1.0 INTRODUCTION

1.1.0 Organophosphorus Compounds:

Organophosphates are the group of compounds with various toxicities to different forms of life. The widest use of these compounds is as insecticides for which purpose organophosphate with toxicity relatively high to insects and low to man and other mammals are chosen. Organophosphorus compounds may be divided into two groups: The first group will inhibit the enzymes "in vitro," and as a general rule, their activity as inhibitors is proportional to their toxicity to mammals. The second group may have little or no activity as inhibitors of cholinesterase in vitro, but once inside the animal body they become changed into active inhibitors of uncertain chemical constitution. The original compound is more stable to hydrolysis and some of the groups are valuable insecticides.

Previously some organophosphates have been used medically for the management of myasthenia gravis and glaucoma, but this use has decreased because of their narrow margin between therapeutic and toxic doses. The most toxic of these compounds has been stockpiled as nerve-gases for possible use in chemical warfare. Some organophosphates are also used as plasticizer in plastic industry or as a lubricant and additive e.g.
tri-orthocresyl phosphate (TOCP), diazinon etc.

According to the W.H.O. report approximately, 1300 organophosphorus compounds have so far been prepared out of which some are not recommended for use due to their high level of mammalian toxicity. The yearly production of organophosphate is now approaching 100,000 tons. The utility of the organophosphate pesticide is undisputed today. It can be expected that they will solve many of the World's nutritional problems. These compounds form the basis of the large number of insecticides, pesticides, nematocides and fungicides widely used in agriculture. Therefore, the increasing use of the organophosphate insecticides and pesticides offers more challenging areas of study.

1.2.0 Historical Overview

Ever since the dawn of civilization, man has been waging a ceaseless war for his survival against an army of pests. Many weapons were deployed in the war against the ravages caused by pests and partial success was achieved in some instances (Table - I).

The organic phosphate pesticides had a rather sinister origin. In 1940, a group of German chemists were looking for an efficient war gas which would be absorbed through the skin and would cause rapid death or incapacity. As a by-product of their scientific devotion they synthesized a series of materials which we refer to collectively now as the organic phosphate pesticides.
<table>
<thead>
<tr>
<th>Remarks</th>
<th>Year</th>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Era of natural products</td>
<td>1690</td>
<td>Use of tobacco, soaps, phosphorus and derris root etc.</td>
</tr>
<tr>
<td>Era of fumigants, inorganics</td>
<td>1854</td>
<td>CS₂, Paris green, HCM petroleum products, lead arsenate etc.</td>
</tr>
<tr>
<td>Era of modern synthetic insecticides</td>
<td>1925</td>
<td>DDT, thiocyanates, BHC, organophosphates.</td>
</tr>
<tr>
<td>Era of hormones and pharomones</td>
<td>1967</td>
<td>First hormone mimic (juvinile) insecticides, DDT trial begins.</td>
</tr>
</tbody>
</table>

The first organophosphate insecticide was tetramethyl pyrophosphate (TEPP). It was developed in Germany as a substitute for nicotine, which was in short supply in that country during World War II. It is remarkable that the first account of the synthesis of TEPP, was published by Clermont in 1854, by alkylating the silver salt of the pyrophosphoric acid with alkyl halides.

\[
\begin{align*}
\text{C}_2\text{H}_5 & - \text{O} - \text{O} - \text{O} - \text{O} - \text{C}_2\text{H}_5 \\
\text{C}_2\text{H}_5 & - \text{O} \quad \text{P} \quad \text{O} \quad \text{O} - \text{C}_2\text{H}_5
\end{align*}
\]
This ester is the bridge between the inorganic and organic chemistry. The insecticidal activity of this compound was discovered after 80 years of its synthesis. TEPP, although an effective insecticide, was highly toxic to mammals and was rapidly hydrolyzed in the presence of moisture.

Holfmann in 1872 synthesized the corresponding phosphoric acid by reacting methyl and ethyl phosphine with nitric acid. Subsequently in 1897 Michaelis and Backer synthesized a phosphonic acid ester by the reaction of dialkyl phosphite with ethyl iodide. This reaction became well known as Michaelis - Backer reaction.

Further in 1898 Michaelis and Kaehne synthesized another compound from tryalkyl phosphite and methyl iodide whose structure was not similar to the compound which was obtained by Michaelis-Backer reaction. Michaelis (1903) again synthesized phosphorus-nitrogen compounds by the reaction of ammonia or amines with phosphorus trichloride, pentachloride, phosphoryl chloride and thiophosphoryl chloride. He synthesized N-alkylamino-dichlorophosphine (II) by the reaction of phosphorus trichloride with alkylamine, which he later oxidized with chlorine to the tetrachlorides (III), and atmospheric moisture sufficed to

\[ R_2N - PCl_2 \quad R_2N - PCl_4 \]

II III

hydrolyze the tetrachloride to dialkylaminophosphorodichloridates (IV).
Subsequently he synthesized O,O-diethyl N,N-Diethyl phosphoroamidate (V) and related compounds.

Modern investigations of the organophosphorus compounds, and the first hint of their toxicity, date from the 1932 publication of Lange and Krueger on the synthesis of dimethyl and diethyl phosphorofluoridates (VI).

It has been reported that the inhalation of the vapors of these compounds cause a persistent choking sensation and blurred vision.
Investigators in the allied countries also followed Lange and Krueger's lead in the search for potentially toxic compounds; diisopropyl phosphorofluoridate (DFP) synthesized by McCombie and Saunders (1946), was the organophosphorus compound studied most extensively by British and American Scientists. They also described the miotic and high inhalation toxicity of these compounds.

\[
\text{VII}
\]

During the synthesis and investigations of approximately 200 compounds, Scrader (1952) defined the structural requirements for insecticidal activity. One compound in this early series, "parathion" (VIII) later became the most widely employed insecticide of this class.

\[
\text{VIII}
\]

Shortly after parathion became available for study,
acute toxicity studies on experimental animals revealed signs of poisoning that resembled excessive stimulation of cholinergic nerves.

During the last two decades the agricultural chemistry industry has developed many other organic triesters of phosphoric acid and phosphorothioic acid that have been registered for use. In the same period (1960) in Germany, oxydemeton-methyl (Metasystox) was first introduced as a separate compound by the Bayer-Levekusen Company.

\[
\begin{aligned}
\text{CH}_3O & \quad \parallel \quad \text{O} \\
\text{P} & \quad \text{S-CH}_2 - \text{CH}_2 - \text{S} - \text{C}_2\text{H}_5 \\
\text{CH}_3O & \quad \parallel \quad \text{O}
\end{aligned}
\]

IX

Since, then numerous studies have been conducted on metasystox because of its wide use as an agricultural insecticide.

1.3.0 Nomenclature:

Several years ago there were four different "official" systems for nomenclature: competing British, Swedish, German and American systems. Fortunately, Anglo-American agreement was reached in 1952 for the naming of the compounds containing one atom of phosphorus and these make up the great majority of economic compounds. But the old habits die hard, and the
"international" system is not universally used.

The compounds are complex in structure, and it is often convenient to use a different part of the molecule as the key in closely related molecules. It does however, lead to situations in which, e.g. \((\text{EtO})_2 \text{Po. S. CH}_2 \cdot \text{CH}_2 \cdot \text{SEt}\) (Demeton-S), is named \(0,0\)-diethyl S-2-ethylthioethyl phosphorothiolate, and \((\text{EtO})_2 \text{PO.S.CH}_2 \cdot \text{CH}_2 \cdot \text{SEt}_2\), its ethylsulphonium derivative is named 2-(diethoxyphosphinylthio)-ethyl-diethyl sulphonium ion. Much can, however, be done by the systematic use of trivial or shortened systematic names and here the following rules are adhered to (Heaths, 1961).

At the first mention of each compound the structural formula is given, and either its systematic name on the Anglo-American system, or a brief name. Brief names have been chosen from those in common use and have been taken from the Journal of Economic Entomology or British Standards.

Groups of compounds are, when considered together, given group names, based on the Anglo-American system (Table-2). The distinction between PS and P.S.C. compounds is made by calling the former thionates and latter thiolates. The alternative, in which both are called thioates, but the substituents are prefixed by O- or S-, is only unambiguous if these substituents are named, which is inconvenient. Thus \(0,0\)-diethyl S-ethylphosphorothioate is unambiguous, but if for brevity
### TABLE - 2: Chemical Structures, Name of the Compounds and Group Names

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name of type</th>
<th>Group name</th>
</tr>
</thead>
<tbody>
<tr>
<td>(RO)(_2)PO(_2)R</td>
<td>phosphonates</td>
<td>phosphonates</td>
</tr>
<tr>
<td>(RO)(_3)PO</td>
<td>phosphates</td>
<td>phosphates</td>
</tr>
<tr>
<td>(RO)(_2)PO(_2)SR</td>
<td>phosphonothionates</td>
<td>thionates</td>
</tr>
<tr>
<td>(RO)(_3)PS</td>
<td>phosphorothionates</td>
<td></td>
</tr>
<tr>
<td>(RO)((R)PO)SR</td>
<td>phosphonothiolates</td>
<td>thiolates</td>
</tr>
<tr>
<td>(RO)(_2)PO(_2)SR</td>
<td>phosphorothiolates</td>
<td></td>
</tr>
<tr>
<td>(RO)(_2)PS(_2)SR</td>
<td>phosphorodithioates</td>
<td>dithioates</td>
</tr>
<tr>
<td>(RO)(_2)PO(OH)</td>
<td>hydrogen phosphates</td>
<td>hydrogen phosphates</td>
</tr>
<tr>
<td>(RO)(_2)PO(_2)OH</td>
<td>hydrogen phosphorothioates</td>
<td>hydrogen thioates</td>
</tr>
<tr>
<td>(RO)(_2)PO(_2)SH</td>
<td>hydrogen phosphorodithioates</td>
<td>hydrogen dithioates</td>
</tr>
<tr>
<td>R(_2)PO(_2)F</td>
<td>phosphinofluoridates</td>
<td></td>
</tr>
<tr>
<td>(RO)((R)PO)F</td>
<td>phosphonofluoridates</td>
<td>fluoride s</td>
</tr>
<tr>
<td>(RO)(_2)PO(_2)F</td>
<td>phosphorofluoridates</td>
<td></td>
</tr>
<tr>
<td>(RNH)(_2)PO(_2)F</td>
<td>phosphorodiamidic fluorides</td>
<td></td>
</tr>
<tr>
<td>(R(_2)N)(_2)PO(_2)F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(RO)((R(_2)N)PO)F</td>
<td>phosphoramidofluoridates</td>
<td></td>
</tr>
</tbody>
</table>
the 'ethyls' are omitted, phosphorothioate includes both triethyl phosphorothiolate, \((\text{EtO})_3\text{PS}\).

Where a thionate has a trivial name ending in "thion", the phosphate analogue is given the same prefix but the ending 'oxon'. Thus \((\text{EtO})_2\text{PS.O.C}_6\text{H}_4-4-\text{NO}_2\) is Parathion, and \((\text{EtO})_2\text{P.O.C}_6\text{H}_4-4-\text{NO}_2\) is Paraoxon. Most of the names formed in this way are accepted names, when the oxygen analogue would have a particularly unpronounceable name, as Gu-Oxon from Guthion, the 't' is retained, giving Gutoxon. When a trivial name exists for one member of a homologous series, other members in which both basic groups are changed are named by putting the formula of the basic group as a suffix to the trivial name.

The multiplicity of organophosphates frequently causes them to be victims of confusion between common and proprietary names. A proprietary name is the one usually allocated by the company which owns the compound; it should always be used with a capital letter and is often followed by an R to show that it is registered trade mark.

1.3.1 Nomenclature of Isomers

Some of the organic phosphorus insecticides contain two asymmetrically substituted, nonterminal atoms joined by a double bond and therefore, occur in two stereoisomeric forms.

It sometimes has occurred that the isomers of a compound were at least partially separated, characterized entomologically
or in some other way, and designated alpha- and beta- before the orientation of their moieties had been defined.

When the orientation was learned the two forms "cis" and "trans" were designated respectively. In considering the orientation of similar moieties in space across the double bond, emphasis generally was placed on carbon chains, but the rules were not precise or failed to cover some situations. This became a source of confusion in organic chemistry. The problem was in no way restricted to phosphorus compounds and had no special implications for toxicology.

A rigid set of rules was developed for determining which moiety on one side of a plane is to be compared to which moiety on the other side. Rather than imposing the new rule on the old terms, new terms were used, presumably on the basis that new wine ought to be put into new bottles. The new terms are Z (from the German Zusammen, meaning together), and E (from entgegen, meaning opposite). The entire matter has been discussed in great detail by the International Union for Pure and Applied Chemistry (IUPAC, 1970). Unfortunately the new rules for defining the precedence of moieties sometimes are opposite to earlier custom. The result is that some isomers formerly designated "cis" are now correctly designated "E", even though the terms themselves have opposite meanings. Examples include the isomers of monocrotophos, dicrotophos, mevinphos and phosphamidon.
1.4.0 **Classification of Organophosphorus Compounds:**

No chemical classification has been proposed that will divide this wide variety of organophosphorus compounds into pharmacologically and toxicologically homogeneous groups with no overlapping.

According to Holmsted (1959), Schrader noted as early as 1937 that the general formula for anticholinesterase organic phosphorus compounds is

\[
\begin{array}{c}
\text{R}_1 \\
\text{P} \\
\text{X} \\
\text{R}_2
\end{array}
\Rightarrow \text{O(or S)}
\]

in which \textit{X} is the leaving group. All the compounds may be placed in four main categories, depending on the character of the \textit{X} constituent, as follows:

(I) \textit{X} contains a quaternary nitrogen

(II) \textit{X} = F

(III) \textit{X} = \textit{CN}, \textit{OCN}, \textit{SCN}, or a halogen other than F

(IV) \textit{X} = Other moieties (Table-3).

The first of these categories are small. Although categories II and III are somewhat larger, only a very few compounds in one of them (II) have been used or even considered as pesticides.
### TABLE - 3: Examples of the Four Main Categories of Organic Phosphorus Compounds

<table>
<thead>
<tr>
<th>Group</th>
<th>X-constituents</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>substituted quaternary nitrogen</td>
<td><img src="C2H5-O_P_O_I_I__C2H5-O_S-CH2-CH2-N%5E+-(CH3)_3" alt="Structure" /> \text{ ecathiopate iodide}</td>
</tr>
<tr>
<td>II</td>
<td>F</td>
<td><img src="CH3_CH-0_P_O_CH3_F" alt="Structure" /> sarin</td>
</tr>
<tr>
<td>III</td>
<td>CN, OCN, SCN, or halogen other than F</td>
<td><img src="CH3_N_O_CH3_P_O_C6N" alt="Structure" /> tabun</td>
</tr>
<tr>
<td>IV</td>
<td>alkyl, alkoxy or alkylthio; aryl or heterocyclic; aryloxy, arylthio, or one of their heterocyclic analogs; nitrogen; or disubstituted phosphoryl groups</td>
<td><img src="C2H5-O_S_P_O_NO2" alt="Structure" /> parathion, <img src="CH3_O_P_O_CH3" alt="Structure" /> triorthocresyl phosphate</td>
</tr>
</tbody>
</table>
There seems to be no need to subdivide these categories at this time. On the other hand, category IV may be subdivided usefully into at least eight groups on the basis of their $R_1$ - and $R_2$ - constituents. Several of these groups differ either quantitatively or qualitatively in toxicity. In each of the first seven groups $R_1$ and $R_2$ are identical, with the exception that thioisomers formed by rearrangement of certain compounds are also classified as belonging to the parent group. For example, isomalathion, S-methyl isomer.

In the last group (mixed constituent compounds), $R_1$ and $R_2$ are different. In many compounds of this group, all four substitutions on the phosphorus are different and the compound is optically active. However, there are exceptions, in which the X-group or leaving group is identical to one of the R-groups, and the compound is not optically active.

1.5.0 Structure Activity Relationship:

There is a relation between the activity and chemical structure of organophosphorus compounds. Slight change in the chemical structure greatly alter the entire spectrum of biological activity. The effects of the structures of inhibitors on the rates at which they inhibit enzyme have only been investigated in any detail on ChE's. Inhibition of ChE's is therefore considered first. A phosphorus inhibitor contains three types of group:
the basic or neutral 'B' groups, the acidic group, X, and the atom from the sixth period of the periodic table, usually oxygen or sulphur, doubly bonded to the phosphorus. Each type of group has a typical effect on activity. In addition there are certain steric and charge effects.

1.5.1 **Basic Groups:** Change in the basic groups in phosphorus compounds, produces a complex variation in rates of inhibition, and this variation is different according to whether the enzyme inhibited is an AChE or a ChE.

There is no general relationship between the rates at which compounds are hydrolyzed and the rates at which they inhibit ChE's, because the effects of the basic groups on hydrolysis rates are mainly 'electronic', and on rates of inhibition mainly 'steric'.

1.5.2 **Acidic Groups:** When changes in structures are confined to the acidic group, X, there is generally close correlation between rates of hydrolysis and rates of inhibition. Rates of hydrolysis depend primarily on the strength of the acidic, HX. Exceptions are cyanidates, thiolates, and phosphate esters containing
vicinyl double bonds (P.O.C:C), which hydrolyse abnormally rapidly.

1.5.3 P:O and P:S Groups: Thionates isomerize or are oxidized readily to more active compounds. The $p_{50}^T$ of 'parathion' is thus about 4 less than the $p_{50}^T$ of its oxygen analogue, 'paraoxon', i.e. paraoxon reacts about 10,000 times faster. Perhaps the P=O oxygen in phosphates is hydrogen bonded to the enzyme surface, and this facilitates the reaction. Sulphur has much less ability to form hydrogen bonds, so the thionates react more slowly. It is interesting in this respect that the thiono-analogues of ACh and related esters are not hydrolysed by AChE and other esterases (Bergmann et al. 1958).

Biological activity of organophosphorus compounds therefore, vary according to the change in structure or even the change in the same group as shown in Table-4.

<table>
<thead>
<tr>
<th>Neurotoxic</th>
<th>Non-Neurotoxic</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>C$_2$H$_5$</td>
<td>BONDY et al., 1961.</td>
</tr>
<tr>
<td>CH$_3$X</td>
<td>C$_2$H$_5$X</td>
<td>BONDY et al. 1960</td>
</tr>
<tr>
<td>(O-o-I)P=O$_2$</td>
<td>(O-o-I)P=O$_2$</td>
<td></td>
</tr>
</tbody>
</table>
1.6.1 Molecular Mechanism of Action: The most significant and unambiguous molecular mechanism of action universally accepted as the model for enzyme inhibition studies is the action of organophosphates through phosphorylation of the hydroxyl groups of serine in the active site of the vital enzyme acetylcholine esterase (AChE) of the target organism. A scheme for substrate and inhibitor interactions with acetylcholinesterase is shown in Fig. 1. The overall reaction can be illustrated by considering the organophosphate insecticides as substrates:

\[
\begin{align*}
  & \text{EOH} + \text{AX} & \xrightarrow{K_1} & \text{EOH} \cdot \text{AX} \xrightarrow{K_2} X^- + H \\
  & \text{EOH} \cdot \text{AX} & \xrightarrow{K_3} & \text{EOH} + A^- + H^+ \\
  & \text{ACETYL CHOLINESTERASE (AChE)} & \text{ANIONIC SITE} & \text{ESTERIC SITE} & G = \text{ACTIVE CENTER} & \text{SERINE} & -\text{glu} - \text{NH} - \text{CH} - \text{CO} - \text{ala} \\
  & & \text{CH}_2 & \text{OH} & \text{ACTIVE CENTER}
\end{align*}
\]
Fig. 1. Active and inactive forms of acetylcholine esterase: Mechanism of inhibition by organophosphate
There are three important steps in the reaction - (1) Complex formation, (2) Phosphorylation, (3) Dephosphorylation. Complex formation is governed by an affinity constant $K_a$ (i.e. $K = 1/K_1$). This is quite small for the organophosphate insecticides as well as the natural substrate acetylcholine; therefore, the enzyme substrate or enzyme inhibitor complex ($EOH\cdot AX$) is favoured. With acetylcholine $K_2$ and $K_3$ are very fast so that total reaction occurs rapidly and new enzyme is regenerated. With organic phosphates is moderately fast, but $K_3$ is extremely slow; so $EOA$ accumulates while $EOX\cdot AX$ is minimal. The third step ($K_3$) is the most critical and slowest.

To summarize, phosphorus inhibitors react chemically with the enzymes, they inhibit by phosphorylation of them. The reaction is progressive (amount of reaction(inhibition) increases with time), non-competitive and not readily reversed. Reaction may be preceded by the formation of an adsorption complex, but, if so, the complex is very unstable.

1.6.2 **Mechanism of Nerve Transmission**: The central role of AChE inhibition in the mode of action of many systemic insecticides and the involvement of some phase of nervous activity in their residual toxicity to the non-target mammalian system, make it obligatory to appreciate the elementary mechanistics of synaptic transmission.
The central nervous system is made up of brain and spinal cord. CNS communicates with the rest of the body by the peripheral nervous system. The communication channel is through cable lines of axon made up of innumerable neurons (Structural and functional unit of the nervous system).

The transmission of the nervous impulse is an electrophysiological process in which the current is carried by monovalent ions (Fig. 2). Propagation of nerve impulse coincides with changes in the permeability of the axon membrane. The axon interior is rich in potassium and poor in sodium ions; the fluid outside has, however, a reverse composition. When a nerve impulse is generated, a flood-gate opens and lets sodium ions pour into the axon making the interior milieu locally positive. Following the impulse, the "sodium gate" closes and opens a "potassium gate" allowing potassium to efflux out, restoring the normal negative potential (Castillodel and Kotz, 1957; Eccles, 1957). Between the meeting point of a neuron and an axon is the synaptic junction 20-30 nm wide. The electrical impulse is sent across by chemical transmitters. The arrival of the nerve impulse at presynaptic membrane triggers the release of acetylcholine from "vesicles" or storage sites located in presynaptic cells. After passage across the synaptic cleft, acetylcholine gets itself attached to a "binding site" on the post-synaptic cell (Fig. 3). The liberated acetylcholine is not allowed to stay too long in the free
Fig. 2 - Diagrammatic representation of the basic principle of nerve impulse transmission.

Fig. 3 - Ion fluxes and membrane involvement in nerve impulse transmission.
state and is immediately broken down to acetic acid choline by the hydrolytic enzyme acetylcholinesterase.

The inhibition of acetylcholinesterase leads to the accumulation of acetylcholine and prevents the smooth transmission of nervous impulses across the synaptic gap. The resulting disturbances in electrophysiology cause loss of muscular coordination, induction of convulsions and ultimately death.

1.7.0 Neurotoxicity of Organophosphorus Compounds

Some organophosphate pesticides do constitute a serious threat of neurotoxicity. Numerous research programs have been directed towards an understanding of the structural requirements of these neurotoxic esters (Davies et al., 1960; Abou-Donia, 1979). It was found that some compounds that produce moderate, inhibition of cholinesterase are neurotoxic while others that produce profound prolonged inhibition are not neurotoxic. When neurotoxicity occurs, its course does not parallel the degree of cholinesterase inhibition (Cavanagh, 1964; 1973). The idea that inhibition of cholinesterase is related to neurotoxicity, has been dropped (Aldridge and Barnes, 1966b), but it is difficult to abandon the idea that some antiesterase activity is of importance.

The esterase that is phosphorylated by neurotoxic organic phosphorus compounds has been called "Neurotoxic esterase" (NTE)
(Johnson, 1969b). Some compounds are direct inhibitors of NTE, others are indirect inhibitors, requiring metabolic change in order to become active. For example, activation of neurotoxic phosphonothionates to the phosphonates is required before they are active inhibitors of NTE (Johnson, 1975).

All neurotoxic compounds examined by the test cause 75% or more inhibition of NTE, usually within 24 hours (Johnson, 1975a). The NTE test has been made both more selective and sensitive and also easier to perform (Johnson, 1977). There is a good correlation between the in vitro reactivity of brain neurotoxic esterases derived from hens and people (Lotti and Johnson, 1978). Demonstration of a correlation coefficient of + 0.88 for inhibition of NTE in brain and in lymphocytes opens the possibility of using the test in man (Dudek et al., 1979).

So far, the NTE test has two limitations. First, results of the test do not explain the delay inherent in classical neurotoxicity (Johnson, 1969a). Second, the test usually is carried out on homogenates of brain, a tissue that shows little if any functional or morphological change in polyneuropathy.

Hence, most of the organophosphorus compounds that produce neurotoxicity of any sort produce either the delayed irreversible or the immediate reversible form leading to a
great spectrum of effects and in spite of some progress, there is still much mystery about why some compounds of phosphorus are neurotoxic and other are not.

1.8.0 Reports Available on the Poisoning due to Organophosphate Insecticides

The incidence of poisoning has been reported from various parts of the world. Statistical surveys of mortality and morbidity resulting from acute exposures to insecticides reveal that children are the victims of a high percentage of accidental fetal poisonings. The greatest incidence has been reported from Japan (population 98.9 million), where there were 19,436 cases of organophosphate insecticide poisoning during seventeen years, including 10,031 accidental and 9,405 suicidal or homicidal cases, with 9,460 deaths by the Ministry of Health and Welfare (Japanese Government, 1954-1970). In Finland (population, 4.7 million) there were 286 deaths during six years, including 237 suicide, forty two accidents and seven homicides (Toivonen et al., 1959). In Denmark 273 deaths occurred during six years including 263 suicides, six accidents and four homicides (Frost and Paulsen, 1964). In California, there were 950 cases of poisoning during four years, including 789 agricultural, ninety one industrial and seventy other causes (Department of Public Health, State of California, 1957-1960). In Dade country, Florida, sixty five deaths were recorded during 12 years,
including twenty seven suicides, twenty four accidents, eleven homicides and three occupational poisoning (Reich et al., 1968). Incidents of mass poisoning by gross contamination of food with organophosphates has been reported from India, Egypt, Singapur and Mexico. An "epidemic" of poisoning due to malathion, an organophosphate that is generally considered safe, occurred among field workers in a malaria control program in Pakistan (Baker et al., 1978). Out of the 75,000 workers involved in this mosquito control program 2,800 were estimated to have had at least one episode of malathion intoxication. Five of these were fatal cases. In United States the incidence of poisoning by organophosphate insecticides has been lower than the incidence of poisoning by some other pesticides such as arsenicals and cyanide compounds (Hayes, 1959). Somehow, approximately 20,000 Americans were paralysed in 1930 when ginger got adulterated with lindol (Aring et al., 1941; Aring, 1942). Eleven cases of paralysis were observed in Holland (Moeschlin, 1952) and sixty in Germany (Creutzfeldt and Orzechwak, 1941-1943). Another outbreak of paralysis occurred in Durban (1957) when eleven Africans used water stored in drums taken from a paints factory. Many other cases of paralysis have been reported from different countries including England, Germany and India (Vora et al., 1962).

According to a report from California, out of the 175 cases of systemic poisoning, organophosphate insecticides
were responsible for 80 percent, and they were involved in 47 percent of another 160 reports of digestive and other non localized symptoms of illness. This high contribution of systemic poisonings due to organophosphates continued the record of several years (from 1956 to 1969 from 125 to 407 reports of systemic occupational poisonings were reported annually in California).

Furthermore, there have been repeated cases of multiple poisoning among agricultural workers engaged in picking fruits sprayed with the highly toxic organophosphate insecticides parathion (Spear et al., 1975). Sachitanand (1983) is of the view that pesticides threaten the welfare of the poor in the Third World Countries, because of their free availability, lack of adequate protection, improper storage, excessive and wasteful use leading to environmental pollution.

1.9.0 Complexity of Environmental Problems

Increasing use of pest control chemicals has generated serious problems of environmental pollution. It is apparent that there are many sources of exposure of humans and other nontarget species to pesticides by direct contact with materials at the site of application. In recent years, however, it has become increasingly apparent that exposures to pesticides far remote from the source of application are also possible. This results from the translocation of the chemical from
their sites of application through the various media of the environment. The extent to which translocation within the environment occurs will depend to a large degree on the physicochemical properties of the pesticides. Perhaps one of the most important factors is the extent of and time required for degradation of chemicals to simpler non-toxic forms.

Other nonbiologic modes of translocation include vaporization and drift by airborne routes so that materials are carried by prevailing wind patterns far remote from their site of application. Subsequently they may be precipitated out by rainfall onto land surface waters in areas in which the pesticides have not been applied directly. Application to the soil may result, ultimately, in suspension of the pesticides, absorbed on soil particles, and airborne translocation as dust. The extent to which pesticides will remain in soil after application depends upon a number of factors: such as soil type, moisture, temperature, pH, micro-organism content, degradability of the pesticide itself, and the extent of cultivation and cover crops (Lichtenstein, 1966). Interestingly the chemical changes of the parent insecticides that result from either physicochemical reactions in the environment or biologically catalyzed reactions may lead to products with either greater or lesser potential for biotransformation. However, the build up of residues of non-biodegradable pesticides in human
Fig. 4: Complexity of environmental problems posed by pest control chemicals.
and animal tissues has caused the greatest concern from health point of view (Newman, 1965 and Upchurch, 1974) (Fig. 4).

1.10.0 **Sign and Symptoms of Toxicity of Organophosphorus Compounds**

The usual symptoms of organophosphate poisoning include: headache, giddiness, nervousness, blurred vision, weakness, nausea, cramps, diarrhoea and discomfort in chest etc.

Signs and symptoms of acute poisoning by organophosphate insecticides are predictable from their biochemical mechanism of action. Thus the inhibition of acetylcholinesterase by organophosphates results in accumulation of endogenous acetylcholine in nerve tissues and effector organs with consequent signs and symptoms that mimic the muscarinic, nicotinic, and central nervous system actions of acetylcholine.

**Muscarinic receptors for acetylcholine are found primarily in smooth muscles, the heart, and exocrine gland.** Signs and symptoms of organophosphorus insecticide poisoning that result from stimulation of these receptors include tightness in the chest and wheezing expiration due to bronchoconstriction and increased gastrointestinal tone etc.

**Nicotinic actions at the neuromuscular junctions of skeletal muscle usually consist in scattered fasciculations, muscular twitching, cramp weakness, fatiguability and paralysis.**
TABLE 5: Sign and Symptoms of Organophosphate Poisoning

**MUSCARINIC MANIFESTATIONS:**
- Bronchial tree: Tightness in chest, bronchoconstriction, dyspnea increased, bronchial secretion, cough, pulmonary edema, cyanosis.

**GASTROINTESTINAL SYSTEM:**
- Sweat Glands: Nausea, vomiting, abdominal tightness, diarrhea, fecal incontinence.
- Salivary Glands: Increased Sweating
- Lacrimal Glands: Increased Salivation
- Cardiovascular System: Increased Lacrimation
- Pupils: Bradycardia, fall in blood pressure, Miosis occasionally unequal
- Ciliary body: Blurring of vision
- Bladder: Frequency, urinary incontinence.

**NICOTINIC MANIFESTATIONS:**
- Striated Muscle: Fasciculation, muscular twitching, cramp, weakness in respiratory muscle.
- Sympathetic Ganglia: Giddiness, tension, anxiety, restlessness, emotional lability, excessive dreaming, insomnia, headache, apathy, drowsiness, coma, convulsions, depression of respiratory and circulatory centres and fall in blood pressure.

Modified by Grob and Harvey (1953).
The most serious consequence of neuromuscular action is paralysis of the respiratory muscles.

Accumulation of acetylcholine in the central nervous system is believed to be responsible for the broad spectrum of effects such as, giddiness, tension, anxiety, restlessness, emotional lability, excessive dreaming, insomnia, headache, etc. Depression of respiratory and circulatory centres and fall in blood pressure are other CNS effects (Table 5). Effects may be localized or systemic (Table 6 and 7).

TABLE - 6 : Signs and Symptoms of Organophosphate Poisoning Following Local Exposure

<table>
<thead>
<tr>
<th>Site of action</th>
<th>Signs and symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupils</td>
<td>Miosis, marked, usually maximal (pin-point), some times unequal.</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>Frontal headache, eye pain on focusing, slight dimness of vision, occasional nausea and vomiting.</td>
</tr>
<tr>
<td>Conjunctivae</td>
<td>Hyperaemia</td>
</tr>
<tr>
<td>Nasal mucous membrane</td>
<td>Rhinorrhoea, hyperaemia</td>
</tr>
<tr>
<td>Bronchial tree</td>
<td>Tightness in chest, some times with prolonged wheezing expiration suggestive of bronchoconstriction or increased secretion, cough.</td>
</tr>
<tr>
<td>Sweat glands</td>
<td>Sweating at site of exposure to the liquid.</td>
</tr>
<tr>
<td>Striated muscle</td>
<td>Fasciculations at site of exposure to the liquid.</td>
</tr>
</tbody>
</table>
TABLE 7: Signs and Symptoms of Organophosphate Poisoning Following Systemic Absorption

<table>
<thead>
<tr>
<th>Site of action</th>
<th>Signs and symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupils</td>
<td>Slight moisis (occasionally unequal), later more marked.</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>Burning of vision.</td>
</tr>
<tr>
<td>Bronchial tree</td>
<td>Tightness in chest, with prolonged wheezing expiration suggestive of bronchoconstriction or increased secretion, dyspnoea, slight pain in chest, increased bronchial secretion, cough.</td>
</tr>
<tr>
<td>Gastro-intestinal</td>
<td>Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness with &quot;heartburn&quot; and eructation, diarrhoea, tenesmus, involuntary defecation.</td>
</tr>
<tr>
<td>system</td>
<td></td>
</tr>
<tr>
<td>Sweat glands</td>
<td>Increased sweating.</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>Increased salivation.</td>
</tr>
<tr>
<td>Lachrymal glands</td>
<td>Increased lachryration.</td>
</tr>
<tr>
<td>Heart</td>
<td>Slight bradycardia.</td>
</tr>
<tr>
<td>Bladder</td>
<td>Frequent or involuntary micturition.</td>
</tr>
<tr>
<td>Striated muscle</td>
<td>Easy fatigue, mild weakness, muscular twitching, fasciculations, cramps, generalized weakness including muscles of respiration, with dyspnoea and cyanosis.</td>
</tr>
<tr>
<td>Sympathetic ganglia</td>
<td>Pallor, occasional elevation of blood pressure.</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Giddiness; tension; anxiety; jitteriness; restlessness; emotional lability; excessive dreaming; insomnia; nightmares; headache; tremor; apathy; withdrawal and depression; bursts of slow waves of elevated voltage in EEG especially on overventilation; drowsiness; difficulty in concentrating; slowness of recall; confusion; slurred speech; ataxia; coma with absence of reflexes; cheynesstokes respiration; convulsion; depression of respiratory and circulatory centres with dyspnoea; cyanosis and fall in blood pressure.</td>
</tr>
</tbody>
</table>
1.11.0 **Diagnosis and Treatment of Poisoning:**

1.11.1 **Diagnosis:** Diagnosis is of three kinds: diagnosis of incipient cases, showing no overt symptoms, diagnosis in casualties, and diagnosis of post-mortem.

Diagnosis of incipient cases is carried out by estimating the AChE activity of blood samples or the ChE activity of plasma samples. Such tests are carried out routinely on many engaged in spraying or manufacturing phosphorus insecticides. They show whether a worker has absorbed a phosphorus compound or not, and consequently whether he is likely to be unusually sensitive to a further dose. ChE is lowered faster than AChE by most phosphorus poisons, and there are no cases reported in which it is not lowered as fast initially, but ChE may recover faster than AChE, so that the ChE level may lead to an underestimate the residual effects of a dose received some time earlier.

Therefore, ChE determinations are a reliable and sensitive method of diagnosing its early stages.

Diagnosis in casualties depends mainly upon recognizing the very general nervous symptoms. It may be confirmed by giving about 2 mg of atropine. In normal subjects this causes marked atropinization, but in a case of phosphorus poisoning relieves the symptoms,
without atropinizing (Golz, 1959). Estimations of ChE are confirmatory.

Diagnosis at autopsy may be of financial importance to dependants of the deceased. There are no gross pathological changes and diagnosis depends mainly upon determinations of AChE either in erythrocytes or histochemically at myoneural junctions (Petty, 1958).

1.11.2 Treatment of Poisoning in Man: Organophosphates if in sufficient doses may have a rapid fatal outcome. It is important therefore, to take emergency action including, removal of the toxic agent and decontamination of exposed skin with an alkaline solution of soap and water, even though complete history may not have been obtained.

In very serious cases of poisoning by organic phosphorus insecticides, the order of treatment should be as follows:-

(1) artificial respiration, preferably by mechanical means;

(2) atropine sulfate (2 to 4 mg) intravenously and repeated at 5 to 10 minute intervals until signs of atropinization appear;

(3) paraadoxime chloride (1gm) slowly, intravenously;
(4) decontamination of the skin, stomach, and eyes and
(5) symptomatic treatment (e.g. inhalation of oxygen).

In usual cases, the procedure should be as follows:-

(1) atropine sulphate (1 to 2 mg) if symptoms appear, and repeated doses as required by excessive secretions, (2) decontamination of the skin, stomach and eyes; (3) paralidoxime chloride (1gm) slowly intravenously if the patient fails to respond satisfactorily to atropine; and (4) symptomatic treatment.

11.1.3 Treatment of Poisoning in Animals: Atropine, enzyme reactivators and symptomatic care, constitute the most important means of treating poisoning by organic phosphorus compounds in man. Their value is so firmly established by pharmacological theory and clinical experience that there is no need to review studies in animals that first explored and later confirmed their value. It is desirable to review animal experiments exploring certain other compounds that may be useful for treating poisoning by organic phosphorus compounds.

Glucose: Glucose apparently gave some protection when 3 ml of a 20% aqueous solution were injected intraperitoneally in rats poisoned by parathion, parathion-
methyl, and demeton (Diechmann and Rakoczy, 1953). Glycogen stores may be depleted during poisoning, but this is more likely to be significant in small animals.

Cysteine: It was reported that cysteine protected animals intoxicated by parathion (Botescu et al., 1970) but the pharmacological basis is not clear, and the finding apparently has not been reinvestigated.

Vitamin C: According to Karimov (1974), there is a significant reduction in the concentration of vitamin C in the blood and urine of people considered to have sequelae of acute poisoning. The same was true of rabbits receiving 3.8 mg/kg/day of dimethoate plus 3.1 mg/kg/day of lindane for 3 months. Vitamin C at the rate of 30 mg/kg/day restored the normal blood level of this vitamin, reduced the abnormally high blood alkaline phosphatase level, and was considered to improve the detoxifying power of the liver. It was recommended that vitamin C, unlike vitamin B₁, should be used in treating chronic poisoning by pesticides.

Reduced Glutathione (GSH): In pigs, plasma choline-sterase activity that had been depressed by parathion-methyl was increased temporarily by GSH (Matsueda et al. 1972b). The reported results do not yet justify use
of multiple doses of GSH in human therapy, but further studies should focus on influences of GSH on the rate of metabolism of different organic phosphorus compounds and on the survival of poisoned animals.

1.12.0 Metasystox- an Organophosphate

Metasystox-R, (O,O-dimethyl-S-2(ethylsulphinyl) ethylthiophosphate) sometimes called Oxydemeton methyl or methylisosystox sulfoxide, is a systemic organophosphate insecticide exhibiting marked specificity in its action against spider, mites, aphids leafhoppers and similar plant sucking insects. It features good plant tolerance. Beneficial insects, such as larvae of Coccinellidae and Syrphidae, remain very largely unharmed by application of metasystox. It is also unique because of its total water solubility as well as being soluble in most organic solvents.

1.13.0 Synonyms

Metasystox is the trade name for Oxydemeton-methyl. The compound can be accurately described as demeton-S-methyl sulfoxide by reference to the ISO-approved name of the parent compound, and this term is sometimes used as a name. However, in Germany, a different convention has been used by which reference is made to O, and thus the term, demeton-O-methyl sulfoxide to the compound. However, the term demeton-S-methyl-
sulfoxide also is used in Germany. Ossidemeton-metile is the term used in Italy, and metilmekaptofrosoksis is used in USSR. Other common names include isomethylsystox-sulfide and meta-isosystox sulfoxide. Code designations for metasystox have included Bayer-21097, ENT-24964, and R-2107, the CAS registry number is 301-12-2.

1.14.0 Chemical and Physical Properties

Metasystox has the following physico-chemical properties:

Chemical name: O,O-dimethyl-S-(2-ethylsulphinyl)ethylthiophosphate
Common name: Demeton-O-methylsulphoxide (Germany) Oxydemeton-methyl (England)

Structural formula:

\[
\begin{align*}
\text{CH}_3\text{O} & \quad \text{O} \\
\text{P} & \quad \text{S} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{S} \\
& \quad \text{C}_2\text{H}_5
\end{align*}
\]

Empirical formula: \( \text{C}_{15}\text{H}_{15}\text{O}_{4}\text{PS}_2 \)

Appearance and odour: Yellowing liquid, practically odourless.
Molecular weight: 246.3
Boiling point: \( 106^\circ\text{C} \) at \( 0.01 \text{ mm.Hg} \)
Volatility:
- \( 0.09 \text{ mg/m}^3 \) at \( 20^\circ\text{C} \)
- \( 0.3 \text{ mg/m}^3 \) at \( 30^\circ\text{C} \)
- \( 0.7 \text{ mg/m}^3 \) at \( 40^\circ\text{C} \)
Specific gravity : 1.289 at $\frac{20^\circ}{40^\circ} C$

Refractive index : $n^20_D = 1.5216$

Solubility : Miscible with water in any ratio.
Soluble in most organic solvents, but practically insoluble in petroleum ether.

Stability : In aqueous solution, the active ingredient slowly hydrolysis.
It is not stable in alkaline media.

1.14.1 Formulations

Metasystox$^R$ is available as a 50% solution concentrate and a 25% emulsifiable concentrate. Formulation retains full biological activity for at least 2 years when stored at 20 to 30$^\circ$C.

1.15.0 Metabolism of Metasystox

The metabolism of metasystox (demeton-methyl) in plants and animals was studied by Fukuto et al. (1955). Similar studies with methyl analog of PO-demeton, were carried out by Muhlmann and Tietz (1956). These investigators showed that metasystox, like PO-demeton, is converted to the corresponding sulfoxide and sulfone. It seemed likely that the action
of metasystox would be due mainly to the sulfoxide and sulfone of the O,O-dimethyl-S-ethyl thioethyl phosphorothiolate produced metabolically both in plants and animals. In addition to isomerizing, it reacts with itself to form sulfonium ions of greatly increased toxicity and anticholinesterase activity as shown in Fig. 5:

\[
\text{(Unstable inhibitor)}
\]

\[
\begin{align*}
\text{(CH}_3\text{O)}_2 \text{P} & \xrightarrow{\text{SCH}_2\text{CH}_2\text{SC}_2\text{H}_5} \text{(CH}_3\text{O)}_2 \text{P} \text{SCH}_2\text{CH}_2\text{SC}_2\text{H}_5 \\
\text{SCH}_2\text{CH}_2\text{SC}_2\text{H}_5 & \rightarrow \text{(CH}_3\text{O)}_2 \text{P} \text{CH}_3 \text{SCH}_2\text{CH}_2\text{SC}_2\text{H}_5
\end{align*}
\]

\[
\text{(methylsulfoniothiollate)}
\]

\[
\begin{align*}
\text{+ CH}_3\text{O} & \xrightarrow{\text{SCH}_2\text{CH}_2\text{SC}_2\text{H}_5} \\
\text{SCH}_2\text{CH}_2\text{SC}_2\text{H}_5 & \rightarrow \text{SCH}_2\text{CH}_2\text{SC}_2\text{H}_5
\end{align*}
\]

\[
\text{(... (2))}
\]

Fig. 5 : Schematic representation of metabolic pathways of metasystox.
1.16.0 Toxicity of Metasystox

1.16.1 Toxicity to Animals: Metasystox (Oxydemeton-methyl) is a compound of moderate acute toxicity, as shown in Table(8). Rats given dietary levels of 200 and 100 ppm (about 10 and 5 mg/kg/day) not only showed marked inhibition of cholinesterase activity but also signs of intoxication during the first 3 weeks (Vandekar, 1958). In another study, no illness or pathology was produced by 5 mg/kg for 3 months (Wirth, 1958). A dietary level of 50 ppm also inhibited cholinesterase but produced no signs of illness and did not interfere with growth (Vandakar, 1958).

1.16.2 Toxicity to Man: Some cases of poisoning by metasystox involved relapse in the course of a long illness. In one instance, a 17 year old girl was found unconscious with an empty bottle beside her. She was in a coma, and had nearly all the signs of anticholinesterase poisoning except pulmonary edema. She was hospitalized 12 to 15 hours after the estimated time of ingestion. She received first aid treatment, including gastric lavage, assisted respiration, atropine and 2 PAM. Her condition began to deteriorate on the second day. In spite of vigorous treatment, there was no more change. A blood transfusion was performed on the 10th day. The
<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (mg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat, m</td>
<td>oral</td>
<td>65</td>
<td>Heath and Vandekar, 1957.</td>
</tr>
<tr>
<td>rat, m</td>
<td>oral</td>
<td>65</td>
<td>DuBois and Plzak, 1962</td>
</tr>
<tr>
<td>rat, m</td>
<td>oral</td>
<td>30-75</td>
<td>Schrader, 1963.</td>
</tr>
<tr>
<td>rat, m</td>
<td>oral</td>
<td>47</td>
<td>Gaines, 1969.</td>
</tr>
<tr>
<td>rat, f</td>
<td>oral</td>
<td>52</td>
<td>Gaines, 1969.</td>
</tr>
<tr>
<td>rat, m</td>
<td>derm</td>
<td>173</td>
<td>Gaines, 1969.</td>
</tr>
<tr>
<td>rat, f</td>
<td>derm</td>
<td>158</td>
<td>Gaines, 1969.</td>
</tr>
<tr>
<td>rat,</td>
<td>iv</td>
<td>47</td>
<td>Heath and Vandekar, 1957.</td>
</tr>
<tr>
<td>mouse, m</td>
<td>ip</td>
<td>12</td>
<td>DuBois and Plzak, 1962.</td>
</tr>
<tr>
<td>g pig</td>
<td>oral</td>
<td>120</td>
<td>DuBois and Plzak, 1962.</td>
</tr>
<tr>
<td>g pig</td>
<td>ip</td>
<td>30</td>
<td>DuBois and Plzak, 1962.</td>
</tr>
<tr>
<td>rabbit</td>
<td>oral</td>
<td>104</td>
<td>Khaitov et al., 1971.</td>
</tr>
</tbody>
</table>
patient was released after 41 days with all clinical and laboratory findings normal (Goebel et al., 1969).

Another poisoning (involving relapse) was the results of injection of a mixture of 20% metasystox and 17.5% parathion with suicidal intent (Knolle, 1970).

A third example involved a suicide attempt by a 40 year old woman. She received gastric lavage 2 hours after ingestion and was hospitalized 4.5 hours after ingestion. Approximate treatment with drugs had led to a regression of miosis, however, the patients suffered cardiac arrest at 48 hours and again at 72 hours after admission. Heart action was restored each time by external cardiac massage. Clinical recovery was essentially complete by the 8th day. Plasma cholinesterase had returned to normal by the 13th day (Gaultier et al., 1975).

1.17.0 Treatment of Metasystox Poisoning

The efficacy of atronine and pyridine-2-aldoxime methiodide (2-PAM) given separately and in combination was investigated in rats acutely poisoned by metasystox (DuBois and Plazak, 1962). The influence of 1,1-trimethylenebis-4-formylpyridinium bromide (TMB-4) on acute poisoning by this organic phosphate was also tested by DuBois and Plazak (1962).
TABLE-9: Protective Effect of Atropine Sulfate, 2-PAM, and TMB-4 Against the Acute Toxicity of Metasystox to Rats (DuBois and Plzak, 1962)

<table>
<thead>
<tr>
<th>Antidote</th>
<th>Dose of antidote (mg/kg)</th>
<th>Number of Rats</th>
<th>Approx. LD$_{50}$ of Metasystox (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>40</td>
<td>21</td>
</tr>
<tr>
<td>Atropine sulfate</td>
<td>100</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>2-PAM</td>
<td>100</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td>TMB-4</td>
<td>75</td>
<td>24</td>
<td>70</td>
</tr>
<tr>
<td>Atropine sulfate</td>
<td>100</td>
<td>26</td>
<td>80</td>
</tr>
<tr>
<td>plus 2-PAM</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These experiments demonstrated that the combination of atropine sulfate with 2-PAM provided mere protection than either agent alone as evidenced by a four-fold increase in the LD$_{50}$ of metasystox. The results of these measurements are summarized in Table-9 (DuBois and Plzak, 1962).
1.18.0 **Brain Lipids**

Lipids are biological materials containing a mixture of compounds of widely differing chemical composition. The brain is rich in lipids, which constitute structural elements of the plasma membrane, components of ion channels, comprise portions of neurotransmitter receptors, and are major constituent of myelin. The range of values for myelin total lipid is 60-80% of dry weight (White et al. 1978). The important lipids of the central nervous system are cholesterol, cerebrocides and phospholipins, lecithin, sphingomyelin and kephalin (Johnson, McNab and Rossiter, 1948).

Waelsch, Sperry and Stoyanoff (1941) have studied that after birth lipid is deposited in the brain as a result of two processes, (a) growth and (b) myelination. Immediately after birth and before myelination is complete there is an active deposition of brain lipids (Fries, Changus and Chaikoff, 1940). The lipid class distribution among animals of the same species is highly variable. Thus, brains of rats and mice frequently have less cerebroside, sulfatide and correspondingly more phosphatidylcholine and phosphatidyl ethanolamine than human brain, but some rodent brains have the same high sphingolipid levels found in human brain. The data of Galli et al. (1970) indicate that rat brain may have sphingomyelin levels as high as in human brain.
Surrounding nerve axons and dendrites in the peripheral nervous system, the cell bodies of sensory ganglion cells and the nerves of the white matter of the central nervous system is a sheath of myelin formed by the neurilemmal or Schwann cells. The complete lipid analysis of myelin from different species has been published by various workers (Autilio et al., 1964; Eichberg et al., 1964; O’Brien, 1965). The protein content is characteristically low, about 22% for light myelin and 26–29% for heavy myelin (Autilio et al., 1964; Fong, 1966b) and conversely the lipid content, about 78% (Autilio et al., 1964) is higher than any other brain subcellular fraction. Early analysis of white matter revealed that cholesterol, sphingomyelin, and cerebroside were present in larger amounts than in the grey matter (Johnson et al., 1948). Furthermore, a comparison of the lipids in the newborn and the adult brain showed little change in the grey matter with maturation, but increasing amounts of free cholesterol, cerebroside, and sphingomyelin in the white matter as a function of age (Johnson et al., 1949a). Cumings (1953, 1955) compared the lipids of demyelinated lesions in multiple sclerosis with those of normal areas of the brain and found a decrease in sphingomyelin, cerebroside, and free cholesterol. Similar findings were made in demyelination resulting from Wallerian degeneration of the peripheral nerve (Rossiter, 1961; Berry et al., 1965).
The available literature on total lipids indicate that the knowledge of organophosphate toxicity on brain lipids is inadequate. Since the brain regions show remarkable heterogeneity in lipid levels, it is reasonable to investigate the neurotoxicants influence on different regions of the brain. The present study deals with the effect of metasystox on lipid concentration in different regions of the rat brain.

1.19.0 Phospholipids in the Brain

Phospholipids are the primary lipid components of plasma membranes, organic membranes and are important in myelin membranes as well. The importance of phosphorus containing lipids of the central nervous system, presumably dependent upon their role as membrane constituents. The analytical figures show that the phospholipid composition of myelin isolated from peripheral nerve is very similar to that of brain myelins (Table 10) but there is a significant increase in the phospholipid:cholesterol ratio as compared with myelin from the brain of the same animal. Certain phospholipids were found to be more concentrated in the white than in grey matter, and since their accretion parallels myelination, it was proposed that these lipids were characteristic components of the myelin sheath (Fumagalli and Pao Letti, 1963). The metabolically active polyphosphoinositides are located in the excitable
membrane of the neurone, particularly the exolemma. This distribution in brain of the enzymes responsible for the biosynthesis of the compounds and the influence of cations (e.g. Ca$^{++}$) on the enzymes suggests that the phosphorylation and dephosphorylation is at least partially responsible for the regulation of axonal membrane permeability to Na$^+$ and K$^+$ ions.

**TABLE-10: Phospholipid Ratios for Myelin from the Central and Peripheral Nerve System**

<table>
<thead>
<tr>
<th></th>
<th>Rat myelin</th>
<th>Guinea pig myelin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNS</td>
<td>PNS</td>
</tr>
<tr>
<td>Choline phosphatides</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>48</td>
<td>37</td>
</tr>
<tr>
<td>phosphatides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine and inositol</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>phosphatides</td>
<td>28</td>
<td>39</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>18</td>
<td>20</td>
</tr>
</tbody>
</table>

Results expressed as percentages of total phospholipid.
Phosphotidyl glycerol is only a minor component of cerebral lipids. Stanacev et al. (1968) and Possmayer et al. (1968) established that the compound is biosynthesized in brain by the following reactions.

1. CPD - diacylglycerol + Sn - glycerol - 3- phosphoric acid $\rightarrow$ 3 - Sn - phosphatidy1 - 1 - glycerol - 3' phosphate + CMP

2. 3 - Sn - phosphatidyl - 1' - glycerol - 3' - phosphate $\rightarrow$ phosphatidy1 glycerol + R

The incorporation of radioactive phosphorus in the phospholipin fraction and of deuterium in both the fatty acid and non-soaponifiable fraction of the brain lipids (Waelsch et al., 1941) was greatest in young rats immediately after birth when growth was greatest, and decreased sharply with increasing age, even although myelination was proceeding rapidly (Johnson, 1949).

Lunt et al. (1971) reviewed the effect of acetylcholine on phosphotidylionositol phospholipids. Nelson and Barnum (1960) have demonstrated the effect of anticholinesterase, DFP on brain phosphotidyl-choline metabolism. Neurochemical studies of regional phospholipid changes of rat brain following the administration of metasystox have not been reported in the literature and present investigation provides information on the effects of organophosphate metasystox on brain phospholipids.
1.20.0 **Cholesterol in the Brain**

Although cholesterol is widely distributed in various body tissues, it is found in highest concentration within the central and peripheral nervous systems. It accounts for about 10% of dry weight of the brain in contrast to less than 1% found in most other organs. This high concentration of cholesterol seems to be characteristic of nervous tissue. The constancy of amount of cholesterol in the brain suggests that the sterol is metabolically stable (Waelsch et al. 1940). Laatsch et al. (1962) demonstrated that cholesterol accounts for 18-20% of the dry weight of the myelin fraction and that about 70% of the total brain cholesterol is present in the myelin. Fetal or neonatal brain prior to myelination contains relatively little cholesterol. Kritchevsky and Holmes (1962) found varying amounts of the sterol in the newborn rat brain. Recently Fumagalli and Paoletti (1963) and Fumagalli et al. (1964) reported that desmosterol accounted for up to 7% of the human and rat fetal and neonatal brain sterol content. Desmosterol is rapidly converted into cholesterol in the brain where it seems probable that it is an important precursor of brain cholesterol (Fish et al., 1962).

Johnson et al. (1949) found more than 4% of cholesterol as ester in infant white matter, while less than 2% was found in infant grey and adult grey and white matter. Pritchard
(1963) has detected small amounts of cholesterol ester in the brain lipids of the 7-day-old rat. More specific localization of cholesterol by histochemical means (Adams, 1961) indicate a strong reaction in the myelin sheath of peripheral nerve and in fiber tracts of the brain. A large portion of adult rat, rabbit, or chicken brain cholesterol undergoes very slow metabolism. Metabolism of cholesterol in the brain was different from that in other tissues (Waelisch et al., 1940). It seemed probable from their experiments that the cholesterol was largely synthesized within the developing brain while suitable precursors, such as $1\text{-C}^{14}$ acetate are readily utilized by young animals. There is a progressive decrease during development in the amount incorporated into central nervous system but not into liver cholesterol (Nicholas and Thomas, 1959a,b). Since about 70% of brain cholesterol is located in the myelin sheath, it is probable that at least part of this structure is metabolically a relatively stable tissue component. No information is available thus far on the cholesterol levels in the rat brain following the administration of organophosphate metasystox.

1.21.0 Fatty Acids of the Brain

The type of fatty acid and percentage composition of the fatty acid content of most brain lipids has been compiled by Eichber et al. (1969). Free fatty acids are present only in small amount in central nervous tissue, in combination, mainly
as long-chain fatty acids they constitute a large portion of the major lipids of the brain and spinal cord.

Fatty acid metabolism in the central nervous system has been studied by D'Adamo (1960). The combined yield of free fatty acids from subcellular fractions of rat brain was greater than that from fresh tissue. Fatty acids in the central nervous system can arise by synthesis in situ and by transport from the bloodstream (D'Amado, 1970). Recent studies have again indicated that both possibilities exist (Dhopeshwarker and Mead, 1970). The enzymes of the fatty acid oxidizing system are \( \beta \)-hydroxyacyldehydrogenase and \( \beta \)-ketoacyl thiolase (Lynen, 1954). An additional enzyme required for long-chain fatty acid degradation, fatty acyl-CoA synthetase (Acyl:CoA ligase (AMP), EC 6.2.1.3), has been studied by Pande and Mead (1968). On a specific activity basis, liver was most active testes and serum least active, and brain somewhere in between. The amount of the fatty acid composition of each lipid class of human brain changes continuously throughout the life (Rouser et al., 1968 and Rouser et al., 1968). Hansen and Clasusen (1968) investigated the fatty acids of the human fetal brain. The predominant acids were palmitic, stearic, and oleic in brain as a whole, and in fetal brain, lecithin, phosphatidylethanolamine, and phosphatidylserine. In adult brain oleic was dominant in all of these phospholipid fractions. Linoleic acid was practically absent in all of these
phospholipid fractions. Linoleic acid was practically absent in fetal brain but represented 4.5% of the total fatty acids present in adult brain.

Following post-decapitation ischemia and after electroconvulsive shock in rats, Bazam (1970) found that a striking rise in the total free acid pool in brain occurred. Several reports (e.g. Witting and Horwitt, 1967), indicated changes in the fatty acid content of various tissues in Vitamin E deficiency. At present, no attempt has been made to evaluate the effect of pesticide metasystox in the various regions of the rat brain and spinal cord. Therefore, it would be of interest to estimate the levels of fatty acids quantitatively after the administration of three graded doses of metasystox.

1.22.0 Gangliosides in the Brain

Ganglioside is the generic term for glycosphingolipids containing sialic acid, which are a group of derivatives of neuraminic acid. Gangliosides are thought to be primarily responsible for specific cellular reactions. It is now generally assumed that exogenous ligands such as bacterial toxins, antigens, calcium ion and so on, bind to specific gangliosides to induce sequential activations of membrane-associated enzymic reactions and cellular metabolism (Fishman and Brady, 1976a; Yamakawa and Nagai, 1978; Hakomori, 1981).
Gangliosides are ubiquitously distributed in neural tissues of vertebrates only, and the composition and content seem to vary among different brain regions. The reported data on the localization of gangliosides have been summarized by Ledeen and Yu (1976). Gray matter contains much more lipid-bound sialic acid than white matter and other myelin rich tissues such as spinal cord peripheral nerve. It is probably safe to say that astroglia and neuronal perikarya show similar patterns of gangliosides (Abe and Norton, 1974). On the other hand myelin has a characteristic pattern with $\text{GM}_4$ and $\text{GM}_1$ as the major ganglioside species (Ledeen et al., 1973; Yu and Iqbal, 1979). The ganglioside pattern of oligodendroglia resembles that of white matter and to some extent that of neuronal perikarya, but oligodendroglia are quite different from neuronal perikarya and astroglia in containing much more $\text{GM}_4$. $\text{GM}_4$ therefore, is considered to be specifically localized in human myelin and oligodendroglia, suggesting that $\text{GM}_4$ as well as $\text{GM}_1$ are synthesized in oligodendrocytes and preferentially incorporated into the myelin sheath in myelinogenesis (Yu and Iqbal, 1979). The myelin is also characterized by an unusually high level of $\text{GM}_4$, which accounts for more than 30% of the total gangliosides.

Gangliosides are principally synthesized in neuronal perikarya and transported to nerve endings together with macromolecules (Rehmann and Rosner, 1973; Ledden et al., 1976b;
Landa et al., 1979), but local synthesis within the axon and nerve endings (Ledeen et al., 1976b; Tettamanti et al., 1980) or even at the plasma membrane level (Preti et al., 1980) can not be excluded. Synaptic membranes are thus supposed to incorporate as a unit most of the gangliosides transported from neuronal cell bodies by axonal flow.

Gangliosides seemed to be gradually degraded in tissues, and only 20-30% of the original activities were lost within 8 days. Two-thirds of the metabolites were removed through the kidney, and one-third through the liver. Lysosomes are thought to be a major locus for the degradation of gangliosides (Sandhoff and Christomanou, 1979).

Ganglioside changes related to development have been extensively studied in the nervous system. The total ganglioside level in rat brain increases rapidly during the period just before myelinogenesis, 7-16 days after birth and the level remains constant thereafter throughout the adult period (Susuki, 1965; Rehmann, 1980). Postnatal pattern changes of human brain gangliosides were also reported by Susuki (1965). Yamakawa and Nagai (1978) discussed the possible role of gangliosides as receptors and modulators of membranes machinery and also mentioned the therapeutic trials with gangliosides in relation to the activating effects of gangliosides on neurite-genesis. To my knowledge, the effect of organophosphate
pesticides on gangliosides are limited and particularly, the effect of organophosphate-metasystox on ganglioside is not known, therefore, it would be of particular interest to evaluate the ganglioside levels in different regions of the rat brain after the administration of three doses of organophosphate-metasystox.

1.23. G Lipid Peroxidation

Lipid peroxidation has been associated in various ways with a number of normal and abnormal physiological processes. Recent interest in lipid peroxidation has probably resulted from the realisation that oxygen radicals and other organic radicals do indeed exist in biological tissues for an appreciable time. The peroxidation of endogenous phospholipids in biological membranes has long been thought to be the basis for a variety of toxicological phenomena.

Lipid peroxidation appears to be a phenomenon that reflects free radical events associated with biological membranes, which contain most of the polyunsaturated fatty acid containing lipids in animal tissues. In addition, biological membranes are filled with active catalysts of oxidation of fatty acids by oxygen with peroxide formation (lipo-peroxidation) such as hemoproteins and nonheme iron, copper, and manganese complexes. The consequences of oxidation of even a small portion of unsaturated fatty acids in the phospholipid bilayer are obviously dramatic: deterioration of barrier and matrix function of the lipid bilayer, enzyme inactivation, toxic effects on cell division, etc. Apparently the formation of membranes in primary cells proceeded in
reduced atmosphere, and later the cells developed specialized systems to protect their membrane substance against oxidation by free oxygen.

Systematic study of the reaction kinetics of lipid peroxidation has shown that the process, is rather complicated (Vladimirov and Archakov, 1972). The chemical process of lipid peroxidation (Mead, 1976 and Poyer, 1976) is defined as the reaction of an oxidant initiator with a polyunsaturated fat (LH) to form a lipid-free radical intermediate (L*), a peroxo free radical (LOO*) then forms when oxygen reacts with this free radical intermediate. A relatively non-specific hydrogen abstraction reaction is initiated when the unpaired electrons of the peroxo free radical react with another lipid molecule:

\[
\begin{align*}
L^* + O_2 & \rightarrow LOO^* \\
LOO^* + LH & \rightarrow LOOH + L^*
\end{align*}
\]

The peroxo free radical can also react with an antioxidant to terminate the reaction chain:

\[
\begin{align*}
LOO^* + \text{Vitamin E} & \rightarrow LOO + \text{Vitamin E Oxidized}
\end{align*}
\]

The nature of the primary free radicals generated in living cells and able to initiate lipid peroxidation reactions remains one of the most challenging problems. Theoretically, there are three types of radicals may be considered as possible candidates for this function: (1) Semiquinones participating in electron transport in respiratory chains, (2) Organic molecular
free radicals, and (3) Free radicals created by sequential addition of electron to oxygen molecule $^\ast O_2^-$ and $^\ast OH$.

The "active forms of oxygen" have become a matter of growing interest (Fridovich, 1974a,b; Beveris et al., 1972). Presence of active oxygen molecule ($O_2^-$, $O_2$ and $OH$) and their involvement in the processes such as hydroxylation of unnatural compounds, lipid peroxidation and cell injury phenomenon have been studied by Rosenthal (1976) and Hamberg et al. (1974). The oxygen radical receiving the most attention is the superoxide radical. In fact superoxide radicals never exist alone in water solution but spontaneously create all forms of "active oxygen" in the sequence of reactions (Fong et al., 1973; Merzljak and Sobolev, 1975):

$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2^- (\text{Single oxygen})$

$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2^- (\text{usual triplet oxygen})$

$H_2O_2 + Fe^{2+} (\text{if available}) \rightarrow Fe^{3+} + OH^- + OH$

It is interesting that moderate amounts of superoxide dismutase slightly increased lipoperoxidation, probably because of an increase of $H_2O_2$ production, which, in turn, would increase the rate of formation of $OH$: 
Superoxide dismutase

\[ *O_2^- + H_2O_2 \rightarrow O_2 + H_2O + OH \]

Any system in cell producing superoxide radicals should initiate the lipid peroxidation as follows:

\[ \text{From Fe}^{2+} \text{ or } O_2^- \]

An antioxidant vitamin E significantly inhibit the lipid peroxidation in organophosphate...
treated brain tissue. To my knowledge, the effect of organophosphate pesticides on the rate of lipid peroxidation are limited and particularly, the effect of organophosphate-metasystox on lipid peroxidation is not known, therefore, it would be of particular interest to investigate the rate of lipid-peroxidation in different regions of rat CNS after the administration of three different doses of organophosphate-metasystox.

1.24.0 Action of Lipases

Enzymes hydrolyzing triglycerides have been studied for well over a hundred years. In 1846 Claude Bernard demonstrated lipase activity in the pancreas and Marcet described gastric lipase in 1858 (Sumner and Somers, 1953). However, White et al. (1978) has described phospholipase 'A' and 'B' activity in brain. In the brain lipase activity is concentrated more in gray matter than in the white (Gozzano, 1934) and certain areas, like postrema and caudate nucleus, contain higher concentrations than other areas (Ishii, 1957). Intracellular distribution experiments have shown that lipases are concentrated considerably more in the cytoplasm than in the nuclei (Gomori, 1946).

Lipases hydrolyze triglycerides and, products are formed in the following sequence.
Rates of lipase reaction can therefore be measured by determining either (a) the rate of disappearance of the substrate, the trilysceride, or (b) the rate of production of the fatty acids. The presence of phospholipase-A-like activity in brain was observed by Gallai et al. (1962). These enzymes have been useful in study of the structure of phosphoglycerides as well as indicating routes of their degradation. The susceptible bonds in phosphotidylcholine are indicated by Fig. 6 below (White et al., 1973).

Fig. 6. Action of Phospholipase
Among different brain regions, human cerebral cortex has the highest phospholipase $A_1$ activity. However, activity in the sciatic nerve is very low. Most phospholipase $A_1$ activity is predominantly localized in the microsomes, whereas the phospholipase $A_2$ in mitochondria (Goracci et al., 1978). On the other hand, the phospholipase $A_1$ activity is nonspecific in its subsynaptic distribution. Both glial and neuronal cell-enriched fractions contain phospholipase $A_1$ and $A_2$ activities. However, activity in neurons is five-to eight fold higher than that in glia.

Activity of the neuronal phospholipase $A_1$ is competitively inhibited by plasmalogens (Woelk et al., 1979). An increase in phospholipase $A_1$ and $A_2$ activities is shown in degenerating nerves as early as 2 days after sectioning (Webster, 1973). Phospholipase $A_1$ and $A_2$ activities are also elevated in the acute stage of experimental allergic encephalomyelitis in rats (Woelk et al., 1974 and Woelk and Kanig, 1974).

There are some indications that the lipase (s) in brain is stimulated by biogenic amines (Vyvoda and Rowe, 1976). The increase in the lipase activity due to organophosphate TOCP intoxication is also reported (Shull and Cheeke, 1973). To date, no previous report is available on the action of lipases in the different regions of the rat brain following the administration of organophosphate-metasystox. It is...
therefore, appropriate to estimate the activity of lipase in cerebral hemisphere, cerebellum, brain stem and spinal cord in the metasystox-induced toxicosis in rats.

1.25.0 Vitamin E an Antioxidant

Some thirty years have passed since the existence of vitamin E was recognized, and some twenty years since the synthesis of \(\alpha\)-tocopherol, the substance with the highest known vitamin E activity. The principal group of compounds having vitamin E activity are the tocopherols. The seven tocopherols which have been found to occur naturally have the structures as shown in formula given below.

![Tocopherol Structure](image)

The biopotencies of various tocopherols differ somewhat, depending on the assay criteria and animals used. \(\alpha\)-Tocopherol has the greatest biological activity however. This could be a reflection of its greater extent of methylation. Such methylation will prevent side reactions and lower the oxidation-reduction potential of hydroquinone-quinone system. The
features of tocopherol chemistry that appear most salient to possible biological function are its lipid solubility and oxidation properties. They play a physiological antioxidant activity (potentiated by ubiquinone Q10) at the cellular level by counteracting lipid peroxidation and increasing membrane phospholipids supplied by PUFAs and preventing the formation of ceroid substance. Vitamin E acts as a H⁺ donor in the kreb's cycle, between the steps of flavin coenzymes (FMH and FAD) and of the cytochrome system (Butturini et al., 1955).

The identification of vitamin E as a fat-soluble vitamin, its occurrence in vegetable oils, its storage in association with body lipids and its possible function as a biological antioxidant suggest that a close relationship exists between vitamin E and various phases of lipid metabolism. When vitamin E is deficient or completely lacking there is an uncoupling of phospholipids and proteins, necessary for the formation of cell membrane. In studies in humans, the administration of tocopherol to healthy male subjects resulted in increased plama cholesterol values (Gray and Loh, 1958). Gray (1959) found higher levels of phospholipids and cholesterol esters in livers of rats fed 100 mg \( \alpha \)-tocopherol acetate daily when compared with livers from unsupplemented rats presumably due to increased hepatic lipid synthesis in the supplemented animals.

After the pioneering works of Kudrjashov (1937) and Davies and Moore (1941) an appreciable number of scientists have been
dealing with the relationship between vitamin E and lipid peroxidation. The contribution of Tappel's laboratory has generally been recognized (Tappel, 1962, 1972, 1973; Vladimirov and Archakov, 1972). The increase in lipid peroxidation resulting from E-avitaminosis was proved beyond question. The activation of lipid peroxidation increase the requirement of vitamin E (Witting, 1970, 1972). Synthetic antioxidants prevent symptoms of E-avitaminosis though not completely (Roels, 1967; Tappel, 1972). The toxic action of lipid peroxidation products (Holman and Greenberg, 1958) which is usually inhibited by \( \alpha \)-tocopherol (Kokathur et al., 1966; Privett and Cortesi, 1972) an antioxidant, on the one hand, and the existence of E-avitaminosis symptoms which can not be removed by synthetic antioxidants (Roels, 1967; Tappel, 1972) on the other hand imply that both the consequences of toco- pherol deficiency may contribute to the observed macroscopic symptoms: the direct dependence of molecular machines of the cell on \( \alpha \)-tocopherol and toxic effects of lipid peroxidation products. Since organophosphates catalyze the formation of an oxidative state in body lipids creating a greater need for vitamin E (Hove, 1955; Hove, 1953; Seward et al. 1968), it is reasonable to postulate the effects of \( \alpha \)-tocopherol on the rate of lipid peroxidation and lipase activity in different regions of brain and spinal cord following the administration of metasystox an organophosphate.
Proteins in the Brain

Protein, one of the many important biochemical components in the vertebrate brain, constitutes 40% of the dry weight (McIlwain and Bachelard, 1971). This protein content of the brain is very constant. There are many kinds of proteins present in the brain. Recent evidences suggest the role of glycoproteins in the number of specific cell-cell interactions, including intercellular adhesion and the mechanisms governing neural histogenesis, regional brain differentiation and the specificity of neuronal associations (Margolis et al., 1975). The changes in the neuronal activity is well accompanied by measurable changes in macromolecules like protein in brain cells. It has also been reported that the increased neuronal activity decreases or inhibits the synthesis of proteins (Hyden and Lange, 1972).

Brain-specific proteins have been described by Moore (1965) and Warecka and Bauer (1967). Takehara (1956/1957) mentioned the existence of a species-specific fraction and an organospecific fraction in the brain proteins. Also Caspara and Field (1963) described a brain-specific antigen. The S-100 protein of Moore (1965) is distributed in all parts of the nervous system, both peripherally and centrally. The author suggested that it is probably a neuronal protein composing no part of the myelin sheath structure. Recent studies
(Uyemura et al., 1967) demonstrated that the S-100 protein is heterogeneous. The proteins in the brain are in a dynamic state. Synthesis and catabolism have been intensively studied by Lajtha (1961). Protein synthesis can probably start from free amino acids, which have to be activated and transferred to a soluble ribonucleic acid or nucleoprotein. The changes in brain cell RNA suggest that the genetic material of neurons and glia is called upon to make adjustments to physiological changes by elaborating information and perhaps different protein molecules which may themselves be directly involved in the nervous activity.

It has been evidenced that many environmental and nutritional factors may bring about the changes in the proteins (McIlwain and Bachelard, 1971). The decrement in the protein concentration in many regions of the brain and spinal cord has been observed in the rats treated with sublethal dose of phosalone (Kirubagarn, 1984; Palanivelu, 1984; Tamilvanan, 1984). The decrement in the protein concentration in the brain regions of the fishes Labeo rohita and Cyprinus carpio has also been reported due to phosalone toxicity (Ravi, 1984).

Brain, in general, has high rate of metabolic activities. It needs more proteins for expected high rate of protein turnover. This view is well correlated with the presence of large amount of cytoplasmic ribosomes, which gives large number of sites for protein synthesis (McIlwain and Bachelard, 1971). Any
change in the protein concentration may influence the metabolic rate of the tissue. It requires rapid synthesis and renewal of protein. To analyse this view, in the present work, an attempt has been made to study the changes in the regional brain protein concentration due to metasystox treatment.

1.27.0 Nucleic acids and Nucleic Enzymes (DNase and RNase) of the Central Nervous System

Nucleic acids in the central nervous system, as in other organs are characterised by their size, composition, and role in protein synthesis. Recent reports suggest that, in addition to the established role of nucleic acids in the biosynthesis of protein (Campbell, 1965), RNA and protein synthesis may be involved in the accrual of sensory information in the brain, thus indicating a possible approach to elucidation of brain function on a molecular basis (Hyden, 1964). Since the majority of the cells in brain are diploid, there is generally a fixed quantity of DNA per cell. Some polyploidy has been reported in brain cells: however, the number of these cells is relatively small (Viola, 1963). In general DNA content in mammalian brain is between 6.1-7.1 Pg/Cell (Heller and Elliott, 1954). The DNA extracted from human cerebral gray matter had an A + T/G+C ratio of 1.48, while the white matter had a ratio of 1.41. The actual content
of DNA was reported to be 0.50 mg/g fresh weight in human gray matter and 0.72 mg/g fresh weight in white matter (Robinson, 1966). The base ratio for DNA does not change in various regions of the rat brain nor even among the various mammalian brain.

Any enzyme that breaks down DNA has been at times, called a deoxyribonuclease. An acid and an alkaline DNase have been identified in infant and adult rat brain (Sung, 1968). The latter enzyme shows a strong preference for denatured DNA. On the other hand the acid DNase prefers native DNA. In the brain of senescent rats, DNase activity was found to be twice as high as that in young adult animals (Asano et al., 1979). With regard to cellular localization, neuronal nuclei prepared from young and adult rat brain have been shown to have a higher DNase activity than glial nuclei (Stanmbolova, 1973).

It has been reported that organophosphate DDVP-induces degenerative changes in neurons and nerve fibres. The results with organophosphate pesticide metasystox in rats show that there is a remarkable decrease in the DNA level in all the brain regions that are analysed. This has been correlated with increased DNase activity (Tayyaba et al., 1981). The available literature on DNA and DNase indicate that the knowledge of organophosphate toxicity on brain nucleic acids and nucleic enzymes is inadequate. It is therefore, appropriate to estimate the level of DNA and the activity of DNase in cerebral hemisphere,
cerebellum, brain stem and spinal cord after metastystox toxicosis in rats.

The studies of RNA concentration are of interest to know the rate of protein synthesis and also to understand the functional status of the nervous tissue (Bergen, et al., 1974). Edstrom (1956) and Edstrom and Pigon (1958) have reported that there is a proportionality between RNA content and the surface area of the cell body. The perikarya contain more RNA than the axons. There is a decrease in RNA concentration along two-thirds of the axon distal to the hillock, then the RNA increases towards the distal end of the axon Edstrom (1956). In contrast to the glia the neurons contain more RNA by a factor of approximately thirteen (Hyden, 1962). Hyden has demonstrated that the content of RNA in neurons varies over a wide range. Jacob et al. (1966) isolated RNA from rat brain combined samples of cerebral cortex, cerebellum, and brain stem.

In vertebrate animals, extra cellular RNases are secreted by the pancreatic and by the salivary glands (Ellem and Colter, 1963). The usual function of an RNase is, rather obviously, to break down RNA. This, however, tells very little about the actual physiological actions of these enzymes. The response of RNA/and RNase to neurotoxicants like organophosphate compounds has been reported (Tayyaba et al., 1981). However, information on the dose dependent effect of organo-
phosphate metasystox on the ribonucleic acid metabolism is still lacking. It is therefore, appropriate to estimate the levels of RNA and RNase activity in different regions of brain and spinal cord after metasystox administration.

1.28.0 Specific Objectives of this Study

The present study was undertaken with the following main aims:

i) To evaluate the normal concentration of various lipid and nucleic acid components in the different regions of the rat brain.

ii) Quantitative evaluation of the effect of metasystox-intoxication on the concentration of total lipids, phospholipids, cholesterol, C/P Ratio, esterified fatty acids, gangliosides, lipid peroxidation and lipase activity.

iii) To estimate the levels of DNA, RNA, protein and the activity of DNase and RNase following the administration of metasystox in various brain regions.

iv) Qualitative evaluation of total lipid, phospholipid, nucleic acid and the activity of lipase in the cerebral hemisphere.

v) To evaluate the effects of vitamin E on the rate of lipid peroxidation and the activity of lipase alone and in combination with metasystox.