Neurotoxic Disorders of Organophosphorus Compounds and Their Managements

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Organophosphorus compounds have been used as pesticides and as chemical warfare nerve agents. The mechanism of toxicity of organophosphorus compounds is the inhibition of acetylcholinesterase, which results in accumulation of acetylcholine and the continued stimulation of acetylcholine receptors. Therefore, they are also called anticholinesterase agents.

Organophosphorus pesticides have largely been used worldwide, and poisoning by these agents, particularly in developing countries, is a serious health problem. Organophosphorus nerve agents were used by Iraqi army against Iranian combatants and even civilian population in 1983–1988. They were also used for chemical terrorism in Japan in 1994–1995. Their use is still a constant threat to the population. Therefore, medical and health professionals should be aware and learn more about the toxicology and proper management of organophosphorus poisoning.

Determination of acetylcholinesterase and butyrylcholinesterase activity in blood remains a mainstay for the fast initial screening of organophosphorus compounds but lacks sensitivity and specificity. Quantitative analysis of organophosphorus compounds and their degradation products in plasma and urine by mass spectrometric methods may prove exposure but is expensive and is limited to specialized laboratories. However, history of exposure to organophosphorous compounds and clinical manifestations of a cholinergic syndrome are sufficient for management of the affected patients.

The standard management of poisoning with organophosphorous compounds consists of decontamination, and injection of atropine sulfate with an oxime. Recent advances on treatment of organophosphorus pesticides poisoning revealed that blood alkalization with sodium bicarbonate and also magnesium sulfate as adjunctive therapies are promising. Patients who receive prompt proper treatment usually recover from acute toxicity but may suffer from neurologic complications.

Introduction

Organophosphorus (OP) compounds have been used as pesticides and also as chemical warfare nerve agents. The mechanism of toxicity of OP compounds is the inhibition of acetylcholinesterase (AChE), resulting in an accumulation of acetylcholine and the continued stimulation of acetylcholine receptors. Therefore, they are also called anticholinesterase agents. Carbamates are also anticholinesterase agents, but they are less toxic than OP pesticides, thus are mainly used as home insecticides and herbicides.

OP compounds are not ideal pesticides because of the lack of target vector selectivity, and severe toxicity and even death in humans and domestic animals. Their toxicities have been recognized since the 1930s, when they were also developed for use as chemical warfare agents. A few OP compounds (glyphosate, merphos) are used as herbicides, but they are structurally different from the OP pesticides and their AChE-inhibiting power is very weak. OP nerve agents are more toxic than...
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OP pesticides and because of their high toxicity, they are also called lethal agents.5

An important step in the synthesis of OP compounds was made by von Hofman, who synthesized methylphosphor chloride in 1873. Michaelis in 1903 introduced a compound with P-CN bond, which led to the synthesis of a number of insecticides and the nerve agent tabun. Lang and von Kreuger synthesized compounds with P-F linkage in 1932. Schrader developed sarin and tabun in 1937 and in 1944 Germans developed soman. Britons developed VX in 1952.6,7

Over the past five decades, numerous structurally different OP compounds have been synthesized.2,8 Presently, more than 100 different OP compounds are used worldwide as insecticides.9 The advantage of a lower environmental stability, compared with organochlorine compounds and a high effectiveness against different insect species is accompanied by the disadvantage of high mammalian toxicity.10 The widespread use and easy accessibility of these compounds result in a huge number of intoxications. It was estimated that between 750,000 and 3,000,000 human OP intoxications occurred worldwide,9,11 resulting in several thousands of fatalities annually.12 Occupational and accidental OP exposure are the main causes for mild poisonings,13–15 whereas severe cases are mostly due to suicidal attempts and self-poisoning.16,17

In some parts of the developing world, pesticide poisoning causes more deaths than infectious diseases.11 Use of pesticides is poorly regulated and often dangerous; their easy availability also makes them a popular method of self-poisoning. In 1985, the UN Food and Agriculture Organization (FAO) produced a voluntary code of conduct for the pesticide industry in an attempt to limit the harmful effects of pesticides.16 Unfortunately, the lack of adequate governmental resources in the developing world makes this code ineffective.16 The World Health Organization (WHO) has recommended that access to highly toxic pesticides be restricted, but it has not been applied in some developing countries such as the Islamic Republic of Iran. OP pesticides are still easily available in this country, thus their exposure either as occupational or intentional oral ingestion are common and induce health problems as well as environmental hazards.1,18

Several highly toxic OP compounds were developed and stockpiled as chemical warfare nerve agents during the last century.19 But its human exposure until the Iraq-Iran war was restricted to one prospective study with VX and sarin at low levels and to case reports of accidental exposure to sarin and soman.6

The first use of nerve agents in the war occurred in February 1984 in Majnoon Island by the Iraqi army against the Iranian troops. Among the chemical warfare agents applied by the Iraqi army, the nerve agent tabun was found in the environmental samples and in the postmortem examination of the patients who died soon after the exposure. More than 300 patients died within 30 min of exposure in the field and several thousands were poisoned by tabun.18 Toxico logical analyses of the blood, urine, skin, and gastric juice of the chemical war gas victims revealed tabun and sulfur mustard.18 Later in 1987 and 1988, particularly during the Halabjah massacre, another nerve agent (sarin) was also identified.1,20

A confirmed terrorism attack with sarin occurred in a residential area of the city of Matsumoto, Japan, on June 27, 1994. About 600 residents and rescue staff were poisoned; 58 were admitted to hospital and 7 died.21 On March 20, 1995, terrorists released sarin at several points in the Tokyo subway, which killed 11 and poisoned more than 5,500 people.22,23

Based on the above information, OP compounds have induced tragedies with lots of human morbidities and mortalities. OP pesticides are still used in most parts of the world and unfortunately are easily available in some developing countries. Thus occupational and accidental exposure and even intentional ingestion are common and can induce health problems. In spite of the establishment of organization for prohibition of chemical weapons (OPCW) and its active role in chemical warfare agents (CWA) control, OP nerve agents are still a big threat to the population worldwide as a chemical war or terrorism agents. Therefore, medical and health professionals should be aware and learn more about the toxicology and proper management of OP poisoning.

Definition, classification, and chemical structures

OP compounds including organophosphates are chemically derived from phosphoric, phosphonic, phosphinic, and thiophosphoric acids.5
Organophosphates are usually esters, amides, or thiol derivatives of phosphoric, phosphonic, or phosphinic acids, which have the general structural formula as shown in Figure 1.24

OP compounds are divided into two main groups; pesticides and chemical warfare nerve agents. Very few OP compounds such as glyphosate and merphos have been used as herbicides. OP herbicides are structurally different from the OP pesticides and their AChE-inhibiting power is very weak.

OP pesticides vary in chemical structures. Variations in the chemical structure of main groups of OP pesticides are summarized in Table 1.

As a group, OP compounds exhibit marked variability of action depending on the specific substituents occurring as R1, R2, and X. For example, extreme toxicity is associated with those compounds in which X is a strongly electronegative group such as a halide, cyanide, or thiocyanate. The structures of these four agents (tabun, sarin, soman, and VX) are presented in Figure 2.25

Nerve agents are divided into two groups of G and V agents. The G agents are fluorine compounds of organophosphate except for tabun (GA), which is a cyanide compound of organophosphate. The V agents are sulfur containing organophosphate compounds. The principal G agents; GA, GB, and GD have common names of tabun, sarin, and soman, respectively. The other G agents and V agents do not have common names. The oldest and main V agent is called VX.18

**Mechanism of action**

The mechanism of action of OP compounds that has well been known for more than 70 years, is the inhibition of cholinesterase. Two types of cholinesterase are involved:

1. Acetylcholinesterase (AChE), which is a specific enzyme for the diagnosis of OP poisoning and is called true cholinesterase. It is usually estimated in red blood cells (RBC) and is thus also called RBC ChE or erythrocyte acetylcholinesterase (EAChE).

2. Butyrylcholinesterase (BChE), which is less specific but more sensitive than AChE and is so called pseudocholinesterase. It is usually estimated in plasma and is thus also called plasma ChE.

The reaction between OP compounds and AChE occurs in three step reactions as shown in Figure 1.
Figure 3.

Step 1 is the formation of a reversible enzyme-inhibitor complex.

Step 2 is the phosphorylation and inactivation of the enzyme molecule.

Step 3 is the aging reaction involving formation of a monophosphoric acid residue bound to the enzyme.\textsuperscript{18}

The toxic manifestations and lethality after nerve agent exposure appear to follow the irreversible phosphorylation of the serine-containing active site of AChE. The time between OP exposure and the irreversible phosphorylation is called aging. The kinetics of aging and spontaneous reactivation of nerve agent-inhibited human cholinesterase is dependent on the agent. The aging varies from a few minutes (soman) to 22 hours (cyclosarin).\textsuperscript{24}

Different aging mechanisms are involved. Both tabun and butyl-tabun appear to be similarly accommodated in the active center, as suggested by molecular modeling via kinetic studies of phosphorylation and aging with a series of HuAChE mutants (E202Q, F338A, F295A, F297A, and F295L/F297V).\textsuperscript{25} A variety of proteolytic enzymes (e.g., chymotrypsin and trypsin) may also be inhibited by OP compounds. Soman and sarin are detoxified in part via a two-step pathway involving bioactivation of the parent compound by the cytochrome P450 system, then hydrolysis of the resulting oxygenating metabolite (oxon) by serum and liver paraoxygeanse (PON1). Serum PON1 has been shown to be polymorphic in human populations.\textsuperscript{26}

Even though all OP compounds have a common mechanism of action, their effectiveness as inhibitors of AChE, vary widely. OP compounds can be classified as direct or indirect inhibitors of AChE. Direct inhibitors are effective without any further metabolic modification after absorption

**Figure 3.** Different steps of AChE reaction with OP. (Separate new JPG file with high resolution is attached).
into the body. Indirect inhibitors need to be transformed in the body to be effective. All thiono OP pesticides, that are those containing a P=S bond (mainly the phosphorothioates and phosphorodithioates), are not active inhibitors of AChE, but require activation by oxidation of the P=S to the P=O group. The practical importance of this classification is that direct inhibitors cause symptoms and signs quickly during or after exposure, whereas in the case of indirect inhibitors symptoms and signs appear later and the effects last longer after cessation of exposure. The insecticide dichlorvos is an example of a direct inhibitor while malathion and parathion are indirect inhibitors.4

People with BChE genetic variations may be at risk. The clinically most important variant is atypical (D70G) BChE, because people with this variation have two hours apnea after receiving a dose of succinylcholine that is intended to paralyze muscles for three to five minutes in anesthesia.27

In addition, OP compounds covalently bind to other serine esterases, namely carboxylesterase (CaE), neuropathy target esterase (NTE), trypsin, and chymotrypsin.28, 29 Furthermore, binding to a tyrosine residue of human serum albumin has also been observed.30

The persistence of unbound OP in the body is dependent on the physico-chemical properties and the activity of endogenous OP hydrolyzing enzymes, primarily paraoxonases.31 Lipophilic OP compounds, e.g., the pesticide parathion and its active form paraoxon, may distribute into deep compartments resulting in long-term toxicologically relevant plasma concentrations.32

Toxicity
The most toxic OP compounds are nerve agents and the least toxic are carbamates.

The vapor pressure of the three G agents (GA, GB, and GD) makes them significant inhalational hazards, especially at warmer temperatures or when droplets are created by explosion or spray. Based on information achieved from animal studies, the lethal inhaled dose of G agents in humans may be about 1 mg.25 The G agents also represent a skin contact hazard, particularly when evaporation is minimized and contact is prolonged by contamination of clothing. However, the percutaneous absorption of G agents is much less rapid and complete than the inhalation form.33

VX does not pose a major inhalation hazard under usual circumstances, but it is well absorbed through the skin.33 The relative lethality as determined in animal studies is VX>soman>sarin>tabun.34 Acute toxic values of nerve agents in humans are summarized in Table 2.

The acute toxicity of nerve agents is due primarily to irreversible inactivation of AChE leading to an accumulation of toxic levels of acetylcholine.35 Like other OP compounds, these agents act by binding to a serine residue at the active site of a cholinesterase molecule, thus forming a phosphorylating protein that is inactive and incapable of breaking down acetylcholine. The resulting accumulation of toxic levels of acetylcholine at the synapse, initially stimulates and then paralyzes cholinergic synaptic transmission. Cholinergic synapses are found in the central nervous system (CNS), at the termination of somatic nerves, in the ganglionic synapses of autonomic nerves, and at the parasynaptic nerve endings such as those in the sweat glands.36

The rate of aging varies greatly among the nerve agents. The half-time of aging is within minutes after soman exposure, about five hours after sarin exposure, and >40 hr after exposure to tabun and VX.34 Inactivation of neurotoxic esterase by some OP pesticides can lead to organophosphate-induced delayed neuropathy (OPIDN). The nerve agents

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**Table 2. Summary of available acute toxic values of nerve agents in humans.**

<table>
<thead>
<tr>
<th>Term</th>
<th>Unit</th>
<th>Route</th>
<th>Tabun</th>
<th>Sarin</th>
<th>Soman</th>
<th>VX</th>
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<tr>
<td>LD₅₀</td>
<td>mg/kg</td>
<td>PC</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>LD₉₀</td>
<td>mg/kg</td>
<td>Inhalation</td>
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<td>—</td>
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<td>PC</td>
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<td>—</td>
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<td>PC</td>
<td>—</td>
<td>23</td>
<td>18</td>
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<td>—</td>
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<td>—</td>
</tr>
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</tr>
<tr>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>30</td>
</tr>
<tr>
<td>TD₉₀</td>
<td>µg/kg</td>
<td>SC</td>
<td>—</td>
<td>—</td>
<td>3.2</td>
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</tr>
</tbody>
</table>

LD₅₀=median lethal dose; LC₉₀=lethal concentration at the lowest dose; LD₉₀=lethal dose at the lowest concentration; TD₉₀=toxic dose at the lowest concentration; Based on Grob (1956)²⁵.
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inhibit neurotoxic esterase at many time the LD$_{50}$, delayed neuropathy with VX is much less than that of G agents. Possible involvement of neurotrophic factor (growth-related enzyme ornithine decarboxylase) during early stages of OPIDN particularly after diisopropyl fluorophosphates (DFP) has been reported. Nerve agents also bind quickly to cardiac muscarinic ($M_2$) receptors at higher than physiologic concentrations, but whether this contributes to cardiac toxicity is unknown. They also interact with the nicotinic acetylcholine receptor-ion channel complexes, but only at tissue concentrations of 10 to 100 times greater than that fully inhibiting AChE. Sarin, soman, and tabun are partial agonists of those channel complexes, whereas VX acts as an antagonist.

There is also evidence that nerve agents affect noncholinergic mechanisms in the CNS at a dose approaching LD$_{50}$. Antagonistic effects of δ-amino butyric acid (GABA)-ergic systems may explain convulsive activity after organophosphate poisoning. Effects of soman and tabun on the uptake and release of GABA and glutamate in the synaptosomes of cerebral cortex of guinea pigs did not support the previous beliefs that nerve agents caused convulsions by affecting the uptake or release of GABA or glutamine. However, indirect evidence was obtained that soman and tabun inhibit catabolism of GABA and glutamine. Acute exposure to tabun, sarin, and/or soman alters brain levels of cyclic AMP and cyclic GMP as a result of effects of adenylcyclase and phosphodiesterase systems. VX at 10 µM produced significant reduction in cell metabolism within two minutes as measured by changes in the acidification rate of medium after four hours of exposure. Two alkali degradation products of VX produced no cytotoxicity.

Toxicokinetics

More data on the toxicokinetics of OP pesticides than nerve agents are available.

Absorption

Absorption varies by route of exposure. OP compounds are absorbed by the skin as well as by the respiratory and gastrointestinal tracts.

Dermal exposure

Absorption by the skin tends to be slow, but because the OP pesticides are difficult to remove, dermal absorption is frequently prolonged. Uptake of active ingredients through the skin from powdered and granulated formulations may be relatively inefficient; the presence of aqueous dispersing agents or organic solvents in a spray concentrate or formulation may greatly enhance uptake. On the basis of radioautographic studies in human and animals, it appears that skin absorption of parathion is transepidermal. The rate of dermal absorption of parathion in the rabbit is 0.059 mg/min/cm$^2$.

When $^{14}$C-malathion was applied to the ventral forearm of 12 volunteers, radioactivity equivalent to a “corrected” average of 8.2% of the total dose was recovered from urine produced during the first five days. This percentage is an essentially accurate indication of the absorption during the period because almost all (90.2%) of the radioactivity was recovered in the urine after intravenous (IV) injection of malathion. Inhalation exposure

Exposure by respiratory and dermal routes were compared in workers spraying parathion, who either breathed a pure air supply but did not wear protective clothing, or who wore total protective clothing but did not have any respiratory protection. Total urinary output of 4-nitrophenyl as derived from the respiratory source, compared with that derived from the dermal source, was 1.2% in one test and 12% in another. The total exposures by the dermal and respiratory routes were in the proportion of 1000:1 and the efficiency of dermal absorption was 1 to 2%. Therefore, the dermal absorption by the respiratory route was significant.

Oral exposure

When $^{32}$P-dimethoate was given orally to volunteers, it was absorbed and excreted rapidly: 76 to 100% of the radioactive substance appeared in the urine in 24 hr.

Distribution

The intrinsically reactive chemical nature of OP pesticides means that any dose entering the body is immediately liable to a number of biotransformations and reactions with tissue constituents, so that the tracing of radiolabeled material alone does not give any clue to the unchanged parent compound. In view of the
inherent instability of the OP pesticides, storage in human tissue is not expected to be prolonged. Experimental animal studies indicate rapid excretion of these compounds. However, some OP pesticides are very lipophilic and may be taken into, and then released from fat depots over a period of many days.

The lipophilic diethyl phosphoryl pesticides are: azinphos-ethyl, bromophos-ethyl, chlorpyrifos, coumaphos, diazinon, parathion, phosalone, and sulfotep. They may remain in the body for many days or weeks in severe cases, and may promote a recurrence of clinical effects after an initial period of apparent recovery. For example, a case of fenitrothion poisoning promptly treated by conventional treatment caused a recurrence of symptoms attributed to mobilization of the organophosphate stored in adipose tissue. In contrast, dichlorvos (a dimethyl phosphate) and omethoate (a dimethyl phosphorothioate) are rapidly hydrolyzed by plasma and tissue esterases to inactive products and are unlikely to cause late clinical effects.

Metabolism

Metabolism occurs principally by oxidation and hydrolysis by esterases and by reaction with glutathione. Demethylation and glucuronidation may also occur. Oxidation of OP pesticides may result in more or less toxic products. In general, phosphorothioates are not directly toxic but require oxidative metabolism to the proximal toxin. The glutathione transferase reactions produce products that are, in most cases, of low toxicity. Hydrolytic and transferase reactions affect both the thioates and their oxons. Numerous conjugation reactions follow the primary metabolic processes, and elimination of the phosphorus-containing residue may be via the urine or feces. Parathion, for example, must be activated by an oxidative conversion via liver cytochrome P450 microsomal enzymes to paraoxon, a potent cholinesterase inhibitor. Both compounds are rapidly hydrolyzed by plasma and tissue esterases, to diethyl thiophosphoric acid, diethyl- phosphoric acid, and p-nitrophenol. These products are excreted mostly in the urine and represent most of a parathion dose. Phosphorothioates containing a P=S bond need to be converted into the analogous oxone before they acquire substantial anticholinesterase activity.

It is possible to determine the rate of disposal of metabolites and thereby to estimate an approximate half-life of an OP pesticide in the body. The half-life of most OP pesticides and their inhibitory metabolites in vivo is comparatively short. For example, the serum half-life of malathion was 2.89 hr in a 24-year-old white male who, in a suicide attempt, injected approximately 3 mL of 50% malathion IV into his right forearm.

Excretion

There is no evidence of prolonged storage of OP pesticide compounds in the body, but the process of elimination can be subdivided roughly according to the speed of the reactions involved. Most OP pesticides are degraded quickly by the metabolic reactions described. The elimination of the products, mostly in the urine and in a lesser amounts in the feces and expired air, is not delayed, so that rates of excretion usually reach a peak within two days and decline quite rapidly. Experimental animal studies have shown that most of a radiolabeled dose of OP pesticides is rapidly excreted in expired air, urine, and feces. Thus, it was reported that from 67 to 100% of the administered radioactivity was recovered within one week in the combined urine and feces of cows, rats, and a goat that were given various doses of 32P-dichlorvos.

Very little data are available on the toxicokinetics of the nerve agents. A two-compartment model, with a biological half-life of one to one and a half minutes, has been described. Toxicokinetics of the four stereo isomers of soman in atropinized rats were reported. The extremely toxic C (±)P(-) isomers could be followed in rat bloods for more than four and two hours at doses of six and three LD50 (82 µg/kg), respectively. The toxicokinetics of P(-) isomers were described with three-compartment model, with terminal half-lives of 40 – 64 and 16 – 22 min at doses of six and three LD50, respectively.

Toxicokinetic studies with nerve agents in different animal species indicate that the elimination half-life of so-called G agents (e.g., soman and sarin) will be rather short (less than one hour), whereas VX was found to persist for several hours after IV exposure and even longer after percutaneous challenge. Accidental or homicidal exposure to nerve agents will occur most likely by the inhalational or percutaneous route. Due to the high toxicity of these compounds,
plasma concentrations in a nano molar range are expected, which represents a considerable challenge for the quantitative analysis of nerve agents in biologic samples.

**Toxicodynamics**

Toxicodynamics of OP compounds were mostly explained under the mechanism of action. The common nerve agents are chiral compounds with two (e.g., sarin) or four enantiomers (soman), having different inhibitory potency with AChE and toxicities in vivo. Unfortunately, the more toxic enantiomers persist substantially longer than the less toxic ones. Chemical or enzymatic decomposition of nerve agents results in the formation of inactive phosphonic acids, which are renally excreted.

**Clinical effects**

Clinical effects after OP exposure are divided into acute poisoning and chronic poisoning.

**Acute poisoning**

Initial clinical manifestations following acute exposure vary according to the route of exposure.

**Clinical manifestations according to the route of exposure**

The acute effects of OP exposure occur after inhalation, contact with skin and eye, and by ingestion. Most often, exposure to OP nerve agents is to vapor (inhalation) or liquid (percutaneous). After small to moderate doses, initial effects and their time of onset are determined by the route of exposure. In contrast, large doses cause similar effects by all exposure routes, although the time of onset varies.

For most OP pesticides, dermal exposure and subsequent absorption through intact skin represents the most important route of entry in case of occupational exposure.

Gastrointestinal absorption occurs following accidental or intentional use of an OP pesticide and may rarely occur through contaminated food ingestion. Parenteral exposure is very rare and only a few cases were reported.

**Inhalation**

Inhalation of OP pesticides depends on volatility of the compound, on the type of formulation, and on the technique of application. However, acute effects appear immediately or shortly after exposure to OP pesticides.

Exposure to low-vapor concentrations may affect only the eyes, nose, and airways. Miosis, visual disturbances, rhinorrhea, and/or some degree of dyspnea develop within seconds to several minutes. The severity of dyspnea is dose dependent. Usually, these effects do not progress significantly once the patient is removed from contamination. After inhalation of high-vapor concentrations, victims lose consciousness within one or two minutes and then have seizures, flaccid paralysis, and apnea. Other early effects of high-vapor concentrations include miosis and copious secretions. Involuntary micturation/defecation may also occur. Unless medical assistance is immediate, the patients may die within 30 min.

**Skin**

In dermal absorption, symptoms and signs usually manifest in about two to three hours. However, it is possible to observe effects within half to one hour, depending on the circumstances of the intoxication. A few OP compounds may be retained in the fat tissue of the body, which may result in delayed symptoms for up to 24 hr.

Percutaneous absorption of OP compounds varies according to the body site exposed and the ambient temperature. VX was absorbed nearly eight times more rapidly from facial skin than it was from the volar forearm and absorption increased markedly as surrounding temperature rose from 18 to 46°C. Initial local effects of liquid, which are seldom noticed, include muscular fasciculations and sweating at the contamination site. A large droplet may also cause gastrointestinal effects and complaints of malaise and weakness. Droplets containing near-lethal or lethal doses can cause loss of consciousness, seizures, flaccid paralysis, and apnea. The onset of these effects is sudden, usually after an asymptomatic interval of 10 to 30 min.

**Eyes**

Miosis rapidly occurs after splash exposure or eye contact with vapor. It will appear later in case of systemic poisoning. Unilateral miosis can occur, if only one has been exposed. Miosis may be accompanied by deep, aching eye pain, conjunctival irritation, and visual disturbances. Dim vision may be because of constricted pupils or inhalation of cholinergic fibers of the retina or central nervous system. The miotic pupil may
improve vision (the pinhole effect), although a complaint of blurred vision is common. Direct installation of diluted nerve agent into the eye does not produce tissue damage.

**Gastrointestinal**

The oral route of entry is important in accidental and intentional OP pesticide poisoning. Occupational accidental ingestion may occur in children and as a result of poor work practices and lack of personal hygiene. Following oral ingestion, nausea and vomiting may occur. Abdominal pain and diarrhea together with a cholinergic syndrome, CNS, and cardiovascular effects can produce in moderate to severe OP poisoning. Clinical features of cholinergic syndromes, CNS, and cardiovascular effects are described in the following relevant sections.

**Parenteral**

Intradermal injection of paraoxon or surface application of maloxon or dichlorvos to human skin produced a long-lasting, local sweating response in a few minutes. Intramuscular administration of DFP to people with schizophrenia, manic-depressive psychosis, and to normal controls at a rate of 2 mg/min per day (about 0.028 mg/kg/day) for seven days caused anorexia, vomiting, and diarrhea, somewhat more severe in normal than in psychotic people.

Suicidal attempts or self-poisoning by parenteral OP pesticides are very rare, but were observed by the first author of this article.

**Cholinergic syndromes**

Parasympathetic stimulations or cholinergic syndromes are due to the acetylcholine accumulation at the nerve endings, stimulating both muscarinic and nicotinic receptors. Muscarinic effects include increased bronchial secretion, excessive sweating, salivation and lacrimation, pinpoint pupils, bronchoconstriction, abdominal cramps (vomiting and diarrhea), urinary frequency, bradycardia, hypotension, and in severe intoxicated patients pulmonary edema may occur. Nicotinic effects comprise of tachycardia, hypertension, mydriasis, twitching and fasciculation of muscles, and in more severe cases, paralysis of diaphragm and respiratory muscles.

**Central nervous system effects**

CNS effects include headache, dizziness, restlessness and anxiety, mental confusion, convulsions, and coma. As a result, depression of the respiratory and vasomotor centers in brain may occur and complicate the clinical picture.

**Cardiovascular effects**

Cardiovascular cholinesterase inhibition increases the vagal nerve influence on heart rate, and the expected result is bradycardia. In a person who is accidentally poisoned, however, other factors, such as fear, hypoxia, and ganglionic stimulation, may contribute to acceleration of heart rate. In experimental studies in animals, nerve agents and other OP compounds have been shown to slow cardiac conduction, leading to a decrease in cardiac output. In 36 of 200 mildly exposed patients only 13 (6.5%) had heart rates of less than 65, and 70 (35%) had heart rates exceeding 90.

ECG abnormalities other than bradycardia have been described in animals exposed to nerve agents and in human beings exposed to organophosphate insecticides. Reported abnormalities include atrial fibrillation, idioventricular dysrhythmias, multiform ventricular extrasystoles, torsades de pointes, ventricular fibrillation, and complete heart block. Sudden death occurring after the patient had appeared to recover from the respiratory and neurologic effects of acute organophosphate insecticide exposure has been reported. However, this has not been described after nerve agent exposure.

**Life-threatening complications**

The most life-threatening complication is respiratory failure, which is mainly due to central effect of the nerve agents. Although in one animal experiment with sarin, the authors concluded that respiratory paralysis could be purely central, purely peripheral, or both central and peripheral, depending on the doses of sarin and atropine employed. Hypoxia is also a major problem in the nerve agent poisoning, which may cause cerebral edema and convulsions and may also induces histopathologic brain damage. Cardiovascular complications are sometimes severe and life threatening. In guinea pigs, the nerve agents like tabun, sarin, soman, or VX at five to ten times the LD₅₀ induced circulatory arrest a few minutes after apnea in nontreated animals. Antidote treatment by atropine (10 mg/kg) and H16 or HLo7 (30 mg/kg) two minutes later, rapidly restored the heart
rate and arterial pressure and respiratory function to various extent. The nerve agent injection caused marked sinus bradycardia and a subsequent complete atrioventricular block within one to two minutes. In guinea pigs with depressed respiratory function (<50%), intermittent ST-T wave alterations and second-degree atrioventricular heart block were observed.\textsuperscript{33} Other reported ECG abnormalities in animal experiments and in humans being exposed to nerve agents include torsades de pointes, atrial fibrillation, idioventricular dysrhythmias, complete heart block, and ventricular fibrillation.\textsuperscript{21,36,52} Histopathologic changes compatible with toxic myocarditis were observed following sarin and soman exposure in animal experiments,\textsuperscript{51} but it has not been reported in humans.

Similar life-threatening complications may occur in severely OP pesticides-intoxicated patients.

**Intermediate syndrome**

The intermediate syndrome consists of marked weakness of the proximal skeletal musculature (including the muscles of respiration) and cranial nerve palsies, which may occur on one to four days after acute OP pesticide poisoning. This syndrome that was observed after certain organophosphate poisoning,\textsuperscript{53,54} has not yet been reported after nerve agent poisoning. Intermediate syndrome is probably a consequence of cholinergic overactivity at the neuromuscular junction and a connection has been made between the intermediate syndrome and OP-induced myopathy. Myopathy has been observed histologically in experimental animals with the nerve agents tabun, soman, and sarin.\textsuperscript{55} It thus can be anticipated that the intermediate syndrome occurs in some cases of nerve agent poisoning.

**Severity grading of intoxication**

Severity grading of OP poisoning can be made based on clinical manifestations, cholinesterase activity, and initial atropine dose required for atropinization.

**Clinical**

The patients with OP poisoning can be divided into four groups of mild, moderate, severe, and fatal as shown in Table 3.

**Inhibition of cholinesterase**

The patients with nerve agent poisoning may be divided into three groups according to their cholinesterase activities (Table 4).

**Atropine dose**

The patients with OP nerve agent poisoning can also be divided into three groups according to the initial dose required for atropinization:
- Mild <2mg
- Moderate  <2 – 10 mg
- Severe >10 mg

Based on the first author’s experience, patients with OP pesticide poisoning require much more atropine doses and thus the above atropine dosing for the severity grading of OP pesticides should be five to ten times higher.\textsuperscript{18}

**Chronic poisoning**

Chronic and/or subacute OP poisoning is usu-
ily occupational and may occur in workers who have daily exposure during production and storing. It is of course more common in agricultural workers who are involved in spraying of OP pesticides or in sheep divers. However, chronic OP poisoning is much less common than acute poisoning and often may not be diagnosed, if the physician does not pay attention to the occupation of the patient.

Most of the OP compounds are rapidly metabolized and excreted, so subacute or chronic poisoning does not occur. However, since several OP compounds cause slowly reversible inhibition of cholinesterase, accumulation of this effect can occur. Thus, signs and symptoms resembling those occurring after a single high dose may be produced by repeated small doses absorbed on prolonged exposure.

Some OP pesticides are able to induce delayed neuropathy in experimental animals and the structure-activity relationship for this effect has been in part identified.

Cases of delayed neuropathy have been reported only as a sequel to acute human poisoning with few OP compounds, most of which are no longer in use. Cases of delayed neuropathy occurring after long-term, low-level exposure are not yet presented.4

**Delayed neuropathy**

Organophosphate-induced delayed neuropathy (OPIDN) is a symmetrical sensorimotor axonopathy, tending to be most severe in long axons and occurring seven to 14 days after exposure. In severe cases, it is an extremely disabling condition.56 Inhibition of NTE appears to be necessary for OPIDN to develop. However, other mechanisms such as a trophic factor (ornithine decarboxylase, a growth-related enzyme) decrease in spinal cord following the neuropathic agent DFP may be involved.57

Although OPIDN has not observed after nerve agent poisoning in experimental studies,58 and in accidental nerve agent poisoning, a case of sensory polyneuropathy seven months after sarin poisoning has been reported.59

Temporary psychological effects such as depression, fatigue, insomnia, irritability, nervousness, and impairment of memory have been described after exposure to nerve agents.6,18

An electroencephalogram (EEG) in a person who was severely intoxicated with sarin showed marked slowing with bursts of high-voltage waves at a rate of five per second.60 Epileptic type changes in EEG were observed following sarin poisoning up to 11 months after exposure.21

**Diagnosis**

Initial diagnosis of OP poisoning can be made based on the history of exposure (intentionally or accidentally oral OP pesticide taken, occupational, terrorism, and chemical warfare attack), and clinical manifestations. In low-level exposure, the route of absorption may affect the clinical features, but in high-level exposure, severe intoxication occurs, although the occurrence time is faster through inhalation than by skin absorption.18

Onset and intensity of symptoms and signs also vary depending on the compound (direct/indirect inhibitors), and on the level of exposure. The first symptoms are usually nausea, headache, tiredness, giddiness, papillary constriction, and blurred vision, which is often described as ‘though a veil has fallen over the eyes’. Depending on the severity of poisoning these symptoms become worse with the onset of vomiting, abdominal pain, diarrhea, sweating, and salivation.6

Progressive worsening is characterized by muscular twitching, which usually commence in the tongue and the eyelids, progressing to tremor, convulsions, and finally paralysis. There is also bronchoconstriction and bronchial hypersecretion and in the final stages, paralysis, convulsions, respiratory depression, and coma are observed. In fatal OP poisoning the immediate cause of death is generally asphyxia resulting from respiratory depression.4

Estimation of AChE in erythrocytes is required to confirm the anticholinesterase exposure and to estimate the severity of intoxication. BChE estimation may also help, although it is not specific and may be low because of genetic variations.27

A reasonable correlation exists between red cell and plasma cholinesterase inhibition and the clinical signs of acute intoxication. The correlation tends to increase as the rate of inhibition is faster. When inhibition occurs slowly and repeatedly, as happens on chronic or repeated exposure, the correlation with illness may be low or totally non-

<table>
<thead>
<tr>
<th>Grade</th>
<th>Butyrylcholinesterase activity (%)</th>
<th>Acetylcholinesterase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>40 – 50</td>
<td>50 – 90</td>
</tr>
<tr>
<td>Moderate</td>
<td>10 – 40</td>
<td>10 – 50</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;10</td>
<td>&lt;10</td>
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</tbody>
</table>
Neurotoxic effects of organophosphorus compounds and their managements

exist. For instance, after continuous exposure, clinical signs of intoxication may appear only at AChE inhibition of 85 – 90% level, whereas after single exposure, they appear at 60 – 70% AChE inhibition.

Diagnosis of a certain nerve agent requires toxicological analyses of the environmental and/or blood samples for the nerve agents. A biosensor that is a potentiometer enzyme electrode for direct determination of organophosphate nerve agent has been developed.62

A fiberoptic enzyme biosensor for the direct measurement of OP nerve agents is also developed. Concentrations as low as 2 µM can be measured in less than two minutes using the kinetic response. When stored in buffer at 4°C, the biosensor shows long-term stability.63

A new method for retrospective detection of exposure to organophosphate nerve agents was applied to estimate serum sarin concentrations of the Matsumoto incident. The concentrations ranged from 0.2 – 4.1 ng/mL of serum.64 Definitive evidence for the acute sarin poisoning of the Tokyo subway was made by detecting sarin-hydrolyzed products from erythrocytes of four victims in postmortem examinations.65

Although diagnosis of an anticholinesterase may be sufficient for the management and administration of atropine, oxime therapy requires the recognition of the agent.66 Cholinesterase activity in postmortem blood as a screening test for OP nerve agent exposure was performed in 53 non-preserved postmortem whole blood specimens. There was a negligible loss of cholinesterase activity by the seventh day of the study. It could therefore be applied as the screening test for anticholinesterase nerve agents.67

Diagnosis of the delayed neurotoxic effects can be made by estimation of NTE, although it is unlikely to occur following the nerve agents poisoning. Marked reduction of the neurotrophic factor (ornithine decarboxylase) during the early stages of the neurotoxicity may also be helpful where it will be possible to perform.68

Toxicological analyses
1. Cholinesterase determination

Determination of AChE and BChE activity in whole blood and plasma, respectively, is a rapid, convenient, and cheap screening method to establish exposure to OP pesticides and nerve agents. Modifications of the original colorimetric Ellman assay,69 allow the sensitive and specific determination of AChE activity in whole blood and of BChE activity in plasma.70 It may be used under field conditions.71 However, serious limitations for the diagnosis of low-level exposure to these compounds have to be considered.

Standard determination of AChE and BChE activity may indicate inhibition of the enzyme, which may be due to OP or other cholinesterase inhibitors such as carbamates. Thus, reduced activity of AChE/BChE points to exposure by a cholinesterase inhibitor without specifying the agent. Low-level exposure to nerve agents with no clinical signs and symptoms of OP poisoning, may not reveal enzyme inhibition. This is because of marked inter-individual variations of AChE and BChE activities and to intra-individual variations of BChE activities.10 Knowing pre-exposure control enzyme activities would improve substantially the diagnosis of low-level exposure to nerve agents. However, determination of cholinesterase activities is a valuable tool for confirming the clinical diagnosis of OP poisoning. Clinical symptoms are expected at inhibition of more than 50% of brain AChE and 70% of diaphragm AChE, respectively.32 Inhibition levels greater than 90% are associated with severe toxicity.

Functional similarities of synaptic and erythrocyte AChE,72 indicate that changes in erythrocyte AChE activity reflect the situation at target tissues in the peripheral compartment (e.g., neuromuscular junction). In the central compartment, the degree of AChE inhibition may be different because of the lipophilicity of the OP and its distribution to the CNS, respectively. This assumption is supported by clinical data achieved from the patients who were poisoned by OP pesticide.32,17

Erythrocyte AChE showed to be a far more reliable parameter than BChE for the assessment of antidotal efficacy, which is due to different kinetic properties of BChE inhibited by pesticides and nerve agents.70,73 The analysis of the cholinesterase status of patients who were poisoned by OP may provide a valuable tool for making the decision on the duration of antidotal oxime therapy in case of rapidly aging (soman) or long persisting (VX) agents.

2. Detection of OP compounds and their metabolites
The identification and quantitative analysis of trace concentrations of OP compounds in plasma after human exposure require sophisticated gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) equipment and well-trained operators. The analysis of unbound nerve agent in biological samples is the most specific method for diagnosis and verification of exposure (limit of detection at \(\sim 1 \text{nmol/L}\)). However, the rather short life-time of nerve agents in the body will limit their detection to a maximum of several hours after exposure.  

OP nerve agents are hydrolyzed in vivo into the substance-specific alkylmethylphosphonic acid, which is further degraded to methyl phosphonic acid. Hydrolysis products of nerve agents may be analyzed in plasma and urine by GC-MS or directly by LC-MS. The applicability of these procedures was demonstrated with samples collected from victims poisoned by sarin and VX in Japan. 

For most OP pesticides, the blood or urine concentration of the substance itself or its metabolites represent an indicator of exposure. The measurement of nitrophenol in urine to assess the internal dose of parathion has been among the oldest tests developed. Exposure to fenitrothion may also be surveyed by measuring \(p\)-nitrocresol in urine.

The alternative is measuring alkylphosphates in urine. The rationale for the use of this method resides in the fact that metabolism of most OP yields alkylphosphates or alkyl(di)thiophosphates as terminal products. Since these metabolites can be produced by several different OP, this method is not compound specific and is only usable to assess exposure to all parent compounds, which may generate these derivatives.

The measurement of alkylphosphates in urine requires rather sophisticated analytical methods such as gas liquid chromatography. So far, the measurement of alkylphosphates has been performed in few studies, mostly for research purposes. Biological assessment of exposure through urinary metabolite determination may be a very sensitive method, which is capable of revealing exposure in a range of doses that are too low to be detected with AChE monitoring.

OP metabolites are usually excreted in the urine within a short time and the peak of emission occurs a few hours after the beginning of exposure. Therefore, in occupational exposure, samples collected soon after the end of the work are suitable for metabolite determination when 24-hr urine collection is impractical. When using spot specimens, creatinine or specific gravity can be determined in order to select and discard those samples that are too diluted or too concentrated.

**Biochemical and hematological changes**

Acid-base and electrolyte disturbances are common during the severe OP poisoning. Arterial blood gas analysis and estimation of serum electrolytes, liver and kidney function tests, amylase, CPK, and LDH may be required for the management of patients. Hypokalemia and hyperglycemia are common and should be considered and corrected. Elevation of serum amylase and lipase may reveal acute pancreatitis. Transient elevation of liver enzymes, hematuria, leukocyturia, and proteinuria may be observed during nerve agent poisoning. Blood cell count and other hematological tests may be performed as clinically indicated. However, transient leukocytosis particularly in polymorphonuclear cells may be observed.

**Other investigations**

Chest radiography, electrocardiography (ECG), EEG, electromyography, nerve conduction velocity, spirometry, computed tomography, magnetic resonance imaging, and further investigations should be performed in OP poisoning as clinically indicated.

**Course and prognosis**

The first four to six hours are the most critical in acute OP poisoning. If there is improvement in symptoms after initial treatment then the patient will be very likely to survive if adequate treatment is continued. Delayed toxicity represents an onset of effects on the central and peripheral nervous systems appearing days to weeks after exposure. This may occur independently of the effects observed in acute poisoning due to cholinesterase inhibition. Death in cases of heavy exposure is usually related to respiratory collapse, reflecting depression of the respiratory center, weakness of the muscles of respiration, bronchoconstriction, and excessive pulmonary secretions. Death may also result from cardiac arrest, because of cardiac dysrhythmias, and various degrees of heart block.

Patients with high exposure to nerve agents
may die within a few minutes in the field. Those with physical and chemical protection (pyridostigmine) who remain in a heavily contaminated area, may become intoxicated after 30 min. Patients with moderate to severe intoxication who receive first aid and emergency medical treatment may survive within a few days to a few weeks, according to the severity of intoxication and type of treatment. However, hypoxia, coma, convulsions, and respiratory failure are the signs of poor prognosis.18

The patients who remain severely hypoxic, cyanotic, and receive atropine, may develop cardiac arrhythmias and die very soon. Those who develop apnea and are not instantly receiving assisted ventilation may develop brain damage and either die or become vegetative. It is unlikely that nerve agents possess the potential to give rise to OPIDN.6,18

Soldiers who are caught unaware and who are exposed to large amounts of nerve agents before put on respirators and other protective clothing, rapidly develop severe symptoms and signs and are unlikely to survive. Those who slip rapidly into respiratory failure and who become incapable of self-administrating their own autoinjector systems/devices will also have a poor prognosis, unless emergency medical treatment is rapidly provided.18

Management

First aid advice for lay persons

The primary principle of first aid is to take care of life-threatening events. In this context the airway should be kept clear to maintain respiration, particularly when the patient is unconscious or has vomited. The mouth and pharynx should be cleared and dentures removed. The jaw should be supported and the patient placed in a face down position with the head down and turned to one side, with the tongue drawn.

First aid advice for medical and paramedical personnel

The first rule for managing chemical casualties is that the emergency responders must protect themselves from contamination resulting from contact with casualties and the environment. This can be done by approximate personal protective equipment and by thoroughly decontaminating the patient. At minimum, rescuers should wear a protective mask (or mask containing a charcoal filter and not a surgical or similar mask) and heavy rubber gloves (surgical gloves offer negligible protection) and avoid skin contact with victims until decontamination has been carried out.36,68

As soon as possible victims should be removed from the contaminated place, and decontamination must be initiated. Antidotes should be given at the onset of effects as appropriate (e.g., autoinjector containing atropine, obidoxime, and diazepam). For unconscious or severely intoxicated patients, priorities must follow the ABCs of resuscitation. Oxygen administration and assisted ventilation should be undertaken as soon as possible in those with respiratory distress. Because atropine will reverse bronchoconstriction within minutes, one might hesitate to intubate a dyspneic, conscious patient who probably will improve quickly. However, in a severely poisoned, unconscious, apneic patient, endotracheal intubation with assisted ventilation should be undertaken as quickly as possible.

Airway resistance may be very high initially, causing some mechanical ventilators to malfunction, but it will return toward normal after atropine administration. Frequent airway suctioning may be required for copious bronchial secretions. Supplemental oxygen through an endotracheal tube with positive end-expiratory pressure is indicated for severely hypoxic patients. It is important to improve tissue oxygenation before atropine administration to minimize the risk of ventricular fibrillation. Advanced life support, including IV line placement, should be provided to all victims with evidence of respiratory compromise or other signs of severe OP exposure.36

Decontamination

Decontamination must be carried out at the earliest opportunity to limit skin absorption of the agent and prevent contamination of the rescuers. Thorough decontamination is essential before casualties enter an emergency department or other site of medical care to avoid contamination of staff and other patients.

If the eyes have been exposed, they should be irrigated as soon as possible with running water or saline. Skin decontamination should be done by pouring on large amounts of a chlorine-liberated solution such as 5.0% hypochlorite solution (household bleach) followed by copious water rinsing. If bleach is not available, the skin should be blotted gently (without rubbing) with generous
amounts of alkaline soap and water followed by a water rinse. Copious amounts of water alone can be used if nothing else is available; water will dilute and physically wash away the agent, but it will not hydrolyze it. Contaminated clothing and jewelry should be removed, and the underlying skin should be thoroughly decontaminated. Care should be taken to clear under the nails, intertriginous areas, axilla, groin, and hair.36

Fetal bovine serum acetylcholinesterase (FBS-AChE) protected mice from multiple LD50 doses of OP nerve agents.37 BChE purified from human plasma (HuBChE) was also effective both in vitro and in vivo in mice and rats as a single prophylactic antidote against the lethal effects of nerve agents.48 Addition of the oxime HI6 to FBS-AChE as a pretreatment drug, amplified the efficacy of enzyme as a scavenger of nerve agents.77

Recombinant DNA-derived AChEs revealed a great improvement over wild-type AChE as bioscavengers; they can be used to develop effective methods for the safe disposal and stored OP nerve agents and potential candidates for pre-or postexposure treatment for OP toxicity.78 By the utilization of cell immobilization technology, immobilized Escherichia coli with surface-expressed OP hydrolase was made to detoxify nerve agents.79 By protein engineering techniques one BChE mutant G117H was made to hydrolyze V and G agents but reacts much too slowly.80

Organophosphate acid hydrolases (OPAH) from two species of Ateronomas were cloned and sequenced to detoxify G agents, which was effective.81 Cholinesterases that were covalently linked to a polyurethane matrix can effectively be used to remove and decontaminate nerve agents from the skin or wounds. This could protect medical personnel from secondary contamination and civilians exposed to highly toxic nerve agents.65

A reactive skin decontamination (RSD) lotion active against classical nerve agents and mustard was developed. Inactivation process was time and agent dependent and also related to ratio of OP to RSD lotion.82

Pretreatment in OP nerve agents poisoning

In animal studies, pretreatment with reversible carbamate AChE inhibitors, such as pyridostigmine and physostigmine, enhances the efficacy of postexposure treatment of soman exposure or soman poisoning with atropine and pralidoxime chloride and permits survival at higher agent challenges. This protection apparently is due to the fact that the more lethal nerve agents cannot attack AChE molecules bound by carbamates. Carbamylation of 20 to 40% of the EACHe molecules is associated with antidotal enhancement. Carbamate pretreatment will not reduce the effects of the agents, and by themselves carbamates provide no benefit. Pretreatment is not effective against sarin and VX challenge and should not be considered a panacea for all nerve agents. It is of value for soman intoxication when agent challenge is followed by atropine and an oxime. Pretreatment is ineffective unless standard treatment is administered after the exposure.18

Because physostigmine is toxic at the amounts required, pyridostigmine is the drug of choice for pretreatment. The standard dosage is 30 mg orally every eight hours for impending nerve agent system attack. Because pyridostigmine does not cross the blood-brain barrier, it causes no central nervous toxicity. Carbamates must never be used after nerve agent exposure; in that setting, carbamate administration will worsen, rather than protect from toxicity.83 Excessive doses cause many of the same toxic effects as do the nerve agents, and the recommended amounts caused annoying side effects in more than half of the population in a war zone.6,83 Administration of eptastigmine IV protected mice better than physostigmine against soman exposure.83

Pretreatment with a drug mixture (pyridostigmine, benactyzine, and trihexyphenidyl), and antidotal treatment (HI6+benactyzine) was investigated in rats. This cholinergic-anticholinergic pretreatment was effective in restoring respiratory and circulatory changes induced by soman.84

Antidotes

Available antidotes (atropine, oximes) do not necessarily prevent respiratory failure or incapacitation.85 However, early aggressive medical treatment with antidotes and intensive care management are the keys to prevention of morbidity and mortality associated with OP poisoning. Recent experience and studies by clinical toxicologists revealed that blood alkalinization by sodium bicarbonate, and also magnesium sulfate should be added to the treatment regime of OP poisoning.86
Atropine

Atropine sulfate should be titrated with the goal of the treatment being drying the secretions and resolution of bronchoconstriction and bradycardia.\textsuperscript{87} Thus there is no actual dose for atropine. The dose (2 mg) of atropine available in autoinjector is not adequate for the moderate to severe exposure to nerve agents. In fact, atropine should be given IV in doses to produce mild to moderate atropinization (dryness of tongue, oropharyngeal, and bronchial tree, tachycardia, mydriasis, and flushing) as soon as possible. At least the same amount as the initial atropinization dose should be infused in 500 mL dextrose 5% constantly to sustain the atropinization and repeat it as needed until the patient becomes asymptomatic. Continuous infusion of atropine effectively antagonizes the muscarinic effects and some of the CNS effects of OP poisoning, but has no effect on skeletal muscle weakness, seizures, unconsciousness, or respiratory failure.\textsuperscript{36}

Large doses of atropine require higher concentration of atropine preparation (e.g., 100mg/10 mL made in Germany) or at least a vast amount of atropine (10 – 100 mg) in dextrose 5% solution, ready made for IV infusion in severely OP-intoxicated patients. However, based on clinical experience of the first author, much lower atropine doses are required for nerve agents than for the severe OP-pesticides poisoning.

Atropine should not be given IV to a hypoxic patient. If the patient is hypotensive, atropine can be given through an endotracheal tube or intratracheally for more rapid absorption through the peribronchial vessels.\textsuperscript{36} Aerosolized atropine has also been used and can be administered quickly by inhalation. Studies suggest that in addition to the local effects in the lungs, it is also absorbed systemically.\textsuperscript{88,89}

Oximes

Oximes are mainly pyridinium compounds, which are divided into mono and bispyridium oximes. Names and suppliers of the common oximes are summarized in Table 5.

Although oximes have been designed to reactivate the inhibited AChE, clinical experience has indicated that they are not always effective as reactivators and none of them can be regarded as a broad-spectrum antidote.\textsuperscript{89} The choice of oximes is based on the data presently available and may also be dependent on factors other than protection against lethality, such as cost and availability of the oxime and its side effects. Obidoxime (Toxogonin) is likely to cause more toxic effects (particularly with high doses) than pralidoxime and HI6.\textsuperscript{90} HI6 is the least toxic, but is less unstable in solution and is not commercially available in many parts of the world.

Pralidoxime (PAM-2Cl), HI6, and HGG-12 were used in dogs with soman and tabun poisoning. PAM-2Cl (in conjunction with atropine and diazepam) revealed the best protection in soman-poisoned dogs, with the respective protective indices of 9, 6.3, and 3.5. None of them were effective against tabun poisoning.\textsuperscript{91} Efficacy of two other oximes, HLo-7 and pyrimidoxime in 3x LD\textsubscript{50} dose of sarin, soman, and GF and 2x LD\textsubscript{50} of tabun were tested in mice. HLo-7 produced significant ($P<0.05$) reactivation of phosphorylated AChE, resulting in 47, 38, 27, and 10% reactivation of sarin, GF, soman, and tabun in inhibited mouse diaphragm AChE, respectively.\textsuperscript{92} In this comprehensive study, the order of effectiveness against soman was HI6, HLo7, and pyrimidoxime. HLo7 was very effective against tabun poisoning, while HI6 and pyrimidoxime were of moderate value. HI6 and HLo7 were extremely effective against GF, while obidoxime was moderately effective and PAM-2Cl and pyrimidoxime were the least effective.\textsuperscript{93}

In soman-intoxicated guinea pigs, HI6 was therapeutically slightly more effective than HLo7.

Table 5. Common oximes used in the treatment of OP nerve agent poisoning.

<table>
<thead>
<tr>
<th>Type of oxime</th>
<th>Generic name</th>
<th>Trade name</th>
<th>Supplier/Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monopyridinium</td>
<td>Pralidoxime chloride(2-PAM)</td>
<td>Protopam</td>
<td>Ayerst/USA/Canada</td>
</tr>
<tr>
<td></td>
<td>Pralidoxime</td>
<td>Contrathion</td>
<td>SERB/France</td>
</tr>
<tr>
<td></td>
<td>Methylsulfate</td>
<td>(P2S)</td>
<td>UK government</td>
</tr>
<tr>
<td></td>
<td>Pralidoxime</td>
<td>UK government</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methanesulfonate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bispyridinium</td>
<td>Trimefoxime</td>
<td>(TMB-4)</td>
<td>Merck/Germany</td>
</tr>
<tr>
<td></td>
<td>Obidoxime</td>
<td>Toxogonin</td>
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<tr>
<td></td>
<td>HI-6</td>
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<td></td>
<td>HLo-7</td>
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<td></td>
<td>HGG-12</td>
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</table>
HLo7 was more effective against tabun intoxication than HI6.94 Pharmacokinetics and effects of HI6 in blood and brain of soman-intoxicated rats have been studied.95 High doses of HI6 can reach the brain in sufficient amount to reactivate inhibited brain AChE. Signs of soman poisoning correlated positively to acetylcholinesterase inhibition and negatively to the concentration of inbound HI6 in the brain and that soman intoxication significantly decreased uptake of HI6 into the brain.95 Reactivating potency of obidoxime, pralidoxime, HI6, and HLo7 in human AChE inhibited by soman, sarin, cyclosarin, and VX were studied in vitro.95 After soman, sarin, cyclosarin, and VX, the reactivating potency decreased in the order of HLo7>HI6>obidoxime>pralidoxime.96 Dose response effects of atropine and HI6 treatment in soman and tabun poisonings were studied in guinea pigs. Atropine had a large effect on the efficacy of HI6 against the nerve agents. They were also more effective against soman than against tabun. Adjunctive treatment with diazepam enhanced the efficacy of HI6 and atropine against soman.97 The effects of common oximes in different nerve agent poisoning are summarized in Table 6.

ProPAM, the tertiary amine analog of pralidoxime penetrates the CNS more readily than pralidoxime. Consequently, proPAM would be expected to have greater beneficial effect in nerve agent poisoning than pralidoxime. This expectation has not in general been realized in experimental studies.18

Dosage regimen of oximes
In spite of many oximes tested in animal experiments, the human experience either in pesticides or war/terrorism limited to pralidoxime and obidoxime. Pralidoxime should be administered IV at a dose of 30 mg/kg initially over 30 min followed by constant infusion of 8 mg/kg/hr in dextrose 5% solution. It could be continued until the full recovery or until atropine is required. Obidoxime was hepatotoxic at high recommended doses of 8 mg/kg initially, and 3 mg/kg/hr. It may be given at a dose not more than 500 mg initially and 750 mg/day. Liver function tests should be checked regularly during obidoxime therapy to avoid severe hepatotoxicity.1

Diazepam
Behavioral efficacy of diazepam against nerve agents in rhesus monkeys was studied. The results revealed that diazepam would be an excellent adjunct to the traditional nerve agent therapy to facilitate behavioral recovery from nerve agent intoxication that might be encountered by the medical military personnel on the battlefield.98 Despite the introduction of diazepam as a symptomatic anticonvulsant, a number of studies have been performed that indicate the effects of diazepam may be more specific. These studies have mainly investigated the effects on cholinergic and GABAergic systems.18,46

Gacyclidine
Gacyclidine is an antiglutamatergic compound that was studied as a complement to the available emergency treatment in organophosphate poisoning. It was used in conjunction with atropine, pralidoxime, and diazepam in nerve agent poisoning in primates.18 Gacyclidine prevents the mortality observed after early administration of the above classical emergency medication. EEG recordings and clinical observations also revealed that gacyclidine prevented soman-induced seizures and motor convulsions. It also markedly accelerated clinical recovery of soman-challenged primates. Gacyclidine prevented the neuropathology observed three weeks after soman exposure in animals.18,99 In case of severe nerve agent poisoning, gacyclidine represented a promising adjuvant therapy to the currently available polymedication to insure optimal management of OP nerve agent poisoning in man. This drug is currently being evaluated in human

<table>
<thead>
<tr>
<th>Table 6. Relative effects of oximes in OP nerve agents poisoning.</th>
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<tbody>
<tr>
<td>Oximes</td>
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<tr>
<td>--------</td>
</tr>
<tr>
<td>Pralidoxime</td>
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<tr>
<td>Pyrimidoxime</td>
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<tr>
<td>Obidoxime</td>
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<tr>
<td>HI6</td>
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<tr>
<td>HLo7</td>
</tr>
<tr>
<td>HGG12</td>
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</table>

NA=no data available; + = least effective; ++ = partially effective; +++ = moderately effective; ++++ = most effective; OP = organophosphorus.
Blood alkalinization by sodium bicarbonate

Effects of sodium bicarbonate in OP pesticide poisoning were investigated in patients with moderate to severe intoxication. It was aimed to make an alkalinization to reach and sustain the arterial blood pH between 7.45 and 7.55. Sodium bicarbonate was administered IV, firstly to correct the metabolic acidosis and then 3 – 5 mg/kg/24hr as constant infusion until recovery or until atropine was required. Arterial blood gas analysis was performed in certain intervals to adjust the dosing. The preliminary results were promising, and the final results were satisfactory.

Since alkalinization products of nerve agents particularly soman are less toxic, it seems that administration of IV infusion of sodium bicarbonate to produce moderate alkalinization, may also be effective in nerve agent poisoning and should be added to the treatment regimen. Roberts and coworkers were concerned about the safety of alkalinization regime but the other investigators provided data about the safety of alkalinization.

Drug interactions

The reported drugs that are contraindicated in severe OP nerve agent poisoning are: morphine and its derivatives, aminophylline, theophylline, and chlorpromazine. Drugs that are known to be hydrolyzed by the enzyme cholinesterase, such as suxamethonium (succinyl choline) and procaine, should also be avoided.

Hemoperfusion

Effects of hemoperfusion (HP) through coated resin adsorbent synachrome E-5 was studied in five anesthetized dogs following intoxication by two to six LD$_{50}$, VX and in another four dogs with two to three LD$_{50}$ sarin. HP therapy prevented the development of serious signs of intoxication provided that the dose of both nerve agents was only 2x LD$_{50}$. It was then concluded that HP in nerve agents of sarin and VX is only partially successful.

Clinical experience of the first author of this study with the management of OP nerve agents and pesticides in humans revealed that HP may be effective in severely intoxicated OP patients.

High-risk groups

1. Pregnant women and mother’s milk

Organophosphate nerve agents may cross the placenta and induce fetal intoxication. The fetus is more sensitive to OP compounds than the mother and also more sensitive to atropine than the mother. Based on clinical experience with pregnant women exposed to sarin during the Iran-Iraq war in Sardasht and Halabjah, and also with pregnant women poisoned with OP pesticides, mortality was higher in fetus than the mother. Some pregnant women survived from sarin poisoning but the fetus died within a few hours to a few days. Atropine and oximes should be administered with caution and at lower doses to pregnant women. Obstetric consultation is required. If fetal death occurs, the dead fetus should be removed as soon as the clinical condition of the mother is improved.

OP compounds may be excreted in the mother’s milk. It would therefore be advisable to stop breast feeding at least for a few days after exposure.

2. Children

Children are more susceptible to OP nerve agents as were seen during the Hallabjah massacre. Mortality was higher in children than in adults. They are also more sensitive to atropine and oximes. Atropine should be administered with caution, by monitoring vital signs, particularly the pulse rate. If the pulse rate exceeds more than 160 beats/min atropine infusion should be stopped and restarted when the pulse rate drops to below 140 beats/min.

Based on clinical experience with children poisoned by OP pesticide, pralidoxime should be administered at 25 mg/kg as an initial loading dose, which should be infused over 15 – 30 min followed by 10 – 20 mg/kg/hr to provide a plasma concentration of >4 mg/L.

3. Elderly

Elderly people are also more susceptible to OP nerve agent intoxication. Experience with the sarin poisoning during Iran-Iraq was in Sardasht and Halabjah revealed that morbidity and mortality were higher among elderly people. Multiple organ failure and complications were more common among the elderly than the other adult age groups. Administration of atropine, oxime, and diazepam, and any other medications should be performed with caution. Depending on the age and clinical
condition of the patient, critical care therapy should be initiated more rapidly. In fact, the elderly patients with OP poisoning should be treated as the priority group as the children.  

4. Rescue staff and hospital personnel

Rescue staff and hospital personnel who are in contact with patients of OP nerve agent poisoning may become intoxicated because of the secondary exposure. Among 59 rescuers and duty doctors, eight had mild symptoms of sarin poisoning during the Matsumoto incident. All the rescue activities had taken place without gas masks or decontamination procedures. Secondary contamination was observed in house staff who treated victims during the Tokyo subway incident. Over 20% of the house staff had symptoms including ocular pain, headache, sore throat, dyspnea, nausea, dizziness, and nasal pain, but none were seriously affected. However, the rescue staff and medical personnel either in the field, during transportation, or in the hospital should be protected by proper, gas masks, clothing, and thick gloves (not surgical gloves).

Iranian experience in management of OP poisoning

Iranian experience in management of acute OP pesticide poisoning, revealed that in addition to the standard treatment, blood alkalinization with sodium bicarbonate and also magnesium sulfate may be effective in moderate to severe intoxication. Observations during the nerve gas attack of the Iraqi army against the Iranian troops in Majnoon Island revealed that the heavily exposed people died within 30 min following coma, convulsions, hypersecretion and respiratory failure, and apnea. Although the medical facilities were not adequate in Majnoon Island, first aid treatment and decontamination were performed. The patients with moderate to severe intoxication were transferred from the filed hospital to medical centers in big cities for further management. Recorded clinical manifestations included: miosis, hypersecretions, hypotension, nausea, vomiting, abdominal cramps, diarrhea, loss of consciousness, respiratory depression, cyanosis, pulmonary edema, muscle twitching, and convulsions. Bradycardia and hypotension were more observed before treatment with atropine whereas tachycardia and normal hypertension together with mydriasis and dryness of the tongue were more recorded following atropinisation. Morbidity and mortality were higher in patients with severe respiratory distress and cyanosis who received large doses of atropine. It was very vital to correct the severe hypoxemia and cyanosis before atropinization (see management). Thus, suctions of naso/oropharyngeal and bronchial secretion (clear airway) and performing adequate ventilation is the first priority. Intermediate syndrome that was described following OP pesticide poisoning, has not been observed with the nerve agent poisoning.

It should be noted that sulfur mustard was the most common chemical warfare agent that was used by the Iraqi army. Thus, mixed poisoning by tabun and sulfur mustard was also common. No exact statistical records of the nerve agents were available. It has been estimated that >2000 patients with nerve agent poisoning (later on diagnosed as tabun) were treated in March 1984. Another massive nerve agent poisoning occurred during the Halabjah massacre. It was also diagnosed as sarin mixed with sulfur mustard.

Based on the first author’s experience, the management of OP nerve agents poisoning is summarized in a flow chart (Figure 3).

Preventive measures

It is essential that persons intending to use OP pesticides are provided with adequate health precautions and other safety instructions before usage. This information should be provided by the manufacturer in the form of either an information leaflet or a label attached to the pesticide container. Protective clothing is important. OP pesticides can be absorbed through the skin, resulting in poisoning. The risk is greater in hot weather when the user is sweating. Protective measures may include a long-sleeved shirt, long trousers or overalls, and a hat of some sort. The more toxic OP pesticides will require gloves, waterproof outerwear (preferably made of heavy PVC), and rubber boots. The label should list these details.

Clothing worn during spraying should be washed daily after use. Contaminated clothing should be washed separately from the general wash to avoid cross-contamination. When working with liquid concentrates, there is often a danger that they will splash the eyes. This not only can damage the eyes, but also can allow a significant amount of the chemical to be absorbed into the bloodstream.
Management of nerve agent (NA) poisoning:

Heavy exposure _____ N.A. exposure _____ Low exposure ___ Asymptomatic

Rapid Death  Moderate exposure  Symptomatic  Discharge

(Application of auto injector containing atropine, oximes & diazepam)

Eye damage ______ Eye  Skin  Inhalation  Ingestion

DECONTAMINATION

Removal  G.A.L. in 1 hr

from contamination

Opthalmologic Consultation

TRIAGE

Symptoms only  Signs of general intoxication  Asymptomatic

Psychiatric evaluation & Discharge

Cholinesterase  ABC’s  Atropinisation

Estimation, Clinical evaluation, oximes, diazepam

Paraclinical Investigations  Alkalisation

Complications (Organ failure)

Severe Respiratory Distress

I.C.U.

Monitoring, antidotes, supportive therapy

Paraclinical investigations

Relevant Consultation

Sequelae

Recovery  Psychiatric assessment ___ Discharge

Figure 3. Flow chart on the management of OP poisoning
Simple goggles or a face shield will protect against this danger. Eye protection is the most important if contact lenses are worn, because the chemical may seep in behind the lenses. The lenses must be removed before the eyes are washed, and in the time it takes to remove them, serious damage can occur. With some pesticides, a respirator may be required. The label should specify when this is necessary. The correct canister or cartridge must be used.

Greater precautions are necessary when mixing the concentrated material than when spraying. Measurements should be accurate and spillages should be cleaned up promptly. Mix the chemicals carefully, using a stick or paddle. Ensure that there is minimal skin exposure by wearing gloves. If any concentrate is spilled on the skin, wash it off as soon as possible. The hazards of spraying increase dramatically on windy days because there is an increased risk of inhaling spray drift or contaminating the skin. Also, the risk of drift on to other properties or crops is increased. Always wash hands before eating, drinking, or smoking. After spraying, take a shower, and change clothing.

By preference, all chemicals should be stored in a locked shed, out of the reach of children and animals. Chemicals should also be kept away from work areas and separate from other stored materials such as animal foods. Always leave chemicals in their original containers. If they must be transferred to another container, ensure that it is one not normally used for food or drink. This secondary container should be labeled properly and be of a variety that is not likely to leak.

Following spillage, empty any of the product remaining in damaged/leaking container(s) into a clean empty container(s), which should then be tightly closed and suitably labeled.

If it is a liquid product, prevent it from spreading or contaminating other products, vegetation, or waterways by building a barrier of the most suitable available material, e.g., earth or sand. Sweep up the spillage with sawdust, sand, or earth (moisten the powders), and place it in a suitable container for disposal. Decontamination and clean-up procedures utilize the instability of OP pesticide products with alkali. The following procedure has been developed for decontaminating spills of OP pesticides:

Apply a 10% sodium carbonate (washing soda solution) to the contaminated area, brush well in, and leave for at least eight hours. Absorb into sawdust, sand, or earth and then rinse.

Contaminated sawdust, sand, earth, and containers should be burnt in a proper incinerator. When no incinerator is available, bury in an approved dump or in an area where there is no risk of contamination of surface water. Before burying, mix liberally in sodium carbonate (washing soda) crystals to hydrolyze the product.

For workers who are exposed on a regular basis to OP pesticides, it is advisable for them to have a pre-employment examination to determine their baseline cholinesterase levels. These tests should be undertaken on a regular basis to determine whether exposure is occurring with subclinical findings. When the RBC or plasma cholinesterase falls below 25% of baseline levels, workers should be taken off the job and should not return to work until their cholinesterase levels return to normal.  

Conclusions and recommendations

OP compounds either as pesticides or nerve agents, have induced a lot of human morphologies and mortalities. OP pesticides are still used in most parts of the world and unfortunately are easily available in some developing countries and thus occupational and accidental exposure and even intentional ingestion are common and induced health problems.

WHO has recommended that access to highly toxic pesticides be restricted and wherever this has been done, suicide rates have fallen. Proper legislations and pesticides control, particularly OP compounds, which are the most commonly used pesticides are recommended. Biological pest control that has recently been applied in some countries should be extended and advanced to replace OP pesticides.

In spite of the establishment of OPCW and its active role in CWA control, chemical nerve agents attack either as a war or terrorism remains a big threat to the population in any part of the world. Therefore, medical and health professionals should be aware and update their knowledge on the toxicology and proper management of OP poisoning.

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Narenjestan-e Ghavam, a beautiful house and garden (now a museum)—19th century, Qajar period, Shiraz, Iran.