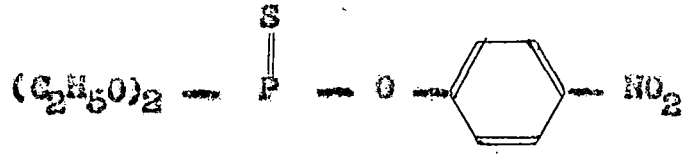
P A R T - I

ORGANOPHOSPHORUS PESTICIDES : GENERAL INTRODUCTION

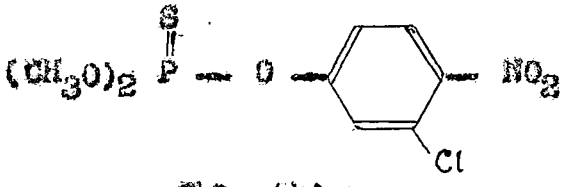
1. INTRODUCTION:

Intensive research in the field of plant protection and pest control has been going on throughout the world. As a result various types of pesticidal compounds are being prepared, and their pesticidal, toxicological and other properties are being studied everyday. Of these, organophosphorus compounds constitute a class in which quite a large number of compounds have been synthesized and examined as effective pesticides, owing to their high activity and bio-degradability, their application in agriculture, public health, and related fields has been going rapidly. Several new compounds of this group are used for insecticidal, acaricidal, nematocidal, anthelmintic, insect sterilizing, fungicidal, harbicidal, rodenticidal and other purposes. The development of new phosphorus compounds was for a long time dominated almost exclusively by one single guiding principle namely the "Acyl Rule" of Schrader[1,2,3]. The great advancement in agricultural practice, scientific knowledge of the structure activity relationship and mode of action of organophosphorus pesticides were achieved by the discovery of parathion by Schrader in 1944. Parathion is extremely toxic to mammals as well as to insects. Many less toxic pesticides have been synthesized by slight structural modification of parathion, for example, chlorthion (in 1952), fenthion (in 1958) and

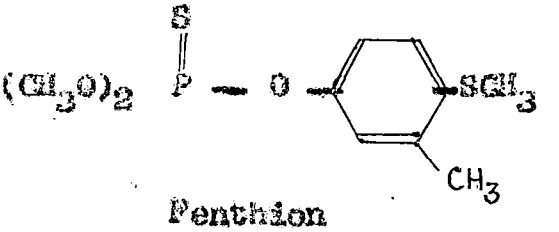
fenitrothion (in 1959) were discovered[3].



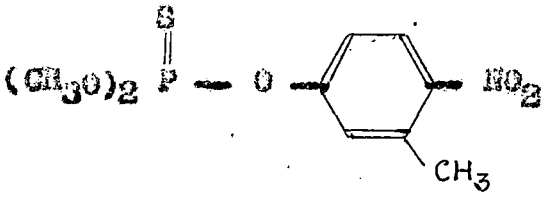
Parathion



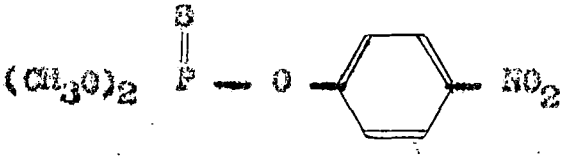
Chlorthion



Penthion

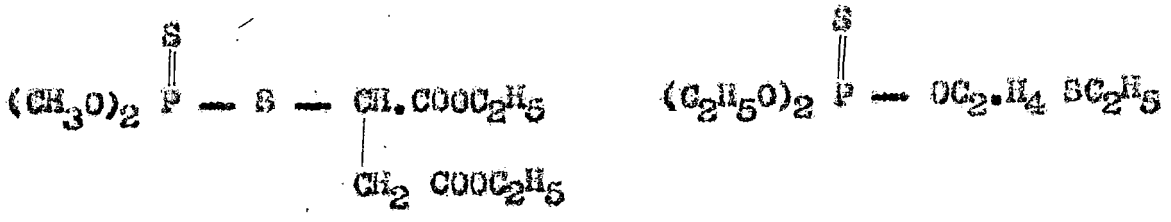


Fenitrothion



Parathion - methyl

Malathion was discovered in 1950 and demeton in 1951.



Malathion

demeton - S

In 1952, the Perkow reaction was discovered, and many important vinyl phosphate esters have been introduced as practical pesticides. Since then several new compounds have been developed and are in commercial use[3].

2. ORGANOPHOSPHORUS FUNGICIDES:

The first studies in which the microbiological action of organophosphorus compounds was noted were made at the beginning of the 1940's, but systematic investigations of their fungicidal and bactericidal properties were begun much later[4-6]. It is only recently they have been gaining importance in the control of pathogenic fungi[2,3]. In comparison to the heavy metal fungicides, the organophosphorus compounds are particularly

favourable as regards to residue problem.

The simplest organophosphate is trimethyl phosphorothioate.

$$(CH_3O)_3 . PS$$

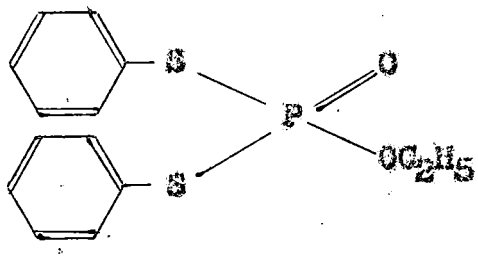
It is an effective, selective soil fungicide to control Pythium sp[7].

Trimethyl phosphorotetrathioate appear to be useful for the control of Pythium sp.[7b,c]

$$(CH_3S)_3 PS$$

The fungicidal activity of trialkyl phosphorotetrathioates decreases with increasing chain length of the alkyl group. These compounds are highly species selective in fungicidal activity. Thus, trimethyl phosphorothionate is almost ineffective against Rhizoctonia, Fusarium, and Verticillium[7c]. On the other hand, an analogous compound, O,O-diethyl S-methyl phosphorodithioate, is a good fungicide against Rhizoctonia solani[7b]. The fungicidal activity of different other compounds are given below:

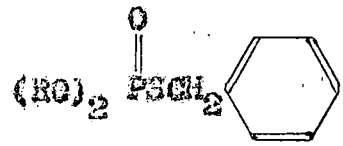
Edifenphos (Hinosan):



This is a fungicide with specific action against Pyricularia oryzae on rice at 30 - 50 g a.i./l water using 800 - 1200 l/ha; 1 or 2 applications to wet paddy in the nursery, 2 or 3 applications after transplanting or in fields of broadcast rice before tillering has ceased. It is also effective against Pellicularia sasakii and ear blight and is well tolerated by rice varieties at effective fungicidal rates. It should not be used within 10 days before or after an application of propanil.

The n-propyl and isopropyl homologs have almost the same fungicidal activity as the ethyl ester edifenphos, but the methyl and butyl homologs are much less active than the later. The introduction of a chlorin atom into the benzene ring causes a remarkable decrease in fungicidal activity.

Kitazin:



R = $(CH_3)_2$ CH Kitazin P.

R = C_2H_5 Kitazin.

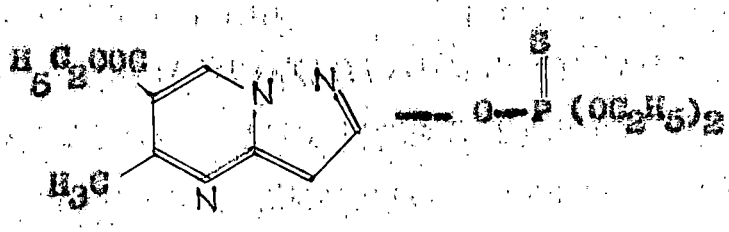
S - Benzyl diethyl phosphorothiolate was first introduced in 1965 as a fungicide under the trade name Kitazin, but was replaced in 1967 by the isopropyl homolog Kitazin P for commercialization.

It is a systemic fungicide used to control Pyricularia oryzae in rice. It is applied at 400 - 600 g a.i. (as e.c.) in 1000 l/ha as soon as the blast lesions appear. One or 2 sprays may be needed during the head-sprouting season. Kitazin and Kitazin P inhibit more strongly the mycelial growth and the spore formation of Pyricularia oryzae than the spore germination. Thus, they are effective curatively rather than prophylactically.

In the homologous series of dialkyl S-benzyl phosphorothiolates, the maximum fungicidal activity is obtained when the number of carbon atoms in the alkyl group is three or four. The dimethyl homolog has poor activity. In the analogous series, the phosphorothiolate esters are much more effective than corresponding phosphorothionate, phosphorothiolothionate, and phosphate esters. Introduction of substituents such as chlorine atom or

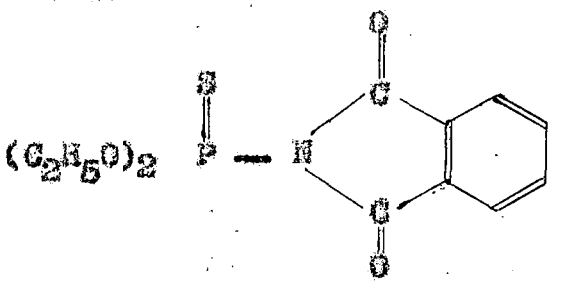
nitro group on the benzene ring has little effect on the increase of the fungicidal activity.

Pyrazophos (Afugen):



Pyrazophos is a systemic fungicide controlling powdery mildews on a wide range of crops at 10 - 30 g a.i./100 l, and on cereals at 500 - 700 g/ha. It has both preventive and curative activity against powdery mildews. It is absorbed by foliage and green stems and translocated within the plant when applied to the soil or a seed dressing uptake by roots is insufficient for effective fungicidal action within the plant.

Ditalimfos (DowCo 199):

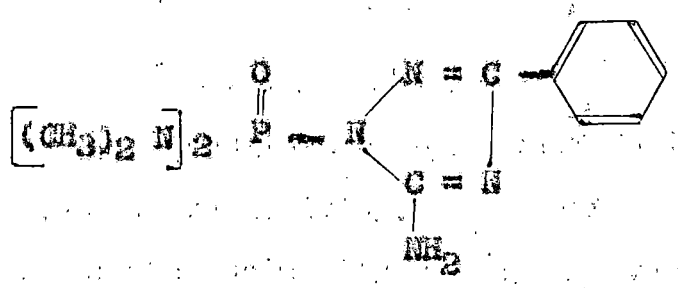


Ditalimfos is a non-systemic foliar fungicide with protectant and curative activity. It is used to control powdery mildew on ornamentals (primarily roses) and vegetables (cucurbits) under glass, as well as under field conditions, at 30 - 50 g. a.i./100 l; on apples (25 - 50 g/100 l) and cereals (500 - 550 g/ha). It is also used against Venturia inaequalis on apples at 37.5 - 100g/100 l. It is liable to 'russet' certain apple cultivars, particularly Golden Delicious.

The isopropyl homolog of Ditalimfos has similar fungicidal activity but is about three times more toxic to mammals. The methyl homolog and the methylamide analog are much less active in fungicidal action. The aromatic ring is necessary for the fungicidal activity, but any substitution on the ring causes a remarkable decrease in fungicidal activity. It is interesting to note that the fungicidal activity of Ditalimfos is lost by replacing the thiophosphoryl sulfur atom with an oxygen atom.

Furthermore, if the phthalimide-N is not directly attached to the phosphorus but through an S-CH_2 bridge or an oxygen atom, the phosphorus compounds are not fungicidal but insecticidal.

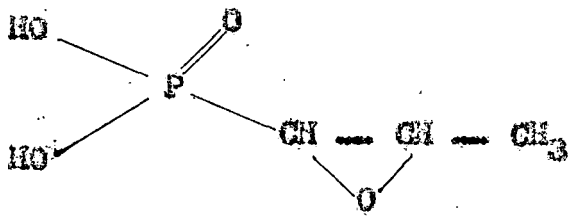
Triamiphos (Wepsyn, Wepsin):



Triamphos is a fungicide for powdery mildew control and shows some systemic activity; it also has systemic insecticidal and acaricidal properties. Rates for powdery mildew control include : for apples 25 g.a.i. (as w.p.)/100 l every 10 d; for roses 25 g.a.i. (as water-miscible)/100 l. At these concentrations, it is non-phytotoxic and presents no hazard to wild life.

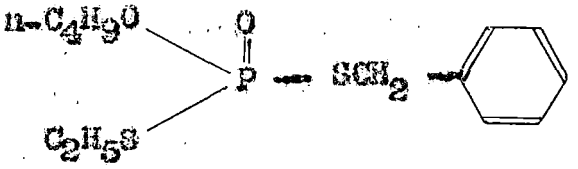
The 5- anilino-3-alkyl analogs of Triamiphos are also active as fungicides.

Phosphonomycin:



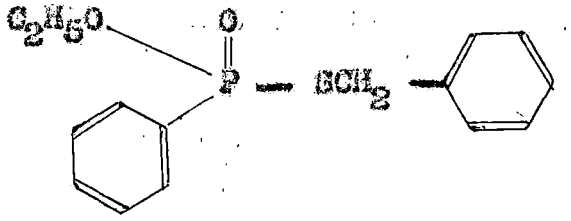
Phosphonomycin is a naturally occuring phosphonate antibiotic recently discovered by Merck & Co. Inc. It was isolated from fermentation broths on which Streptomyces fradiae was grown. Its structure was demonstrated by synthesis[8]. This new antibiotic has a broad spectrum of activity and inhibits irreversibly pyruvate-uridine diphospho-N-acetylglucosamine transferase in extracts of gram-positive and gram-negative micro-organisms. It compares favourably with tetracycline and chloramphenicol.

Conen:



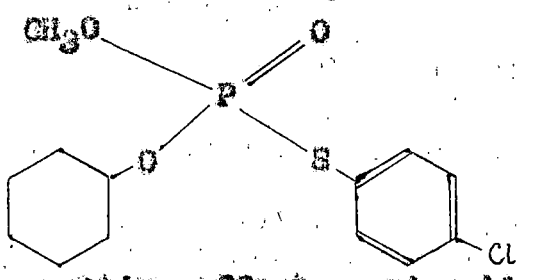
It is a fungicide to control rice blast disease.

Inezin:



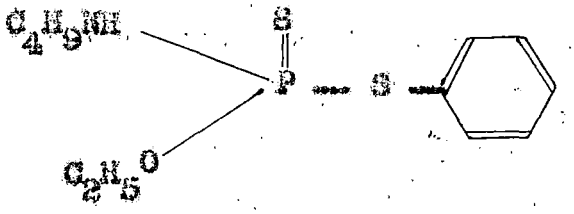
It is a fungicide to control rice blast and rice sheath blight.

Cerezin:



Cerezin has a curative effect on rice blast disease. It has also insecticidal activity against two hopper species, Nephotettix cincticeps and Delphacodes striatella, which transmit virus disease to rice plants.

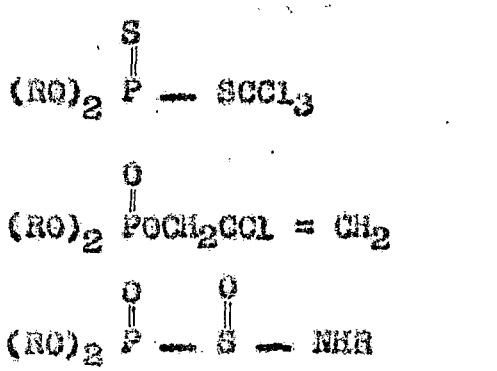
Phosbutyl:

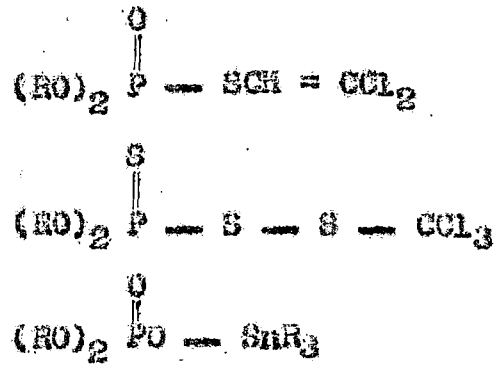


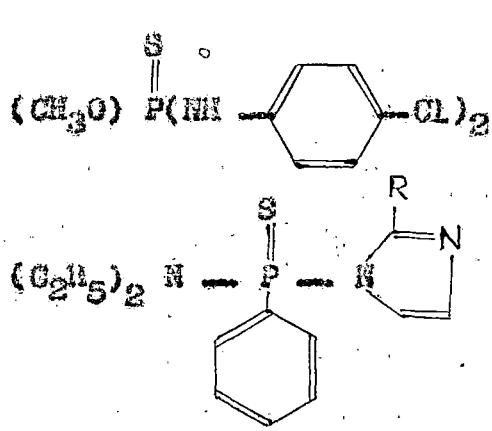
It is highly active against mycelial cells, but not active against

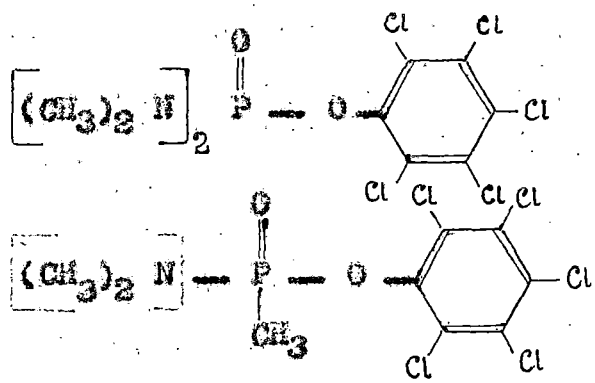
spore germination. Being absorbed rapidly by the plant, it thus shows a good curative activity for many plants infected with pathogenic fungi.

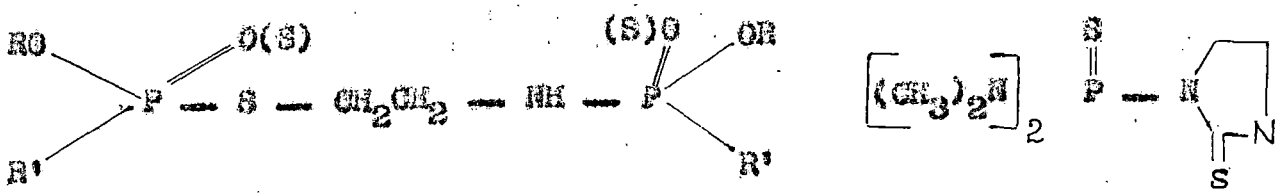
Certain organophosphorus compounds are known to have fungicidal activity. Structures of some selected compounds are given below:











There are certain reviews on organophosphorus fungicides (7b,9,10). There is an interesting correlation among the alkylating activity,

the inhibitory activity against 'SH enzymes', and the antifungal activity of some cyclic organophosphorus esters[11]; many fungicides are known as the inhibitors of 'SH enzymes'[12].

3. REACTION WITH CHOLINESTERASE:

It is generally accepted that the organophosphorus compounds are toxic because they phosphorylate vital esterases, thus forming complexes that are either irreversible or do not readily release the enzymes[3]. The enzyme mainly affected is accepted to be cholinesterase, an enzyme that plays a vital role in hydrolysing acetylcholine. The reaction between acetyl choline (ACh) and cholinesterase (ChEH) takes place in three stages:

$$(CH_3)_3 \overset{+}{N}C_2H_4O - \overset{O}{\overset{\|}{C}} - CH_3 + ChEH \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} (CH_3)_3 \overset{+}{N}C_2H_4O - \overset{O}{\overset{\|}{C}} - CH_3/ChEH$$

Acetyl Choline — Enzyme Complex

At this stage there is an equilibrium between the enzyme and its substrate on the one hand and a complex of the two on the other.

$$(CH_3)_3 NC_2H_4O - \overset{O}{\overset{\|}{C}} - CH_3/ChEH \xrightarrow{K_2} (CH_3)_3 NC_2H_4OH$$

Enzyme Complex — Choline + CH_3CoECh Acetylated enzyme

The complex yields choline and acetylated enzyme in the second stage.

$$CH_3CO.ECh \xrightarrow[K_3]{H_2O} CH_3COOH + ChEH$$

Acetylated enzyme Acetic acid enzyme

The final stage is the deacetylation in which the acetylated enzyme is hydrolysed to give the free enzyme and acetic acid.

The active centre of acetylcholinesterase (AChE) is structurally complementary to its substrate acetylcholine which contains a trimethyl ammonium group with a positive charge on N and an ester linkage. The enzyme's active centre contains a negatively charged anionic site, which binds the trimethylammonium group, and a relatively "non-specific" esteratic site, which catalyzes the hydrolysis of the ester linkage. In the esteratic site there are basic (histidine, imidazole, serine hydroxyl) and acidic (tyrosine hydroxyl) groups (Fig. 1). The reaction between an organophosphorus compound and AChE is represented in Fig. 2. When the two chemicals interact there is a nucleophilic attack of the serine hydroxyl on the phosphorus atom that is aided by the acidic and basic groups present in the esteratic site of the enzyme. This results in the formation of a "reversible" complex that finally yields phosphorylated enzyme and nitro-saligenin. Aldridge[13] investigated the inhibition of cholinesterase by parathion and related compounds and found that the complex did not show significant reversibility. In other words, the inhibition of cholinesterase in this case followed first order kinetics and was bimolecular, i.e.;

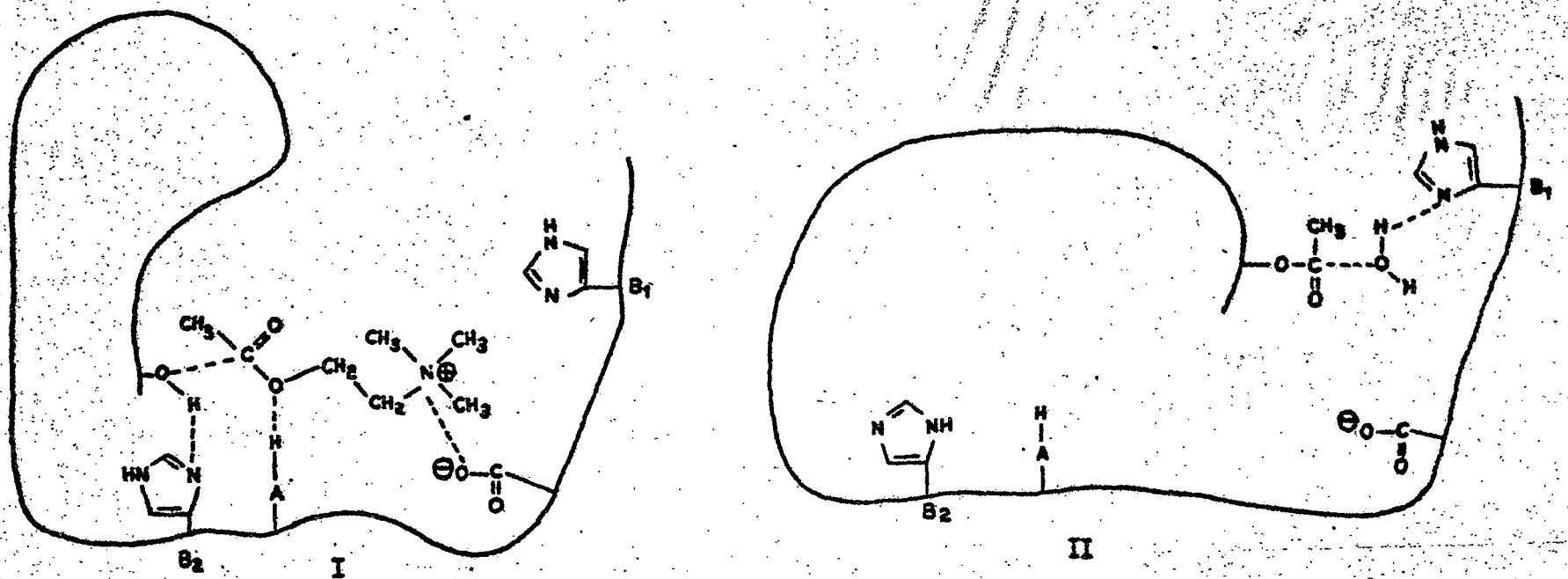


Fig. 1. Schematic Mechanism of action of AChE, after Krupka.
(I) Enzyme - substrate complex in AChE.
(II) Deacetylation of acetyl - AChE.

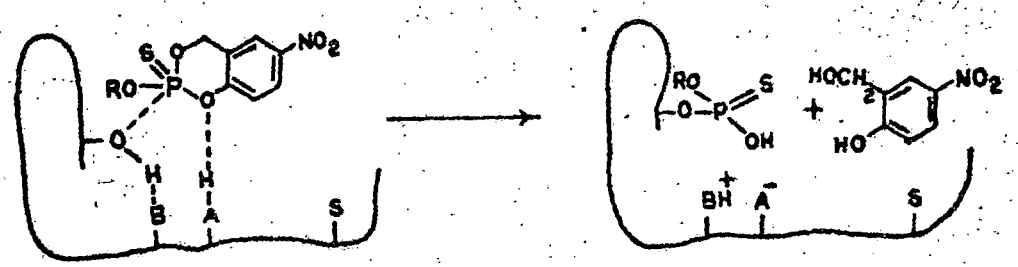


Fig. 2. Schematic mechanism of reaction of organophosphate with AChE.

$$K = \frac{1}{tI} \cdot I_n \frac{100}{b}$$

Where, K = bimolecular rate constant

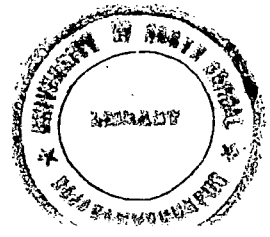
t = time in minutes,

I = molar inhibitor concentration,

and, b = percentage residual activity

Correlation between the reactivity of a organophosphorus compound and its cholinesterase inhibition, however, has not been ideal, and Main[14] introduced a kinetic treatment for the reaction that takes into account the reversibility of the complex. This reversibility is dependent on the affinity of the inhibiting compound for the active site of cholinesterase as well as on the rate of phosphorylation (Fig. 2). By utilizing different kinetic methods the values for K_1 (affinity constant), K_p (Phosphorylation constant), and K_3 (bimolecular inhibition constant) may be determined[12,15].

If the acelylcholinesterase is destroyed, is irreversible bound, or forms a complex from which it is released more slowly than under normal condition, its substrate, acetylcholine, is not easily removed from the receptor surface of the muscle. This causes the muscle to be depolarised longer than usual and gives rise to several action potentials passing through the muscle. The result is a twitching of the muscle leading to tetanus and eventual



paralysis of the muscles. Death in mammals occurs as a result of asphyxia caused by the paralysis of the respiratory muscles.

4. CHEMICAL HYDROLYSIS:

Since most organophosphorus pesticides hydrolyse their persistant and/or appearance of hydrolysis products may be obtained from kinetic studies. Hydrolysis rates of these compounds and their metabolites are of interest since chemical hydrolysis determines whether or not toxic residues will persist. The first-order half-lives of some common organophosphorus pesticides [redacted] including some metabolites are listed in Table - 1 (16).

Table - 1

Half-lives of some organophosphorus pesticides in ethanol (temp. 70°C pH 6.0, buffer Solution 1:4)

Compound	Half-life hours	Compound	Half-life hours
Thimet oxon	0.50	Demeton-S	18.0
Dichlorvos	1.35	Morphothion	18.4
Thimet	1.75	Baytex	22.4
Trichlorphon	3.2	Vamidothion	25.4
Mecarbam	5.9	Menazon	27.6
Maleoxon	7.0	Paraoxon	28.0
Demeton-S-methyl	7.6	Thionazin	29.2
Malathion	7.8	Disulfoton	32.0
Thionazin-oxon	8.2	Diazinon	37.0

.....Contd

Table-1 (Contd.)

Compound	Half-life hours	Compound	Half-life hours
Parathion methyl	8.4	Ethion	37.5
Fenchlorphos	10.4	Parathion	43.0
Azinphos methyl	10.4	Phenkapton	92.0
Sumithion	11.2	Chlorfenvinphos	93.0
Dimethoate	12.0	Carbophenothion	110.0
Thiometon	17.0	Dimefox	212.0
Methyl Oxy-demeton	17.1		

The hydrolysis rate is dependent upon the chemical structure and reaction conditions such as pH, temperature, the kind of solvent used, and the existence of catalytic reagents [3]. In aqueous solution, between the pH range 1 to 5 many organophosphorus pesticides are most stable [117], and in this range (pH 1 to 5), the variation in pH of the solution has practically no effect on the hydrolysis rate. But the hydrolysis rate increases steeply at pH higher than 7, and all organophosphorus pesticides are much more unstable under alkaline conditions. Very good discussions on chemical structure and hydrolyzability of various organophosphorus pesticides are given by Eto [3] and Faust [16].

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