.0	2.50	-4.44	-37.08	115.56	51.67	10.0	0.0	-42.50
12 Hr	46.67	0.0	-25.0	-46.67	-14.28	73.33	60.0	20.00	-41.67	-13.81	102.78	130.0	3.33	-7.78	-20.0	106.67	100.0	45.0	-14.44	-23.33
24 Hr	83.78	220.0	13.33	-33.3	-5.71	78.38	303.3	33.33	-17.78	-31.9	106.48	113.33	44.0	0.0	-5.71	68.65	300.0	36.89	0.0	-20.0
48 Hr	52.22	80.0	50.0	0.0	-26.67	120.0	130.0	19.16	-41.67	6.67	180.0	138.33	45.0	0.0	-7.78	213.33	40.00	50.0	0.0	-33.05
72 Hr	58.24	0.0	20.0	50.0	0.0	97.80	40.0	51.67	25.00	-25.0	163.74	28.89	54.17	51.67	-11.11	197.80	-19.44	41.66	66.67	-33.33
96 Hr	35.00	-13.3	18.57	17.65	-42.86	60.0	25.0	21.74	31.37	-19.05	80.0	0.0	32.81	76.47	-35.24	287.5	-11.11	37.55	96.08	-58.81
120 Hr	34.40	25.0	40.0	50.0	-33.3	77.78	50.0	4.44	0.0	-35.56	145.56	110.83	100.0	-15.0	-94.35	137.22	108.33	59.44	51.67	-49.07
240 Hr	26.67	75.0	20.0	-16.67	-64.29	63.33	50.0	13.33	-33.33	8.57	46.67	126.66	34.44	-30.56	-25.71	131.11	125.0	106.67	0.0	-23.33

Percent changes for Hyperpolarisation frequency were not calculated

II (b). EFFECT OF MONOCROTOPHOS ON THE DURATIONS OF ELECTRICAL ACTIVITIES

The effect of monocrotophos on the durations of electrical activities of spinal cord of mice are tabulated in table 2.11 and the changes in the durations expressed as percentages are presented in table 2.12. Fig 2.4 (b) – 2.8 (b) represents the changes in duration electrical activity.

Changes in the durations of action activities

The durations of spikes of action potentials were not altered significantly in response to various doses and time intervals except at 100 ppm. The durations of plateau phases increased significantly at a few time intervals under the influence of a few doses. The durations of spikes remained constant at 0.04 ± 0 sec at all the time intervals in control animals, while that of plateau phases was equivalent to 0.60 ± 0 sec.

1 ppm dose of monocrotophos did not induce any change in the durations of spikes at all the time intervals under study except for a very marginal decrease (2.5%) at the end of 120 hours (Table 2.12). The duration of spike was equivalent to 0.39 ± 0.02 sec at the end of 120 hours while it remained at 0.40 ± 0 sec at rest of the time intervals (Table 2.11).

Mice treated with 1 ppm dose of monocrotophos showed durations of plateau phases in the range of 0.60 ± 0 to 0.86 ± 0.20 sec (Table 2.11). The durations of plateau phases elevated at all the time intervals except at the end

of 96 and 240 hours and the durations remained similar to that observed for control. The increases in the durations of plateau phases ranged between 16.67 to 43.33 %. The maximum hike in durations of plateau phase was observed at the end of 12 hours while the minimum hike was observed at the end of 48, 72 and 120 hours (Table 2.12).

5 ppm dose of monocrotophos induced increase in the durations of the spikes at the end of 12, 72 and 120 hours while it remained unaltered at the rest of the time intervals. The duration of spike was 0.43 ± 0.02 sec at the end of 12 hours and 0.42 ± 0.04 sec at the end of 72 and 120 hours (Table 2.11). The increase in the durations of the spikes was by 7.5 % at the end of 12 hours and 5.0 % at the end of 72 and 120 hours (Table 2.12).

Under the influence of 5 ppm dose of monocrotophos the durations of plateau phases ranged from 0.60 ± 0 to 0.74 ± 0.16 sec (Table 2.11). The durations of plateau phase were elevated at all the time intervals except at the end of 240 hours where it remained similar to that observed in control. The increases in the durations of plateau phases were in the range of 0.8 to 23.33%. The maximum hike in the durations of plateau phases was observed at the end of 6 hours while the minimum hike was noted at the end of 72 hours (Table 2.12).

Mice treated with 10 ppm dose of monocrotophos showed durations of inward current spikes in the range of 0.40 ± 0 to 0.45 ± 0.05 sec (Table 2.11). The durations of the spikes were elevated from the end of 6 hours to the end

72 hours and the increases in the range of 5.0 to 12.5 % (Table 2.12). At the rest of the time intervals the durations of spikes remained unaltered and similar to that observed for control. The maximum hike in the duration of the spike was observed at the end of 72 hours while the minimum hike was observed at the end of 6 and 12 hours.

10 ppm dose of monocrotophos promoted elevations in durations of plateau phases and the durations were in the range of 0.60 ± 0 to 0.75 ± 0.11 sec (Table 2.11). The durations of plateau phases were elevated at all the time intervals except at the end of 240 where it remained similar to the control plateau phase duration. The increases in the durations of plateau phases were in the range of 8.33 to 25.0 %. The maximum hike in the duration of plateau phases was observed at the end of 120 hours while the minimum was observed at the end of 24 and 96 hours (Table 2.12).

In response to treatment to 100 ppm dose of monocrotophos the durations of spikes were in the range of 0.40 ± 0 to 0.53 ± 0.08 sec (Table 2.11). The durations of spikes were elevated at all the time intervals except at the end of 120 hours where it remained similar to that observed for controls. The increases in the durations of the spikes were in the range of 5.0 to 32.5%. The maximum elevation in the duration of spike was observed at the end of 24 hours while the minimum hike was noted at the end of 6 and 240 hours.

100 ppm dose of monocrotophos induced elevations in durations of plateau phases and the durations were in the range of 0.63 ± 0.05 to $0.71 \pm$

0.08 sec (Table 2.11). The durations of plateau phases increased at all the time intervals and the increases were in the range of 5.0 to 18.33 %. The maximum hike in the duration of plateau phase was observed at the end of 48 hours while the minimum hike was observed at the end of 24 hours.

Changes in durations of inward currents

The durations of spike of the inward current were not significantly altered except at few time intervals. Animals exposed to monocrotophos showed elevations in durations of plateau phases at almost all time intervals barring a few exceptions. However, the increases in the durations of plateau phases fluctuated. The control animals exhibited duration of spike equivalent to 0.40 ± 0 sec at all the exposure periods while the durations of plateau phases remained at 0.60 ± 0 sec at all the time intervals.

Mice treated with 1 ppm dose of monocrotophos showed no change in the durations of spikes and remained similar to that observed for control at all the time intervals i.e. 0.40 ± 0 sec. (Table 2.11).

1 ppm dose of monocrotophos induced changes in durations of plateau phases and they were in the range of 0.60 ± 0 to 0.82 ± 0.20 sec (Table 2.11). The durations of plateau phases at the end of 12, 96 and 240 hours remained almost similar to that observed for control while at the rest of the time intervals they increased. The increases in the durations were in the range of 1.67 to 36.67 %. The maximum increase in the durations of plateau phases was

observed at the end of 6 hours while the minimum increase was noted at the end of 120 hours (Table 2.12).

In response to 5 ppm dose of monocrotophos the durations of spikes of inward currents increased at the end of 6, 24 and 72 hours while it remained similar to that observed for control at rest of the time intervals. The durations of spikes at the end of 6, 24 and 72 hours were equivalent to 0.46 ± 0.05 , 0.44 ± 0.08 and 0.42 ± 0.04 sec respectively (Table 2.11) and the increases were by 15.0, 10.0 and 5.0 % respectively (Table 2.12). The maximum hike in the duration of the spike was observed at the end of 6 hours.

Under the influence of 5 ppm dose of monocrotophos the durations of plateau phases of inward current were in the range of 0.58 ± 0.14 to 0.82 ± 0.10 sec (Table 2.11). The durations of plateau phases remained similar to that noted for controls at the end of 6 and 48 hours while it declined below the control at the end of 12 hours. The durations were elevated at the end of 24, 72, 96, 120 and 240 hours and the increases were in the range of 10.0 to 36.67%. The maximum hike in the durations of plateau phases was observed at the end of 72 hours while the minimum hike was noted at the end of 24 hours.

Mice administered with 10 ppm dose of monocrotophos showed increase in durations of spikes at all time intervals except at the end of 12 and 240 hours where the spike duration was similar to the controls i.e. $0.40 \pm$ (sec. The durations of spikes remained in the range of 0.41 ± 0.04 to 0.45

0.08 sec for rest of the exposure periods (Table 2.11). The increases in durations of spikes in terms of percentages were in the range of 2.5 to 12.5%. The maximum hike in the duration of spike was observed at the end of 48 and 96 hours while the minimum hike was observed at the end of 120 hours (Table 2.12).

Under the influence of 10 ppm dose of monocrotophos the durations of plateau phases of inward current were in the range of 0.60 ± 0 to 0.72 ± 0.10 sec (Table 2.11). The durations of plateau phase was similar to that of control at the end of 72 hours while it elevated at rest of the time intervals. The increases in the durations of plateau phases were in the range of 3.33 to 20.0%. The maximum elevation in the durations of plateau phases was observed at the end of 120 hours while the minimum hike was noticed at the end of 96 hours.

100 ppm dose of monocrotophos elevated the durations of spikes of inward currents at all the time intervals except at the end of 240 hours. The spike durations were in the range of 0.40 ± 0 to 0.50 ± 0.05 sec (Table 2.11). The increases in the durations of spikes ranged between 5.0 to 25.0 % and it remained unaltered at the end of 240 hours. The maximum hike in the duration of spike was observed at the end of 96 hours while the minimum hike was observed at the end of 12, 24 and 120 hours (Table 2.12).

Mice subjected to 100 ppm dose of monocrotophos showed durations of plateau phases in the range of 0.60 ± 0 to 0.72 ± 0.04 sec (Table 2.11). The

durations of plateau phase was similar to that observed for control at the end of 96 hours while at rest of the time intervals it increased. The increases in the durations of plateau phases ranged between 8.33 to 20.0 %. The minimum increment in the durations of plateau phase was observed at the end of 72, 120 and 240 hours while the maximum hike was noted at the end of 48 hours (Table 2.12).

Changes in durations of isoelectric phase

The durations of isoelectric phase fluctuated under the influence of various doses of monocrotophos. The durations of isoelectric phases were higher than those observed for the controls except at a few time intervals wherein they were below the control level. The durations of isoelectric phase observed in the control animals were in the range of 0.45 ± 0.10 to 0.64 ± 0.20 sec.

Under the influence of 1 ppm dose of monocrotophos the durations of isoelectric phases were in the range of 0.65 ± 0.10 to 10.37 ± 0.21 sec (Table 2.11). The durations of isoelectric phases elevated at all the time intervals and the increases were in the range of 25.0 to 100.0%. the maximum hike in the durations of isoelectric phase was observed at the end of 12 hours while the minimum hike was observed at the end of 120 hours (Table 2.12)

5 ppm dose of monocrotophos induced changes in the durations of isoelectric phases and they were in the range of 0.58 ± 0.20 to 1.06 ± 0.13 sec (Table 2.11). The durations of isoelectric phase were below that observed

for control at the end of 240 hours while they were elevated at rest of the time intervals. The increases in duration of isoelectric phase were in the range of 4.84 to 75.47%. The maximum hike in the durations of isoelectric phase was observed at the end of 72 hours while the minimum hike was noted at the end of 24 hours (Table 2.12).

Under the influence of 10 ppm dose of monocrotophos the durations of isoelectric phases fluctuated in the range of 0.44 ± 0.16 to 0.95 ± 0.24 sec (Table 2.11). The durations of isoelectric phases declined as compared to those found in controls at the end of 6, 12 and 24 hours and they elevated at rest of the time intervals. The decline in the durations of isoelectric phases ranged between 3.23 to 31.25 % while the increases were in the range of 4.84 to 48.44 %. The maximum hike in the durations of isoelectric phase was observed at the end of 12 hours while the minimum hike was observed at the end of 120 hours (Table 2.12).

100 ppm dose of monocrotophos induced changes in the durations of isoelectric phases and the durations were in the range of 0.40 ± 0.07 to 1.06 ± 0.20 sec (Table 2.11). The durations of isoelectric phases were below that observed for control at the end of 6, 12 and 24 hours and the reduction was by 33.33, 3.64 and 22.58 % respectively. At the rest of the time intervals the durations of isoelectric phases increased and were in the range of 4.84 to 43.33%. The maximum decline in the durations of isoelectric phase was observed at the end of 6 hours while the maximum hike was noted at the end of 240 hours (Table 2.12).

**TABLE 2.11: EFFECT OF MONOCROTOPHOS ON THE DURATION OF ELECTRICAL ACTIVITIES OF SPINAL
CORD OF MOUSE (*Mus musculus*)**

Time intervals	Control					1 ppm					5 ppm					10 ppm					100 ppm				
	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec
	S	P	S	P		S	P	S	P		S	P	S	P		S	P	S	P		S	P	S	P	
	±0	±0	±0	±0	±0.20	±0	±0.20	±0	±0.20	±0.36	±0	±0.16	±0.05	±0.10	±0.09	±0.02	±0.04	±0.04	±0.11	±0.16	±0.03	±0.08	±0.4	±0.09	±0.07
6Hr	0.40	0.60	0.40	0.60	0.64	0.40	0.72	0.40	0.82	0.13	0.40	0.74	0.46	0.60	0.77	0.42	0.67	0.42	0.70	0.44	0.42	0.68	0.44	0.68	0.40
12Hr	0.40	0.60	0.40	0.60	0.55	0.40	0.86	0.40	0.6	0.11	0.43	0.72	0.40	0.58	7.1	0.42	0.66	0.40	0.70	0.48	0.43	0.69	0.42	0.70	0.53
24Hr	0.40	0.60	0.40	0.60	0.62	0.40	0.72	0.40	0.66	0.78	0.40	0.68	0.44	0.66	0.65	0.43	0.65	0.42	0.65	0.60	0.53	0.63	0.42	0.70	0.48
48Hr	0.40	0.60	0.40	0.60	0.45	0.40	0.70	0.40	0.73	0.65	0.40	0.65	0.40	0.60	0.73	0.43	0.69	0.45	0.70	0.53	0.45	0.71	0.44	0.72	0.52
72Hr	0.40	0.60	0.40	0.60	0.53	0.40	0.70	0.40	0.80	0.17	0.42	0.61	0.42	0.82	0.93	0.45	0.68	0.44	0.60	0.64	0.44	0.70	0.46	0.65	0.68
96Hr	0.40	0.60	0.40	0.60	0.62	0.40	0.60	0.40	0.60	0.76	0.40	0.73	0.40	0.75	1.06	0.40	0.65	0.45	0.62	0.65	0.46	0.70	0.50	0.60	0.65
120Hr	0.40	0.60	0.40	0.60	0.64	0.39	0.70	0.40	0.61	0.80	0.40	0.65	0.40	0.75	0.82	0.40	0.75	0.41	0.72	0.70	0.04	0.70	0.42	0.65	0.81
240Hr	0.40	0.60	0.40	0.60	0.60	0.40	0.60	0.40	0.60	1.07	0.40	0.60	0.40	0.80	0.58	0.40	0.60	0.40	0.70	0.72	0.42	0.66	0.04	0.65	0.76
	±0	±0	±0	±0	±0.12	±0	±0	±0	±0	±0.21	±0	±0	±0	±0	±0.20	±0	±0	±0	±0	±0.08	±0.04	±0.05	±0	±0.05	±0.28

AP – Action Potential

IC – Inward Current

S – Spike

IP – Isoelectric phase

P – plateau phase

Table 2.12: EFFECT OF MONOCROTOPHOS ON THE DURATIONS OF ELECTRICAL ACTIVITIES OF SPINAL CORD OF MOUSE (*Mus musculus*) EXPRESSED AS PERCENT CHANGES.

Time intervals	1 ppm					5 ppm					10 ppm					100 ppm				
	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec	AP sec		IC sec		IP sec
	S	P	S	P		S	P	S	P		S	P	S	P		S	P			
6 Hr	0.0	20.0	0.0	36.67	98.44	0.0	23.33	15.0	0.0	20.31	5.0	11.67	5.0	16.66	-31.25	5.0	13.33	10.0	13.33	-33.33
12 Hr	0.0	43.33	0.0	0.0	100.0	7.5	20.0	0.0	-3.33	29.09	5.0	10.0	0.0	16.66	-12.72	7.5	15.0	5.0	16.67	-3.64
24 Hr	0.0	20.0	0.0	10.0	25.8	0.0	13.33	10.0	10.0	4.84	7.5	8.33	5.0	8.33	-3.23	32.5	5.0	5.0	16.67	-22.58
48 Hr	0.0	16.67	0.0	21.67	44.44	0.0	8.33	0.0	0.0	62.22	7.5	15.0	12.5	16.66	17.78	12.5	18.33	10.0	20.0	15.55
72 Hr	0.0	16.67	0.0	33.33	32.07	5.0	0.8	5.0	36.67	75.47	12.5	13.33	10.0	0.0	20.75	10.0	16.67	15.0	8.33	28.30
96 Hr	5.0	0.0	0.0	0.0	22.58	0.0	21.0	0.0	25.0	70.96	0.0	8.33	12.5	3.33	4.84	15.0	16.67	25.0	0.0	4.84
120 Hr	-2.5	16.67	0.0	1.67	25.0	5.0	8.33	0.0	25.0	28.13	0.0	25.0	2.5	20.0	48.44	0.0	16.67	5.0	8.33	26.56
240 Hr	0.0	0.0	0.0	0.0	78.33	0.0	0.0	0.0	33.33	-3.33	0.0	0.0	0.0	16.66	35.0	5.0	10.0	0.0	8.33	43.33

II (c). EFFECT OF MONOCROTOPHOS ON THE ELECTRICAL POTENTIAL

The alterations in the electrical potentials of spinal cord of mice including the baseline potential, amplitudes of action potential and inward currents in response to monocrotophos are tabulated in table 2.13 and the percentage of changes are presented in table 2.14.

Changes in baseline potentials

Monocrotophos induced nearly dose-dependent elevations in the baseline potentials barring a few exceptions. The baseline potentials in the control animals were in the range of 14.83 ± 2.5 to 18.20 ± 1.5 mv.

1 ppm dose of monocrotophos induced a steady increase in the baseline potentials from the end of 6 hours to the end of 48 hours and subsequently promoted decline upto the end of 240 hours. The baseline potentials were in the range of 15.20 ± 2.5 to 28.20 ± 4.5 mv (Table 2.13). In comparison to the controls the baseline potentials increased at all the time intervals except at the end of 240 hours where it declined below the control by 5.59%. The increases in the baseline potentials ranged between 14.59 to 90.75 %. The maximum hike in the baseline potential was observed at the end of 48 hours (Table 2.14). The changes of base line potential were statistically significant at the end of 24 and 48 hours ($P < 0.005$).

Under the influence of 5 ppm dose of monocrotophos, the baseline potentials were in the range of 19.40 ± 2.8 to 38.20 ± 3.0 mv (Table 2.13). The baseline potentials increased at all the time intervals and the increases were in the range of 14.03 to 148.14 %. The maximum hike in the baseline potential was observed at the end of 48 hours while the minimum hike was noted at the end of 6 hours (Table 2.14). The changes of baseline potential was statistically significant at the end of 24, 48, 72 and 96 hours.

Mice treated with 10 ppm dose of monocrotophos showed baseline potentials in the range of 24.70 ± 2.5 to 52.80 ± 6.6 mv (Table 2.13). The increases in the baseline potentials ranged between 58.84 to 256.03 %. The maximum hike in the baseline potentials of more than 3 folds (256.03%) was observed at the end of 48 hours while the minimum hike was observed at the end of 12 hours (Table 2.14). the alterations of baseline potential were statistically significant all time intervals except at he end of 6 and 12 hours

100 ppm dose of monocrotophos induced a sharp increase in the baseline potentials at all the time intervals. The baseline potentials were in the range of 87.0 to 367.85 %. The maximum increase in baseline potential was observed at the end of 72 hours while the minimum hike was noted at the end of 6 hours (Table 2.14). All the elevations of baseline potentials were statistically significant.

Changes in the amplitude of action potentials

The amplitudes of action potentials increased in general in response to monocrotophos treatment. The changes were more evident in response to 5, 10 and 100 ppm doses. The amplitudes of action potentials in the control animals remained constant at 2.5 mv at all the time intervals.

1 ppm dose of monocrotophos did not alter the amplitudes of action potential at all the time intervals except for an insignificant change (4.0%) at the end of 96 and 240 hours (Table 2.14).

Under the influence of 5ppm dose of monocrotophos the amplitudes of action potentials were unaltered and remained similar to controls at the end of 6 and 12 hours (2.5 ± 0 mv). The amplitudes of action potentials at rest of the time intervals were in the range of 2.6 ± 0.5 to 3.12 ± 0.4 mv (Table 2.13). The increases in the amplitudes were in the range of 4.0 to 24.8 %. The minimum hike in the amplitude of action potential was observed at the end of 240 hours while the maximum hike was observed at the end of 48 hours (Table 2.14).

Mice treated with 10 ppm dose of monocrotophos showed elevations in amplitudes of action potentials at all the time intervals. The amplitudes ranged between 2.60 ± 0.3 to 3.0 ± 0.5 mv (Table 2.13). The increases in the amplitudes of action potentials were in the range of 4.0 to 20.0 %. The maximum hike in amplitudes of action potentials was observed at the end of

96 hours while the minimum hike was noted at the end of 48 and 240 hours (Table 2.14).

Under the influence of 100 ppm dose of monocrotophos the amplitudes of action potentials elevated at all the time intervals. The amplitudes were in the range of 2.6 ± 0.4 to 4.70 ± 0.3 mv (Table 2.13). The increases in the amplitudes of action potentials ranged between 4.0 to 88.0 %. The maximum increase in the amplitudes of action potential was observed at the end of 48 hours and the minimum hike was observed at the end of 120 and 240 hours (Table 2.14).

Changes in amplitude of inward currents

Monocrotophos did not cause significant alterations in the amplitude of inward currents except at a few time intervals. The amplitude of inward currents in the control animals remained constant for all the time intervals and was equivalent to 2.5 ± 0 mv.

The mice treated with 1 ppm dose of monocrotophos did not show any change in the amplitude of inward currents at the end of all the time intervals except at the end of 48 hours. The amplitude of inward currents was elevated to 3.0 ± 0.4 mv at the end of 48 hours and the increase was by 20.0% (Table 2.14), while at the rest of the time intervals the amplitude remained at 2.5 ± 0 mv.

In response to 5 ppm dose of monocrotophos, the amplitude of inward currents remained similar to that observed for control at the end of 6 and 12 hours. The amplitude of inward currents fluctuated from the end of 24 hours to the end of 240 hours and they ranged between 2.6 ± 0.2 to 3.0 ± 0.5 mv (Table 2.13). The amplitudes were well above the control level and the increases were in the range of 4.0 to 20.0%. The maximum hike in amplitude of inward currents was observed at the end of 24 hours while the minimum hike was observed at the end of 48, 96 and 240 hours (Table 2.14).

Mice subjected to 10 ppm dose of monocrotophos revealed increase in the amplitude of inward currents at all the time intervals except at the end of 12 hours. The elevated amplitudes were in the range of 2.6 ± 0.3 to 3.00 ± 0.3 mv and at 12 hours it was similar to that observed for control (Table 2.13). The increases in the amplitude of inward currents were equivalent to 4.0% at the end of 6, 72 and 96 hours and the amplitudes elevated by 8.0% at the end of 48, 120 and 240 hours. The maximum of 20.0% elevation of inward current amplitude was observed at the end of 24 hours.

Under the influence of 100 ppm dose of monocrotophos the amplitude of inward currents were elevated at all the time intervals and were in the range of 2.60 ± 0.3 to 3.12 ± 0.6 mv (Table 2.13). The increases in the amplitude were in the range of 4.0 to 20.0%. The minimum elevation in amplitude of inward current was observed at the end of 48 and 120 and 240 hours while the maximum hike was observed at the end of 24 hours.

**Table 2.13 : EFFECT OF MONOCROTOPHOS ON THE ELECTRICAL POTENTIAL
OF SPINAL CORD OF MOUSE (*Mus musculus*)**

Time intervals	Control			1 ppm			5 ppm			10 ppm			100ppm		
	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv
6Hr	17.54 ± 2.3	2.5 ±0	2.5 ±0	20.10 ±5.8	2.5 ±0	2.5 ±0	20.00 ±2.9	2.5 ±0	2.5 ±0	28.20 ±5.9	2.70 ±0.4	2.60 ±0.2	32.80 ^c ±6.8	3.14 ±0.6	2.65 ±0.2
12Hr	16.18 ± 2.0	2.5 ±0	2.5 ±0	20.60 ±4.2	2.5 ±0	2.5 ±0	23.80 ±2.8	2.5 ±0	2.5 ±0	24.70 ±2.5	2.70 ±0.3	2.50 ±0	30.80 ^c ±7.2	2.70 ±0.2	2.70 ±0.2
24Hr	18.20 ± 1.5	2.5 ±0	2.5 ±0	26.30 ^c ±3.01	2.5 ±0	2.5 ±0	38.20 ^a ±3.0	2.7 ±0.2	3.0 ±0.5	36.80 ^c ±5.3	2.90 ±0.4	3.00 ±0.3	40.8 ^b ±5.4	3.24 ±0.8	3.12 ±0.6
48Hr	14.83 ± 2.5	2.5 ±0	2.5 ±0	28.20 ^c ±4.5	2.5 ±0	3.0 ±0.4	36.80 ^a ±4.6	2.8 ±0.4	2.6 ±0.2	52.80 ^a ±6.6	2.60 ±0.3	2.70 ±0.3	54.50 ^a ±4.6	4.70 ±0.3	2.60 ±0.2
72Hr	15.71 ± 2.6	2.5 ±0	2.5 ±0	21.60 ±5.3	2.5 ±0	2.5 ±0	29.8 ^c ±6.4	3.0 ±1.0	2.6 ±0.2	39.50 ^a ±2.3	2.80 ±0.2	2.60 ±0.2	73.50 ^a ±8.7	3.10 ±0.9	2.90 ±0.5
96Hr	17.33 ± 2.8	2.5 ±0	2.5 ±0	20.90 ±5.6	2.6 ±0.2	2.5 ±0	31.60 ^b ±5.4	3.12 ±0.4	2.6 ±0.2	37.40 ^c ±3.5	3.00 ±0.5	2.60 ±0.3	39.5 ^b ±6.2	3.60 ±1.0	2.70 ±0.2
120Hr	15.38 ± 2.0	2.5 ±0	2.5 ±0	18.10 ±4.3	2.5 ±0	2.5 ±0	25.40 ±5.2	2.7 ±0.3	2.8 ±0.3	41.30 ^a ±5.8	2.90 ±0.4	2.70 ±0.2	42.6 ^b ±5.7	2.60 ±0.4	2.60 ±0.3
240Hr	16.10 ±1.8	2.5 ±0	2.5 ±0	15.20 ±2.5	2.6 ±0.1	2.5 ±0	19.40 ±2.8	2.6 ±0.5	2.6 ±0.1	36.00 ^b ±5.5	2.60 ±0	2.70 ±0.4	44.4 ^a ±8.2	2.60 ±0.3	2.60 ±0.3

BP – Baseline Potential
AP – Action Potential
IC – Inward Current

a= p< 0.001
b=p< 0.01
c = p<0.05

**Table 2.14 : EFFECT OF MONOCROTOPHOS ON ELECTRICAL POTENTIALS OF SPINAL CORD
OF MOUSE (*Mus musculus*) EXPRESSED AS PERCENT CHANGES.**

Time intervals	1 ppm			5 ppm			10 ppm			100 ppm		
	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv	BP mv	AP mv	IC mv
6 Hr	14.59	0.0	0.0	14.03	0.0	0.0	60.77	8.0	4.0	87.00	25.6	6.0
12 Hr	27.32	0.0	0.0	46.91	0.0	0.0	58.84	8.0	0.0	90.36	8.0	8.0
24 Hr	44.50	0.0	0.0	109.89	8.0	20.0	102.20	16.0	20.0	124.17	29.6	20.0
48 Hr	90.75	0.0	20.0	148.14	24.8	4.0	256.03	4.0	8.0	267.50	88.0	4.0
72 Hr	37.49	0.0	0.0	89.69	20.0	8.0	151.43	12.0	4.0	367.85	24.0	16.0
96 Hr	20.60	4.0	0.0	82.34	12.0	4.0	115.81	20.0	4.0	127.98	44.0	8.0
120 Hr	17.68	0.0	0.0	65.15	8.0	12.0	168.53	16.0	8.0	176.98	4.0	4.0
240 Hr	-5.59	4.0	0.0	20.50	4.0	4.0	123.60	4.0	8.0	175.78	4.0	4.0

FIG 2.5(a): Electrical activity of spinal cord of mice in response to

Monocrotophos treatment (1 ppm dose)

(segments of actual recordings for 10 secs)

S - spike

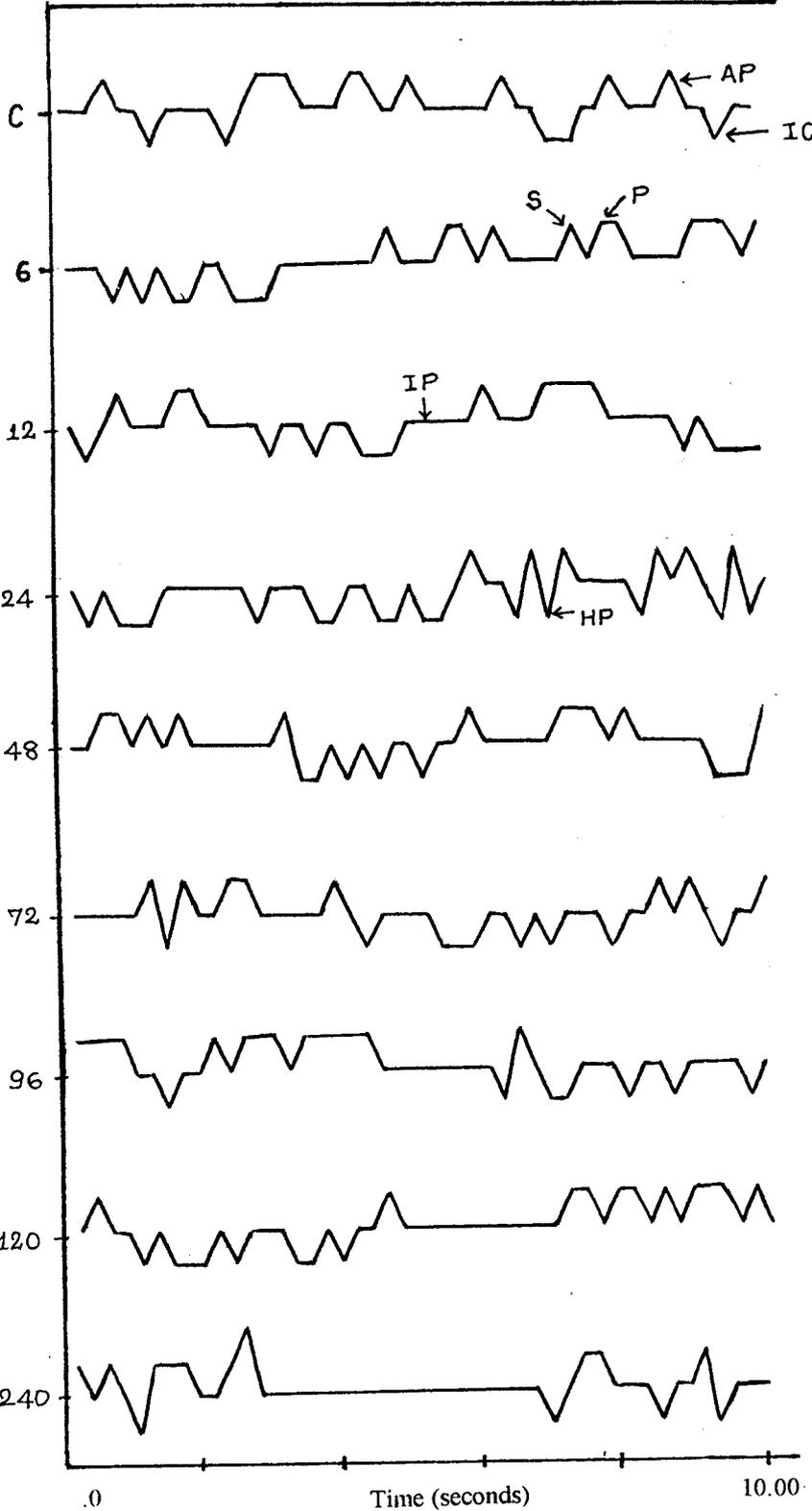
P- plateau phase

AP – action potential

IC- inward current

P- isoelectric phase

HP- hyperpolarisation



**FIG 2.5 (b): Electrical activity of spinal cord of mice in response to
Monocrotophos treatment (1 ppm dose)**

**Enlarged recordings indicating the changes in the durations
and amplitudes of the action potentials**



CONTROL



6 hr



12 hr



24 hr



48 hr



72 hr



96 hr



120 hr



240 hr

┌ 1 mV
└ 0.2 sec

**FIG 2.6 (a): Electrical activity of spinal cord of mice in response to
Monocrotophos treatment (5 ppm dose)
(segments of actual recordings for 10 secs)**

S - spike

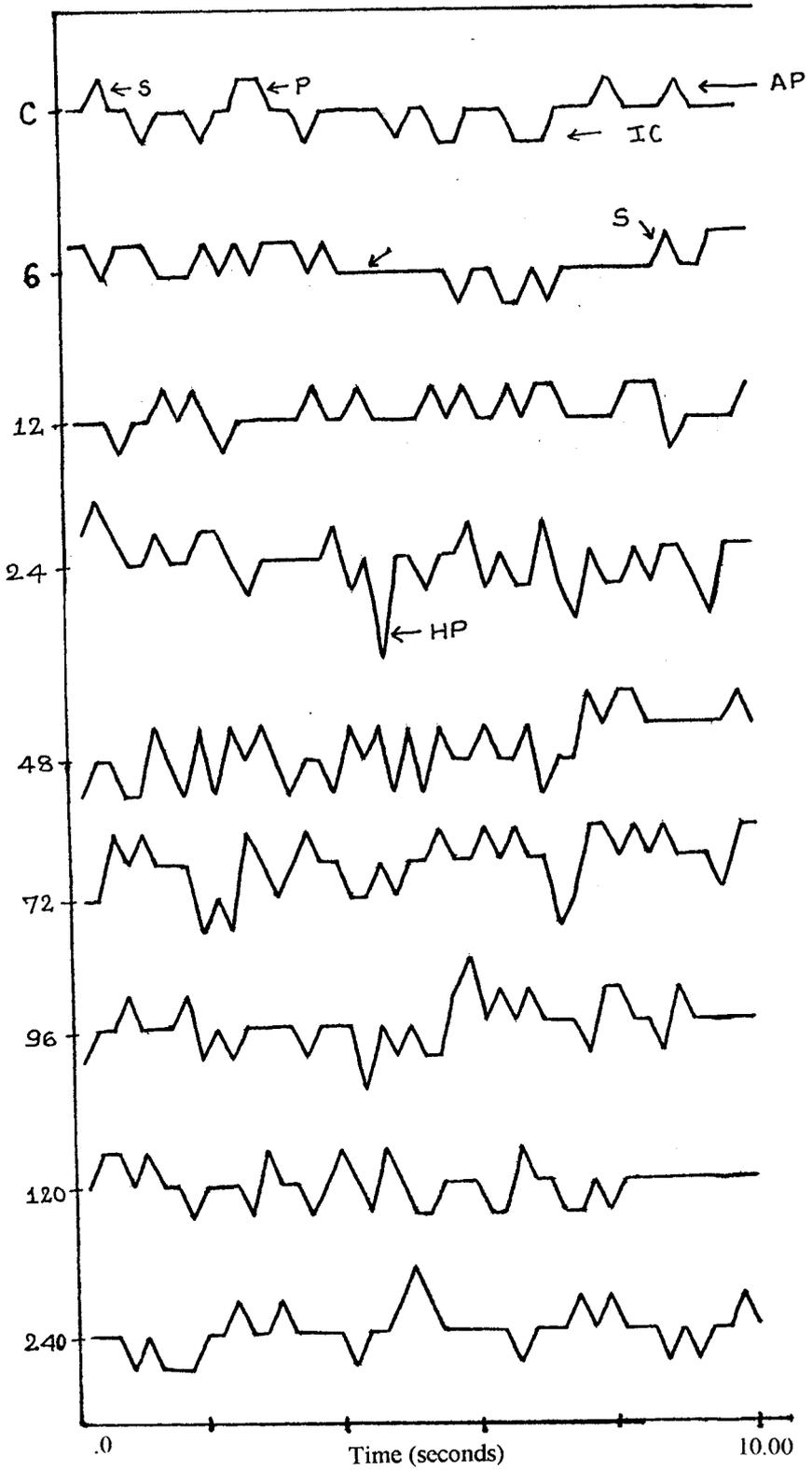
P- plateau phase

AP – action potential

IC- inward current

IP- isoelectric phase

HP- hyperpolarisation



**FIG 2.6 (b): Electrical activity of spinal cord of mice in response to
Monocrotophos treatment (5 ppm dose)**

**Enlarged recordings indicating the changes in the durations
and amplitudes of the action potentials**



CONTROL



6 hr



12 hr



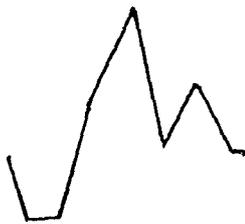
24 hr



48 hr



72 hr



96 hr



120 hr



240 hr

1mv
0.25sec

**FIG 2.7 (a): Electrical activity of spinal cord of mice in response to
Monocrotophos treatment (10 ppm dose)
(segments of actual recordings for 10 secs)**

S - spike

P- plateau phase

AP – action potential

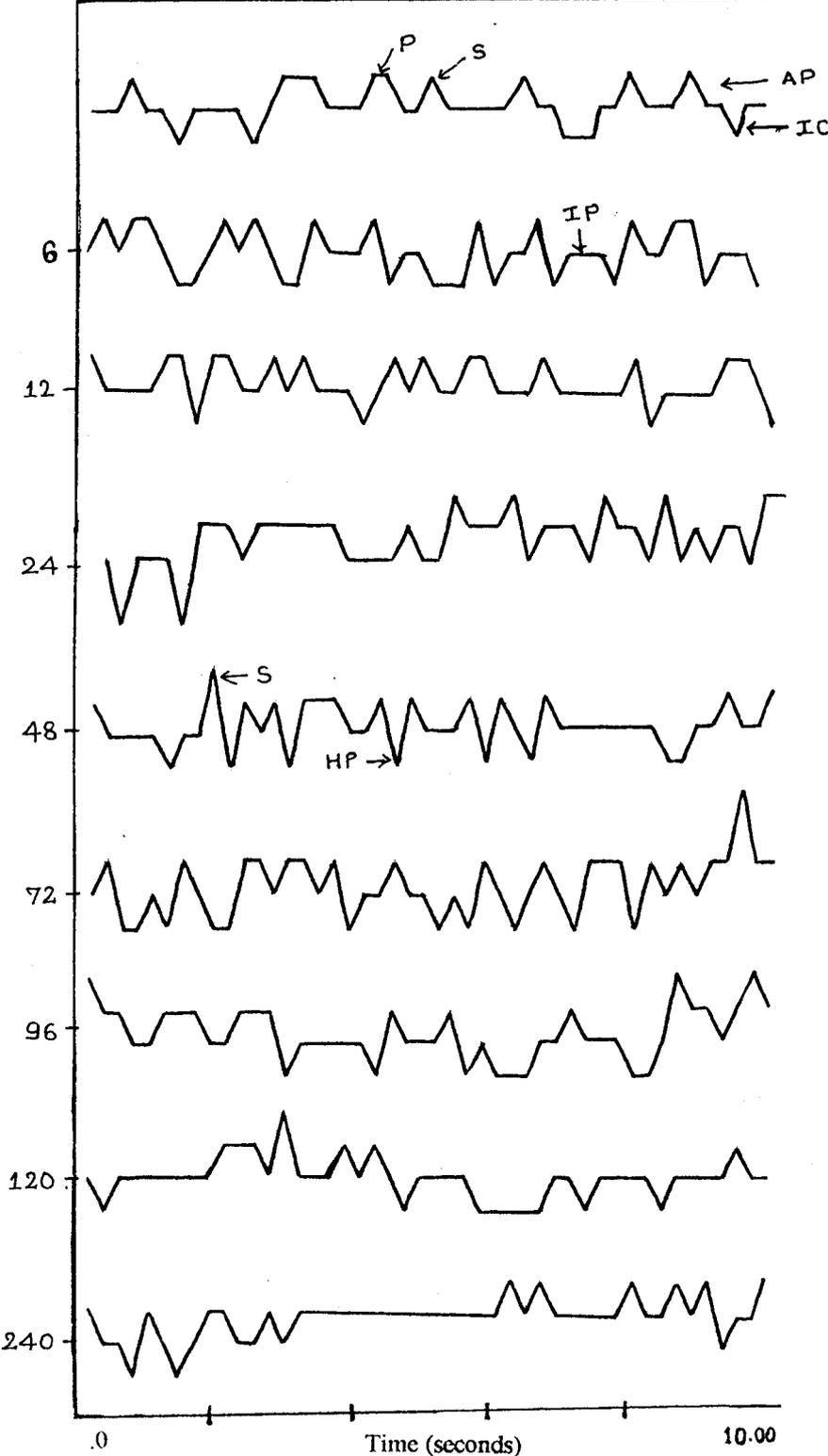
IC- inward current

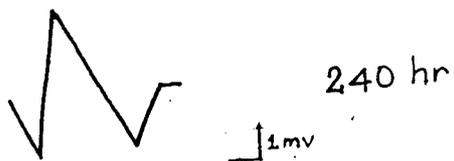
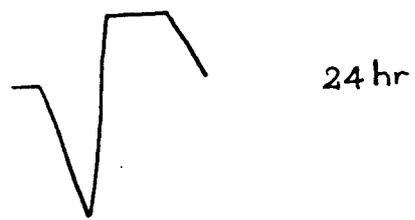
IP- isoelectric phase

HP- hyperpolarisation

**FIG 2.7 (b): Electrical activity of spinal cord of mice in response to
Monocrotophos treatment (10 ppm dose)**

**Enlarged recordings indicating the changes in the durations
and amplitudes of the action potentials**





↑ 1mv
0.2sec

**FIG 2.8(a): Electrical activity of spinal cord of mice in response to
Monocrotophos treatment (100 ppm dose)
(segments of actual recordings for 10 secs)**

S - spike

P- plateau phase

AP – action potential

IC- inward current

IP- isoelectric phase

HP- hyperpolarisation

II (d) EFFECT OF MONOCROTOPHOS ON CONDUCTION VELOCITY

Monocrotophos induced alterations in conduction velocity of spinal cord are tabulated in table 2.15 and the percent changes are presented in table 2.16. The control mice exhibited conduction velocity in the range of 15.3 ± 3.1 to 16.0 ± 2.4 m / sec.

1 ppm dose of monocrotophos induced conduction velocity in the range of 15.5 ± 4.2 to 19.4 ± 2.8 m / sec (Table 2.15). At the end of 6 hours the conduction velocity was declined below that observed for the corresponding control and the decline was by 1.89 %. At the rest of the time intervals the velocity was elevated and ranged between 1.29 to 21.25 % (Table 2.16). The maximum elevation was observed at the end of 24 hours.

Under the influence of 5 ppm dose of monocrotophos the conduction velocity at the end of 6 hours was below that observed for the corresponding control. However, it elevated steadily from the end of 12 hours to the end of 48 hours and subsequently declined till the end of 240 hours as compared to the preceding time intervals. The conduction velocity was in the range of 15.20 ± 3.6 to 23.7 ± 5.1 m / sec (Table 2.15). The increases in conduction velocity ranged between 0.63 to 54.90 %. The maximum elevation was observed at the end of 48 hours while the decline in conduction velocity at the end the 6 hours was by 3.80 % (Table 2.16).

10 ppm dose of monocrotophos induced conduction velocity in the range of 15.10 ± 3.8 to 23.8 ± 5.5 m /sec (Table 2.15). The conduction velocity elevated at all the time intervals except at the end of 240 hours. The increases in conduction velocities were in the range of 0.65 to 52.94 %. The maximum conduction velocity was observed at the end of 48 hours while the decline observed at the end of 240 hours was by 1.31 % (Table 2.16).

Mice treated with 100 ppm dose of monocrotophos revealed conduction velocity in the range of 14.70 ± 2.8 to 28.5 ± 6.4 m / sec (Table 2.15). The conduction velocity was elevated at all the time intervals except for a decline at the end of 240 hours. The increases were in the range of 7.09 to 86.27 %. The maximum elevation in conduction velocity was observed at the end of 48 hours while a decline of about 3.26 % was evident at the end of 240 hours.

**TABLE 2.15: EFFECT OF MONOCROTOPHOS ON THE
CONDUCTION VELOCITY OF SPINAL CORD OF MOUSE**

(*Mus musculus*)

Time intervals	Control	1 ppm	5 ppm	10 ppm	100 ppm
6 hours	15.8 ±2.7	15.5 ±4.2	15.20 ±3.6	19.8 ±2.7	17.4 ±2.3
12 hours	16.0 ±3.3	16.30 ±2.5	16.10 ±2.1	18.0 ±2.5	17.8 ±2.7
24 hours	16.0 ±2.4	19.4 ±2.6	23.10 ±4.3	19.4 ±3.2	17.30 ±2.1
48 hours	15.3 ±3.1	17.8 ±4.2	23.70 ±5.1	23.40 ±6.2	28.50 ±6.4
72 hours	15.8 ±2.0	18.2 ±3.7	20.90 ±4.6	23.80 ±5.5	22.90 ±4.5
96 hours	15.5 ±2.5	16.6 ±2.3	20.2 ±1.9	17.50 ±1.5	16.6 ±3.5
120 hours	15.4 ±2.5	15.6 ±2.3	17.8 ±1.9	15.50 ±1.5	16.60 ±3.5
240 hours	15.3 ±2.4	15.70 ±3.1	16.0 ±2.3	15.10 ±3.8	14.70 ±2.8

TABLE 2.16: EFFECT OF MONOCROTOPHOS ON THE CONDUCTION VELOCITY OF SPINAL CORD OF MOUSE (*Mus musculus*) EXPRESSED AS PERCENT CHANGES

Time intervals	1 ppm	5 ppm	10 ppm	100 ppm
6 hours	-1.89	-3.80	29.41	13.73
12 hours	1.87	0.63	12.50	11.25
24 hours	21.25	44.37	21.25	8.13
48 hours	16.34	54.90	52.94	86.27
72 hours	15.18	32.28	50.63	44.93
96 hours	7.09	30.32	12.90	7.79
120 hours	1.29	15.6	0.65	7.79
240 hours	2.61	4.57	-1.31	-3.26

EFFECT OF CHANNEL BLOCKERS

The effect of potassium channel blocker (BaCl_2) and calcium channel blockers (CdCl_2) on the electrical properties of spinal cord of mice were almost same for all the doses and time intervals of DDVP as well as monocrotophos. Hence the changes are discussed in general and are represented in fig. 2.09 and 2.10.

Potassium channel blocker

The effect of potassium channel blocker on the electrical activities of spinal cord of mice are shown in fig 2.09.

The blocking of potassium channel by BaCl_2 revealed a steady state of electrical activity. The exposure of spinal cords of the control mice to BaCl_2 caused no significant changes in the frequencies of spikes and plateau phases of action potentials. However, the inward currents were abolished. A non-significant reduction in the isoelectric phases were noted. The durations of action potentials spike and plateau phases of action potentials were not altered. The baseline potentials were elevated by 10-20 %. The amplitudes of the action potentials were unaltered.

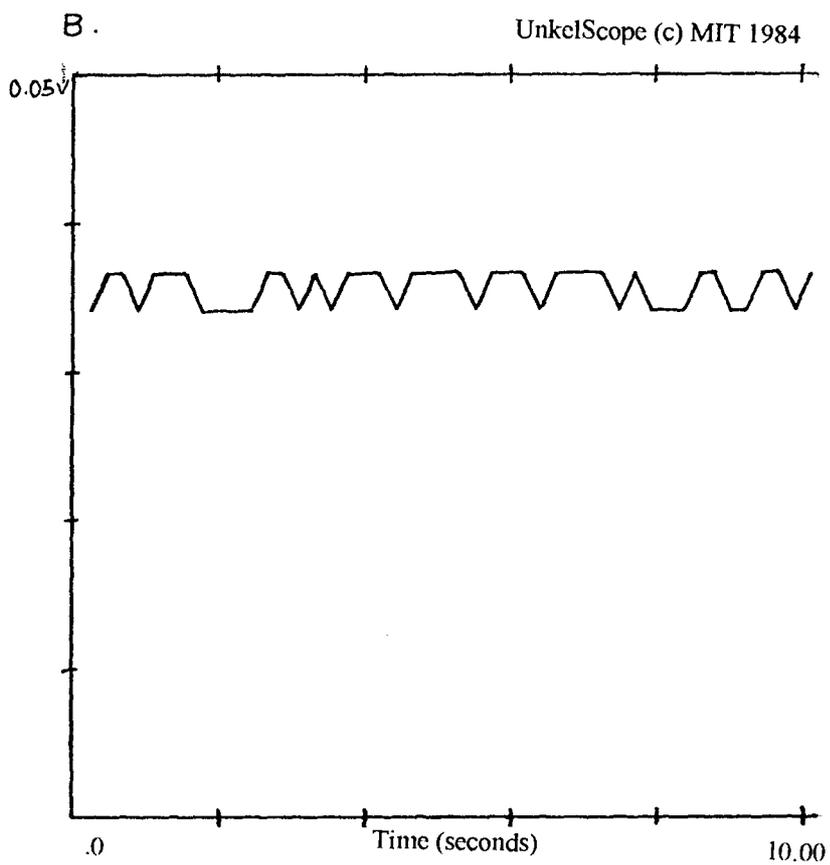
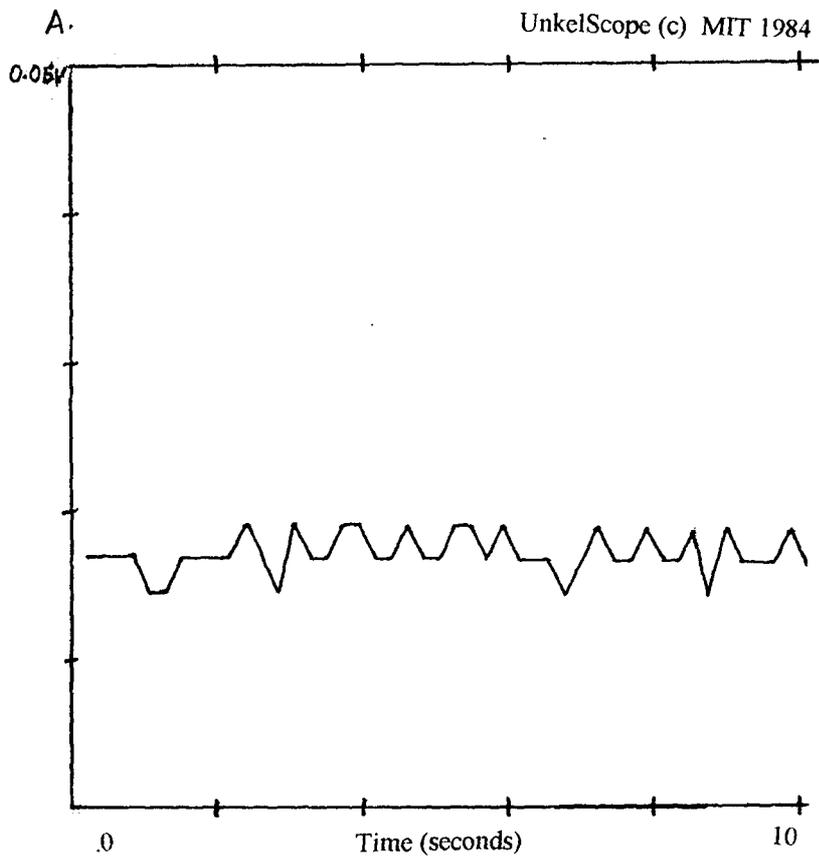
The exposure of spinal cord of pesticide treated mice resulted in a non-significant change in the frequencies of spike and plateau phases of action potentials. The inward currents were abolished and if present their

frequency was negligible. Hyperpolarisations were not evident in any of the recordings. The durations and amplitudes of spike and plateau phases of action potential were reduced and remained almost similar to that observed for control. The baseline potential however elevated slightly by about 10-20 % as compared to that observed in spinal cord of pesticide treated mice prior to the channel blocker effect.

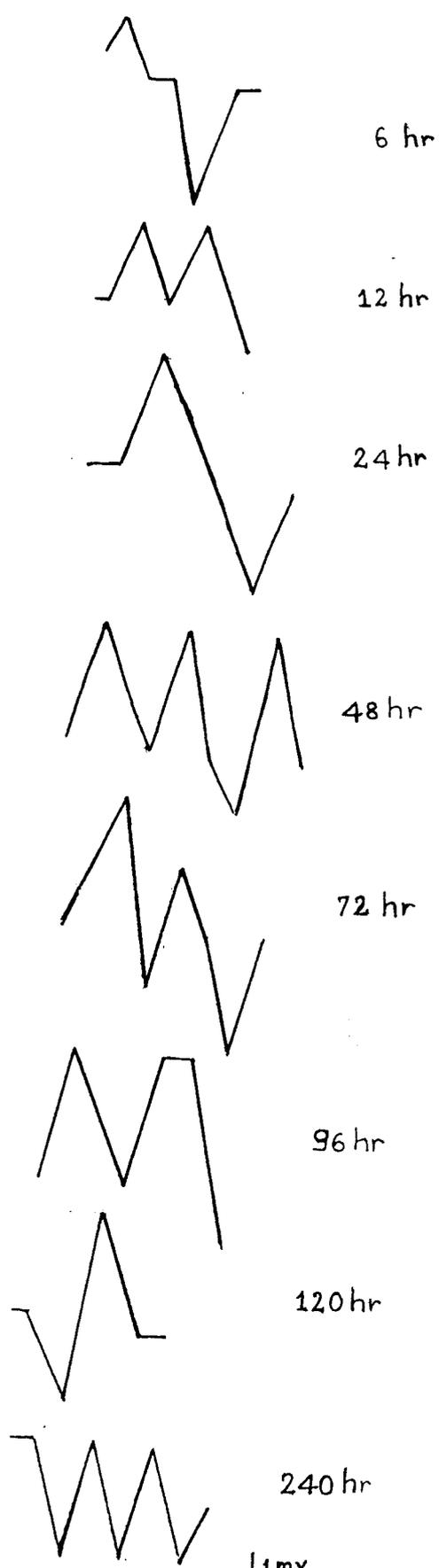
Calcium channel blocker

The recording of the effect of calcium channel blocker is represented in fig. 2.10.

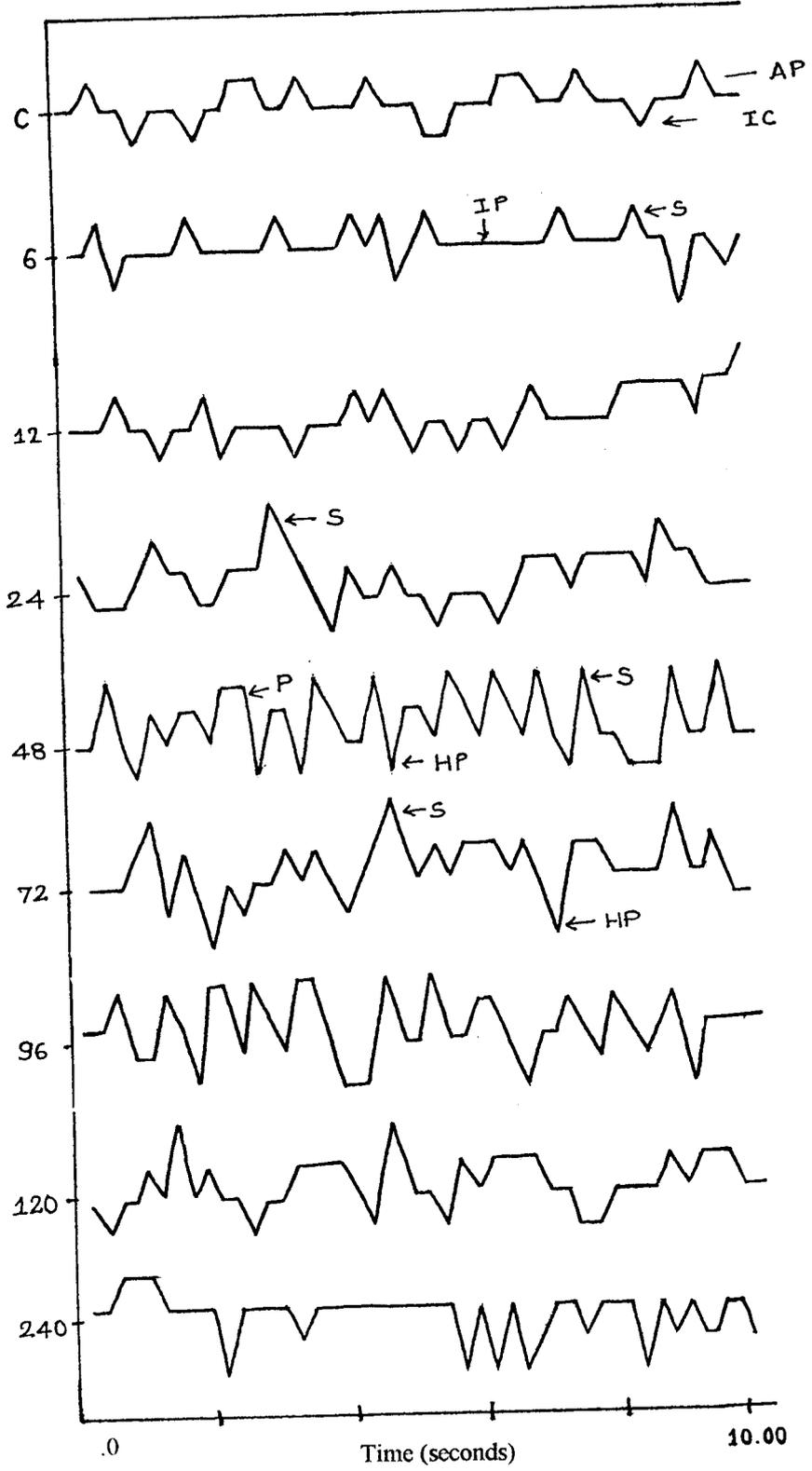
The blocking of calcium channel of the spinal cord of control as well as pesticide treated mice promoted only action potentials while inward currents were abolished. The spike and plateau phase frequencies were reduced by almost 30-40 %. Hyperpolarisations were not observed. In spinal cords of control mice, CdCl_2 did not alter the durations and amplitudes of spikes and plateau phases. While in the spinal cord of pesticide treated mice the elevated durations and peaks of spike phases induced by the pesticides reduced and were similar to that observed for control. The baseline potentials in the spinal cords of control and pesticide treated mice declined sharply and reached to almost a zero level.



CONTROL



1mv
0.2Sec



**FIG 2.8 (b): Electrical activity of spinal cord of mice in response to
Monocrotophos treatment (100 ppm dose)**

**Enlarged recordings indicating the changes in the durations
and amplitudes of the action potentials**

**FIG 2.9 : Electrical activity of spinal cord of mice in response to
Potassium channel blocker (BaCl₂)**

- A. Control**
- B. With BaCl₂ effect**

S - spike
P- plateau phase
AP – action potential
IC- inward current
IP- isoelectric phase

FIG 2.10 : Electrical activity of spinal cord of mice in response to calcium channel blocker (CdCl₂)

- A. Control**
- B. With CdCl₂ effect**

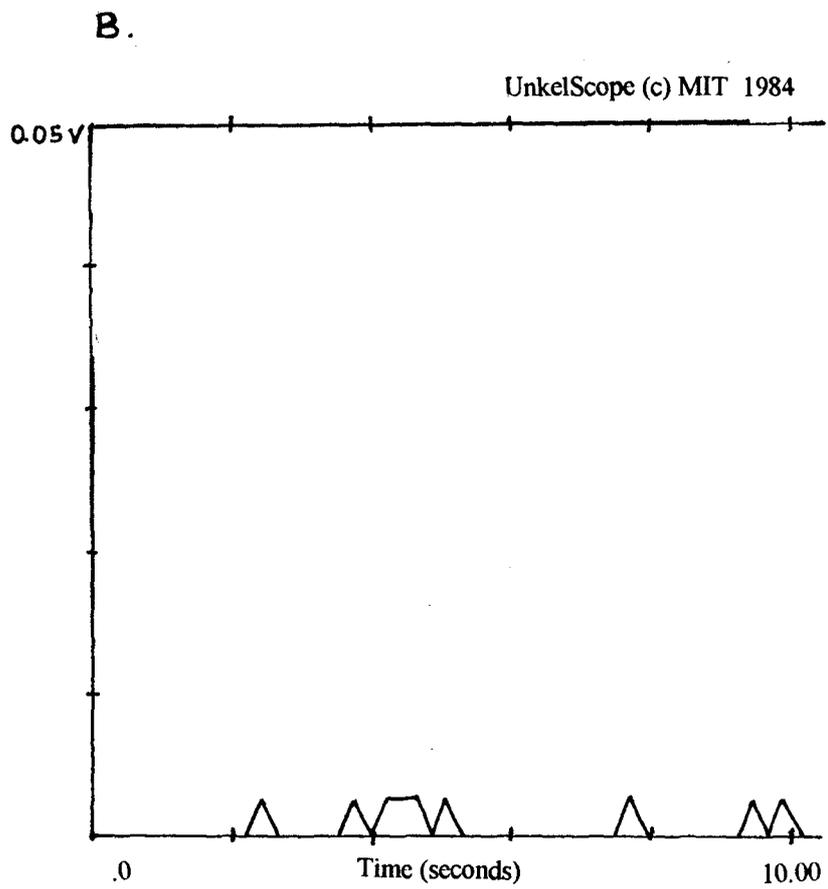
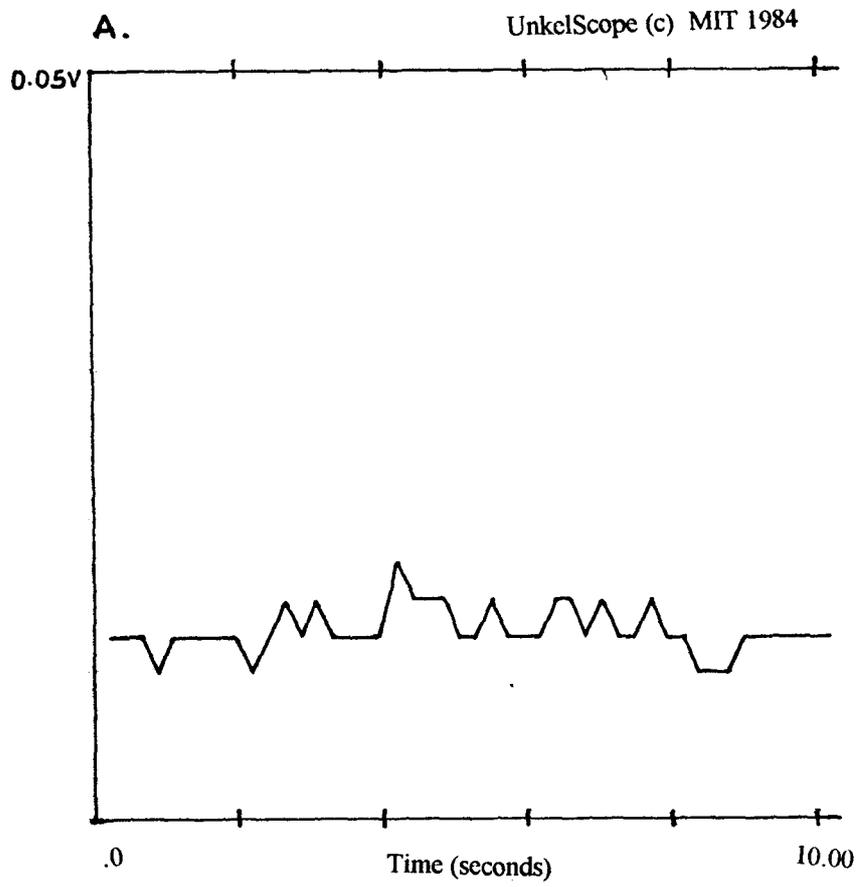
S - spike

P- plateau phase

AP – action potential

IC- inward current

IP- isoelectric phase



BIOCHEMICAL CHANGES IN SPINAL CORD OF MICE UNDER THE INFLUENCE OF DDVP

Mice exhibited alterations of enzyme activities as well as alterations in the biochemical constituents of the spinal cord, under the influence of various doses of DDVP at different time intervals.

ACID PHOSPHATASE (AP)

The alterations in the AP activities of the spinal cord of mice exposed to DDVP are compiled in table 3.01 and are graphically presented in graph 3.01. The control animals exhibited AP activity in the range of 20.28 ± 4.78 to 30.21 ± 6.09 μ mols of p-nitrophenol / g wet wt of tissue. Nearly time progression dependent elevations in AP activities were observed under the influence of all the doses of DDVP except 1 ppm.

Under the influence of 1 ppm dose, a non-significant increase in the AP activity was observed at the end of 6 and 12 hours and AP activity was equivalent to 28.24 ± 7.55 and 26.22 ± 3.85 μ mols / g wet wt of tissue respectively. However, the AP activity steadily elevated from the end of 24 hours to the end of 120 hours except for a slight decline at the end of 72 hours as compared to that observed at the preceding time interval. At the end of 240 hours the activity was equivalent to 35.17 ± 5.05 μ mols / g wet wt of tissue. The AP activity was in the range of 26.22 ± 3.85 to 40.03 ± 6.82 μ mols

/ g wet wt of tissue. The maximum elevation of AP activity was observed at the end of 120 hours.

5 ppm dose induced elevations in AP activity from the end of 6 hours to the end of 48 hours. At the end of 72 hours a marginal decline was noted but subsequently the AP activity elevated upto the end of 240 hours. The AP activity was in the range of 30.01 ± 9.55 to 99.08 ± 10.77 μ mols / g wet wt of tissue and the maximum activity was observed at the end of 240 hours. The elevations in AP activities were statistically significant at all time interval except at the end of 6 and 12 hours.

10 ppm dose of DDVP promoted a time progression dependent elevations in AP activity from the end of 6 hours to the end of 240 hours. The increases in AP activity were not significant at the end of 6 and 12 hours, while for the rest of the time intervals they were highly significant. The AP activity ranged between 38.79 ± 10.66 to 137.0 ± 14.49 μ mols / g wet wt of tissue. The maximum AP activity was observed at the end of 240 hours.

Under the influence of 100 ppm dose of DDVP, the AP activity steadily elevated from the end of 6 hours to the end of 24 hours. At the end of 48 hours the AP activity decreased marginally as compared to the preceding time interval but subsequently it increased gradually to a maximum level at the end of 240 hours. The AP activity ranged between 59.27 ± 11.8 to 214.03 ± 32.26 μ mols / g wet wt of tissue. The elevations in AP activities were statistically significant at all the time intervals.

**Table 3.01 : ACID PHOSPHATASE ACTIVITY OF THE SPINAL CORD OF
MICE EXPOSED TO DDVP**

	6	12	24	48	72	96	120	240
control	26.63 ±5.28	25.94 ±6.38	20.28 ±4.78	21.67 ±5.14	23.74 ±3.87	27.17 ±2.71	23.05 ±4.67	30.21 ±6.09
1ppm	28.24 ±7.55 ns	26.22 ±3.85 ns	28.43 ±6.97 b	34.78 ±5.32 b	33.40 ±5.39 a	37.40 ±6.46 a	40.03 ±6.82 a	35.17 ±5.05 c
5ppm	30.01 ±9.55 ns	34.78 ±5.32 ns	55.33 ±7.48 c	72.02 ±9.02 a	70.06 ±5.04 a	84.04 ±6.76 a	86.66 ±8.40 a	99.08 ±10.77 a
10ppm	38.79 ±10.66 ns	47.17 ±8.39 ns	61.41 ±10.95 b	77.14 ±9.31 a	86.66 ±8.40 a	91.77 ±10.31 a	95.63 ±6.27 a	137.0 ±14.49 a
100ppm	59.27 ±11.8 b	73.27 ±12.44 b	98.53 ±15.58 b	97.43 ±8.50 a	100.31 ±4.60 a	116.75 ±13.69 a	138.07 ±7.56 a	214.03 ±32.26 a
µmols / g wet wt. of tissue								

Values represent mean ± S.D.

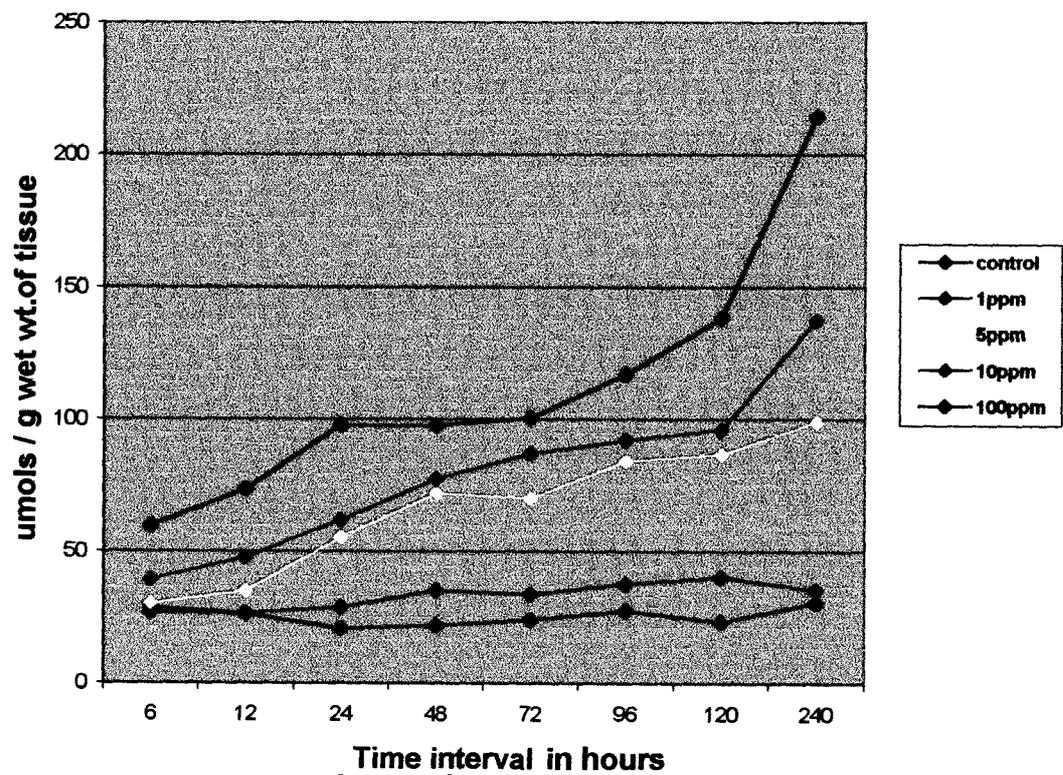
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.01 : Acid phosphatase activity of spinal cord of mice exposed to DDVP



ALKALINE PHOSPHATASE (ALP)

DDVP induced decline in the ALP activity of the spinal cord of mice. The changes in ALP activities are presented in table 3.02 and represented graphically in graph 3.02. The ALP activity in the control animals was in the range of 1.85 ± 0.24 to 2.15 ± 0.40 μ mols / g wet wt of tissue.

1 ppm dose of DDVP induced fluctuations in the ALP activity from the end of 6 hours to the end of 24 hours. However the ALP activities were below the control level at all the time intervals. The ALP activity ranged between 0.34 ± 0.09 to 1.46 ± 0.32 μ mols / g wet wt of tissue. ALP activity declined non-significantly at the end of 6 hours while at the rest of the time intervals the decline was statistically significant. The maximum decline of ALP activity was noted at the end of 240 hours.

5 ppm dose of DDVP decreased the ALP activity steadily from the end of 6 hours to the end of 120 hours except for an increase in the activity at the end 48 hours as compared to that observed at the preceding time intervals. At the end of 240 hours the ALP activity was equivalent to 0.19 ± 0.05 μ mols / g wet wt of tissue. The ALP activities were in the range of 0.18 ± 0.05 to 1.01 ± 0.19 μ mols / g wet wt. of tissue. The maximum decline in ALP activity was observed at the end of 120 hours. All the reductions in the ALP activities were statistically significant.

**Table 3.02 : ALKALINE PHOSPHATASE ACTIVITY OF THE SPINAL CORD
OF MICE EXPOSED TO DDVP**

	6	12	24	48	72	96	120	240
Control	1.85 ±0.24	2.08 ±0.43	2.08 ±0.60	2.05 ±0.51	2.00 ±0.38	2.15 ±0.42	2.13 ±0.40	1.94 ±0.30
1ppm	1.46 ±0.32 ns	1.39 ±0.48 c	0.95 ±0.24 c	0.98 ±0.12 c	0.79 ±0.08 b	0.87 ±0.21 c	0.62 ±0.09 b	0.34 ±0.09 a
5ppm	1.01 ±0.19 c	0.47 ±0.09 b	0.36 ±0.08 a	0.43 ±0.14 b	0.21 ±0.07 a	0.20 ±0.05 a	0.18 ±0.05 a	0.19 ±0.05 a
10ppm	0.95 ±0.10 a	0.52 ±0.06 a	0.24 ±0.06 a	0.23 ±0.06 a	0.17 ±0.02 a	0.13 ±0.02 a	0.13 ±0.02 a	0.11 ±0.03 a
100ppm	0.57 ±0.08 a	0.24 ±0.07 a	0.11 ±0.02 a	0.11 ±0.03 a	0.12 ±0.04 a	0.09 ±0.03 a	0.10 ±0.02 a	0.09 ±0.04 a
µmols / g wet wt. of tissue								

Values represent mean ± S.D.

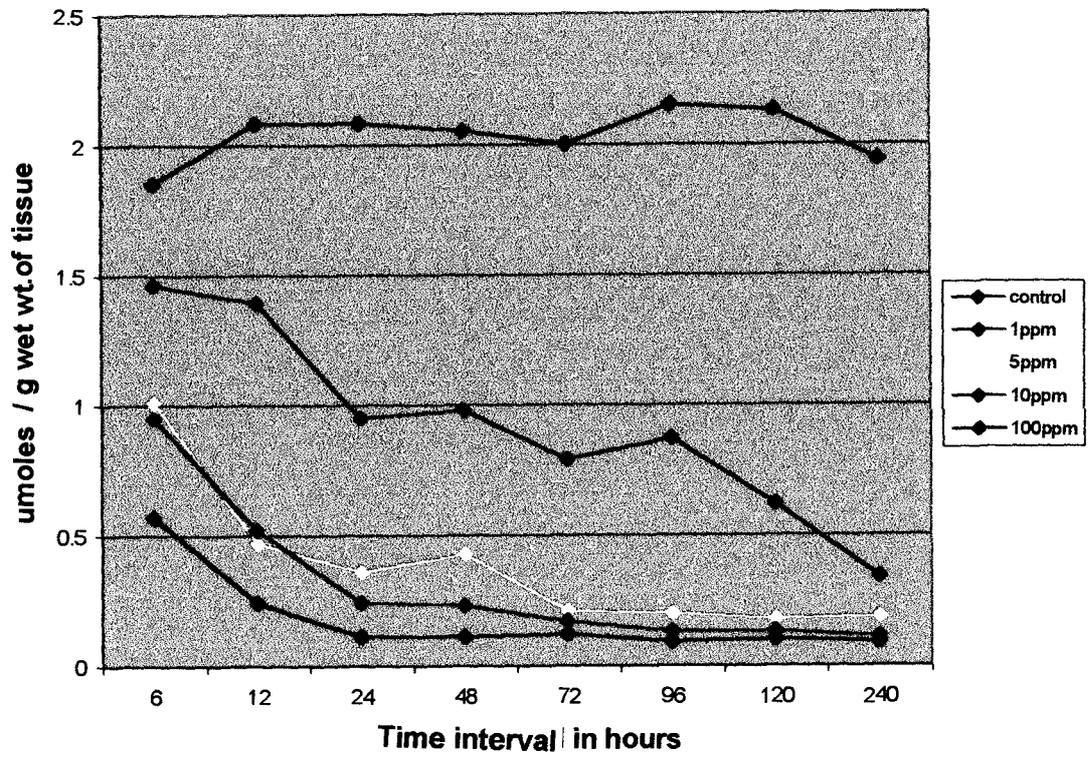
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

**Graph 3.02 : Alkaline phosphatase activity of spinal cord
of mice exposed to DDVP**



Mice treated with 10 ppm dose of DDVP exhibited a decline in ALP activity from the end of 6 hours to the end of 96 hours and further remained unaltered till the end of 120 hours to decline once again at the end of 240 hours. The ALP activity ranged between 0.95 ± 0.10 to 0.11 ± 0.03 μ mols / g wet wt of tissue. The maximum decline of ALP activity was observed at the end of 240 hours. All the declines of ALP activity were statistically significant ($P < 0.001$).

100 ppm dose of DDVP decreased the ALP activity from the end of 6 hours to the end of 24 hours and it remained unchanged up to the end of 48 hours. Further the ALP activity fluctuated from the end of 72 hours to the end of 240 hours. The ALP activity ranged between 0.09 ± 0.04 to 0.57 ± 0.08 μ mols / g wet wt of tissue. The maximum decline in ALP activity was evident at the end of 240 hours. All the changes in ALP activity were highly significant ($P < 0.001$).

NON-SPECIFIC ESTERASES (NSE)

DDVP induced a nearly dose dependent increase in the NSE activity at all the time intervals. The NSE activity data are compiled in table 3.03 and graphically presented in graph 3.03. The control animals exhibited NSE activity in the range of 3.01 ± 0.60 to 3.25 ± 0.50 μ mols of p-nitrophenol / g wet wt of tissue.

The mice exposed to 1 ppm dose of DDVP showed fluctuations in NSE activity from the end of 6 to 240 hours. The NSE activity was non – significantly elevated as compared to control at the end of 6, 12, 24 and 48 hours while the elevations were significant at the end of 72, 96 and 120 hours. A non-significant decline in the NSE activity with reference to the corresponding control was observed at the end of 240 hours. The NSE activity ranged between 3.18 ± 0.50 to 4.28 ± 0.76 μ mols / g wet wt of tissue. The maximum increase in NSE activity was observed at the end of 72 hours.

5 ppm dose of DDVP induced a steady elevation in the NSE activity from the end of 6 hours to the end of 240 hours. The NSE activity ranged between 5.15 ± 1.03 to 6.88 ± 0.82 μ mols / g wet wt of tissue. The maximum NSE activity was observed at the end of 240 hours. All the elevations of NSE activity were statistically significant.

Under the influence of 10 ppm dose of DDVP, the NSE activity elevated well above the controls at all the time intervals and showed a steady increase from the end of 6 hours to the end of 72 hours and subsequently fluctuated till the end of 240 hours. The NSE activity ranged between 5.70 ± 0.70 to 7.87 ± 0.87 μ mols / g wet wt of tissue. The NSE activity reached to a maximal level at the end of 120 hours. The increases in NSE activities were statistically significant at all time intervals.

Animals subjected to 100 ppm dose of DDVP, revealed an time intervals dependent elevation in NSE activity from the end of 6 hours to the

**Table 3.03: NON – SPECIFIC ESTERASES ACTIVITY OF THE SPINAL
CORD OF MICE EXPOSED TO DDVP**

	6	12	24	48	72	96	120	240
Control	3.25 ±0.50	3.13 ±0.30	3.01 ±0.60	3.15 ±0.45	3.20 ±0.32	3.18 ±0.09	3.08 ±0.40	3.28 ±0.30
1ppm	3.94 ±0.89 ns	3.62 ±0.90 ns	3.24 ±0.08 ns	3.36 ±0.60 ns	4.28 ±0.76 c	4.20 ±0.44 c	3.90 ±0.42 c	3.18 ±0.50 ns
5ppm	5.15 ±1.03 b	5.21 ±0.78 b	6.12 ±0.76 a	6.44 ±0.90 a	6.57 ±0.42 a	6.83 ±0.09 b	6.86 ±0.60 c	6.88 ±0.82 b
10ppm	5.70 ±0.70 a	5.93 ±0.80 a	6.78 ±0.90 a	6.81 ±0.76 a	7.42 ±0.94 b	7.35 ±0.90 b	7.87 ±0.87 a	7.83 ±1.25 a
100ppm	6.27 ±1.12 a	7.65 ±1.40 a	8.27 ±0.16 a	8.43 ±0.80 a	8.47 ±1.02 a	8.87 ±1.29 a	8.78 ±1.84 a	8.82 ±2.07 a
μmols / g wet wt. of tissue								

Values represent mean ± S.D.

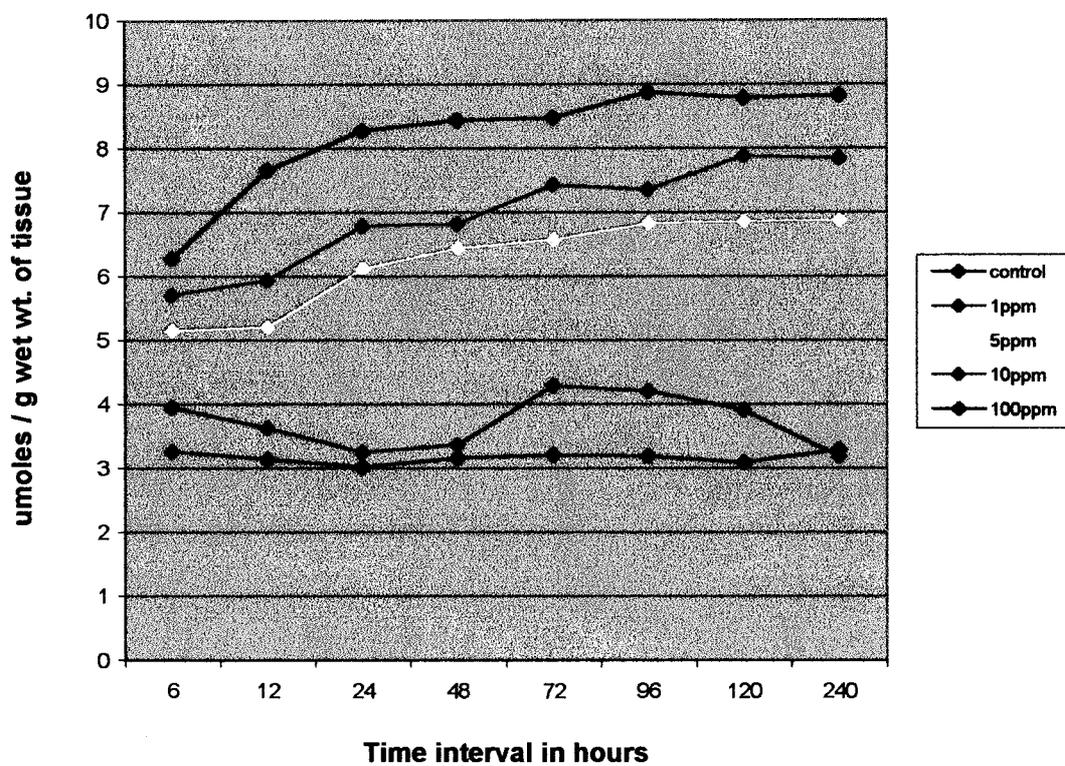
ns= not significant

a= P <0.001

b= P< 0.01

c = P< 0.05

Graph 3.03 : Non-specific esterases activity of spinal cord of mice exposed to DDVP



end of 96 hours while at the end of 120 hours and 240 hours the NSE activity decreased as compared to that observed at the preceding time interval. The activity was in the range of 6.27 ± 1.12 to 8.87 ± 1.29 μ mols / g wet wt of tissue. The maximum activity was observed at the end of 96 hours. All the elevations in NSE activities were statistically significant. ($P < 0.001$).

XANTHINE OXIDASE (XOD)

The XOD activity was elevated in response to DDVP administration. The alterations in XOD activities are tabulated in table 3.04 and presented graphically in graph 3.04. The control group of animals exhibited the XOD activity in the range of 6.2 ± 1.3 to 7.1 ± 1.8 nmols / mg of protein.

Under the influence of 1 ppm dose of DDVP, the XOD activity increased from the end of 6 hours to the end of 24 hours and then remained almost unchanged upto the end of 48 hours but increased again at the end of 72 hours. At the end of 96 hours, the XOD activity decreased negligibly and further declined gradually upto the end of 240 hours. The XOD activity was in the range of 17.5 ± 2.1 to 11.0 ± 3.3 n mols / mg of protein. The highest activity was observed at the end of 72 hours. The elevations in XOD activity were non-significant at the end of 6, 12, 48, 96 and 240 hours while it was significant at the rest of the time intervals.

5 ppm dose of DDVP promoted a time progression dependent elevation in XOD activity from the end of 6 hours to the end of 48 hours and

subsequently the XOD activity fluctuated from the end of 72 hours to the end of 240 hours. The XOD activity was in the range of 13.9 ± 3.0 to 24.4 ± 4.2 n mols / mg of protein. The maximum XOD activity was observed at the end of 48 hours. The increase in XOD activity was statistically significant at all time intervals except at the end of 6 and 96 hours.

Mice administered with 10 ppm dose of DDVP exhibited elevations of XOD activity at all the time intervals. The XOD activity was equivalent to 16.3 ± 2.8 n mols / mg of protein at the end of 6 hours and then it declined a little at the end of 12 hours. However it subsequently elevated gradually from the end of 24 hours to the end of 240 hours. The XOD activity was in the range of 15.5 ± 2.7 to 43.5 ± 4.3 nmols / mg of protein. The highest was observed at the end of 240 hours. The hike in XOD activities was statistically significant at all time intervals except at the end of 12 hours.

In response to 100 ppm dose of DDVP, the XOD activity increased steadily from the end of 6 hours to the end of 240 hours. The XOD activity ranged from 26.5 ± 5.5 to 55.9 ± 5.4 n mols / mg of protein. The maximum elevation of XOD activity was observed at the end of 240 hours. All the elevations of the XOD activity were statistically significant.

Table 3.04: XANTHINE OXIDASE ACTIVITY OF THE SPINAL CORD OF MICE EXPOSED TO DDVP

	6	12	24	48	72	96	120	240
Control	7.1 ±1.8	6.2 ±1.3	6.8 ±2.6	6.8 ±1.2	6.3 ±1.2	6.8 ±2.0	7.0 ±1.8	6.9 ±1.0
1ppm	11.8 ±3.8 ns	13.0 ±2.3 ns	16.3 ±2.0 c	16.3 ±4.0 ns	17.5 ±2.1 c	17.4 ±3.5 ns	15.4 ±3.0 b	11.0 ±3.3 ns
5ppm	13.9 ±3.0 ns	16.7 ±3.5 c	19.9 ±3.0 c	24.4 ±4.2 c	21.5 ±3.7 c	18.8 ±4.0 ns	19.4 ±3.5 c	20.2 ±4.2 b
10ppm	16.3 ±2.8 c	15.5 ±2.7 ns	20.1 ±4.0 c	33.0 ±4.1 c	36.2 ±4.4 b	38.1 ±4.6 c	42.7 ±3.5 c	43.5 ±4.3 b
100ppm	26.5 ±5.5 c	32.3 ±8.7 c	41.5 ±9.3 b	47.9 ±9.0 a	52.5 ±5.0 a	53.3 ±9.1 a	54.5 ±4.7 c	55.9 ±5.4 c
nmols of urate / mg of protein								

Values represent mean ± S.D.

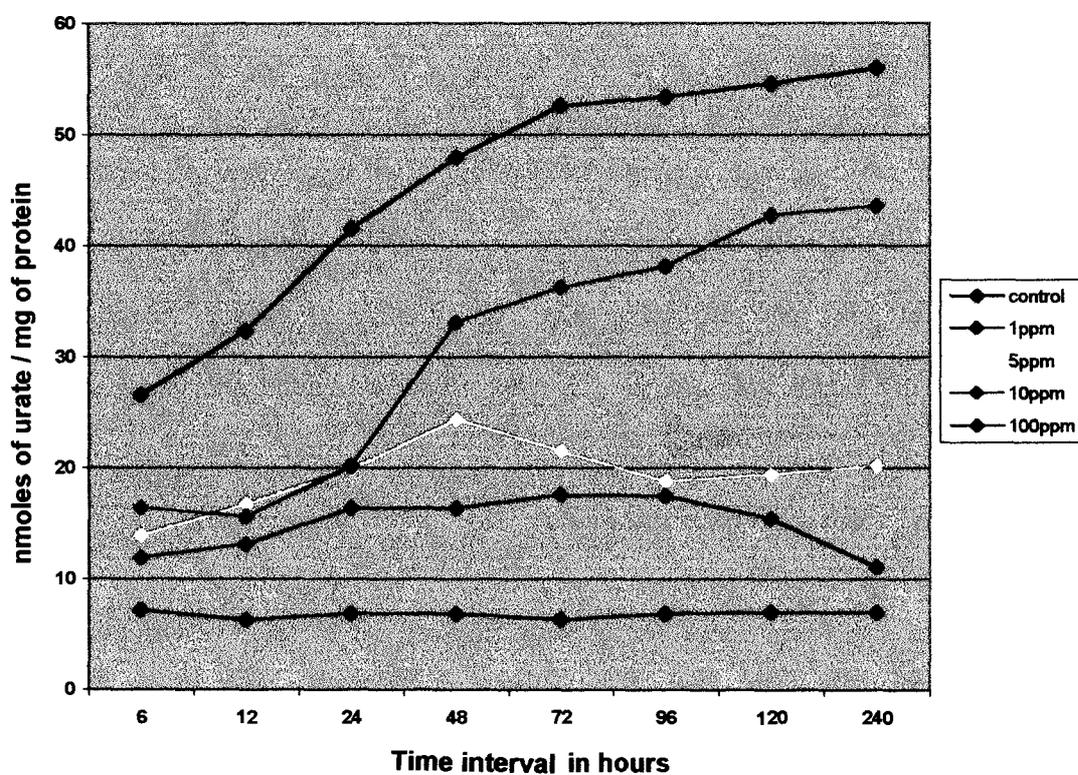
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.04 : Xanthine oxidase activity of spinal cord of mice exposed to DDVP



SUPER OXIDE DISMUTASE (SOD)

DDVP induced a dose dependent elevation of the SOD activity in the spinal cord of mice at all the time intervals. The changes in SOD activities are tabulated in table 3.05 and graphically presented in graph 3.05. The SOD activity in the control animals ranged between 30.64 ± 3.3 and 33.28 ± 3.7 units / mg of protein.

1 ppm dose of DDVP elevated the SOD activity from the end of 6 hours to the end of 96 hours and the activity was in the range of 37.82 ± 3.4 to 43.21 ± 4.7 units / mg of protein. The SOD activity declined slightly at the end of 120 and 240 hours with reference to that observed at preceding time interval and was equivalent to 41.35 ± 3.7 and 41.10 ± 5.9 units / mg of protein. All the elevations in SOD activity were statistically significant. The maximum hike in SOD activity was observed at the end of 96 hours.

In response to 5 ppm dose of DDVP, the SOD activity elevated from the end of 6 hours to the end of 72 hours and it subsequently declined from the end of 96 hours to the end of 240 hours with reference to that observed at the end of 72 hours. The SOD activity ranged between 42.93 ± 3.4 to 51.54 ± 5.76 units / mg of protein. The maximum activity was observed at the end of 72 hours. All the elevations in SOD activity were statistically significant ($P < 0.001$).

10 ppm dose of DDVP induced fluctuations in the SOD activity from the end of 6 hours to the end of 240 hours. The activity ranged from 54.44 ± 5.4 to 62.52 ± 7.2 units / mg of protein. The maximum hike in SOD activity was observed at the end of 240 hours. All the changes in SOD activity were statistically significant ($P < 0.001$).

Animals administered with 100 ppm dose of DDVP promoted an increase in the SOD activity from the end of 6 hours to the end of 240 hours. The SOD activity was in the range of 62.01 ± 5.4 to 78.53 ± 6.8 units / mg of protein. All the hikes in SOD activity were statistically significant ($P < 0.001$). The maximum elevation in SOD activity was observed at the end of 240 hours.

TOTAL PROTEINS

In response to various doses of DDVP the total protein content of the spinal cord decreased at all the time intervals. The changes in protein concentrations are tabulated in table 3.06 and graphically presented in graph 3.06. The control animals exhibited protein concentrations in the range of 0.230 ± 0.04 to 0.249 ± 0.03 g / g wet wt. of tissue. A nearly dose dependent decrease in total protein concentrations was induced by DDVP at all the time intervals except at the end of 72 and 240 hours.

TABLE 3.05: SUPEROXIDE DISMUTASE ACTIVITY OF THE SPINAL CORD OF MICE EXPOSED TO DDVP

	6	12	24	48	72	96	120	240
Control	31.01 ±3.4	31.81 ±4.5	30.64 ±3.3	33.28 ±3.7	31.56 ±1.6	32.53 ±2.9	31.74 ±2.2	31.40 ±3.2
1ppm	37.82 ±3.4 c	38.51 ±4.2 c	40.83 ±3.9 a	42.93 ±3.7 a	42.92 ±3.8 a	43.21 ±4.7 a	41.35 ±3.7 a	41.10 ±5.9 a
5ppm	42.93 ±3.4 a	45.46 ±3.6 a	48.35 ±2.8 a	49.03 ±5.4 a	51.54 ±5.76 a	50.62 ±4.3 a	49.97 ±6.8 a	49.81 ±7.5 a
10ppm	54.44 ±5.4 a	55.71 ±7.9 a	55.02 ±3.5 a	58.46 ±3.1 a	60.64 ±7.60 a	58.70 ±5.4 a	62.40 ±4.7 a	62.52 ±7.2 a
100ppm	62.01 ±5.4 a	63.31 ±4.7 a	66.28 ±5.5 a	69.53 ±5.2 a	74.31 ±7.7 a	75.83 ±6.9 a	77.22 ±7.1 a	78.53 ±6.8 a
Units / mg of protein								

Values represent mean ± S.D.

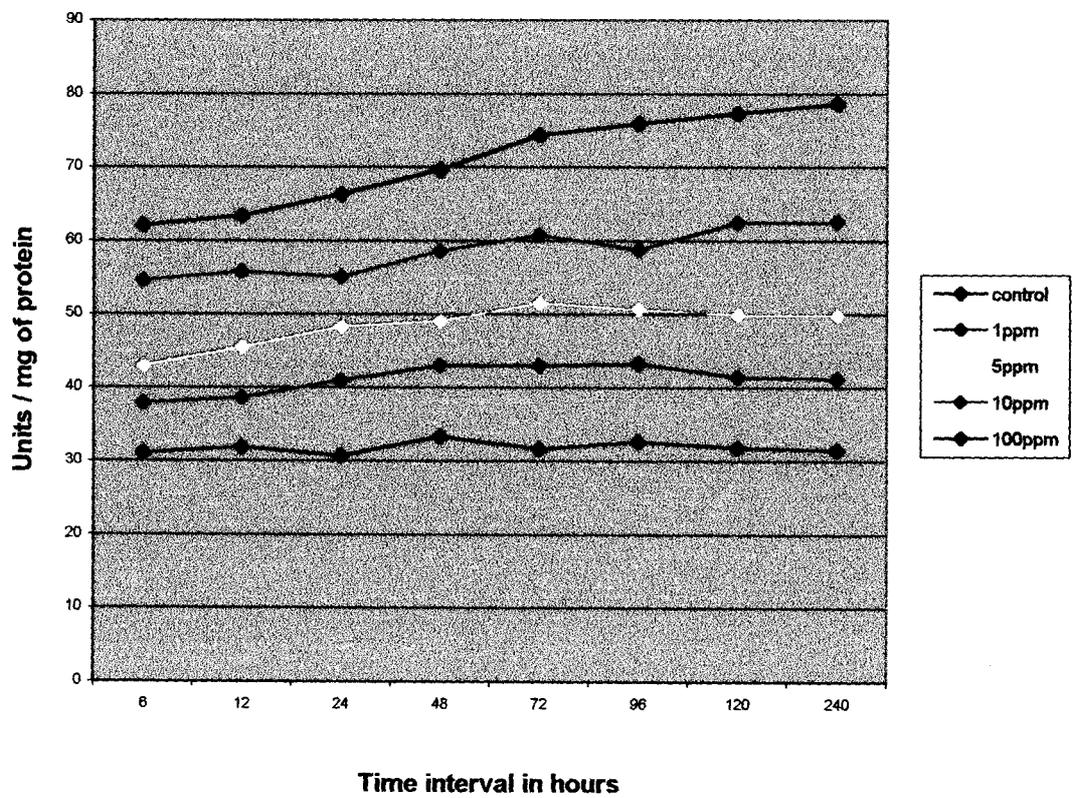
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.05 : Superoxide dismutase activity of spinal cord of mice exposed to DDVP



1 ppm dose of DDVP induced a steady decline in total protein content of spinal cord from the end of 6 hours to the end of 120 hours and the protein contents were in the range of 0.218 ± 0.08 to 0.169 ± 0.09 g / g wet wt. of tissue. At the end of 240 hours there was a slight recovery in protein concentration in comparison to that observed at the preceding time intervals and the total protein concentration was equivalent to 0.174 ± 0.08 g / g wet wt. of tissue. The maximum decline in protein content was found at the end of 120 hours. The reductions in total protein contents were statistically significant except at the end of 6 and 12 hours.

Under the influence of 5ppm dose of DDVP, the total protein contents declined steadily from the end of 6 hours to the end of 96 hours and there was no further decline at the end of 120 hours but subsequently it declined at the end of 240 hours. The protein contents ranged from 0.152 ± 0.07 to 0.196 ± 0.07 g / g wet wt. of tissue. The maximum reductions of protein contents were noticed at the end of 240 hours and all the reductions of protein contents were statistically significant.

10 ppm dose of DDVP induced a decline of proteins at all the time intervals and the total protein content were in the range of 0.155 ± 0.05 to 0.177 ± 0.05 g / g wet wt. of tissue. The maximum reduction in total protein contents was observed at the end of 240 hours. All the changes in the protein concentrations were statistically significant.

TABLE 3.06: TOTAL PROTEIN CONTENT OF THE SPINAL CORD OF

MICE EXPOSED TO DDVP

	6	12	24	48	72	96	120	240
Control	0.246 ±0.09	0.243 ±0.04	0.244 ±0.04	0.232 ±0.06	0.230 ±0.04	0.242 ±0.05	0.245 ±0.03	0.249 ±0.03
1ppm	0.218 ±0.08 ns	0.204 ±0.08 ns	0.198 ±0.09 c	0.186 ±0.07 c	0.184 ±0.07 c	0.171 ±0.08 c	0.169 ±0.09 c	0.174 ±0.08 b
5ppm	0.196 ±0.07 a	0.189 ±0.08 a	0.177 ±0.09 b	0.172 ±0.08 b	0.167 ±0.06 a	0.163 ±0.09 a	0.163 ±0.07 a	0.152 ±0.07 a
10ppm	0.177 ±0.05 a	0.175 ±0.08 a	0.176 ±0.06 a	0.168 ±0.06 a	0.168 ±0.07 a	0.161 ±0.05 a	0.157 ±0.08 a	0.155 ±0.05 a
100ppm	0.085 ±0.05 a	0.084 ±0.03 a	0.082 ±0.05 a	0.083 ±0.04 a	0.077 ±0.03 a	0.075 ±0.04 a	0.068 ±0.02 a	0.062 ±0.02 a
g / g wet wt. of tissue								

Values represent mean ± S.D.

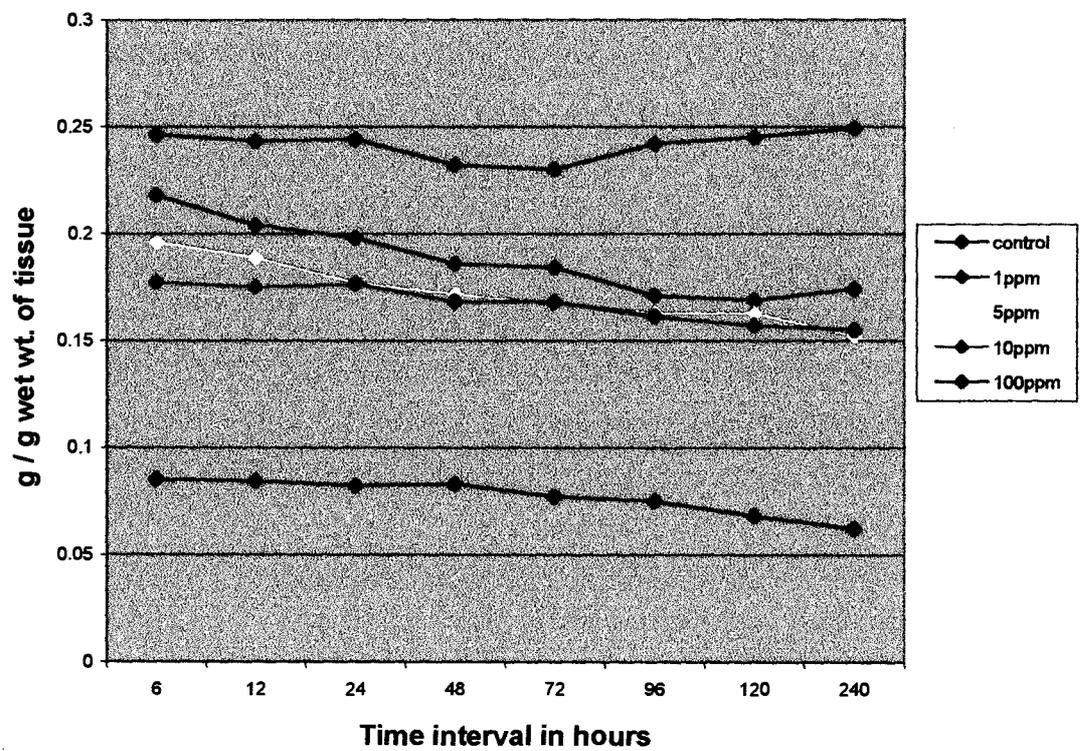
ns= not significant

a= P <0.001

b= P< 0.01

c = P< 0.05

Graph 3.06 : Total protein content of spinal cord of mice exposed to DDVP



The exposure of mice to 100 ppm dose of DDVP resulted in a precipitous decline in the total protein concentrations of the spinal cord at all the time intervals. The protein contents of the spinal cord ranged from 0.062 ± 0.02 to 0.085 ± 0.05 g / g wet wt. of tissue. The maximum reduction in the total protein concentration was found at the end of 240 hours. All the reductions in total protein contents were statistically significant ($P < 0.001$).

Mitochondrial Proteins (m-protein)

The mitochondrial protein concentrations (m-protein) decreased in response to various doses of DDVP in nearly a dose dependent manner. The changes in the concentrations of m-proteins are compiled in table 3.07 and graphically presented in graph 3.07. The m-protein contents of the control animals were in the range of 0.080 ± 0.029 to 0.087 ± 0.030 g / g of mitochondrial pellet.

1 ppm dose of DDVP induced a decline of m-protein contents at all the time intervals. A steady decline in m-protein contents was observed from the end of 6 hours to the end of 72 hours and subsequently it fluctuated upto the end of 240 hours. The m-protein concentrations were in the range of 0.064 ± 0.012 to 0.073 ± 0.008 g / g of mitochondrial pellet. The maximum decline in the m-protein concentrations was observed at the end of 72 hours while the minimum decline was observed at the end of 6 hours. All the changes in the m-proteins were statistically significant.

Under the influence of 5 ppm dose of DDVP, the m-protein concentrations fluctuated from the end of 6 hours to the end of 240 hours but they were less than those observed in the controls. The m-protein concentrations were in the range of 0.057 ± 0.008 to 0.062 ± 0.012 g / g of mitochondrial pellet. The maximum decline in m-protein concentration was observed at the end of 48 hours. The reductions in protein content were statistically significant at all the time intervals.

In response to 10 ppm dose of DDVP, the m-protein contents decreased from the end of 6 hours to the end of 240 hours and they were in the range of 0.042 ± 0.008 to 0.055 ± 0.016 g / g of mitochondrial pellet. All the changes in m-protein concentrations were highly significant ($P < 0.001$).

Mice treated with 100 ppm dose of DDVP, exhibited a sharp decline in the m-protein contents at all the time intervals. The maximum decline in m-protein concentrations was observed at the end of 240 hours while a relatively minimum decrease in m-protein concentrations was observed at the end of 6 hours. The m-protein concentrations were in the range of 0.037 ± 0.008 to 0.052 ± 0.012 g / g of mitochondrial pellet. All the reductions in m-protein concentrations were statistically significant.

**TABLE 3.07: MITOCHONDRIAL PROTEIN CONTENT OF THE SPINAL
CORD OF MICE EXPOSED TO DDVP**

	6	12	24	48	72	96	120	240
Control	0.086 ±0.032	0.084 ±0.027	0.083 ±0.033	0.086 ±0.027	0.085 ±0.028	0.087 ±0.030	0.081 ±0.031	0.080 ±0.029
1ppm	0.073 ±0.008 c	0.072 ±0.015 c	0.067 ±0.008 c	0.065 ±0.008 b	0.064 ±0.012 b	0.065 ±0.007 b	0.068 ±0.019 b	0.067 ±0.017 b
5ppm	0.060 ±0.016 a	0.061 ±0.015 a	0.059 ±0.013 b	0.057 ±0.008 b	0.060 ±0.020 b	0.061 ±0.009 b	0.061 ±0.014 a	0.062 ±0.012 a
10ppm	0.055 ±0.016 a	0.053 ±0.012 a	0.053 ±0.007 a	0.051 ±0.007 a	0.049 ±0.008 a	0.048 ±0.007 a	0.047 ±0.006 a	0.042 ±0.008 a
100ppm	0.052 ±0.012 a	0.050 ±0.009 a	0.048 ±0.012 c	0.046 ±0.007 b	0.046 ±0.005 a	0.043 ±0.008 a	0.042 ±0.010 a	0.037 ±0.008 a
g / g of mitochondrial pellet								

Values represent mean ± S.D.

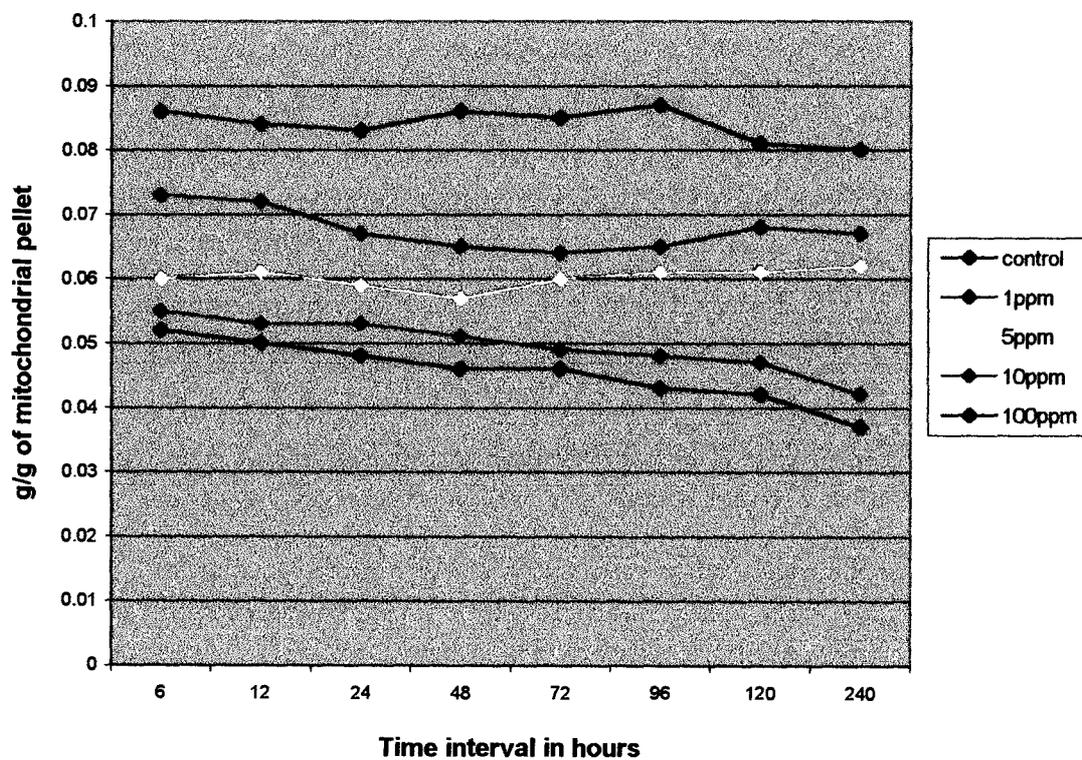
ns= not significant

a= P <0.001

b= P< 0.01

c = P< 0.05

Graph 3.07: Mitochondrial proteins of spinal cord of mice exposed to DDVP



CREATININE

The creatinine contents elevated in a dose dependent manner at all the time intervals except 12, 24 and 48 hours in the mice treated with DDVP. The changes in creatinine level are tabulated in table 3.08 and presented graphically in graph 3.08. The control animals exhibited creatinine contents in the range of 0.019 ± 0.005 to 0.025 ± 0.007 mg / g wet wt of tissue.

1 ppm dose of DDVP did not induce significant change in the creatinine level of spinal cord at almost all the time intervals except at the end of 96 and 240 hours. The creatinine contents were in the range of 0.019 ± 0.003 to 0.026 ± 0.004 mg / g wet wt of tissue. A significant increase in creatinine content was observed at the end of 72 and 240 hours.

5 ppm dose of DDVP induced an increase in creatinine contents from the end of 6 hours to the end of 96 hours and during this period the creatinine concentrations were in the range of 0.025 ± 0.005 to 0.047 ± 0.004 mg / g wet wt of tissue. The creatinine concentrations were equivalent to 0.039 ± 0.006 and 0.046 ± 0.003 mg / g wet wt of tissue at the end of 120 and 240 hours respectively. The maximum elevation of creatinine level was evident at the end of 96 hours. All the elevations in creatinine concentrations were statistically significant.

In response to 10 ppm dose of DDVP, the creatinine level elevated at all the time intervals. A steady elevation in creatinine content was observed

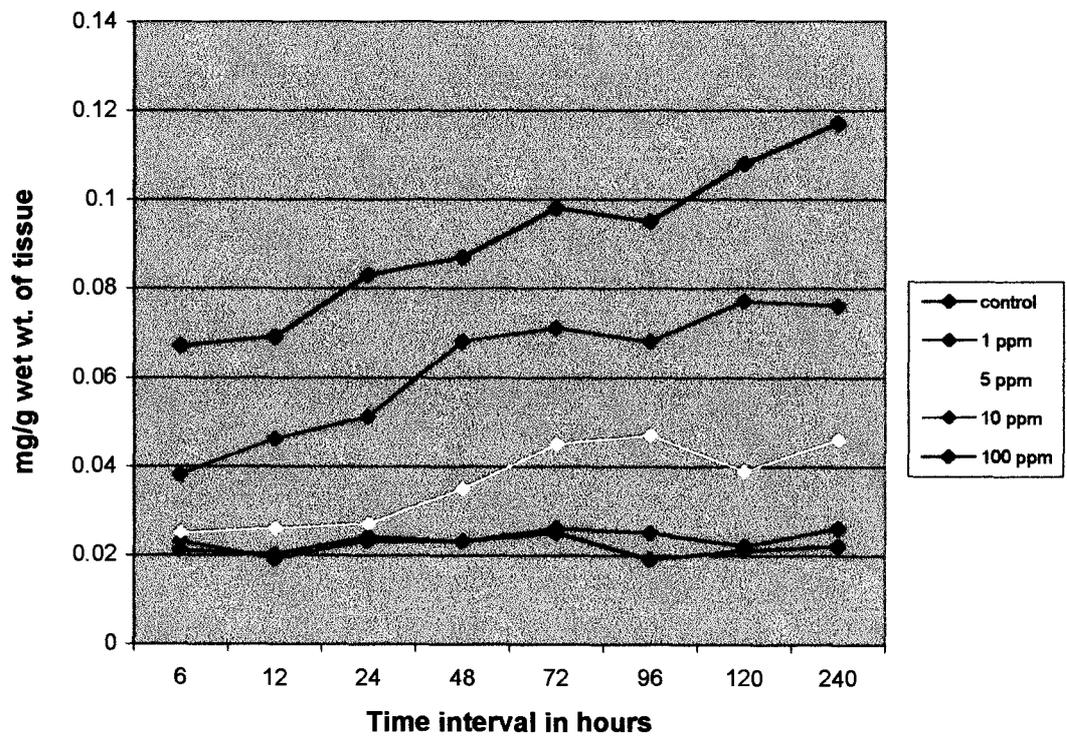
**TABLE 3.08: CREATININE CONTENT OF THE SPINAL CORD OF
MICE EXPOSED TO DDVP**

	6	12	24	48	72	96	120	240
Control	0.021 ±0.008	0.020 ±0.007	0.024 ±0.009	0.023 ±0.004	0.025 ±0.007	0.019 ±0.005	0.021 ±0.009	0.022 ±0.007
1ppm	0.023 ±0.005 ns	0.019 ±0.003 ns	0.023 ±0.006 ns	0.023 ±0.005 ns	0.026 ±0.005 ns	0.025 ±0.008 c	0.022 ±0.002 ns	0.026 ±0.004 c
5ppm	0.025 ±0.005 c	0.026 ±0.004 c	0.027 ±0.007 b	0.035 ±0.006 c	0.045 ±0.007 a	0.047 ±0.004 a	0.039 ±0.006 a	0.046 ±0.003 a
10ppm	0.038 ±0.006 b	0.046 ±0.008 a	0.051 ±0.009 b	0.068 ±0.007 a	0.071 ±0.007 a	0.068 ±0.006 c	0.077 ±0.007 a	0.076 ±0.007 a
100ppm	0.067 ±0.007 a	0.069 ±0.005 a	0.083 ±0.007 a	0.087 ±0.009 a	0.098 ±0.007 a	0.095 ±0.008 a	0.108 ±0.015 a	0.117 ±0.012 a
mg / g wet wt. of tissue								

Values represent mean ± S.D.

ns= not significant
a= P < 0.001
b= P < 0.01
c = P < 0.05

Graph 3.08: Creatinine content of spinal cord of mice exposed to DDVP



from the end of 6 hours to the end of 72 hours and subsequently it fluctuated upto the end of 240 hours. The creatinine contents were in the range of 0.038 ± 0.006 to 0.077 ± 0.007 mg / g wet wt of tissue. The maximum increase in creatinine concentrations was observed at the end of 120 hours. All the changes in creatinine concentration were statistically significant.

The mice administered with 100 ppm dose of DDVP, showed elevations of the creatinine contents at all time intervals. The creatinine contents ranged between 0.067 ± 0.007 to 0.117 ± 0.012 mg / g wet wt of tissue. All the changes in creatinine concentration were highly significant ($P < 0.001$).

CHOLESTEROL

The alterations in the cholesterol contents of the spinal cord of mice exposed to DDVP are tabulated in table 3.09 and graphically presented in graph 3.09. The cholesterol content in the control animals were in the range of 7.52 ± 1.30 to 8.08 ± 2.10 mg / g wet wt of tissue.

1ppm dose of DDVP induced a steady elevation in the cholesterol contents from the end of 6 hours to the end of 72 hours and subsequently the concentration fluctuated. The cholesterol contents were in the range of 10.45 ± 1.38 to 12.78 ± 1.54 mg / g wet wt of tissue. The maximum elevation in the cholesterol contents was observed at the end of 120 hours.

5 ppm dose of DDVP induced fluctuations in the cholesterol content from the end of 6 hours to the end of 240 hours. However, they were well above those observed in controls. The cholesterol contents were in the range of 9.92 ± 0.70 to 15.83 ± 0.95 mg / g wet wt of tissue. All the changes in cholesterol concentration were highly significant ($P < 0.001$). The maximum increase in cholesterol content was observed at the end of 120 hours.

Under the influence of 10 ppm dose of DDVP, the cholesterol contents increased above the level found in controls at all the time intervals. The cholesterol contents were in the range of 13.75 ± 1.60 to 18.5 ± 1.50 mg / g wet wt of tissue and the maximum increase was observed at the end of 120 hours. All the changes in the cholesterol concentrations were statistically significant.

In response to 100 ppm dose of DDVP, the mice showed sharp increase in the cholesterol concentrations of the spinal cord at all the time intervals. A steady increase in cholesterol content was observed from the end of 6 hours to the end of 120 hours while it relatively declined a little at the end of 240 hours. The cholesterol contents ranged from 17.38 ± 2.50 to 25.99 ± 1.85 mg / g wet wt of tissue. The maximum increase in cholesterol content was observed at the end of 120 hours. All the elevations in the cholesterol concentrations of the spinal cord were highly significant ($P < 0.001$).

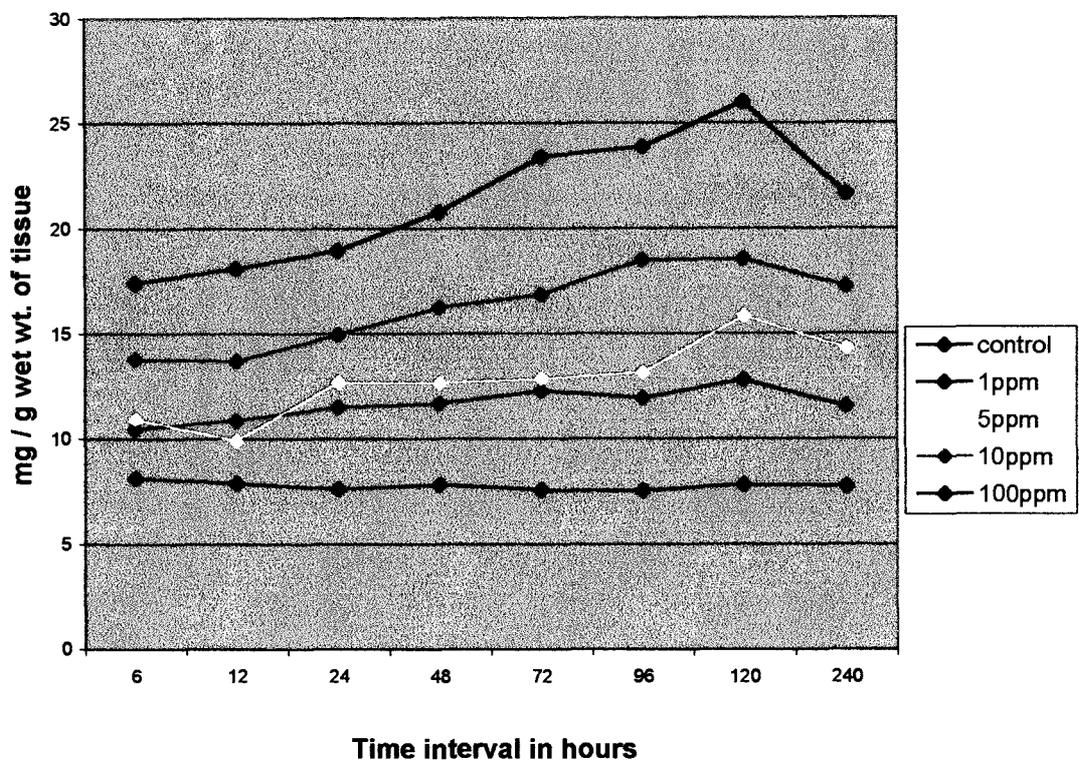
**TABLE 3.09 : CHOLESTEROL CONTENT OF THE SPINAL CORD OF
MICE EXPOSED TO DDVP**

	6	12	24	48	72	96	120	240
Control	8.08 ±2.10	7.84 ±1.20	7.59 ±1.10	7.78 ±1.40	7.55 ±1.12	7.52 ±1.30	7.80 ±1.41	7.73 ±1.77
1ppm	10.45 ±1.38 a	10.82 ±1.33 a	11.47 ±2.02 a	11.64 ±1.80 a	12.26 ±1.43 a	11.94 ±1.70 a	12.78 ±1.54 a	11.52 ±1.48 a
5ppm	10.93 ±0.85 a	9.92 ±0.70 a	12.68 ±1.20 a	12.67 ±0.80 a	12.85 ±1.05 a	13.13 ±0.60 a	15.83 ±0.95 a	14.30 ±1.14 a
10ppm	13.75 ±1.60 a	13.66 ±0.80 a	14.90 ±1.40 a	16.23 ±0.53 a	16.84 ±1.69 a	18.48 ±2.10 a	18.53 ±1.50 a	17.22 ±1.26 a
100ppm	17.38 ±2.50 a	18.08 ±2.12 a	18.94 ±1.60 a	20.74 ±1.50 a	23.37 ±1.75 a	23.85 ±2.30 a	25.99 ±1.85 a	21.64 ±2.12 a
mg / g wet wt. of tissue								

Values represent mean ± S.D.

ns= not significant
a= P < 0.001
b= P < 0.01
c = P < 0.05

Graph 3.09 : Cholesterol content of spinal cord of mice exposed to DDVP



TRIGLYCERIDES

DDVP induced pronounced elevations in the triglyceride contents of the spinal cord. The changes in triglyceride concentrations are tabulated in table 3.10 and presented graphically in graph 3.10. The triglyceride contents of the spinal cord of control animals ranged from 0.085 ± 0.4 to 1.23 ± 0.01 mg / g wet wt of tissue.

Under the influence of 1ppm dose of DDVP, the triglyceride contents increased at all the time intervals. The triglyceride concentrations were in the range of 1.91 ± 0.2 to 3.21 ± 0.4 mg / g wet wt of tissue. The maximum elevation in the triglyceride concentrations was observed at the end of 240 hours. All the changes in the triglyceride concentration were statistically significant.

In response to 5 ppm dose of DDVP, a time progression dependent elevations in the triglyceride contents were observed and they were in the range of 2.35 ± 0.4 to 4.46 ± 0.4 mg / g wet wt of tissue. The maximum increase in triglyceride concentrations was observed at the end of 240 hours. All the elevations in the triglyceride concentrations were highly significant.

Mice treated with 10 ppm dose of DDVP showed sharp elevations in triglyceride content of the spinal cord from the end of 6 hours to the end of 240 hours. The triglyceride contents were in the range of 4.03 ± 0.2 to 5.33 ± 0.8 mg / g wet wt. of tissue. The maximum increase in triglyceride

TABLE 3.10: TRIGLYCERIDE CONTENT OF THE SPINAL CORD OF

MICE EXPOSED TO DDVP

	6	12	24	48	72	96	120	240
Control	1.23 ±0.1	1.22 ±0.68	1.03 ±0.1	0.93 ±0.02	0.85 ±0.04	1.00 ±0.07	1.15 ±0.03	1.20 ±0.07
1ppm	1.92 ±0.1 a	1.91 ±0.2 a	2.34 ±0.2 a	2.37 ±0.12 a	2.84 ±0.3 a	3.02 ±0.5 a	3.13 ±0.4 a	3.21 ±0.4 a
5ppm	2.35 ±0.4 a	2.59 ±0.3 a	3.18 ±0.5 a	3.37 ±0.7 a	3.82 ±0.5 a	4.01 ±0.4 a	4.31 ±0.5 a	4.46 ±0.4 a
10ppm	4.03 ±0.2 a	4.39 ±0.5 a	4.76 ±0.8 a	4.71 ±1.1 a	4.84 ±1.0 a	5.33 ±0.8 a	5.26 ±1.2 a	5.30 ±0.9 a
100ppm	4.76 ±0.9 a	4.63 ±1.2 a	4.91 ±1.3 a	5.21 ±1.4 a	5.52 ±1.5 a	5.63 ±1.6 a	5.67 ±1.2 a	5.69 ±1.3 a
mg / g wet wt. of tissue								

Values represent mean ± S.D.

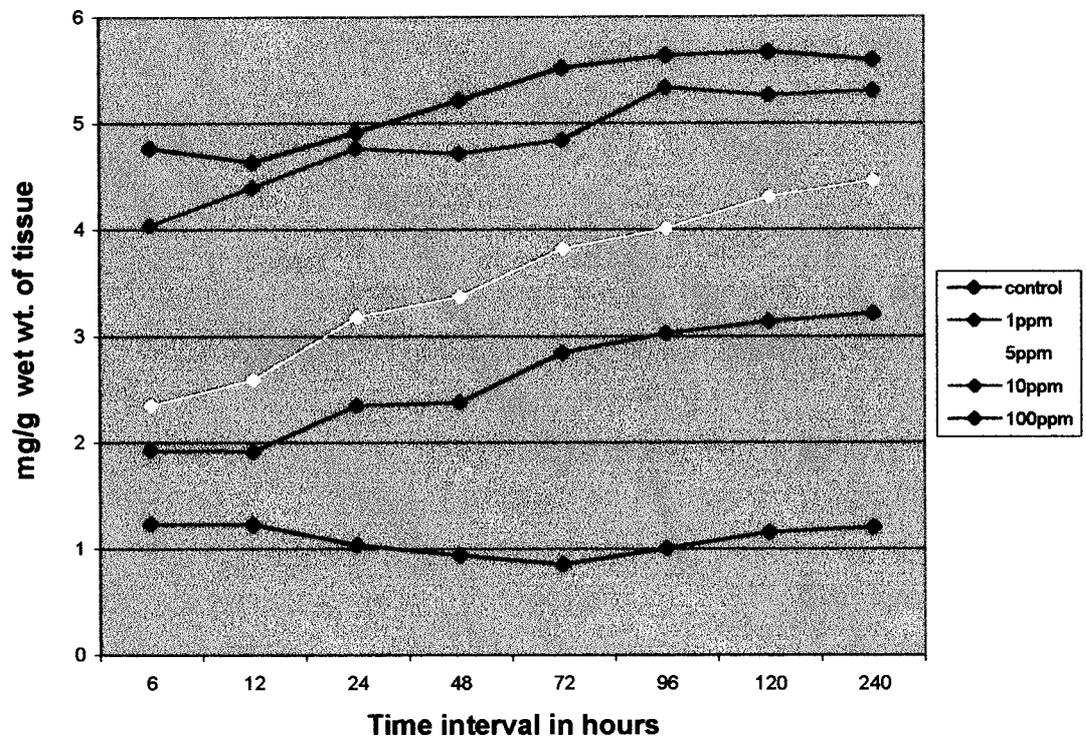
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.10 : Triglyceride content of spinal cord of mice exposed to DDVP



concentration was observed at the end of 96 hours. All the elevations in triglyceride concentrations were statistically significant.

100 ppm dose of DDVP promoted pronounced elevations in the triglyceride contents of the spinal cord at all the time intervals. The triglyceride contents were in the range of 4.63 ± 1.2 to 5.69 ± 1.3 mg / g wet wt of tissue. The elevations in the triglyceride concentrations were statistically significant at all the time intervals.

GLUCOSE

The glucose contents of spinal cord of mice decreased in response to DDVP treatment. The changes in the glucose concentrations are tabulated in table 3.11 and graphically presented in graph 3.11. The glucose contents of the spinal cord of control animals ranged between 6.94 ± 0.90 to 7.72 ± 0.82 mg / g wet wt of tissue.

1 ppm dose of DDVP induced fluctuations in the glucose content from the end of 6 hours to the end of 240 hours and the glucose contents were in the range of 2.27 ± 0.20 to 7.83 ± 0.63 mg / g wet wt of tissue. The maximum decline in glucose content was observed at the end of 240 hours while the glucose contents of the spinal cord elevated non-significantly at the end of 6 and 12 hours.

Under the influence of 5 ppm dose of DDVP, the glucose contents steadily declined from the end of 6 hours to the end of 240 hours except at the end of 12 hours. The glucose contents ranged between 2.63 ± 0.30 to 3.84 ± 0.70 mg / g wet wt of tissue. The decline was highly significant at all the exposure periods ($P < 0.001$). The maximum decline in the glucose concentration was observed at the end of 240 hours.

10 ppm dose of DDVP caused sharp reductions in the glucose contents of the spinal cord at all the time intervals. The glucose contents were in the range of 2.08 ± 0.47 to 3.25 ± 0.90 mg / g wet wt of tissue. The maximum decline in glucose content of spinal cord was observed at the end of 24 hours. All the changes in glucose concentrations of the spinal cord were statistically significant.

The mice subjected to 100 ppm dose of DDVP, showed a pronounced decline in glucose contents of the spinal cord at all the time intervals. The glucose contents of the spinal cord were in the range of 2.33 ± 0.32 to 2.58 ± 0.38 mg / g wet wt of tissue. The maximum decline in the glucose concentration was observed at the end of 48 hours. All the changes in glucose concentrations were highly significant ($P < 0.001$).

**TABLE 3.11: GLUCOSE CONTENT OF THE SPINAL CORD OF
MICE EXPOSED TO DDVP**

	6	12	24	48	72	96	120	240
Control	7.36 ±0.90	6.94 ±0.90	7.72 ±0.82	7.60 ±0.54	7.37 ±0.42	7.00 ±0.50	7.42 ±0.73	7.51 ±0.63
1ppm	7.83 ±0.63 ns	7.78 ±0.67 ns	6.19 ±0.99 c	5.42 ±0.80 c	6.23 ±0.65 b	4.51 ±0.90 c	5.21 ±0.55 a	2.27 ±0.20 a
5ppm	3.84 ±0.70 a	3.52 ±0.64 a	3.74 ±0.66 a	3.58 ±0.73 a	3.39 ±0.40 a	3.36 ±0.40 a	3.03 ±0.90 a	2.63 ±0.30 a
10ppm	3.25 ±0.90 a	2.85 ±0.68 a	2.08 ±0.47 a	2.34 ±0.69 a	2.79 ±0.53 a	3.19 ±0.71 a	2.96 ±0.70 a	2.35 ±0.82 a
100ppm	2.58 ±0.52 a	2.58 ±0.38 a	2.34 ±0.40 a	2.33 ±0.32 a	2.48 ±0.30 a	2.39 ±0.21 a	2.54 ±0.30 a	2.34 ±0.19 a
mg / g wet wt. of tissue								

Values represent mean ± S.D.

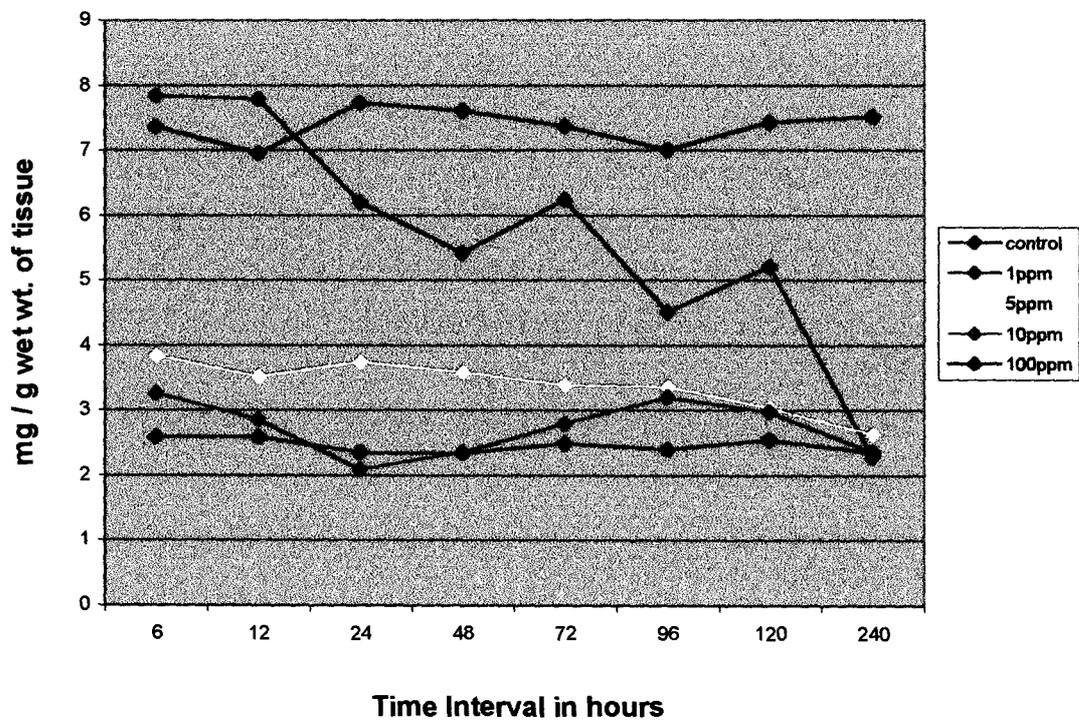
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.11 : Glucose content of spinal cord of mice exposed to DDVP



GLYCOGEN

DDVP induced a dose dependent decline in the glycogen contents of the spinal cord at all the time intervals. The changes in glycogen contents are tabulated in table 3.12 and presented in graph 3.12. The control animals revealed glycogen content of the spinal cord in the range of 6.39 ± 0.60 to 6.81 ± 0.90 mg / g wet wt of tissue.

1 ppm dose of DDVP decreased the glycogen content from the end of 6 hours to 240 hours except at the end of 12 hours. The glycogen contents were in the range of 2.43 ± 0.25 to 6.58 ± 1.02 mg / g wet wt of tissue. At the end of 12 hours the glycogen contents of spinal cord were higher than the control level. The maximum decline in glycogen contents was seen at the end of 240 hours and this decline was quite pronounced. All the changes in glycogen contents of the spinal cord were significant except at the end of 6 and 12 hours.

5 ppm dose of DDVP induced fluctuating reductions in the glycogen contents of the spinal cord from the end of 6 hours to the end of 48 hours but subsequently the glycogen contents reduced gradually upto the end of 240 hours. The glycogen contents were in the range of 2.29 ± 0.43 to 4.37 ± 0.52 mg / g wet wt of tissue. The minimum decline in glycogen was observed at the end of 48 hours while the maximum decrease was observed at the end of 240 hours. All the changes in glycogen concentrations were statistically significant.

In response to 10 ppm dose of DDVP the glycogen contents of the spinal cord reduced at all the time intervals. The glycogen contents were in the range of 2.21 ± 0.30 to 3.94 ± 0.75 mg / g wet wt of tissue. The maximum decline in glycogen contents was observed at the end of 240 hours and this decline was very sharp. The reductions in glycogen contents were statistically significant at all time intervals.

100 ppm dose of DDVP induced decrement in the glycogen content, from the end of 6 hours to the end of 240 hours. The glycogen contents were in the range of 1.75 ± 0.22 to 3.82 ± 0.63 mg / g wet wt of tissue. The maximum decline in glycogen contents was found at the end of 240 hours. All the reductions in glycogen contents were statistically significant ($P < 0.001$).

**TABLE 3.12: GLYCOGEN CONTENT OF THE SPINAL CORD OF
MICE EXPOSED TO DDVP**

	6	12	24	48	72	96	120	240
Control	6.48 ±0.70	6.39 ±0.60	6.52 ±1.10	6.81 ±0.90	6.47 ±1.20	6.67 ±0.90	6.53 ±0.75	6.50 ±0.70
1ppm	6.20 ±0.68 ns	6.58 ±1.02 ns	5.86 ±0.60 c	5.50 ±0.64 b	5.71 ±0.40 c	4.82 ±0.90 b	4.53 ±0.70 b	2.43 ±0.25 a
5ppm	4.07 ±0.50 a	4.22 ±0.52 a	4.00 ±0.40 a	4.37 ±0.52 a	4.18 ±0.48 a	4.06 ±1.20 a	3.85 ±0.80 a	2.29 ±0.43 a
10ppm	3.92 ±0.63 a	3.94 ±0.75 a	3.82 ±0.20 a	3.89 ±0.34 a	3.86 ±0.42 a	3.74 ±0.60 a	2.52 ±0.37 a	2.21 ±0.30 a
100ppm	3.82 ±0.63 a	3.86 ±0.40 a	3.79 ±0.50 a	3.73 ±0.90 a	3.71 ±0.67 a	3.06 ±0.70 a	2.20 ±0.6 a	1.75 ±0.22 a
mg / g wet wt. of tissue								

Values represent mean ± S.D.

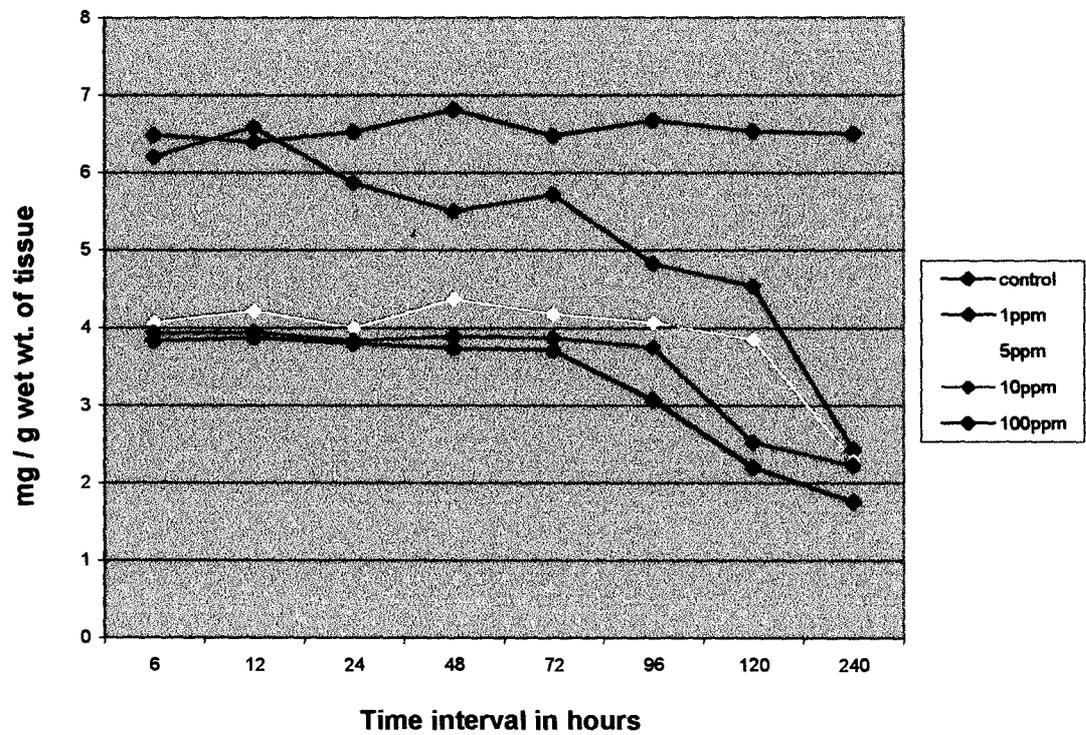
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.12 : Glycogen content of spinal cord of mice exposed to DDVP



BIOCHEMICAL CHANGES OF SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS :

Monocrotophos induced alterations in the enzyme activities as well as in the biochemical constituents of the spinal cord to varying degrees.

ACID PHOSPHATASE (AP)

The AP activities increased in response to monocrotophos treatment in a dose and time progression manner barring a few exceptions. The changes in AP activities are tabulated in table 3.13 and graphically presented in graph 3.13. The AP activity in control animals was in the range of 27.88 ± 4.72 to 30.08 ± 7.20 μ mols / g wet wt of tissue.

Mice treated with 1 ppm dose of monocrotophos, exhibited a slight decline in AP activity as compared to the corresponding control at the end of 6 hours. The AP activity elevated steadily from the end of 12 hours to the end of 240 hours and was in the range of 34.84 ± 4.21 to 44.85 ± 8.0 μ mols / g wet wt of tissue. The maximum elevation in the AP activity was observed at the end of 240 hours. All the increases were statistically significant.

Under the influence of 5 ppm dose of monocrotophos, the AP activity steadily increased from the end of 6 hours to the end of 240 hours except for a relatively marginal decline with respect to the preceding level of activity at

the end of 96 hours. The AP activity was in the range of 38.07 ± 6.10 to 114.54 ± 10.88 μ mols / g wet wt of tissue. The maximum AP activity was observed at the end of 240 hours. All the increases in AP activity were statistically significant except at the end of 6 hours.

10 ppm dose of monocrotophos induced elevations in AP activity at all the time intervals and the activity increased steadily from the end of 6 hours to the end of 240 hours except at the end of 72 hours where it declined a little with respect to that found in preceding time interval. The AP activity ranged between 57.82 ± 10.38 to 167.26 ± 15.70 μ mols/ g wet wt of tissue. The maximum elevation in the ACP activity was observed at the end of 240 hours. The elevations of AP activities were statistically significant at all the time intervals.

Mice subjected to 100 ppm dose of monocrotophos showed a sharp increase in the AP activity at all the time intervals. The AP activity revealed a steady increase from the end of 6 hours to the end of 240 hours except for a marginal decline at the end of 48 hours in comparison to that observed at the end of 24 hours. The AP activity ranged between 101.29 ± 10.60 to 429.59 ± 25.70 μ mols / g wet wt of tissue. The highest AP activity was observed at the end of 240 hours. All the elevations of AP activity were statistically significant ($P < 0.001$).

Graph 3.13 : Acid phosphatase activity of spinal cord of mice exposed to monocrotophos

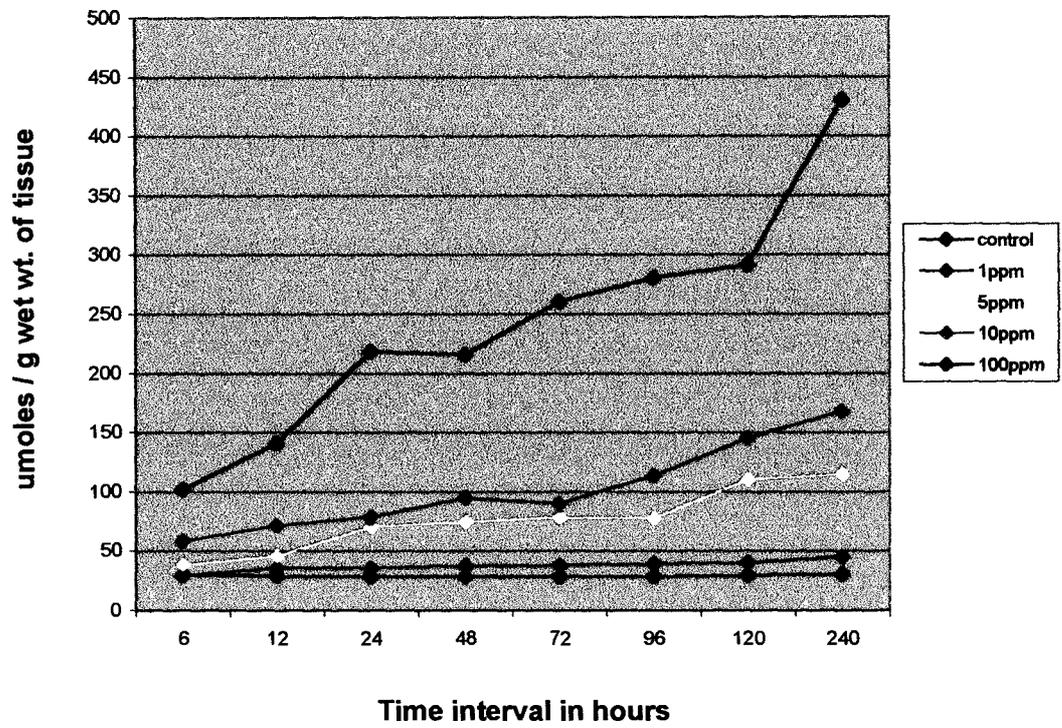


TABLE 3.13: ACID PHOSPHATASE ACTIVITY OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	29.73 ±5.78	28.72 ±6.30	27.89 ±5.01	28.28 ±4.52	28.02 ±5.10	27.88 ±4.72	29.12 ±4.54	30.08 ±7.20
1ppm	28.84 ±3.20 ns	34.84 ±4.21 c	35.18 ±6.58 c	37.12 ±5.54 c	37.53 ±4.72 c	39.05 ±5.48 c	39.83 ±6.05 c	44.85 ±8.00 b
5ppm	38.07 ±6.10 ns	45.82 ±10.30 b	70.65 ±9.50 a	74.38 ±9.30 a	78.38 ±7.96 a	77.97 ±8.40 a	110.40 ±9.40 a	114.54 ±10.88 a
10ppm	57.82 ±10.38 b	70.66 ±11.50 a	78.34 ±6.40 a	94.46 ±4.10 a	89.77 ±7.30 a	112.75 ±12.40 a	144.71 ±9.07 a	167.26 ±15.70 a
100ppm	101.29 ±10.60 a	140.21 ±14.40 a	218.32 ±11.8 a	215.60 ±7.40 a	260.40 ±17.40 a	280.42 ±22.09 a	290.78 ±21.60 a	349.59 ±32.70 a
µmols / g wet wt. of tissue								

Values represent mean ± S.D.

ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

ALKALINE PHOSPHATASE (ALP)

The ALP activity of the spinal cord of mice exposed to various doses of monocrotophos for different time intervals declined as a function of dose. The alterations of ALP activities are tabulated in table 3.14 and graphically presented in graph 3.14. The ALP activities in the control animals were in the range of 1.42 ± 0.28 to 1.91 ± 0.40 μ mols / g wet wt of tissue. The highest decline of ALP activity was induced by 100 ppm dose at the end of 240 hours and no other dose of monocrotophos could induce such a decline at any of the time intervals.

1 ppm dose of monocrotophos induced decline in the ALP activities at all the time intervals. The ALP activity showed a steady decline from the end of 6 hours to the end of 96 hours and subsequently fluctuated. The ALP activity was in the range of 0.23 ± 0.04 to 0.50 ± 0.01 μ mols / g wet wt of tissue. The maximum reduction of ALP activity was observed at the end of 96 hours while the minimum decline was observed at the end of 6 hours. The declines of ALP activities were statistically significant at all the time intervals.

Mice exposed to 5 ppm dose of monocrotophos revealed decline in ALP activity at all the time intervals. A steady decline of ALP activity was observed at the end of 6 hours to the end of 12 hours and showed no further change upto the end of 24 hours but once again the activity continued to decrease gradually upto the end of 240 hours. The ALP activities were in the range of 0.14 ± 0.02 to 0.23 ± 0.07 μ mols / g wet wt of tissue. The maximum

decrease of ALP activity was observed at the end of 240 hours while the minimum decline was noted at the end of 6 hours. the decreases were statistically significant at all the time intervals.

Under the influence of 10 ppm dose of monocrotophos, the ALP activities were declined and remained almost steady at a level of about 0.14 μ mols / g wet wt of tissue at the end of 6, 24 and 48 hours and subsequently declined upto the end of 240 hours. At the end of 12 hours the ALP activity was slightly elevated as compared to that found at the preceding time intervals. The ALP activities were in the range of 0.08 \pm 0.03 to 0.15 \pm 0.02 μ mols / g wet wt of tissue. The maximum decline of ALP activity was observed at the end of 240 hours while the minimum decline was observed at the end of 12 hours. All the reductions of ALP activities were statistically significant (P < 0.001).

The treatment of mice with 100 ppm dose of monocrotophos resulted in a precipitous decline of the ALP activity and the decline was gradual from the end of 6 hours to the end of 240 hours. The ALP activities were in the range of 0.04 \pm 0.03 to 0.13 \pm 0.02 μ mols / g wet wt of tissue. The maximum decline in the ALP activity was observed at the end of 240 hours. All the reductions of ALP activities were highly significant (P < 0.001).

TABLE 3.14: ALKALINE PHOSPHATASE ACTIVITY OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	1.54 ±0.20	1.42 ±0.28	1.65 ±0.52	1.74 ±0.43	1.68 ±0.31	1.70 ±0.24	1.84 ±0.30	1.91 ±0.40
1ppm	0.50 ±0.01 b	0.44 ±0.03 a	0.36 ±0.03 a	0.35 ±0.03 a	0.34 ±0.02 a	0.23 ±0.04 a	0.27 ±0.02 a	0.25 ±0.06 a
5ppm	0.23 ±0.07 a	0.20 ±0.06 a	0.20 ±0.04 a	0.18 ±0.05 a	0.17 ±0.03 a	0.16 ±0.01 a	0.15 ±0.02 a	0.14 ±0.02 a
10ppm	0.14 ±0.01 a	0.15 ±0.02 a	0.14 ±0.02 a	0.14 ±0.03 a	0.13 ±0.02 a	0.11 ±0.04 a	0.09 ±0.03 a	0.08 ±0.03 a
100ppm	0.13 ±0.02 a	0.12 ±0.01 a	0.11 ±0.01 a	0.10 ±0.02 a	0.08 ±0.02 a	0.08 ±0.03 a	0.05 ±0.30 a	0.04 ±0.03 a
µmols / g wet wt. of tissue								

Values represent mean ± S.D.

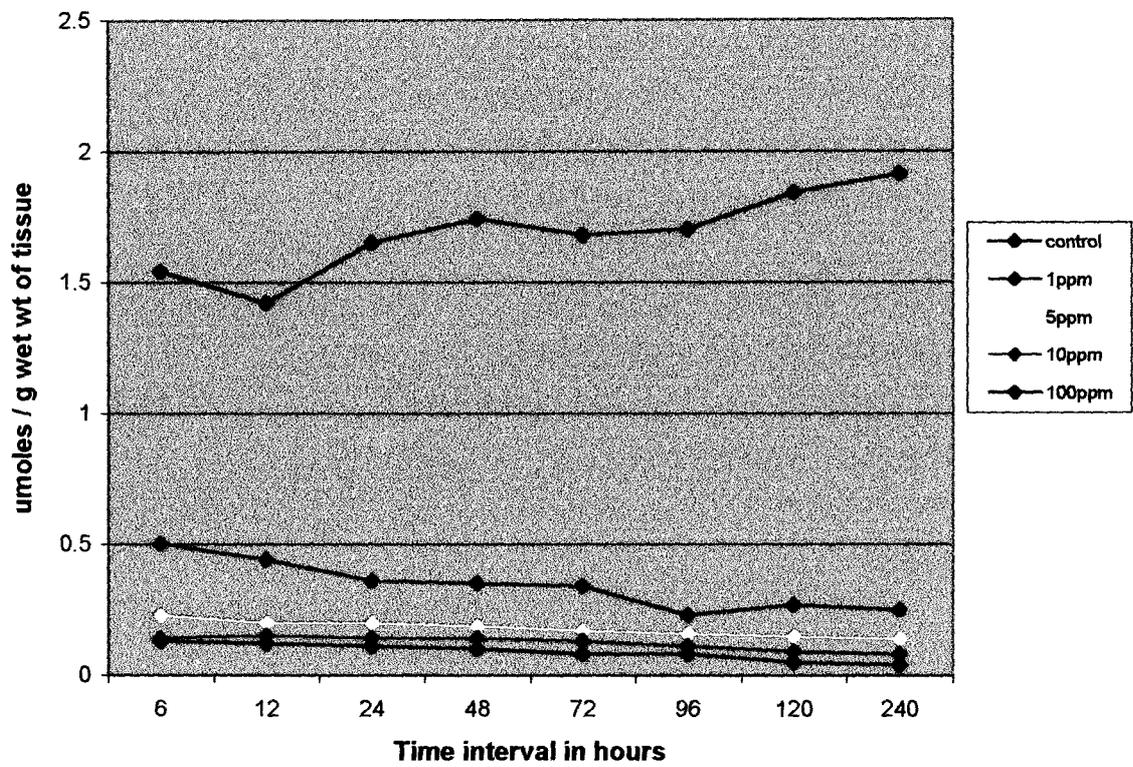
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.14 : Alkaline phosphatase activity of spinal cord of mice exposed to monocrotophos



NON SPECIFIC ESTERASES (NSE)

Monocrotophos induced a nearly dose dependent elevations in the NSE activities at all the time intervals except at the end of 48 hours. The changes of the NSE activities are compiled in table 3.15 and graphically presented in graph 3.15. The control animals exhibited NSE activity in the range of 3.48 ± 0.40 to 3.85 ± 0.30 μ mols / g wet wt of tissue.

Under the influence of 1 ppm dose of monocrotophos the NSE activities were elevated at all the time intervals. However, the elevations NSE activities fluctuated from the end of 6 hours to the end of 240 hours. The NSE activity was in the range of 4.70 ± 0.37 to 5.69 ± 0.50 μ mols / g wet wt of tissue. The maximum hike in the NSE activity was observed at the end of 120 hours while the minimum hike was observed at the end of 12 hours. All the declines in the NSE activities were statistically significant.

The treatment of mice with 5 ppm dose of monocrotophos led to elevation of NSE activity at all the time intervals and showed a steady increase from the end of 6 hours to the end of 240 hours. The NSE activity was in the range of 5.26 ± 0.64 to 7.42 ± 1.30 μ mols / g wet wt of tissue. The maximum hike of NSE activity was observed at the end of 240 hours. The elevations of NSE activities were statistically significant at all the time intervals ($P < 0.001$).

Graph 3.15 : Non-specific esterase activity of spinal cord of mice exposed to monocrotophos

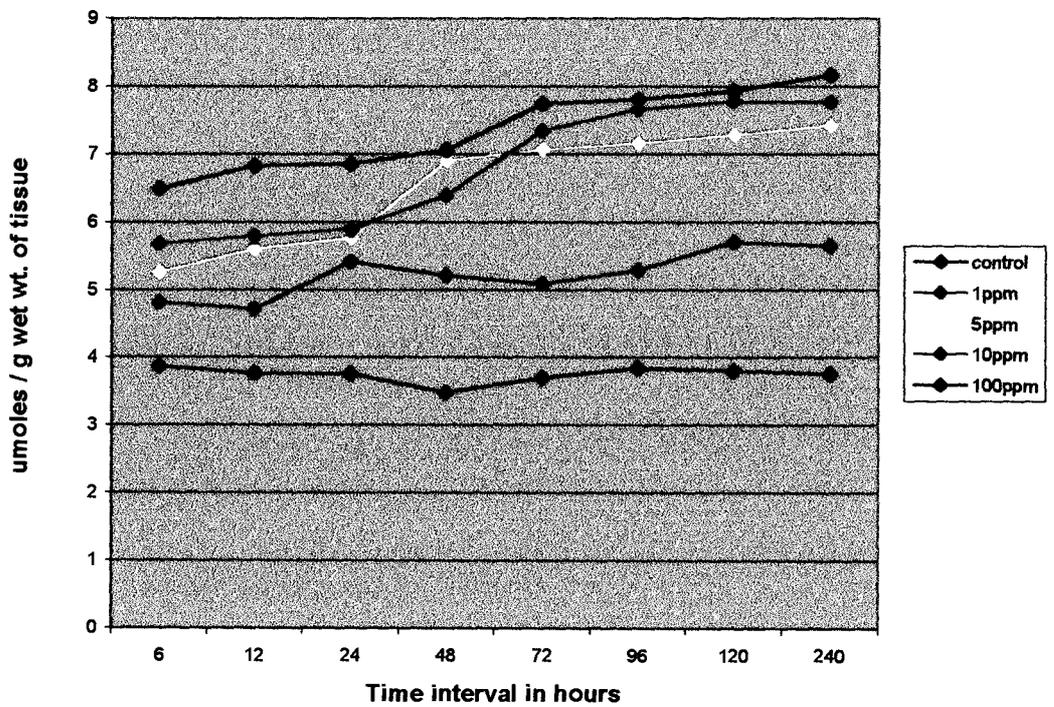


TABLE 3.15: NON SPECIFIC ESTERASES ACTIVITY OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	3.85 ±0.30	3.76 ±0.40	3.75 ±0.30	3.48 ±0.40	3.69 ±0.50	3.83 ±0.40	3.80 ±0.50	3.75 ±0.40
1ppm	4.80 ±0.35 b	4.70 ±0.37 b	5.40 ±0.40 a	5.20 ±0.27 a	5.08 ±0.50 a	5.28 ±0.50 a	5.69 ±0.50 a	5.64 ±0.70 a
5ppm	5.26 ±0.64 a	5.60 ±0.48 a	5.76 ±0.80 a	6.90 ±0.67 a	7.06 ±0.50 a	7.16 ±0.81 a	7.28 ±0.85 a	7.42 ±1.30 a
10ppm	5.67 ±0.50 a	5.78 ±0.70 a	5.88 ±0.92 a	6.38 ±0.79 a	7.34 ±1.14 a	7.65 ±0.85 a	7.77 ±1.20 a	7.86 ±0.68 a
100ppm	6.48 ±0.90 a	6.82 ±1.02 b	6.85 ±0.57 a	7.05 ±0.78 a	7.73 ±0.90 a	7.80 ±1.22 a	7.92 ±.87 a	8.16 ±1.24 a
µmols / g wet wt. of tissue								

Values represent mean ± S.D.

ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

10 ppm dose of monocrotophos induced a steady elevation of NSE activity from the end of 6 hours to the end of 240 hours. The NSE activity was in the range of 5.67 ± 0.50 to 7.85 ± 0.68 μ mols / g wet wt of tissue. The maximum elevation of the NSE activity was observed at the end of 240 hours. All the increments of the NSE activities were statistically significant ($P < 0.001$).

Mice administered 100 ppm dose of monocrotophos exhibited a steady rise of the NSE activity from the end of 6 hours to the end of 240 hours. The NSE activities were in the range of 6.48 ± 0.90 to 8.16 ± 1.24 μ mols / g wet wt of tissue. The maximum hike of the NSE activity was observed at the end of 240 hours. All the elevations of NSE activities were statistically significant.

XANTHINE OXIDASE (XOD)

A pronounced elevation of the XOD activity was observed in response to monocrotophos treatment. The changes of the XOD activities are compiled in table 3.16 and presented graphically in graph 3.16. The XOD activity in the control animals ranged between 5.77 ± 1.20 to 7.15 ± 0.80 n mols / mg of protein. The increases in XOD activity were nearly dose dependent.

In response to 1ppm dose of monocrotophos the XOD activities were elevated at all the time intervals, however they showed fluctuations. The XOD activity was in the range of 15.35 ± 2.40 to 24.56 ± 2.80 n mols / mg of

protein. The maximum XOD activity was observed at the end of 72 hours while a relatively minimum rise in XOD activity was observed at the end of 6 hours. The changes in the XOD activity were statistically significant at all the time intervals.

5 ppm dose of monocrotophos induced a steady elevation in the XOD activity from the end of 6 hours to the end of 72 hours and subsequently XOD activity continued to decline upto the end of 240 hours in comparison to that observed at the end of 72 hours. The XOD activity was in the range of 18.47 ± 2.50 to 27.90 ± 3.60 n mols / mg of protein. A relatively less increase in the XOD activity was observed at the end of 6 hours while the minimum elevation was noted at the end of 72 hours. The increases in XOD activity were statistically significant at all the time intervals.

Under the influence of 10 ppm dose of monocrotophos, the XOD activities were elevated at all the time intervals and showed a steady increase from the end of 6 hours to 48 hours and then fluctuated further. The XOD activity was in the range of 20.28 ± 2.50 to 34.45 ± 4.00 n mols / mg of protein. The maximum elevation in XOD activity was observed at the end of 240 hours while the relatively minimum elevations of XOD activity was observed at the end of 6 hours. All the increases of XOD activity were statistically significant.

The treatment of mice with 100 ppm dose of monocrotophos resulted in a sharp increase in the XOD activity and showed an time progression

**TABLE 3.16: XANTHINE OXIDASE ACTIVITY OF THE SPINAL CORD
OF MICE EXPOSED TO MONOCROTOPHOS**

	6	12	24	48	72	96	120	240
control	7.00 ±1.40	6.43 ±0.90	6.24 ±0.90	5.77 ±1.20	6.54 ±0.80	6.54 ±1.10	7.15 ±0.80	6.25 ±0.90
1ppm	15.35 ±2.40 c	17.45 ±1.80 c	21.34 ±3.30 b	20.82 ±2.70 b	24.56 ±2.80 b	20.70 ±1.90 b	19.30 ±1.70 c	17.80 ±0.90 c
5ppm	18.47 ±2.50 b	19.71 ±3.00 b	24.78 ±4.10 b	26.47 ±3.80 b	27.90 ±3.60 b	27.64 ±3.30 b	26.52 ±4.70 b	25.08 ±2.00 a
10ppm	20.28 ±2.50 b	21.54 ±2.80 c	28.07 ±3.20 b	31.83 ±4.20 c	30.82 ±3.50 b	32.42 ±3.70 b	31.88 ±2.90 a	34.45 ±4.00 a
100ppm	38.35 ±4.60 b	41.41 ±4.30 b	54.36 ±4.00 a	65.84 ±5.80 a	64.73 ±4.40 a	66.80 ±6.00 a	55.60 ±5.80 a	58.80 ±5.40 a
n mols /mg of protein								

Values represent mean ± S.D.

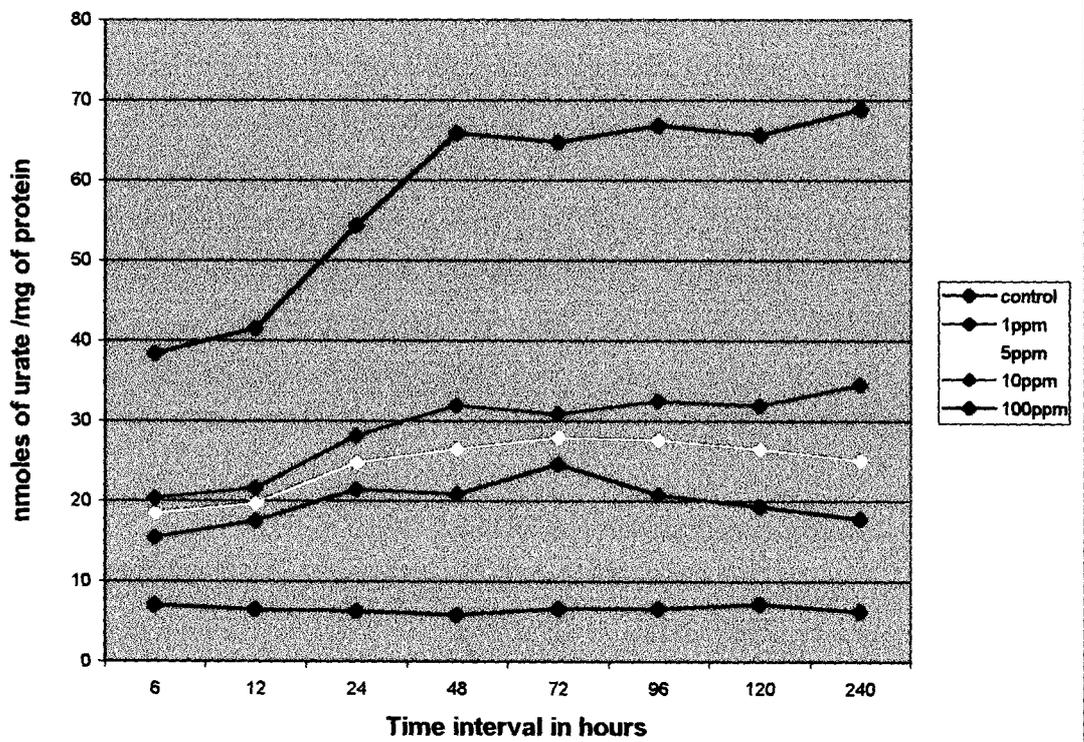
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.16 : Xanthine oxidase activity of spinal cord of mice exposed to monocrotophos



dependent elevation from the end of 6 hours to the end of 48 hours and it further fluctuated till the end of 240 hours. The XOD activity was in the range of 38.35 ± 4.60 to 68.80 ± 5.40 n mols / mg of protein. The maximum hike in XOD activity was observed at the end of 240 hours while relatively minimum rise of XOD activity was noted at the end of 6 hours. The increases in XOD activity were statistically significant at all the time intervals.

SUPEROXIDE DISMUTASE (SOD)

Monocrotophos induced a dose dependent and nearly exposure period dependent elevations in the SOD activities. The alterations of SOD activities are tabulated in table 3.17 and graphically presented on graph 3.17. The SOD activity in the control animals ranged between 32.78 ± 2.05 to 34.56 ± 1.82 units / mg of protein.

Mice treated with a dose of 1 ppm of monocrotophos showed elevations in the SOD activity at all the time intervals and the activity steadily elevated from the end of 6 hours to the end of 120 hours with a negligible decline at the end of 240 hours. The SOD activity was in the range of 36.85 ± 2.02 to 42.73 ± 3.21 units / mg of protein. The maximum hike in SOD activity was observed at the end of 120 hours while the relatively minimum hike was noted at the end of 6 hours. The increases in SOD activity at the end of 6, 12 and 240 hours were non-significant while it was statistically significant ($P < 0.005$) at the rest of the time intervals.

5 ppm dose of monocrotophos elevated the SOD activity at all the time intervals and it gradually increased from the end of 6 and 120 hours. At the end of 240 hours the SOD activity declined slightly as compared to that observed for the preceding time interval. The SOD activity was in the range of 40.37 ± 2.18 to 49.52 ± 4.58 units / mg of protein. The maximum increase of the SOD activity was observed at the end of 120 hours. The increases in SOD activity were statistically significant.

In response to 10 ppm dose of monocrotophos the SOD activity steadily elevated from the end of 240 hours. The SOD activities were in the range of 46.54 ± 2.76 to 61.17 ± 5.21 units / mg of protein. The maximum SOD activity was observed at the end of 240 hours. The changes in the SOD activities were statistically significant at all the time intervals.

100 ppm dose of monocrotophos induced steady elevations in the SOD activities from the end of 6 hours to the end of 240 hours. The SOD activity was in the range of 59.34 ± 2.53 to 74.15 ± 7.87 units / mg of protein. The maximum SOD activity was observed at the end of 240 hours. All the changes in SOD activities were statistically significant ($P < 0.001$).

TABLE 3.17: SUPEROXIDE DISMUTASE ACTIVITY OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	33.53 ±2.72	34.05 ±2.25	34.56 ±1.82	34.44 ±1.75	33.65 ±2.43	32.95 ±1.88	33.75 ±1.54	32.78 ±2.05
1ppm	36.85 ±2.02 ns	37.25 ±1.87 ns	39.41 ±2.25 c	39.88 ±2.32 c	41.08 ±3.34 c	41.85 ±3.67 c	42.73 ±3.21 c	41.25 ±5.01 ns
5ppm	40.37 ±2.18 c	42.51 ±2.11 c	45.33 ±4.25 c	46.17 ±4.52 b	48.20 ±4.12 b	48.85 ±4.04 b	49.52 ±4.58 b	47.67 ±5.34 c
10ppm	46.54 ±2.76 b	49.31 ±4.65 b	50.78 ±3.10 b	53.41 ±3.87 b	55.17 ±3.87 a	57.41 ±4.85 b	58.76 ±6.75 b	61.17 ±5.21 a
100ppm	59.34 ±2.53 a	64.85 ±4.57 a	66.95 ±3.87 a	68.36 ±4.26 a	68.79 ±3.98 a	71.22 ±6.74 a	72.58 ±7.54 a	74.15 ±7.87 a
units / mg of protein								

Values represent mean ± S.D.

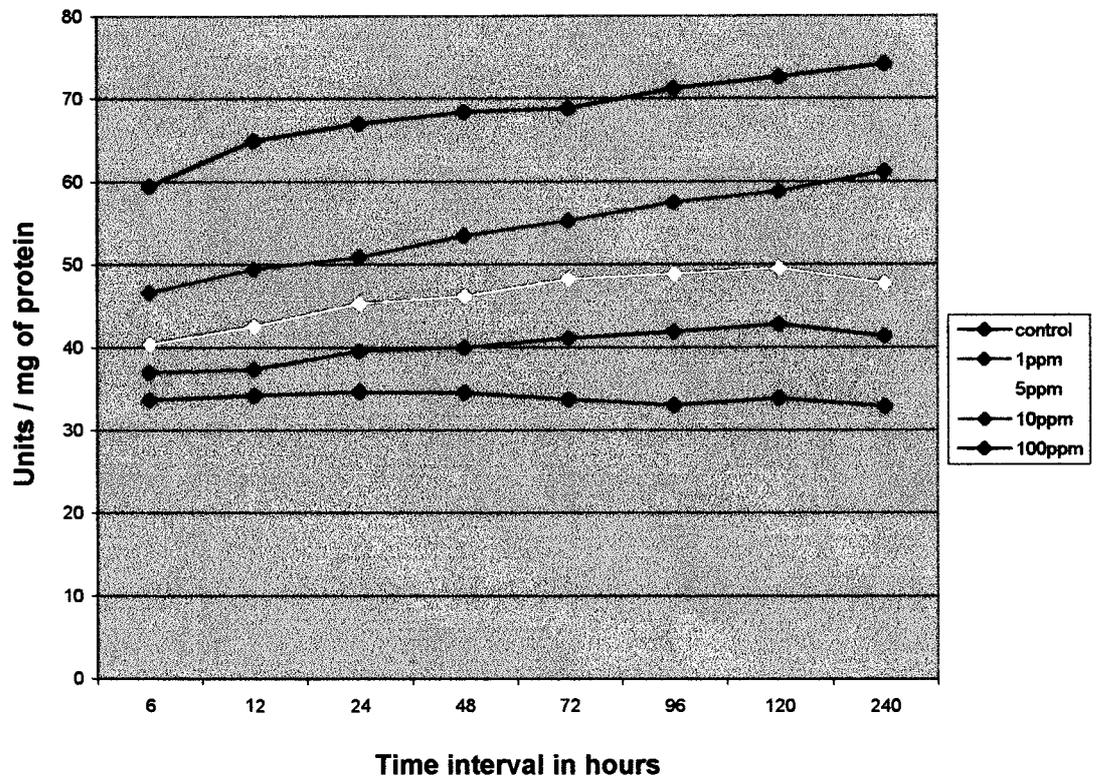
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.17 : Superoxide dismutase activity of spinal cord of mice exposed to monocrotophos



TOTAL PROTEINS

The total protein concentrations were declined at all the time intervals under the influence of monocrotophos and the decline was in a dose dependent manner. The changes in the total protein contents are tabulated in table 3.18 and presented graphically in graph 3.18. The control animals exhibited protein concentrations in the range of 0.225 ± 0.07 to 0.233 ± 0.07 g / g wet wt of tissue.

Under the influence of 1 ppm dose of monocrotophos the total protein concentrations were declined at all the time intervals. The total protein concentrations declined almost gradually from the end of 6 hours to 96 hours with a slight recovery at the end of 120 and 240 hours. The protein concentrations were in the range of 0.140 ± 0.08 to 0.188 ± 0.07 g / g wet wt of tissue. The maximum reduction in total protein content was observed at the end of 96 hours. All the reductions in protein concentrations were statistically significant.

5 ppm dose of monocrotophos induced a steady decline in the total protein concentrations from the end of 6 hours to the end of 240 hours and they were in the range of 0.081 ± 0.007 to 0.126 ± 0.04 g / g wet wt of tissue. The maximum reduction in the total protein concentration was observed at the end of 240 hours.

Mice subjected to 10 ppm dose of monocrotophos showed a steady decline in the total protein concentration from the end of 6 hours to the end of 12 hours with no further decline at the end of 24 hours. But it subsequently continued to decline till the end of 240 hours, except for a slight elevation at the end of 72 hours as compared to that observed at the preceding time intervals. The protein contents were in the range of 0.072 ± 0.003 to 0.095 ± 0.004 g / g wet wt of tissue. The maximum decline in the total protein content was observed at the end of 240 hours. The reductions in the total protein concentrations were statistically significant at all the time intervals ($P < 0.001$).

Under the influence of 100 ppm dose of monocrotophos, the total protein content gradually declined from the end of 6 hours to the end of 240 hours except for a negligible elevations at the end of 48 hours as compared to that observed at the preceding time intervals. The protein contents were in the range of 0.057 ± 0.007 to 0.086 ± 0.003 g / g wet wt of tissue. The maximum decline in protein concentrations was observed at the end of 240 hours and no other dose of monocrotophos could decline the protein content of spinal cord to this extent at any of the time intervals studied. The reductions in total protein contents were statistically significant at all time intervals ($P < 0.001$).

**TABLE 3.18: TOTAL PROTEIN CONTENT OF THE SPINAL CORD OF
MICE EXPOSED TO MONOCROTOPHOS**

	6	12	24	48	72	96	120	240
Control	0.228 ±0.08	0.229 ±0.070	0.225 ±0.07	0.232 ±0.08	0.230 ±0.07	0.227 ±0.05	0.233 ±0.07	0.227 ±0.06
1ppm	0.188 ±0.07 c	0.182 ± 0.07 c	0.160 ±0.05 c	0.161 ±0.05 c	0.141 ±0.07 b	0.140 ±0.08 b	0.152 ±0.07 b	0.157 ±0.08 c
5ppm	0.126 ±0.04 a	0.120 ±0.08 a	0.116 ±0.04 a	0.103 ±0.03 a	0.092 ±0.007 a	0.084 ±0.07 a	0.083 ±0.008 a	0.081 ±0.007 a
10ppm	0.095 ±0.004 a	0.087 ±0.005 a	0.087 ±0.005 a	0.081 ±0.004 a	0.083 ±0.004 a	0.080 ±0.003 a	0.075 ±0.003 a	0.072 ±0.003 a
100ppm	0.086 ±0.003 a	0.080 ±0.009 a	0.077 ±0.008 a	0.078 ±0.012 a	0.071 ±0.008 a	0.065 ±0.01 a	0.064 ±0.005 a	0.057 ±0.007 a
g / g wet wt. of tissue								

Values represent mean ± S.D.

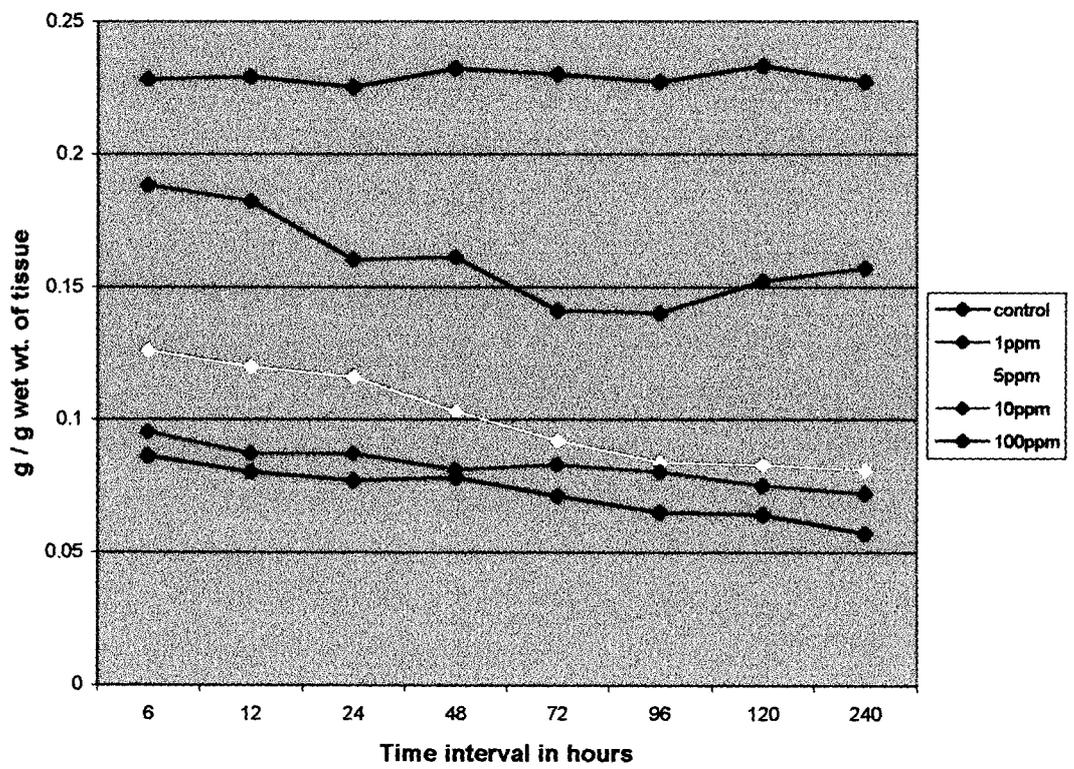
ns= not significant

a= P <0.001

b= P< 0.01

c = P< 0.05

Graph 3.18 : Total protein content of spinal cord of mice exposed to monocrotophos



MITOCHONDRIAL PROTEINS (m-proteins)

Monocrotophos induced decrement in the m-proteins at all time intervals, in nearly a dose dependent manner. The changes in the concentrations of m-proteins are tabulated in table 3.19 and graphically presented in graph 3.19. The mprotein contents in the control animals were in the range of 0.080 ± 0.029 to 0.087 ± 0.03 g / g of mitochondrial pellet.

Under the influence of 1ppm dose of monocrotophos the mprotein contents declined at all time intervals and an apparent steady decline was observed from the end of 6 hours to end of 72 hours, with no further decline till the end of 96 hours and showed a trend towards slight recovery at the end of 120 to 240 hours. However the changes from one time intervals to other though appear insignificant, they were infact significant with reference to the control for the respective time intervals. The m-protein concentrations were in the range of 0.072 ± 0.013 to 0.078 ± 0.004 g / g of mitochondrial pellet. The maximum decline in m-protein content was observed at the end of 72 hours and 96 hours while a minimum decline was observed at the end of 6 hours. All the reductions in m-protein were statistically significant.

Treatment of mice with 5 ppm dose of monocrotophos resulted in an apparent steady decline in m-protein concentrations from the end of 6 hours to the end of 48 hours but further the concentrations of m-protein elevated slightly compared to that observed at the end of 48 hours. The m-protein concentrations were in the range of 0.057 ± 0.006 to 0.050 ± 0.015 g / g of

mitochondrial pellet. The maximum reduction of m-protein contents was observed at the end of 48 hours. The reductions in m-protein contents were significant at all time intervals with reference to the respective controls but the m-protein levels did not vary significantly at any time intervals with reference to the level observed at the preceding time point.

10 ppm dose of monocrotophos induced a steady decline in the m-protein concentrations from the end of 6 hours to the end of 240 hours. The m-protein contents were in the range of 0.055 ± 0.006 to 0.040 ± 0.010 g / g of mitochondrial pellet. The maximum decline in mprotein concentrations was observed at the end of 240 hours. All the declines of m-proteins content were statistically significant ($P < 0.001$).

Under the influence of 100 ppm dose of monocrotophos the m-proteins concentrations declined at all the time intervals. A gradual decline in m-protein concentration was observed from the end of 6 hours to the end of 240 hours except for an insignificant increase at the end of 24 hours with reference to the concentration found at the end of 12 hours. The m-protein concentration were in the range of 0.047 ± 0.005 to 0.033 ± 0.008 g / g of wet wt. of tissue. The maximum decrease in m-protein concentration was observed at the end of 240 hours. The reductions in the m-protein concentration were statistically significant at all time intervals ($P < 0.001$).

TABLE 3.19: MITOCHONDRIAL PROTEIN CONTENT IN THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	0.086 ±0.032	0.084 ±0.027	0.083 ±0.033	0.086 ±0.027	0.085 ±0.028	0.087 ±0.030	0.081 ±0.031	0.080 ±0.029
1ppm	0.078 ±0.004 c	0.075 ±0.005 c	0.074 ±0.010 c	0.073 ±0.008 b	0.072 ±0.008 b	0.072 ±0.013 c	0.074 ±0.009 c	0.074 ±0.008 b
5ppm	0.057 ±0.006 a	0.055 ±0.008 a	0.054 ±0.005 b	0.050 ±0.015 b	0.051 ±0.010 b	0.053 ±0.006 b	0.053 ±0.012 a	0.054 ±0.007 a
10ppm	0.055 ±0.006 a	0.053 ±0.005 a	0.052 ±0.005 a	0.050 ±0.005 a	0.048 ±0.007 a	0.044 ±0.007 a	0.041 ±0.0071 a	0.040 ±0.010 a
100ppm	0.047 ±0.005 a	0.044 ±0.006 a	0.045 ±0.008 a	0.041 ±0.008 a	0.037 ±0.002 a	0.036 ±0.000 a	0.035 ±0.005 a	0.033 ±0.008 a
g / g of mitochondrial pellet								

Values represent mean ± S.D.

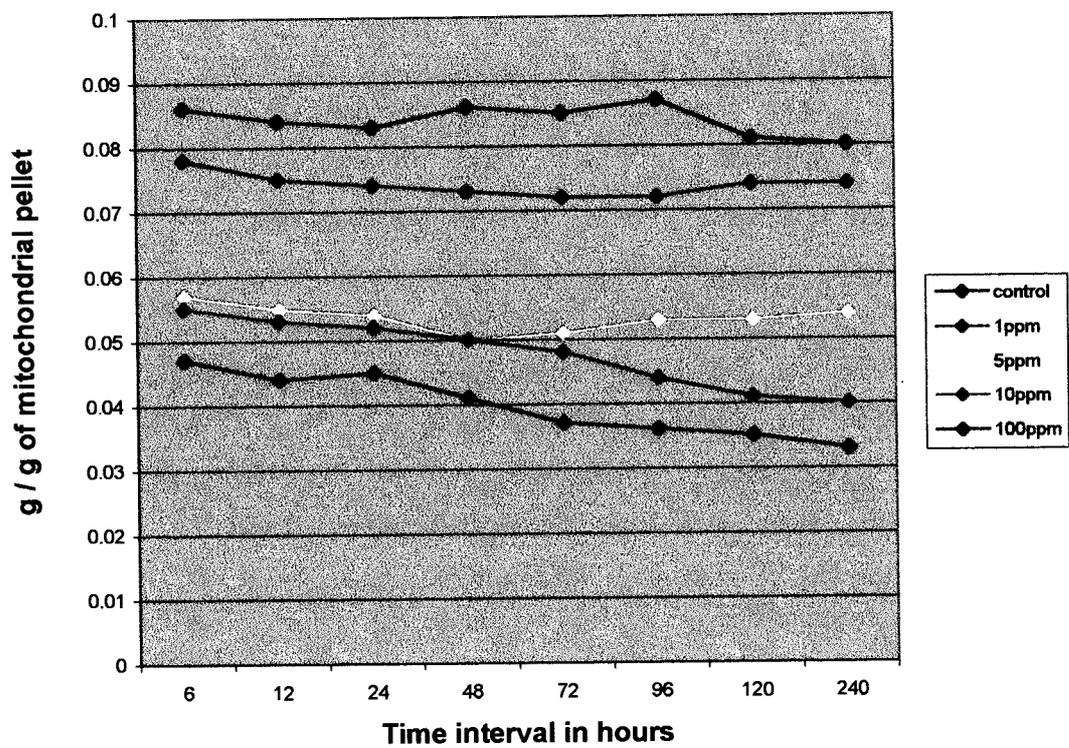
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.19 : Mitochondrial proteins of spinal cord of mice exposed to monocrotophos



CREATININE

Monocrotophos induced a dose-dependent increase in the creatinine content at all the time intervals except at all the end of 240 hours. The alterations in the creatinine levels are compiled in table 3.20 and presented in graph 3.20. The creatinine levels in the control animals ranged between 0.018 ± 0.004 to 0.026 ± 0.003 mg / g wet wt. of tissue.

1 ppm dose of monocrotophos failed to induce significant elevations in creatinine concentration at the end of 24 and 48 hours while it promoted a decline at the end of 240 hours. At the rest of the time intervals the creatinine levels increased significantly. The creatinine levels were in the range of 0.019 ± 0.006 to 0.028 ± 0.003 mg / g wet wt. of tissue. A relatively higher increase in the creatinine content was observed at the end of 96 and 120 hours.

Mice treated with 5 ppm dose of monocrotophos revealed a steady elevation in the creatinine level from the end of 6 hours to the end of 120 hours and a marginal decline at the end of 240 hours as compared to the creatinine level observed at the preceding time intervals. The creatinine levels ranged between 0.043 ± 0.006 to 0.079 ± 0.011 mg / g wet wt. of tissue. The maximum increase in the creatinine level was noted at the end of 120 hours. All the elevations in creatinine concentrations were statistically significant ($P < 0.001$).

TABLE 3.20: CREATININE CONTENT OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
control	0.018 ± 0.004	0.023 ± 0.005	0.026 ± 0.003	0.024 ± 0.005	0.019 ± 0.05	0.025 ± 0.007	0.023 ± 0.007	0.022 ± 0.005
1ppm	0.021 ± 0.12 b	0.026 ± 0.004 b	0.027 ± 0.002 ns	0.025 ± 0.000 ns	0.026 ± 0.006 b	0.028 ± 0.004 c	0.028 ± 0.003 b	0.019 ± 0.006 ns
5ppm	0.043 ± 0.006 a	0.046 ± 0.007 a	0.053 ± 0.005 a	0.057 ± 0.011 a	0.060 ± 0.008 a	0.074 ± 0.008 a	0.079 ± 0.011 a	0.076 ± 0.009 a
10ppm	0.065 ± 0.006 a	0.063 ± 0.009 a	0.071 ± 0.006 a	0.069 ± 0.003 a	0.086 ± 0.009 a	0.120 ± 0.017 a	0.140 ± 0.015 a	0.180 ± 0.008 a
100ppm	0.086 ± 0.009 a	0.088 ± 0.008 a	0.102 ± 0.009 a	0.108 ± 0.008 a	0.114 ± 0.010 a	0.123 ± 0.012 a	0.138 ± 0.014 a	0.158 ± 0.027 a
mg / g of wet wt. of tissue								

Values represent mean ± S.D.

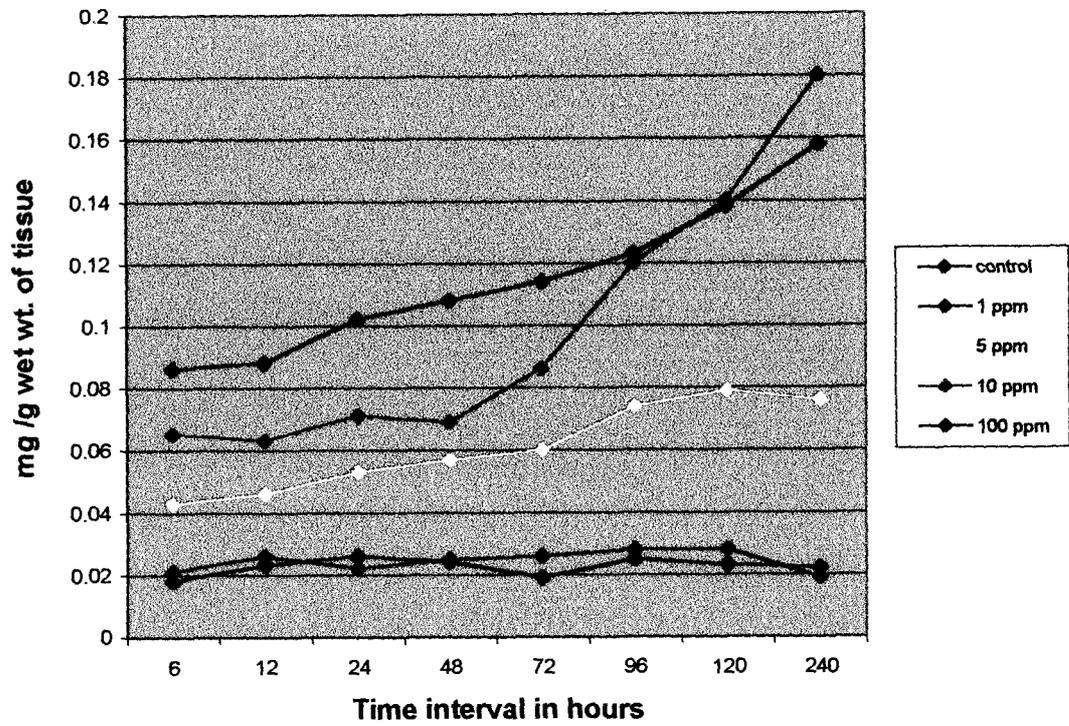
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.20: Creatinine content of spinal cord of mice exposed to monocrotophos



Under the influence of 10 ppm dose of monocrotophos the creatinine concentrations elevated at all the time intervals and ranged between 0.063 ± 0.009 to 0.18 ± 0.008 mg / g wet wt. of tissue. The maximum increase in the creatinine concentration was observed at the end of 240 hours. The elevations in the creatinine concentrations were statistically significant at all the time intervals ($P < 0.001$).

100 ppm dose of monocrotophos promoted a steady increase in the creatinine concentrations from the end of 6 hours to the end of 240 hours and they were in the range of 0.086 ± 0.009 to 0.158 ± 0.027 mg / g wet wt. of tissue. The highest increase in the creatinine concentration was observed at the end of 240 hours. All the elevations in creatinine concentrations were statistically significant ($P < 0.001$).

CHOLESTEROL

The cholesterol content of the spinal cord of mice elevated at all the time intervals under the influence of monocrotophos. The cholesterol contents elevated in a nearly dose dependent manner at all time intervals except 6 and 12 hours. The alterations in the cholesterol contents are tabulated in table 3.21 and presented graphically in graph 3.21. The cholesterol contents in the control animals were in the range of 9.15 ± 1.80 to 10.13 ± 1.34 mg / g wet wt. of tissue.

1 ppm dose of monocrotophos elevated cholesterol concentrations of the spinal cord at all the time intervals, however, the elevations were not uniform. The cholesterol concentrations were in the range of 10.83 ± 1.34 to 14.87 ± 1.24 mg / g wet wt. of tissue. The highest increase in cholesterol content was observed at the end of 72 hours. The elevations in cholesterol content were statistically significant at all the time intervals except at the end of 6 and 12 hours.

5 ppm dose of monocrotophos induced a steady increment in the cholesterol content from the end of 6 hours to the end of 120 hours while it decreased a little at the end of 240 hours with reference to cholesterol level observed at the end of 120 hours. The cholesterol contents ranged between 12.82 ± 1.54 to 19.05 ± 1.54 mg / g wet wt. of tissue. The highest increase in the cholesterol content was observed at the end of 120 hours. The increases in cholesterol contents were statistically at all the time intervals.

Mice treated with 10 ppm dose of monocrotophos showed increases in cholesterol contents at all the time intervals. The cholesterol content elevated steadily from the end of 6 hours to the end of 120 hours and declined marginally at the end of 240 hours as compared to the cholesterol level found at the preceding exposure period. The cholesterol contents were in the range of 12.75 ± 1.80 to 19.63 ± 1.02 mg / g wet wt. of tissue. The maximum hike in cholesterol contents was observed at the end of 120 hours. All the changes in cholesterol contents were statistically significant.

TABLE 3.21: CHOLESTEROL CONTENT OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	9.89 ±1.26	9.98 ±1.66	9.16 ±1.70	9.36 ±1.37	10.13 ±1.34	9.79 ±1.29	9.15 ±1.80	9.63 ±1.20
1ppm	10.96 ±1.63 ns	10.83 ±1.34 ns	11.85 ±1.40 c	13.14 ±2.00 c	14.87 ±1.24 b	13.87 ±1.07 b	14.53 ±1.50 b	11.57 ±1.44 c
5ppm	12.82 ±1.54 c	14.41 ±1.91 b	14.87 ±1.65 b	17.15 ±1.71 a	17.77 ±1.40 a	18.35 ±1.36 a	19.05 ±1.54 b	17.94 ±2.50 a
10ppm	12.75 ±1.80 b	13.94 ±0.90 a	16.78 ±1.82 a	17.92 ±1.24 a	18.67 ±1.40 a	18.93 ±0.80 a	19.63 ±1.02 a	18.16 ±1.10 a
100ppm	18.15 ±1.04 a	20.12 ±1.54 a	19.78 ±1.03 a	22.48 ±2.04 a	25.29 ±1.84 a	24.62 ±1.39 a	26.37 ±2.21 a	22.63 ±1.42 a
mg /g of wet wt. of tissue								

Values represent mean ± S.D.

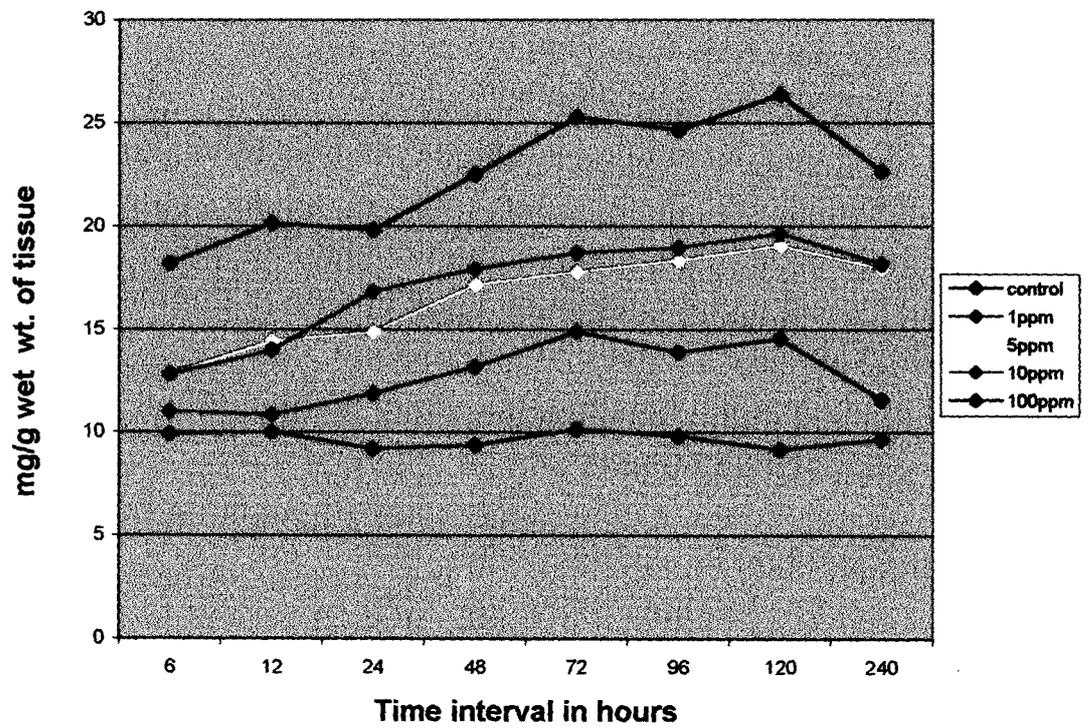
ns= not significant

a= P <0.001

b= P < 0.01

c = P < 0.05

Graph 3.21 : Cholesterol content of spinal cord of mice exposed to monocrotophos



Under the influence of 100 ppm dose of monocrotophos the cholesterol contents were significantly elevated at all the time intervals. The cholesterol contents were in the range of 18.15 ± 1.04 to 26.37 ± 2.21 mg / g wet wt. of tissue. The maximum increase in the cholesterol contents was observed at the end of 120 hours.

TRIGLYCERIDES

Monocrotophos promoted a dose dependent elevations in the triglyceride contents of the spinal cord at all the time intervals. The alterations of the triglyceride contents are tabulated in table 3.22 and graphically presented in graph 3.22. The control animals exhibited triglyceride contents in the range of 0.95 ± 0.08 to 1.2 ± 0.16 mg / g wet wt. of tissue.

Under the influence of 1 ppm dose of monocrotophos an time progression dependent elevation in the triglyceride content was noticed from the end of 6 hours to the end of 240 hours. The triglyceride contents were in the range of 1.64 ± 0.17 to 3.13 ± 0.29 mg / g wet wt. of tissue. The maximum increase in triglyceride content was observed at the end of 240 hours. The alterations of triglyceride content were statistically significant at all the time intervals except at all the end of 6 and 12 hours.

Mice administered with 5 ppm dose of monocrotophos showed a gradual elevation in the triglyceride contents from the end of 6 hours to the

TABLE 3.22: TRIGLYCERIDE CONTENT OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	1.17 ± 0.23	1.20 ± 0.16	0.96 ± 0.18	0.95 ± 0.08	1.04 ± 0.08	0.98 ± 0.07	1.12 ± 0.15	0.95 ± 0.07
1ppm	1.64 ± 0.17 ns	1.68 ± 0.21 ns	1.84 ± 0.15 b	2.05 ± 0.27 a	2.29 ± 0.18 a	2.56 ± 0.24 a	2.77 ± 0.32 a	3.13 ± 0.29 a
5ppm	2.14 ± 0.19 a	2.34 ± 0.26 a	2.72 ± 0.27 a	2.83 ± 0.31 a	2.85 ± 0.22 a	3.17 ± 0.25 a	3.43 ± 0.37 a	3.62 ± 0.41 a
10ppm	3.56 ± 0.37 a	3.74 ± 0.42 a	3.95 ± 0.38 a	4.21 ± 0.43 a	4.19 ± 0.33 a	4.47 ± 0.42 a	4.78 ± 0.63 a	5.13 ± 0.68 a
100ppm	4.85 ± 0.73 a	4.88 ± 0.65 a	5.09 ± 0.77 a	5.33 ± 0.80 a	5.58 ± 0.75 a	5.76 ± 0.64 a	5.87 ± 0.92 a	6.05 ± 0.87 a
mg /g of wet wt. of tissue								

Values represent mean ± S.D.

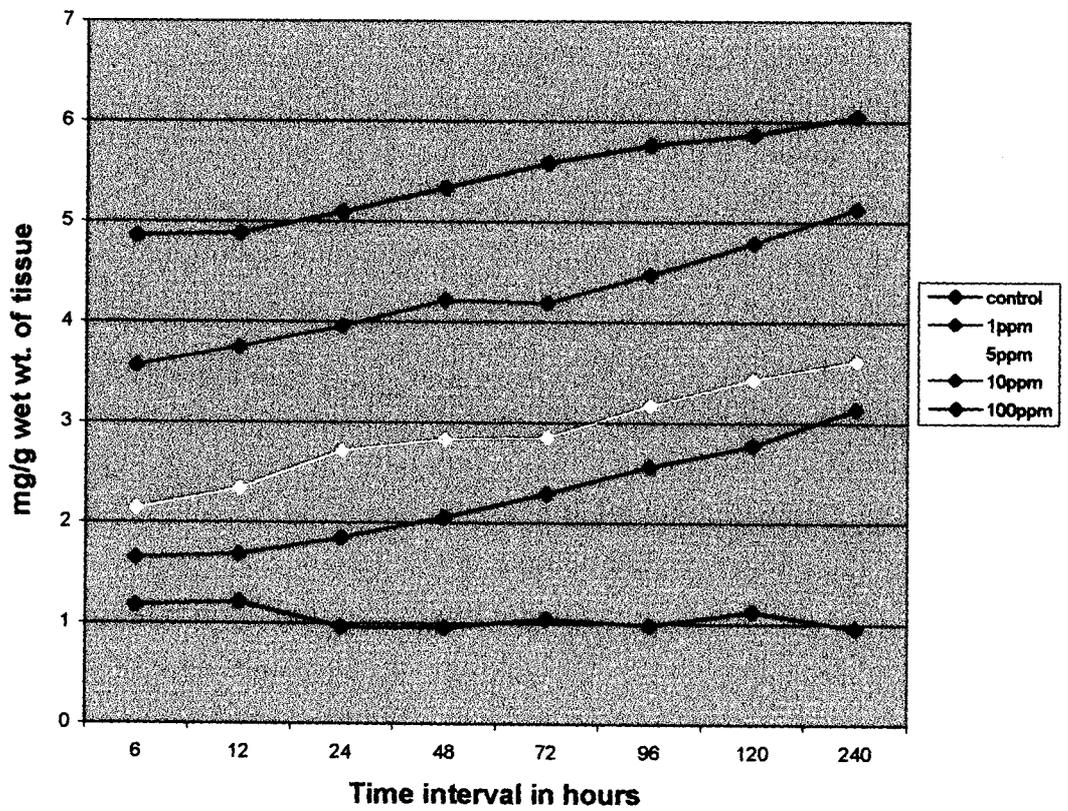
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.22: Triglyceride content of spinal cord of mice exposed to monocrotophos



end of 240 hours. The triglyceride contents were in the range of 2.14 ± 0.19 to 3.62 ± 0.41 mg / g wet wt. of tissue. The maximum increase in triglyceride content was observed at the end of 240 hours. All the elevations of triglyceride contents were statistically significant ($P < 0.001$).

In response to 10 ppm dose of monocrotophos the triglyceride contents elevated at all the time intervals and showed a steady elevation from the end of 6 hours to the end of 240 hours except for a comparatively marginal decline at the end of 72 hours. The triglyceride contents were in the range of 3.56 ± 0.37 to 5.13 ± 0.68 mg / g wet wt. of tissue. The maximum increase in the triglyceride content was observed at the end of 240 hours. The elevations in triglyceride contents were statistically significant at all the time intervals ($P < 0.001$).

100 ppm dose of monocrotophos induced steady elevations in the triglyceride contents from the end of 6 hours to the end of 240 hours and they were in the range of 4.85 ± 0.73 to 6.05 ± 0.87 mg / g wet wt. of tissue. The maximum hike in triglyceride content was observed at the end of 240 hours. All the hikes in triglyceride contents were highly significant ($P < 0.001$).

GLUCOSE

Monocrotophos induced a dose dependent decline in the glucose contents of the spinal cord at all the time intervals. The alterations in glucose contents are tabulated in table 3.23 and graphically presented in graph 3.23.

The glucose contents in the control animals were in the range of 7.56 ± 0.7 to 7.78 ± 0.8 mg / g wet wt. of tissue.

1 ppm dose of monocrotophos induced decline in the glucose contents at all the time intervals, however the degree of decrement varied from one exposure period to the other. The glucose contents were in the range of 6.85 ± 0.7 to 7.70 ± 0.7 mg / g wet wt. of tissue. The maximum decline in glucose content was observed at the end of 72 hours. The decline in glucose contents were statistically significant at the end of 24, 48 and 96 hours ($P < 0.005$).

5 ppm dose of monocrotophos induced decline in glucose contents at all the time intervals and they were in the range 6.22 ± 0.7 to 6.84 ± 0.8 mg / g wet wt. of tissue. The maximum reduction in the glucose content was noted at the end of 240 hours. All the reductions in glucose contents were statistically significant.

Mice subjected to 10 ppm dose of monocrotophos exhibited decline in glucose concentration of spinal cord at all the time intervals and they were in the range of 5.56 ± 0.7 to 6.45 ± 0.9 mg / g wet wt. of tissue. The maximum decline was observed at the end of 24 hours. The reductions in glucose contents were statistically significant at all the time intervals.

100 ppm dose of monocrotophos induced a precipitous decline in glucose contents at all the time intervals. The glucose contents were in the range of 3.63 ± 0.4 to 4.34 ± 0.7 mg / g wet wt. of tissue. The maximum

TABLE 3.23: GLUCOSE CONTENT OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	7.64 ± 1.1	7.78 ± 0.8	8.02 ± 1.3	7.61 ± 1.3	7.68 ± 1.0	7.59 ± 1.2	7.59 ± 0.9	7.56 ± 0.7
1ppm	7.43 ± 1.2 ns	7.51 ± 0.9 ns	7.70 ± 0.7 c	6.93 ± 0.6 c	6.85 ± 0.7 ns	7.05 ± 0.7 c	7.35 ± 0.8 ns	6.98 ± 0.8 ns
5ppm	6.62 ± 1.1 c	6.84 ± 0.8 c	6.71 ± 0.8 c	6.72 ± 1.2 c	6.35 ± 0.9 c	6.28 ± 0.8 c	6.30 ± 0.9 b	6.22 ± 0.7 c
10ppm	6.45 ± 0.9 c	6.03 ± 1.1 b	5.56 ± 0.7 b	6.25 ± 0.8 c	6.20 ± 0.5 c	5.82 ± 0.6 b	5.75 ± 0.5 b	5.64 ± 0.9 b
100ppm	4.34 ± 0.7 a	4.21 ± 0.5 a	3.98 ± 0.5 a	4.05 ± 0.5 a	3.77 ± 0.6 a	3.75 ± 0.3 a	3.80 ± 0.5 a	3.63 ± 0.4 a
mg /g of wet wt. of tissue								

Values represent mean ± S.D.

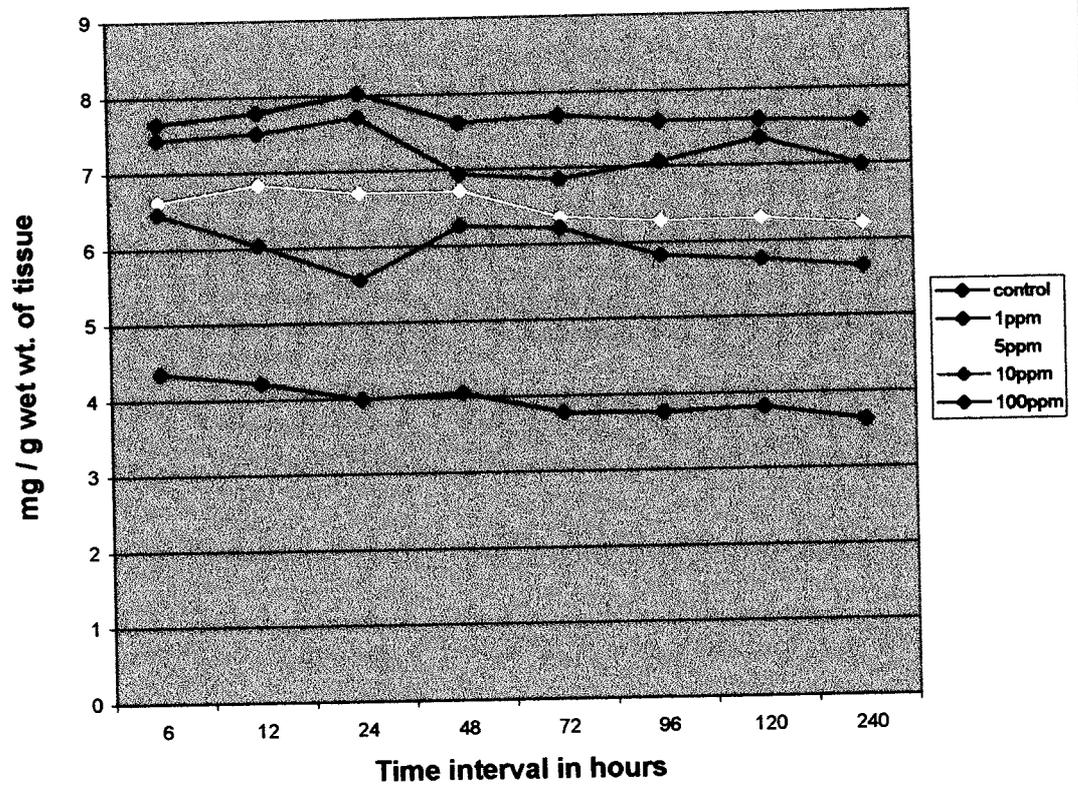
ns= not significant

a= P < 0.001

b= P < 0.01

c = P < 0.05

Graph 3.23: Glucose content of spinal cord of mice exposed to monocrotophos



decline was observed at the end of 240 hours. The reductions in glucose contents were statistically significant at all time intervals ($P < 0.001$).

GLYCOGEN

The glycogen contents of the spinal cord of mice declined in a dose dependent manner in response to monocrotophos treatment at all the time intervals. The changes in the glycogen contents are compiled in table 3.24 and graphically presented in graph 3.24. The glycogen contents in the control animals ranged between 6.47 ± 1.12 to 6.54 ± 0.91 mg / g wet wt. of tissue.

Under the influence of 1 ppm dose monocrotophos the glycogen contents gradually declined from the end of 6 hours to the end of 240 hours except for a negligible increase at the end of 72 hours as compared to the preceding time intervals. The glycogen contents were in the range of 5.25 ± 0.60 to 6.38 ± 0.73 mg / g wet wt. of tissue. The maximum reduction in glycogen content was observed at the end of 240 hours. The reductions in glycogen contents were significant at all the exposure periods except at the end of 6 and 12 hours.

5 ppm dose of monocrotophos promoted an time progression dependent decline in the glycogen content from the end of 6 hours to the end of 240 hours. The glycogen contents ranged between 3.87 ± 0.68 to 5.34 ± 1.03 mg / g wet wt. of tissue. The maximum decline in the glycogen content

was observed at the end of 240 hours. All the reductions in glycogen contents were statistically significant.

Mice exposed to 10 ppm dose of monocrotophos showed steady reduction in glycogen contents from the end of 6 hours to the end of 240 hours. The glycogen contents were in the range of 3.35 ± 0.64 to 4.28 ± 0.53 mg / g wet wt. of tissue. The maximum decrease was observed at the end of 240 hours. The decreases in glycogen contents were statistically significant at all the time intervals.

100 ppm dose of monocrotophos induced a gradual decline in the glycogen content from end of 6 hours to the end of 240 hours except for a negligible increase at the end of 12 hours as compared to that observed at the end of 6 hours. The glycogen contents were in the range of 2.23 ± 0.30 to 3.78 ± 0.63 mg / g wet wt. of tissue. The maximum decrease in the glycogen content was noted at the end of 240 hours. The reductions in glycogen contents were statistically significant at all time intervals ($P < 0.001$).

TABLE 3.24: GLYCOGEN CONTENT OF THE SPINAL CORD OF MICE EXPOSED TO MONOCROTOPHOS

	6	12	24	48	72	96	120	240
Control	6.52 ±1.13	6.51 ±0.95	6.53 ±.93	6.48 ±0.95	6.47 ±1.12	6.52 ±0.83	6.54 ±0.91	6.50 ±0.85
1ppm	6.38 ±0.73 ns	6.34 ±0.68 ns	6.08 ±0.96 c	6.03 ±0.84 c	6.04 ±0.72 c	5.86 ±0.75 b	5.72 ±0.77 c	5.25 ±0.60 b
5ppm	5.34 ±1.03 b	5.27 ±0.85 b	5.11 ±0.68 b	4.86 ±1.03 b	4.64 ±0.95 b	4.38 ±0.74 b	4.09 ±0.67 b	3.87 ±0.68 a
10ppm	4.28 ±0.53 b	4.25 ±0.55 a	4.15 ±0.63 a	4.13 ±0.68 a	4.03 ±0.71 a	3.81 ±0.68 a	3.62 ±0.73 a	3.35 ±0.64 a
100ppm	3.77 ±0.62 a	3.78 ±0.63 a	3.53 ±0.57 a	3.47 ±0.58 a	3.15 ±0.55 a	2.85 ±0.50 a	2.47 ±0.38 a	2.23 ±0.30 a
mg /g wet wt. of tissue								

Values represent mean ± S.D.

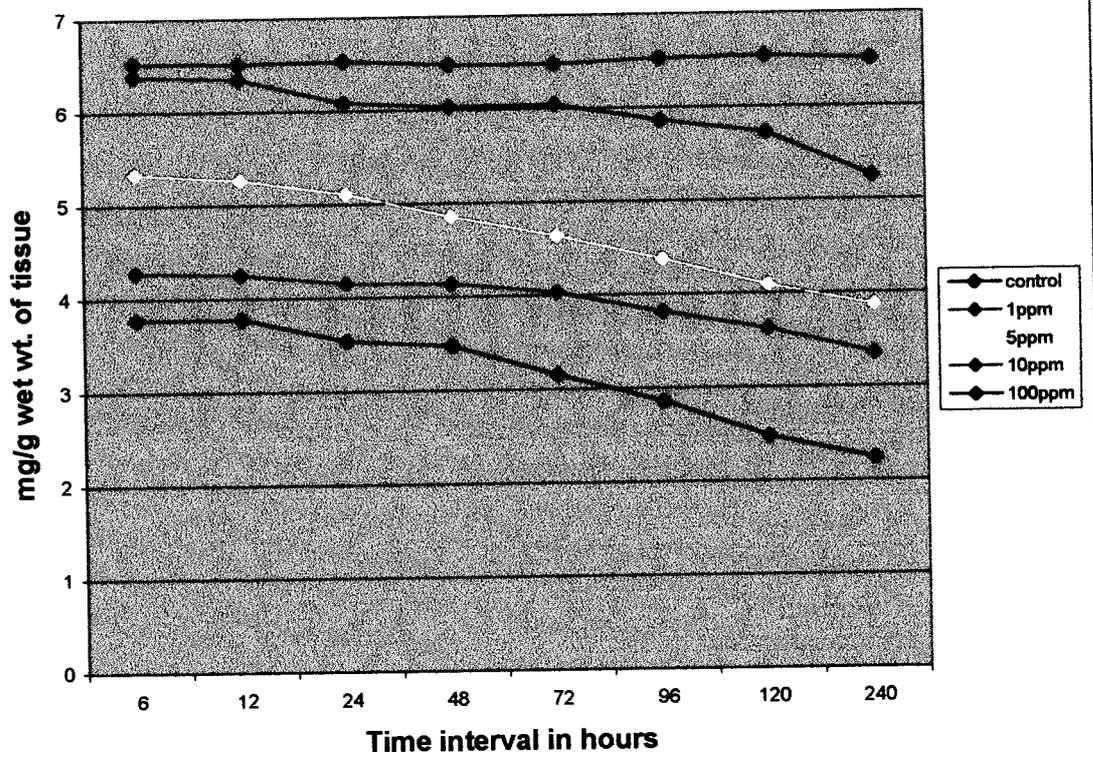
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a= P <0.001

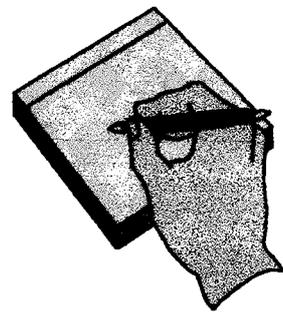
b= P < 0.01

c = P < 0.05

Graph 3.24: Glycogen content of spinal cord of mice exposed to monocrotophos



DISCUSSION



The extensive use of any biologically active chemical implies a potential hazard to the environment as well as the inhabitants alike and hence its effects need to be evaluated from various angles. The organophosphorus compounds due to their propensity for relatively less persistent and acute toxicity are widely used by agriculturists and hygienists and hence attract due attention of toxicologists. It is axiomatic that organophosphorus compounds execute their toxic potentiality by inhibiting acetylcholinesterase (WHO, 1986). Besides their anticholinesterase activity resulting in a typical cholinergic crisis, the organophosphorus compounds are capable of producing several subacute or chronic neurological deficits (Bleecker *et al.*, 1992).

DDVP, an organophosphate compound, owing to its low systematic toxicity is widely used as insecticide and schistosomicide (WHO, 1988). ~~Monocrotophos is widely used due to fast acting systemic action and high toxicity~~ (Kumar *et al.*, 1998). A review of literature reveals that both DDVP and monocrotophos produce cholinergic stimulation symptoms typical of organophosphate toxicity. The effect of DDVP has been studied most extensively as compared to monocrotophos. Both cause divergent effects in various organs, however, most toxicologists have investigated chronic effect of these pesticides.

The efficacy of any pesticide action mainly depends on its mode of administration. From the literature available on toxicity data of DDVP and monocrotophos it is evident that, they are more toxic when given intraperitoneally followed by oral mode while the dermal exposure is

comparatively less toxic. As compared to DDVP, monocrotophos appears to be highly toxic and this is substantiated by the present toxicity evaluation studies which indicate that the LD₅₀ concentration of monocrotophos (241.63 to 476.00 ppm) is lower than that of DDVP (3378.00 to 5736.00 ppm).

The neurotoxicity of DDVP and monocrotophos has been investigated. However, most neurotoxicologists emphasize the effect of DDVP / monocrotophos on the brain regions except for a few reports on the effect on spinal cord. Ali and Hasan (1977) reported diminution of the amino acids contents while Ali *et al* (1979), reported alterations in catecholamines and 5 – HT in brain and spinal cord of rats given daily intraperitoneal dose of DDVP for 15 and 10 days respectively. In another report Hasan and Ali (1979) studied ultrastructural changes in spinal cord of rats exposed to DDVP. The lipid levels and alterations of lipid peroxidation in brain and spinal cord of fish exposed to DDVP for 7 days has been documented (Vadhva and Hasan, 1986). Apart from this report no information is currently available on the acute neurotoxic effect of DDVP / monocrotophos on spinal cord. The systemic effect of monocrotophos with respect to spinal cord has not been evaluated.

The present report is first of its kind on the ability of DDVP and monocrotophos to induce electrophysiological as well as biochemical changes in the spinal cord of mice, administered with acute intraperitoneal doses of DDVP and monocrotophos.

ELECTROPHYSIOLOGICAL ALTERATIONS OF SPINAL CORD

The spontaneous electrical activities recorded from the spinal cord of mice administered with 1, 5, 10 and 100 ppm doses of DDVP and monocrotophos individually show alterations in frequencies, durations and amplitudes of the electrical activities as well as conduction velocities of impulses.

The organophosphate compounds are known to affect excitable membranes directly. At higher concentrations, the organophosphorus compounds block peripheral nerve conduction (Woodin and Wieneke, 1970). At lower concentrations these compounds promote their effect on the central respiratory neurons (Bay *et al.*, 1973) and cortical activity of brain (Van Meter *et al.*, 1978). Compounds like DFP and soman are reported to alter peripheral nerve excitability (Anderson and Dunham, 1985).

The review of literature shows a lacuna as far as effect of organophosphate compounds especially DDVP and monocrotophos on the electrophysiological activity of mice is concerned. The present work clearly shows that DDVP and monocrotophos induce subtle changes in electrophysiological activity of the spinal cord even when administered as single acute low doses which indicates that the spinal cord is sensitive to DDVP and monocrotophos.

The changes induced by DDVP and monocrotophos are nearly in a dose dependent fashion. The effect is more pronounced after 24 hours to the end of 96 hours after pesticide treatment. At the end of 240 hours, a slight recovery in a few electrical activities is seen in comparison to those observed at the preceding time intervals.

Effect of ionic channels

Ionic channels play pivotal roles in normal physiological function of the nervous system. In excitable cells they are the critical sites for generation of action potentials, neurotransmitter release and muscle contraction (Narahashi, 1989). Thus, the ionic channels are the target sites of a variety of neurotoxicants. The ionic channels can be chemically modulated or their kinetics altered by specific chemicals. The channels can be blocked by specific blockers and presently are used as important tools in neurotoxicological studies.

Barium ions are known to block delayed rectifying, inward rectifying or anomalous rectifying potassium channels from inside as well as outside of the membrane (Hagiwara *et al.*, 1978; Armstrong and Taylor, 1980; Eaton and Brodwick, 1980 and Ohmori *et al.*, 1981, Hille, 1989). In the present study, the use of BaCl₂ to block potassium channels of spinal cord cells resulted in abolishment of inward spike and plateau phases as well as hyperpolarisations indicating involvement of potassium channels / ions in expression of these phenomena.

Polyvalent ions like Cd^{2+} are known to block calcium channels (Kostyuk and Kristal, 1977; Narahashi, 1992). Walton and Fulton (1986) have reported the blocking of calcium channels in spinal motoneuron by Cd^{2+} . In the present study the blocking calcium channels resulted in the decline of the baseline potential and decrease in the number of spikes and plateau phases and also their amplitudes indicating the involvement of calcium channels in producing them.

Alterations in frequencies of electrical activities

DDVP induces increase in the frequencies of spike and plateau phases barring a few exceptions. The spike frequencies of action potentials elevate as the concentration of DDVP increases. Monocrotophos too elevate the spike and plateau phase frequencies at almost all the time intervals with a few exceptions. The increases in frequencies are higher till the end of 96 hours to 120 hours at most of the dosing schedules and thereafter show a slight recovery at the end of 240 hours. However, the spike frequencies do not return to a normal pattern.

The increase in spike frequencies indicate the excitation of spinal cord viz-a- viz the sensory and motor fibres including slow as well as fast conducting nerve fibres. The occurrence of spike phases are due to Na^+ and K^+ channels while the peak spike phase is due to Ca^{2+} channels (Hille, 1984; Yarom *et al.*, 1985; Llinas, 1988). This indicates that DDVP and

monocrotophos influence Na^+ , K^+ and Ca^{2+} channels. Similar increase in spike frequency is reported by Desi and Nagymajt (1988) for brain of rats treated with DDVP.

The non inactivating or persistent Na^+ conductance, sometimes coupled with low and slow Ca^{2+} generate long plateau phase potentials, which helps in regulation of membrane excitability in a delicate manner (Llinas 1988). Because of their low kinetics and small unitary conductance, activation of these channels does not generate spike potentials but rather serve as trigger for spike initiation by fast Na^+ channels. This conductance has been reported in dorsal horn and spinal cord motoneurons (Llinas, 1988). Such plateau phase potentials are observed in the present study under the influence of DDVP and monocrotophos indicating that Na^+ channel kinetics are also modulated by these pesticides to a certain extent. The elevations in the number of spikes and plateau phases by DDVP and monocrotophos indicate induction of excitation and the rise in the number of spike phases could be a result of rise of plateau phases as plateau phases serve as trigger for spike initiation by fast Na^+ channels (Llinas,1988). Thus, it appears that DDVP and monocrotophos influence fast Na^+ channels as well as inhibit acetylcholinesterases (WHO, 1988a; WHO and IPCS, 1993) thereby promoting over excitation, which could pave way for the occurrence of neuropathy. Trichlorfon induced nerve membrane excitability in sciatic nerve of rats following repeated doses are reported by Averbook and Anderson (1983).

The increase in the frequency of inward current spike and plateau phases could be attributed to the activation of calcium and potassium channels or it could be also due to the activation of calcium activated potassium channels. This is indicated by the elimination of inward spikes and plateau phases after blockade of Ca^{2+} and K^+ channels. These observations indicate the influence of DDVP and monocrotophos on external Ca^{2+} and K^+ channels which are known for the genesis of inward currents (Walton and Fulton, 1986)

The isoelectric phase frequencies decline under the influence of all the doses of DDVP and monocrotophos at all the time intervals. The isoelectric phase indicates a period of non-excitability hence the reduction of these phases as the dosage increases indicates that the excitability of the membranes are increased by DDVP and monocrotophos and these observations are in agreement with the elevations in the frequencies of action potentials and inward currents noted in the present work which indicate excitation of spinal cord.

Hyperpolarisations are introduced in the spinal cord of animals subjected to DDVP and monocrotophos and increase is nearly dose-dependent. Calcium mediated hyperpolarisations have been reported in mammalian spinal motoneurons. Calcium activated increase in K^+ conductance is also reported to be responsible for producing hyperpolarisations in neurons (Hotson and Prince, 1980). The elimination of hyperpolarisations by CdCl_2 and BaCl_2 observed in the present study

indicates the involvement of Ca^{2+} and K^+ channels in the hyperpolarisations. This observation also supports the views of Hotson and Prince (1980).

Hence, the increase in hyperpolarisations promoted by DDVP and monocrotophos could be partly due to opening of Ca^{2+} channels and opening of calcium mediated K^+ channels. The increases in hyperpolarisations of spinal cord under the influence of DDVP and monocrotophos has physiological implications. As the aforementioned pesticides promote excitation of spinal cord as evidenced by the elevation of the action potential and inward current frequencies, the introduction of large number of hyperpolarisations could be considered as compensatory process to reduce the number of excitatory spikes. However, the excessive rises in hyperpolarisations could eventually be detrimental to the normal functioning of spinal cord. It is not known how many hyperpolarisations could be useful in preventing / delaying elevations in the number of excitatory spikes and how many hyperpolarisations are detrimental as well as responsible for slowing down the normal or abnormal spontaneous bursts / firing of electrical potentials.

Alterations in durations of electrical activities

Both DDVP and monocrotophos fail to promote significant changes in the durations of spikes of action potentials and inward currents except at a few occasions especially with doses of 1 and 10 ppm. But 100 ppm doses do

change the durations of spikes at some time intervals. These results indicate that low doses of DDVP and monocrotophos do not promote opening of Na^+ and K^+ channels for longer durations. If Na^+ channels are opened and Na^+ is retained for longer duration the spinal nerve fibres would remain depolarised for longer time and when Na^+ is transported out bringing K^+ (which had moved out earlier) back into the nerve fibres it would repolarise. Only in such events the spike durations would increase. The present findings of less significant or almost negligible changes in durations of spike are due to increased spike frequencies. But it is difficult to know how the spike frequencies are increased without affecting the durations. This could be possible only by decreasing the number of isoelectric phases. The present studies show such adjustments in isoelectric phases at many time intervals. However at a few time intervals and doses this adjustments is not observed.

In general, DDVP has promoted significant elevations in plateau phase durations of action potentials and inward currents at some of the time intervals, especially for 5 to 100 ppm doses. In comparison monocrotophos has induced pronounced changes in plateau phase durations of action potentials and inward currents. The elevations in plateau phase durations indicate the retention of Ca^{2+} inside the nerve fibres as their channels are closed after their entry. The retention of Na^+ and Ca^{2+} inside the nerve fibres could trigger the Ca^{2+} dependent degenerative processes promoting the activation of xanthine oxidase which is responsible for formation of reactive oxygen species. In the present study the activation of xanthine oxidase is observed. The increases in the durations of plateau phases are an indication

of influence of pesticides on the calcium channels. Though increase in plateau phases are believed to be associated with triggering of spike frequencies (Llinas, 1988) the associated retention of Ca^{2+} could be deleterious and may pave way for induction of delayed neuropathy.

Exposure of spinal cords of pesticide treated mice to CdCl_2 reduce the spike and plateau phase duration and are noted to be similar to that obtained for control, indicating that this elevation in duration is largely due to calcium conductance. Similar findings are reported by Walton and Fulton (1986).

The durations of isoelectric phases show fluctuations in response to DDVP and monocrotophos treatment. However, the isoelectric phase durations are declined in general. 100 ppm dose of DDVP and monocrotophos induce maximum reductions at almost all the time intervals.

The reductions of isoelectric phase durations could be considered as a compensatory mechanism to accommodate elevated spike frequencies induced by DDVP and monocrotophos.

Alterations in the electrical potentials

DDVP elevates the baseline potential of spinal cord in nearly a dose dependent manner at all the time intervals, however, after 96 hours time interval the baseline potentials tend to decline a little with respect to what it

was at the end of 96 hours. But such a decline is not obtained with 100 ppm dose.

Monocrotophos promotes elevations in baseline potentials at all the time intervals. However, baseline potential shifts towards normalcy, though it fails to reach to the control level, under the influence of 1 and 5 ppm doses. The baseline potentials get prominently elevated under the influence of 10 and 100 ppm doses of monocrotophos.

The baseline potential is maintained by the complex interaction of ionic channels (Hille, 1984). The blocking of K^+ channels elevates the baseline potential partially which sometimes is not significant. But the blocking of Ca^{2+} channels result in a drop of the baseline potential. These findings suggest that the baseline potential is maintained largely due to calcium conductance but the role of potassium conductances as well as other channel conductance can not be ruled out.

The baseline potentials are the reflections of resting membrane potentials. The resting membrane potentials are reported to be elevated under the influence of DFP and soman (Anderson and Dunham, 1985). Therefore, elevations of baseline potentials could be considered due to the increases in resting membrane potentials. The elevations in the resting membrane potentials are reported to be due to the inhibition of $Na^+ - K^+$ ATPase and / or decreased K^+ channel conductance by the pesticides (Anderson and Dunham, 1984). Besides, the resting membrane potentials are known to

increase due to the paucity of nutrient / energy supply (Anderson and Dunham, 1984). The elevation of baseline potential under the influence of DDVP and monocrotophos could also be due to either the inhibition of Na^+ - K^+ ATPases which are responsible for transport of nutrients. However in the present study a decline of alkaline phosphatase , an enzyme responsible for membrane transport is observed which may disturb the transport of nutrients, resulting into elevation of baseline potential. Besides the excessive excitation of spinal cord (under the influence of DDVP and monocrotophos) as evidenced by the elevated spike frequencies could deplete the energy reserves and / or the nutrients of spinal cord, thereby creating energy crisis which when coupled with inhibition of transport enzyme, would elevate baseline potential in a manner identical to that reported for resting membrane potential by Anderson and Dunham (1984, 1985) in rats exposed to pesticides. However, it requires further investigation to throw more light on the development of energy crisis and involvement of Na^+ - K^+ ATPase. The observations of decline in glycogen and glucose levels of spinal cords under the influence of DDVP and monocrotophos indicates the possibility of development of energy crisis.

DDVP induces increase in the amplitudes of action potentials at almost all the time intervals. The increases are more significant in animals treated with 5, 10 and 100 ppm doses. The elevations in amplitudes of inward currents are significant only at few time intervals especially in response to 5, 10 and 100 ppm dose.

Similarly, monocrotophos induces significant increase in amplitude of action potential especially in mice administered with 5 to 100 ppm doses. However, the increases are significant at only few exposure periods. Except for significant elevations at few time intervals, the inward current amplitude was not much affected by monocrotophos treatment.

The amplitudes of action potential are more affected than amplitudes of inward currents and DDVP is more effective than monocrotophos with respect to the changes in amplitudes of action potential.

The peak amplitude of action potentials are caused due to activation of Ca^{2+} channels (Llinas, 1988). In the present work the pesticide induced spike elevations (amplitudes) are abolished when subjected to CdCl_2 . This suggests the involvement of Ca^{2+} in the production of peak elevation of elevated amplitudes of spikes. An identical effect is demonstrated by Walton and Fulton (1986) in rat motoneurons. Thus, the elevations in amplitudes of spikes under the influence of DDVP and monocrotophos could be considered due to the opening of calcium channels. However it remains to be seen if these pesticides are opening voltage gated Ca^{2+} channels or independent Ca^{2+} channels sensitive to pesticides or leaky Ca^{2+} channels. Anderson and Dunham (1985) have reported initial elevations in amplitudes of action potentials followed by fluctuations under the influence of DFP and soman.

Alterations in conduction velocity

The conduction velocity of spinal cord of mice gets significantly elevated in response to nearly all the doses of DDVP from the end of 12 hours to 120 hours. But subsequently the conduction velocity decreases at the end of 240 hours. Monocrotophos induces significant elevations in the conduction velocity only at a few time intervals while it reduces considerably, even below the normal level at the end of 240 hours.

Conduction velocity is an index of nerve membrane stability (Anderson, 1983). Alterations in the conduction velocity following peripheral neuropathy promoted by toxicants is well known (Hopkins and Gilliat, 1971).

The increase in the conduction velocity till the end of 96 hours / 120 hours in the present study suggests that DDVP and monocrotophos cause initial excitation and speedy operations of ionic channels. Similar elevations in conduction velocities are reported by Nachtman and Couri (1981) and Ruff *et al.*, (1981) in rats exposed to hexacarbon. Similarly, Anderson and Dunham (1985) have reported elevations in conduction velocities with fluctuations in peripheral nerves of rats exposed to DFP and soman. The initial excitation noted till 96 / 120 hours probably are due to the anticholinesterase property of DDVP and monocrotophos (WHO, 1988a; WHO and IPCS, 1993). The inhibition of acetyl cholinesterase allows accumulation of acetylcholine initiating hyperexcitability of the membranes. Cholinergic effects are usually

manifested after 1-3 days of intoxication (Senanayake and Karalliedde, 1987). In the present study too the excitation is evident from the end of 12 hours to 96 / 120 hours after pesticide treatment. This effect could also be due to the blocking of acetylcholine receptors, however, as reported for some organophosphate compounds (Fitzergard, 1991, Nagata *et al.*,1997). The excitability of the neuronal membrane can also be attributed to the actions of organophosphorus compounds on nerve membrane inducing faster sodium flux or earlier activation of potassium conductance (Anderson and Dunham, 1985). This view is substantiated by the present study as the excitability is diminished when potassium channel blockers are used in spinal cord of pesticide treated animals.

The initial excitation or rise in conduction velocity subsequently declines and further by the end of 240 hours it declines below that obtained for control in response to certain doses. This shift in the nerve excitation or nerve conduction velocity as the time progresses to normal or below normal in some case reveals a possibility of an operation of a compensatory mechanism of the neuronal membrane. The observed decreases in conduction velocity could be attributed to the setting in of degenerative changes in neuronal membrane as evidenced by elevated activity of lysosomal membrane, activation of xanthine oxidases as well superoxide dismutases which indicate the genesis of reactive oxygen species responsible for neuropathy in response to DDVP and monocrotophos toxicosis. Previous reports have correlated conduction velocity changes to structural nerve damage (Takeuchi *et al.*,1981; Ono *et al.*, 1981). Similar decline in conduction

velocities are reported in peripheral nerves of rats treated with DDVP for six weeks (Desi and Nagymajt, 1988). Anderson and Dunham (1984) have reported a slower conduction velocity in peripheral nerves of rats treated with hexacarbon for 35 consecutive days.

Maxell and LeQuesne (1982) found no change in motor nerve conduction velocity in rats chronically exposed to organophosphates. In a study conducted in workers exposed to fenthion, the nerve conduction velocities were altered (Misra *et al.*, 1988). Chronic exposure of workers to organophosphate compounds causes slowing of motor nerve conduction velocity (Verberk and Salle, 1977) and also in the sensory nerves (Stalberg *et al.*, 1978)

Therefore, the initial elevations of conduction velocities under the influence of DDVP and monocrotophos can be considered as a result of rapid influx of Na^+ or due to early conductance of K^+ and subsequent reductions in conduction velocity could be attributed to the energy crisis, impairment of membrane transport, increase in lysosomal enzymes, and formation of reactive oxygen species resulting into setting in degenerative changes of the neuronal membranes.

BIOCHEMICAL ALTERATIONS OF SPINAL CORD

Acid phosphatase (AP)

DDVP induces nearly a time progression dependent elevations in AP activity under the influence of all the doses except 1 ppm. 1 and 5 ppm doses of DDVP failed to induce significant increases in AP activity at the end of 6 and 12 hours. Similarly 10 ppm dose can not promote significant rise of AP activity at the end of 6 hours. However, 100 ppm dose of promotes maximum elevation of AP activity at the end of 240 hours.

Monocrotophos elevates AP activity in a dose and time progression dependent manner barring a few exceptions. The elevations are significant at all exposure periods, except at the end of 6 hours in response to 1 and 5 ppm dose. The present study shows that monocrotophos is more potent than DDVP in promoting elevations of AP activity.

AP is a typical lysosomal enzyme generally found in the lysosomes in a latent state which gets activated in response to stressors including pesticides (Dingle and Fell, 1969). AP is precisely located in the lysosomes of the neuronal cell body especially of motor neurons and spinal ganglion cells (Hogan and Banik, 1985). Becker and Barron (1963) have reported an

increase in AP activity in the injured neurons. Holtzman and Novikoff (1965) and Friede (1966) have reported increases in AP activity in the axons undergoing changes subsequent to injury. Glees (1967) reported increase in AP activity in nerves and neuroglia of TOCP treated hens. The relationships between elevated AP activity and necrosis are well-documented (Dingle and Fell, 1969, Tietz, 1970). Tappel (1970) opined that lysosomal enzymes disturb membrane integrity leading to neurodegenerative changes while Morre (1988) viewed that elevations in AP activities are associated with labilisation of lysosomes. The process of necrotic changes that encountered in organophosphate toxicity is similar to that of wallerian degeneration (Bouldin and Cavanagh, 1974) and the AP concentration is elevated during wallerian degeneration, indicating its role in autolytic process (Bell *et al.*, 1972). Lysosomal hydrolyses that mediate cellular digestion, phagocytosis and cytolysis are prime candidates for release and activation of processes of tissue degeneration, necrosis and breakdown of myelinated axons (Kao and Chang, 1977).

Th high AP activity in the present study indicates the possibility of invivo labilisation of the lysosomes in the spinal cord when administered with a single acute dose of DDVP and monocrotophos. This may further induce degeneration of cellular membranes as suggested by Tappel (1970). And the reductions in protein concentrations of the spinal cord observed in the present work indicate the probability of this event. This view is substantiated by the presence of altered lysosomes in electron microscopic studies of rat spinal cord exposed to DDVP (Hasan and Ali, 1980).

Kakari *et al.*, (1976) have reported elevated AP activity during spinal cord trauma leading to impairment of motor, sensory and sphincter function and in the present study symptoms like drowsiness, immobility of hind limbs and excessive salivation were observed in mice given at 100 ppm dose. Swamy *et al.*, (1992) reported elevation of AP activity in brain of rats given chronic oral dose of monocrotophos.

The gradual elevations of AP activities after acute exposure to DDVP and monocrotophos, especially after 12 hours treatment upto the end of 240 hours indicate the possibility of development of delayed neuropathy. Similar observations are reported by Abou- Donia (1978) in hens chronically exposed to oral doses of leptophos.

The present work suggests that a single dose ranging from 1 ppm to 100 ppm of DDVP and monocrotophos is sufficient enough to elevate AP activity, which can promote neuropathy.

Alkaline Phosphatase (ALP)

DDVP promotes nearly dose as well as time progression dependent decrease of ALP activity, expect at the end of 12 hours. Similarly also monocrotophos induces decline in ALP activity as a function of dose and progression of time except for 1 ppm dose.

ALP is known to be present at the cell membranes (Bretaudiere and Spillman 1984) and is involved with metabolic activities such as permeability and transport of nutrients through plasma membranes (Seth *et al.*, 1969).

The decreases in ALP activity in the present study indicate the probable disruption of permeability and transport of spinal cord. This may in turn hamper the axonal flow of metabolites. Further, disruption of normal metabolite flow, may deprive the axon of nourishment, which may initiate activation of lysosomes, resulting in hydrolysis of cellular components to provide nutrition to the dying cells (Edwards and Hassal, 1971) or it may be proceeding towards apoptosis, if its compensatory mechanism fails to enable the organ to sustain energy supply. The elevation of lysosomal enzymes observed in the present work suggest this chain of events. Besides, the electrophysiological changes especially excitatory changes in early stages of exposure to DDVP and monocrotophos could demand more energy thereby depleting the energy sources and reduction of ALP activity could further deprive the spinal cord of nutrients which could culminate in induction of apoptosis or delayed neuropathy of spinal cord with activation of lysosomal enzymes.

Engstron (1964) is of the opinion that decreased ALP activity indicates alteration of phosphate transport. Shaikila *et al.*, (1993) have reported sevini induced inhibition of ALP activity in fishes due to their interactions of regulatory factor of ALP.

Non – specific Esterases (NSE)

DDVP induces a nearly dose dependent increase in NSE activity at all the time intervals however time progression dependent elevation was observed only for 5 ppm dose. 1 ppm dose does not activate NSE significantly except at few time intervals.

Monocrotophos promotes elevations in NSE activity at all the time intervals in a dose dependent manner except at the end of 48 hours. However, time progression dependent elevation of NSE is promoted by 5, 10 and 100 ppm doses of monocrotophos.

The mode of action of organophosphorus compounds is mainly by inhibition of several splitting enzymes particularly cholinesterases. However, certain non-oxidative enzymes with esteric property have the ability to hydrolyse organophosphate compounds (Ahmad and Forgash, 1976). Kao *et al.*, (1985) reported non-specific esterase mediated hydrolysis of malathion and paraxon. Shibko and Tappel (1964) opined that NSE is bound to the lysosomal membrane and in contrast to other esterases these lysosomal membrane bound enzymes are resistant to organophosphorus inhibitors. They further suggested that under normal physiological condition the products of intracellular hydrolysis of some of the naturally occurring esters eg: cholesterol esters and vitamin A esters might be involved in control of permeability of lysosomal membrane. Ntiforo and Stein (1967) put forth their

results indicating the possibility that organophosphorus pesticides are able to interact with the structural component of the lysosomal membrane and thereby alter the permeability and lead to the release of NSE.

The increase in NSE activity in response to DDVP and monocrotophos stress corroborates the above view. This is further supported by the fact that the AP (lysosomal enzyme) activity is also concurrently elevated in spinal cord in the present study. But it is difficult to say whether the elevation in NSE activity is for hydrolysis of DDVP and monocrotophos like that reported by Kao *et al.*, (1985) or is a result of interaction of these pesticides with the lysosomal membranes as suggested by Ntiforo and Stein (1967). But the release and increase of NSE activity could definitely lead to onset of degeneration of spinal cord.

The changes in the electrophysiology of spinal cord as the time progresses indicate the onset of delayed neuropathy and NSE being lysosomal enzymes is likely to play some role in the development of neuropathy. It needs further investigation to see how rise in NSE activity could lead to neuropathy.

Xanthine Oxidase (XOD)

Both DDVP and monocrotophos promote activation of XOD activity. 1 ppm dose of DDVP does not induce significant elevation in XOD activity at

all the time intervals and 5 ppm dose induces maximum elevation of XOD activity at the end of 72 hours. However, a nearly time progression dependent elevation of XOD activity is seen in animals treated with 10 ppm and 100 ppm doses of DDVP.

Monocrotophos induces significant elevations of XOD activity at all the time intervals. 1 ppm and 5 ppm doses elevate the XOD activity to a maximum level at the end of 72 hours while 10 ppm and 100 ppm doses produce maximum increase in XOD activity at the end of 240 hours.

Kehrer *et al.*(1988) propose the involvement of chemical toxicants in production of reactive oxygen species / free radicals in the tissue. These ROS have the ability to induce oxidative stress (Halliwell and Gutteridge, 1986) by causing an imbalance of cellular redox status which may lead to several kinds of genomic function by alteration of DNA or impairment of membrane properties by attacking proteins and lipids. Thus, the high lipid content of myelin makes the nervous system especially susceptible to oxidative stress (Bondy, 1994).

Superoxide radicals are undoubtedly the most abundant oxygen radical generated *in vivo*. Biological sources of superoxide in CNS include the mitochondrial electron transport chain, activated leukocytes and tissue oxidases, most notably XOD (Salaris *et al.*, 1991; Pazdernik *et al.*, 1992). XOD is reported to be a prime generator of superoxide and may be a significant factor in several pathological states (Bondy, 1994). The increase in

XOD activity in the present study is hence an indication of genesis of reactive oxygen species in response to DDVP and monocrotophos induced stress. Any chemical, disrupting membrane structure or mitochondrial function by diverse means has the potential for induction of oxygen radicals. This is especially true of neurotoxic agents causing hyperexcitation.

Most workers are of the opinion that XOD does not exist in CNS. Except for the report of Muller *et al* (1985) and Betz (1985) who demonstrated the presence of XOD activity in rat caudate and brain capillaries respectively, no other report evidence is available. The present study reveals the presence of XOD in the spinal cord (probably in neurons) which is activated by pesticidal stress. The elevations in XOD activities under the influence of DDVP and monocrotophos especially after longer lapse of time indicates the possibility of delayed formation of reactive oxygen species responsible for delayed neuropathy. It is known that impaired membrane transports result in the increase in intracellular calcium overload causing activation of calcium dependent processes which contribute to cell damage (Pazdernik *et al.*, 1992) and XO is one of the calcium dependent processes responsible for production of superoxide radicals associated with cell damage. Therefore activation of XOD activity could be considered as initiation of a process of cell / tissue degeneration. However, it needs further investigation to see if pesticides like DDVP and monocrotophos promote elevation of intracellular calcium.

Superoxide dismutase (SOD)

DDVP induces a dose dependent elevation in SOD activity at all time intervals. 1ppm and 5 ppm doses induce maximum elevation in SOD activity at the end of 72 and 96 hours. 10 ppm and 100 ppm doses induce time progression dependent elevations in SOD activity. Monocrotophos induces a dose dependent and nearly time progression dependent elevation in SOD activity. 1 ppm and 5 ppm dose treated animals show slight recovery at the end of 240 hours.

The toxicant mediated release of reactive oxygen species produce ill effects on living components (Kehrer *et al.*, 1988) and has to be dismutated spontaneously or enzymatically to prevent the onslaught of oxidative insults to the tissue. The role of reactive oxygen species scavengers or antioxidants such as SOD (Deby and Goutier, 1990) thus assumes importance as an endogenous defensive adaptation against xenobiotic toxicity. The CNS is known to have somewhat low - levels of antioxidant enzymes and the localisation of these enzymes primarily in glia rather than neuron (Raps *et al.*, 1989) providing a first line of defense which may render the neurons susceptible to toxicants only when they traverse this first line defense. Increased levels of SOD, are generally taken as an indirect evidence of an increased oxidant milieu (Bondy, 1994). The increase in SOD activity in the present study indicates that DDVP and monocrotophos cause oxidative stress in the spinal cord through induction of reactive oxygen species, which may further prove deleterious. The reactive oxygen species are known to induce

lipid peroxidation (Pederson and Aust, 1973) and this is supported by the findings of Hasan and Ali (1980) who reported DDVP induced lipid peroxidation in different regions of rat brain.

The SOD activity in the present study shows a pesticide dose related increase which is in agreement with reports of Matkovics *et al.*(1983) who reported increased SOD activity in rat liver treated with trichlorfon and that of Kakkar and Vishwanath (1990) who demonstrated increased SOD activity in brain of aniline treated rats.

The present findings do not endorse the general view of several authors who are of the opinion that toxicants reduce antioxidant activity (Fujiyata *et al.*, 1998; Sahoo and Chainy, 1998). The biphasic fluxes of SOD levels are common and a change in either direction relates to the presence of superoxide radicals (Bondy, 1994).

In the present study a pesticide dose dependent and / or time progression dependent elevations of SOD activity, especially after 72/ 96 hours could be considered for scavenging the reactive oxygen species or oxygen related metabolites but if the formation of reactive oxygen species or oxygen related metabolites supercede the level of SOD activation, the defense of the cells against these oxygen radicals would fail thereby promoting delayed neuropathy. Thus, the increase in SOD activity indicate the formations of reactive oxygen species under the influence of DDVP and monocrotophos as SOD is known to be responsible for scavenging reactive

oxygen species (Ryter *et al.*, 1990) and the activation of xanthine oxidase should be considered as a factor responsible for production reactive oxygen species. The reduction of antioxidant enzyme activity reported by a few researchers (Fujiyata *et al.*, 1998; Sahoo and Chainy, 1998) under the influence of toxicants could be due to the interaction between the toxicants and the antioxidant enzyme leading to the changes in configuration or conformation of the enzyme. Such changes in the enzyme molecules would inhibit their activity. The elevations of SOD activities under the influence of DDVP and monocrotophos could be for quenching the reactive oxygen species and also could be due to the failure of DDVP and monocrotophos to interact with SOD for rendering it inactive. However, it needs further investigation to throw more light on the insensitivity of SOD of spinal cord of rat to the pesticides under study.

Total proteins

The results indicate that the exposure of mice to DDVP causes a nearly dose related decrements in total proteins concentrations at all the time intervals except at the end of 72 and 240 hours. The maximum reduction in total protein concentration are observed at the end of 240 hours under the influence of 5, 10 and 100 ppm doses and at the end of 120 hours for 1 ppm dose treatment.

Monocrotophos induces decline in the total proteins at all the time intervals as a function of dose. A time progression dependent decrease in protein concentration was observed in animals administered with 5, 10 and 100 ppm doses.

Richardson (1981) reported rapid loss of proteins during pesticide toxicity. Perturbations in protein metabolism are reported in rat brain after daily oral dosing of monocrotophos (Swamy *et al.*, 1992) while reduction in total protein content by dichlorvos in fishes is reported by Sarkar *et al.* (1996). The reduced proteins levels probably indicate their metabolic utilization during pesticidal stress and decrements in protein concentrations may be also due to the catabolic activity of increased acid phosphatase (AP) levels in the spinal cord either by breakdown of proteins (Shaikila *et al.*, 1993) or by impairment of protein synthesis through RNA depletion by AP enzymes (Jovic and Milosevic, 1970) which may gradually lead to functional and metabolic depression of spinal cord.

The decrease in total proteins concentration under the influence of DDVP and monocrotophos suggest the rapid breakdown of proteins by AP as a concomitant increase in AP activity is observed in the present study. Besides the reductions in total protein concentrations also indicate degeneration of nervous tissue. A thorough biochemical analysis is required to know which components of spinal cord are degenerated or have lost proteins. Further studies on various protein profiles and protein metabolism would ascertain the mode of damage induced by the pesticides.

Mitochondrial proteins (m-proteins)

DDVP and monocrotophos cause a pronounced decline in the m-protein concentrations of spinal cord. The decreases in m-protein concentration are nearly dose dependent in response to DDVP treatment. A time progression dependent decline of m-proteins is observed in animals administered 10 and 100 ppm doses. All the doses of monocrotophos promote decrease of m-protein concentrations. Animals treated with 1 ppm and 5 ppm doses of monocrotophos show recovery of m-protein content from the end of 48 and 96 hours, however, 10 ppm and 100 ppm doses induce decline in m-protein concentration in a time progression manner.

Most mitochondrial defects are caused to the protein part of mitochondria which constitutes enzymes and translocases (Scholte *et al.*, 1988). The breakdown of cellular components like mitochondria and lysosomes are known to be associated with chemical mediated cell injuries and secondly the metabolic stress results into oxidation of cellular enzymes and loss of mitochondrial function (WHO, 1986). The pesticides like DDVP and monocrotophos in the present study cause activation of AP, which may hydrolyse m-proteins as the excitation of spinal cord lead to high energy demand which could not be met by the natural and normal nutrient resources. After the natural resources are exhausted, the AP could be turning to the m-proteins. Besides the stimulation of apoptic processes can also contribute to mitochondrial degeneration. Another possibility is that the oxidative energy – producing system in mitochondria may be susceptible to oxidative damage by

reactive oxygen species as suggested by Richter *et al.* (1988). This view is endorsed by the findings in the present study, which reveals an increase in the free reactive oxygen species as evidenced by the increase of XOD and SOD. However, it needs further investigation to throw more light on mitochondrial degeneration and m-protein degradation.

Creatinine

The present study shows that the creatinine level of spinal cord elevates in response to DDVP and monocrotophos treatment. DDVP and monocrotophos induce nearly a dose dependent elevation in creatinine levels.

Creatinine is the product of endogenous protein metabolism or creatine metabolism and it arises in the body from the spontaneous breakdown of creatine phosphate. It serves practically no apparent function in the body and the creatinine level increases due to excessive tissue destruction or due to failure of creatinine being properly phosphorylated (Chatterjee, 1994b). Creatine phosphate is reported to be present as a source of energy in neuronal tissue (Chatterjee, 1994b). Under stress the creatinine phosphate is depleted in the CNS (Duffy *et al.*, 1972). In hypoxic conditions the phosphocreatine / creatinine ratio is reduced (Bachelard *et al.*, 1974). Organophosphates are reported to lower creatinine phosphate levels in brain (Miller and Medina, 1986).

The elevation of creatinine levels in the present study shows that DDVP and monocrotophos induce stress and the high metabolic activity of spinal cord depletes the high – energy phosphates sources such as creatinine phosphate releasing creatinine. The increase in the electrical activity observed in the present study requires more energy, which is probably drawn from these sources when the existing normal nutrient resources get exhausted. Thus, due to energy crisis, the creatine phosphates could be utilized thereby promoting the release and elevation of creatinine besides, the overexcitation of spinal cord may step up metabolism which is likely to create a hypoxic condition and such local hypoxic condition could also contribute to the drop in creatine / creatinine ratio a rise in creatinine as suggested by Bachelard *et al.*, (1974). However, it needs further investigation to ascertain the exact cause of elevation of creatinine level in spinal cord.

Cholesterol

DDVP and monocrotophos promote an increase in the cholesterol content at all the time intervals. Both the pesticides promote nearly dose-dependent elevations except at the end of 6 and 12 hours. By the end of 240 hours, the cholesterol content shows a relative reduction as compared to the preceding time intervals.

Cholesterol is quantitatively the most significant lipid component of mammalian spinal cord especially of the myelin (Hogan and Banik, 1985) and

excitable cell membranes (Hille, 1989). Cholesterol is an important constituent of cell membrane and a precursor to several stress hormones (corticosteroids)(Chatterjee, 1994). Augmentations of cholesterol synthesis leading to formation of corticosteroids to protect the organism from pesticide toxicity are known (Ramamurthy, 1984). An increase in net amount of esterified cholesterol subsequent to wallerian type nerve injury is reported (Mezei, 1970; Yao *et al.*, 1980 and Jurevics *et al.*, 1998).

The elevations in cholesterol levels in the present study may be due to increased synthesis of cholesterol to confer protection from pesticidal effect via corticosteroid production as reported by Ramamurthy (1984). Similar increases in cholesterol contents are reported by Vadhva and Hasan (1986) in DDVP treated fish brain and spinal cord and also by Prasada and Ramana (1984) in organophosphate treated fishes. Increases concentration of total lipids after organophosphate after organophosphate administration has also been reported (Tayyaba and Hasan, 1980) and this increase is suggested to be due to reduced lipo- protein lipase activity under pesticidal stress (Hazzard *et al.*, 1969). The increases in cholesterol concentrations could be either due to increased synthesis or perturbed metabolism or due to reduction in lipo- protein lipase activity.

However, the reductions observed at later stages i.e. at the end of 240 hours indicates utilization of cholesterol to meet the metabolic energy demands as other sources such as glucose and glycogen get exhausted. The analysis of spinal cord for glucose and glycogen concentrations under DDVP

and monocrotophos stress, reveal their depletion thereby suggesting reduction of normal nutrients which possibly might have resulted in utilization of cholesterol. Besides the degenerative changes occurring in the membranes of nerve fibres and neurons under pesticide stress could also result in release of cholesterol from the cholesterol rich nerve membranes. The elevation of xanthine oxidase, an enzyme responsible for formation of reactive oxygen species, suggests the occurrences of degenerative changes. Also the elevation of lysosomal enzyme activities indicate the possibility of degeneration of spinal cord components.

Further studies on specific neuronal lipids are essential to elucidate the perturbations induced in lipid profiles and lipid metabolism.

Triglycerides

DDVP and monocrotophos elevate triglyceride concentrations in a dose-dependent and nearly time progression dependent manner.

Small amounts of triglycerides have been found in the myelin sheath of the spinal cord (Fredman *et al.*, 1982). Triglycerides, which form the major lipid sources, are essentially fatty acid esters of glycerol. Triglycerides are also called as neutral lipids and are mobilized to different parts of the body for storage or degradation depending upon the energy demands.

In the present work the triglyceride concentrations of the spinal cord appear to be elevated under the influence of DDVP and monocrotophos. DDVP and monocrotophos are observed to promote stress on the spinal cord, especially promoting excitations as evidenced by alterations in the electrical activities of spinal cord. The elevations in the electrical activities appear to induce energy crisis and in order to provide the increasing demand in a situation like depleted natural resources (glucose, glycogen and creatine phosphate), the animals might be promoting mobilization of neutral lipids to the spinal cord as neutral lipids are major fuels for animals. Besides the degradative changes, if any induced in the spinal cord, especially in the myelinated nerve fibres owing to the activation of lysosomes and formation of reactive oxygen species which appears to happen under the influence of DDVP and monocrotophos, the triglycerides present in myelin would be released. Therefore, elevation of triglycerides in the spinal cord could be attributed to the mobilization of neutral fats to the spinal cord. However, it requires more research to throw light on this aspect. The survey of literature reveals that there is information on the mobilization of triglycerides and degenerative releases of triglycerides from the myelin sheath or myelinated nerve fibres as well as degenerative membranes.

Glucose

The present study shows reduction in the glucose contents under the influence of all the doses of DDVP and monocrotophos and time intervals barring a few exceptions. DDVP induces dose dependent decrease in glucose

concentration. An initial time progression dependent increase in glucose in glucose concentration is noticed at few instances with 1 ppm doses which subsequently declines with minor fluctuations.

The CNS depends mainly on carbohydrates, especially glucose for energy supply (Bradford, 1968). The utilization of glucose is higher as compared to glycogen in spinal cord (Hogan and Banik, 1985). The reductions in glucose level in the spinal cord of mice exposed to DDVP and monocrotophos is probably due to the rapid utilization of glucose to meet the high metabolic demands of the spinal cord under pesticidal stress as evidenced by elevated electrical activities of the spinal cord. Altered carbohydrate metabolism in rat liver chronically exposed to DDVP is reported (Teichert and Szymczyk, 1979). Increased cerebral glucose utilization is reported in response to soman intoxication in rats (Samson *et al.*, 1984; Pazdernik *et al.*, 1983).

Considering the glucose profile in the present study, the increases in glucose concentrations at certain time intervals probably may be due to supply of glucose through blood or due the glycogenolysis to compensate to some extent for the rapid declines in spinal cord glucose level. Similar findings are reported by Omkar *et al.*, (1984). Glucose is the primary source of energy for the neurons and must pass through the blood brain barrier. The transport of glucose has an exceedingly high capacity. Glucose combines with carrier protein on the luminal surface of the endothelial cells and is translocated across the cell membrane. Glucose transport across CNS capillary endothelia

is not coupled with Na^+ . Therefore, the increases in glucose concentrations at some time intervals and with a few low doses of pesticides could partly be due to the enhanced transport of glucose as well as due to the enhanced breakdown of glycogen and in the present work glycogen level of the spinal cord is found to decrease.

The changes in glucose profile under DDVP and monocrotophos toxicity reflects an altered carbohydrate metabolism. Further work on carbohydrate metabolic changes at molecular level needs to be conducted in spinal cord.

Glycogen

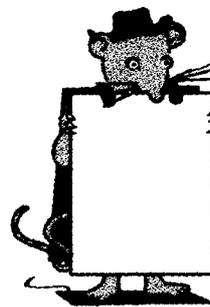
DDVP and monocrotophos induce a dose dependent decline in glycogen contents at all the time intervals. The decline is more pronounced after the end of 96 hours in mice treated with DDVP while a nearly time progression dependent decrease is induced by monocrotophos.

The disturbance in the glycogen profile is one of the most outstanding biochemical lesions due to the action of chemicals (DeBruin, 1976). The glucose and glycogen concentration declines and a few elevations observed in the present study could be attributed to the pesticides induced disruptions of carbohydrate metabolism. The possible mechanisms involved with alterations of glycogen metabolism are diverse in nature and such changes are generally interpreted as non-specific/ general metabolic disturbances due

to secondary stress conditions. The toxic manifestations of DDVP and monocrotophos as observed in the general glycogen depletion may be also due to activation of enzymes involved in glycogen metabolism. Kakimoto *et al.*, (1964) and Nakona and Tomilson (1967) opined that stress conditions increase the secretions of catecholamines, which in turn promote the breakdown of glycogen. This view is substantiated by the findings of Ali *et al.*, (1979), who reported DDVP induced disturbances in catecholamines in spinal cord of rats. Therefore, the decline in glycogen concentrations of the spinal cord could be considered as due to the rapid depletion of glucose and other natural resources to meet increasing energy demand under the pesticide promoted over excitatory state. The glycogen breakdown for yielding glucose might be promoted by the elevated catecholamine levels under the influence of pesticides (Kakimoto *et al.*, 1964; Nakona and Tomilson, 1967 and Ali *et al.*, 1979).

The present study reveals that single low acute dose of DDVP / monocrotophos are capable of inducing changes in electrophysiological properties and biochemical changes of the spinal cord which in turn may alter the motor neurons causing impairment of limbic functions over a period of time. The effect of DDVP is more pronounced on the electrical properties of the spinal cord, while, monocrotophos affects the biochemical profile more severely than DDVP. This property may contribute to the toxicity differences in promoting delayed neuropathy by these two pesticides.

SUMMARY



In view of their high toxicity and wide spread use the effects of organophosphorus compounds assume great importance in the study of neurotoxicology. The toxicity of DDVP and monocrotophos are well documented in terms of symptomology and metabolism. Their potentiality as anticholinesterases is well known and they promote multivariate physiological, morphological and pathological effects in nearly all organisms.

The present study focuses on 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 which is their characteristic mechanism, in contributing to the overall toxicity.

DDVP and monocrotophos promote alterations in frequency, duration and amplitude of the intrinsic electrical activities of the spinal cord as well as conduction velocities of the impulses.

DDVP and monocrotophos elevate the spike and plateau phase revealing their excitatory effect on the neuronal membrane. It appears that DDVP and monocrotophos influence Na^+ , K^+ and Ca^{2+} channels responsible for spike phase and also persistent Na^+ conductance responsible for plateau phases. The simultaneous decrease of isoelectric phase frequencies, representing the period of non-excitability also indicates that DDVP and monocrotophos promotes increased neuronal excitations.

The elevations in hyperpolarisation frequency reveals the activation of calcium channels and calcium activated K^+ channels by DDVP and monocrotophos. The increase in hyperpolarisations by the aforementioned pesticides could be considered as compensatory process to reduce the excitatory spikes. However, further study to delineate the detrimental effect of increased hyperpolarisations is essential to draw accurate conclusions.

DDVP and monocrotophos fail to promote significant changes in the duration of spike phases at lower dose regimen. However, higher dose (100 ppm) promotes increase in spike duration at some time intervals. DDVP and monocrotophos may not influence the opening of Na^+ and K^+ channels for longer durations. But at higher doses these channels may be retained open for longer duration. However, this aspect needs further investigation.

The durations of plateau phases are increased by DDVP and monocrotophos, with monocrotophos being comparatively more effective. This increase are opined to be due to the retention of Ca^{2+} and Na^+ inside the nerve fibres and this retention would further initiate a cascade of calcium – dependent degenerative processes or subsequently trigger faster spikes. The fact that the durations changes are mediated through Ca^{2+} channels is confirmed by the addition of $CdCl_2$ (calcium channel blocker), which diminishes the peaks of spike.

The durations of isoelectric phases are reduced by DDVP and monocrotophos and this can be considered as a compensatory mechanism to

accommodate elevated spike frequency induced by DDVP and monocrotophos.

The baseline potentials are a reflection of resting membrane potential and they are maintained by the interaction of ionic channels. The elevated baseline potential in response to DDVP and monocrotophos especially at all time intervals in mice treated with higher doses could be mediated via the activation of Ca^{2+} channels. Development of energy crisis due to depletion of energy sources and / or nutrients could elevate the baseline potential. It could also be due to decrease in Na^+ - K^+ ATPases and / or decrease in K^+ conductance.

Conduction velocity is initially increased upto the end of 96/ 120 hours after DDVP / monocrotophos treatment .The initial hike in conduction velocity could be attributed to the anticholinergic property of these two pesticides which leads to hyperactivation. The activation could be also due to the faster Na^+ flux or early activation of K^+ conductance by these pesticides. However, subsequently the velocity reduces and in some instances it is below the normal level as time progresses i.e. by the end of 240 hours, which indicates compensatory mechanism of the neuronal membrane to restore the velocity to normal or could be due to onset of neurodegenerative changes in the spinal cord.

DDVP and monocrotophos alter the enzyme activities and the biochemical profiles to varying degrees.

DDVP and monocrotophos treatment to mice resulted in increased fragility of lysosomal membranes and a loss of latency of the content hydrolytic enzymes which is evident by the marked enhancement of acid phosphatase activity, which induces subsequent neuronal degeneration. The increase in non-specific esterases in response to both the pesticides reveals the disruption of lysosomal membranes under pesticidal stress.

DDVP and monocrotophos have a dichotomous effect on the phosphatase activity. While the activity of acid phosphatase is elevated the alkaline phosphatase activity is declined in spinal cord. This could lead to impairment of membrane transport and thereby initiate degeneration in the neuronal cells due to lack of essential metabolites.

The elevations in xanthine oxidase activity promoted by DDVP and monocrotophos is indicative of genesis of reactive oxygen species and implies the role of reactive oxygen species as a contributing factor in mediating neurodegeneration. The intrinsic defensive antioxidant enzyme, superoxide dismutase is elevated in spinal cord of mice treated with DDVP and monocrotophos, which also reveals the presence of reactive oxygen species.

The total proteins are degraded by DDVP and monocrotophos in spinal cord of mice either through direct effect or due to the secondary effect via elevations of lysosomal enzyme, acid phosphatase. The mitochondrial proteins are also declined in response to DDVP and monocrotophos

treatment, which probably reflects the susceptibility of the mitochondrial oxidative system to the action of lysosomal enzymes and reactive oxygen species, which are elevated by DDVP and monocrotophos.

The elevations of creatinine levels in spinal cord of mice treated with DDVP and monocrotophos is indicative of excessive breakdown of creatine phosphate to meet the high metabolic demands of the hyperexcited spinal cord.

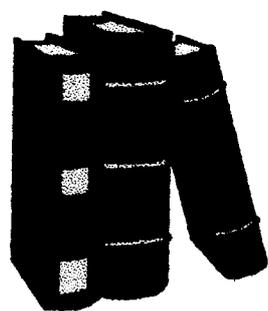
The lipid constituents i.e. cholesterol and triglycerides concentrations are elevated by DDVP and monocrotophos. The increase in cholesterol content may be a compensatory mechanism of the neuronal membrane to resist the effect of pesticides. Increase in the triglyceride contents indicates the mobilization of stored lipid reserves to meet the high metabolic demands of the spinal cord under pesticidal stress in an energy crisis as other normal energy sources are depleted.

The decreases in the glucose and glycogen profile of the spinal cord of mice treated with DDVP and monocrotophos reveals the rapid utilization of these carbohydrates resources to meet the demands of the excitatory activities of the spinal cord in response to DDVP and monocrotophos. The decrease of glycogen probably is due to the increased glycogenolysis activity yielding glucose.

RECOMMENDATIONS FOR FUTURE WORK

DDVP and monocrotophos have shown to induce alterations in the electrophysiological properties and biochemical profiles of the spinal cord. It would be of interest to further investigate the effect of these two pesticides on the electrical properties of whole cell / single ionic channels using patch-clamp technique to elucidate the exact toxicity mechanism of these pesticides on the kinetics of specific ions and modulation of ionic channels. DDVP and monocrotophos , appear to influence K^+ channels but further study to ascertain the specific type of K^+ channels being affected , is recommended. Future investigations on effect of these pesticides on intracellular calcium mobilisation and calcium accumulation is suggested. Effect of these pesticides on the spinal cord metabolism and the qualitative biochemical profile like that of various proteins, lipids and carbohydrates specific to mammalian spinal cord should be carried out.

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