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THE LONG TERM NEUROTOXICITY OF ANTICHOLINESTERASES

Report of a DSAC Working Party

CONTENTS

WORKING PARTY

Terms of Reference :

1. To review the results of research in animals and man on the long term toxic effects of anticholinesterase compounds, with particular emphasis on organophosphate nerve agents and carbamate prophylactics.

2. To advise on the health risks to service personnel, including exposed volunteers, and the need for continued health surveillance.

3. To make recommendations on policy and further research requirements.

EXECUTIVE SUMMARY

Background

Anticholinesterases inhibit the cholinesterase enzymes which break down acetylcholine (ACh), the chemical transmitter at a number of neural and neuromuscular synapses. Inhibition of the enzyme leads to accumulation of ACh at both muscarinic and nicotinic receptors in the central and peripheral nervous systems, prolonging the acute effects of acetylcholine.

Carbamates and organophosphorus (OP) pesticides and nerve agents inhibit cholinesterases in general and acetylcholinesterases in particular. Inhibition by carbamates is, in most cases, spontaneously reversible whereas that by OPs is relatively irreversible and functional recovery following exposure is the result of the synthesis of new enzyme. Monitoring of blood acetylcholinesterase (AChE) or cholinesterase (ChE) has been used as a marker of exposure in man and up to 60-70% inhibition of red blood cell AChE can occur before clinical symptoms are detectable.

Historically carbamates were the first recognised inhibitors of cholinesterases to be used for pharmacological purposes. They are reversible inhibitors of AChE with half lives of inhibition ranging from 15 min to several hours depending on the structure of the molecule. Physostigmine (eserine) the toxic alkaloid of the Calabar bean (*Physostigma venenonsum)* was for many years the drug of choice for the treatment of atony of the g.i. tract and for the induction of miosis. Pyridostigmine, a synthetic carbamate, has been used for many years in the treatment of myasthenia gravis and more recently, as a pretreatment drug for protection against the lethal effects of OP inhibitors of this enzyme. Research since WWII has led to the development of carbamates for use as drugs and pesticides.

OP inhibitors of cholinesterases were developed in Germany by Schrader before and during WWII as pesticides and chemical warfare agents. After the war there was considerable

exploitation of these materials for agricultural and horticultural pest control. At the same time both western and FSU nations conducted much research on those OPs with extremely high toxicities as potential chemical warfare agents.

OP pesticides are in general characterised by their low acute mammalian toxicity and high acute insect toxicity. This selective toxicity has been designed into the molecule and exploits differences between the metabolism of the OP between mammals and insect pests. Structurally most commercial pesticides are phosphates or phosphorothioates with O,O-dimethyl or O,O-diethyl substituents on the phosphorus atom. To exploit further metabolic differences between species some OPs are delivered as "pro-pesticides" which are, in the main, thio-phosphate derivatives which are metabolically activated to the proximate phosphate inhibitors by the target animal species. Oxime reactivators of AChE in general, readily reactivate OP pesticides. Most commercial pesticides have acute toxicities (LD_{50}) within the range 1 mg kg^{-1} to 1-2g kg^{-1} and have dose effects curves which are notably shallower than those of the chemical warfare OPs.

Chemical warfare (CW) OPs (frequently termed nerve agents) have a different chemical structure. In general these are phosphonates in which there is a direct chemical bond between the alkyl substituent and the phosphorus atom. Phosphoramidates (e.g. tabun) have a P-N linkage. The P-C bond confers a high degree of stability to the OP-inhibited cholinesterase in the body and is in large part responsible for the high toxicity of the nerve agents in vivo. Nerve agents have acute toxicities (LD_{50}) within the range 5-500 μg kg^{-1} and are characterised by having extremely steep dose-effects curves with slopes above 10.

Biological effects of anticholinesterases

Some OPs have the potential to inhibit other acyl-transferase enzymes that contain a serine hydroxyl at the active site. Whether a biological response is elicited as a consequence of such inhibition is a complex function of the chemical structure and reactivity of the OP. OPs have been selected for their ability to inhibit ChE in general and AChE in particular and the ratio of the concentration of OP required to inhibit other enzymes and that of AChE is usually of several orders of magnitude. Other factors such as its rate of absorption and metabolism within the target organism will also have a major influence on the ultimate expression of any effects.

During the development of the OP pesticides it was noted that several members of this group of compounds induced a delayed neuropathy of peripheral motor nerves. Almost all of these compounds were identified within the commercial pesticides either during development or, regrettably in some cases, after use. Not all organophosphorus compounds have the same propensity, but even some of those which are considered safe for commercial and domestic use (e.g. chlorpyrifos) have been found to produce a neuropathy (Kaplan et al 1993). Clinical signs develop 2-3 weeks after exposure and consist of a distal mixed neuropathy (i.e.predominantly motor, though with variable sensory involvement), principally affecting the lower limbs, and ataxia. Severely affected cases may develop a flaccid paralysis. The pathogenesis of this neuropathy is associated with inhibition of neuropathy target esterase (NTE). However, this causal link has not been directly confirmed and the putative mechanism so far has been supported by association of events only. Neuropathy target esterase is also present in many peripheral tissues including blood lymphocytes and platelets. Inhibition of this enzyme in lymphocytes has been used as a marker of exposure to neuropathic organophosphates (e.g. Mutch et al 1992) and recovery of the enzyme inhibition paralleled clinical recovery from an overdose of chlorpyrifos (Lotti et al 1986). The neuropathology includes Wallerian degeneration with an initial focal lesion in large myelinated fibres leading to axonal death distal to the lesion. The precise

pathogenic mechanism has not been determined but appears to be non-cholinergic and involve phosphorylation of neuronal sites.

The classical nerve agents, GA (tabun), GB (sarin), GD (soman), and VX, together with several close analogues, have been assessed for their ability to induce delayed peripheral neuropathy and to inhibit NTE (Gordon, J. J. et al; 1983). Delayed neuropathy associated with high inhibition of NTE was found at 30-60 LD_{50} for GB but not at 38 and 82 LD_{50} for GD and GA. To achieve any indication of activity with GB heroic measures had to be taken to ensure that treated animals survived the administration of such high multiples of the lethal doses of these agents. It was estimated that the minimum neuropathic doses of GD and GA were between 100-150 LD_{50}s of agent.

An entirely different, short term, condition termed the *intermediate syndrome* has been described (Senanayake & Karalliedde, 1987). This syndrome is only apparent after incidents of severe acute poisoning leading to acute cholinergic crises. It is characterised by an onset 24-96 hours after any acute AChE inhibition and lasts about 18 days when there is resolution with no apparent residual neurological deficits. The clinical findings are predominantly of a proximal neuropathy with involvement of cranial nerves and brain stem. There may be acute ventilatory failure due to paralysis of respiratory muscles. The pathological mechanism is not understood, but may involve a combination of neuropathic and myopathic, as well as neuromuscular junction, abnormalities. Electrophysiological studies suggest that there is a post-synaptic abnormality but the site has not been determined.

Follow-up studies of individuals exposed to high levels of OP pesticides suggest that neurobehavioral changes may develop which include drowsiness, confusion, anxiety, emotional lability, depression, fatigue and irritability. However these studies are limited by the non-specific nature of many of the symptoms and the reliability, validity and sensitivity of the various neuropsychological and neurophysiological tests used.

Other reports have suggested that long-term effects on the central and peripheral nervous systems may be associated with relatively low level organophosphate exposure. These effects range from neurobehavioral and EEG abnormalities to changes in the latencies of action potentials in skeletal muscles (the "jitter" of muscle impulses) (Kelly et al, 1990). Some studies have shown psychological, behavioural or electrophysiological changes after exposure of humans to low doses of organophosphates (Levin et al, 1976). Rodnitzky (Rodnitzky et al 1975) studied 23 subjects (12 farmers and 11 pesticide sprayers) and a control group, using a variety of psychometric tests and found no abnormalities. Acutely poisoned individuals were not included. Levin (Levin et al, 1976) found elevated levels of anxiety in commercial sprayers, but not farmers, although in this study the population had been potentially exposed about 2 weeks before testing. Some of these long-term effects may be mediated by cholinergic mechanisms although other suggestions have been made (Duffy et al, 1979). Phosphorylation of neuronal protein sites may contribute to the underlying disorder. It is suggested that genetic differences in activation of detoxification enzymes account for some of the inter-individual variations in susceptibility to anticholinesterases (Mutch et al, 1997) so that it is not inconceivable that the development of long term effects may have a genetic component or involve altered gene expression.

Electroencephalographic (EEG) changes have been described (Duffy et al, 1979) after symptomatic exposure to sarin. These changes were small and have only been identified after computer spectral analysis. Burchfiel (Burchfiel et al, 1982) described a relative increase in ß activity that persisted for over a year in the EEG of rhesus monkeys after a single large dose

(5µg/kg) or repeated small doses (1µg/kg weekly to a total dose 10 µg/kg) of sarin. Previous studies at CBD Porton Down supported this finding that there were small changes in β_2 energy which were present 6 - 12 months after an acute dose of sarin in rhesus monkeys. The functional significance of these small changes is unknown.

A more recent study in which data were recorded remotely from conscious marmosets (Pearce et al. 1999) was specifically designed to facilitate determination of the functional significance of any changes in EEG observed following acute administration of a single dose of sarin. No statistically significant changes in EEG were observed nor was there any behavioural decrement up to 15 months after exposure. Methodological differences may account for the disparity between this and earlier studies in rhesus monkeys. Further work is required to investigate the effect of a range of exposure levels.

It is difficult to extrapolate the civilian observations on pesticides directly to military exposure to nerve agents. The available data suggest that high dose acute organophosphate poisoning can result in long term neurological effects, changes in behaviour, deficits in neuropsychological performance and alterations in EEG. Information on low dose exposure, either acute or chronic, in a volunteer setting or otherwise, is less robust. The findings need to be considered in the context of the biochemical activities of anticholinesterases and other proteins.

Work at CBD has also demonstrated a temporary peripheral change in the Single Fibre Electromyogram (SFEMG) in human volunteers. However, men dosed with pyridostigmine bromide (Nerve Agent Pretreatment Set, NAPS) over an eight week period only exhibited small, clinically insignificant changes in SFEMG which remained within normal limits and which returned to normal within one week of the cessation of dosing. Many service personnel involved in the Gulf War were required to take NAPS as a prophylactic treatment and some may have been exposed to organophosphates. The long term neurotoxicity of anticholinesterases remains, therefore, an important military health issue that requires an objective review.

Scope

The Terms of Reference of the Working Party were: -

1. To review the results of research in animals and man on the long term toxic effects of anticholinesterase compounds with particular emphasis on chemical warfare (CW) nerve
   agents and carbamate prophylactic administration.

2. To advise on the health risks to service personnel, including exposed volunteers, and the need for continued health surveillance.

3. To make recommendations on policy and further research requirements.

Conclusions

1. There is good robust evidence that high doses of OPs may have long term toxic effects on the peripheral nervous system (PNS) and skeletal muscle in man and animals, but the toxic mechanisms have not been established.

2. There is more limited information about the long term toxic effects of low doses of OPs on the

central nervous system (CNS). Nevertheless, evidence does exist of neurobehavioral changes and the implications of potential long term adverse health effects of organophosphates remain unanswered.

3. Susceptibility to these toxic effects may be limited to a subgroup of the general population defined, for example, by genetic factors and may differ with different OPs.

4. There is sufficient uncertainty in the interpretation of the evidence for potential long term toxicity of organophosphate nerve agents to justify continued caution in authorising exposure of human volunteers to these materials.

5. There is little evidence that the prophylactic use of carbamates (e.g. as NAPS) is associated with any long term adverse health effects alone or by interaction with other medication. However, this possibility does need to be fully explored.

Recommendations

A series of recommendations for further priority research have been made.

1. INTRODUCTION

The acute toxic effects of anticholinesterases are well recognised. However, recent reports have begun to support a growing public concern that long term toxic effects may result from either an acute exposure or repeated low level exposures to these compounds. The issue has mainly involved the civilian use of organophosphate sheep dips, but recently other organophosphate pesticides, CW nerve agents and carbamate prophylactic treatment have been considered in the aetiology of some cases of illness described in former military personnel who were involved in the 1990-91 Gulf War.

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The Working Party has reviewed MOD research on the neurotoxicity of anticholinesterases and relevant research in the public arena. An evaluation has been made of the health risks to human volunteers and the need for follow-up studies in volunteers. The issue is very sensitive in view of the possible medico-legal repercussions, the implications for prophylactic policy and the future volunteer programmes.

Recommendations are made for further research needs and policy implications.

The report has considered the scientific and medical evidence under separate categories of effects:

Biochemical and neurochemical effects
Clinical neurological aspects
Psychiatric and psychological effects
Electrophysiological effects
Conclusions
Recommendations

BIOCHEMICAL AND NEUROCHEMICAL EFFECTS

Introduction

The following questions are addressed in this section: -

1. What are the biochemical long term toxic effects of anticholinesterase nerve agents, organophosphorus compounds (OPs) and carbamate prophylactics (CBs), and might these develop following low level exposure?
2. Are the changes demonstrated in animals also seen in man ?
3. What is the mechanism, and is it triggered by prolonged low level acetylcholinesterase inhibition ?

These questions will be considered under the following headings:

- Background
- Recognised toxic effects of anticholinesterase compounds
- Monitoring exposure to anticholinesterases: exposure, metabolism and inter-individual variability
- Other neurochemical changes associated with OP exposure
- Conclusions

Background

There are increasing reports of chronic health effects following multiple exposures to organophosphorus pesticides (e.g. Steenland, 1996, Blain 1996). These may include symptoms suggestive of effects on peripheral nerve transmission and central nervous system function that are not necessarily accompanied by acute clinical signs at the time of exposure. There is conflicting evidence on the long-term sequelae of acute OP exposure or of the possible effects of chronic exposure not causing overt clinical signs of cholinergic toxicity.

Recognised toxic effects of anticholinesterase compounds

Three categories of anticholinesterases are considered: -

1. Direct acting and irreversible inhibitors	(e.g. nerve agents)
2. Direct acting but reversible inhibitors	(e.g. carbamates)
3. Require metabolic activation to an irreversible inhibitor	(e.g. some OP pesticides)

Acute poisoning with organophosphates (nerve agents or pesticides) or carbamates is the result of inhibition of acetylcholinesterase (AChE, EC 3.1.1.7.), an important enzyme which by hydrolysing acetylcholine regulates transmission of nerve impulses across some nerve synapses or the neuromuscular junction. OPs and carbamates interact at the serine residue of the active site of the enzyme and the nature of the biochemical reaction with the AChE is similar to that of the natural substrate acetylcholine. The main difference is that the rate of enzyme reactivation is extremely fast for the natural substrate but is extremely slow for OPs and they are classed as irreversible inhibitors. In general carbamates are classed as reversible inhibitors since the half-lives of reactivation of the enzyme-inhibitor complex are relatively short (min-hours).

Most organophosphate inhibited AChEs may be reactivated by administration of an oxime which

effectively removes the OP from the active site of the enzyme to restore its functionality in nerve transmission. However some OP-inhibited AChE complexes may undergo chemical modification (ageing) after the enzyme-inhibitor complex has formed and they become refractory to reactivation by oxime. In such circumstances nerve transmission is only restored by resynthesis of new enzyme. Different OPs vary in their potency to inhibit AChE as well as the rates of spontaneous reactivation and ageing. In general pesticidal OPs are the least potent inhibitors of AChE and are less toxic than nerve agents which are highly potent inhibitors. Inhibition of red blood cell AChE (60-70%) may be associated with acute central and peripheral cholinergic effects consequent upon the excess acetylcholine at the nerve synapse or neuromuscular junction. The major features of these effects are well understood.

Some OP pesticides and their analogues also produce a delayed peripheral neuropathy (Organo Phosphorus Induced Delayed Neuropathy, OPIDN) associated with phosphorylation and ageing at the active site of neuropathy target esterase (NTE), a membrane bound protein (Johnson 1990). Many of these compounds also inhibit AChE however some, such as the lubricant tri-orthocresol phosphate, inhibit NTE and produce OPIDN but do not inhibit AChE (Zech and Chemnitius 1987).

CW nerve agents, although extremely potent inhibitors of acetylcholinesterase, are in general poor inhibitors of NTE. Only sarin had some ability to inhibit NTE and cause OPIDN and then only at doses greatly exceeding the LD_{50}. The I_{50S} (inhibitory concentration 50%)(in vitro) against AChE were shown to be 0.1 - 1.0 nM for the classical nerve agents, sarin, soman, tabun and VX whereas the I_{50S} against NTE were between 178 - 2000 fold higher for sarin, soman and tabun and 10^6 greater for VX. Only sarin induces an overt neuropathy in hens but they had to be protected against the acute lethal effects of sarin by pretreatment with physostigmine, atropine and an oxime, since a dose of 30-60 times the LD_{50} was required to induce the response (Gordon et al 1983).

Carbamates are widely used as pesticides and therapeutic agents and their pharmacological action is a short-term reversible inhibition of AChE. The carbamate, pyridostigmine, is used as a prophylactic medical countermeasure against organophosphate chemical warfare agents. Protection is achieved when the AChE is in an unstable equilibrium with pyridostigmine. An adequate level of pre-treatment with pyridostigmine is achieved when the activity of red cell AChE is reduced by between 20 and 40% of its original level. This level of reserved enzyme is produced with an oral dose of 30 mg pyridostigmine bromide every 8 hours. Pyridostigmine, which is a quaternary ammonium compound, does not penetrate the CNS although a recent, but much criticised study has suggested that stress may compromise the integrity of the blood-brain barrier and thus allow access of compounds normally excluded (Friedmann et al 1996). However, this view is not widely accepted and the experimental design in this study was not robust. Physostigmine, a tertiary carbamate, crosses the blood brain barrier and inhibits acetylcholinesterase in the CNS.

Neural AChE exists in functional and non-functional pools of several molecular forms. The functional forms are located extracellularly in the cleft of the neuromuscular junction and comprise the lipophilic asymmetric form (A12, collagen tailed), which is bound to the basal lamina, and some water soluble globular AChE (G4) present in the extracellular matrix in the cleft (Younkin et al 1982). The non functional precursor pool contains the monomeric precursor from, G1, together with G4 and are found intracellularly. Drugs and OPs, which inhibit AChE, differentially affect the AChE molecular forms (Ogane et al, 1992; Taylor et al 1994). Aggregation of the monomeric globular forms differs between slow and fast muscle, diaphragm

and areas of the brain. Several studies have shown that A12 is absent from the brain, and G4 may be the functional form.

Monitoring exposure to anticholinesterases: Exposure, metabolism and inter-individual variability

Exposure to organophosphates

The major routes of exposure to nerve agents are by inhalation and dermal absorption. OP pesticides are used in liquid droplet form or exposure is to the concentrate so that the dermal route is the major route of entry into the body. Assessment of occupational exposure to OPs is normally by measurement of the blood AChE or plasma butyryl cholinesterase (BuChE) activity. However since OPs are rapidly hydrolysed and excreted in the urine, the presence of hydrolysis products in the urine may also be used as a marker of exposure in man.

OP pesticides and nerve agents are detoxified by hydrolysis by A-esterases (aryldialkylphosphatase E.C. 3.1.8.1.) and by binding to serine containing carboxylesterases (EC 3.1.1.1.). The relative affinities of OPs for AChE, paraoxonase, carboxylesterase and other hydrolases influence the toxicity and the route of detoxification. OPs containing the P-N bond, such as tabun and mipafox, are poor substrates for A-esterases but nerve agents such as sarin are rapidly hydrolysed by other blood and liver enzymes. Carboxylesterase binding has particular importance for detoxification of nerve agent OPs and in rodents the binding of soman to plasma carboxylesterase is a major determinant of the overall toxicity in each species (Jokanovic et al 1996, Gaustad, Johnsen and Fonnum, 1991, Due, Trap, Langenberg and Benschop 1994).

Unlike nerve agents which are intrinsically toxic and potent inhibitors of AChE, most commercial pesticides are designed to exhibit low mammalian toxicity and high insect toxicity. This is achieved by exploiting differences between species in the ability to metabolise the administered pesticide to a toxic product which inhibits AChE. In general the administered pesticide is a poor inhibitor of AChE, and hence of intrinsic low toxicity, but is metabolised once absorbed, to a toxic AChE inhibitor. The balance between activation of the pesticide and its degradation determines the overall toxicity to the species exposed. In mammals, including humans, this conversion normally occurs in the liver and a phosphorothionate group is oxidised to a toxic oxon - the proximate inhibitor of AChE. Detoxification also occurs in parallel in the liver and is mediated by cytochrome P450 enzymes for some phosphorothionates (mainly by CYP 3A - the most abundant human form of P450). Metabolism may also occur locally at the site of action e.g. the brain. Differences in these pathways may contribute to inter-individual differences in susceptibility in man to OP pesticides which require activation (Mutch et al 1996a, 1997, Butler and Murray 1997).

Human serum PON1 has been shown to have a polymorphic distribution and different individuals also express widely different levels of this enzyme (Davies et al 1996). The amino acid polymorphism is at position 192 and results in two alloenzymes which differ in their hydrolytic activity towards paraoxon (Humbert et al 1993). The Arg 192 (R) isoform hydrolyses paraoxon five times more rapidly than the Gln 192 (Q) form. Both isoforms hydrolyse chlorpyrifos oxon at approximately the same rate. However, the effect of PON1 polymorphism is reversed for the hydrolysis of diazinon oxon, soman and sarin. The study carried out by Davies et al showed that the RR homozygotes (high paraoxonase activity) had lower hydrolytic activity towards diazinon oxon than the QQ homozygotes. Similarly, the Q homozygotes hydrolysed sarin about ten times more rapidly than the RR individuals.

Several studies have shown ethnic variations in the frequency of the PON1 polymorphism (e.g. La Du, 1996 ; Yamasaki et al, 1997). Fifty percent of Caucasians are homozygous for the Q allozyme, 40% are QR heterozygotes and the rest are RR homozygotes (La Du, 1996). Of interest, 41.4% of Japanese subjects were shown to be homozygous for the Arg 192 allele (Yamasaki et al, 1997). A second PON1 polymorphism has now been disclosed at position 55 involvoing a Leu->Met substitution (Mackness et al, 1997). The results of this study of 279 Caucasian individuals showed that the 192 polymorphism and the 55 polymorphism both had an independent effect on PON1 activity. Multiple regression analysis indicated that the 192 polymorphism, 55 polymorphism and serum PON1 concentration were responsible for 46%, 16% and 13% of the variation in PON1 activity, respectively.

For paraoxon, detoxification by paraoxonase may be of little importance in vivo at the low concentrations found following exposure to parathion occupationally because of the high K_m of the enzyme. Detoxification of some OPs may occur following P 450 mediated activation, to an unstable phosphooxythiran intermediate, and then spontaneous breakdown and binding to carboxylesterases (Mutch et al 1998). However, monitoring of detoxification enzymes may be a predictive marker of susceptibility. A balance exists between activation and the detoxifying hydrolytic pathways and this may differ between brain and liver.

In man pyridostigmine has an elimination half life of less than 2 hours. 75-90% of the absorbed dose is excreted in the urine unchanged after an intravenous dose although it is poorly and irregularly absorbed from the gastrointestinal tract with first pass metabolism. Less than 20% of the dose is excreted unchanged and the metabolites included 3-hydroxy methylpridinium. Physostigmine is also rapidly hydrolysed and most of a dose is eliminated by 2 hours.

Single dose compared to multiple dosing

Much of the information on the toxicity profiles of individual OPs is derived from single doses designed to produce an acute effect on marker enzymes. However, many exposures to OP pesticides are multiple low level exposures at doses which do not inhibit acetylcholinesterase sufficiently to produce classical cholinergic effects. Because of the possibility of long term effects in farmers exposed to low level OP pesticides whilst dipping sheep, and soldiers exposed to carbamates and nerve agents, a number of studies have been initiated to address the effects of multiple low level exposures on biochemical and effect markers.

It has been shown that single doses of ecothiopate (a pure anticholinesterase) or mipafox (a neuropathic weak anticholinesterase slowly reversible) have differential effects on the electrophysiological parameter “jitter”, pre and post junctional, in a mouse hemidiaphragm preparation (Kelly et al 1994). Effects on jitter after a single dose appear to relate to inhibition of total acetylcholinesterase and NTE measured in brain and diaphragm homogenate at early times. (Mutch et al 1996b, Williams et al 1997). However multiple low doses of both mipafox and ecothiopate, and paraoxon, which do not inhibit NTE or produce OPIDN in the hen, produced similar effects on electrophysiology. Furthermore electrophysiological effects were elicited following additive inhibition. This suggests that some long term effects of organophosphate exposure may result from prolonged acetylcholinesterase inhibition (Kelly et al 1997) and that this effect on the nerve is related to the time profile /degree of inhibition of acetylcholinesterase and so may be shared by other organophosphates.

The relevance to man of these findings in the mouse is supported by the report of similar effects

on SFEMG measured in the forearm in human volunteers following exposure to sarin at a dose which inhibited acetylcholinesterase by about 40% (Baker and Sedgwick 1996). These effects lasted for up to two years when they had returned to normal values.

For mipafox and paraoxon which cross the blood brain barrier, inhibition of brain AChE was similar to the diaphragm. However, following multiple doses of ecothiopate there was an increase in brain AChE at 3 hour following each dose. Ecothiopate, which is a quaternary ammonium salt, does not readily cross the blood brain barrier and probably remains extracellularly at the neuromuscular junction. Consistent with this it had previously been shown that ecothiopate did not inhibit brain acetylcholinesterase following a single dose *in vivo,* although nonsignificant increases in brain activity were measured at 7 and 28 days. Ecothiopate did inhibit brain acetylcholinesterase *in vitro* (Mutch et al 1996b). Secondary effects at the brain following peripheral inhibition may have resulted in an upregulation of brain enzymes, which was only detectable in the absence of direct central inhibition. Subsequent studies (Lintern, Wetherell and Smith,1998) have examined the profile of the multiple forms of acetylcholinesterase in different guinea pig tissues (diaphragm v brain) following a single dose of soman (27µg/kg). They showed differential inhibition of the molecular isoforms in the different tissues and differential rates of recovery.

The studies using OPs in mouse muscle are consistent with the results of Lintern and Smith and Ferry (1997, a, b) with pyridostigmine. Multiple low doses of carbamates have not been shown to produce overt adverse effects in man, although they induce biochemical changes in an animal model (Lintern, Wetherell and Smith 1997; Lintern, Smith and Ferry, 1997, a,b). These authors showed that increased total AChE levels and changes in molecular form distribution occurred in mouse muscles after single and multiple doses of pyridostigmine. When a single dose of pyridostigmine (100µg/kg) was administered there was an initial inhibition of AChE in some, but not all, muscles at 3 hours which was followed by an increase in enzyme levels. In studies in which pyridostigmine was administered twice a day for three weeks at 100µg kg^{-1}, AChE levels were initially depressed in some, but not all tissues. When the enzyme levels had apparently returned to normal, a single subsequent challenge dose of pyridostigmine (100µg kg^{-1}) two weeks later, caused a rapid rise in enzyme levels suggesting that the upregulation mechanism had become sensitised by the earlier inhibition. These changes were possibly due to upregulation of expression of globular forms of AChE following pyridostigmine inhibition of functional AChE. It is probable that there is feed back control following the increased levels of acetylcholine in the cleft.

These consequential effects upon the regulation of enzyme levels following repetitive or prolonged inhibition of AChE are supported by the work of Milatovic and Dettbarn (1994,1996). These authors showed that rats develop tolerance to low doses of paraoxon that is associated with changes in affinity and phosphorylation of acetylcholinesterase in brain and diaphragm(Milatovic and Dettbarn, 1996, 1994, Dettbarn and Milatovic 1994). Similarly continuous injections of DFP up to 14 days did not produce additional inhibition of AChE but a recovery of the enzyme in the diaphragm (Gupta et al 1986). It is possible that these changes are merely the consequence of normal homeostatic mechanisms that come into play when levels of ACh are sustained as a result of prolonged inhibition of AChE. However the long term significance of these changes on function remain to be resolved.

Other Neurochemical changes associated with OP exposure

The primary effect of organophosphate exposure is inhibition of AChE by phosphorylation of the

serine at the active site resulting in increased levels of acetylcholine. In parallel with this, OPs may phosphorylate serine in other enzymes which may produce secondary effects. For example, pirimiphos methyl has been shown to inhibit proteases at levels that had little effect on acetylcholinesterase (Mantle et al, 1997). Also, elevated acetylcholine levels in the neural system may exert feedback control on transmitter synthesis, reduce receptor density and produce secondary effects on other transmitters and enzymes.

Studies in vitro have indicated secondary effects of acetylcholinesterase inhibition. Functional AChE was measured in whole hemidiaphragm from animals exposed to GB in parallel with other biochemical markers such as adenosine triphosphate (ATP), cylic adenosine monophosphate (c AMP), cylic guanosine monophosphate (c GMP) and calcium. Functional AChE was reduced, which resulted in increased levels of ACh. cAMP and cGMP were also elevated, possibly due to ACh activation of guanylate and adenylcyclases in an inhibitory feedback loop. Ca^{2+} was elevated and proteases activated leading to myofibril damage and myopathy.

Abou Donia has shown that neurotoxicity is associated with increased autophosphorylation of calcium/calmodulin kinase II and enhanced phosphorylation of cytoskeletal proteins. The effects were produced by neuropathic OPs, hexane and acrylamide and therefore did not depend on acetylcholinesterase inhibition (Gupta and Abou-Donia, 1995)

Central biochemical effects of anticholinesterases were summarised by Lotti (1992). Cholinergic muscarinic receptors are decreased with OPs, nicotinic receptors decreased after chronic cholinergic stimulation. It is of interest that symptoms of excessive cholinergic stimulation gradually reduce during chronic OP exposure, despite significant inhibition of AChE. The development of this tolerance has been, in part, associated with effects on cholinergic muscarinic receptors caused by prolonged AChE inhibition and ACh stimulation (Russel and Overstreet, 1987).

Studies at CBD Porton (e.g.Fosbraey, Wetherell and French (1989) showed there was a correlation between central neurotransmitter changes and incapacitation produced by soman. Acute pre-treatment with physostigmine and hyoscine prevented changes in brain neurochemistry measured as acetylcholine, noradrenaline, dopamine turnover (DOPAC/DA), 5 hydroxy tryptamine (5HT) turnover (5HIAA/5HT), aspartate (ASP) and gamma amino butyric acid (GABA). Increases in neurotransmitters are probably a secondary effect to raised acetylcholine levels.

Conclusions

A number of biochemical effects occur as the result of AChE inhibition but it has not been confirmed whether these are also manifest following low-level chronic exposure to certain anticholinesterases, when the level of inhibition is low. Confirmation of these toxic effects in man and their implications for the health risks following prolonged low level exposure to these compounds are more difficult to determine. Similarly it is not clear whether the biochemical changes that lead to the electrophysiological or histological changes produce any clinical effects.

CLINICAL NEUROLOGICAL ASPECTS

The neurological effects of pesticide and nerve agent OPs will be considered under the following headings :

- Introduction
- Acute effects
- Delayed peripheral neuropathy
- Intermediate syndrome (myopathy)
- CNS pathology of acute poisoning
- Chronic opthalmological effects
- Neurotoxic effects of pyridostigmine and oximes

Introduction

The acute and sub-acute effects of organophosphorus (OP) poisoning are well documented. For the neurologist, the main clinical interest has been in the neuropsychological and neuropsychiatric aspects of OP exposure, and the majority of the available literature relates to pesticide OP poisonings. The sub-acute and chronic physical neuropathological effects of OPs that are documented are virtually all neuromuscular i.e. the intermediate syndrome and delayed neuropathy. Leaving aside the claimed long term neuropsychological and neuropsychiatric effects of OPs, there is virtually no available evidence concerning possible long term central nervous system effects of OPs, either as pesticides or CW nerve agents. However, sequential EEG studies in animals have demonstrated definite, if subtle, chronic changes after nerve agent exposure, and although the significance of such changes is at present far from clear, this evidence has heightened fears that anticholinesterase nerve agents may be capable of producing irreversible CNS damage.

This section is limited to consideration of clinical neurological effects of OPs, and does not discuss the extensive neuropsychological/neuropsychiatric literature, nor the neurophysiological literature which are reviewed separately.

Acute Effects

Acetylcholine is a major transmitter in the central nervous system (CNS) and peripheral nervous system (PNS). After release from nerve terminals it is inactivated by acetylcholinesterase (AChE) which is largely responsible for the termination of its action. Inhibition of AChE by OPs leads to rapid accumulation of ACh at cholinergic junctions. Two major subtypes types of cholinergic receptor exist: the muscarinic receptor (parasympathetic nervous system) and the nicotinic (sympathetic and motor nervous system). Symptoms and signs of acute poisoning are classified into muscarinic, nicotinic and CNS manifestations (table 1) dependent on the major receptor type involved and whether the effects are largely peripheral or central in origin. The time interval between exposure to inhibitor and the onset of symptoms varies with the route of exposure, the dose of OP and the chemical nature of the OP.

Table 1: Signs and symptoms of inhibition of acetylcholinesterase

Peripheral Effects

Muscarinic	Blurring of vision, miosis, lachrymation, bronchoconstriction, hypersalivation, urination, defecation, bronchial hypersecretion.
Nicotinic	Muscles: twitching, fasciculation cramps, paralysis, including respiratory muscles
	Sympathetic ganglia: pallor tachycardia, increase in blood pressure

Central Effects

Confusion	Dizziness
Anxiety	Headache
Apathy	Hypothermia
Convulsions	Impaired concentration
Depersonalisation	Irritability
Respiratory depression	Restlessness
Circulatory collapse	Tremor
Drowsiness	Cognitive impairment
Emotional lability	Dysarthria
Generalised weakness	Opsoclonus
Coma	

Delayed Peripheral Polyneuropathy

Two types of peripheral neuropathy may be induced by OPs. The first, a delayed peripheral polyneuropathy is induced by a limited number of OPs developed for pesticide use, whilst the second, the so-called Intermediate Syndrome, occurs following severe cholinergic crises induced by high acute doses of some OP pesticides.

A number of pesticide OPs have been shown to cause peripheral polyneuropathy, either as a result of suicidal ingestion, or accidental poisoning (Minton and Murray, 1988; Karalliedde and Senanayake, 1989). After poisoning by such high dose, an acute cholinergic crisis is followed by the development of a polyneuropathy two to three weeks later. The neuropathy is predominantly motor, though with variable sensory involvement. In some patients, there is also involvement of pyramidal tracts (Senanayake and Johnson, 1982).

Polyneuropathy may also develop after chronic, low dose repeated exposure to some OPs (pesticides or plasticisers). That due to triortho-cresyl phosphate (TOCP) has been extensively studied. The pathology is a "dying back" process, or distal axonopathy (Cavanagh, 1954). Central processes of sensory axons may also be affected, and this is a pattern seen with a variety of toxic neuropathies, in which central and peripheral processes may be differentially affected. Sub-acute myelo-optic neuropathy (SMON) due to clioquinol poisoning, is an example. These central / peripheral distal axonopathies were reviewed by Thomas (1982). Recovery from pesticide OP induced delayed neuropathy is variable (Morgan and Penovich, 1978).

The delayed neuropathy is not a function of AChE inhibition, but associated with the

phosphorylation of an enzyme which has been termed "neuropathy target esterase" (NTE). Transformation of the phosphorylated enzyme to an aged form then occurs. This may be prevented by phosphinates or carbamates, which may in turn prevent the development of a neuropathy (Johnson, 1975; Johnson and Lauwerys, 1969).

The classical nerve agents GA (tabun), GB (sarin), GD (soman) and VX together with several analogues and pesticides have been assessed for their ability to induce delayed peripheral polyneuropathy and inhibit NTE (Gordon, et al; 1983). Delayed neuropathy associated with inhibition of NTE was found at 30-60 LD_{50} for GB but not at 38 and 82 LD_{50} for GD and GA. To achieve any indication of activity with GB in vivo, heroic measures had to be taken to ensure that treated hens (the recognised test species) survived the administration of such high multiples of the lethal doses of these agents. It was estimated that the minimum neuropathic doses of GD and GA were between 100-150 LD_{50}s of agent. The high potency of nerve agents as inhibitors of AChE mean that the dominant effects of nerve agents are immediate and at these doses humans would have died from the effects of the agent before the effects of possible neuropathy would have become apparent.

Intermediate Syndrome

In addition to a delayed polyneuropathy, an intermediate syndrome has been described (Senanyake and Karalliedde, 1987). In this, patients with acute pesticide OP poisoning develop paralysis of proximal muscles, cranial nerve-innervated muscles, and respiratory muscles 24 to 96 hours after poisoning, and following a well-defined cholinergic phase. The paralytic symptoms last up to 18 days. A delayed neuropathy may subsequently develop in some patients. Pyramidal signs and dystonia developed in some of the patients described by Senanyake and Karalliedde (1987). Neurophysiological studies showed normal motor and sensory nerve conduction, with marked fade with tetanic stimulation, but not to low frequency stimulation, indicative of a post-synaptic defect at the neuromuscular junction (Senanayake and Karalliedde, 1987).

Muscle necrosis occurs in the intermediate syndrome, peaking at a few days, and recovering at about 3-4 weeks (see Karalliedde and Senanayake, 1987). It is thought that the necrosis results from excessive entry of calcium ions into muscle cells due to prolonged transmitter-receptor interaction. The intermediate syndrome is more common in severe OP poisoning, as judged by the severity of the initial cholinergic phase (see Karalliedde and Henry, 1993).

CNS Pathology in Acute OP Poisoning

It has been shown that large single doses of soman (McLeod et al, 1984) and more recently sarin (Kadar et al, 1995) are capable of producing marked and progressive neuropathological damage in experimental animals. Although there appear to be differences in the site of cerebral damage with these two compounds, in general, there is similarity in the type of damage. In the study by Kadar et al (1995), rats were injected with 1 x LD_{50} of sarin, observed acutely for convulsions, and then sacrificed at intervals of up to 90 days. Cerebral damage was found in 70 % of surviving animals, being most marked in those with prolonged seizures. Minimal changes or no changes were found in animals with seizures lasting less than two hours. At 4 hours following sarin injection severe changes were already present in the hippocampus and thalamus, and at later intervals, lesions (including degeneration, vacuolar necrosis, haemorrhage, gliosis, ventricular enlargement, calcification, and hyaline plaques) were found in the piriform cortex, hippocampus, amygdala and thalamus. Cortical damage was less marked than that reported with soman. In conjunction with a previous study, (Kadar et al 1992), Kadar et al (1995) concludes that these

changes might be related to the severity of the initial seizures and that the duration of these seizures plays a major role in initiating these lesions. Animals having short episodes of convulsions (2h) displayed only minimal brain damage.

The implication of these observations is that if seizures could be prevented then neuropathological damage might also be prevented. Support for this interpretation comes from extensive investigations in the USA and France (Shih et al. (1997), Shih and MacDonough (1997); MacDonough and Shih (1997); Lallelment et al (1997); Lallement et al (1998). Treatment of guinea pigs poisoned by nerve agents with the anticonvulsant diazepam, or its pro-drug, Avizafone, will block nerve agent induced seizures/convulsions and enhance survival. There is significantly reduced neuropathology compared with those animals which did not receive diazepam. The protection was not complete since levels of diazepam in the brain are not sustained beyond the point when seizure activity ceases to be driven by cholinergic stimulation and becomes driven by other excitatory amino acids. These systems are recruited and eventually assume control of the seizure process, independent of cholinergic involvement. If this process can be prevented by early administration of diazepam then neuropathological damage is minimised.

Chronic Ophthalmological Effects of OPs

In addition to the well-recognised acute effects of OPs on the eye (miosis), long term changes have been reported, both in experimental animals and in man. Chronic exposure to OPs can produce pathological changes in the ciliary muscle and optic nerve in animals and man (Ishikawa, 1973; Uga et al, 1977), reduced retinal sensitivity in man (Ohto, 1974), and macular degeneration in man (Misra et al, 1982). There is no evidence of long term ophthalmological damage following brief nerve agent OP exposure to the eye. The ophthalmological evidence with chronic organophosphate exposure indicates that potentially irreversible damage can occur to retina and optic nerve. There is no such evidence with short term organophosphate nerve agent exposure to the eye.

Neurotoxic Effects of Pyridostigmine and Oximes

The potential neurotoxicity of pyridostigmine in acute (short term, up to 4 weeks) administration to human volunteers at CBD, has been reviewed by Wetherell (Sept. 1996, presentation to Working Party). As judged by the measures used, which included psychometry, personality assessment, subject diaries and tests of performance, no adverse effects were identified in 305 subjects. However, there are no published studies of long term clinical neurological damage, either peripheral or central, with prolonged administration of pyridostigmine, although it is used routinely, and often for periods of many years, in the treatment of patients with myasthenia gravis. These data would be useful for carbamate toxicity long term effects.

Toxicity of oximes used in the treatment of OP poisoning is reviewed by Marrs (1991). Treatment is usually for short periods, though some patients may require longer treatment. Although relatively few data exist, there is no evidence of long term adverse clinical neurological effects.

PSYCHIATRIC AND PSYCHOLOGICAL EFFECTS

The purpose of this section is to provide an overview of the psychiatric and psychological effects of acute and chronic exposure to anticholinesterase substances, in particular organophosphate pesticides and nerve agents. The effects will be considered under the following headings:

- Methodological considerations
- Organophosphorus pesticides
- Organophosphorus nerve agents
- Carbamate prophylaxes

Methodological considerations

In much of the literature, reports of psychiatric and psychological disturbances are woefully inadequate, frequently to the point of being uninformative. Phrases such as "anxiety and depression", "memory disturbance" abound, and obsolescent diagnoses such as "neurasthenia" and "cerebral decline" persist. Other problems relate to changes in how psychologists codify behavioural, cognitive and emotional functions, so that the interpretation of standard research instruments may shift. But the main reason is the limited expertise of many medical practitioners in the assessment and diagnosis of psychiatric disorders and psychological abnormalities.

Psychiatric sequelae to environmental toxins can occur at the symptomatic, the syndromal or the disorder level. A very wide range of symptoms is encountered in psychiatry, both psychological and somatic. Many are exaggerations of normal. For example, anxiety is a ubiquitous emotion, experienced by us all, and only when it becomes too severe, too frequent or too all-pervasive for the individual to tolerate can it be regarded as a symptom. Elicitation of symptoms is not as easy as it may appear. The interviewer is dependent on the verbal skills of the interviewee and on the "labelling" ascribed to a particular feeling.

The bodily symptoms induced by a toxin can be primary or secondary. Primary symptoms are directly induced, for example, sweating or anorexia with organophosphate pesticides. Secondary symptoms accompany central emotional states, for example, sweating as a symptom of anxiety or loss of appetite with depression.

At the syndromal level, a recognisable constellation of symptoms and signs is encountered. For example, the syndrome of "delirium" is characterised by clouding of consciousness, disorientation in time and space, anxiety and agitation and frightening visual hallucinations. There is usually partial or complete amnesia for the episode.

Many other syndromes can be induced by environmental toxins. Amnesic syndromes may be severe and global or subtle and localised, the latter requiring expert testing. The deficits themselves may be restricted to memory function or be part of a wider deterioration with dementia. In the latter case, tests for aphasia, apraxia, agnosia and executive functions are needed to establish the profile of impairments. Even the dementia can be part of a more general syndrome with emotional flattening or lability, sleep rhythm inversion, and behavioural disturbances such as aggressive outbursts and nocturnal wanderings.

The highest level of diagnosis involves well-specified disorders such as Major Depressive Disorder and Alzheimer's Type Dementia. The distinction between syndrome and disorder is often an artificial one in psychiatry but generally speaking, epidemiological studies will have

supported the status of disorder for a well-defined condition. The entire psychiatric diagnostic schema is constantly being revised as new research data accrue. Nevertheless, it is important to use a generally accepted system such as DSM-IV (1994) or ICD-10 (1992) in order to facilitate communication between researchers and clinicians. The headings of the section of the ICD-10 dealing with disorders secondary to psychoactive substance use are set out in table 2.

Table 2. Mental and behavioural disorders due to psychoactive substance use.
Source: ICD-10, WHO, 1992, pp.70-71.

Acute intoxication
- Uncomplicated
- With trauma or other bodily injury
- With other medical complications
- With delirium
- With perceptual distortions
- With coma
- With convulsions
- Pathological intoxication

Harmful use

Dependence syndrome
- Currently abstinent
- Currently abstinent, but in a protected environment
- Currently on a clinically supervised maintenance or replacement regime (controlled dependence)
- Currently abstinent, but receiving treatment with aversive or blocking drugs
- Currently using the substance (active dependence)
- Continuous use
- Episodic use (dipsomania)

Withdrawal state
- Uncomplicated
- With complications

Withdrawal state with delirium
- Without convulsions
- With convulsions

Psychotic disorder
- Schizophrenia-like
- Predominantly delusional
- Predominantly hallucinatory
- Predominantly polymorphic
- Predominantly depressive symptoms
- Predominantly manic symptoms
- Mixed

Amnesic syndrome

Residual and late-onset psychotic disorder
- Flashbacks
- Personality or behaviour disorder
- Residual affective disorder
- Dementia
- Other persisting cognitive impairment
- Late-onset psychotic disorder

Other mental and behavioural disorders

Unspecified mental and behavioural disorder

The lack of objective validating criteria is a major handicap in psychiatry. Very few objective tests exist and those that do relate to some primary condition, for example a hormonal dysfunction. Others are non-specific, for example measurement of heart-rate or sweat-gland activity in states of arousal such as anxiety, aggression and embarrassment.

With behavioural and cognitive abnormalities, controlled observation and formal testing is routine and highly informative. However, personnel administering these tests must be properly trained. In the UK, this usually means the involvement of a Chartered Clinical Psychologist. A wide range of tests are available (Bolla, 1996) covering the spectrum of psychological abilities (see table 3).

Reactions induced by environmental toxins are typically non-specific in psychiatric or psychological terms with anxiety, depression, memory disturbances and so on at the symptomatic or syndromal level (Spurgeon et al., 1996). Sometimes, however, specific defined disorders are introduced which closely resemble spontaneously occurring conditions. Perhaps the best example is amphetamine-induced paranoid psychosis which is almost indistinguishable from schizophrenic disorder of the paranoid type.

Predisposition to psychiatric disorders is an important factor in the induction of reactions to environmental toxins. A detailed previous psychiatric history and assessment of previous psychological and other functioning is essential in assessing reactions to environmental insults. Often psychological symptoms have occurred chronically or sporadically prior to the exposure. Ascription of causation can then become very difficult particularly when multiple agents and a complex response are involved. As well as psychological predispositions, physical predispositions are operative, for example, polymorphisms in metabolising enzymes for organic chemicals. Similar polymorphisms are suspected of influencing susceptibility to psychiatric disorders.

Organophosphate pesticides

Organophosphates are extensively used as pesticides, in spraying crops and in dipping sheep. Much controversy has attending the use of these compounds, particularly as sheep-dips. It is obviously important to establish whether any link exists between such usage and psychiatric/neuropsychological dysfunction.

The acute toxic effects of exposure to OP pesticides are well known but the first symptoms are mainly psychological with anxiety, tension, irritability, restlessness, emotional lability and insomnia with excessive dreaming. Other non-specific symptoms include drowsiness, headaches, dizziness, and tremor which may be marked. Disturbances of attention, concentration and memory are noted both subjectively and on formal testing. Less common psychiatric symptoms are depression and social withdrawal.

Some neuropsychological test procedures have been conducted on workers exposed acutely to OPs. An inherent difficulty in many such studies is that there are few, if any, measures of the dose or frequencies of exposure to the OP. In some studies workers have been exposed to more than one compound and to other ingredients within the technical formulations.

Table 3. Examples of neuropsychological tests for use in neurotoxicology examinations.

(From Bolla, 1996)

Intellectual ability
- Wechsler Adult Intelligence Scale-Revised (WAIS-R)
- Vocabulary Subtest of the WAIR-R
- Raven's Progressive Matrices
- Shipley Institute of Living Scale

Language
- Peabody Picture Vocabulary Test-Revised (PPVT-R)
- Boston Naming Test
- Wide Range Achievement Test-Revised (WRAT-R); Reading and Spelling Subtests
- Boston Diagnostic Aphasia Examination (BDAE)
- Western Aphasia Battery (WAB)

Memory
- Wechsler Memory Scale-Revised (WMS-R)

Verbal memory
- Rey Auditory Verbal Learning Test (RAVLT)
- Logical Memory Passages of the WMS-R
- California Verbal Learning Test (CVLT)

Nonverbal memory
- Visual Reproduction Subtest of the WMS-R
- Rey-Osterrieth Complex Figure Test
- Symbol Digit Paired Associate Learning Test

Visuoperception/visuoconstruction
- Judgement of Line Orientation
- Hooper Visual Organization Test
- Block Design of the WAIS-R
- Rey-Osterrieth Complex Figure Test (copy)

Manual dexterity/motor
- Finger Tapping Test
- Purdue Pegboard
- Grooved Pegboard

Executive/psychomotor functions
- Wisconsin Card Sorting Test
- Stroop
- Category Test
- Digit Symbol Subtest of the WAIS-R
- Symbol Digit Modalities Test
- Trail Making Test
- Reaction Time
- Continuous Performance Test

Psychological functioning - Mood
- Minnesota Multiphasic Personality Inventory-2 (MMPI-2)
- Symptom Checklist (SCL-90-R)
- Profile of Mood States (POMS)
- Center for Epidemiologic Studies Depression Scale (CES-D)

Computerized batteries
- Neurobehavioral Evaluation System (NES-2)
  - Milan Automated Neuropsychological System (MANS-WHO)
- Automated Neuropsychological Assessment Metrics (ANAM)

One of the first studies was that of Durham et al (1965). They used various tests of "mental alertness", including complex reaction time and paced vigilance tests. The group studied was crop sprayers with varying degrees of exposure; the controls had no history of exposure. Immediately after exposure, testing revealed decrements in performance which did not persist. No dose effect relationships were uncovered.

Abnormalities of memory, signal processing, vigilance, semantic performance and proprioceptive feedback were sought in 23 workers exposed to OP pesticides (Rodnitzky et al., 1975). Compared with unexposed individuals, no deficits were detected leading the authors to conclude that higher CNS functions are relatively resistant to the acute effects of OPs.

The OP, diazinon, was used by 99 pest control workers who were tested before and after their work shift using a computerised battery of tests (Maizlish et al., 1987). Again no unequivocal decrements in performance were detected but the diazinon exposure was fairly low level.

Delayed effects of OP exposure have been described. Symptoms begin 1 - 3 weeks after acute exposure and generally resemble those of acute exposure itself. Psychiatric symptoms have not been described other than those in reaction to the physical symptoms such as pain, numbness and limb weakness. One study of 229 Egyptian pesticide workers detected reduced tactile sensitivity but no impairment on the block design and Santa Ana Dexterity tests (Solinan et al., 1993).

Attempts have been made to discover any chronic sequelae resulting from one or more episodes of undoubted acute toxicity. Savage and his colleagues (1988) compared 100 individuals with previous acute exposure to OPs with carefully matched individuals not so poisoned. A physical examination with neurological evaluations, EEG recordings and various neuropsychological and personality tests were incorporated in the battery. The groups did not differ with respect to special sense function or the EEG. However, the poisoned subjects performed worse than the controls on 4 out of 5 summary measures - memory, abstraction, depressed mood and motor reflexes. More specifically, deficits were found with respect to widely differing abilities such as intellectual functioning, academic skills, flexibility of thinking and simple motor skills. The authors conclude that their careful matching of cases and controls and the exclusion of other possible sources of neuropsychological impairment "make it likely that the excess deficits recorded in the poisoned subjects are due to their previous OP poisoning". However, close analysis of their data shows that pre-morbid intelligence, as assessed by verbal IQ, was significantly higher in the controls but was not used as a covariate in analysing the other variables. This criticism can be levelled at some other studies as well.

A retrospective study of agricultural workers in Nicaragua compared 36 men previously poisoned with OPs with men without episodes of poisoning although possibly exposed to OP use (Rosenstock et al., 1990, 1991). A WHO neuropsychological core test battery was used and differences were found in the Digit Span, Digit Vigilance, Benton Visual Retention, Digit Symbol, Trails A, Block Design, Pursuit Aiming and dexterity tests. Again the poisoned groups had lower vocabulary scores than the non-poisoned group: including this in the analysis of the other variables only lessened the contrast to a minor degree. No psychiatric symptoms were reported.

A study in California compared 45 agricultural pesticide applicators with a known prior history of cholinesterase inhibition (short of frank toxicity) with 90 without such a history (Ames et al., 1995). Nerve conduction and vibration sense were normal in both groups. Of a range of psychomotor, cognitive and mood measures, only one differed between the groups with better

performance on the serial digit test in those with prior history of cholinesterase inhibition. The authors are reassured that preventing acute OP poisoning may obviate chronic sequelae.

The effects of chronic exposure to low OP levels are difficult to detect (Beach et al., 1996). Psychiatric abnormalities on chronic exposure have long been believed to occur. In 1961, Gershon and Shaw (1961) reported on 16 cases in Australia in whom psychiatric sequelae to chronic exposure to OP pesticides were claimed. Of these, 11 had experienced acute episodes of schizophrenic and depressive type symptoms as well as more chronic symptoms. Psychological symptoms included severe impairment of memory and difficulty in concentration. By 12 months post exposure- "almost all reverted to normal". An epidemiological study, also in Australia, conducted in response to the Gershon and Shaw report evaluated the incidence of psychiatric admissions in Victoria (Stoller et al., 1965). No relationship to sales of OP pesticides was found, a crude measure of possible exposure. Furthermore, patients with OP-related symptoms, not admitted to hospital, would have been missed.

Another early study involved 2 crop-spraying pilots who developed psychiatric symptoms such as depression, phobia, and acute anxiety (Dille and Smith, 1964). It is interesting to note that it was common practice at that time for such pilots to take atropine to suppress the acute symptoms of exposure in order to continue work.

The study by Davignon and her co-workers (1965) compared 441 apple-growers exposed to OP pesticides with 170 persons living in the same environment and 162 other people with no OP exposure. Some neurological but no psychiatric differences were found.

An index of chronic exposure levels to OPs was developed and used to dichotomise 59 male workers (Korsak and Sato, 1977). Outcome measures included tests from a neuropsychological test battery and power-spectral analysed EEG. Plasma cholinesterase estimates were also made. Visuo-spatial impairment was detected in the Trail Making Test and the Bender Visual Motor Gestalt Test, with respect to the high- and low-exposure groups. Some EEG differences were also noted. The authors concluded that left frontal lobe function was particularly vulnerable to OPs.

A cross-sectional comparison was made of neuropsychological performance in 146 sheep farmers exposed to OP during sheep-dipping and 143 non-exposed quarry workers (Stephens et al., 1995). The General Health Questionnaire was used to assess "vulnerability to psychiatric disorder" and a battery of 8 neuropsychological tests was used. Exposed subjects were 1.5 times more likely to reach criteria for "caseness" on the GHQ ($p = 0.035$). The farmers performed significantly worse than controls on tests of sustained attention and speed of information processing. Covarying out pre-existing differences with respect to numerous inter-group differences still left test performance differences, but this post-hoc procedure is less rigorous than very careful propter hoc matching. Another criticism is that time-of-day and place-of-test effects may have acted differently on the two groups as the farmers were visited at home in the evening and the quarrymen were tested during the day at work. Furthermore, despite reluctance to wear adequate protective clothing, sheep farmers usually manage to avoid significant OP toxicity (Rees, 1996).

The data from the OP-exposed farmers were analysed further (Stephens et al., 1996). Correlations were sought between change in total reported symptoms following acute OP exposure through sheep-dipping and neuropsychological test performance at the time of assessment for chronic effects. None were found; indeed, all the correlations are close to zero. Although the investigators interpret this as indicating that chronic OP effects do not necessarily follow acute symptoms, a more conservative interpretation is that chronic effects are trivial.

Eyer (1995) stated that it is probable that neuropsychological and psychiatric effects (those most frequently occurring are in Table 4) can occur after OP poisoning. However, the same reviewer concluded that "the presently available data do not indicate that asymptomatic exposure to organophosphates is connected with an increasing risk of neuropsychopathological sequelae". This is essentially the conclusion of a team of psychologists who also reviewed the topic and called for more sophisticated and complex research (Mearns et al., 1994). Factors such as individual predisposition, type of compound, use of alcohol or concurrent illness or disability have not been explored. Longitudinal studies would be invaluable (Steenland, 1996), together with meticulous neurophysiological investigations. Thus, the question as to possible psychiatric and neuropsychological effects of low-level OP exposure remains unresolved. Despite long-standing interest in the topic (Kraybill, 1969), a recent conference involving doctors, farmers and others was unable to reach a conclusion backed by any adequate data (Seminar, 1995).

Table 4. Most frequent long-term symptoms following acute OP poisoning (Eyer, 1995).

Impaired vigilance and reduced concentration
Reduced information processing and psychomotor speed
Memory deficit
Linguistic disturbances
Depression, anxiety and irritability.

Organophosphate nerve agents

These are a rather diverse group of rapidly acting cholinesterase inhibitors (for review, see Marrs and Maynard, 1994). Many of the neuropsychiatric features of acute and subacute poisoning resemble those of OP pesticide poisoning but few data exist.

The study of Duffy et al. (1979) involved 77 workers with one or more documented exposures to sarin, but not within the previous year. The authors used both clinical and computer-analysed EEG to seek persistent brain abnormalities following acute exposure to the nerve agent, sarin. The controls were 38 workers from the same factory, not so exposed. The investigations comprised spectral analysis of recorded EEGs, visual analysis of routine clinical EEGs, and visual inspection of an all-night EEG. Some differences were found both during waking and sleeping and may be interpreted to reflect long-term sequelae. A range of statistical differences were found which tended to differ between computer and visual analysis and included increased beta activity, increased delta and theta slowing, decreased alpha and increased rapid eye movement (REM) sleep in the exposed population. It was concluded that acute exposure to sarin can result in persisting EEG changes.

In another study, 235 individuals reported as having been poisoned by an OP were interviewed (Tabershaw & Cooper, 1966). About a third reported persisting effects including unspecified neuropsychiatric symptoms.

Burchfiel et al. (1976) administered single doses (5µg/kg i.v.) or repeated doses (1µg/kg x 10 at weekly intervals) of sarin or single doses (4 mg/kg i.v.) or repeated doses (1 mg/kg i.m. x 10) of dieldrin to small groups of rhesus monkeys. EEG changes were induced which persisted for 1 year. The single large dose of sarin or dieldrin induced convulsions which may have accounted for the changes, a relative increase in beta (fast-wave) activity.

In a series of limited studies at CBD Porton Down, rhesus monkeys given a single, acute injection of sarin, to induce a 40% inhibition of red blood cell AChE, were shown to exhibit a long term change in EEG. The most consistent and greatest effect was a progressive increase in β_2 activity which was statistically significant after 26 weeks and was continuing to increase at the end of the experiment at 63 weeks (CDE Technical Paper (TP) 627,1991; CDE Technical Note (TN) 916, 1988). These changes were opposite to those seen in another study in which the a total dose of 10μg kg^{-1} of sarin was administered over a 10 week period at 1μg kg^{-1} per week (CDE Technical Paper 544, 1989). This higher dose produced a 60% inhibition of red blood cell AChE and the long term changes were characterised by shifts from high frequency β to low frequency θ activity. It was speculated that these changes were different to that of the single dose administration because of the production of tolerance and down regulation of cholinergic receptors. No visual signs of poisoning were observed after any exposure in these studies and there were no morphological changes apparent in any of the histopathological examinations of the brains from these animals. (CDE TP 627, 1991). All of these studies had significant experimental constraints in that the animals were tranquillised before restraint to measure EEG.

Subsequent to these studies methodology has been developed to permit long term monitoring of EEG by means of radiotelemetry with concurrent behavioural testing. All monitoring takes place in conscious unrestrained subjects in their home cage environment with minimal human intervention. These procedures have been adopted in a recent study in marmosets (Pearce et al 1999). Patterns of behaviour and EEG in a group of marmosets up to 15 months following administration of a single dose of sarin, which inhibited erythrocyte cholinesterase by 36.4-67.1%, were compared to behaviour and EEG in a parallel control group. No significant changes in EEG patterns were observed, although changes in β_2 energy approached statistical significance over time in that a mean increase of 11% was observed in sarin treated animals compared with controls ($p=0.07$). At this dose level, sarin induced no deleterious effects on long term performance of complex cognitive tasks.

In an early study of acute and multiple exposure of humans to anticholinesterase agents, Holmes and Gaon (1956) described the features of 449 cases of acute poisoning. They related these features to the index of severity of the exposure as assessed by the % reduction in RBC acetylcholinesterase. Some symptoms such as perspiration and dizziness were clearly exposure-severity-related; others including "nervous and irritable, mood changes" and fatiguability were not. After multiple exposures, some workers showed features of forgetfulness, irritability and impaired concentration. The authors also reported eliciting psychiatric symptoms in workers not directly exposed to OPs in whom physical symptoms and changes in cholinesterase levels were absent.

It is highly probable, on theoretical and experimental grounds, that severe nerve agent poisoning will lead to long-term irreversible changes in brain function. This should be manifest on behavioural, emotional and neuropsychological assessment. EEG changes will be present but their interpretation will remain unclear. In less severe, low-dose chronic poisoning, the data are less clear: nevertheless, the possibility of milder changes cannot be excluded. Nor can the possibility that such changes are slowly progressive.

Carbamate prophylaxes

Carbamates, such as pyridostigmine and physostigmine, are used as prophylactic medication against exposure to OP nerve gases. The question has been raised as to whether they, themselves, can cause long term neurotoxicity. A series of studies using a wide range of EEG and

neuropsychological tests have been carried out at CBD to investigate this possibility. In one set of studies, the only effects of pyridostigmine that were detected were rhinorrhoea and gastro-intestinal symptoms: anticipated effects of the drug. In a study of physostigmine, an increase in EEG percentage of theta activity (eyes closed) was detected. However, temporal effects might account for this, subjects being more relaxed on the second occasion, having been through the procedure before. Also, the possibility of a chance finding cannot be ruled out as multiple comparisons were made.

An intriguing but highly criticised and poorly controlled study has suggested that stress may alter the properties of the blood-brain barrier (Friedman et al., 1996). Of the carbamates used as prophylactics against nerve gas attack, pyridostigmine is believed not to enter the brain. However, in mice under stress (forced swimming) an increase in blood-brain permeability reduced the pyridostigmine ED_{50} for brain cholinesterase inhibition to a hundredth of normal. The implication is that central effects might be expected in personnel given pyridostigmine under combat conditions but this concept is not widely accepted.

Physostigmine is a tertiary carbamate which crosses the blood-brain barrier. In a limited study with a group of four monkeys, treated subcutaneously with this carbamate for 14 days, three showed no significant EEG changes. However one monkey had an increase in β2 activity which was apparent after seven days and persisted for up to 12 months. The significance of this change is unknown.

ELECTROPHYSIOLOGICAL EFFECTS

The electrophysiological effects of pesticides and nerve agents will be considered under the following headings:

- Introduction
- Carbamates
- Single fibre electromyography

Introduction

The acute electrophysiological effects of exposure to organophosphates on the nervous system are well recognised and have been documented in great detail. Both the central and peripheral nervous systems are affected, and it is likely that the effects are mediated by inhibition of acetylcholinesterase (AChE). Initially, excess ACh causes excitation, followed by inhibition of cholinergic transmission, probably by depolarisation block, although other more complex mechanisms may come into play. This leads to the expression of some or all of the acute CNS and PNS related symptoms, depending upon the degree of AChE inhibition.

An important question which remains unanswered, is whether or not repeated exposure to small amounts of organophosphorus compounds can produce delayed neurotoxic effects. A study of Stålberg et al (1978) would seem to suggest that repeated low-dose exposure in workers using organophosphorus pesticides can produce a mild motor and sensory neuropathy (see below).

It has been demonstrated that significant protection against the action of these CW OP irreversible nerve agents is afforded by carbamates such as pyridostigmine. It is, therefore, important to ascertain whether short term or long term exposure to carbamates might have any significant brief or longer lasting toxic effects on the central and peripheral nervous systems.

Single Fibre Electromyographic (SFEMG) Studies In Man And Animals

Organophosphate compounds irreversibly block the action of acetylcholine esterase and as a result produce a disturbance of neuromuscular transmission, which in the immediate phase is due to muscle fibre depolarisation. Other more complex changes of the physiology and structure of the neuromuscular junction may ensue, but these are highly speculative in nature and have not been studied in depth. Characteristically, in the acute toxic illness a repetitive muscle response is seen following a single shock to the nerve. Repetitive nerve stimulation produces an immediate drop in amplitude of the first and succeeding responses, particularly at relatively high rates of stimulation. Single fibre electromyography (SFEMG**)** also reveals abnormal jitter in these circumstances, both in humans and animal in vitro models. Studies on mouse diaphragm muscle preparations, pre-treated in vivo with sarin and soman, show variation in the latency or jitter of muscle action potentials following stimulation of the nerve. The effects are seen maximally three days after soman administration and 7 days after sarin administration, and the effects last up to 28 days. They can be ameliorated by pyridostigmine pre-treatment and prevented by treatment with oximes. These findings confirm that the early changes at least are likely to be due to the AChE inhibition and the consequent high levels of ACh at the junction. There is evidence from animal studies that necrosis may be induced in some muscle fibres, but this is unlikely to account for the jitter abnormality persisting for up to 28 days. This time scale probably reflects the time needed to replace the blocked acetylcholinesterase. However, there could well be other secondary changes occurring both pre-synaptically and post-synaptically, affecting the physiological events

controlling transmitter release and also structural arrangements of the post synaptic complex. It has not been confirmed that it is possible to distinguish between pre and post synaptic causes of jitter.

The studies by Baker and Sedgwick (1996) showed that a single exposure to a low dose of sarin can produce slight but significant increases in jitter which may be present for up to 30 months, but which returns to normal within two years. No explanation was forthcoming for the long term effects, but is unlikely to be due to continuing inhibition of the cholinesterase, which should have been replenished fairly quickly after the initial exposure. In the congenital myasthenic syndrome associated with acetylcholinesterase deficiency, secondary myopathic damage to the motor end-plate has been described, with reduction of the post-synaptic folds, which contain abundant voltage dependent sodium channels responsible for magnifying the effects of the AChR channel sodium influx for the production of the end-plate potential. The possibility of secondary excessive calcium influx (known to produce local muscle fibre damage) at the motor end-plate could be considered as a putative mechanism.

Lintern, Smith and Wetherell's studies on the effects of carbamates on acetylcholinesterase levels in mouse muscle, showed an eventual increase (120 hrs post exposure) in activity of the various forms of the enzyme following exposure to pyridostigmine. This excessive acetylcholinesterase activity could reduce the safety factor at some neuromuscular junctions and thereby account for an increase in the jitter.

The study of Stålberg et al (1978) on subjects exposed to organophosphorus pesticides showed no consistent differences in jitter between pre and post exposure (within 1 to 24 hours) studies, although abnormal jitter values were seen in some cases. Four out of 11 cases showed increased fibre density values before exposure and there was no significant change afterwards. It was felt that the increase in fibre density could reflect an underlying neuropathy which led to previous denervation and subsequent re-innervation, perhaps as a result of previous exposure to the organophosphates. A slight reduction in the sensory conduction velocities after work exposure was noted in 7 subjects, which was interpreted as indicating a mild sensory neuropathy in some of the workers. Thus, although this study did not demonstrate any significant immediate disturbance of neuromuscular transmission related to exposure, it did raise the possibility of a subclinical motor and sensory axonal neuropathy in subjects repeatedly exposed to organophosphorus pesticides.

Myasthenia gravis patients on treatment with pyridostigmine also show an increase in fibre density which is more marked than that seen in other myasthenic patients who are not on this drug (Hilton-Brown et al 1982). This was thought to indicate the presence of denervation and reinnervation changes in muscle, although a possible direct neurotoxic effect of the drug, as well as other mechanisms, were suggested.

CONCLUSIONS

Task 1.
To consider the results of research in animals and man on the long term toxic effects of anticholinesterase compounds with particular emphasis on organophosphate nerve agents and carbamate prophylactics.

1. The body of evidence in man and experimental animals on the acute toxic effects of OPs, and to a lesser extent carbamates, is considerable. There is evidence of short term and intermediate effects on cholinergic synapses as well as longer lasting effects on the neuromuscular junction with some compounds.

2. The neurophysiological evidence for long term effects in man of OPs, and physostigmine, on the central nervous system suggests that these changes are relatively minor and non-specific and, therefore, of uncertain functional and clinical relevance. Nevertheless, in view of the possible consequent neuropsychological and psychiatric effects, these changes cannot be dismissed. The crucial question of whether or not even single low dose nerve agent exposure may lead to subtle neurological changes, which could be progressive, still remains unanswered.

3. It remains unclear whether psychiatric and/or neuropsychological sequelae may follow repeated exposure that is below the threshold for causing acute detectable toxicity. Therefore, a high degree of alertness should be maintained since at present no definite conclusions can be reached.

4. The mechanisms whereby OPs and carbamates may produce the central nervous system changes are not fully understood but could be related to :

- inhibition of acetylcholinesterase activity and (resultant) changes in ACh receptors
- altered gene expression following chronic cholinesterase inhibition
- inhibition of neuropathy target enzyme and any possible non-enzymatic functions
- other mechanisms, such as effects on cytoskeletal proteins, which may lead to neuronal malfunction

Summary of Conclusions on Task 1

There is good robust evidence that high doses of organophosphates could have long term toxic effects on the peripheral nervous system (PNS) and skeletal muscle in man and animals, but the toxic mechanisms have not been established. There is more limited information about the long term toxic effects of organophosphates on the central nervous system (CNS). Nevertheless, evidence does exists of neurobehavioural changes following repeated exposures and the implications of potential long term adverse health effects of organophosphates is of considerable concern. Susceptibility to these toxic effects may be limited to a subgroup of the general population defined, for example, by genetic factors and may differ with different OPs.

Task 2.
To advise on the health risks to service personnel, including exposed volunteers and the need for continued health surveillance.

1.Despite the evidence for minimal toxic effects of pyridostigmine on the CNS and PNS in man, it

is unlikely that these compounds will cause any significant long term damage when administered as NAPS, particularly as they have been used therapeutically in the management of myasthenia gravis for many years, without any reports of significant untoward side effects.

2.The continued experimental use of these agents in human volunteers is, therefore, acceptable.

3. So far there has been no indication that carbamates can interact to cause long term adverse reactions or toxic potentiation with any other drug or bioactive agent such as antibiotics, antimalarials, vaccines or organophosphates. A role for NAPS in the aetiology of Gulf War Illness is, therefore, unlikely.

4. As a result of the current uncertainties concerning the long term toxic effects of organophosphates, and in the light of the neuropsychiatric, neurological and neuropathological evidence reviewed by the Working Party, the present suspension of human volunteer multiple exposure to systemic organophosphate nerve agents should continue.

5. Low dose single exposure to an organophosphate nerve agent is probably safe, but in the present climate of public opinion, and with increasing concern about the effects of organophosphate sheep dips, the political wisdom of conducting further human exposure needs to be seriously considered, and very strong military justification for such experiments would be required. Quite apart from any stance taken by this Working Party, the MOD's Ethics Committee will, of course, take an independent view on any new research protocols submitted. The question of whether human experimentation with low dose single exposure is ethical depends on a careful assessment of the risks of the procedure and possible benefits to military personnel and cannot be prejudged.

Summary of Conclusions on Task 2

As a result of current uncertainties regarding the potential long term toxicity of organophosphate nerve agents the suspension of human volunteer exposure studies should continue.

There is little evidence that the prophylactic use of carbamates (e.g. as NAPS) is associated with any long term adverse health effects alone or by interaction with other medication. However, this possibility does need to be fully explored.

RECOMMENDATIONS FOR FURTHER RESEARCH

The recommendations of the Working Party for research into putative toxic mechanisms and to further inform health risk assessments have been subdivided into :-

A. Biochemical Research

There are three model systems that could be utilised for obtaining the required biochemical data :

1. In vivo animal models : which relate biochemical measures to kinetics and neurophysiological effects :

> Studies of the effects of organophosphate dosing on NTE activity and molecular forms of acetylcholinesterase will help to elucidate the physiological roles of these enzymes, the different forms of acetylcholinesterase and the mechanism of altered responses to multiple exposures.
>
> Mechanism of upregulation of RNA and protein synthesis following acetylcholinesterase inhibition should be determined.

2. *In vitro* models : which are suitable for mechanistic studies, such as those relating effects on the functional AChE form to ACh levels, receptor density and to markers of secondary effects and apoptosis (calcium proteases). *In vitro* systems could be used to modulate the effects.

3. More precise studies on the turnover of the various molecular forms of AChE at the neuromuscular junction, using fluorescent antibody or radioisotope techniques, if they are available and suitable for this type of investigation, are required.

B. Physiology and Pathology Research

Any physiological studies will need to be linked to detailed histological examination of brain tissue, neurones, neuromuscular junctions and cholinergic synapses as well as enzyme activities: -

1. Histological and ultrastructural examinations of the neuromuscular junctions are needed in animal models as well as motor point biopsy studies in humans suspected of suffering from acute, subacute or chronic organophosphate poisoning. These would complement parallel studies on the mechanism of the changes in “jitter” in animals and the SFEMG in man. A more comprehensive electrophysiological study of the effects of organophosphates on a broad range of parameters, including membrane conductances and fibre densities, is required. It is possible that some of the long term jitter changes reflect neuropathic damage, and examination of the nerves for evidence of terminal and ultraterminal sprouting and light microscopic examination of the motor end-plates for changes in gross structure as a result of repair processes, would help clarify this issue.

2. The use of data on exposure to, and effects of, organophosphate pesticides as a "surrogate model" for nerve agent poisoning is worth pursuing. However, the toxicity differences between organophosphate pesticides and organophosphate nerve agents, and differences within subject groups, together with lack of information regarding dosage, exposure etc., may render such comparisons impossible at this stage e.g. the interindividual differences in activation of pesticides.

3. Human biomonitoring studies are required to develop improved and validated biomarkers. Observations following exposure to multiple low doses of organophosphates in the mouse have implications for the toxicity of organophosphates to man, who is generally exposed

occupationally to low levels over an extended period of time. This would lead to the development and validation of biomarkers of central effects for use *in vivo* in man.

4. Chronic administration of sarin resulted in a shift from high frequency β to low frequency θ activity in the EEG. These changes are nonspecific and their functional significance is unknown. Nevertheless they do indicate that OPs may have a long term effect on the central nervous system, and because of this, further studies should be undertaken to try and elucidate the possible pathophysiological mechanisms. Studies utilising evoked potentials in animal models may be useful. Correlates between neurophysiological, behavioural, cognitive and EEG changes in a higher primate species should be established.

C. Clinical Research

Clinical studies would help answer several outstanding questions :-

- What is the evidence that exposure to anticholinesterases may lead to chronic neurological damage, both peripheral and central ?
- What differences exist, comparing pesticide organophosphates and nerve agent organophosphates, in susceptibility to neurological damage ?
- Can acute exposure, or low level chronic exposure, to nerve agent organophosphates, lead to a state of lowered resistance to the later development of neurological disease, particularly degenerative conditions such as Alzheimer's disease ?
- Are drugs, such as pyridostigmine and the oximes, used in the management of organophosphate poisoning, themselves capable of causing long term neurological effects ?
- Significant protection against the action of OP nerve agents is provided by carbamates, such as pyridostigmine, so it is important to determine whether short or long term exposure to carbamates has any significant brief or longer lasting toxic effects on the central and peripheral nervous systems. Such research could be carried out initially using model systems (see above).

There are many ethical constraints on the nature of any clinical studies which could address these issues, especially in the light of the recommendation that the suspension of human volunteer studies should continue. Consequently, it is recommended that experimental and animal research takes priority to inform a human health risk assessment and indicate the most relevant protocol for confirmatory studies in man.

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