INTRODUCTION

1.1 Organophosphorus compounds

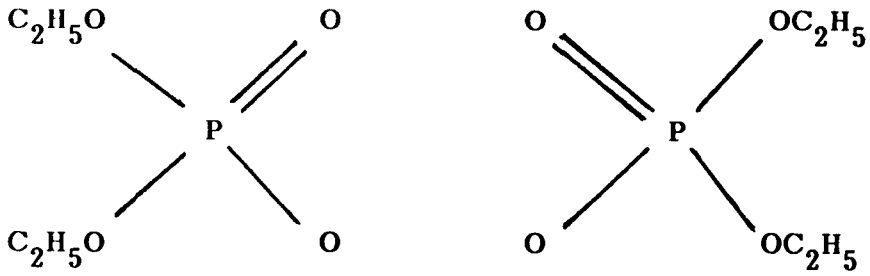
Organophosphates, one of the most versatile group of pesticides, are being increasingly preferred because of their rapid biodegradability (Moriarty, 1975). The indiscriminate use of organophosphorus pesticides to boost the crop production, is now known to be largely responsible for the contamination of the aquatic environment, which gradually becomes causative for reduction in the density of aquatic biota in general and fish population in particular. The gross impact of such a situation on fish, is well established (Eichelberger and Lichtenberg, 1971; Maziarka, 1975; Basak and Konar, 1976; Rao et al., 1979 and Gopalkrishna et al., 1981). Ideally, their effects should be highly selective in affecting the undesirable target animals. However, some experimental studies have evidently shown that excessive use of pesticides threatens destruction of several kinds of non-target organisms, sometimes the practice is also liable to cause catastrophic mortality of the affected organisms (Bhuyan, 1967 and Konar, 1975).

The deterioration in the water quality of the aquatic environment by organophosphorus compounds is more alarming due to the great possibility of their access through rain water as surface run off. The sprayed chemicals may also get mixed up directly with the accumulated water of the fields and the contamination may pass over to the adjoining water areas. The contaminant may likewise find access to the inland water sources through irrigated water. In all these stipulated situations, thus there is possibility of contamination of the inland water sources

like ponds, tanks, lakes, reservoirs or even rivers and streams to varying extents and thus inhibiting the survival potency of fish (Faust and Aly, 1964; Johnson, 1968; Sreenivasan and Soundararaj, 1968; Holden, 1973; Mellanay, 1975 and Jacob et al., 1982). Koundinya and Ramamurthy (1978) have reported that pesticide contamination in sediment, water and biota of the freshwater and estuarine portions of the water body affects not only the aquatic life but also the people who consume the contaminated food products. Whereas on the credit side, the use of organophosphates has increased the yield of agricultural products. They have also proved helpful in controlling vectors of certain diseases such as malaria, filaria, leishmaniasis, etc. (Casida and Baron, 1976).

1.2 Historical overview

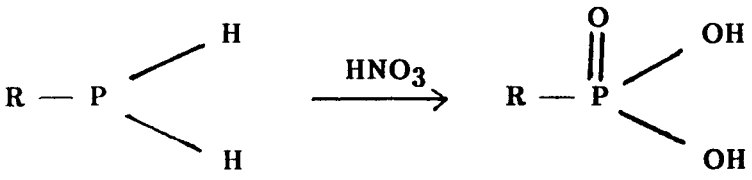
In 1820, Lassaigne for the first time reacted alcohol with phosphoric acid in reaction analogous to that with sulfuric acid and thereby launched the chemistry of the organophosphorus compounds. Later, in 1847, Thenard synthesized phosphines and in the same year Cloez (1847) discovered a thiophosphoric acid ester. Clermont (1854) synthesized tetraethyl pyrophosphate (TEPP, I) by alkylating the silver salt of the pyrophosphoric acid with alkyl halides. The compound was developed in Germany as a substitute for nicotine, which was in short supply in that country during World War II.



(I)

The insecticidal activity of this ester (TEPP), which may be regarded as a link between inorganic and organic chemistry, was discovered after 80 years of its synthesis. TEPP, although an effective insecticide, was highly toxic to mammals and was rapidly hydrolyzed in presence of moisture.

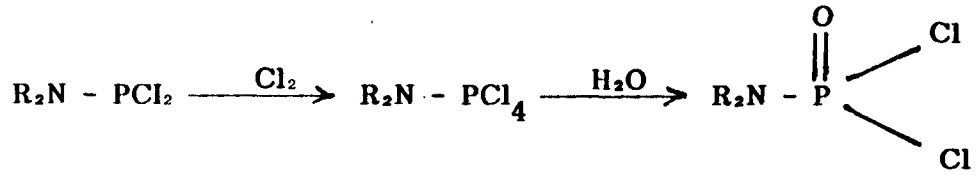
Hofmann in 1872, synthesized the corresponding phosphoric acid by oxidation of methyl and ethyl phosphine with nitric acid (II).



(II)

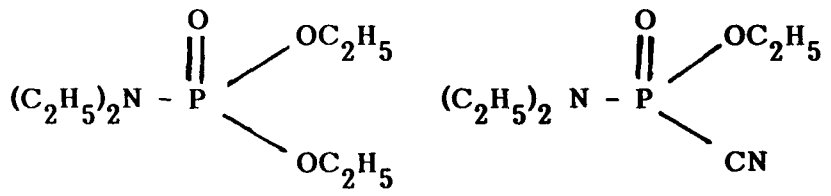
In 1897, Michaelis and Becker synthesized a phosphonic acid ester by the reaction of sodium dialkyl phosphite with ethyl iodide. This reaction became known as Michaelis-Becker reaction. Further, in 1898, Michaelis and Kaehne isolated a compound from trialkyl phosphite and methyl iodide, whose structure was not analogous to the compund

which was obtained by Michaelis-Becker reaction. In early 19th century, Michaelis repeated the reaction of phosphorus trichloride with sodium ethylate. At the same time, in 1903, Michaelis also synthesized phosphorus-nitrogen compounds from phosphorus trichloride, pentachloride, phosphoryl-chloride, thiophosphorylchloride and ammonia or amines. In the reaction of phosphorus trichloride with alkyl amines, he obtained N-alkyl-aminodichlorophosphine (III), which he oxidized with chlorine to tetrachloridates (IV), and atmospheric moisture sufficed to hydrolyze these tetrachlorides to dialkyloaminophosphorodichloridates (V).



(III) (IV) (V)

Subsequently, Michaelis also synthesized O,O-diethyl N,N-diethyl phosphoroamidate (VI) and O-ethyl N,N-diethyl phosphoroamidocyanidate (VII) by the reaction of N,N-diethyl phosphoroamidodichloridate with potassium cyanide in absolute alcohol.

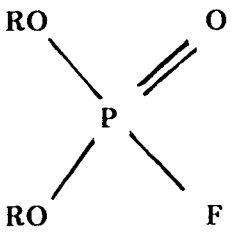


(VI) (VII)

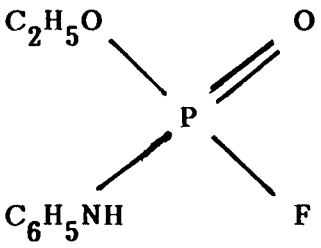
In 1932, Lange and Krueger prepared the esters of monofluorophosphoric acid from its silver salts with alkyl iodide. They, for the

first time, reported the highly toxic properties of these compounds, including respiratory distress, clouding of the consciousness, temporary blindness and photophobia.

Saunders and his group (1945-1946) during the second World War, worked on esters and ester amides of phosphoric acid fluoride (VIII and IX).



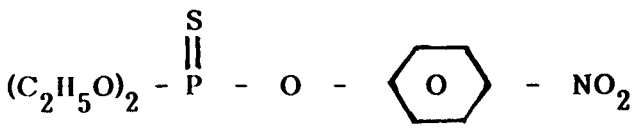
(VIII)



(IX)

They also discovered the miotic action and high inhalation toxicity of these substances.

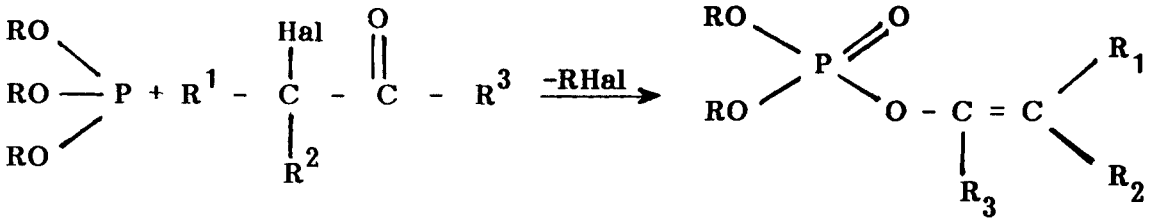
During the synthesis and investigation of approximately 2000 compounds, Schrader (1952) defined the structural requirements for their insecticidal activity. One compound in this early series, parathion (X), later became the most widely employed insecticide of this class.



(X)

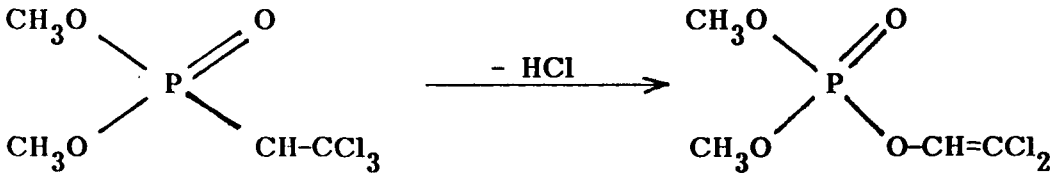
In 1952-54 Perkow synthesized a number of dialkylvinyl phosphates (XI) by the reaction of triethyl phosphite with the α-halogen carbonyl

compounds. To honour its discoverer the reaction is known as Perkow reaction.



(XI)

Dichlorvos (dichlorovinyl phosphate) is one of the important products thus obtained. It is synthesized by treating triethylphosphite with chloral. A different route to such compound was discovered by Lorenz (1954) who showed that trichlorfan (XII) is hydrolyzed to dichlorvos (XIII).



(XII) (XIII)

It has been estimated that over 50,000 organophosphorus compounds have by now been synthesized and screened for their insecticidal potency, of which over 3 dozen have been produced commercially (Chadwick, 1963).

1.3 Nomenclature

There seems to be considerable confusion concerning the naming

of organophosphorus compounds. This often resulted in several chemical and trade names for each compound. However, the 1952 agreement between England and the United States on the nomenclature of organophosphates is followed by most scientists. In this system, esters are named as derivatives of the corresponding parent compound. A 'phosphate' is a derivative of phosphoric acid, a 'phosphonate' a derivative of phosphonic acid. Esters containing a nitrogen, sulfur or halide attached to the phosphorus have appropriate suffixes. The structures of these derivative base names are illustrated in Table 1. Of these simplest derivatives, there can be various combinations such as, phosphonothionate, or phosphorodiamidofluoridate.

Table 1. Derivative base names of organophosphorus compounds

phosphate	$(RO)_3$ P = O
phosphonate	(R) $(RO)_2$ P = O
phosphinate	$(R)_2$ (RO) P = O
phosphoramidate	(RNH) $(RO)_2$ P = O
phosphorothionate	$(RO)_2$ P = O
phosphorothiolate	(RS) $(RO)_2$ P = O
phosphorofluoridate	(F) $(RO)_2$ P = O

Once the derivative base name is established, the R-substituent groups are included in the name with an indication of the atom to which they are attached. The structural formulae, chemical names

and common names of several organophosphates are given in the Table 2.

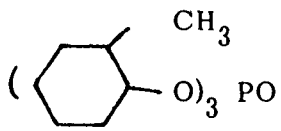
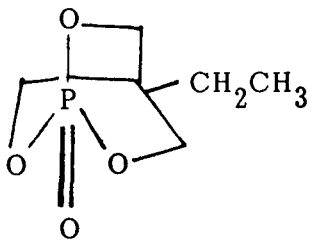
The multiplicity of organophosphates frequently causes them to be victims of confusion between common and proprietary names. A proprietary name is the one usually allocated by the company which owns the compound; it should always be used with a capital letter, and is often followed by an ® to show that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

1.4 Mechanism of action of organophosphates

The mode of action of organophosphate insecticides in vertebrates is generally regarded as disruption of nerve impulse transmission in the central and peripheral nervous system by inhibition of acetyl-cholinesterase, the enzyme that modulates the amounts of neurotransmitter acetylcholine.

The central nervous system is made up of brain and spinal cord. It 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. Neuron is the basic structural unit of the nervous system and comprises the nerve cell body together with all its processes. The simplest neural action in vertebrates involves the participation of several neurons which form a common functional pathway. This functional conjunction is established in the synapses. They consist of a pre-synaptic membrane (usually an axon or rarely a dendrite) and a post-synaptic membrane (cell body, a dendrite or occasionally an axon) and the distance between these two membrane

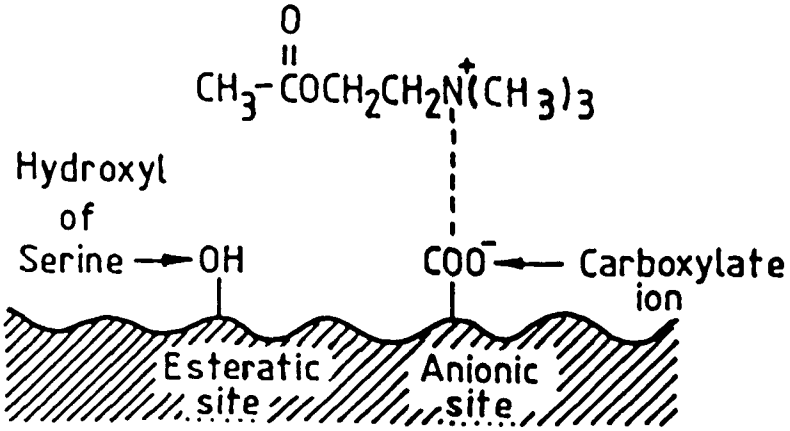
Table 2 . Representative organophosphorus nomenclature

Common name	Chemical Name	Structure
Paraoxon	Diethyl 4-nitrophenyl phosphate.	$(C_2H_5O)_2P(O)$ O  NO_2
Parathion	Diethyl 4-nitrophenyl phosphorothionate	$(C_2H_5O)_2P(S)$ O  NO_2
EPN	Ethyl 4-nitrophenyl phenyl phosphono-thionate	(C_2H_5O) $(C_6H_5)P(S)$ O  NO_2
Mipafox	N,N'-diisopropyl phosphorodiamido-fluoridate	$[(CH_3)_2CHNH]_2$ P (O) F
Dichlorvos	2-2-dichlorovinyl dimethyl phosphate	$(CH_3O)_2P(O)$ $(OCH = CCl_2)$
DFP	Diisopropyl phosphoro-fluoridate	$[(CH_3)_2$ $CHO]_2$ P (O) F
TOCP (TOTP)	Tri-ortho-tolyl phosphate	
EPTBO	4-ethyl-l-phospha-2,6,7-trioxabicyclo-(2-2-2) octane-l-oxide	

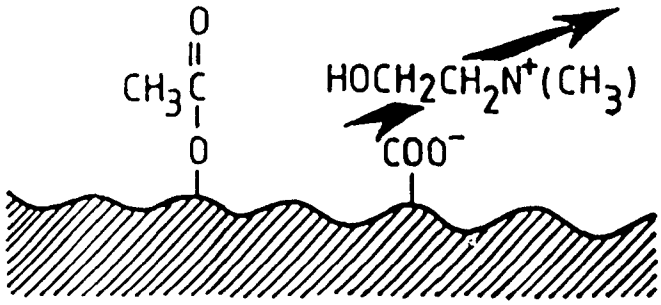
is called synaptic gap which is about 100-200 $\mathring{A}$. The action potential (pre-synaptic impulse) is built up by selective Na^+ and K^+ concentration changes ('ion pump'), and arriving at the synapse induces a corresponding post-synaptic reaction. The impulse is transmitted in the synapse by a chemical mechanism. On the pre-synaptic side, acetylcholine or noradrenaline is liberated and absorbed by receptors on the post-synaptic side, thus altering the permeability of the membrane to ions. In order to bring acetylcholine induced action potential back to the resting potential, in other words to facilitate the transmission of a new impulse, the stimulant in the synapse must be degraded. In organophosphate poisoning, degradation is affected by the acetylcholinesterase, an enzyme which hydrolyzes acetylcholine to acetic acid and inactive choline. Another enzyme, choline-O-acetyl-transferase, is capable of esterifying both compounds to acetylcholine again. ATP and COA are required for this reaction.

The active organophosphates function by blocking acetylcholinesterase. This inhibition results in an accumulation of acetylcholine at the post-synaptic membrane which is then unable to return to its original (resting) state and prevents the smooth transmission of nervous impulses across the synaptic gap, which results in the loss of muscular coordination, induction of convulsions and ultimately death.

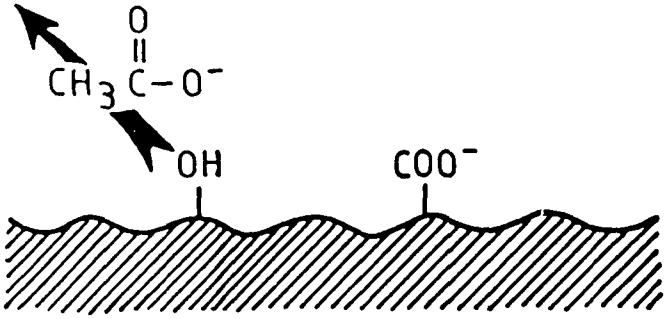
The overall hydrolysis of a molecule of acetylcholine by acetylcholinesterases is a three-step process. First the acetylcholine molecule is bound to the active site (Fig. 1a). The binding is believed



(a) Binding of Acetylcholine.



(b) Acetylation of the enzyme cholinesterase.



(c) Deacetylation of the enzyme.

Fig. 1. REACTION OF ACETYLCHOLINE WITH ACETYLCHOLINESTERASE.

to involve a negatively charged site, the anionic site of the enzyme, which promotes the binding by attraction to the cationic nitrogen. The second step involves the chemical reaction of the acetylcholine with a part of the enzyme called the esteratic site. It is the hydroxyl group of serine which is acetylated by the acetylcholine, and the result is to transfer an acetyl group from acetylcholine to the serine hydroxyl, giving an acetylated enzyme, and to permit the choline to drift away, choline is the leaving group (Fig. 1b). The final step involves the attack of water upon the acetylated enzyme, leaving the acetyl-serine bond to give acetate ion and the enzyme in its original form (Fig. 1c).

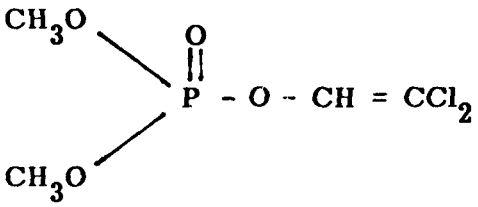
1.5 Dichlorvos, an organophosphate

Dichlorvos or Dimethyl 2,2-dichlorovinyl phosphate (DDVP) is an important commercial insecticide with low residual activity because of its hydrolytic instability in an aqueous environment. It is used mainly for the control of insects in tobacco, warehouses, mushroom houses, greenhouses, animal shelters, homes, restaurants and other food-handling establishments. It was recommended by the 21st World Health Assembly for the control of flies, mosquitoes and other disease vectors in aircraft.

1.6 Physical and chemical properties

Dichlorvos has the following physico-chemical properties :

Common name	Dichlorvos, DDVP
Market name	Nuvan, Nogos, Vapone
Manufacturer	Ciba-Geigy India Ltd.

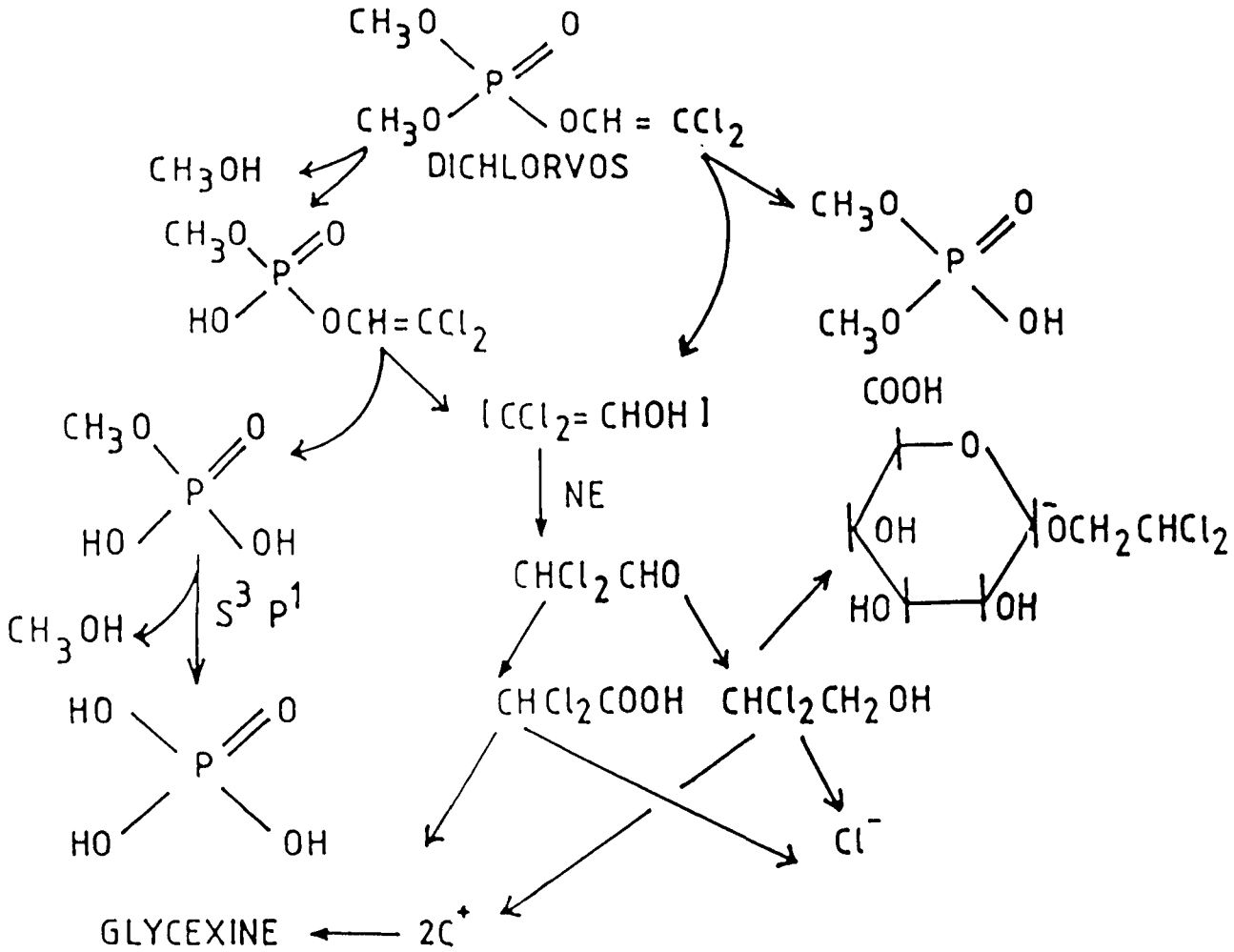
Chemical name	O,O-dimethyl-2,2-dichlorovinyl phosphate.
Structural formula	
Empirical formula	$C_4H_7Cl_2O_4P$
Purity (Technical grade)	93% (determined by the iodometric method).
Byproducts (Technical grade)	7% or less
Colour (Technical grade)	Yellowish to colourless.
Molecular weight	220.98
Specific gravity	1.420 at 15°C
Boiling point	35°C at 0.05 mm Hg
	53°C at 0.2 mm Hg
Vapour pressure	1.2×10^{-2} mm Hg at 20°C
	3.0×10^{-2} mm Hg at 30°C
	7.0×10^{-2} mm Hg at 40°C
Volatility	145 mg/m^3 at 20°C
	350 mg/m^3 at 30°C
	800 mg/m^3 at 40°C
Flash point	$>$100°C
Characteristics	Pleasent smelling, colourless liquid.

Solubility	1% in water, soluble in most organic solvents.
Stability (Pure)	Hydrolyses slowly in neutral and acid solutions, but rapidly in alkaline media.
Stability (Technical grade)	Dichlorvos is stable when stored in glass and certain plastic materials, e.g. polythene.
Corrosiveness	Dichlorvos corrodes mild steel; in the absence of moisture it does not corrode aluminium, nickel, Hastelloy B or stainless steel.

1.7 Metabolism of dichlorvos

The major in-vitro pathways for the metabolism of dichlorvos were defined first by Hodgson and Casida (1962) in an schematic manner (Fig. 2).

Later, in 1967 O'Brien described in detail the chemical degradation of dichlorvos. According to him the degradation of dichlorvos occurs by hydrolytic routes involving phosphatases, which are able to split the P-O vinyl linkage and to a smaller extent to the P-O methyl bond (as shown in Figs. 3 and 4), whereas dimethyl phosphate is not metabolised, but rapidly excreted by the animal body. Methyl phosphate, presumably the product of hydrolysis of desmethyl dichlorvos is slowly hydrolysed to phosphoric acid by a soluble enzyme of animal liver.


CH3O
O
P
CH3O
OCH = CCl2
DICHLORVOS
CH3OH
CH3O
O
P
HO
OCH=CCl2
CH3O
O
P
CH3O
OH
[CCl2= CHOH]
NE
CHCl2CHO
CH3O
O
P
HO
OH
CH3OH
S3 P1
HO
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HO
OH
CHCl2COOH
CHCl2CH2OH
COOH
O
OH
OCH2CHCl2
HO
OH
Cl-
2C+
GLYCEXINE

Fig. 2

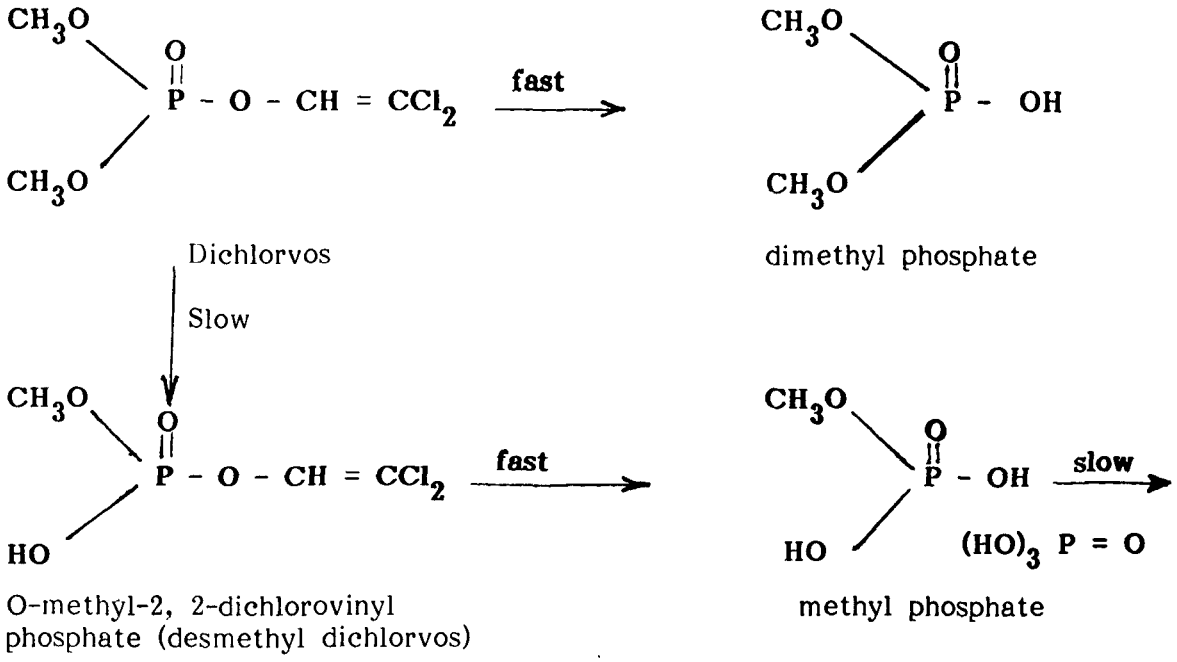


Fig. 3

In animal tissue when dichlorvos or desmethyl dichlorvos is hydrolysed it releases a non-phosphorous moiety, which is subsequently degraded as follows (Fig. 4) :

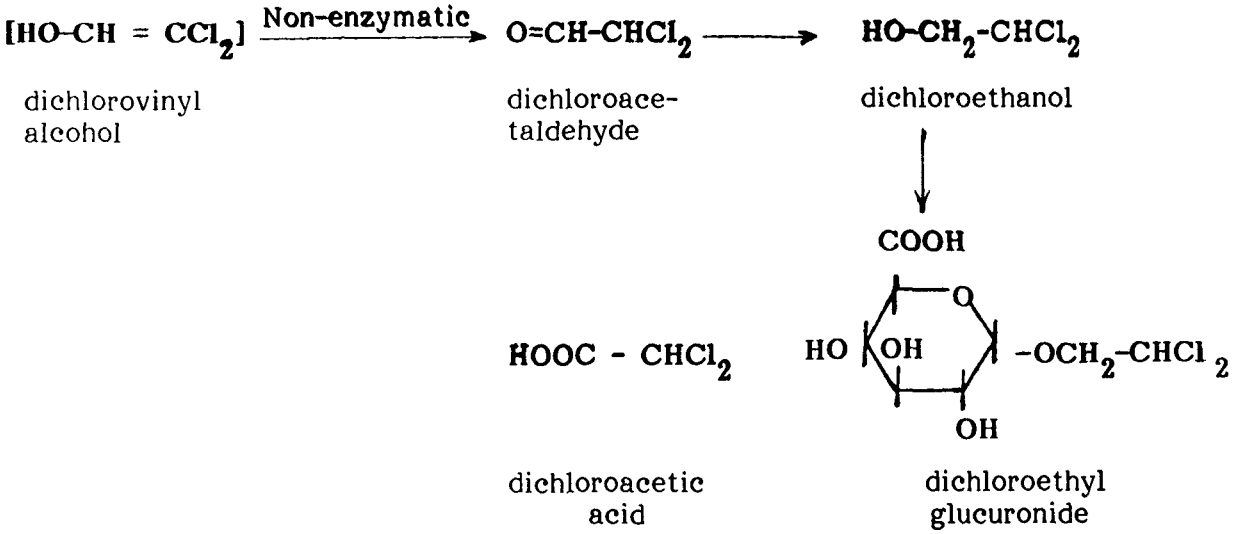


Fig. 4

Dichloroacetaldehyde appears to be formed by non-enzymatic transformation of the unstable dichlorovinyl alcohol. Subsequent reduction of dichloroacetaldehyde to dichloroethanol is accomplished by liver-soluble preparations or purified alcohol dehydrogenase, and requires reduced nicotinamide adenine dinucleotide (NADH). Formation and excretion of dichloroethyl glucuronide are consistent with an elimination pathway as demonstrated by Williams (1959) but the mechanism which leads to the formation of small amounts of dichloroacetic acid has not been investigated (Ciba Monograph, 1971).

1.8 Brain lipids

Study of lipids of the nervous system forms an important aspect of neurochemical investigation. Among various body organs, the brain is one of the richest in lipids. Lipids account for half the dry weight and most of the structural architecture of membranes in the brain (Ordy and Kaack, 1975). It contains a unique structure, the myelin sheath, which has the highest lipid concentration of any normal tissue of subcellular components, except for adipose tissue, and which has been the subject of intensive and extensive studies in recent years. The important lipids of the central nervous system are cholesterol, sphingolipids and glycerophospholipids.

The lipid composition of the brain is relatively stable throughout adult life. However, substantial changes in lipid composition take place during the period of active myelination. During active myelination, the brain loses water, predominantly in white matter, the lipid content

increases rapidly, and the differences between gray and white matter become more apparent (Wells and Dittmer, 1967; Cuzner and Davison, 1968 and Norton and Poduslo, 1973).

There is a good deal of experimental evidence to show that organophosphates influence lipid synthesis in the brain (Majno and Karnovsky, 1955). Tayyaba and Hasan (1980) have reported that DDVP administration to rats significantly increases the total lipid concentration in all the brain regions. Perturbation of total lipid concentration following metasystox in different region of CNS have been observed by Islam et al. (1983) and Tayyaba and Hasan (1985). However, Hasan and Khan (1985) have reported increment in the level of total lipid in the discrete areas of the CNS after administration of methyl parathion. Rao and Rao (1984) also observed changes in the lipid profiles of fish, Oreochromis mossambicus during methyl parathion toxicity. So far, no attempt has been made to evaluate the effect of organophosphorus compound, dichlorvos, on fish brain lipids. Therefore, it would be of particular interest to investigate the total lipid concentration in different regions of the fish (Heteropneustes fossilis) brain after the exposure to three different doses of organophosphate dichlorvos.

1.9 Phospholipids in the brain

Phospholipids in adult brain account for about 20-25% of the dry weight (White, 1973). The total amount of phospholipids are higher in white matter (3.1 to 4.6 g) than in gray matter (6.2 to 9.3 g) per 100 g fresh tissue. Large amounts of phospholipids in white matter

evidently reflect their occurrence as a component of the molecular structure of myelin sheaths. The importance of phosphorus-containing lipids of the central nervous system presumably depends upon their role as membrane constituents. They not only constitute the back bone of biomembranes, but also provide the membrane with a suitable environment, fluidity and ion permeability. Porcellati (1983) and Porcellati et al. (1983) reported that different phospholipids turnover at different rates with respect to their structure and localization in various cells and membranes. Newly synthesized phospholipids are transported to membranous structures by phospholipid exchange and transfer proteins (Demel et al., 1984 and Brammer, 1983) which are found in the cytosol. The distribution of phospholipids in a biologic membranes is thus regulated not only by the activities of enzymes involved in their metabolism but also by the transport and incorporation processes into the membrane.

Nelson and Barnum (1960) have demonstrated the effect of anticholinesterase DFP on brain phosphotidyl-choline metabolism. Decrement of phospholipid in the fish Oreochromis mossambicus, after exposure to methyl parathion has been observed by Rao and Rao (1984). Inhibition of phospholipid content following organophosphate administration has also been reported earlier from this laboratory (Tayyaba and Hasan, 1980; Islam et al., 1983; Tayyaba and Hasan, 1985; Vadhva and Hasan, 1986 and Naqvi et al., 1988). Neurochemical studies of regional phospholipid changes in fish brain following exposure to dichlorvos have not been reported in literature. The present investigation provides information on the effect of organophosphate dichlorvos on the brain phospholipids.

1.10 Cholesterol in the brain

Cholesterol is the only sterol present in significant amounts in the normal adult brain. It accounts for about 10% of dry weight of the brain in contrast to less than 1% found in most other organs. The presence of higher cholesterol content in nervous tissue is well established (Ansell and Hawthorne, 1964). Unesterified cholesterol has been suggested as a lipid characteristic of myelin sheaths because of its occurrence in the white matter in concentration exceeding that in the gray matter (Yasuda, 1937; Randall, 1938; Johnson et al., 1949 and Brante, 1949). The widespread distribution of cholesterol is an indication of its importance in various life processes. Cholesterol is thought to act as a conveyer in the absorption of fats. Bloor (1943) reported a parallelism between the cholesterol content of blood and the fatty acid. Due to the abundance of cholesterol in nervous tissue, and its variation in mental diseases, it may function as an insulating medium for the myelin sheaths. Sterols are thought to have a role in maintaining the balance between the cell permeability and the membrane equilibrium of living cells. Further studies of incorporation of labelled cholesterol or labelled acetate into brain tissue indicate that the cholesterol of adult brain is relatively inert (Bloch et al., 1943; Waelsch et al., 1940; Srere et al., 1950 and Van Bruggen et al., 1953). Paoletti (1971) has substantiated the observation that microsomes are the subcellular site of brain cholesterol biosynthesis.

Exposure to dichlorvos (Tayyaba and Hasan, 1980), sumithion (Nag and Ghosh, 1984) and methyl parathion (Rao and Rao,

1984 and Hasan and Khan, 1985) have been found to increase the cholesterol content in rats and fishes. Alteration in cholesterol concentration in different brain regions after organophosphate administration has also been observed (Islam et al., 1983 and Tayyaba and Hasan, 1985). No report is, however, available on variations in the cholesterol levels of fish brain following dichlorvos intoxication.

1.11 Esterified fatty acid of the brain

Fatty acids are rich source of energy. The excess of those fatty acids which do not fulfill the immediate nutritional requirements is taken up in the adipose tissue and stored as triglyceride. A portion of those which enters the other extrahepatic tissues is oxidized immediately after its uptake, but a part may also be stored, possibly to provide a reserve source of energy which is immediately available to the tissues (Fritz, 1961 and Fritz and Kaplan, 1961). The concentration of esterified fatty acids depends upon the contents of the fatty acids present in triglyceride and phospholipids (Stern and Shapiro, 1953). Alterations in the concentration of esterified fatty acids after organophosphate intoxication have been observed in all the regions of CNS (Islam et al., 1983 and Tayyaba and Hasan, 1985). To my knowledge, the effect of organophosphate pesticides on esterified fatty acid levels are limited and particularly, the effect of organophosphate-dichlorvos on esterified fatty acids is not known, therefore, it would be of particular interest to evaluate the esterified fatty acid levels in different regions of the fish brain after exposure to three different doses of organophosphate-dichlorvos.

1.12 Gangliosides in the brain

Gangliosides are the sialic acid-containing glycosphingolipids which are found in highest concentration in nervous tissues (Klenk, 1942; Wiegandt, 1967; Ledeen, 1978 and Svennerholm, 1980). In the brain at the cellular level, gangliosides seem to be more highly concentrated in neurons (Yu and Iqbal, 1979) than in glial cells (Roberts et al., 1975) and myelin (Ledeen, 1978). Regional differences in ganglioside patterns of the nervous system have also been recognized. Many investigators have thought that each component of neural tissues would possess a specific set of gangliosides, and that a definite set of gangliosides might be required for particular membrane functions. In adult nervous system, the individual gangliosides have been suggested to play a role as membrane-bound receptors or co-receptors for toxins, drugs, viruses, hormones, transmitters etc. (Svennerholm, 1980). Evidence, linking gangliosides to specific neuronal function, is not yet convincing but because of their binding capacity for Ca^{2+} (Behr and Lehn, 1973 and Probst et al., 1979) and transmitters (Richardson et al., 1982), they may be involved in synaptic transmission (Rahmann et al., 1982).

It has been reported that the metasystox administration decreases the ganglioside concentration in all the brain regions (Islam et al., 1983 and Tayyaba and Hasan, 1985). Recently, Khan and Hasan (1988) have also observed decrement in gangliosides concentration in different regions of CNS following organophosphate methyl parathion intoxication. To date, no report is available on the ganglioside concentration in fish brain following dichlorvos treatment. Therefore, the present study

deals with the effect of dichlorvos on ganglioside concentration in different regions of the fish brain.

1.13 Lipid peroxidation

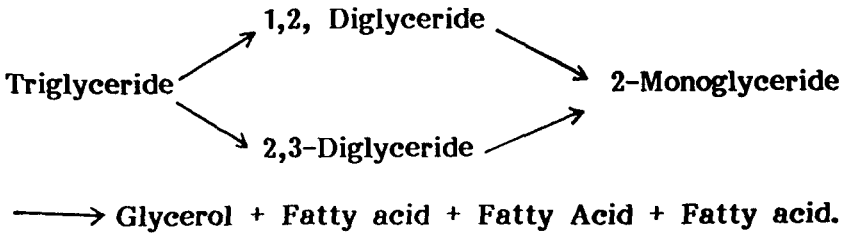
Lipid peroxidation is a basic deteriorative reaction that is involved in many disease processes and chemical toxicities (Tappel and Dillard, 1981). Recent interest in lipid peroxidation has probably resulted from the realization that oxygen radicals and organic radicals do indeed exist in biological tissues for an appreciable time (Tien et al., 1981). Lipid peroxidation is damaging because of subsequent reactions of free radicals, mainly peroxy radicals, $LOO^{\cdot}$ that are produced. Because of their unpaired electrons, free radicals react energetically and initiate relatively non-specific hydrogen abstraction and chemcial addition reactions.

The peroxidation of endogenous phospholipids in biological membranes has long been thought to be the basis for a variety of toxicological phenomena. Phospholipid constituents of biological membranes are subject to oxidative degradation, leading to structural damage causing disruption of cell integrity. Lability to lipid peroxidation in membranes is a function of polyunsaturated fatty acid content of constituent phospholipids. The rate of peroxidation varies directly with the number of double bonds in the fatty acids present in phospholipids (Witting, 1965). Thus, membranes that contain higher levels of polyunsaturated fatty acids in their phospholipids are more labile to lipid peroxidation.

Lipid peroxidation has been given attention in connection with various metabolic disorders or symptoms of senility (Strehler et al., 1959 and Oeriu, 1969). Studies also suggest that radiation hazards (Inouye et al., 1979) or influence of various environmental pollutants (Mudd and Freeman, 1977) are closely related to lipid peroxidation. Haider et al. (1980 and 1981) have reported that exposure of guinea pigs and rats to air pollutants, SO_2 and H_2S, induce significant increase in the rate of lipid peroxidation. Enhancement of lipid peroxidation following exposure to organophosphorous compounds have been noted in different regions of brain and spinal cord (Hasan and Ali, 1980; Islam et al., 1983; Tayyaba and Hasan, 1985; Hasan and Khan, 1985; Vadhva and Hasan, 1986 and Naqvi et al., 1988). To date, no report is available on variations in the rate of lipid peroxidation of different regions of fish brain following exposure to organophosphate dichlorvos. It would, therefore, be of interest to investigate this aspect in fish (H. fossilis).

1.14 Lipase activity

In the brain, lipase activity is concentrated more in gray than in the white matter (Gozzano, 1934), and certain areas, like postrema and caudate nucleus, contain higher concentrations than the others (Ishii, 1956). Lipases hydrolyze the triglycerides in the following sequence:



Rates of lipase reaction can therefore be measured by determining either the rate of disappearance of the substrate, the triglyceride, or the rate of production of the fatty acids.

Cabot and Gatt (1976; 1977 and 1978) found three lipase activities in brain. The lipase activity at the acidic pH (pH 4.8) hydrolyzing di-and triacylglycerols, whilst that at the alkaline pH (pH 8.0 - 8.6) hydrolyzing mono - and diacylglycerols. There are some indications that the lipase in brain is stimulated by biogenic amines (Vyvoda and Rowe, 1976).

Haider et al. (1982) have shown depletion of lipase activity in different regions of CNS after exposure to air pollutants to rats. Effect of water pollutants and organophosphorous compounds (malathion, diazinon and methyl parathion) on lipase activity in vitro was observed by Christensen and Riedel (1981). The alteration in the lipase activity due to organophosphate TOCP intoxication has also been reported (Shull and Cheeke, 1973). Caley and Jensen (1973) have observed a decline in the lipase activity following parathion intoxication. Depletion of lipase activity after metasystox administration has also been reported by Islam et al. (1983) and Tayyaba and Hasan (1985). The effect of organophosphorus compound, dichlorvos, on lipase activity in different regions of fish brain and spinal cord is not well known. It is therefore, appropriate to estimate the activity of lipase in different regions of fish CNS following exposure to dichlorvos.

1.15 Glycogen in the brain

Glycogen is the main storage polysaccharide of animal cells, made up of highly branched, chain like strings of glucose molecules. Although present in relatively low concentration (approximately 0.1%) in brain, glycogen is a unique energy reserve that requires no energy (ATP) for initiation of its metabolism. Glycogen granules function as a reserve fuel source, utilized when glucose falls below the requirement level.

It is known that fish under stress secrete catecholamines in increased amounts which deplete glycogen reserves (Nakano and Toulinson, 1967 and Larsson, 1973). Several workers (Koundinya and Ramamurthy, 1979 and Srivastava and Singh, 1981) have reported that sublethal concentration of certain organophosphate pesticides cause decrement of glycogen content which produced hyperglycemia in the African food fish (Tilapia mossambica) and the Indian cat fish (Heteropneustes fossilis). Decrement of glycogen level has also been observed in muscles and liver of the fishes exposed to various concentration of thiotox and dichlorvos and sublethal concentration of formothion (Singh and Srivastava, 1982 and Verma et al., 1983). Husain and Matin (1986) and Matin and Husain (1987a) have showed that depletion of cerebral glycogen in malathion treated animals was accompanied by an increase in the activity of glycogen phosphorylase which decomposes glycogen to glucose-1-phosphate. Reduction in cerebral glycogen content was also observed in diazinon treated hyperglycaemic animals (Matin and Husain, 1987b). Recently,

Khan and Hasan (1988) reported decreased concentration of glycogen after organophosphate administration. Neurochemical studies of regional glycogen changes of fish brain following the exposure of dichlorvos have not been reported in the literature and present investigation provides information on the effect of organophosphate dichlorvos on brain glycogen.

1.16 Nucleic acids in the brain

In brain, the nucleic acids provide for the storage and transmissions of genetic information as well as translation of this information leading to the synthesis of cellular proteins (White et al., 1978).

The only well-authenticated sites at which DNA is found in all animal cells, including brain and nerve, are the nucleus and the mitochondria. Since the average amount of DNA per diploid nucleus is constant for all normal tissues of the body, including brain tissue (Heller and Elliott, 1954), measurements of DNA quantity provides a convenient method for estimating total cell population in entire brain or in its various regions. The amount of DNA in white matter approximately equals to that in the cortex, and that regional differences in the amount of brain DNA are relatively small (Bodain and Dziewiatkowski, 1950; Logan et al., 1952 and Elliott and Heller, 1957). However, only cerebellum shows exceptionally high amounts of DNA (Mihailovic et al., 1958; Palladin, 1955; Grenell, 1958 and May and Grenell, 1959). It has been reported that the organophosphate DDVP induces degenerative changes in neurons and nerve fibres (Hasan et al., 1979a). The loss

of myelin during degeneration and the subsequent myelination of the newly growing fibres bring changes in most of the cell constituents including nucleic acids (McIlwain and Bachelard, 1971). Significant changes were marked in nucleic acids and protein content of DDT and dieldrin exposed rats (Bhatia et al., 1973 and Kohli et al., 1975). Some pesticides induced DNA damage in human cell culture (Ahmed et al., 1977). The results with organophosphate pesticide metasystox in rats show a remarkable decrease in the DNA level in all the brain regions. This has been correlated with increased DNase activity (Tayyaba et al., 1981).

The studies of RNA concentration are of interest to assess the rate of protein synthesis and also to understand the functional status of nervous tissue (Bergen et al., 1974). The amount of RNA in gray matter usually exceeds that in white matter (Bodian and Dziewaitkowski, 1950; Logan et al., 1952; Mihailovic et al., 1958 and Grenell, 1958). RNA is highly concentrated in the nucleolus and in the Nissl substance of the cytoplasm of nerve cells (Caspersson, 1936, 1940; Landström et al., 1941 and Hyden, 1943). The response of RNA to neurotoxicants like organophosphates has earlier been documented (Rath and Misra, 1980). RNA concentration has been reported to increase significantly in rat brain after metasystox administration (Tayyaba et al., 1981). It has been demonstrated that RNA synthesis increases due to damage of the neurons, in which, the RNA level remains high between 10 to 30 days after which it returns to a normal level (McIlwain and Bachelard, 1971). The available literature on nucleic acids, however,

indicate that knowledge of organophosphate toxicity on brain nucleic acids is inadequate. The present work deals with the effect of dichlorvos on nucleic acids concentration in different regions of the fish brain and spinal cord.

1.17 Proteins in the brain

Protein, one of the important biochemical components in vertebrate brain, constituents 40% of the dry weight of whole brain and 8% of the weight of whole fresh brain (McIlwain and Bachelard, 1971). A larger amount of protein is present in the gray than in the white matter. This difference probably affects the large volume of tissue occupied by myelin sheaths in white matter.

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 provides large number of sites for protein synthesis (McIlwain and Bachelard, 1971). Any change in protein concentration may influence the metabolic rate of tissue. It requires rapid synthesis and renewal of protein. It has also been reported that increased neuronal activity decreases or inhibits the synthesis of proteins (Hyden and Lange, 1972). The specific neuronal functions such as conduction of action potentials and synaptic transmission are extensively mediated by proteins (Bock, 1978).

Significant changes were observed in the protein contents of DDT and dieldrin intoxicated rats (Bhatia et al., 1973 and Kohli et al., 1975). The decrement in the protein concentration of different regions of brain following metasystox treatment has been reported by Tayyaba et al., (1981). Sub-lethal concentration of dichlorvos is reported to induce significant decrement in the levels of protein in fish, Tilapia mossambica (Rath and Misra, 1980). However, information on the dose-dependent effect of organophosphate dichlorvos on the protein content is still lacking in fishes. It is therefore, appropriate to estimate the protein content in different regions of fish CNS after dichlorvos treatment.

1.18 Sulfhydryl groups

Sulfhydryl groups act as active enzymatic sites in a number of important enzymes (Hoch and Vallee, 1959). Many enzymes contain sulfhydryl groups derived from the side chains of cysteine residues. The sulfhydryl group (-SH) of cysteine and the disulfide bond (-SS-) of cysteine are highly reactive and apparently involved in the maintenance of the conformation and biological activity of certain proteins. Because receptors are protein in nature, the use of reagents which modify these groups may influence the interaction of neurotransmitters with their recognition sites (Karlin and Bartels, 1966; Del Castillo et al., 1971; Sobrino and Del Castillo, 1972 and Barrantes, 1980).

Sulfhydryl groups have also been related to lipid peroxidation. Chio and Tappel (1969) have shown that sulfhydryl enzymes are most susceptible to lipid peroxidation induced inactivation. Moreover, Tappel

(1970) has suggested that deficiency of total and free sulfhydryl groups may lead to deficient degradation of lipid peroxides to hydroxy acids, causing accumulation of peroxides in various regions of the brain. However, no information is available on the neurobiochemical perturbations of sulfhydryl groups in the various regions of fish brain following dichlorvos intoxication. Therefore, it would be of paramount importance to evaluate the total and free sulfhydryl groups in different brain regions of dichlorvos treated fishes.

1.19 Light and electron microscopy

Light microscopic study was carried out to observe the changes in the total lipid and phospholipid cencentration in rat brain after organophosphate intoxication (Tayyaba and Hasan, 1980). Organophosphate-treated rats showed sudanophillic lipid deposits. However, at frequent sites mild deposits of phospholipid were seen. Neurotoxic effect of organophosphates have been attributed to changes in peripheral nerves, degeneration or demyelination (Cavanagh, 1954). Glees and White (1961) showed nerve fibres degeneration in the anterior medial tract of the cord of hen after painting 0.1 ml triorthocresyl phosphate (TOCP)/kg on the comb. Motor neurons did not show significant changes but silver preparations demonstrated characteristics terminal degenerations, fragmentation and granulations of axons.

In an ultrastructural investigation by Le Vay et al. (1971) on neurons of spinal ganglia in TOCP-poisoned hens, light neurons were shown to react to the poisoning by an increase in the number of cytoplasmic filaments, whereas the darker cells showed a hypertrophy of the endoplasmic reticulum. Glees and Janzik (1965) and Janzik and

Glees (1966) reported that organophosphate intoxication induced fibre degeneration in the CNS and chromatolysing spinal neurons. On the other hand, Ahmed and Glees (1971) observed large number of laminated cytoplasmic inclusion bodies in the spinal cord neurons intoxicated with insecticides. The postulated that phospholipids become unmasked during degenerative process and arrange themselves in the form of laminated dense bodies. Vij and Kanagasuntheram (1972) showed the damage caused by TOCP intoxication on the peripheral nerve endings of loris. Krishnamurthy et al. (1972) have showed electron microscopic evidence of toxic effects of triorthocresyl phosphate on digital Pacinian corpuscles of this animal. Evidence of mitochondrial degeneration in the form of swollen profiles and loss of cristae, together with neurofilamentous accumulation, were also reported. After 10 days of TOCP ingestion in spinal ganglia, Spoerri and Glees (1979) have clearly demonstrated enlarge and swollen mitochondria with complete to partial disruption of cristae and loss of matrix. Lipofuscin granules of various sizes and shapes were found to be associated with the altered mitochondria. Their internal structure were heterogenous, showing dark granules, laminations and vacuoles. The cisternae of the endoplasmic reticulum were elongated and arranged in parallel arrays. Hasan et al. (1979 a,b & c) observed remarkable myelin figures in a few axons and dendritic profiles after organophosphate administration. In many instances, the axonal profile was found to be stuffed with synaptic vesicles. A few mitochondria were detectable within the dense axons. The most remarkable feature of the organophosphate treated spinal neurons was, however, the increased incidence of pleomorphic electron dense bodies in the perikaryon. Most of

these organelle were uniformly electron-dense but occasionally electron lucid vacuoles were also detected. Hasan and Ali (1980) reported that after 1.5 mg dichlorvos/kg body wt. treatment, cerebellar neurons, particularly Purkinje cells of the animals exhibit electron dense pigment granules revealing internal complexity in the form of a couple of translucent or almost translucent vacuoles. The specimens obtained from the animals given 3 mg dichlorvos/kg body weight daily for ten days showed a remarkable increase in the incidence of electron dense bodies. In the perikaryon of a Purkinje cells a string of 3 pleomorphic granules was observed. The neuropathological studies of cases with organophosphate pesticide dichlorvos intoxication are limited and electron microscopy has, so far, not been practiced to investigate the effects on the fish brain. Therefore, it would be of great importance to investigate the histochemical and ultrastructural changes in the fish brain following dichlorvos exposure.

1.20 Objectives of the present study

The present study on the fresh water teleost, Heteropneustes fossilis, was undertaken with the following main aims:

i) Quantitative evaluation of the effect of organophosphate dichlorvos intoxication on the concentration of total lipids, phospholipids, cholesterol, esterified fatty acids, gangliosides, lipid peroxidation and lipase activity in different regions of CNS.

ii) To estimate glycogen content in various regions of brain and the spinal cord following dichlorvos exposure.

iii) To evaluate the effect of dichlorvos on the levels of DNA, RNA and protein after dichlorvos treatment in discrete areas of the brain.

iv) Qualitative evaluation of total lipids, phospholipid and RNA content following dichlorvos treatment.

v) To estimate total and free sulfhydryl groups in different regions of CNS after dichlorvos exposure.

vi) To evaluate ultrastructural changes in the brain after dichlorvos intoxication.