A BRIEF REVIEW ON ROLE OF ORGANOPHOSPHORUS IN PESTICIDES

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Abstract

One of the significant classes of pesticides is that of the organophosphates (OPs). Starting improvements date back very nearly 2 centuries yet it was distinctly during the 1940s that OPs arrived at a noticeable status as insect poisons, a status that, yet declining, is as yet continuous. Operations are profoundly poisonous to nontarget species including people, the essential impacts being an intense cholinergic harmfulness and a postponed polyneuropathy. Several issues of current debate and investigation on the toxicology of OPs are discussed in this brief review. These include (1) Organophosphorus Pesticides Exposure, (2) Absorption, Metabolism, Biotransformation and Excretion of Organophosphorus Pesticides (Mechanism of Action of Organophosphorus pesticides: Acetylcholinesterase. A portion of these issues have been discussed and read up for quite a while, while others are more up to date, proposing that the investigation of the toxicology of OPs will stay a significant logical and general medical problem for a really long time in the future.

Key words: organophosphates, poisonous, Pesticides, Acetylcholinesterase

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History of Organophosphorus Pesticides

In "Pesticides and Neurological Diseases" it was noted that in 1932, Lange in Berlin synthesized some compounds containing a phosphorus-fluoride bond (esters of monofluorophosphoric acid from silver salts and alkyl halides). During the synthesis of dimethyl- and diethyl phosphorofluoridate, Lange and his graduate student, Gerda von Krueger, noted toxic effects of the vapors on themselves, the pertinent observations being included in a published chemical paper. Lange was unable to convince the chemical industry and I. G. Farben industries, in particular, that the alkyl esters synthesized might be useful insecticides. In 1934, Gerhard Schrader was appointed by Otto Bayer to pursue the development of synthetic insecticides for I. G. Farbenindustrie, but it was not until 1936 that Schrader began working on phosphorus and sulfur acid fluorides in search of aphicidal and acaricidal compounds, initially discovering methane sulfonyl fluoride which was used as a fumigant. From 1938 to 1944, Schrader developed a series of fluorine-containing esters including DFP (di- isopropyl fluorophosphate) and Sarin (l-methylethyl methyl phosphono

fluoridate), pyrophosphate esters including TEPP and OMPA (octamethylpyrophosphortetramide) and thio- and thionophosphorus esters including parathion and its oxygen analog paraxon. He was aware of the toxic signs produced by these esters and, while the potency of some of these chemicals prevented their development and use as "insecticides", they were of immediate interest to the German Ministry of Defense which recognized their value as chemical warfare agents. Production of stocks of Tabun and Sarin were carried out in a factory outside of Duhernfurt, near Breslau. Soman (1,2,2-trimethylpropyl methylphosphonofluoridate), another nerve gas was also synthesized at this factory. The pharmacological and toxicological studies of these compounds were carried out in a number of industrial and military laboratories. British scientists had taken note of the comments of Lange and Krueger concerning the toxicity of acylphosphorofluoridates, and during World War II they were paying particular attention to fluorine-containing compounds. With this lead, it is interesting to note that studies conducted by these two protagonists were almost parallel, DFP and other alkyl phosphorofluoridates being the prime test chemicals. A similar line of investigation was being followed at Edgewood Arsenal in the U. S., again DFP being a compound of choice in such studies. Scientists on both sides of the Atlantic were well aware of the potent, irreversible, anticholinesterase properties of these esters. When the structures and properties of the German nerve gases Tabun and Sarin became known, it was realized that they were more potent than DFP by an order of two of magnitude. With the cessation of hostilities and the exchange of information in the post-war period, the chemistry of organophosphorus insecticide poisons developed at a rapid rate. The decade from 1950 to 1960 can well be said to have been the era of the organophosphate poisons. Malathion [diethyl(dimethoxyphosphinothioyl) thiobutanedioate] was introduced by the American Cyanamid Company in 1950; this ester contains carboxy ester groups. In 1951, G. Schrader continued developing new insecticide poisons including Systox (demeton or mercaptophos, a mixture of the thiono- and thioloisomers of O,O-diethyl-2-ethylmercaptoethyl phosphorothioate), thereby introducing a new class of insecticide poisons having a thioether group. In 1952, the Perkow reaction was first described in which alpha-halogen carbonyl compounds were reacted with triethyl phosphite, resulting in the synthesis of a number of new dialkylvinyl phosphate esters such as dichlorvos (2,2-dichlorovinyl dimethyl phosphate) and trichlorfon (O,O-dimethyl [2,2,2-trichloro-1-hydrox". Today, a wide range of organophosphorus esters having a variety of biological properties are available for such equally diversified range of uses as various "registered" poisons, e.g., insecticides, nematocides, acaricides, etc.

INTRODUCTION:

Pesticides are described as chemicals that kill or slow down the growth of undesirable organisms. Pesticides include herbicides, insecticides, fungicides, and nematocides. Nowadays, it is believed that application of synthetic pesticides is one of the most effective methods for controlling insects that affect crop growth. Organophosphorus pesticides constitute the most widely used insecticides available today. This class of compounds has achieved enormous commercial success as a key component in the arsenal of agrochemicals, and is currently an integral element of modern agriculture across the globe. According to the EPA, about 70% of the insecticides in current use in the US are organophosphorus pesticide . They were developed to replace organohalide pesticides in the late 1950's because organophosphorus pesticides are relatively easier to degrade via microbial or environmental processes. Unlike organohalide pesticides, the organophosphorus pesticides do not bioaccumulate due to their rapid breakdown in the environment and they are thus preferred over organohalides for insecticide and/or pesticide use.[6]

Although organophosphorus compounds are considered safer than organohalides, they are still highly neurotoxic to humans and in some cases their degradation products have the potential to be more toxic with chronic exposure. Organophosphorus compounds (OP) are efficiently absorbed by inhalation, ingestion, and skin penetration.

They are strong inhibitors of cholinesterase enzymes that function as neurotransmitters, including acetylcholinesterase, butylcholinesterase, and pseudocholinesterase. These enzymes are inhibited by binding to the organophosphorus compound. Upon binding, the organophosphorus compound undergoes hydrolysis leading to a stable phosphorylated and a largely unreacted enzyme. This inhibition results in the accumulation of acetylcholine at the neuron/neuron and neuron/muscle junctions or synapses. Each year organophosphorus pesticides poison thousands of humans across the world. In fact, in 1994, an estimated 74,000 children were involved in common household pesticide related poisoning or exposures in the United States .[7]

In a more recent study, it was found that children exposed to organophosphorus pesticides were more likely to be diagnosed with attention deficit hyperactivity disorder (ADHD). Exposure has been attributed to frequent use of organophosphorus pesticides in agricultural lands and their presence as residues in fruits, vegetables, livestock, poultry products and municipal aquifers (Liu and Lin, 1995). For example, typical pesticide concentrations that flow into aqueous waste range from 10,000 ppm to 1ppm Pesticides are influenced by a number of biological, chemical and physical processes once they enter the environment. While many OP pesticides can degrade via microbial or environmental processes, some of the pesticides are consumed by organisms, or they could leach into ground water.[8] Once a pesticide enters ground water it can remain there for considerable periods of time. In ground water, there is little sunlight exposure, which slows down the degradation of OP pesticides and increases their potential risks to the environment and human health.[9]

Organophosphorus Pesticides Exposure

Intoxication may occur following absorption via the gastrointestinal system, respiratory tract or skin. Deliberate or accidental ingestion is a common mode of poisoning [10]. Deliberate ingestion, which is usually of suicidal intent, is common in developing countries [11] where these agents are relatively more readily available and cheaper compared with the more sophisticated agents used for suicidal purposes in developed countries. Inhalation may occur when spraying is carried out under improper conditions. Intoxication by inhalation may occur also during chemical warfare and following accidents during storage, particularly when stocks catch fire. Absorption through skin may occur in handlers and sprayers. Exposure during normal operational production and use depends mainly on the quality of personal clothing and the physical state of the pesticides.

Absorption, Metabolism, Biotransformation and Excretion of Organophosphorus Pesticides:

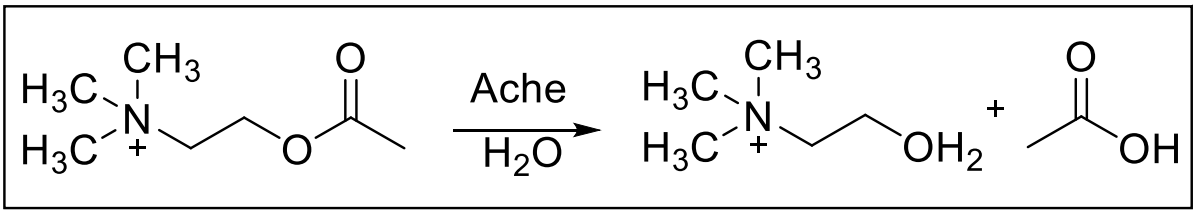
Organophosphorus insecticides are lipophilic compounds and are readily absorbed from the oral mucousa, skin membrane, conjunctiva, respiratory, gastrointestinal tracts and are rapidly distributed to the tissues of the body. Organophosphorus Pesticides (OP) absorption into the bloodstream is fast through any of the routes mentioned but dermal uptake is slower than other routes. The onset to duration of toxicity is determined by physiochemical properties (e.g. partition coefficient, lipid solubility and Pka,) dose, route of exposure, rate of metabolism and t1/2 of the type of organophosphorus insecticide involved. OP metabolism is dependent on the type of group attached to the OP backbone structure and is highly specie specific. Biotransformation of

organophosphorus Pesticides occurs in the liver, where cytochrome P- 450 converts P = S to P = O (reactive metabolite) in toxic or oxon form. OP readily undergo oxidation, reduction, hydrolysis (phase I biotransformation) and conjugation with glutathione (GSH) liver (Phase II biotransformation). Malathion provides a good illustration of the mechanisms involved in acute toxicity. OP is mainly excreted through urine. For instance, conversion of methyl parathion to methylparaoxon occurs within minutes of administration. Methyl parathion or methylparaoxon are mainly detoxified in the liver through Phase I (hydrolysis, oxidation,) and dearylation with reduced glutathione (GSH). One of its metabolites is p-nitrophenol. Therefore, measuring the urinary excretion of p-nitrophenol would throw some light into method of elimination. The elimination of methyl parathion and its reactive metabolites occurs primarily via the urine. Studies conducted on mice with radiolabelled (% P- methyl parathion) revealed 10% radioactivity in the faeces and 75% radioactivity in the urine.[12]

Mechanism of Action of Organophosphorus pesticides:

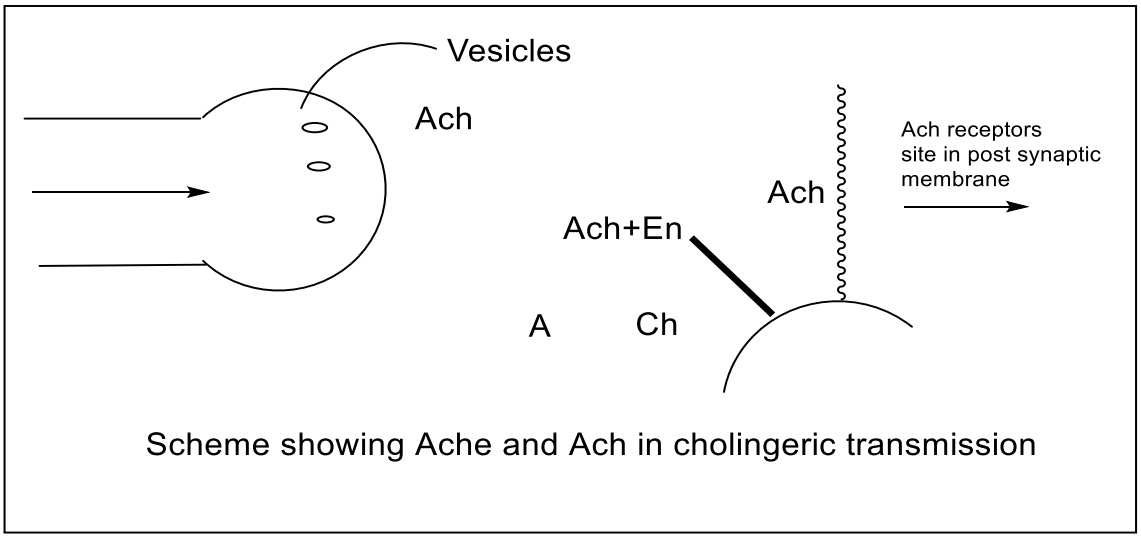
Acetylcholinesterase

AChE is present and has been isolated from a wide range of animals, including mammals, birds, fish, reptiles, and insects. It is responsible for the rapid hydrolytic degradation of the neurotransmitter Ach into the inactive products choline and acetic acid, as indicated by Equation. Acetylcholine is one of a number of physiologically important neurotransmitting agents and is involved in the transmission of nerve impulses to effector cells at cholinergic, synaptic, and neuromuscular junctions.[13]



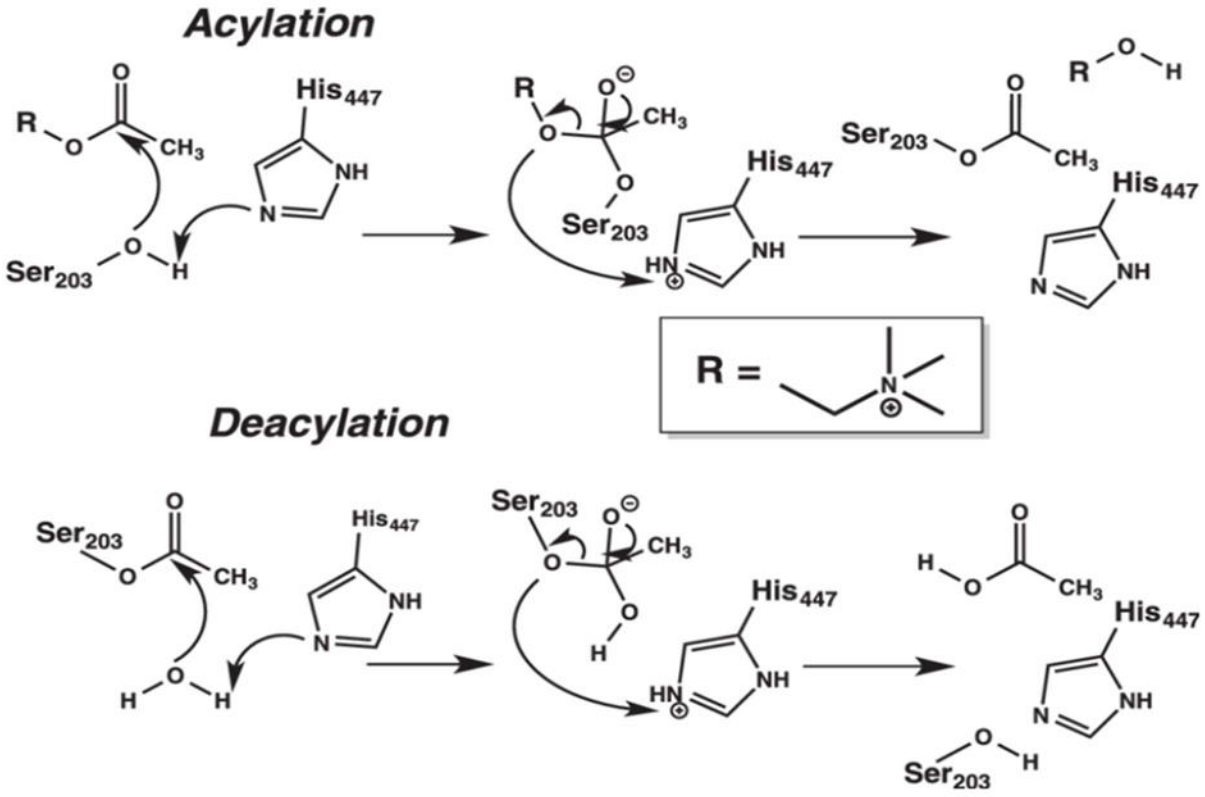
AChE is virtuallya ubiquitous enzyme in vertebrates and invertebrates, and in mammals, it is localized in certain areas of the central nervous system and in organs and glands that are controlled by the parasympathetic division of the autonomic nervous system.

The role of AChE in cholinergic transmission at a synaptic or cholinergic junction is depicted in the elementary scheme given in Figure. When a nerve impulse moves down parasympathetic neuron and reaches a nerve ending, the ACh stored in vesicles in the ending is released into the synaptic or neuromuscular junction. Within 2 to 3 msec the released Ach interacts with the ACh receptor site on the postsynapticmembrane, causing stimulation of the nerve fiber or muscle. AChE serves as a regulating agent of nervous transmission by reducing the concentration of ACh in the junction through AChE catalyzed hydrolysis of Ach into choline (Ch) and acetic acid (A)



Scheme showing Ache and Ach in cholingeric transmission

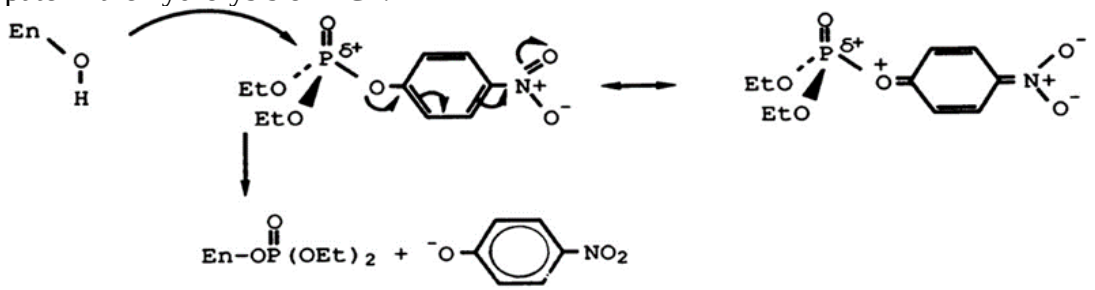
These products do not stimulate the postsynaptic membrane. In the scheme En denotes the enzyme AChE; AChi En- is the enzyme-substrate complex formed prior to hydrolysis of ACh into choline and acetic acid. When AChE is inactivated, e.g., by an organophosphorus or carbamate ester, the enzyme is no longer able to hydrolyze ACh; the concentration of ACh in the junction remains high, and continuous stimulation of the muscle or nerve fiber occurs, resulting eventually in exhaustion and tetany. Based on a number of studies, a plausible mechanism for AChE-catalyzed hydrolysis of ACh is presented in Figure 2. ACh is drawn to the active site of the enzyme by electrostatic attraction between the positive charge on the ACh nitrogen atom and negative charge in the anionic site (structure E + S) resulting in the enzyme-substrate complex (ES). Acetylation of a serine hydroxyl (OH) in the esteratic site is catalysed by the basic imidazole moiety B (histidine) and acidic moiety AH (tyrosine hydroxyl), leading to the acetylated enzyme EA. Deacetylation then takes place very fast, resulting in the free enzyme (E) within milliseconds. As presented, ACh hydrolysis by AChE has the elements of an acid-base catalyzed reaction, including both the acetylation and deacetylation reaction. The negative charge at the anionic site is attributed to the carboxylate anion of aspartic or glutamic acid. The reaction steps given in Figure 2 provide an elementary presentation of the AChE active site and a reasonable mechanism for the hydrolysis of ACh. The enzyme is in reality a highly complex protein, having in addition to the esteratic and anionic sites, a number of peripheral sites and hydrophobic areas. While the preceding discussion has focused on AChE, it should be pointed out that there is at least one other type of cholinesterase enzyme beside AChE, namely pseudocholinesterase. AChE has the highest specificity for ACh of any other choline ester and pseudocholinesterase has the highest specificity for butyrylcholine. The physiological role of pseudocholinesterase in animals is not as well defined as that of AChE (4). However, both enzymes are inhibited by organophosphorus esters.[14]



The mechanism of acetylcholine hydrolysis catalyzed by acetylcholinesterase. There are two steps to the reaction, both of which occur via a mechanism of type (A N +D N) [22] with a metastable oxyanion intermediate whose formation the enzyme favors.

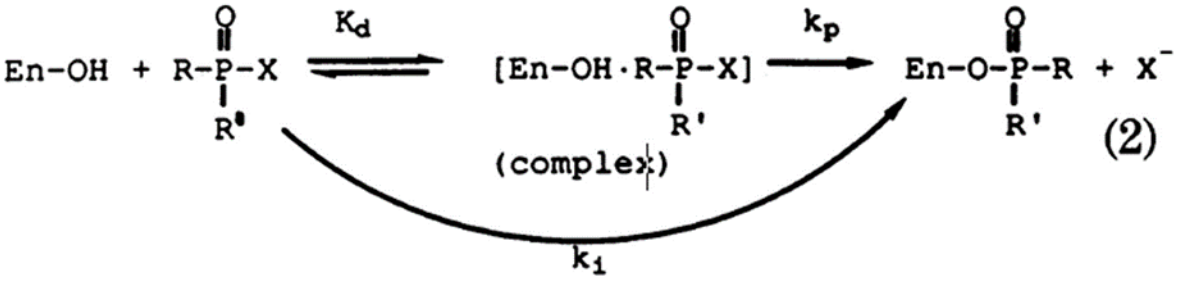
Common Mechanism of Action

The inhibition of AChE by an organophosphorus ester takes place via a chemical reaction in which the serine hydroxyl moiety in the enzyme active site is phosphorylated in a manner analogous to the acetylation of AChE . In contrast to the acetylated enzyme, which rapidly breaks down to give acetic acid and the regenerated enzyme, the phosphorylated enzyme is highly stable, and in some cases, depending on the groups attached to the phosphorus atom (R and R'), it is irreversibly inhibited. The serine hydroxyl group, blocked by a phosphoryl moiety, is no longer able to participate in the hydrolysis of ACh.

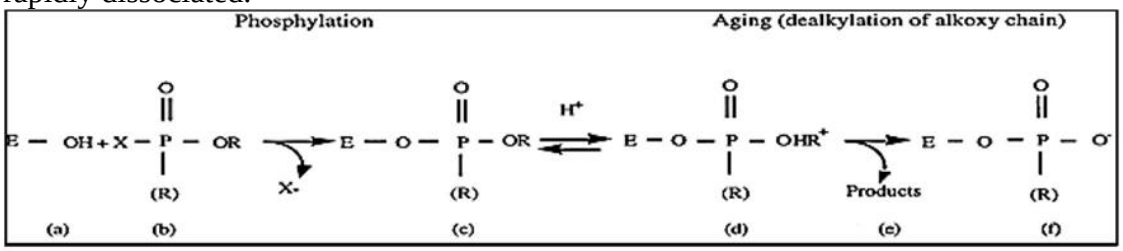


The effect of an electron-withdrawing substituent on the reactivity of paraoxon.

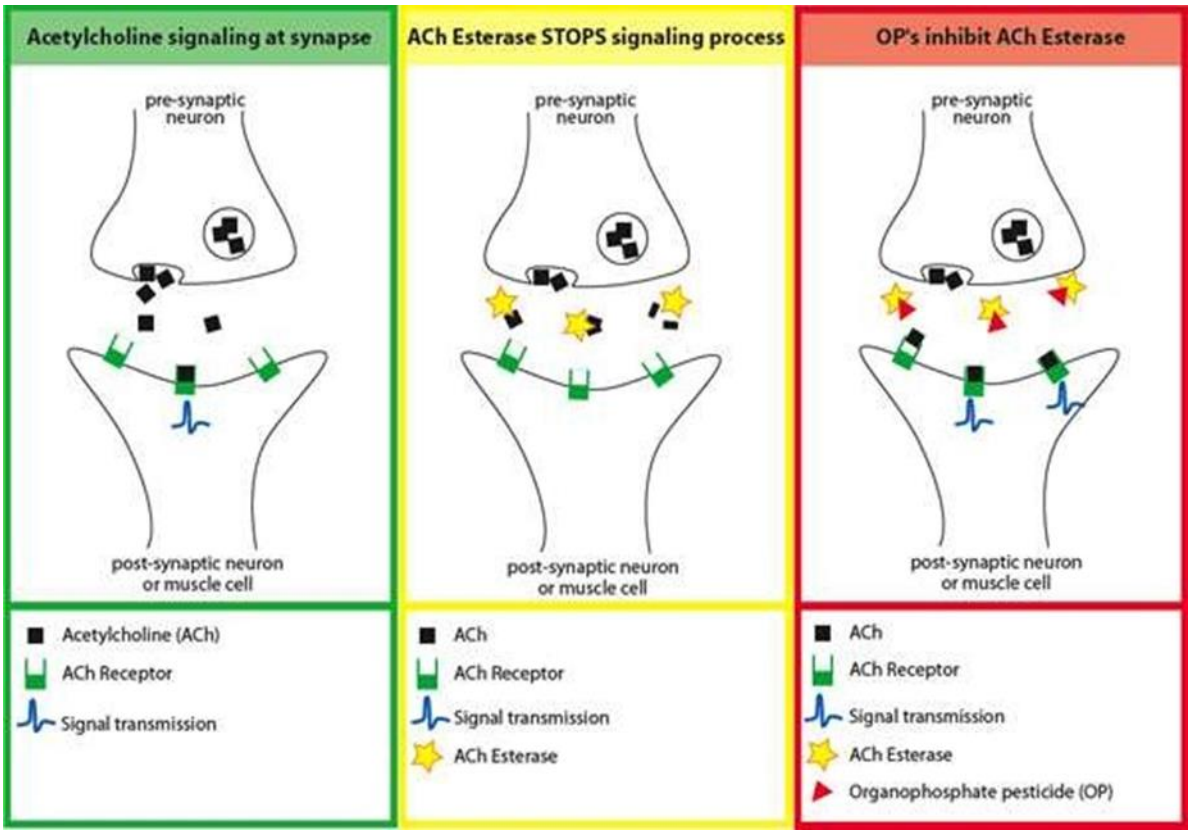
The inhi-bition reaction takes place in a two-step process, asindicated by Eq. (2). In this equation.



En-OH represents AChE in which the serine hydroxyl moiety (-OH) is emphasized, R and R' are a variety of different groups (alkoxy, alkyl, amino, thioalkyl, etc.), X is the leaving group, Kd is the dissociation constant between the enzyme-inhibitor complex and reactants, kp is the phosphorylation constant, and ki is the bimolecular rate constant for inhibition and is equal to k/IKd. Since Kd provides a measure of the dissociationof the enzyme-inhibitor complex, it is regarded as an estimate for binding and is dependent on the structural and steric properties of the molecule. In contrast, the phosphorylation constant kp is regarded as an estimate of the reactivity of the organophosphorus ester. The bimolecular rate constant ki is dependent on the values of Kd and kp and is generally regarded as the most useful parameter for the estimation of the inhibitory potency of an organophosphorus (and carbamate) anticholinesterase. According to Eq. (2), the moiety X is displaced from the phosphorus atom by the serine hydroxyl of the enzyme and is, therefore, referred to as the leaving group. OP exerts their acute effect by acetylcholinesterase (AChE) inhibition in the nervous system (NS). The covalent reaction between the organophosphorus ester with the serine hyrodxyl group an active site for in the AChE protein, leads to the formation of an intermediate, which partially hydrolyses the X group of OP (Figure 2) . [15] This forms an irreversible inhibited enzyme when the phosphorylated AChE ages. Hence, leading to the accumulation of high levels of acetylcholine (ACh) at the cholinergic synapse with over stimulation of nicotinic and muscarinic receptors some of the cholinergic signs and symptoms includes salivation, sweating, and muscular twitching and could lead to death due to flaccid paralysis of the pulmonary muscle. However, some recently introduced OP like dicholorus and temephos are less tenacious AChE inhibitors. The phosphorylated enzyme is spontaneously and rapidly dissociated.



Illustrates the mechanism of phosphorylation and aging of cholinesterase. (a) free enzyme;(b) organophosphorus compound (c) phosphorylated enzyme; (d) intermediate; (e) dealkylation products and (f) aged enzyme



Conclusion:

he continuous lowering of the maximum residue limits of the OP pesticides in food and in the environment calls for the development of sensitive methods for their determination. Such high effective techniques, well suited for testing complex matrices, are the chromatographic ones. Nevertheless, the review of the advances in OP pesticides analysis.

Conflict of Interest:

The authors declare no conflict of interest.

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Reference:

1. Alam AU, Bishop SH. Growth Of Escherichia Coli On Some Organophosphonic Acids. Can J Microbiol. 1969 Sep;15(9):1043–1046.
2. Cook AM, Daughton CG, Alexander M. Phosphonate Utilization By Bacteria. J Bacteriol. 1978 Jan;133(1):85–90.
3. Daughton CG, Crosby DG, Garnas RL, Hsieh DP. Analysis Of Phosphorus-Containing Hydrolytic Products Of Organophosphorus Insecticides In Water. J Agric Food Chem. 1976 Mar-Apr;24(2):236–241.

4. Daughton CG, Hsieh DP. Accelerated Parathion Degradation In Soil By Inoculation With Parathion-Utilizing Bacteria. Bull Environ Contam Toxicol. 1977 Jul;18(1):48–56.
5. Daughton CG, Hsieh DP. Parathion Utilization By Bacterial Symbionts In A Chemostat. Appl Environ Microbiol. 1977 Aug;34(2):175–184.
6. Forrest Is. Enzymatic Hydrolysis Of O, O-Diisopropyl Thionothiolphosphate. Arch Biochem Biophys. 1955 Sep;58(1):178–180.
7. Gerlt JA, Whitman GJ. Purification and properties of a phosphohydrolase from Enterobacter aerogenes. J Biol Chem. 1975 Jul 10;250(13):5053–5058.
8. Harkness DR. Bacterial growth on aminoalkylphosphonic acids. J Bacteriol. 1966 Sep;92(3):623–627.
9. Koch AL. Turbidity Measurements Of Bacterial Cultures In Some Available Commercial Instruments. Anal Biochem. 1970 Nov;38(1):252–259.
10. Labeyrie S, Neuzil E. Influence Des Ions Zinc Et Cobalt Sur La Chromogenèse De Pseuodomonas Aeruginosa En Présence D'acid Méthylphosphonique. C R Seances Soc Biol Fil. 1977;171(4):755–759.
11. Mastalerz P, Wieczorek Z, Kochman M. Utilization Of Carbon-Bound Phosphorus By Microorganisms. Acta Biochim Pol. 1965;12:151–156.
12. Munnecke DM. Properties Of An Immobilized Pesticide-Hydrolyzing Enzyme. Appl Environ Microbiol. 1977 Mar;33(3):503–507.
13. Rosenberg H, La Nauze JM. The Metabolism Of Phosphonates By Microorganisms. The Transport Of Aminoethylphosphonic Acid In Bacillus Cereus. Biochim Biophys Acta. 1967 Jun 13;141(1):79–90.
14. Wolfenden R, Spence G. Derepression Of Phosphomonoesterase And Phosphodiesterase Activities In Aerobacter Aerogenes. Biochim Biophys Acta. 1967 Sep 12;146(1):296–298.
15. Zeleznick Ld, Myers Tc, Titchener Eb. Growth Of Escherichia Coli On Methyl- And Ethylphosphonic Acids. Biochim Biophys Acta. 1963 Nov 15;78:546–547.

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