Br. J. Anaesth. (1989), **63**, 736–750

ORGANOPHOSPHORUS INSECTICIDE POISONING

L. KARALLIEDDE AND N. SENANAYAKE

During the past four decades, some 15000 individual compounds and more than 35000 different formulations have come into use as pesticides [11]. Amongst these, insecticides constitute an important group, and organophosphorus (OP) compounds are possibly the insecticides most widely used in the world. OP compounds are anticholinesterase (anti-AChE) agents. They are of particular interest to anaesthetists, as patients in the acute and intermediate phases of OP poisoning may present with severe disturbances of cardiorespiratory function requiring critical or intensive care.

The first account of the synthesis of a highly potent anti-AChE compound, tetraethyl pyrophosphate (TEPP), was given by Clermont in 1854. It is remarkable that the investigator survived to report on the taste of the compound, a few drops of which placed on the tongue usually proves to be rapidly fatal [36]. Modern investigations of OP compounds date from 1932 when Lange and Krueger recorded the synthesis of dimethyl and diethyl phosphorofluoridates. They noted that inhalation of these compounds caused a persistent choking sensation and blurring of vision. These observations led Schrader of I. G. Farbenindustrie to develop OP compounds, first as agricultural insecticides and later as potential chemical warfare agents. Consequently, during World War II, several highly toxic compounds were developed as nerve gases in Germany, *viz.* ethyl N-dimethyl phosphoroamidocyanidate (Tabun), isopropyl methyl phosphonofluoridate (Sarin), pinacolyl methyl phosphonofluoridate (Soman). The Allied countries also followed Lange and Krueger's lead and developed compounds such as diisopropyl phosphorofluoridate (DFP) [91].

LAKSHMAN KARALLIEDDE, F.F.A.R.C.S.; NIMAL SENANAYAKE, F.R.C.P.; Departments of Anaesthesiology and Medicine, Faculty of Medicine, Peradeniya, Sri Lanka.

Correspondence to L.K.

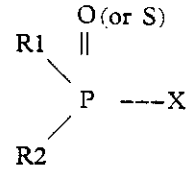
In 1944, Schrader synthesized parathion and its oxygen analogue—paraoxon. Parathion exhibited a wide range of insecticidal activity and desirable chemical and physical properties such as low volatility and sufficient stability in water; it continues to be one of the most widely used OP insecticides [70]. OP insecticides used in crop production have made a major contribution to improvements in agricultural output. Few of the less hazardous agents have been evaluated for disease vector control.

In addition to its use as an insecticide, TEPP underwent extensive clinical trials in the therapy of glaucoma and myasthenia gravis. Ecothiopate, a quaternary ammonium OP compound was used widely in therapy of glaucoma. In the U.S.A., more than 900 chemicals with 25000 brand names are registered as pesticides; these are produced in quantities in excess of 0.5 million tonnes annually [90]. In Great Britain, in 1984, a total of 29543000 kg of pesticides were produced [5].

In 1974, the World Health Organization (WHO) used data from 19 countries to estimate that approximately 500000 cases of acute pesticide poisoning were occurring annually. Of the resulting 9000 or more deaths, 99% were in the Third World [98]. In 1981, the estimate was 750000 cases annually [7], whilst in 1983 the figure was an astonishing 2 million, of which 40000 could be fatalities [18]. Organophosphorus insecticides account for more than 50% of all acute poisonings in hospital practice in Sri Lanka [47, 83]. The majority of the patients (91% in one study) are younger than 30 yr [48]. Self poisoning by ingestion is the commonest mode of intoxication. Outbreaks of mass poisoning caused, for example, by contamination of food such as wheat flour or by accidents during storage, have complicated the picture of OP poisoning [81].

Chemistry

OP insecticides are usually esters, amides or thiol derivatives of phosphoric or phosphonic acids, the general formula being:



R1 and R2 are usually simple alkyl or aryl groups. Group X, referred to as the "leaving group" may be one of a wide variety of substituted or branched aliphatic, aromatic or heterocyclic groups linked to phosphorus via a bond of some lability, usually —O— or —S—. The double bonded atom may be O or S and the related compound termed a phosphate or phosphorothioate. The P=S form is intrinsically more stable and many insecticides are manufactured in this form, which may be converted subsequently *in vivo* to the biologically active oxon.

Oxidation of phosphorothioates to phosphates is potentially dangerous, as phosphates are more volatile and directly toxic. Oxidation may take place at increased temperatures. Isomerization of some formulations (e.g. malathion) may occur during storage under warm humid conditions with a concomitant increase in toxicity [101].

Commercial formulations of OP insecticides may contain more than one OP compound. In addition to deliberately added substances, commercial products may also contain production impurities, sometimes in high concentrations. The biological behaviour and toxicity to man of OP combinations and impurities may be different from those of the single compound.

Mode of intoxication

Intoxication may occur following absorption via the gastrointestinal system, respiratory tract or skin. Deliberate or accidental ingestion is a common mode of poisoning [47]. 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 insecticides.

Metabolism

After absorption, OP insecticides and their metabolites distribute quickly in all tissues, maximum concentrations usually being reached in the liver and kidney. Lipophilic compounds may reach high concentrations in neural and other lipid-rich tissues. Plasma half-life after a single administration ranges from a few minutes to a few hours, depending on the compound and route of administration. Metabolism occurs principally by oxidation, hydrolysis by esterases and transfer of portions of the molecule to glutathione. Most thiophosphorus compounds with P=S, such as parathion, are converted to the biologically active oxon, paraoxon (P=O), by microsomes in the liver.

Detoxification of OP insecticides occurs either by biochemical modification of their structure or by linkage to binding sites without toxicological significance. Malathion is metabolized to inactive products more rapidly in higher animals than in insects; consequently it is less dangerous to man, other mammals and birds in insecticidal concentrations. Elimination of OP compounds and their metabolites occurs mainly via urine and faeces. Urinary and faecal excretion is usually rapid, 80–90% of most compounds being eliminated within 48 h. A very small proportion of OP compounds and their active forms (oxons) are eliminated unchanged in the urine. Some compounds (e.g. fenthion, fenitrothion) are known to persist in the body for longer periods [63].

MECHANISM OF ACTION AND TOXICOLOGY

OP insecticides exert their biological actions mainly by inhibition of enzymes. Esterases are the target responsible for OP toxicity to insects and mammals, the toxicity depending primarily on the power of the OP compound to inhibit the esterases.

Inhibition of acetyl cholinesterase (AChE)

AChE is responsible for hydrolytic cleavage of acetylcholine (ACh) to choline and acetic acid. ACh acts as neurotransmitter for all preganglionic autonomic fibres, all post-ganglionic parasympathetic fibres and a few post-ganglionic sympathetic fibres. Moreover, ACh is the neurohumoral transmitter at the skeletal muscle motor endplates and some interneuronal synapses in the central

nervous system (CNS). Synaptic conduction is mediated by the pre-synaptic release of ACh into the interneuronal space. The arrangement of the synapse is such that ACh comes into contact with AChE before the post-synaptic receptor sites. ACh which is not degraded by AChE binds to the post-synaptic receptors, resulting in generation of an excitatory post-synaptic potential and propagation of the impulse.

The transient nature of this event is caused by degradation of ACh by AChE. AChE is an enzyme located mainly in the nervous system and in the motor end-plates of skeletal muscle. When AChE is inhibited, the post-synaptic cholinergic transmission time is extended and this results in protracted cholinergic overstimulation.

Organophosphorus compounds react with AChE and the latter becomes firmly, sometimes irreversibly, phosphorylated and therefore inhibited in the process. Cleavage of the carbon–enzyme bond from ACh is complete in a few microseconds. However, the breaking of the phosphorus–enzyme bond requires a period varying from 60 min to several weeks, depending on the OP compound involved. Reactivation of the inhibited enzyme may occur spontaneously, the rates of reactivation depending on the species and the tissue, in addition to the chemical group attached to the enzyme. In most mammals O-O dimethyl phosphorylated AChE undergoes substantial spontaneous reactivation within 1 day, which facilitates recovery from intoxication. Reactivation of O-O diethyl phosphorylated AChE is much slower [30]. The process of reactivation of inhibited AChE may be induced by some oxime reagents which thus provide opportunities for therapy [10, 32, 33].

Response to reactivating agents declines with time; this process is caused by "ageing" of the inhibited enzyme. Ageing is probably a result of loss of one alkyl or alkoxy group, leaving a much more stable monoalkyl- or monoalkoxy-phosphoryl AChE [63]. The rate of this reaction depends on the nature of phosphorylation of the enzyme. The rates of ageing for mammalian AChE increase in the order diethyl < diisopropyl < dimethyl < isopropyl-methyl [30]. The aged phosphorylated enzyme cannot be reactivated by oximes [10, 105].

Neuropathy target esterase inhibition

Delayed neurotoxic action of OP insecticides is independent of AChE inhibition, but related to the phosphorylation of a specific esteratic enzyme in the nervous tissue [42, 43] which has been termed "neurotoxic esterase" or "neuropathy target esterase" (NTE) [44, 45]. The initial biochemical reaction is phosphorylation of NTE. The essential second step responsible for the neuropathy (organophosphate-induced delayed polyneuropathy (OPIDP)) is the transformation of the phosphorylated enzyme to an aged form. If compounds such as phosphinates and carbamates link to NTE before contact with a neuropathic OP, ageing does not occur, preventing the development of a neuropathy [46].

Inhibition of other enzymes

A number of other enzymes including lipases, trypsin and chymotrypsin are phosphorylated by OP insecticides. The rate of reaction with these enzymes is generally slower than with AChE, and the clinical consequences of these reactions are not known as yet [30].

Myopathic effects

Muscular weakness following OP intoxication in animals had been known to experimental scientists for many years, the earliest observations being made by Carey [8]. Paralysis appearing within 24 h of poisoning and lasting a few days or weeks had been described in hens [27, 104]. Myopathic changes were described in the diaphragm, gastrocnemius and psoas muscles of the rat following sublethal doses of OP compounds such as DFP, tabun and paraoxon [1]. Necrotic changes have been shown to begin and to be most extensive in the region of the motor endplate [76]. The peak of necrosis was seen at 1–3 days; at 7 days, signs of muscle recovery were evident. Full recovery occurred within 2–3 weeks. This myopathy was different from the delayed neuropathy which began at nerve terminals approximately 3 weeks after exposure to DFP when muscle contractile strength was returning to normal [27].

Inability to sustain tetanic stimulation has been observed in the experimental animal following OP intoxication [1]. Whilst normal muscle is capable of sustaining tetanic stimulation at 25, 50, 100 and 200 Hz for 10 s, 2–4 h after OP intoxication, contractions were subnormal at 100 Hz and were not observed at 200 Hz. Similar observations that the ability of the muscle to sustain tetanus in response to nerve stimulation was eliminated were made when the esterases of the neuromuscular junction were saturated with the OP compound

DFP [76]. There was a positive correlation between the frequency of stimulation at which the tetanic response could be maintained and the extent of AChE recovery. Tetanic responses at 100 Hz appeared indistinguishable from controls with only approximately 25% of normal AChE.

Muscle paralysis occurring in man after apparent recovery from the cholinergic crisis but before the expected onset of the delayed polyneuropathy has been identified by us recently as the "Intermediate Syndrome" (IMS) [82]. Onset, progression and recovery of muscle weakness in the IMS corresponds closely to the sequence of myopathic changes observed in animal experiments and is distinguishable from the muscle weakness that follows the delayed polyneuropathy which sets in 2–4 weeks after poisoning. The diaphragm was affected most severely in most instances in the animal experiments and this finding is consistent with the cardinal feature of respiratory failure in the IMS.

Of the 93 patients with OP poisoning seen by us over a period of 30 months, beginning mid-1986, 16 (17%) required ventilatory care following development of IMS. There were 13 other patients with varying degrees of muscle weakness and respiratory difficulty who were managed with oxygen inhalation therapy. In our practice, ventilatory care is offered and instituted on a very selective basis because of constraints related to equipment and staff.

The mechanism underlying the myopathic changes following OP intoxication has been the subject of study and discussion [15, 22–25, 59, 60, 99]. Abnormally high concentrations of ACh at the end-plate created a prolonged depolarization resulting from a long-lasting change in ion permeability of the junctional membrane, and this was considered to be the cause of the necrosis [1]. An excessive amount of ACh was capable of inducing skeletal muscle necrosis even when the AChE system was intact; the myopathy was considered to result from disturbances of the trophic effect mediated by ACh rather than excessive depolarization and contraction [23, 24]. Organophosphorus agents caused antidromic firing of motor nerve action potentials. This increased activity in the presence of accumulated ACh was considered to have a causal relation with the disorganization of subsynaptic fibre structure [99]. The severity of the myopathy appeared to depend on a critical degree and duration of AChE inhibition which triggered neurally mediated events, including increased neurotransmitter release and antidromic nerve activity. Paraoxon did not have an effect on K^+ conductance channels [52]. Interference with receptors controlling Na^+ conductance channels was suggested as a cause for the myopathy. However, the observations of dissolution of Z bands of the sarcoplasmic reticulum strongly suggested disturbances of Ca^{2+} flux following OP intoxication [76], and this disturbance was considered to be responsible for the myopathy. Ferry and Townsend [25] observed that anticholinesterase agents caused myopathy at the endplate region of the diaphragm, associated with depletion of creatine kinase from this region and with an increase in serum creatine kinase activity.

Several factors have been found to influence the myopathic reaction to OP agents in the experimental animal [1, 52]. Increased muscle activity, such as that produced by phrenic nerve stimulation, caused aggravation, whereas inactivity produced by prior denervation prevented the myopathic reaction. Hemicholinium and tetrodotoxin had an inhibitory effect, whilst alpha bungarotoxin and curare totally prevented the myopathic reaction [52, 76]. Initially, it was observed that if pralidoxime was administered within 2 h, the myopathic reaction was prevented. However, later it was noted that if pralidoxime was to be fully effective, it should be administered within 10 min [15].

It is of interest that during the past two decades there have been several reports in man (often solitary case reports) of respiratory difficulty arising after the cholinergic phase [13, 26, 62, 73, 95, 96].

Carcinogenicity and teratogenicity

As OP agents are alkylating agents, it has been implied that they may be responsible for mutagenic and carcinogenic effects. However, animal studies to date have not shown dose-related carcinogenic effects and the evidence suggests that these compounds are unlikely to persist long enough *in vivo* to exert any alkylating effect they may possess [21].

OP poisoning and pregnancy

In experimental animals, OP poisoning during pregnancy causes pre-natal and post-natal death and congenital abnormalities, *viz.* vertebral deformities, limb defects, polydactyly, intestinal herniae, cleft palate and hydroureter [30]. Fol-

TABLE I. *Signs and symptoms of organophosphate poisoning. (Reprinted by permission of T. Namba and the* American Journal of Medicine *[72])*

Muscarinic manifestations	
Bronchial tree	Tightness in chest, wheezing suggesting of bronchoconstriction, dyspnoea, increased bronchial secretions, cough, pulmonary oedema, cyanosis
Gastrointestinal system	Nausea, vomiting, abdominal tightness and cramps, diarrhoea, tenesmus, faecal incontinence
Sweat glands	Increased sweating
Salivary glands	Increased salivation
Lachrymal glands	Increased lachrymation
Cardiovascular system	Bradycardia, hypotension
Pupils	Miosis, occasionally unequal
Ciliary body	Blurring of vision
Bladder	Frequency, urinary incontinence
Nicotinic manifestations	
Striated muscle	Muscle twitching, fasciculation, cramp, weakness (including muscles of ventilation)
Sympathetic ganglia	Pallor, tachycardia, hypertension
CNS manifestations	Giddiness, tension, anxiety, restlessness, emotional lability, excessive dreaming, insomnia, nightmare, headache, tremor, apathy, withdrawal and depression, drowsiness, difficulty in concentrating, confusion, slurred speech, ataxia, generalized weakness, coma with absence of reflexes, Cheyne–Stokes respiration, convulsion, depression of respiratory and circulatory centres with dyspnoea, cyanosis, hypotension

lowing OP intoxication during the third month of human pregnancy, abortion has been performed as continuation of pregnancy was considered hazardous [26]. However, successful management of the poisoning during the second and third trimesters may allow the pregnancy to continue to term unaffected [49] and result in the delivery of normal, healthy babies.

CLINICAL MANIFESTATIONS

Cholinergic phase

Cholinergic manifestations are summarized best under three categories based on the site of cumulation of ACh: muscarinic (all post-ganglionic nerve endings), nicotinic (autonomic ganglia and skeletal muscle endplates) and central (synapses in the CNS) (table I).

These symptoms may arise in varying combinations. Their severity and time of onset depend on the chemical composition of the agent and the mode of intoxication. Following massive ingestion, symptoms arise within minutes. Death has occurred within 5 min after ingestion of concentrated TEPP. However, in most instances symptoms appear within 30 min of exposure and almost always in less than 12 h.

The most serious manifestation and the usual cause of death is respiratory failure which results from weakness of the muscles of ventilation and depression of the respiratory centre, aggravated by excessive tracheobronchial secretions and bronchospasm. Loss of consciousness in severe intoxication and the accompanying vomiting predisposes to aspiration of gastric contents into the lungs. Bradycardia may be severe and may progress to heart block. The clinical presentation is often one of acute medical emergency which requires urgent cardiorespiratory resuscitation.

Intermediate syndrome (IMS)

After recovery from the cholinergic crisis, but before the expected onset of the delayed polyneuropathy, some patients develop a state of muscle paralysis which we have described recently as the "Intermediate Syndrome". The syndrome is of acute onset, often seen 24–96 h after poisoning, affecting conscious patients without fasciculations or other cholinergic manifestations. The cardinal feature of the syndrome is muscle weakness affecting predominantly the proximal limb muscles and neck flexors. Muscles innervated by motor cranial nerves III–VII and X are affected also in different combinations. The

syndrome carries a risk of death, because of respiratory paralysis, if not recognized early and treated adequately. The agents commonly responsible are fenthion, monocrotophos and dimethoate.

Respiratory insufficiency drew attention to the onset of the IMS in most of our patients. These patients were conscious and showed marked anxiety, sweating and restlessness caused by progressive hypoxia. They would often try to sit up in bed, bathed in sweat with all their accessory muscles of ventilation in use. A constant feature was marked weakness of neck flexors and patients were unable to raise the head from the pillows. They also showed moderate to severe weakness of shoulder abduction and hip flexion. However, normal strength in the distal muscles gave a false impression that the limbs were spared. As the weakness is balanced and usually symmetrical, mild weakness of the muscles affected may be overlooked easily unless sought specifically. The muscles are free from fasciculations; however, we have occasionally observed spasticity of limbs and hyperreflexia and sometimes dystonic reactions. The tendon reflexes were usually decreased or absent in most patients. There was no sensory impairment.

Respiratory insufficiency develops over approximately 6 h. Initially, the patient uses accessory muscles of ventilation. There is increase in ventilatory rate, sweating, restlessness and later cyanosis. Unattended, the patient soon becomes unconscious and death follows.

In a preliminary study [82], tetanic stimulation of the abductor pollicis brevis 24–48 h after the onset of the IMS showed marked fade at 20 and 50 Hz. The muscle was stimulated via the median nerve at the wrist and the muscle action potentials recorded with surface electrodes placed over the muscle. At 50 Hz a fade of 30–75 % was observed in 5 s, and at 20 Hz there was a fade of 20–30 % in 10 s. There was no post-tetanic facilitation.

The IMS is likely to result from post-synaptic neuromuscular junction dysfunction.

Delayed polyneuropathy (OPIDP)

Most reports of OPIDP involve intoxication with non-insecticidal OP agents. These include an early report in the 19th century when phosphocreosote was used to treat pulmonary tuberculosis [57]. In the 1930s more than 50000 U.S. citizens became paralysed after drinking Jamaica ginger contaminated with TOCP [2, 64, 68]. An outbreak involving approximately 10000 persons occurred in Morocco in 1959 after a mixture of olive oil and aircraft lubricating oil was sold as food [86]. Several other outbreaks have occurred in Durban, Fiji, Vietnam and Sri Lanka, where the poisoning was traced in most instances to accidental contamination or adulteration of cooking oils with mineral oils [14, 79, 87, 89]. There have been reports of OPIDP with insecticides mipafox, leptophos, trichlophon, trichlornat, chlorpyrifos and methamidophos [4, 31, 39, 80].

The neuropathy develops following a latent period of 2–4 weeks after the cholinergic crisis. The cardinal symptoms are distal weakness of feet and hands, calf pain preceding the weakness and in some cases paraesthesiae in the distal parts of the limbs.

Wasting of distal muscles, particularly the small muscles of the hand and those of the anterior and peroneal compartments of the leg, is an inevitable consequence. In many, pyramidal tract signs (spasticity and increased tendon reflexes) appear after a few weeks or few months. Recovery from OPIDP is variable [67, 78].

Behavioural effects

Behavioural changes have been documented following acute or chronic OP poisoning. These symptoms may take months to regress [50]. In human subjects exposed to OP agents to an extent sufficient to depress plasma or erythrocyte ChE, some or all of the following observations have been made:

(1) Impairment of vigilance, information processing, psychomotor speed and memory.
(2) Poor performance and perception of speech.
(3) Increased tendency to depression, anxiety and irritability.
(4) A tendency to faster frequencies and higher voltages in EEG records.

The EEG abnormalities were related positively to the level of AChE inhibition during the initial stages of intoxication [55].

DIAGNOSIS

Acute cholinergic crisis

Clinical diagnosis is relatively easy and is based on the characteristic symptoms and signs, and the history of exposure to OP agent. Helpful signs include miosis and muscle fasciculations. Excessive secretions in the mouth and respiratory tract, sweating and lachrymation are other useful

TABLE II. *Comparison of the intermediate syndrome and delayed neuropathy. (Reprinted by permission of the* New England Journal of Medicine *[82])*

	Intermediate syndrome	Delayed neuropathy
Time of onset after poisoning	1–4 days	2–3 weeks
Sites of weakness		
Limb muscles	Proximal	Distal
Neck muscles	–	–
Cranial nerves	–	–
Ventilatory muscles	–	–
Electromyogram	Tetanic fade	Denervation
Recovery, from time of onset	4–18 days	6–12 months
Organophosphorus agents commonly involved	Fenthion Dimethoate Monocrotophos	Methamidophos Trichlorphon Leptophos

signs. History may be denied in attempts at suicide or unavailable in patients who are found unconscious. In these situations the pungent garlic-like odour in breath, vomitus or faeces may suggest OP intoxication.

The response to atropine therapy may also be a useful aid to diagnosis. Patients with OP poisoning show tolerance to atropine and failure to produce signs of atropinization (mydriasis, tachycardia, flushing of skin, dryness of mouth and skin) with 1–2 mg i.v. indicates OP poisoning.

Intermediate syndrome (IMS)

The diagnosis is clinical and should be suspected when a patient who is recovering from the cholinergic crisis develops respiratory difficulty. The presence of muscle weakness in the absence of muscle fasciculations and other cholinergic features differentiates it from cholinergic crisis. The early onset of muscle weakness distinguishes the IMS from the delayed polyneuropathy which appears 2–3 weeks after poisoning. There is also an obvious contrast between the distribution of muscular weakness in the two conditions (table II).

Delayed polyneuropathy

History of intoxication with OP agents and the time of onset and distribution of muscle weakness differentiate OPIDP from other causes of acute polyneuropathy.

Cholinesterase inhibition

AChE is present in human erythrocytes (RBC) and is the same as the enzyme present in the target synapses. Thus changing concentrations of AChE in RBC are assumed to mirror the effects in the target organs, provided the OP agent has equal access to blood and synapses. In acute poisoning, high inhibition of RBC-AChE might not correlate with severity of the symptoms [101]. Plasma contains a related enzyme, pseudocholinesterase, which has no known physiological function. The sensitivities of AChE and of pseudo-ChE to OP agents differ [30]. Thus the use of whole blood samples in analysis gives only an approximate estimate of the activity of RBC-AChE. However, in many field situations and in clinical practice, procedures using whole blood are more practical than those using separated RBC. Physiological variations in blood concentrations of ChE occur both within and between individuals [30]. Further, there are genetic influences and changes associated with many disease processes, in particular liver disease [9, 100]. Results of ChE activity should be interpreted, therefore, with due consideration of the possible variations.

Genetic variation in plasma concentrations of ChE does not appear to influence susceptibility to poisoning by OP agents, but this may not be the case in disease states with low enzyme activity. The determination of percentage inhibition of plasma ChE by dibucaine indicates if the low cholinesterase activity is genetic in origin.

Methods used for assay of ChE activity vary in sophistication [19, 20, 65, 101]. They include the classical electrometric method, the colorimetric method and a titrometric assay. The WHO has developed a field method and kit for measurement of ChE concentration in whole blood and plasma.

Fast methods exist for the measurement of ChE concentration in serum using paper tests [101].

Measurements of ChE activity in blood are of definitive value if serial blood samples are taken and if pre-exposure values are known. Caution is necessary in interpreting the results, as there is no uniformly accepted standard technique; each method has its own "normal range".

In chronic exposure, depression of ChE activity in blood to 80% is generally considered diagnostic of intoxication. Reduction to 70% or less indicates a hazard. In acute poisoning, mild exposure may cause a reduction to 50% of normal activity of ChE, moderate exposure causing a further reduction to 20% activity. Very severe poisoning may reveal only 10% activity. Clinical recovery correlates well with RBC-AChE recovery to 30% of normal [51]. Plasma ChE recovers quickly, usually within 4 weeks; RBC-AChE takes longer and may not be restored to normal for several months.

OP agents in blood and urine

Measurement of OP compounds in plasma or of metabolites in urine is a more sensitive indicator of exposure than measurement of ChE activity in the blood. The information derived from these two methods differs, as excretion of OP agents occurs rapidly, whilst enzyme activity recovers more slowly. Thus ChE observations, RBC-ChE in particular, provide a summary of the physiological effect of exposure, whereas blood and urine assays of OP agents or their metabolites provide accurate quantifiable data relating to the time course of intoxication.

Although the analysis of urine for concentrations of intact pesticides and their metabolites is useful, because of rapid hydrolysis of OP agents by the body, it is often not possible to detect the parent compound, except in cases of severe intentional poisoning [66]. Measurement of metabolites may be useful in low level chronic exposure to pesticides in cases where blood concentrations of ChE have not been depressed [94]. Detection may be most useful within 24 h of the most recent contact, but urinary metabolites may also persist for several days. Urinary concentrations of paranitrophenol are used widely to provide evidence of exposure to parathion [106].

Assay of OP agents in blood and urine is very slowly being developed for general clinical work [66].

TABLE III. *Management of acute organophosphorus (OP) insecticide poisoning*

System	Management
Cardiac function	
Monitor:	
Heart rate	
Arterial pressure	
ECG	
Abnormalities:	
Cardiac arrest	External cardiac massage Cardiac resuscitation
Arrhythmias (bradycardia, heart block)	Anti-arrhythmics (atropine i.v. for bradycardia)
Respiratory function	
Monitor:	
Rate of ventilation	
Tidal volume	
Pa_{O_2}, Pa_{CO_2}	
Abnormalities:	
Airway obstruction	Remove secretions Oropharyngeal suction Oropharyngeal airway
Depressed ventilation	Tracheal intubation Oxygen therapy Ventilatory care
Central nervous functions	
Monitor:	
Level of consciousness	
Size of pupils	
Occurrence of fits	
Abnormalities:	
Coma	Care of unconscious patient
Restlessness	Diazepam 10 mg i.v.
Convulsions	Diazepam 10 mg i.v.
Prevention of further absorption of OP	Remove clothing Wash skin, eyes Gastric lavage Activated charcoal
Antagonism of effects of OP agent	
At muscarinic sites	Atropine 2–4 mg i.v. Repeat every 5–10 min
At nicotinic sites	Pralidoxime 1 g slowly i.v. twice daily
At central nervous sites	Above + diazepam
Maintenance of adequate vital functions	Monitoring i.v. fluids Oxygen therapy supportive measures (intensive care)

TREATMENT

Acute Cholinergic Crisis

All patients should be managed as emergencies in hospital (table III). Treatment is based on

correcting disturbances of and maintaining vital functions, minimizing further absorption of insecticide and pharmacologically countering effects of OP intoxication. Successful management depends on the rapid and simultaneous implementation of the above principles.

Respiratory failure is the usual cause of death in the acute phase; resuscitation and artificial respiration may be required suddenly. Mouth-to-mouth resuscitation should not be attempted, as the medical attendant could absorb dangerous quantities of the OP agent via the ventilatory tract. Cardiac arrhythmias include various degrees of heart block and should be managed in the conventional manner. Atropine i.v. usually corrects severe bradycardia.

Gastric lavage is most effective within 30 min of ingestion, but is advised also at the time of admission after the necessary precautions have been taken to protect the airway. Induction of emesis with syrup ipecacuanha should be avoided, as rapid unconsciousness may supervene before emesis [66]. Activated charcoal may be administered to reduce further absorption of the OP agent from the stomach.

Atropine

Atropine acts as a physiological antidote, effectively antagonizing the muscarinic receptor-mediated actions of OP agents (increased tracheobronchial secretions, salivation, bronchoconstriction and bradycardia). Atropine is virtually without effect against the peripheral neuromuscular dysfunction and subsequent paralysis induced by OP agents. It may counter the central actions to some extent. A recommended dose is 2–4 mg i.v., repeated at intervals of 5–10 min initially and continued until signs of atropinization (dry mouth, dilated pupils, flushing of skin and a heart rate > 100 beat min^{-1}) appear. Paediatric treatment comprises doses of 0.02–0.05 mg kg^{-1} every 10–30 min [69]. Continuous observation is necessary to ensure that a state of mild atropinization is maintained. Atropine may have to be administered at frequent intervals following admission of the patient to hospital, and several hundreds of milligrams may be required during the first 24 h [28, 97]. Infusions of atropine are used in some centres in doses of 0.02–0.08 mg kg^{-1} h^{-1} [17]. A heart rate exceeding 140 beat min^{-1} should be avoided. ST segment abnormalities in the electrocardiogram may be induced by large doses of atropine. These may be corrected with propranolol, eliminating any need to reduce the rate of administration of atropine [93].

Concern has been expressed regarding administration of atropine to hypoxic patients [30]. Ventricular tachycardia was observed in hypoxic dogs given atropine. Correction of hypoxia in any situation should precede drug therapy and this treatment regimen should be followed.

Oximes

The observation that oximes reactivate phosphorylated AChE more rapidly than does spontaneous hydrolysis led to the development of pralidoxime (pyridine-2-aldoxime methyl chloride-2PAM) and, later, obidoxime [102, 103]. Pralidoxime has three main actions [85]:
(1) A direct reaction converting the inhibitor to a harmless compound.
(2) A transient reaction protecting the enzyme from prolonged inhibition.
(3) Reactivation of the inhibited alkyl phosphorylated enzyme to free the active unit.

The reactivating action of pralidoxime is most marked at the skeletal neuromuscular junction. Pralidoxime does not reverse the muscarinic manifestations of OP intoxication; it has a short elimination half-life of 1.2 h when given i.v. [84], and is thought not to cross the blood–brain barrier easily [91]. The reactivating and antidotal actions of N.N'-trimethylenebis (pyridinium-4-aldoxime) (TMB–4) and N.N'-oxydimethylenebis (pyridinium-4-aldoxine) (Toxogonin) have been examined with particular reference to their effect on phosphorylated AChE in the brain. Reactivation in the brain of mice requires much higher doses of the oximes, and even with these very high doses reactivation amounts to less than 10%. There was evidence that 10% reactivation overestimated the reactivation which occurs in the brain *in vivo* [34]. However, some experimental work suggests that there may be limited passage, and this may have a significant, albeit small effect. A more beneficial direct effect on the medullary centres cannot be excluded. Prompt improvement has been reported in the level of consciousness and in the EEG of an intoxicated child with an i.v. infusion of pralidoxime chloride [58].

Pralidoxime therapy has been reviewed extensively [16, 29, 35, 53, 71, 72, 75, 92]. Pralidoxime is available as the chloride (2-PAM), iodide, mesylate (methanesulphonate) and methyl sulphate [6]. The availability of these preparations

varies in different countries, the mesylate salt being available in the U.K. and the chloride salt in the U.S.A. The chloride has been shown to be more stable than the iodide in the dry state and is preferable for i.m. use. The chloride is much used in many developing countries. Effects of pralidoxime are apparent usually within 10–40 min [72]. Pralidoxime may be administered by slow i.v. injection as a 5% solution in water or in 100 ml of normal saline infused over 15–30 min. Pralidoxime may also be given i.m. or s.c. [6]. Sublingual administration has also been suggested as first aid.

For acute OP poisoning, we use 2-PAM 1 g i.v. every 12 h for 5–7 days. Those who develop the IMS are given the drug for longer periods until they are weaned from ventilatory care. Two separate injections of pralidoxime 1 g followed by an infusion of 2.5% pralidoxime 0.5 g h^{-1} has been suggested in severe poisoning. In view of the short elimination half-life of 1.2 h following i.v. injection, it has been suggested that a continuous infusion of 0.5 g h^{-1} would be more likely to provide constant therapeutic concentrations. This has yet to be tested. However, Grob [29] doubted any possible benefit of infusions compared with repeated injections. We have repeated pralidoxime in 1-g doses at 6-h intervals in severe poisoning with good clinical results. The maximum recommended dose in adults is 12 g in 24 h [6].

High doses of pralidoxime and related compounds may cause neuromuscular block and other effects, including inhibition of AChE [91]. Such actions are minimal at doses of 1–2 g i.v. Pralidoxime injected i.v. at a rate more rapid than 500 mg min^{-1} can cause mild weakness, blurred vision, diplopia, dizziness, headache, nausea and tachycardia. Animal work has demonstrated that, when pralidoxime is combined with atropine, signs of atropinization may occur earlier than might be expected when atropine is used alone [30]. In children younger than 12 yr, the dose is 25–50 mg kg^{-1} i.v. over 5–30 min [69].

Pralidoxime should be administered as early as possible in acute OP poisoning, at least within 24–36 h, as regeneration of AChE is dependent primarily on the lifespan of the red cell when ageing of the enzyme has occurred. Thus regeneration of AChE may take weeks.

A dose of 7.5–10 mg kg^{-1} i.m. hourly until improvement is observed has been recommended. Therapeutic monitoring is possible to confirm adequate plasma concentrations of pralidoxime, but this procedure is not carried out routinely. Most analyses of this type have been carried out for pharmacokinetic studies [66]. Animal studies suggest that plasma concentrations of at least 4 mg $litre^{-1}$ are required for optimal therapeutic effect [88]. Such concentrations are produced in humans within 5 min after i.m. injection of 500 mg of the drug [35]. I.v. injections of at least 7.5 mg kg^{-1} produce comparable concentrations [84].

Pralidoxime is not equally antagonistic to all OP insecticides. For instance, effects of diethyl compounds is countered more effectively than those of dimethoxy compounds [30].

Obidoxime chloride is a more potent cholinesterase reactivating agent than pralidoxime, but its toxicity is slightly greater [91]. It is given in doses of 250 mg i.m. or i.v. slowly. Adverse effects include pain at the site of injection, mild to moderate tachycardia and hypertension.

Benzodiazepines

Several reports have indicated that benzodiazepines are potentially useful as antidotes to poisoning by ChE inhibitors [40, 41, 56]. Diazepam produced an immediate decrease in cerebral electrical activity and terminated the convulsions produced by the OP agent, soman, in Rhesus monkeys [56]. The combination of atropine and diazepam was more effective in protecting rabbits against the OP agent than atropine alone. These experiments suggest that diazepam has some anticholinergic effects. Diazepam has been shown also to increase the therapeutic effect of atropine and obidoxime in rats poisoned by phosphamidon. Diazepam appears to counteract some aspects of CNS-derived symptoms, which are not affected by atropine.

Phenothiazines

The use of phenothiazines in the management of OP poisoning is controversial [30]. The potentiation of OP insecticides by phenothiazine derivatives has been suggested [3]. On the other hand, phenothiazines have been used in the treatment of patients poisoned by OP compounds without observed ill effect and with the clinical impression that they were beneficial. It is unlikely that decisive evidence for or against phenothiazines will be forthcoming, as diazepam has proved to be a very satisfactory and popular alternative.

Respiratory stimulants

A recent survey of the literature has concluded that respiratory stimulants should not be used in the treatment of acute OP poisoning in humans, particularly in view of the bronchospasm, neuromuscular block and convulsions that are associated frequently with intoxication [54].

Other measures

Dialysis of blood against activated charcoal (haemoperfusion) is effective in demeton-S-methyl sulphoxide, dimethoate and parathion poisoning [61]. The practical value of haemoperfusion in the treatment of poisoning by other OP compounds remains to be determined.

Prompt clinical improvement has been reported following repeated injections of purified human cholinesterase [30]. The usefulness of such therapy requires further study. Corticosteroids, camphor, potassium chloride and vitamin C have been used with varying degrees of success. However, all these regimens need further evaluation.

Intermediate Syndrome

Prompt and effective management of respiratory insufficiency is the cornerstone of treatment of IMS. The respiratory difficulty may appear suddenly in a patient who is recovering from the cholinergic crisis, even whilst receiving conventional therapy. Therefore, all patients should be observed in hospital for up to 5 days after poisoning. Should early signs of respiratory insufficiency develop (increase in rate of ventilation, use of accessory muscles of ventilation, decreased tidal volume, reduced Pa_{O_2}), facilities for ventilatory care should be made available. Oxygen should be administered in the early stages of respiratory insufficiency. Clinical or biochemical evidence of hypoxia or respiratory failure is an indication for ventilatory support, which may vary from a few days to several weeks. Diazepam in 10-mg doses i.v. may be given if the patient becomes anxious or restless, as it assists in stabilizing and calming the patient during mechanical ventilation, particularly when restlessness is marked. It is necessary to assess daily the patient's ability to breathe spontaneously. Frequent blood-gas analyses are useful in monitoring and weaning from ventilatory care. Therapy with pralidoxime 1 g twice daily, initiated during the acute cholinergic phase, should be continued until the patient recovers adequate spontaneous ventilation.

The role of atropine therapy in IMS needs further evaluation. Although 80–90 % of most OP compounds and their active forms are eliminated within 48 h, some compounds such as fenthion and fenitrothion are known to persist in the body for longer periods. Accordingly, our patients continue to receive atropine therapy to maintain mydriasis and a heart rate exceeding 100 beat min^{-1} for the same duration as treatment with pralidoxime.

During the IMS, some patients may develop a profuse, offensive diarrhoea. Monitoring and correction of fluid and electrolyte status is an important part of management [47].

Delayed Polyneuropathy

No specific drug therapy has proved useful. The use of atropine and oximes during the acute and intermediate phases does not prevent development of delayed polyneuropathy. The muscle weakness benefits from regular exercises and other forms of physiotherapy.

PREVENTION

Preventive measures should be considered at all the links of the chain of insecticide movement through the environment—formulation, manufacture, mixing, application and disposal. Very little manufacture is undertaken in Third World countries, hence the first locale of potential hazard is the port where active ingredients of formulations are unloaded and stored. During transport and storage, strict guidelines should be followed to prevent contamination of food, clothing, drugs, toys, cosmetics and furnishing. Hazards caused by fires or floods should be prevented. Storage sites should be located to prevent contamination of low lying areas and sources of water. A well studied and effective pesticide infrastructure is recommended for all countries handling these agents in large quantities.

SUMMARY AND CONCLUSIONS

Organophosphorus agents are used world wide in increasing quantities for the control of insects affecting agriculture. These agents are used also for disease vector control. OP agents became

important during World War II for their potential use in chemical warfare. Disease in man caused by these agents is causing much concern, particularly in the developing agricultural countries.

OP compounds produce toxicity following systemic absorption from the skin and mucous membranes. Ingestion with suicidal intent or accidentally following contamination of food by these agents is a common mode of intoxication. Intoxication associated with occupational exposure (formulating, mixing, handling, spraying) usually follows absorption either through the skin or by inhalation.

The acute cholinergic crisis which immediately follows intoxication is caused by phosphorylation and inhibition of AChE. The muscarinic and nicotinic manifestations of ACh accumulation produce a clinical syndrome which frequently requires urgent resuscitation and therapy in intensive care units.

The need for ventilatory care following recovery from the cholinergic phase was emphasized recently following recognition of the "Intermediate Syndrome" (IMS). The IMS corresponds closely to the sequence of myopathic changes observed in animal experiments following OP intoxication. Delayed polyneuropathy caused possibly by ageing of the phosphorylated NTE occurs usually 2–4 weeks after intoxication. The distribution of muscle weakness of the delayed polyneuropathy is distinct from that seen in IMS. Cholinergic crisis and IMS present with life threatening complications. Ventilatory care is required in both situations; in view of the development of IMS it is necessary to observe all patients for at least 5 days after intoxication.

There is growing concern regarding the effects of chronic exposure in man. The International Agency for Research in Cancer (IARC) concluded in 1983 [38] that there was little evidence of strong mutagenic or carcinogenic effects in mammals from five widely used insecticides (malathion, methyl parathion, parathion, tetrachlorvinphos and trichlorfon). However, controversy exists in interpretation of the studies on which the IARC conclusions were based [37, 74]. With the widespread use of these agents, effects of prolonged exposure on teratogenicity, carcinogenicity and reproductive function will be important areas of study. In view of the effect of OP agents on enzymes, drug sensitivities such as those observed with suxamethonium will become evident [77]. Another group of drugs that may be affected similarly are the local anaesthetics, as the amino esters are usually hydrolysed in plasma by pseudocholinesterase.

There is great emphasis on preventive measures and on the use of alternatives to insecticides in agriculture. At present, the goal of safe and effective use of insecticides is achieved best by an agromedical approach to pesticide management [12] — integrated, interdisciplinary application of the skills and knowledge of agriculture, applied chemistry and medicine.

REFERENCES

1. Ariens AT, Meeter E, Wolthuis OL, Van Benthem RBJ. Reversible necrosis at the end-plate region in striated muscles of the rat poisoned with cholinesterase inhibitors. *Experimentia* 1969; **25**: 57–59.
2. Aring CD. The systemic nervous affinity of triorthocresyl phosphate (Jamaica ginger palsy). *Brain* 1942; **65**: 34–47.
3. Arterberry JD, Bonifaci RW, Nash EW, Quinby GE. Potentiation of phosphorus insecticides by phenothiazine derivatives. *Journal of the American Medical Association* 1962; **182**: 848–850.
4. Bidstrup PL, Bonnell JA, Beckett AG. Paralysis following poisoning by a new organic phosphorus insecticide (mipafox). *British Medical Journal* 1953; **1**: 1068–1072.
5. *British Agrochemicals Association, Annual Report and Handbook*. London: British Agrochemicals Association, 1985–86.
6. *British National Formulary*, 17th Edn. London: British Medical Association and the Royal Pharmaceutical Society of Great Britain, 1989; 45–46.
7. Bull D. A growing problem: pesticides and the Third World poor. Oxford: Oxfam, 1983.
8. Carey FJ. Studies on amoeboid motion and secretion of motor end-plates. III Experimental histopathology of motor end-plates produced by quinine, curare, prostigmine, acetylcholine, strychnine, tetraethyl lead and heat. *American Journal of Pathology* 1944; **20**: 393–431.
9. Coye MJ, Lowe JH, Maddy KT. Biological monitoring of agricultural workers exposed to pesticides. 1. Cholinesterase activity determinations. *Journal of Occupational Medicine* 1986; **28**: 619–627.
10. Davies DR, Green AL. The kinetics of reactivation by oximes of cholinesterase inhibited by organophosphorus compounds. *Biochemical Journal* 1956; **63**: 529–535.
11. Davies JE. Changing profile of pesticide poisoning. *New England Journal of Medicine* 1987; **316**: 807–808.
12. Davies JE, Smith RF, Freed VH. An agromedical approach to pesticide management. *Annual Review of Entomology* 1978; **23**: 353–366.
13. Dean G, Coxon J, Brereton D. Poisoning by an organophosphorus compound; a case report. *South African Medical Journal* 1967; **41**: 1017–1019.
14. Dennis DT. Jake Walk in Vietnam. *Annals of Internal Medicine* 1977; **86**: 665–666.
15. Dettbarn WD. Pesticide induced muscle necrosis: Mechanisms and prevention. *Fundamental and Applied Toxicology* 1984; **4**: 518–526.
16. Durham WF, Hayes WJ jr. Organic phosphorus poisoning

and its therapy. *Archives of Environmental Health* 1962; 3: 27–53.
17. Du Toit PW, Muller FO, Van Tonder WN, Ungerer MJ. Experience with the intensive care management of organophosphate insecticide poisoning. *South African Medical Journal* 1981; 60: 227–229.
18. Economic and Social Commission of Asia and the Pacific (ECSAP). *Development/Environmental Trends in Asia and the Pacific: a Regional Overview*. Bangkok: Economic and Social Commission of Asia and the Pacific (ESCAP), 1983.
19. Edson EF. Blood tests for users of OP insecticides. *World Crops* 1958; 40: 49–51.
20. Ellman GL, Courtney KD, Andres V jr, Featherstone FM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 1961; 7: 88–95.
21. FAO/WHO 1977. *Evaluations of Some Pesticide Residues in Food (FAO Plant Production and Protection Paper 10 Sup)* Rome: Food and Agriculture Organization of the United Nations, 1978.
22. Fenichel GM, Dettbarn WD, Newman TM. An experimental myopathy secondary to excessive acetyl choline release. *Neurology (New York)* 1974; 24: 41–45.
23. Fenichel GM, Kebler WB, Olson WH, Dettbarn WD Chronic inhibition of cholinesterase as a cause of myopathy. *Neurology (Minneapolis)* 1972; 22: 1026–1033.
24. Ferry CB. The origin of the anticholinesterase-induced repetitive activity of the phrenic nerve–diaphragm preparation of the rat *in vitro*. *British Journal of Pharmacology* 1988; 94: 169–179.
25. Ferry CB, Townsend HE. Anticholinesterase induced myopathy and changes in creatine kinase in mice. *Journal of Physiology (London)* 1986; 373: 29P.
26. Gadoth N, Fisher A. Late onset of neuromuscular block in organophosphorus poisoning. *Annals of Internal Medicine* 1978; 88: 654–655.
27. Gaines TB. Acute toxicity of pesticides. *Toxicology and Applied Pharmacology* 1969; 14: 515–534.
28. Golsousidis H, Kokkas V. Use of 19590 mg of atropine during 24 days of treatment after a case of unusually severe parathion poisoning. *Human Toxicology* 1985; 4: 339–340.
29. Grob D. Anticholinesterase intoxication in man and its treatment. In: Koelle GB, ed. *Handbook of Experimental Pharmacology*. Berlin: Springer Verlag, 1963; 989–1027.
30. Haves WJ jr. *Pesticides Studied in Man*. Baltimore: Williams & Wilkins, 1982; 284–413.
31. Hierons R, Johnson MK. Clinical and toxicological investigations of a case of delayed neuropathy in man after acute poisoning by an organophosphorus pesticide. *Archives of Toxicology* 1978; 40: 279–284.
32. Hobbiger F. Effect of nicotinhydroxamic acid methoxide on human plasma cholinesterase inhibited by organophosphates containing a dialkylphosphate group. *British Journal of Pharmacology* 1955; 10: 356–362.
33. Hobbiger F. Chemical reactivation of phosphorylated human and bovine true cholinesterase. *British Journal of Pharmacology* 1956; 11: 295–303.
34. Hobbiger F, Vojvodic V. The reactivating and antidotal actions of N,N'trimethylenebis (pyridinium-4-aldoxime) (TMB-4) and N,N'-oxydimethylenebis (pyridinium-4-aldoxime) (Toxogenin) with particular reference to their effect on phosphorylated acetylcholinesterase in brain. *Biochemical Pharmacology* 1966; 15: 1677–1690.
35. Holland P, Parkes DC. Plasma concentrations of the oxime pralidoxime mesylate (P2S) after repeated oral and intramuscular injection in humans. *British Journal of Industrial Medicine* 1976; 33: 43–46.
36. Holmstedt B. Structure–activity relationship of the organophosphorus anticholinesterase agents. In: Koelle GB, ed. *Handbuch der Experimentellen Pharmakologie*. Berlin: Springer Verlag, 1963; 428–485.
37. Huff JE, Bates R, Eustis SL, Haseman JK, McConnell EE. Malathion and malaoxon: histopathology reexamination of the National Cancer Institutes carcinogenic studies. *Environmental Research* 1985; 37: 154–173.
38. International Agency for Research on Cancer (IARC). Miscellaneous chemicals. *Monographs on the Carcinogenic Risk of Chemicals to Humans*. Lyons: IARC, 1983; 30.
39. Jedrzejowsksa H, Rowinska-Marcinska K, Hoppe B. Neuropathy due to phytosol Agritox): report of a case. *Acta Neuropathologica* 1980; 49: 163–168.
40. Johnson DD, Lowndes HE. Reduction by diazepam of repetitive electrical activity and toxicity resulting from soman. *European Journal of Pharmacology* 1974; 28: 245–251.
41. Johnson DD, Wilcox CW. Studies on the mechanism of the protective and antidotal actions of diazepam in organophosphate poisoning. *European Journal of Pharmacology* 1975; 34: 127–132.
42. Johnson MK. A phosphorylation site in brain and the delayed neurotoxic effect of some organophosphorus compounds. *Biochemical Journal* 1969; 111: 487–495.
43. Johnson MK. The delayed neurotoxic effect of some organophosphorus compounds: Identification of the phosphorylation site as an esterase. *Biochemical Journal* 1969; 114: 711–717.
44. Johnson MK. The delayed neuropathy caused by some organophosphorus esters: mechanism and challenge. *Critical Reviews in Toxicology* 1975; 3: 289–316.
45. Johnson MK. The target for initiation of delayed neurotoxicity by organophosphorus esters: biochemical studies and toxicological applications. *Reviews in Biochemical Toxicology* 1982; 4: 141–212.
46. Johnson MK, Lauwerys R. Protection by some carbamates against the delayed neurotoxic effects of diisopropyl phosphorofluoridate. *Nature (London)* 1969; 222: 1066–1067.
47. Karalliedde L, Senanayake N. Acute organophosphorus insecticide poisoning: a review. *Ceylon Medical Journal* 1986; 31: 93–100.
48. Karalliedde L, Senanayake N. Acute organophosphorus insecticide poisoning in Sri Lanka. *Forensic Science International* 1988; 36: 97–100.
49. Karalliedde L, Senanayake N, Ariaratnam A. Acute organophosphorus insecticide poisoning during pregnancy. *Human Toxicology* 1988; 7: 363–364.
50. Karczmar AG. Acute and long lasting actions of organophosphorus agents. *Fundamental and Applied Toxicology* 1984; 4: 1–7.
51. Kipling RM, Cruickshank AN. Organophosphate insecticide poisoning. *Anaesthesia* 1985; 40: 281–284.
52. Laskowski MB, Dettbarn WD. The pharmacology of experimental myopathies. *Annual Review of Pharmacology and Toxicology* 1977; 17: 387–409.
53. Lehman RA, Bloch LP. Effect of aging aqueous pralidoxime solutions on assay, toxicity and antidotal activity. *Journal of Pharmaceutical Sciences* 1965; 54: 1035–1038.
54. Lerman Y, Gutman H. The use of respiratory stimulants

in organophosphorus intoxication. *Medical Hypotheses* 1988; **26**: 267–269.
55. Levin HS, Rodnitzky RL. Behavioural effects of organophosphate pesticides in man. *Clinical Toxicology* 1976; **9**: 391–405.
56. Lipp JA. Effect of diazepam upon soman induced seizure activity and convulsions. *Electroencephalography and Clinical Neurophysiology* 1972; **32**: 557–561.
57. Lorot C. Les combinations de la cresote dans le traitement de la tuberculose pulmonaire. Thesis, Paris 1899. Cited by Johnson MK, in: Organophosphorus esters causing delayed neurotoxic effects. *Archives of Toxicology* 1975; **34**: 259–288.
58. Lotti M, Becker CE. Treatment of acute organophosphate poisoning, evidence of a direct effect on nervous system by 2-PAM (pyridine-2-aldoxime methyl chloride). *Journal of Toxicology: Clinical Toxicology* 1982; **19**: 121–127.
59. Lowdnes HE, Baker T, Reker WF jr. Motor nerve dysfunction in delayed DFP neuropathy. *European Journal of Pharmacology* 1974; **29**: 66–73.
60. Lowdnes HE, Baker T, Reker WF jr. Motor nerve terminal response to edrophonium in delayed DFP neuropathy. *European Journal of Pharmacology* 1975; **30**: 69–72.
61. Luzhnikov EA, Yaroslavsky AA, Molodenkov MN, Shurkalin BK, Ewseer NG, Barsukov UF. Plasma perfusion through charcoal in methyl parathion poisoning. *Lancet* 1977; **1**: 38–39.
62. Mahieu P, Hassoun A, van Bist R, Lauwerys R, Deheneffe Y. Severe and prolonged poisoning by fenthion: significance of the determination of the anticholinesterase capacity of plasma. *Journal Toxicology: Clinical Toxicology* 1982; **19**: 425–432.
63. Maroni M. Review of toxicological properties and biotransformation of organophosphorus esters. In: WHO Manual of Analytical Methods. Cremona: WHO Collaborating Centre for Occupational Health, 1985; 3–39.
64. Merritt HH, Moore M. Peripheral neuritis associated with ginger extract ingestion. *New England Journal of Medicine* 1980; **203**: 4–12.
65. Michel HO. An electrometric method for the determination of red blood cell and plasma cholinesterase activity. *Journal of Laboratory and Clinical Medicine* 1949; **34**: 1564–1568.
66. Minton NA, Murray VSG. A review of organophosphate poisoning. *Medical Toxicology* 1988; **3**: 350–375.
67. Morgan JP, Penovich P. Jamaica ginger paralysis: 47-year follow up. *Archives of Neurology* 1978; **35**: 530–532.
68. Morgan JP, Tulloss TC. The Jake walk blues. *Annals of Internal Medicine* 1976; **85**: 804–808.
69. Mortensen ML. Management of acute childhood poisonings caused by selected insecticides and herbicides. *Pediatric Clinics of North America* 1986; **33**: 421–445.
70. Murphy SD. Pesticides. In: Klaassen CD, Amdur MO, Doull J, eds. *The Basic Science of Poisons*. New York: MacMillan, 1985; 519–581.
71. Namba T, Hiraki K. PAM (Pyridine-2 aldoxime methiodide) therapy for alkylphosphate poisoning. *Journal of the American Medical Association* 1958; **166**: 1834–1839.
72. Namba T, Nolte CT, Jackrel J, Grob D. Poisoning due to organophosphate insecticides, acute and chronic manifestations. *American Journal of Medicine* 1971; **50**: 475–492.
73. Perron R, Johnson BB. Insecticide poisoning. *New England Journal of Medicine* 1969; **281**: 274–275.
74. Reuber MD. Carcinogenecity and toxicity of malathion and malaoxon. *Environmental Research* 1985; **37**: 119–153.
75. Reynolds JEF. *Martindale. The Extra Pharmacopoeia*. London: London Pharmaceutical Press, 1982; 389–390.
76. Salpeter MM, Kasprzak H, Feng H, Fertuck H. End-plates after esterase inactivation *in vivo*: correlation between esterase concentration, functional response and fine structure. *Journal of Neurocytology* 1979; **8**: 95–115.
77. Selden BS, Curry SC. Prolonged succinylcholine induced paralysis in organophosphate insecticide poisoning. *Annals of Emergency Medicine* 1987; **16**: 215–217.
78. Senanayake N. Tri-cresyl phosphate neuropathy in Sri Lanka, a clinical and neurophysiological study with a three year followup. *Journal of Neurology, Neurosurgery and Psychiatry* 1981; **44**: 775–780.
79. Senanayake N, Jeyaratnam J. Toxic polyneuropathy due to gingili oil contaminated with tri-cresyl phosphate affecting adolescent girls in Sri Lanka. *Lancet* 1981; **1**: 88–89.
80. Senanayake N, Johnson MK. Acute polyneuropathy after poisoning by a new organophosphate insecticide. A preliminary report. *New England Journal of Medicine* 1982; **306**: 155–157.
81. Senanayake N, Karalliedde L. Acute poisoning in Sri Lanka: an overview. *Ceylon Medical Journal* 1986; **31**: 61–71.
82. Senanayake N, Karalliedde L. Neurotoxic effects of organophosphorus insecticides: an intermediate syndrome. *New England Journal of Medicine* 1987; **316**: 761–763.
83. Senanayake N, Karalliedde L. Pattern of acute poisoning in a medical unit in Central Sri Lanka. *Forensic Science International* 1988; **36**: 101–104.
84. Sidell FR, Groff WA. Intramuscular and intravenous administration of small doses of 2-pyridinium aldoxime methochloride in man. *Journal of Pharmaceutical Sciences* 1971; **60**: 1224–1228.
85. Sim VM. Chemicals used as weapons in war. In: Di Palma JR, ed. *Drill's Pharmacology in Medicine*. New York: McGraw Hill, 1971: 1232.
86. Smith HV, Spalding JMK. Outbreak of paralysis in Morocco due to ortho-cresyl phosphate poisoning. *Lancet* 1959; **2**: 1019–1021.
87. Sorokin M. Orthocresylphosphate neuropathy: report of an outbreak in Fiji. *Medical Journal of Australia* 1969; **1**: 506–509.
88. Sunderwall A. Minimum concentrations of N-methyl pyridinium methyl methane sulphonate (P2S) which reverse neuromuscular block. *Biochemical Pharmacology* 1961; **8**: 413–417.
89. Susser M, Stein Z. An outbreak of triortho-cresyl phosphate (TOCP) poisoning in Durban. *British Journal of Industrial Medicine* 1957; **14**: 111–120.
90. Tafuri J, Roberts J. Organophosphate Poisoning. *Annals of Emergency Medicine* 1987; **16**: 193–202.
91. Taylor P. Anticholinesterase agents In: Gilman AG, Goodman LS, Rall TW, Murad F, eds. *The Pharmacological Basis of Therapeutics*. New York: MacMillan, 1985; 110–129.
92. Vale JA, Meredith TJ. Antidotal therapy: pharmacokinetic aspects. *Developments in Toxicology and Environmental Science* 1986; **12**: 329–338.
93. Valero A, Golan D. Accidental organic phosphorus poisoning: The use of propranolol to counteract vagolytic

cardiac effects of atropine. *Israel Journal of Medical Sciences* 1967; 3: 582–584.
94. Vasilic Z, Drevenkar V, Frobe Z, Stengl B, Tikalievic B. The metabolites of organophosphorus pesticides in urine as an index of occupational exposure. *Toxicological and Environmental Chemistry* 1987; 14: 111–127.
95. Wadia RS, Bhirud RH, Gulavani AV, Amin RB. Neurological manifestations of three organophosphate poisons. *Indian Journal of Medical Research* 1977; 66: 460–468.
96. Wadia RS, Chitra S, Amin RB, Kiwalkar RS, Sardesi HV. Electrophysiological studies in acute organophosphate poisoning. *Journal of Neurology, Neurosurgery and Psychiatry* 1987; 50: 1442–1448.
97. Warriner RA III, Nies AS, Hayes WJ jr. Severe organophosphate poisoning complicated by alcohol and turpentine ingestion. *Archives of Environmental Health* 1977; 32: 203–105.
98. Wasilewski A. The quiet epidemic: pesticide poisoning in Asia. *IDRC Reports* 1987; 16: 18–19.
99. Wecker L, Laskowski MB, Dettbarn WD. Neuromuscular dysfunction induced by acetylcholinesterase inhibition. *Federation Proceedings* 1978; 37: 2818–2822.
100. Wescoe WC, Hunt CC, Riker WF, Litt IC. Regeneration rates of serum cholinesterase in normal individuals and in patients with liver damage. *American Journal of Physiology* 1947; 149: 549–551.
101. WHO/ILO/UNEP, Environmental Health Criteria-63. *Organophosphorus Insecticides: A General Introduction.* Geneva: World Health Organisation (WHO) 1986, 17–111.
102. Wilson IB. Acetylcholinesterase. Reversibility of tetraethyl pyrophosphate inhibition. *Journal of Biological Chemistry* 1951; 190: 111–117.
103. Wilson IB, Ginsburg SA. A powerful reactivator of alkyl phosphate inhibited acetylcholinesterase. *Biochemica Biophysica Acta* 1955; 18: 168–170.
104. Witter RF, Gaines TB. Relationship between depression of plasma cholinesterase and paralysis in chickens caused by certain organic phosphorus compounds. *Biochemical Pharmacology* 1963; 12: 1377–1386.
105. Witter RF, Gaines TB. Rate of formation *in vivo* of the unreactivable form of brain cholinesterase in chickens given DDVP or malathion. *Biochemical Pharmacology* 1963; 12: 1421–1427.
106. Wolfe HR, Durham WF, Armstrong JF. Excretion of paranitrophenol and DDA as indicators of exposure to parathion. Urinary excretion of insecticide metabolites. *Archives of Environmental Health* 1970; 21: 711–716.