CHAPTER II

REVIEW OF LITERATURE
REVIEW OF LITERATURE

Relatively large number and huge amount of synthetic chemicals of economic importance (pesticides) have been introduced into our environment especially during the last three decades. For the very purpose these chemicals have been developed when these come into contact with biological system causing toxic manifestations posing as health hazard to the living organism including pests as well as beneficial life forms.

A large number of organisms including microorganisms and insects under certain circumstances become noxious, destructive or otherwise troublesome. These are classified as pests. Their proper control is essential for man's survival. The chemical control is linked with the use of wide variety of pesticides. Pesticides constitute the largest group of poisonous chemicals that are widely used today (Edwards, 1986). Pesticides include chemicals or mixtures of such substances intended for preventing, destroying or mitigating insects, rodents, nematodes, fungi, weed or any other form of life declared as pests. According to the type of pest for which these are used are labeled as insecticides, acaricides, nematocides, rodenticides, herbicides and fungicides. Pesticides have contributed in manifold increase in crop yields. These are used for eradication or control of vector borne diseases such as malaria, filaria, yellow fever, dengue, viral encephalitis and louse borne typhus etc.. However in all these operations, invariably there is a risk and potential danger to the environment and human health.
HISTORICAL DEVELOPMENT

Hassan (1982) reviewed the history of pesticides. The use of inorganic chemicals to control insects possibly dates back to Greece and Roman civilization. The Chinese were employing moderate amounts of arsenicals as insecticides by the 16th century. In 19th century both pyrethrum and soap had been used for insect control and also a combined wash of tobacco, sulphur and lime to combat insects and fungi. The middle of 19th century marked the beginning of first systematic scientific studies into the use of chemicals for crop protection. Perhaps the most significant discovery leading to the proliferation of new synthetic insecticide was that of DDT (Dichloro Diphenyl Trichloroethane). This unusual compound was first synthesized by Zeidler in 1874 but its insecticidal properties were first discovered in 1939 by Muller of Switzerland (Hart, 1989). The use of DDT revolutionized the control of insects and pests. Other chlorinated hydrocarbon insecticides such as BHC (Benzene Hexachloride), toxaphene, endrin, chlordane, aldrin and dieldrin followed immediately thereafter. The second wave of introduction of new insecticides was started following efforts of a German worker Gerhard Schrader, a pioneer in chemistry who developed organophosphorus compounds as nerve gas during World War II (Schrader, 1963 and Hart, 1989), which later on were commercialized as OP insecticides. The number of organophosphorus compounds used for insect control today is unmatched by any other group of insecticides. The most widely used OP insecticides include Parathion, Syntox, Malathion, EPN (O-ethyl O-4-nitrophenyl phenyl-phosphonothioate), Diazinon, Monocrotophos, Phorate, Phosalone, DDVP (dichlorvos), Phenthioate, Elsan etc. The carbamates group of synthetic insecticides include Carbophuran, Carbaryl and Methomyl etc. Thereafter,
An important group of insecticides comprising synthetic light stable pyrethroids were developed. These include Permethrin, deltamethrin, cypermethrin etc.

**CLASSIFICATION**

Pesticides can be classified in many different ways, according to the target pest organism, the chemical structure of the compound used or according to the degree or type of health hazard involved (WHO 1990) and based on the nature of pests they control. However, from the point of view of clinicians, public health specialists & WHO classification is ideal, based on their acute toxicity and formulation.

Table: WHO (1992) recommended criteria for classification

<table>
<thead>
<tr>
<th>Class</th>
<th>LD 50 for the rat (mg/kg body weight)</th>
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<tbody>
<tr>
<td></td>
<td>Oral</td>
</tr>
<tr>
<td></td>
<td>Solids</td>
</tr>
<tr>
<td>Ia. Extremely hazardous</td>
<td>5 or less</td>
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<tr>
<td>Ib. Highly hazardous</td>
<td>5 – 50</td>
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<tr>
<td>II. Moderately hazardous</td>
<td>50 – 500</td>
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<tr>
<td>III. Slightly hazardous</td>
<td>Over 500</td>
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</table>

Plestina (1984) proposed that availability of insecticides be restricted to hazard class. Organophosphorus insecticide have high effectiveness, low persistency and rapid hydrolysis after application. Besides insecticidal activity some OP compounds also exhibit fungicidal, herbicidal, and household pest control properties. OP insecticides are usually esters of alcohol with phosphoric acid or anhydrides of...
phosphoric acid with another acid. These can be easily degraded hydrolytically, enzymatically or biologically.

CHEMICAL AND PHYSICAL PROPERTIES

These compounds are generally esters, amides or thiol derivatives of phosphoric or phosphonic acid.

There are three main groups:

- Without a sulphur atom such as phosphates.
- With one sulphur atom such as phosphorothioates
- With two sulphur atoms such as phosphorodithioate.

Chemical structures of different organophosphorus insecticides are illustrated in the table.
Variations in the chemical structure of organophosphorus insecticides.

(Kaloyanova and Batawi, 1991)

<table>
<thead>
<tr>
<th>Type of phosphorous group</th>
<th>Outline structure</th>
<th>Common or other names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>O ( \text{II} ) ( (R-O) \text{-P-O-X} ) 2</td>
<td>Chlorfenvinphos, crotoxyphos, dichlorphos, dicrotophos, heptenphos, mevinphos, monocrotalophos, naled, phosphamidon, TEPP, tetrachlorvinphos, triazophos.</td>
</tr>
<tr>
<td>0-alkyl phosphorothioate</td>
<td>O ( \text{II} ) ( (R-O) \text{-P-S-X} ) 2</td>
<td>Omethoate, amiton, Varidithion, demeton ( -S- ) methyl, oxydemetopmethyl.</td>
</tr>
<tr>
<td></td>
<td>S ( \text{II} ) ( (R-O) \text{-P-O-X} ) 2</td>
<td>Axothoate, bromophos, bromophos ( - ) ethyl, chlorpyriphos, chlorpyriphos-methyl, coumaphos, diazinon, dichlofenthion, fenchlorphos, fenitrothion, fenthion, fensulfothion, iodofenphos, parathion, parathion-methyl, phoxim, pyrasophos, pirimiphos-methyl, sulprofen, temephos, thionazin.</td>
</tr>
<tr>
<td>Phosphorodithioate</td>
<td>S ( \text{II} ) ( (R-O) \text{-P-S-X} ) 2</td>
<td>Amidithion, azinphos-ethyl, azinphos-methyl, dimethoate, dioxathion, disulfoton, ethion, formothion, malathion, mecarbam, menazone, methidathion, morphothion, phenthoate, phorate, phosalon, prothoate, phosmet, thimetion.</td>
</tr>
<tr>
<td>S-alkyl phosphorothioate</td>
<td>O ( \text{II} ) ( R-S-P-O-X ) / ( R-O )</td>
<td>Profenofos, trifenofos</td>
</tr>
<tr>
<td>S-alkyl phosphorodithioate</td>
<td>S ( \text{II} ) ( R-S-P-O-X ) / ( R-O )</td>
<td>Prothiofos, sulprofos</td>
</tr>
<tr>
<td>Phosphoramidate</td>
<td>O ( \text{II} ) ( (R-O) \text{-P-NR} ) 2</td>
<td>Cruformate, fenamifos, fosthiestan</td>
</tr>
<tr>
<td>Type of phosphorous group</td>
<td>Outline structure</td>
<td>Common or other names</td>
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<td>--------------------------</td>
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<tr>
<td>Phosphorothioimidate</td>
<td>O II R-N (\text{P-N} ) (\text{NR} ) (\text{I} ) (\text{2} )</td>
<td>Triamifos</td>
</tr>
<tr>
<td>Phosphorothioimidate</td>
<td>O II R-O (\text{P-NR} ) (\text{I} ) (\text{2} ) S-alkyl</td>
<td>Isofenfos</td>
</tr>
<tr>
<td>Phosphorus (R-O) (\text{P-NR} ) (\text{I} ) (\text{2} ) 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphonate</td>
<td>O II RO (\text{P-X} ) / RO</td>
<td>Butonate, trichloron (tribyton)</td>
</tr>
<tr>
<td>Phosphonothioate</td>
<td>S II R-O (\text{P-O-X} ) / R</td>
<td>EPN, Trichlormat, leptophos, cyanofenphos</td>
</tr>
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</table>

Toxicity of OP insecticides is influenced by a number of factors as follows:

**Effects of light**

Parathion, an OP insecticide, showed experimentally an increase in anticholinesterase activity in vitro and on exposure to Ultra violet radiation (UVR) and sunlight. However, the acute toxicity of parathion decreased under UVR although in vitro anticholinesterase activity increased due to the formation of more polar products. The metabolites were identified as paraoxon and the S-ethyl and S-phenyl isomers of parathion, together with unknown products (Dauterman, 1971). Studies with seven organophosphorus pesticides containing sulphur in the thioether indicated that exposure to Ultra violet radiation (254 nm) resulted in a variety of oxidation products.
SOLUTES OF SOLUTES AND SOLVENTS

The hydrolysis of organophosphorus compounds is influenced by solutes e.g. amino acids, hydroxylammonium derivatives, whereas metal ions such as Cu ++ acts as catalyst. Solvents used in formulating organophosphorus insecticides influence their stability. It has been observed that dimethoate in certain hydroxylic solvents e.g. 2-alkyxyethanols increased its toxicity on storage (Casida and Sanderson, 1963). Heath and Vandekar (1957) observed that a 1% solution of demetan-s-methyl increased its toxicity spontaneously at 35 °C, during the course of the day. This increase was found to be due to the formation of a transalkylated sulfonium derivative, the toxicity of which was more than 1000 times that of the parent compound. Most pesticide formulations include carrier substances in addition to the active ingredients and also solvents and compounds that improve absorption etc. These “inert” ingredients are not usually included in any discussions of the effects on health although they frequently comprise a large part of a pesticide products and their adverse effects may exceed those of active ingredients. The adverse effects of pesticides on health may also be caused by impurities such as dioxins in certain phenoxyacid herbicides, ethylene thiourea in ethylene bisdithiocarbamate fungicide and isomalathion in malathion.

IDENTIFICATION AND CHARACTERISATION

Procedures consists of sampling, extraction, clean up of extract and determination of compounds. Separation and clean up usually involve partition between solvents and chromatography. Detection may be by partially specific colour reagents or by enzyme inhibition tests applied to spots on thin layer chromatographic plates (Stefance et al 1976) or by formation of volatile derivatives suitable for detection by gas chromatography (Shafik et al 1973).
RAME AND METABOLISM

Metabolism and Mode of action

OP pesticides are absorbed by the skin, as well as by the respiratory and gastrointestinal tract. Skin absorption is somewhat greater at higher temperatures and may be much greater in presence of dermatitis. Dermal uptake largely depends on persistence time (related to volatility, clothing, coverage and thoroughness of washing after exposure) and also on the presence of solvents and emulsifiers that may facilitate uptake. Ecobichon et al (1977) referred a case of acute fenitrothion poisoning and concluded that treatment with atropine and oxime reactivator was reversing the recent inhibition of acetylcholinesterase by a compound that had been stored in the body and was entering the circulation over a period of many days. In view of inherent instability of OP pesticides the storage in human tissue is not anticipated to be prolonged. Experimental animal studies have shown that most of the radio labeled dose of OP insecticides is rapidly excreted in expired air, urine and feces. Blair et al (1975) reported the recovery of 67 to 100% of radioactivity in cows, rats and goats given various doses of $^{32}$P-dichlorvos.

Alternative metabolic pathways pertaining to bio transformation reactions of OP pesticides can be divided into three distinct classes e.g. mixed function oxidases, hydrolases and tranferases. Many unrelated substrates can be oxidized by mixed function oxidases (MFO) systems associated typically with liver endoplasmic reticulum and also present in other tissues such as intestine, lung and kidney. Within liver, there appears to be a family of MFO’s possibly some enzymes in common but utilizing slightly different cytochromes of which cytochrome P-450 is the best known. The MFO activity in the liver can vary greatly according to the nutritional and hormonal status of the animal and also according to stimuli arising
From the ingestion of some foreign compounds. The particular enzyme system has an essential requirement for NADPH and molecular oxygen in order to modify a xenobiotic. The in vivo activation of parathion to paraoxon has been demonstrated in both insects and mammals. (Dauterman, 1971). Evidence for oxidative desulfuration has been demonstrated in both plants and animals for a wide variety of OP pesticides e.g. parathionmethyl (Hollingworth et al 1967), malathion (O’Brien, 1967) and dichlorvos (WHO, 1989).

Bioaccumulation and degradation

Hydrolysis, at ester linkages constitutes the major means of decomposition of several organophosphorus pesticides in soil and water systems. The hydrolysis can be chemical, biological or both. With the exception of dichlorvos, most organophosphorus pesticides possess low volatility. Dichlorvos degraded in humid air, water and soil, both by abiotic and biotic processes. It degrades mainly into dichloro-ethanol, dichloroacetaldehyde dichloroacetic acid, dimethyl phosphate, diethyl phosphoric acid and other water soluble compounds which are eventually mineralized (WHO,1989). Exposure of food materials to OP pesticides occurs chiefly at the crop growing stage. Spear et al (1977) observed under exceedingly hot and dry conditions high residues of paraoxon on citrus plants that had been sprayed with parathion 28 days previously. Degradation in the environment involves both hydrolysis and oxidation to mono- and di-substituted phosphonic or phosphoric acids or their thio analogous Leptophos is converted to its desbromo analogue in sunlight and the product is considerably more active than the parent in causing delayed neuropathy (Johnson, 1975). Daughton et al (1979) observed the bacterial cleavage of carbon phosphorous bond of a alkyl phosphonate.
Mechanism of action in mammals

The OP compounds exert their biological action in mammals and arthropods by attacking the system of neural transmission and thus interfering the function of the target organs. The synapses and myoneural junctions of the motor and parasympathetic system as well as the ganglia of the sympathetic system transmit the pre-synaptic impulse by means of acetylcholine to the post-synaptic side. 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. Depending upon the part of nervous system in which the synapses are thus kept in a state of permanent stimulation, symptoms of predominantly nicotine, muscarine and sometimes CNS poisoning are noted.

Physical and Kinetic properties of acetylcholinesterase are reviewed by La Motta and Woronick (1971). Leuzinger et al (1971) succeeded in purifying acetylcholine acetylhydrolase in large quantities and found the molecular weight by sedimentation equilibrium, a value of 2,60,000 +/- 10,000 in addition to acetylcholinesterase, a second cholinester - hydrolysing enzyme is found in the serum of mammals as well as in insects, viz. Acetylcholine- acylhydrolase (E.C. Trivial name: cholinesterase) also referred as pseudocholinesterase or serum-ChE. A third complex of aliphatic ester hydrolyzing enzymes are the carboxyl esterases (‘ali-esterases’). The active centre of the enzyme has two active sites. The ‘anionic site’ binds to the cationic part of the substrate. The strong positive charge on the quaternary nitrogen of the choline moiety forms and electrostatic linkage with the anionic site of the enzyme. The ionic bond is reinforced by auxiliary binding forces between two of the methyl groups on the nitrogen and the surface of the protein. Acetylation of the enzyme is rapidly followed by breakage of the ester linkage and
The elimination of choline. The acetylated enzyme then reacts with water to regenerate the enzyme and release acetic acid. Compounds that mimic one or more actions of acetylcholine are said to be cholinomimetic. This property is shared by:

(a) Compounds that act on cholinergic receptors and produce an effect similar to that of acetylcholine and

(b) Compounds that potentiate or preserve acetylcholine so that its own action is increased. The only known mechanism for “Potentiating or preserving” the neurotransmitter is through inhibition of the enzyme that normally hydrolyzes it. Compounds that mimic acetylcholine by direct action on cholinergic sites include nicotine and muscarine. In acute OP poisoning, clinical manifestations generally occur only when more than 50% serum cholinesterase activity is inhibited. Thus severity of poisoning may or may not be proportional to the inhibition of serum AChE.

**EFFECTS ON HUMAN**

**Epidemiology and Field Survey**

The largest group of pesticides used nowadays is the group of organophosphorus compounds. More than 100 individual compounds of this group are well known and largely used in many countries. OP degradation in the environment is very fast. A disadvantage of OP pesticides is their high acute toxicity and the resulting large number of fatal acute intoxication.

Organophosphates were introduced in 1854 but their toxicity was not known till 1931. In India, they were introduced in 1960's and their toxicity reported in 1962. (Patial, 1993)
There are 79 registered technical grade manufacturers of pesticides, out of which 61 are active in recent years. Out of these, only 16 units account for about 94.3% of the total technical grade production of pesticides. (Srivastava and Patel, 1990). Installed production capacity in India is greatest for the organophosphates monocrotophos (2,500 tonnes per year), malathion (10,000 tonnes per year), parathion (2,700 tonnes per year). (Repetto and Baliga, 1996)

Epidemiological studies merit valuable significance in the identification of high risk groups of populations at risk following pesticides exposure. In Dade County, Florida, 145 cases were reported during 1963 - 1968 and the majority of the hospitalized patients were due to organophosphate intoxication and parathion being responsible for over 70% of cases (Davies and Freed, 1982). This study mentioned that 50 to 60 child poisoning could be equated with the accidental ingestion of parathion by a 2 ½ year old black male toddler.

In view of the ecological catastrophe experienced by the use of pesticides in developed countries like US, Canada, FRG, and UK, their use have been restricted in these countries, however their use is still growing rapidly in developing countries including India. (Repetto and Baliga, 1996)

Jayaratnam et al, 1982, evaluated a sample survey of the clinical records of patients admitted to the different hospitals in Sri Lanka, and observed that approximately 13,000 patients of pesticide poisoning admitted to the hospital annually and out of that 1,000 of them die. Namba (1974) quoted the Japanese government hospital records on OP poisoning of 7 years and found 3,311 accidental and occupational poisoning cases due to parathion including 188 deaths, while for malathion, the numbers were 63 and 10 respectively. (WHO, 1986).
Vietnam et al (1987), studied the magnitude of acute pesticide poisoning in selected agricultural communities in Indonesia, Malaysia, Srilanka, and Thailand and observed that pesticide poisoning is a major problem in developing countries, but not in the industrialized countries despite extensive use of pesticides. Baker, Jr. et al (1978) has reported an epidemic of malathion intoxication affecting 2,800 workers in Pakistan Malaria control programme. Ganapathi and Rao (1966) in “A study on Suicide in Madurai” reported 415 cases (45.5%) of suicide due to organophosphorus compounds. Bami (1981) has reported in his study, a widespread utilization of organophosphorus insecticides for the last 13 – 14 years in respect of 10 states and 2 union territories. Adlakha et al (1988) have reported that in the state of Punjab, the organophosphorus and carbamates poisoning was the second most common poisoning (13.12%) in their 5 year study. Yadav et al (1991) have also supported the same view point by his study in the state of Harayana. Agarwal (1993, postulated that the poisoning due to OP insecticides is steadily increasing in India due to their easy availability and potent toxicity. Seth (1991) have reported organophosphorus and carbamate pesticides poisoning cases admitted to Christian Medical College and Brown Memorial Hospital, Ludhiana, Punjab during the period 1981 - 1986, Baygon (a carbamate) was the most common (18.57%) followed by diazinon (5.55%) and parathion (3.7%). They also reported 44.5% cases of organophosphorus poisoning at Government Medical College, Patiala in a six month study in 1989. The pattern of organophosphorus and carbamate poisoning documented during a period from 1980 – 1988 in a regional study from West Bengal, out of 721 cases of acute poisoning attended in a subdivisional
of Murshidabad district of West Bengal, 88% cases presented with
Organophosphorus poisoning.

Cholinergic effects

(i) Acute toxicity: Apart from non-ester herbicidal compounds, the acute
toxicity of all OP insecticides shares the basic mechanism involving
inhibition of AChE, accumulation of AChE and over stimulation of some
central cholinergic neurons and of the sympathetic and parasympathetic
nervous system. Death is usually caused by respiratory failure due to a
combination of blocking of the respiratory centre, bronchospasm and
paralysis of respiratory muscles.

(ii) Chronic toxicity: The cholinergic effects brought about by repeated
administration of less than a single fatal dose are similar in type to the
acute single dose effects. For majority of OP insecticides long term feeding
tests have been conducted to establish no observed adverse effects. In
every case except bromophos-ethyl, the most sensitive indicator was the
depression of ChE activity in plasma or erythrocytes.

The prolonged illness in the patients who either recover or die rapidly
include liver dysfunction (Barckow et al, 1969; Boelcke and Erdmann, 1969;
Prinz, 1969; Boelcke and Gaaz, 1970) heart dysfunction (Singh et al. 1969;
Schorn, 1972); Psychosis (Seybold and Braeutigam, 1968; Wender and O
W Sianowski, 1969) and delayed convulsions (Pukach and Shakov, 1971).
There is a considerable evidence that many of the complications are the
results of hypoxia and are not directly related to ChE inhibition (Heering,
1970).
Inhalational Effects

Decrements in clarityness and memory have been associated with chronic industrial and agricultural exposure to organic phosphorus insecticides (Hayes and Laws, 1991). Increased irritability memory deficits, lethargy and lack of energy have been associated with multiple or severe acute exposure.

Visual and other local effects

Both the well recognized usual effects of OP compounds and atypical effects were reviewed in detail by Plestina and Piukovic-Plestina (1978). Local effects on sweat glands and muscles from dermal absorption in area involved indicate the peripheral nature of signs frequently seen in systemic poisoning. McLaughlin and Sonnenschein (1960) reported local sweating response following intra dermal injection of paraoxon. Prolonged local sweating followed dermal application of technical parathion (Funckes et al, 1963).

Clinical Manifestations

The following different types of clinical manifestations of OP insecticide poisoning have been extensively reviewed and described by Srinivasan (1962); De and Chatterjee (1967); Balani et al (1968); Gupta and Patel (1968); Mehta et al (1971); WHO (1986); Adlakha et al (1988); Hayes and Laws (1991); Kaloyanova and Batawi (1991); Agarwal (1993).

A. Muscarinic manifestations

- Bronchial Tree: Tightness in the chest, wheezing, dyspnoea, increased bronchial secretion, cough, pulmonary oedema, cyanosis.
- Gastrointestinal system: Nausea, vomiting, abdominal tightness and cramps, diarrhoea, tenesmus, fecal incontinence.
† Glandular glands: Increased sweating, salivation and lacrimation.

- Cardiovascular system: Bradycardia, Hypotension
- Pupil: Miosis, occasionally unequal.
- Ciliary body: Blurring of vision
- Urethral Bladder: Frequency of urine, urinary incontinence.

3. Nicotinic Manifestations

- Striated muscles: Muscular twitching, fasciculations, cramps, weakness including muscles of respiration.
- Sympathetic ganglia: Pallor, tachycardia, hypertension.

C. CNS Manifestation

Giddiness, tension, anxiety, restlessness, insomnia, headache, tremors.
Drowsiness, confusion, slurred speech, ataxia, coma with absent reflexes.
Convulsions, depression of respiratory and circulatory centre with dyspnoea.
Cyanosis and fall in blood pressure.

Balani et al (1968) classified the toxic manifestation in to 5 grades based on progressive severity:

- **Grade I**: No symptoms and no signs.
- **Grade II**: Symptoms of GI tract and mild CNS symptoms like headache and giddiness.
- **Grade III**: Pupillary constriction with or without symptoms of Grade II.
- **Grade IV**: Pulmonary oedema with or without any finding of Grade II and III.
- **Grade V**: Unconsciousness with or without any component of Grade II, III and IV.
Very high doses, death results from paralysis of the striated respiratory muscles, from paralysis of respiratory centre, cardiac arrest or pulmonary oedema. In a few instances, death has followed the profound brain damage that occurred, usually early in the course of poisoning as a result of severe anoxia (Hayes, 1982). Limaya (1989) suggested that OP compounds exert a toxic action on the myocardium leading to toxic myocarditis and explained this as a genesis of rebound or second phase of pulmonary oedema and death.

Clinical manifestations of poisoning are generally observable only after more than 50 % of serum cholinesterase is inhibited (Namba et al., 1971B). The acute symptoms may appear within a few minutes or may occur after inhalation and in 30-60 minutes after oral ingestion or several hours following cutaneous absorption. These periods are, however, very much dependent upon the structure of the organophosphate compound, amount and type of formulation and also upon secondary factors such as stomach contents.

In ordinary occupational cases, relatively incapacitating symptoms of nausea, cramps, discomfort in chest, muscular twitching etc. often follow the initial giddiness, blurred vision and headache only after a period of 2-8 hours. Bhatnagar et al (1982) compared plasma cholinesterase values in 75 pesticide factory workers with 15 non exposed controls. The difference between the mean was statistically significant (P<= 0.01). About 69% of the workers reported symptoms related to intoxication e.g. loss of appetite, nausea, headache, giddiness, vertigo, muscular cramps, and watering of eyes.

Delayed Neurotoxicity

Some organophosphorus compounds with anticholinesterase activity initially can also produce delayed neurotoxic effects, distal degeneration of nerve axons and concomitant ataxia and paralysis which can be irreversible. This is designated as
Hemato delayed neuropathy (OPIDN) which is totally independent of cholinesterase activity (Johnson, 1975). The initial biochemical event in the toxic action of NTE is the phosphorylation of "neuropathy target esterase", a specific enzyme occurring with other esterases in the nerve tissue. Wadia et al (1974) used the term "type II paralysis" and in 1977 used the term "delayed paralysis" in referring to the profound weakness that characterizes some human cases of acute poisoning. NTE activity has been found in hen spinal cord, sciatic nerve (Olajos et al, 1978; Olajos and Rosenblum, 1978) lymphocytes (Dudek et al, 1979). However, human NTE activity appears to have a comparatively wide tissue distribution (Moreto et al, 1983).

The inhibition and modification of NTE is an "aging" process (Johnson, 1987). The pesticides that have caused classical neurotoxicity in people are mephos, mipafon, trichlorfon, and perhaps leptophos and chlorpyrifos.

Narcotic effects

Some organophosphorus compounds produce an immediate narcotic effect ranging from coordination to deep anesthesia following intravenous injection. The mechanism of narcotic actions is not known, however, effects were marked only after injection into the blood has resulted in concentration estimated to be about $10^{-3}$ M which is high enough to block nerve conduction and motor end plates and thus to produce anesthesia and paralysis. The action probably depends on a block in sodium ion transfer across membranes (Heath, 1961). Apparently the narcotic action has not been observed in clinical cases.
EFFECTS

Mutagenic and Carcinogenic effects

IARC monograph (1983) included evaluations of five widely used OP pesticides (malathion, methylyparathion, parathion, tetrachlorvinphos and trichlorfon). In several cases the conclusions were that acceptable tests had been performed with no evidence of carcinogenic effects or mutagenic effects in mammals. None of these compounds were to be a strong mammalian mutagen or carcinogen. Reuber (1985) evaluated the carcinogenic potential of malathion and malaoxon and stated both compounds as carcinogenic.

Teratogenic effects

Defects in the development of fertilized hen eggs injected with various OP insecticides were known but many of them are associated with the inhibition of the enzyme kynurine formamidase and depression of NAD level (Seifert and Casida, 1980). Kimbrough and Gaines (1968) reported deaths and that resorptions were increased in pregnant rats given single high dose of parathion or diazinon on the 11th day of gestation. Staples and Goulding (1979) observed in offspring with cholinergic symptoms in rats, mice and hamsters given trichlorfon (400 mg per day). Knox et al (1978) noted a specific defect consisting of hypoplasia of the cerebellum in offspring of pigs administered neguvon (a veterinary grade of trichlorophon).
EFFECTS ON IMMUNE SYSTEM

The organophosphorus compounds effect on the immunological systems which may influence morbidity are very important. Milby and Epstein (1964) found that agricultural workers exposed to malathion were sensitized to the intermediary product diethyl fumarate and when this compound was decreased in the manufactured product, the incidence of subsequent sensitization also decreased. Allergic effect due to exposure to OP compounds are described. Nevertheless, Ganelin et al (1964), Davignon et al (1965) and Gardner and Iverson (1968) observed that for asthmatic patients such exposure should not be considered, an additional risk of increased bronchial sensitivity. Ercecovc (1973) published a review on pesticide immunological interactions. He concluded that sensitization and high doses of pesticides as evidenced by dermatitis may be more prevalent than previously supposed. Shtenberg et al (1974) observed that oral doses of fenitrothion or trichlorfon at 5 mg/l/kg per day suppressed haemagglutinin levels in rats immunized against sheep red blood cells. Desi et al (1978) demonstrated the malathion or dichlorvos may decrease antibody titres in experimental animals. Zackov (1983) stated that most OP pesticides elicit auto immune reactions and suppress the production of antibodies against vaccines. Katsenovich et al (1981) studied the immune status of patients with different degrees of combined insecticide intoxication. Modern methods were used to assess the T & B systems of immunity. The quantitative deficit in T-Lymphocytes was accompanied by a reduction of their functional activity. The ratio of T to B lymphocytes was altered to varying degrees, depending on the grade of intoxication. Some suggestions were made for developing an auto immune process. Dermatitis due to sensitization from multiple contacts from OP compounds was described by Kambo et al (1970).
Karimov (1970) studied skin sensitivity of 233 cotton growers to different pesticides. In 27 (4.2%) of 633 samples, he found positive reactions to OP pesticides. Changes in immunological pattern has also been reported by Chezzo et al (1968); Janicki (1972); Bezuglyi (1980); Zolotnikova (1980); Pol’chenko and Zinchenko (1987).

Anticholinesterase pesticides inhibit breakdown of the neurotransmitter acetylcholine by inhibiting acetylcholinesterase, a serine hydrolase enzyme. The impairing immune processes could be dependent on serine hydrolase activity. In a series of experiments over the last several years, Casale (1995) demonstrated that carbaryl and other common anticholinesterase insecticides inhibit serine hydrolase dependent immune processes, such as interleukin 2 (IL-2) signaling.

MISCELLANEOUS EFFECTS

Effects on Hormones
Changes in the diurnal pattern of plasma ACTH and adrenal levels of some related enzymes have been reported in rats exposed to dichlorvos in drinking water at 2 mg/litre, Civen et al (1980).

Lipid Metabolism
Few organophosphate esters decrease the activities of some triglyceridases and lipases in vivo and in vitro (WHO, 1986). In a preliminary study, Buchet et al. (1977) observed rise in cholesterol content in aorta of rats exposed to triamiphos, however, later on, no changes were noticed in hormone sensitive lipase and lipoprotein lipase in adipose tissue, the free and total fatty acids, total glycerol and total cholesterol in serum of rats fed triamiphos for one year.
Selective inhibition of thermogenesis

Ray (1980) observed that defoliant DEF (S,S,S-tri-n-butyl phosphorotrithiolate) acted as an anticholinesterase at high doses but at lower doses (60-200 mg/kg in rats and mice) it caused a profound fall in body temperature (as much as 10° C over a few hours) without marked sedation. Death occurred mostly after the depression had persisted for a day. Ray and Cunningham (1985) further demonstrated that the effect was a selective action on a central thermogenic control mechanism rather than on peripheral thermogenic processes and it was probably due to a metabolite of DEF rather than to the parent compound.

Effects on the retina

Imai (1977) demonstrated that electroretinogram of rats was affected by fenitrothion (50 mg/kg) administered intramuscularly for one year. Ishikawa and Miyata (1980) observed some changes in optical function of beagle dogs administered fenitrothion or ethyliothiomelon for 5 days per week for 2 years.

Porphyric effects

Daily application of technical diazinon (20 or 40 mg/kg b.w.) to skin of rats just above tail produced a four fold increase in fecal porphyrins level (Bleakley et al. 1979). There was no increase in urinary porphyrins when diazinon was given in food (about 8 mg/day to rats). Nichol et al (1982) reported that an impurity in stored technical material isodiazinon was very effective in causing porphyrin accumulation when added to cultures of chick hepatocytes. Although human poisoning with diazinon is not uncommon, there has only been one report implicating technical diazinon in few cases of porphyria cutanea tarda in occupationally exposed workers (Bopp and Kosminsky, 1975).
EFFECTS ON ORGANISMS IN THE ENVIRONMENT

Aquatic organisms

OP insecticides are not very stable in aqueous media. However, accidental leaking may occur from treated areas into rivers and lakes where they may exert toxic effects on organisms prior to degradation. In laboratory conditions, toxic effects are pronounced in several aquatic organisms when exposed to various OP insecticides ranging from 0.01 to 1 mg/litre for 48 hours (Nishiuchi, 1981). Yoshida and Nishiuchi (1972, 1976) reviewed toxic characteristics of various insecticides including OP compound on small aquatic organisms such as Daphnia.

Effect on Animals

Insecticides are designated as lethal agents, although they may be designed to be less toxic for animals than for insects, all OP insecticides present a toxic hazard to some extent. The acute LD<sub>50</sub> (oral and dermal) for rat of various OP insecticides ranges from less than 10 to more than 3000 mg/kg body weight (WHO, 1986). The dose response line for OP insecticides is usually steeper than that of carbamates though both kill by their anti cholinesterase action. The precise reason regarding the difference lies in the faster rate of spontaneous reactivation of carbamylated AChE when compared with phosphorylated AChE. Variability in the LD<sub>50</sub> of a compound depends largely on the route, species and also the nature of the vehicle in which the pesticide is applied which may influence its uptake into the body.
MANAGEMENT OF OP POISONING

The following management of OP insecticide poisoning have been extensively reviewed and described by De and Chatterjee (1967); Gupta and Patel (1968); Wadia et al. (1971); WHO (1986); Adlakha et al. (1988); Sunder Ram et al. (1991); Hayes and Laws (1991); Kaloyanova and Batawi (1991); Patial (1993); Watson and Pharm. (1995); Johnson et al (1996); Cherian et al (1997); Goel et al (1998).

An important aspect in the management of OP poisoning is what to do, in what order to do and what not to do. Prompt and correct decision is mandatory to initiate management.

The treatment described in literature suggest that when dermal exposure occurs, decontamination procedures adopted should be removal of contaminated clothes and washing of the skin with alkaline soap or with a sodium bicarbonate solution. Venepuncture area to be cleaned with extreme care. Extensive eye irrigation with water or saline should be performed. In the case of ingestion, vomiting might be induced, if the patient is conscious, the administration of ipecacuanha syrup (10-30 ml) followed by 200 ml of water. Gastric lavage (with addition of 1.3 % potassium permanganate or 0.5% sodium bicarbonate solution or activated charcoal) may be prescribed, particularly in unconscious patients, taking care to prevent aspiration of fluids into the lungs (i.e. only after a tracheal tube has been placed).

Recently the use of activated charcoal (AC) is emerging as a primary therapeutic approach in the management of acute poisoning. AC effectively absorbs a large variety of poisons thereby decreasing their bioavailability and enhancing elimination and even shown to be superior than Ipecac or gastric lavage in terms of efficacy and complications.
High doses of atropine in the form of an intravenous infusion in all OP poisoning cases. The results of continuous infusion of high doses of atropine showed a statistically significant reduction in mortality (23.5% to 8.8%; P < 0.05).

**Oxime Reactivators**

Oximes release the enzyme acetylcholinesterase from combination with most organic phosphorous insecticides and thus restores the enzyme to normal function, sometimes with dramatic benefit to the patient. The other desirable properties of oximes are:

a) reactivating inactivated cholinesterase
b) reacting directly with organic phosphorus molecules and thus detoxifying them
c) possibly having an anticholinergic effect similar to that of atropine
d) depolarise the neuromuscular junction
e) produce a sympathomimetic effect and potentiate the pressure effect of epinephrine
f) inhibit cholinesterase

Cholinesterase reactivators (e.g. pralidoxime, obidoxime) specifically restores AChE activity inhibited by organophosphorus. The treatment should be started at the earliest because oximes are not effective on "aged" phosphorylated ChE's. The combination of atropine plus oximes is far more effective in most cases than the mere summed effect, WHO (1986).

Pralidoxime is the most widely used oxime (1 g, I/M or I/V 2 to 3 times daily usually for 1 to 3 days). Obidoxime 3 mg/kg body weight by I/M injection has been reported to be more effective than Pralidoxime as it has a faster action and crosses the blood brain barrier, Davidson (1991). Luzhnikov and Pankov (1969)
recommended the use of sublingual administration of oximes as first aid measure although it is not available in India.

Other remedies

Fresh exposure to organic phosphorus insecticides of any sort should be avoided in cases, where there is already heavy exposure of OP insecticides enough to produce symptoms and signs.

Ganglionic blocking agents have been used for treating OP poisoning patients (Luzhnikov, 1966) but information is inadequate for evaluating them either separately or in combination with other kinds of drugs.

Phenothiazines have been used in the treatment of OP poisoning patients (Luzhnikov, 1966) but the specificity of this treatment is yet to be proved.

Lopez (1970) gave a very glowing account of the benefit of various corticosteroid compounds in treating acute OP poisoning cases. He administered atropine and P2 AM only to patients who failed to respond promptly to corticosteroids.

Prompt clinical improvement has been reported following repeated injections of purified human cholinesterase (Klose and Gutensohn, 1976; Jax et al., 1977). However, it remains unproved.

Diazepam should be included in the therapy of all mild OP poisoning cases. Besides relieving anxiety it appears to counteract some aspects of CNS derived symptoms which are not affected by atropine. (WHO, 1986).

Use of Glycopyrrolate and Atropine in Acute Organophosphorus poisoning has been reported by Tracey and Gallagher (1990). Two cases of acute Organophosphorus poisoning were treated with a combination of atropine and glycopyrrolate as well as benzodiazepine and pralidoxime and concluded that the use of combination therapy of anticholinergic agents can provide adequate control
and heart rate while avoiding the central toxic effects which can occur when large doses of atropine are used.