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IN-DEPTH REVIEW

Organophosphate toxicity and occupational exposure

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The ubiquitous organophosphates present a continuing health hazard in agriculture, public health eradication programmes and as chemical warfare agents. Despite significant progress in understanding the potential mechanisms of toxicity far beyond the commonly accepted mechanism of cholinesterase inhibition in intentional exposures, the precise health effects following occupational exposures are yet to be completely defined. A much greater understanding exists of the clinical features of organophosphate poisoning. These are characterized by a triphasic response involving an initial acute cholinergic phase, an intermediate syndrome (both associated with high mortality) and a disabling but non-lethal delayed polyneuropathy. The delayed polyneuropathy may occur in the absence of the cholinergic or intermediate phases. However, progress is still required in order to improve the quantification and assessment of occupational exposures and the implementation of appropriate preventive measures. Finally, evidence-based guidelines for appropriate or optimal therapeutic interventions following poisoning are required urgently and collaborative work with colleagues in developing countries, where the occurrence of organophosphate exposures is more frequent, may provide the answers.

Key words Assessments; mechanisms; occupational; organophosphates; toxicology.

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Introduction

This review on the toxicology of organophosphates is intended to be complementary to the review by Coggon [1], which focused on regulatory risk assessment, monitoring exposed populations and other epidemiological aspects of occupational exposure on farms. In this article, the emphasis will be on the mechanisms of toxicity and the clinical features and investigations currently used in individual subjects after organophosphate intoxication, the last being a critical overview.

Organophosphorus compounds are usually esters, amides or thiol derivatives of phosphonic acid. They form a large family of ~50 000 chemical agents with biological properties that have important and sometimes unique implications for man.

Most of the ill-health following exposure to organophosphorus compounds has been attributed to the inhibition of cholinesterases. However, the current literature [2] has justifiably challenged this view, as the inhibition of cholinesterases by itself cannot account for the wide range of disorders that have been reported following organophosphorus poisoning. It is becoming apparent that, although inhibition of cholinesterases plays a key role in the toxicology of organophosphates, individual susceptibility, the inhibition of other enzyme systems and the direct effects of organophosphates on tissues are also important.

However, as inhibition of cholinesterases does occur, assays of plasma butyryl cholinesterase and red blood cell acetylcholinesterase (AChE) are widely used for confirming and assessing exposure.

Exposure to organophosphorus agents causes a sequential triphasic illness in man. In most instances the

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earliest cholinergic phase may only be observed. This cholinergic phase progresses to the intermediate syndrome in ~20% of subjects. Both the acute cholinergic phase and the intermediate syndrome are associated with a high risk of mortality and subjects are best managed in an intensive care unit unless the poisoning has been very mild. The final phase, organophosphate-induced delayed polyneuropathy, which does not carry the risk of death, sets in 7–21 days after exposure to an organophosphorus agent and may not be preceded by either the cholinergic phase or the intermediate syndrome.

Mechanism of action [3]

Most organophosphates are highly lipid-soluble agents and are well absorbed from the skin, oral mucous membranes, conjunctiva and gastrointestinal and respiratory routes. The onset, severity and duration of poisoning is determined by the dose, route of exposure, physicochemical properties of the organophosphate (e.g. lipid solubility), rate of metabolism (whether transformation in the liver is required before the compound becomes toxic) and whether the organophosphorylated cholinesterase ages rapidly [4].

The inactivation of the cholinesterases occurs in the blood and in a wide range of nerve, neuromuscular (skeletal, smooth and cardiac) and glandular tissues where these enzymes have a role in cell-to-cell communication and the hydrolysis of xenobiotics. These enzymes have possibly as yet unidentified roles such as cell development and growth.

The inhibition of AChE leads to the accumulation of acetylcholine, the neurotransmitter at all ganglia in the autonomic nervous system and at many synapses in the brain, skeletal neuromuscular junctions, at some postganglionic nerve endings of the sympathetic nervous system and adrenal medulla. The role of butyryl cholinesterase in the body is yet to be fully identified, but it is known to be involved in the hydrolysis of many therapeutic agents (e.g. suxamethonium, esmolol, procaine and cocaine). There are many other roles speculated for butyryl cholinesterase and these include cellular differentiation and growth, as a scavenger in xenobiotic exposure and as a modulator in lipid metabolism.

The key reactions taking place between organophosphates and AChE are indicated in Figure 1.

The consequences of inhibition of other enzyme systems by organophosphorus compounds are as yet uncertain. A variety of tissue carboxylesterases exist in the serum, liver, intestine and other tissues. Although inhibition of one specific carboxylesterase (neuropathy target esterase) has toxic sequelae [5], no direct deleterious effects of inhibition of other carboxylesterases have been demonstrated. However, carboxylesterases may contribute markedly to the metabolic degradation of organophosphorus insecticides and inhibition of these enzymes may potentiate the toxicity of organophosphorus compounds such as the nerve agents. The search for effects of inactivation or changes in other physiological systems continues. The following effects of organophosphorus agents have been demonstrated in animals and are theoretically possible effects in man [6].

1. Inactivation by phosphorylation of other beta esterases.
2. Altering the release of neurotransmitters, e.g. γ-aminobutyric acid (GABA) and glutamate.
3. Increasing the number of GABA and dopamine receptors.
4. Acting as agonists at M2/M4 muscarinic receptors.
5. Inhibition of mitochondrial enzymes, respiration and ATP generation.
6. Induction of mast cell degranulation, probably causing the release of histamine or histamine-like compounds.

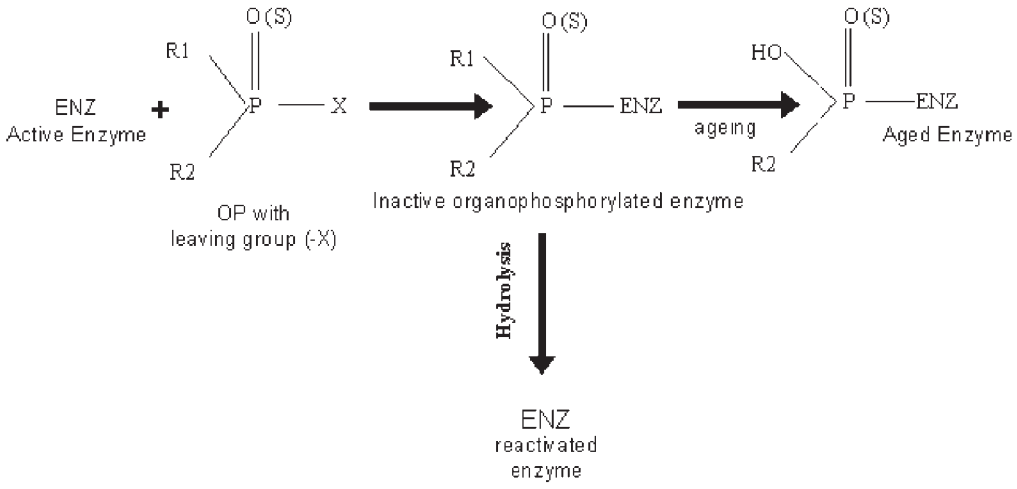

Figure 1. Key reactions occurring between organophosphates and AChE.

7. Inhibition of nitric oxide.
8. Interference with surfactant in the lung.
9. Inhibition of phospholipase A_2.
10. Interference with humoral and cellular immunity, e.g. the function of T lymphocytes.

Clinical features

Acute cholinergic phase [7]

The inactivation of AChE by alkyl phosphorylation of a serine hydroxyl group at the esteratic site of this enzyme leads to accumulation of acetylcholine at the following locations.

1. Muscarinic sites, which causes an increase in secretions (bronchorrhoea, salivation, tearing and sweating), bronchoconstriction (tightness in the chest and wheezing), bradycardia, vomiting and an increase in gastrointestinal motility (abdominal tightness, cramps and diarrhoea). Organophosphates cause the diagnostic miosis in the eye, which results in blurring of vision.
2. Nicotinic sites (e.g. neuromuscular junctions), which causes muscle fasciculations and a flaccid paralysis in severe exposures.
3. Within the central nervous system, which causes headache, insomnia, giddiness, confusion, drowsiness and, in severe exposures, slurred speech, convulsions, coma and respiratory depression.

The intermediate syndrome [8]

This occurs 1–4 days after the acute cholinergic phase and is characterized by the onset of muscle weakness (proximal muscles of the limbs) and cranial nerve palsies. Difficulty in breathing may progress to respiratory failure following paralysis of the diaphragm and other muscles of respiration. Complete recovery occurs within 4–21 days after appropriate ventilatory care. Although the exact pathogenesis of the intermediate syndrome is unknown at present, there is probably altered function and activity of the nicotinic receptors at the neuromuscular junction. Some workers have attributed the intermediate syndrome to either inadequate or delayed oxime therapy [9].

Organophosphate-induced delayed polyneuropathy [10]

This syndrome appears 7–21 days after exposure to an organophosphorus agent and predominantly affects the long nerves or tracts in the nervous system causing symmetrical weakness of peripheral muscles in the hands and feet, with a variable degree of sensory impairment. Disability may be permanent, though recovery has taken place in a few instances. At present, the phosphorylation of an enzyme in nerve tissue, neuropathy target esterase, is considered responsible for the dysfunction.

Other sequelae of exposure

A greater frequency of upper respiratory tract infections was demonstrated in workers occupationally exposed to organophosphates in whom a decrease in serum and red blood cell cholinesterase activity was observed [11]. Twenty-three patients with occupational organophosphate exposure developed 'influenza-like' symptoms [12].

Hyperamylasaemia and acute pancreatitis have been reported after oral or dermal organophosphate exposure. The hyperamylasaemia was closely related to the clinical severity and the presence of shock.

Cardiac complications are often associated with organophosphate poisoning, with the symptoms ranging from hypotension or hypertension to arrhythmias and cardiac arrest. A case of congestive cardiomyopathy has been reported following long-term organophosphate exposure [13].

Profuse and offensive diarrhoea often develops in patients following organophosphate ingestion [14].

Animal data suggested that organophosphate poisoning may cause severe teratogenicity and, consequently, a termination of pregnancy following exposure in the first trimester was carried out [15]. However, normal childbirth has been reported following severe poisoning in the second and third trimesters [16].

Some humans exposed to organophosphates experience a fever that may last several days. One patient manifested a biphasic thermoregulatory response with a rectal temperature of 33°C almost immediately post-ingestion that responded to rewarming. However, a hyperthermic response developed after 18 days that lasted for 48 h [17].

Diagnosis

Clinical

Diagnosis of the cholinergic syndrome in most instances is based on clinical features. Miosis in combination with fasciculations is pathognomonic of this syndrome, particularly in adults. Lachrymation, salivation, bronchorrhoea and excessive sweating along with bradycardia provide supportive evidence. Sulphurated organophosphorus agents possess a pungent garlic-like odour that is easily recognized by clinicians. This odour when present in the breath, vomitus or clothing is often the main diagnostic tool in developing countries where in the majority of instances the agent implicated in poisoning is not known with certainty [18].

Biochemical

Measurement of cholinesterases (butyryl cholinesterase and red blood cell AChE) continues to be used widely for diagnosing exposure to organophosphates. However, the interpretations of these estimations are not without problems. There are many causes of decreased activity of cholinesterases that are not related to exposure to organophosphates and carbamates (anticholinesterases). These are genetic, physiological (age, gender, pregnancy, etc.), iatrogenic (therapeutic agents), disease states, exposure to smoke fumes and in some instances of uncertain origin [19]. There are suggestions that dietary factors can influence cholinesterase levels. Low levels of cholinesterases have been observed in malnutrition. Further, with the increasing popularity of traditional medicines containing plants or plant extracts capable of lowering cholinesterase activity (e.g. solanine and chaconine in potatoes that are often used in African societies for human immunodeficiency virus infection or Huperazine A that is used in Chinese folk medicine), other causes of lowered cholinesterase levels need to be considered [20]. In addition, there is considerable inter-individual variation. The coefficients obtained from butyryl cholinesterase measurements range between 15 and 25%, whilst for red blood cell AChE the range is rather less, at 10–15%. The above findings are compounded by the observation that successive monthly measurements or successive daily measurements in healthy individuals revealed variations exceeding 20% and on occasions reaching 40% [21]. There are also other causes of raised levels of AChE, e.g. in the presence of neural defects in early pregnancy. Changes in cholinesterase levels in patients with multiple myeloma may provide reliable indicators of disease state changes such as remission fulfilment. The mean butyryl cholinesterase levels of chronic spinal pain patients were significantly higher than the mean levels in normal control volunteers. Following myocardial infarction, AChE estimations have enabled classification of patients into four groups with defined prognostic value and estimations prior to the discharge of such patients have been recommended. Some correlations have also been reported during the course of tetanus infections and following liver transplantation [19].

As assays for blood cholinesterase activity have become relatively simple procedures, they continue to be used for assessing the extent of human exposure to organophosphates (Table 1). The role of such assays in diagnosis is more accepted than their role in estimating severity, prognosis or recovery. In industrial or occupational exposures, cholinesterase assays have a clear advantage over estimations of atmospheric contamination of pesticides in that estimations of contamination and of rates of inhalation are difficult under agricultural or farming conditions. Further, the equipment for atmospheric contamination measurements is much less freely available. Moreover, atmospheric analysis does not take into consideration absorption by other routes such as the skin, which is a real hazard amongst those who mix and apply pesticides. Cholinesterase assays have to some extent ensured that prescribed safety precautions during manufacture and application have been implemented.

Another important consideration is the variable response of butyryl cholinesterase and AChE to different organophosphates (Table 2).

Finally, whatever the limitations, cholinesterase estimations remain the only useful biochemical tool in organophosphate exposure at present. However, after nearly four decades, there are doubts that it can be described as a 'trusted tool'. The most important consideration for the reluctance to accept cholinesterase estimations as the sole measurement of value in organophosphate exposure is the obvious inability to attribute all aspects of ill-health following such exposures solely to the inhibition of cholinesterases. Certainly, the development of organophosphate-induced delayed polyneuropathy is not associated with cholinesterase depression.

Therefore, critical assessment is needed soon and, if necessary, the search for a more sensitive and reliable biochemical marker for organophosphate exposure needs to be accelerated. Critical evaluation of alternatives such as carboxylesterase estimations and plasma beta-G levels is also required with some sense of urgency.

Table 1. Relationship between the levels of red blood cell AChE inhibitions and biological/clinical effects and advised intervention measures [22]

Significance	AChE inhibition	Measures required
Values indicative of or compatible with minor and reversible effects	<30[a] <50[b]	Medical surveillance needed Working conditions to be examined to avoid exceeding such a level
Values indicative of or compatible with minor damage (initial symptoms and mild alterations of sensitive clinical indexes)	30–60[a] 50–70[b]	Temporary removal from exposure and analysis of working conditions needed

[a]Based on individual pre-exposure baselines.

[b]Based on normal reference values.

Table 2. Differential sensitivity of plasma cholinesterase and red blood cell AChE inhibition by organophosphates [23]

Compound red blood cell AChE most inhibited	Compound plasma cholinesterase most inhibited
Dimefox	Chlorfenvinphos
Mevinphos	Chlorpyrifos
Parathion	Demeton
Parathion-methyl	Diazinon
	Dichlorvos
	Ecothiophate iodide
	Fenitrothion
	Malathion
	Monocrotophos
	Trichlorfon

Measurement of organophosphates in plasma

Analytical methods exist that allow the determination of plasma levels for many organophosphates. These determinations are difficult or expensive for the biological monitoring of occupational exposures and are less frequently used than the determination of urinary metabolites.

Measurement of urinary metabolites

An alternative or complementary approach to biological monitoring for organophosphates (by measurement of reduction in blood cholinesterases) is based on the analysis of metabolites in urine. These methods can either use metabolites specific to the organophosphate under study or the dialkyl phosphate metabolites that are common to a large number of different organophosphates [24]. In an informative review of such analyses over 10 years, Cocker *et al.* [24] found that, in non-occupationally exposed people, 95% of urinary alkyl phosphates do not exceed 72 μmol/mol creatinine. In occupationally exposed people, the corresponding 95th percentile of total urinary alkyl phosphates is 122 μmol/mol creatinine. In volunteer studies with 1 mg oral doses of chlorpyrifos, diazinon and propetamphos, the mean peak values were 160, 750 and 404 μmol/mol creatinine, respectively, and were not associated with any reduction in blood cholinesterase activity. They concluded that the levels of organophosphate metabolites in the urine from workers potentially exposed to organophosphates are generally low and unlikely to cause a significant reduction in blood cholinesterase activity. This is the probable explanation for the lack of correlation in many instances when both urinary metabolites and red blood cell AChE activity have been measured, and, in most cases, no inhibition of red blood cell AChE was found. Thus, no correlation could be made between urinary metabolites and organophosphate levels associated with cholinesterase inhibition [25].

The only established correlation between urinary metabolite excretion and effects on red blood cell AChE is that for urinary *p*-nitrophenol in parathion-exposed workers [26]. In the USA, the American Conference of Government Industrial Hygienist set a Biological Exposure Index for workers exposed to parathion of 0.5 mg of *p*-nitrophenol per gram of creatinine in urine at the end of a shift [27].

Depending on the compound, metabolism and absorption route, the peak excretion might be reached at different times after exposure. Absorption after dermal exposure is generally slower than after ingestion or inhalation.

Environmental monitoring [23]

Patch monitoring, air sampling and hand washing are examples of environmental monitoring methods routinely used in the assessment of pesticide exposure. A number of exposure models have been proposed for risk assessment requiring default assumptions and extrapolations that are not applicable for the assessment of a single applicator's exposure.

Monitoring of dermal exposure

Several different methods are used for assessing dermal exposure, most of which are used for research or investigative purposes rather than for routine monitoring.

Surrogate skin techniques

The most common approach is the patch technique [28], where 10 patches are applied on the skin and/or clothing on defined areas of the body regions, namely the chest (one patch), back (one patch) and upper and lower limbs (four patches each). The amount of skin exposure (μ/m^2) is then extrapolated from the amount of chemical found in the patches and corrected for the skin surface actually exposed (i.e. not covered by protective equipment). The deficiency is that distribution of the compound may not be uniform across each body region where the patches are attached [29,30]. Further, the assumption that the patch captures and retains the compound in a manner similar to the skin has not been validated for many compounds. Nevertheless, patch techniques might be useful for comparing exposures owing to different application techniques or protective equipment [31] or for quantifying protective clothing penetration by placing patches outside and inside the clothing [32,33].

Chemical removal techniques

Chemical deposits in selected parts of the body can be removed by washing or wiping and concentrations then measured. Washing with water/alcohol or water/surfactants are generally used for assessing hand exposure

(for instance after immersion exposure). The amount of compound determined in the hand wash has been found to correlate with urinary excretion of metabolites in several instances [25].

Fluorescent tracer techniques

Fluorescent tracers are introduced into the formulation and exposed workers are subsequently observed in a dark area using long-wave ultraviolet illumination. This technique allows qualitative determination of skin deposition patterns, the efficiency of protective clothing and the acceptability of work practices [34]. This technique has several limitations including the requirement of introducing the tracer into the formulation and the demonstration (still lacking) of correspondence between skin deposition or protective clothing penetration of the active ingredient and the tracer.

Monitoring of respiratory exposure

Although it is generally recognized that skin uptake is usually the most important route of entry for most occupational situations, fieldworkers may also be exposed to organophosphates as solid particulates or water-based aerosols. The air concentrations of the compound may be measured in samples collected either by a portable air sampling pump worn by the worker or by a static sampling pump. In the former case, the sampled air is the same as that inhaled. The particles' size should also be taken into account, since they may be too large and may deposit in different parts of the airways [35]. A trapping system is chosen on the basis of the chemico-physical characteristics of the compound to be measured. The United States National Institute of Occupational Safety and Health, Occupational Safety and Health Administration and Environmental Protection Agency periodically publish the official/suggested methods for a number of pesticides. In the UK, for risk assessment purposes the respiratory dose is calculated from the air concentration and the estimated values for lung ventilation in adults at different degrees of physical activity. This does not take into account the bioavailability after inhalation, which can be quite different among compounds and formulations [23].

Summary

The ubiquitous organophosphates present a continuing health hazard in agriculture, public health eradication programmes and as chemical warfare agents. The major concern is that there is insufficient information for implementing appropriate preventive measures in occupational exposures. Further evidence-based guidelines for appropriate or optimal therapeutic interventions following poisoning are currently unavailable. The assessment and quantification of exposure remains an area of contentious debate. Research that combines the technology and expertise of the developed countries with the abundant clinical material in developing countries may provide information for eliminating the vast gaps in our knowledge.

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