MRC Institute for Environment and Health

Organophosphorus esters:

An evaluation of chronic neurotoxic effects

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1 General introduction

1.1 Background to this review

This review of the available scientific and medical literature presents an evaluation of the possibility of chronic neurotoxicity resulting from exposure to organophosphorus pesticides and medicines. In addition to reviewing those publications that have directly addressed this matter, an attempt has been made to provide a broader practical and theoretical toxicological context. It was felt important to do this because the available studies did not provide an unambiguous answer, and need to be discussed in a wider context. In addition, the nature of organophosphorus agents, the extent of their distribution and the conditions leading to exposure vary widely; these factors should be considered if comparisons between studies are to be made.

This review is based on a comprehensive search for reports of acute and chronic toxicity linked to organophosphates in general, and to any of the specific agents used for sheep dipping in the UK. Those primary references judged relevant were reviewed, and are discussed here. However, it should be noted that only a very limited number of non-English language references are included. A number of minor case reports are briefly mentioned for the sake of completeness and historical interest, although they are of much less significance than the major collections of cases included in other papers. A number of reviews were also consulted, and any references mentioned which had not appeared in the primary searches were examined. Animal studies are mentioned only where relevant. All comments apply to human subjects unless otherwise stated.

Many of the key studies discussed in this review are epidemiological. Most are retrospective and cross-sectional in design. Particular attention has been paid to a number of key factors crucial to the conduct and interpretations of such investigations, in particular to the selection and matching of exposed and control subjects. Where possible, the size of the sampled population, the selection criteria and the participation rate are given. However, in many cases, particularly in earlier publications, this information was not available. Several authors have discussed potential bias introduced by selection, several have mentioned (and attempted to deal with) confounding variables, and a few have restricted their conclusions to the establishment of an *association* between organophosphorus pesticide exposure and a biological effect. However in a number of studies these qualifications were not made, and some (for example, Savage *et al.,* 1988) appear to draw unreasonably specific conclusions. Hence the conclusions drawn in this review may differ from those of the authors.

To enable the reader more readily to evaluate the biological as well as statistical significance of findings, most changes are expressed as percentages. This may distort the presentation of the results in some cases and, where necessary, problems are pointed out in the text. A number of neuropsychological tests of varied design are grouped together in summary tables as a necessary simplification. In these tables the studies with the largest group size are presented first.

1.2 Organophosphorus esters

Organophosphorus esters were first shown to have biocidal uses in 1937, and are now widely used as pesticides in agricultural, public health, veterinary, domestic and medicinal applications. Other organophosphorus esters are used as lubricants and plasticisers, and there is human exposure via all of these applications. Organophosphorus warfare agents represent a further very specialised group. The hazards associated with acute exposure to any of these classes of organophosphorus agents are now well recognised. The only agents with which this review will concern itself are organophosphorus insecticides. These are in use throughout the world, and although for some uses they have been displaced by the synthetic pyrethroids, their efficacy, low environmental persistence and low production cost will probably ensure their continuing use well into the future as an important component of integrated schemes for pest control. The acute mammalian toxicity of some agents can, however, present problems; this and the evolution of resistant pests represent their major disadvantages.

The toxicology of organophosphorus pesticides has been intensively studied, and several distinct forms of toxicity have been recognised. Most forms of toxicity are well understood and can be explained at the mechanistic level, and in this report these well-recognised forms of toxicity will only be summarised. The possibility that other hazards may exist as a result of single or repeated exposures to organophosphorus pesticides, which may produce lasting and previously unrecognised harmful effects, is reviewed in more detail. A number of studies have already been made with the objective of testing such hypotheses. Finally, consideration is given to how generalisable observed effects are, that is are effects:

- ***common to all organophosphorus pesticides (as is acetylcholinesterase (AChE) inhibition);***
- ***common to a number of organophosphates but not others (as is delayed polyneuropathy); or***
- ***restricted to just one or two specific agents (as is isodiazinon porphyria).***

Organophosphorus agents intended to act as pesticides are selected for their common (class) action on AChE, and all, therefore, produce similar anticholinergic effects at toxic doses. However it is important to realise that actions on other non-selected targets (such as plasma pseudo-cholinesterase) can be much more variable and may be strongly dependent on the individual agent. Hence no general class effect can be assumed for these non-cholinergic effects.

Much of the present concern in the UK relates to sheep dips. The spectrum of agents used in the UK for sheep dipping is limited and differs from that used for other applications. The only agents approved for current UK use are propetamphos and diazinon. However licences have expired for a number of agents only relatively recently (Browning, 1995; Table 1.1). In producing this report it has been assumed that reports of toxicity from any organophosphorus pesticide are potentially relevant to organophosphates used in dips. However it is obvious that some compound-specific effects may not be relevant, given the relatively limited range of organophosphorus agents used in the UK.

Table 1.1 Organophosphorus agents no longer licensed

Agent	Year of licence expiry
Bromophos	1988
Chlorpyrifos	1989
Iodophenos	1990
Coumaphos	1991
Chlorfenvinphos	1994

2 Mechanisms of action

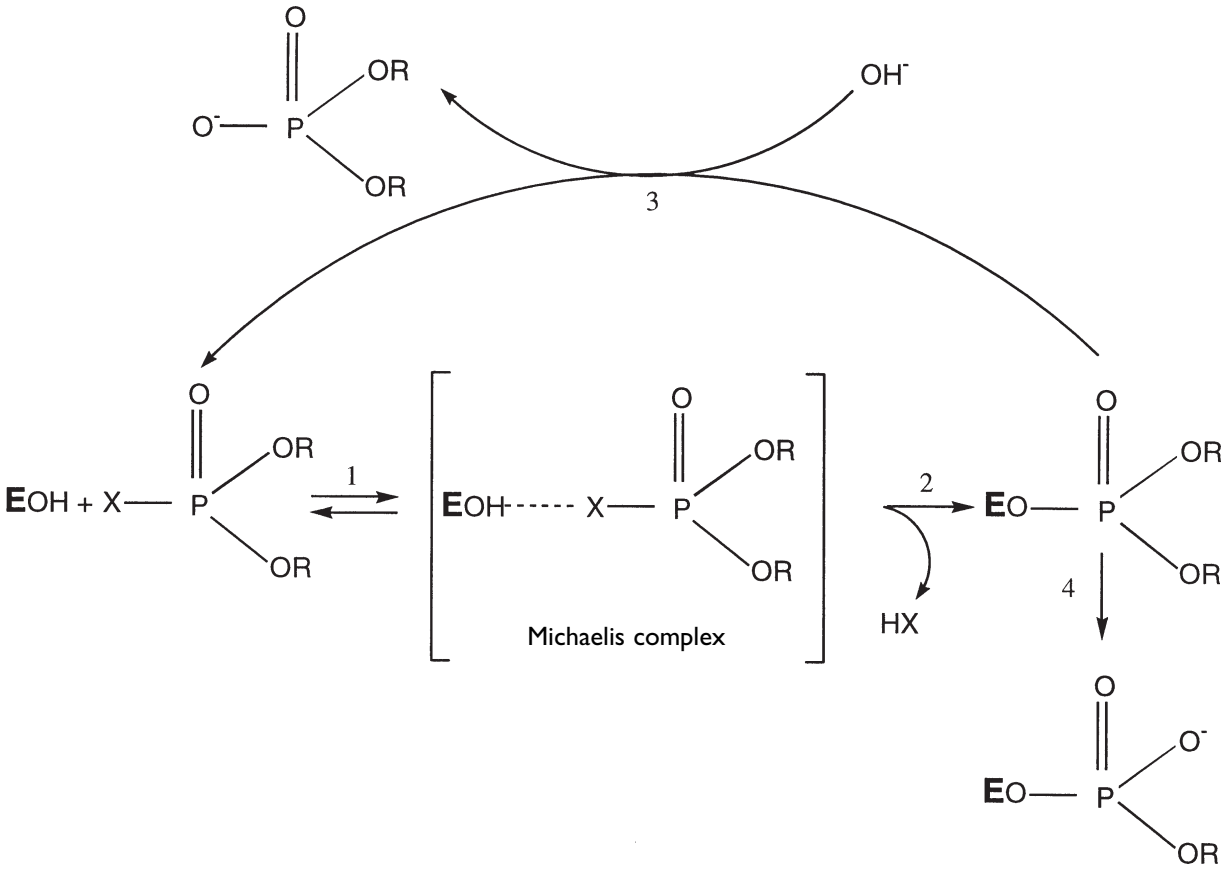
2.1 Background

The toxicology of organophosphorus and carbamate pesticides has been reviewed extensively (WHO, 1986; Marrs, 1993; Richardson, 1995). These man-made esters mimic normal biological ester substrates (such as acetylcholine; ACh) and interfere specifically with the function of ester-hydrolysing enzymes, for which they form a 'surrogate substrate'. The basic steps involved in the interaction of organophosphorus pesticides with esterases are summarised in Figure 2.1. After the reversible formation of the Michaelis–Menten complex by reaction 1, the leaving group is eliminated in the covalent reaction 2. For the hydrolysis of ACh by AChE the leaving group is choline. Reactivation of the enzyme by reaction 3 takes place within a fraction of a second for the acetyl group left from ACh, but is very much slower if the substrate is an organophosphate. This delay leads to prolonged inhibition. For example, the overall half lives for hydrolysis (essentially determined by reaction 3) of the AChE–organophosphorus adducts are 4.6 hours for dimethoxy phosphorylated enzyme in human plasma and 1400 hours for diethoxy phosphorylated enzyme (Mason *et al.,* 1992). For N-alkyl phosphoramidates, little or no spontaneous reactivation may occur, but for S-alkyl agents it may be rapid (Johnson, 1992). Di-isopropylphosphorofluoridate (DFP) — sometimes used in experimental studies — similarly shows little or no reactivation. There are different structure–activity relations with other esterase targets, including the neuropathy target esterase (NTE), but the general principle is the same.

A further complicating effect is introduced by a second covalent reaction, that is 'ageing' of the various phosphorylated forms of the inhibited enzyme (reaction 4). This ageing involves covalent cleavage of a bond within the enzyme–substrate adduct, and the formation of a negative charge which stabilises it. Once this ageing reaction has occurred no equivalent of reaction 3 can then take place, the enzyme being irreversibly inactivated. The rate of this ageing reaction is also highly enzyme and agent specific. The half lives of the ageing reaction on AChE are 4.3 hours for dimethoxy compounds and 10.7 hours for diethoxy compounds (Mason *et al.,* 1992). This means that the ageing reaction can be much faster than the spontaneous reactivation reaction for diethoxy compounds. Phosphinylated and carbamylated esterases do undergo reaction 3, but do not age (reaction 4). After ageing has occurred the only way to regain activity is by *de novo* synthesis of the enzyme. Synthesis of brain AChE or NTE occurs with a half life of about 5 days. Since reactions 3 and 4 determine the duration of enzyme inhibition, the nature of the leaving group (X) is important only in determining which enzyme is targeted. Hence organophosphorus pesticides can usefully be classified on the basis of their basic structure and 'R' groups. To aid the reader, all of the agents mentioned in this review are classified in this way in the Appendix.

Most of the targets of organophosphates are therefore proteins with either this ester-hydrolysing or an analogous capacity (Figure 2.2). The principal targets are AChE, NTE, those A-esterases and carboxyesterases important in the xenobiotic metabolism of organophosphorus or other esters, the blood clotting

Figure 2.1 General scheme of interactions of an organophosphate with an esterase (EOH)



Steps in the interaction of an esterase with an organophosphorus inhibitor: 1, formation of Michaelis complex; 2, phosphorylation of enzyme; 3, reactivation (spontaneous or forced by oximes); 4, ageing

cascade, and a number of esterase and protease activities of unknown biological significance (such as plasma pseudocholinesterase). Not all of these potential targets are equally vulnerable to specific agents, and not all will be affected at non-lethal doses of the agent.

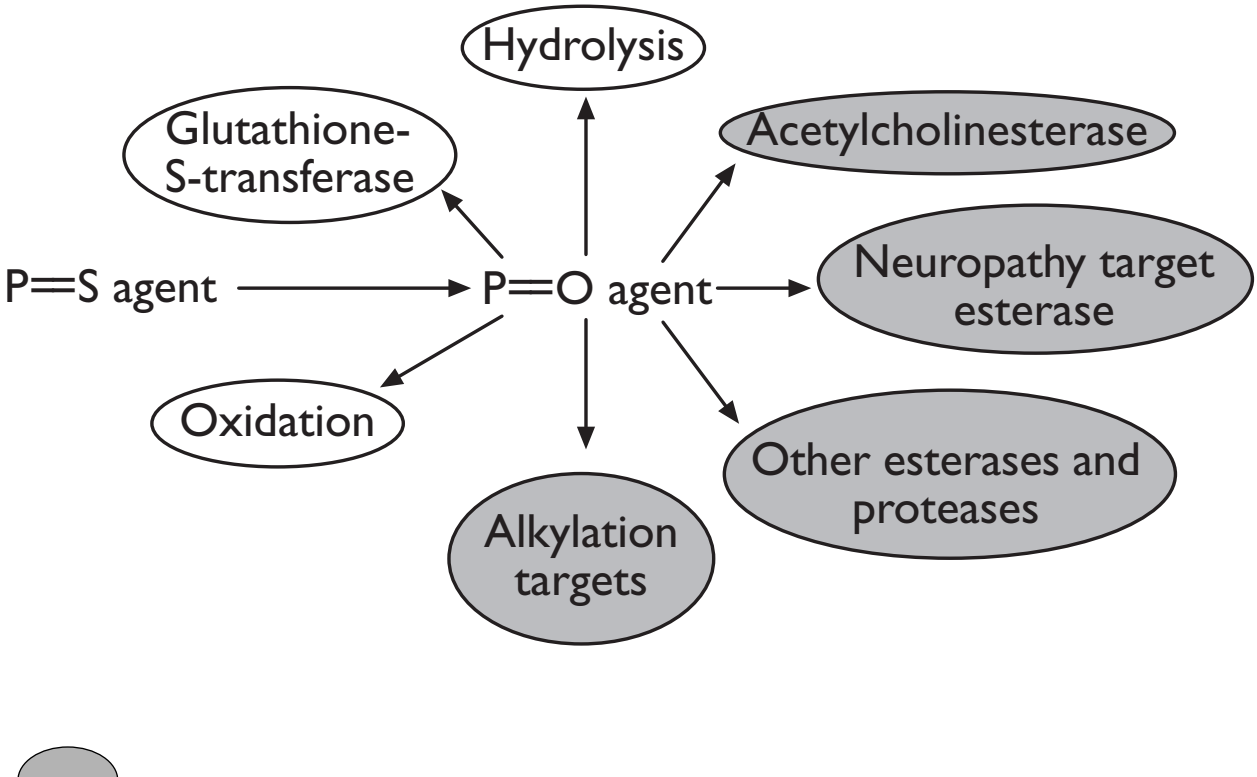
There is good evidence from *in vitro* studies of an additional, direct action of some organophosphorus pesticides and carbamates on cholinergic receptors (see Camara *et al.,* 1997) which can result in the blockade of muscarinic receptors, activation and blockade of nicotinic receptors, and action at glutamatergic and γ-aminobutyric acid (GABA)ergic synapses. However, the relevance of these findings to the clinical or even the experimental animal situation is far from clear. It is difficult to relate these essentially reversible, time-independent effects on receptors to the irreversible, time-dependent effects on cholinesterase (ChE) (Richardson, 1995). The nominal concentrations of organophosphorus agents that are needed to act on receptors are in the range of those which inhibit AChE *in vitro*, but whereas the receptor effects would be stable with time, the *in vitro* ChE inhibition would become progressively greater (until metabolism removed the agent). Hence it is difficult to compare the effective dose across preparations with different target concentrations and metabolic capacities. It is therefore a matter of conjecture whether the tissue dose levels needed to act on receptors in this way would be survivable. This fundamental question could be answered relatively simply by making AChE measures in the same tissues and at the same time points as the receptor measures. It is of interest that methamidophos, which failed to show any such direct action on receptors in a range of *in vitro* systems is, however, still capable of producing the same full range of acute, intermediate, and delayed polyneuropathic syndromes in man as other agents

(Camara *et al.,* 1997). This finding argues against a significant role for non-anticholinesterase effects in the major forms of toxicity produced by organophosphorus agents.

The efficacy of organophosphorus agents as pesticides relates to inhibition of AChE in the target species. The resistance of mammals to organophosphorus pesticides is due to mammals' higher capacity for metabolic inactivation and, in the case of phosphorus thion (P=S) agents, the time provided for this by the process of bioactivation. These P=S agents have little or no inhibitory activity in the absence of oxidative bioactivation. It is important to note that mammalian resistance is almost wholly a function of detoxification capacity, rather than any inherent resistance of the mammalian target proteins. This means that metabolic and pharmacokinetic factors dominate the toxicology of these agents (Figure 2.2).

Another distinctive feature of the toxicology of organophosphorus agents is that, because of the progressive covalent nature of the enzyme–organophosphate reaction and of the irreversible nature of the ageing reaction, the extent of their biological effects is dependent on the product of concentration and time (Johnson, 1992). This is in contrast to the reversible equilibrium reactions commonly seen in pharmacology, which eventually come to a time-independent steady state. Thus with organophosphorus agents *in vitro*, high concentration-short time effects and low concentration–long time effects can be equivalent. Thus the rate of biological disposal of organophosphorus esters *in vivo* is especially important since this not only limits the duration but also the severity of effects. For agents such as chlorpyriphos or demeton-S-methyl, which persist for a relatively long time in the body (particularly after dermal exposure), the inhibitory oxon product may continue to react with target enzymes over several days. This pharmacokinetic effect does not, however, alter the relative sensitivity of the various enzyme targets.

Figure 2.2 The principal biological interactions of organophosphorus agents



2.2 Acute toxicity

Acute intoxication with organophosphorus agents involves a complex mixture of muscarinic and nicotinic responses which vary in relative severity with target organ, dose and agent (McLeod, 1985; Davies, 1990; Marrs, 1993). The duration of intoxication depends on the pharmacokinetics of the agent. Typically it lasts from 1 to 5 days, but may extend beyond 14 days depending on the stability of the agent, the degree of sequestration in adipose tissue (which lacks detoxifying enzymes), and the rate of absorption. There is good evidence that tolerance can develop to the acute cholinergic effects of ChE inhibitors (Schwab & Murphy, 1981; Hudson *et al.,* 1986; Richardson, 1995). This leads to diminishing cholinergic signs during continued exposure, due to a combination of down-regulation of cholinergic receptors and functional adaptation to increased cholinergic tone. There are both good theoretical grounds and some experimental animal evidence (Schwab & Murphy, 1981) to suggest that this functional adaptation can also occur in response to sustained subsymptomatic levels of AChE inhibition. Furthermore, it is a common clinical observation that a given level of continuous ChE inhibition has less adverse effect than an acute fall (Gallo & Lawryk, 1991). Such a protective mechanism would be relevant in cases of sustained or gradually increasing exposure profiles, and would have the effect (under these specific conditions) of partially dissociating ChE inhibition from anticholinergic signs, and of slightly decreasing the ratio of acute to any delayed effects. Acute toxicity is further described in Section 4.

2.3 Intermediate syndrome

Intermediate syndrome has been described as a late complication of some cases of severe acute poisoning (Senanayake & Karalliedde, 1987) usually of suicidal, but, in at least one case, occupational origin. It is characterised by the development of *proximal* muscle weakness immediately after the acute cholinergic crisis (1–4 days), which can lead to respiratory failure. The proximal limb, neck and respiratory muscles are involved, yet grip strength may be preserved. The effect lasts for 5–18 days, and may relate, at least in part, to the muscle end-plate necrosis effects described in Section 4.4.1. In one case of fenthion poisoning in man where measurements were made (Sedgwick & Senanayake, 1997) there was an increased single fibre jitter and discharge failure rate (blocking) at the time of maximal weakness on day 7. Since the necrosis effect is enhanced by motor activity in experimental studies, this would explain the particular involvement of the respiratory muscles in man. Alternatively, intermediate syndrome may be a consequence of enhanced phagocytosis of chronically desensitised receptors (Sedgwick & Senanayake, 1997). This latter explanation would not require morphological changes to take place in affected muscles, simply a large decrease in receptor density.

2.4 Organophosphorus pesticide induced polyneuropathy (OPIDPN)

Organophosphorus pesticide induced polyneuropathy (OPIDPN; also designated OPIDN or OPIDP) is characterised by a distal distribution and delayed onset of polyneuropathy (Cavanagh, 1982; Richardson, 1995). Younger persons and animals are relatively resistant to OPIDPN. There are few morphological changes seen prior to the onset of axonal degeneration and functional loss 10 to 14 days after a single, short-term exposure, other than an accumulation of smooth endoplasmic reticulum in axons. The nature of the subsequent damage closely resembles Wallerian degeneration, and the lesion is highly selective. There is damage only to the distal axons of sensory, motor and autonomic neurones, with the perikarya and proximal axons being unaffected. In mild cases degeneration may be confined to the extreme distal processes of motor axons within the muscles. Some larger-diameter fibres are particularly sensitive, especially the proprioceptive afferents from muscle spindles, although nerve diameter is not the only factor influencing vulnerability. Within the central nervous system the longer spinal tracts in the spinal cord, medulla and cerebellum are involved. Again only the distal ends of tracts (i.e. central ends of ascending tracts and peripheral ends of descending tracts) are involved. The distal nature of the damage is reflected in a distinctive ‘stocking and glove’ distribution of the tingling and numbness, and by foot and wrist drop preceding knee and hip weakness. Although a sensory syndrome with little motor involvement has been reported to be associated with chlorpyrifos exposure

(Kaplan *et al.* 1993; see Section 4.4.2), more comprehensive investigations have found that OPIDPN is primarily a motor phenomenon. Thus Moretto & Lotti (1998) found that, of a series of 11 patients severely poisoned with a range of organophosphorus pesticides including chlorpyrifos, three developed OPIDPN. Sensory involvement (either paraesthesiae, loss of vibration sense, or decreased sensory nerve conduction velocity) was seen in only two patients, both of whom developed a pattern of OPIDPN characterised by more severe motor than sensory effects. The remaining case of OPIDPN showed a purely motor involvement, and none of the nine patients who failed to develop OPIDPN showed any sensory effects once the acute cholinergic crisis was over.

Damage to the lower motor neurone is reversible, but not that to the upper motor neurone. Thus in a series of 12 high-dose intoxications with neuropathic agents (Vasilescu & Florescu, 1980), a marked clinical improvement was seen in the peripheral (lower motor neurone) function over one to two years, but none in the central (upper motor neurone) effects. This damage leads to a progression from flaccid to spastic paralysis. There do not appear to be any direct effects of OPIDPN on cognitive function, although the trauma of the associated acute intoxication may produce its own consequences (see Section 4.4.2).

Many studies have shown that the nature of the toxicity (i.e. acute cholinergic toxicity or OPIDPN) produced by an agent can be predicted by its relative ability to interact with AChE or NTE. This has been demonstrated for both pesticidal (Lotti & Johnson, 1978) and non-pesticidal agents (Gordon *et al.,* 1983; Freundenthal *et al.,* 1993). Absolute neuropathic potential is determined by a combination of this specific interaction with NTE and by general pharmacokinetic factors. A number of agents which have a relatively low potency to react with NTE still have the potential to produce OPIDPN, but only at dose levels which would normally be acutely lethal. Such agents have produced OPIDPN in people who have survived severe intoxication by receiving intensive care therapy. No current pesticides produce OPIDPN at dose levels which fail to produce severe acute cholinergic signs. Pesticides known to be neuropathic at some dose on the basis of NTE inhibition and clinical or animal effects are amiprophos, chlorpyriphos, coumaphos, cyanofenphos, S,S,S-tri-n-butyl phosphorotrithioate (DEF), dioxabenzophos, O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN), dichlorvos, haloxon, isofenphos, leptophos, merphos, methamidophos, metrifonate, mipafox, merphos and trichloronat (WHO, 1986; Marrs, 1993). A number of non-pesticidal organophosphorus agents, such as DFP and tri-o-cresylphosphate are also neuropathic.

Several regulatory studies of organophosphates currently or previously used in UK sheep dip have shown them to have no potential to produce delayed polyneuropathy in animal tests. As an illustration of this, a typical high-dose test for delayed neuropathic potential (WHO, 1998) involved 20 hens given a single oral dose of 100 mg/kg diazinon. Intensive prophylactic treatment with physostigmine and atropine therapy for up to 48 hours resulted in survival of 17 hens, despite a peak inhibition of brain ChE of 83%. This level of ChE inhibition would have rapidly proved lethal without anticholinergic therapy. No ataxia was seen over a 28-day monitoring period, and no inhibition of either brain or spinal cord NTE was seen at either 24 or 48 hours in five hens. Long-term feeding trials have similarly failed to show neuropathic effects, but these cannot be carried out at such dramatically high dose levels since it is not possible to protect hens from the lethal anticholinesterase effects over longer periods.

Although all the agents clearly linked with OPIDPN in man have also produced neuropathy in the hen, there is a possibility that metabolic differences between hen and man in bioactivation or bioinactivation may lead to over- or underestimation of risk, particularly where stereoisomers are involved. However, the application of findings from OPIDPN tests in hens to human risk assessment is rendered more certain by the additional mechanistic information that can be derived from studies of NTE inhibition and ageing. Human lymphocyte NTE, as well as hen NTE, is available for such assessments *in vitro*. Although hen studies can rarely be conducted at much above what would be the lethal dose level in unprotected birds, ratio measures of NTE : AChE can be used to calculate safety margins for the bioactivated (oxon) forms independent of such limitations. Consequently the safety assessments

currently carried out for acute neuropathic potential can be viewed with confidence, and the NTE index can be used to calculate a margin of safety. This margin of safety is larger for some agents than for others, and methamidaphos and chlorpyrifos might be mentioned as agents with an adequate, but measurably lower margin of safety for neuropathic effects. With regard to chronic effects, experimental studies in hens (Lotti & Johnson, 1973) have shown that chronic multiple dose exposure to neuropathic organophosphates yields an NTE inhibition threshold for neuropathy which is only about 10% lower than that seen following a single acute exposure. This very modest additional effect of chronic over acute exposure is much as would be predicted from the relatively rapid rate of removal and resynthesis of fresh target protein in nervous tissue, which has a half life of about 5 days.

The severity of OPIDPN can be enhanced by a subsequent exposure to so called 'promoters', that is, agents which are not neuropathic in themselves but enhance the degree of neuropathy produced by other agents. Phenylmethanesulphonyl fluoride is capable of increasing the severity of experimental OPIDPN lesions by a factor of three (Randall *et al.*, 1997), possibly by inhibiting repair of the lesions. The nature and distribution of the lesions produced remain the same as they would have been had a higher (non-promoted) dose of the original agent been given. This phenomenon is of considerable theoretical interest, but does not represent a major clinical problem as the decrease in neuropathic dose levels reported are relatively modest, and exposure to promoters is quite rare.

2.5 Possible contribution of OPIDPN to chronic low-level effects

A number of studies have reported changes in exposed populations which might be consistent with a very mild OPIDPN, of threshold severity. Thus the study of McConnell *et al.* (1994) showed relatively poor vibrotactile response in workers who had been poisoned with methamidaphos (Section 4.4.2), that of Stokes *et al.* (1995) showed lesser vibration sensitivity in non-poisoned workers, and that of Kaplan *et al.* (1993) showed a range of effects after domestic chlorpyrifos exposure. In none of these studies were the indices measured sufficient to provide an unambiguous diagnosis of OPIDPN, and many other studies have failed to detect neuropathic effects. Hence, given the far lower sensitivity of NTE than AChE to all approved organophosphorus pesticides, it must be considered most unlikely that either acute or chronic OPIDPN could follow exposures to any of these which were insufficient to produce clear cholinergic signs. The safety margin may be lower for some agents, and the ratio of cholinergic to neuropathic effects may be decreased by cholinergic habituation with some chronic exposure patterns (see Section 2.2), but it is still unlikely that classical OPIDPN could be produced by low-level exposure. When considering the potential for low-level exposures to cause OPIDPN, it must also be remembered that in a survey of many clinical studies of unambiguous OPIDPN, those cases which showed the least severe effects also showed the greatest degree of functional reversibility (Cavanagh, 1973). Consequently it is less likely that any very mild OPIDPN would be a chronic condition.

2.6 Other effects

AChE is known to have additional actions in non-cholinergic systems. AChE is transiently expressed in developing non-cholinergic neurones at the time of neurite extension, and is a soluble secreted product of several cell types including dopaminergic neurones in the substantia nigra and adrenal chromaffin cells (Appleyard, 1994). Secreted AChE has a partially characterised neuromodulatory action in the substantia nigra and cerebellum that is not reproduced by butyrylesterase. It is therefore possible that these developmental and neuromodulatory functions of AChE may be affected by organophosphorus esters at similar dose levels to those which affect cholinergic transmission, and that these might complicate the relatively simple pattern of cholinergic hyperactivity.

Several actual or potential toxic actions of certain organophosphorus agents have been described which cannot as yet be related to a surrogate-substrate-like effect on identified enzymes, or surrogate-neurotransmitter-like effect on receptors. Their molecular mechanism of toxicity is as yet unknown. Examples include the *porphyria cutanea tarda* produced

by isodiazinon (Collins *et al.,* 1982), the pulmonary toxicity of malathion impurities (Verschoyle & Cabral, 1982), and the selective inhibition of non-shivering thermogenesis produced by DEF (Ray, 1980). It is also possible that high doses of organophosphates may have a direct (non-cholinergic) action on nerve transmission. Anderson & Durham (1985) gave rats a daily dose of 17% of the acute LD_{50} of DFP for up to 20 days and found that DFP decreased sciatic nerve action potential duration at days 10 and 20, and increased conduction velocity by 17% ($p<0.05$). However, soman and parathion had no significant effect; the effect may be limited to OPIDPN-inducing agents.

A distinctive pattern of ataxia and flaccid paralysis, appearing after 9 to 25 days and associated with axonal degeneration in the fore-brain, has been described in rats given triphenyl phosphite (Lehning *et al.* 1996). In the rat this effect of triphenyl phosphite predominated, whereas, although the effect was also seen in hens, OPIDPN was the predominant effect in this species. The central axonal degeneration produced by triphenyl phosphite was most prominent in the rat neocortex, thalamus and brainstem, and terminal degeneration was still more widespread. In contrast, DFP, an agent producing classical OPIDPN in both species, only produced central damage in the axons of the spinal tracts. Triphenyl phosphite is an NTE inhibitor, but the mechanism whereby triphenyl phosphite might produce this novel neuropathic effect is as yet uncertain. However, from the animal studies so far conducted it appears that classical OPIDPN would probably be the main effect in man.

Metrifonate is known to enhance cognitive performance in rats, but it has been hypothesised that this is not related to AChE inhibition (van der Staay *et al.*, 1996). A dose level of metrifonate which failed to inhibit brain AChE significantly (10–30 mg/kg given 60 minutes before the daily learning trial) did produce significantly accelerated learning in the Morris water maze. Dichlorvos (the active metabolite of metrifonate) and DFP had a similar but smaller effect at sub-AChE inhibiting doses. Higher doses impaired learning, presumably by a cholinergic mechanism (see Section 4.4.2). The ChE inhibitors THA, E2020 and physostigmine, however, failed to affect learning at or below brain AChE inhibiting doses. Paraoxon failed to enhance maze learning at sub-AChE inhibiting doses. The putative non-cholinergic mechanism by which learning is enhanced is unidentified, but a number of potential targets exist in the brain (see Section 6.4).

Persisting ocular effects of chronic, low-level exposure to organophosphorus agents have been described in a number of Japanese language publications reviewed by Dementi (1994). Myopia and other often slowly reversible effects, plus occasional more severe retinal damage, were found in a number of clinical and experimental investigations conducted in the 1960s and 1970s when occupational exposure levels were sometimes high. Other contemporary studies, however, failed to find such changes. These effects have not been described in surveys conducted outside Japan, although exposures associated with marked inhibition of ChE can produce acute ocular effects in man and in experimental animals; such effects can sometimes be slow to resolve (Bouma & Nesbit, 1995). Nohara & Segawa (1996) reported marked pupil constriction and ocular pain associated with cillary spasm in 51 patients exposed to sarin. The constriction slowly resolved over 7 days, and was attributable to ChE inhibition. No lasting effects were seen in this study. It is most unlikely that retinal damage would have been missed in such studies, and hence effects were probably either not causally linked to organophosphate exposure at all, or linked to a solvent or impurity no longer in use. However, an association between organophosphorus pesticide exposure and the lesser effect of myopia would not be so obvious, especially if only seen in one ethnic group. The sometimes rather protracted after-effects of intoxication on vision might perhaps be the result of an action on second messenger systems peculiar to the retina, as was proposed by Tandon *et al.* (1994). They gave rats a dose of fenthion sufficient to produce inhibition of neocortical AChE by 85% and retinal AChE by 89%, and found that receptor-mediated inositolphosphate release was still depressed in the retina at 56 days, although there was no effect on neocortical release at any time. AChE was still significantly depressed at 56 days but had shown marked recovery, whereas inositolphosphate release showed little sign of recovery. These changes in inositolphosphate release were not, however, accompanied by any retinal damage.

In a single case report (Michotte *et al.,* 1989) of a suicide attempt with bromophos, plasma ChE fell by 90% but there were no associated cholinergic signs, and after five weeks there was development of a cerebellar type ataxia which largely resolved by 10 weeks. This time course and nature of effects have not been reported elsewhere.

As with other pesticides, it has been suggested that organophosphorus agents produce effects on the immune system (Richardson, 1995). Given the putative role of cholinergic signalling in the control of immune function, this is not an implausible suggestion. However, the results of many investigations are difficult to interpret because of the non-specific stress produced by pesticide intoxication and the marked immunosuppressive effect of stress itself (via corticosteroids). An action at subcholinesterase inhibiting doses has, however, been described for malathion (Rodgers & Xiong, 1997). Mice given daily oral doses for 14 days showed mast cell degranulation in the peritoneum, skin and heart, as well as the small intestine (the latter being probably a local effect). This degranulation was associated with enhanced peritoneal macrophage function, but no increase in serum histamine was seen. Actions on the neuroimmune axis are beyond the scope of this review.

Some organophosphorus agents have the capacity to alkylate other molecules, including DNA, but this latter effect is not thought to have any significance *in vivo*, since lethal effects occur at much lower doses and shorter times. The question of the possible existence of novel targets is further discussed in Section 6.4.

2.7 Individual susceptibility

Since mammalian resistance to organophosphates is largely a matter of rapid metabolic inactivation and excretion, and since P=S oxidation plays an important part in determining vulnerability, individual susceptibility is strongly influenced by individual metabolic capacity. This individual susceptibility would apply to both acute and chronic exposure, and relates to two factors: inherent metabolic capacity and interactions with other xenobiotics. The latter effect has been recognised for some years (WHO 1986); heavy co-exposure to other agents which share the same metabolic disposal route enhances organophosphorus pesticide toxicity by competition for, or inhibition of A-esterases. The question of inherent metabolic capacity is more complex, since organophosphorus esters are broken down by spontaneous hydrolysis, and by esteratic, de-alkylating and oxidative metabolism (Figure 2.1). Hence it is not always possible to determine which process is the most important for a given target tissue (Richardson, 1995). In mice and rats the developmental rise in serum paraoxonase levels seen at 20 or 25 days after birth coincides with increased resistance to organophosphorus esters (Li *et al.*, 1997); this issue has been clarified by recent experiments with a paraoxonase (PON1) knockout mouse (Furlong *et al.,* 1997, and personal communication, July 1997). Such mice have no ability to hydrolyse paraoxon, and have 20 times less ability to hydrolyse chlorpyrifos than normal mice. They also showed a dramatic increase in brain AChE inhibition on dosing with chlorpyrifos, which suggests that paraoxonase is rate limiting for chlorpyrifos disposal, at least in mice.

In a survey of 127 normal human subjects, serum paraoxonase varied sixfold (Mutch *et al.,* 1992). Most human studies have demonstrated a minority population with a plasma enzyme possessing a markedly lower ability to hydrolyse paraoxon. In an extensive survey Kalow (1982), found the low-activity group to be 50% of British, 21% of Indian, 14% of Kenyan, 14% of Malay and 33% of Chinese subjects. Nevin *et al.* (1996) found 42% of American subjects to be in the low-activity group. The enzyme is termed paraoxonase, but can hydrolyse a wide range of organophosphorus agents. The minority (poor paraoxon hydrolysing) isoform is actually, and somewhat confusingly, better at hydrolysing diazinon than the normal isoform. All individuals show age-related differences, paraoxonase activity being low at birth and not reaching adult levels until 21 days in rats or 2 months in man.

Individual susceptibility is also affected by a wide, but incompletely characterised, variation in liver detoxification capacity. However it is important to note that any decreased systemic detoxification capacity, either with age or across individuals, would confer similar sensitivity to *all* manifestations of toxicity. Thus it

would not alter the ratio of acute toxicity to other effects. This ratio could only change if there were to be individual variation in local target tissue metabolism or target organ vulnerability, and no such effects have been described.

Developmental toxicity is usually associated with similar levels of exposure to those producing adult toxicity, suggesting that there is no particular predisposition in young animals. However, Song *et al.* (1997) produced effects on adenyl cyclase by treating neonatal rats with doses of chlorpyrifos on postnatal days 1–4, resulting in only 25% inhibition of brain AChE. This dose was 20% of the LD_{50}, but produced no weight loss. Brain adenyl cyclase activity was decreased, an effect which outlasted the depression of AChE. This effect on adenyl cyclase was less apparent at a later developmental stage, despite an undiminished anticholinergic effect. Hence the neonatal adenylate cyclase system may be more sensitive either to disruption of cholinergic signalling, or to chlorpyrifos *per se*, than it is later in development.

3 Exposure measures

3.1 Background

In any evaluation of the causation of biological effect it is just as important to have a measure of exposure as it is to have a measure of effect. It is also important to know the identity of the agent since, although many biological effects are class effects, some specific actions and all relative potencies are highly agent specific. Since organophosphorus agents can degrade to more or less toxic products on storage, the identity of the agent can be difficult to determine in practice. An instance of this was the outbreak of apparent malathion poisoning in Pakistan, eventually attributed to an impurity introduced during manufacture of malathion (Baker *et al.,* 1978). Where acute effects of organophosphorus agents occur a number of appropriate measures of exposure are available, but unfortunately this is not the case for chronic effects.

3.2 Cholinesterases

Cholinesterase activity may be measured using a number of substrates and in a number of tissues. In this review ChE activity in whole blood, erythrocytes or plasma/serum will be referred to as blood, red cell, or plasma ChE, and changes will be reported as percentage inhibition, rather than as a percentage of the normal value. Irreversible inhibition of ChE remains detectable as long as the target is still present in the body, which means that it gradually declines (over the course of about seven days in the case of plasma ChE, and about 120 days in the case of red cell ChE) as a result of resynthesis of these enzymes. However inhibition by agents (such as carbamates) which allow spontaneous reactivation is much more short lived (see Section 2.1).

Inhibition of erythrocyte AChE can be used to monitor exposure, and has the additional advantage of being a good surrogate for the more biologically relevant target, brain AChE. Plasma ChE is somewhat more convenient to measure than red cell ChE, but does not so closely parallel brain ChE in terms of structure–activity relations. In man, the ratio of red cell to plasma ChE inhibition varies widely (Mason *et al.,* 1992), from 0.12 : 1 (for chlorpyrifoxon) to 1.64 : 1 (for maloxon). Therefore whilst plasma ChE can provide a convenient and often sensitive measure of exposure, and enable comparison between studies of the same agent, it is far less useful for making comparisons between agents or for studies of mixed exposure. Lymphocyte NTE is another surrogate target, being closely similar to brain NTE but, like brain NTE, it is only sensitive to the small number of specifically neuropathic organophosphorus agents.

All of these surrogate targets have the advantage of being sensitive and responding to a range of agents, but they also have the disadvantage of providing a measure which is inherently transient. Spontaneous reactivation of the pseudo-target, or new synthesis to replace covalently inhibited enzyme, limits their usefulness. A further problem of all of these measures is that the normal baseline value of the pseudo-target enzyme varies between individuals. This means that, unless this individual baseline value can be determined by making a pre-exposure or post-recovery determination, only inhibition in excess of the inter-individual variability (about 25% in the case of human erythrocyte AChE) can be considered significant. In a survey of 127 subjects, normal serum and red cell ChE varied 2-fold, and lymphocyte NTE 4.5-fold (Mutch *et al.,* 1992). In addition, a technical problem may occur if samples are stored, as

the inhibited enzyme can undergo spontaneous reactivation if ageing has not already occurred. The rate of this reactivation can be significant for organophosphates if the blood sample is kept at room temperature, dimethoxy compound inhibited AChE reactivating with a half life of 14.6 hours at 22°C (Mason *et al.,* 1992). Reactivation in stored blood also represents a major problem for the monitoring of carbamate effects, although this problem can be overcome by freezing the sample in liquid nitrogen or storage in acidic buffer.

3.3 Urinary metabolites

Organophosphorus agents themselves can be measured in blood but as many (though not all) biological half lives in blood are short and the concentrations are always very low it is more practical to measure urinary metabolites. Urinary metabolites have been used as monitors of exposure in a number of investigations, and the topic has been reviewed recently (Gompertz & Verschoyle, 1996). There are, however, some difficulties with this approach. Firstly, since the metabolites are rapidly excreted, the time of sampling needs to be well matched to exposure. With transient dermal exposure complete excretion can take as long as two days. If samples are collected too early or too late then exposure will be underestimated, although this is less of a problem for truly continuous exposure which reaches a steady state. A further difficulty relates to the nature of the metabolites, which varies with the structure of the agent, making comparisons of mixed agent exposures very uncertain. Urinary metabolites also register P=S agents even if excreted without bioactivation to the P=O analogue. Although measurement of the sum of alkyl phosphates and thiophosphate gives a wholly valid measure of total internal dose, thiophosphates do not contribute to the toxic dose if excreted intact. This problem does not arise when ChE inhibition is used as an index, since ChE is insensitive to any P=S agents that might be excreted before bioactivation. Urinary metabolites do, however, provide a more sensitive index of exposure.

A wide range of urinary metabolites has been measured, possibly owing to such factors as different agents generating different metabolites, differences in the timing of sample collection, and also because some putative metabolites can be generated by non-pesticidal sources (Niven *et al.*, 1996). Such complications make interpretation of low-dose exposure data very difficult, although in the case of higher exposures (see Table 3.1) the findings are much clearer. The data also show that it is unwise to make too direct a comparison between studies, even when the nature of the exposure appears superficially similar.

3.4 Exposure modelling

Because of the transient nature of blood and urine markers, the only estimates of low or moderate level exposure to organophosphorus agents that are available for retrospective studies are those based on exposure modelling. Such models draw on information such as the frequency and nature of use of the agent, the adequacy of protective clothing or other countermeasures to exposure, and the rate of skin or respiratory uptake. Such exposure estimates are reasonably accurate in defined circumstances, such as controlled field trials, and form an important component of regulatory risk assessment. However, when based on subjective recall over a period of years, they are likely to give an inaccurate estimate of actual exposure. Some activities, such as the mixing and diluting of pesticides, take relatively little time but can account for most of an individual's exposure. Similarly, biologically significant but (from the viewpoint of the subject) incidental exposures, such as those caused by eating or smoking whilst handling pesticides, (Niven *et al.* 1996) may be forgotten. Such errors in estimating individual dose would decrease the power of any study which relied on them as a measure of exposure. In the case of occupational exposure these errors might be biased in the direction of underestimation in circumstances where an implication of lack of care for recommended safety measures might be made.

Table 3.1 Urinary metabolites reported in studies of occupational exposures to pesticides[a]

Group	Pesticide	Metabolite(s) nmol/mole creatinine	mean values for specified metabolites and (range)	Reference
Sheep dippers, morning after	diazinon	DEP+DETP	43	Stephens *et al.* (1995a)
Controls	–	"	5	
Sheep dippers, immediately post-dipping	diazinon	DMP, DMTP, DEP, DETP	8, 7, 14, 11 = 40 (0–106)	Rees (1996)
Sheep dippers, pre-dipping	–	"	7, 10, 6, 3 = 26 (0–79)	
Sheep dippers, immediately post-dipping	propetamphos	"	6, 4, 4, 1 = 15 (0–40)	
Sheep dippers, pre-dipping	–	"	13, 0, 5, 0 = 18 (0-42)	
Sheep dippers, post-dipping	diazinon & chlorfenvinphos	DMP, DEP, DMTP, DETP	13 (1–51),12 (1–63),9 (1–38),19 (1–98)	Nutley & Cocker (1993)
Sheep dippers, pre-dipping	"	"	3 (1–8),4 (5–7) ,17, 8 (1–12)	
Sheep dippers post-shift	diazinon	DEP+DETP+DEDTP	25 (6–57)	Niven *et al.* (1996)
Sheep dippers pre-shift	–	"	14 (3–54)	
Sheep dippers post-shift	diazinon	DMP+DMTP+DMDTP	26 (6—72)	
Sheep dippers pre-shift	–	"	16 (6–43)	
Sprayers, post-shift	diazinon	DETP, DMTP	6 (0–239), 2 (0–25)	Maizlish *et al.* (1987)
Sprayers, pre-shift	–	"	3 (0—30), 2 (0–32)	
Controls	–	"	1 (0–21), 2 (0–21)	
Farmers, spray season	methidathon, parathion, pyrimiphos-methyl	DEP, DMP	138 (<5–380), 410 (<6–1815)	Richter *et al.* (1992a)
Farmers, pre-season	"	"	64 (10–108), 84 (12–260)	
Controls	–	"	<5, <6	
Sprayers, highest workload	guthion, phosphamidon, chlorpyrifos, phosmet, diazinon	DMTP	65 (4–212)[b]	Stokes *et al.* (1995)
Sprayers, medium workload	"	"	39 (2–147)[b]	
Sprayers, lowest workload	"	"	21 (1–77)[b]	
Formulation workers	malathion etc.	DMP, DEP, DMTP, DETP	21 (2–352),30 (1–386),39 (1–320), 22 (1–93)	Nutley & Cocker (1993)
Bystanders, complaining of symptoms	methidathon, parathion, pyrimiphos-methyl	DEP, DMP	207 (84–458), 332 (283–603)	Richter *et al.* (1992a)
Bystanders, not complaining	–	"	74 (10–197), 66 (30–422)	
Infant, domestic exposure	diazinon	DEP, DETP	30, 9	Wagner & Orwick (1994)

[a] Values are means, but this can be misleading as most distributions are highly non-linear, with a few high values and many low ones
[b] values have been normalised to a standard urinary creatinine of 13 mmol/l

DMP, dimethylphosphate; DMTP, dimethylthiophosphate (O,O-dimethylphosphorothioate); DMDTP, dimethyldithiophosphate
DEP, diethylphosphate; DETP, diethylthiophosphate (O,O-diethylphosphorothioate); DEDTP, diethyldithiophosphate

3.5 Subjective measures of exposure

A final factor which is important only in subjective exposure assessment is the distinctive smell associated with organophosphorus agents. Sometimes the pure agent has little smell, but minor breakdown products, such as mercaptans, which in themselves represent little threat to health, can be both very odiferous and very volatile. Hence smell can be dissociated from exposure. This may be an important source of confusion in subjective assessments of non-occupational or bystander exposure, as illustrated by the evacuation of 196 pupils from an elementary school due to the smell of an organophosphorus pesticide being correctly used in a field 100 metres away. Of a sample of 65 pupils and teachers, 71% claimed illness (upset stomach, headache and dizziness) lasting between 3 minutes and 2 days. Of the 35 cases where blood/plasma ChE was examined, none was outside normal limits, clinical examination showed none had cholinergic signs, and 63% were wholly asymptomatic (Baker & Selvey, 1992). It appears that the subjects suffered an acute stress reaction as a result of an inappropriate hazard alert triggered by smell — there being no significant exposure.

4 Acute syndromes

4.1 Background

Many of the studies of frank intoxication with organophosphates address issues relevant to this review. Most such poisoning relates either to high exposures associated with unsafe working practices or to suicide attempts. Although not a direct concern of this review, selected aspects of these high-dose effects will be described for two reasons. Firstly, it is important to establish dose thresholds in order to define low-level exposure; sometimes the presence or absence of signs of acute toxicity provide the only indicator of exposure in studies of putative chronic or low-level effects. Secondly, the degree of reversibility of poisoning can be used to provide some measure of the nature of any persisting hazard associated with lower-level exposure. Thus, for example, if individuals were found to recover uneventfully from severe intoxication it could reasonably be presumed that the same would be true of lower-level exposures of the same nature. It would also be expected that any adverse effects would be more obvious and easier to detect in cases of high exposure. However, the occurrence of an effect at high dose does not necessarily imply its presence at lower doses, although the absence of an effect at high dose does provide a reasonable indication that it is unlikely to be seen at lower doses of a similar nature and duration in a similar population.

4.2 Incidence of poisoning

Exposure to frankly toxic levels of organophosphorus pesticides is regularly reported in the world literature; for example 41 000 cases were reported from 27 Chinese provinces in 1993 (He, 1996). In Poland, organophosphates accounted for 8.5% of all occupational poisonings in 1978 (Kedzierski, 1990). Given that the effects of such exposures are now well described, only those which are clinically unusual or in some other way notable are likely to be published. Furthermore, since most poisonings now occur in developing countries, the published literature probably greatly underestimates the worldwide incidence of poisoning. For example, in a survey of 633 Nicaraguan farm workers, 83% of whom reported current pesticide use, 25% reported having had signs of poisoning over the previous year. Of these poisonings, only 35% were reported to the Pesticide Poisoning Registry (Keifer *et al.,* 1996).

It was estimated that 2800 cases of intoxication occurred in the 1976 episode of poisoning in Pakistan, caused by the manufacture of malathion containing isomalathion and other agents as impurities, which markedly potentiated toxicity (Baker *et al.,* 1978). The falls in red cell ChE in the individuals spraying the pesticide correlated well with the level of isomalathion impurity in the sample that they used.

A dramatic example of the incidence of moderate acute intoxication in some tropical countries is given by a prospective cohort study of farmers spraying organophosphorus pesticides (Kishi *et al.,* 1995). A sample of 228 farmers was selected from a population of 625. All agreed to participate, with only a 6% drop out. Unfortunately no measures of dose were made, but farmers worked 3–4 hour shifts with few precautions, and often with wetted skin. Three or more signs of

intoxication per shift were seen in 21% of the sample when compared with the same sample later in the non-spraying season. Salivation increased 17-fold, dizziness 4.1-fold, blurred vision 4.2-fold, excess sweating 7.3-fold and hand tremor 9.2-fold. Incidence was related to spray frequency.

In the UK the situation is different. Pesticide deaths represented 1.1% of all UK poisonings over 1945–1989 (Casey & Vale, 1994), and organophosphates accounted for only 7% of pesticide deaths over this period (68 in total), most of these being suicides. Acute intoxication with organophosphorus pesticides is rare in the UK, where there were less than 20 incidents per year over 1994–7 (Weir *et al.*, 1992). It is estimated that in 1991 300 000 people were potentially exposed to sheep dip pesticides (Cook, 1992), and of those there were 34 reports of poisoning, 29 occupational and three accidental (Murray *et al.* 1992). Most involved non-specific influenza-like symptoms, and of nine cases where blood was examined only three showed a significant decrease in red cell ChE.

The reason for this low incidence is the relatively low exposure potential of UK agricultural pesticide use. Thus in a study of exposure of sheep dippers Niven *et al.* (1996) found no significant fall in plasma or red cell ChE in 32 individuals, the largest fall being 9%. Diethylphosphate (DEP) and diethylthiophosphate (DETP) were found in urine at 227 and 138 nmol/mmole creatinine (DEP+DETP) in the two most highly exposed individuals, but concentrations increased by less than 20 nmol/mmole creatinine in 28 of 32 cases. Exposure was relatively low for all but a few individuals, who were exposed at a similar level to spray workers (see Table 3.1).

In the study of Stephens *et al.* (1995a; see also Section 5.3.1) there were no specific indications of acute or delayed organophosphorus pesticide intoxication after sheep dipping. A health questionnaire showed a generally low incidence of effects, with the exposed population having lower scores than controls, including lower behavioural arousal, both before and after dipping. Many of the measures (notably global symptoms and the distractor items) in the exposed group showed less of a fall at a second interview (24 hours after dipping) than in the controls. As the exposed population showed no specific indications of classical acute organophosphorus toxicity, the authors attributed most of the differences seen to normal fatigue associated with the hard physical work of dipping. Urinary metabolites indicated a similar level of exposure to that reported by Nutley & Cocker (1993), but greater than that reported by Rees (1996). It is likely that few of the participants in these studies of sheep dippers wore the recommended protective clothing, and this is specifically stated by Rees (1996); none of his subjects did so. Stephens *et al.* (1995a) reported that although 90% of the dippers in their study wore foot and leg protection, only 23% wore gloves, and that 32% drained the sheep dip by manual bailing. Furthermore, 87% reported being splashed by dip and 6% being soaked.

Acute intoxication is not as uncommon in other countries as in the UK. It is also likely that pesticide poisoning is not always recognised as such (Biskind & Mobbs, 1972), and may go unrecognised and unreported.

4.3 Acute toxicity and duration of effects

Many experimental animal studies have addressed the question of threshold doses for acute effects. One typical but particularly thorough example is discussed here. Nostrandt *et al.* (1997) gave a range of single doses of chlorpyrifos to rats and measured behaviour, brain, muscle and red cell ChE, and muscarinic receptor density. Motor activity was unaffected at 60% inhibition of brain AChE, but impaired at 60–70% inhibition (80% for red cell AChE). Compensatory down-regulation of muscarinic receptors was seen only at a dose producing 90% inhibition of brain AChE, although other workers have produced this effect after lower but repeated doses.

A number of studies have addressed the question of dose threshold and duration of poisoning in man. These studies fall into three groups: studies of organophosphate warfare agents, human medicines, and pesticides. All, however, show similar effects. Several of the pesticide studies have involved very large groups of subjects. An important caution for these studies must be made: although very high-dose poisoning is

unambiguous, moderate or mild acute poisoning shows some similarities with other conditions, such as influenza or the neurological form of Lyme disease. Furthermore, those signs and symptoms normally considered to be classically cholinergic, such as headache, eye irritation, tiredness, memory and concentration problems, gastrointestinal disturbance and anxiety, are also reported in a number of other unrelated conditions heavily influenced by demographic and psychosocial pressures, such as sick building syndrome, video display terminal use and proximity to overhead power lines (Spurgeon *et al.,* 1996). Hence signs such as these must often be categorised as only 'possible' intoxications in the absence of more specific indicators, especially in studies where exposure is poorly defined. A typical example of such a study where causation is unclear is the report of a man with a pre-existing psychological disorder who experienced nausea, headaches, fatigue and anxiety for more than two years after treatment of his home with organophosphate pesticides (Rosenthal & Cameron, 1991).

4.3.1 Cholinesterase inhibition and acute toxicity

Data are discussed below and summarised in Table 4.1, which indicate that signs and symptoms are usually experienced in individuals once red cell ChE falls by more than 30–50% for either long-term or short-term exposure, but that population surveys have generally shown symptoms associated with lower exposure levels. Evidence of causation is weaker in population studies, and the degree of specificity of the effects is less for lower doses. It also should be noted that some researchers have found a poor correlation between ChE inhibition and signs (Wadia *et al.,* 1974), and this may be related to the analytical problems discussed in Section 3.2. The specific investigations are described below. As a precaution it is normal to remove pesticide workers from the risk of further exposure if their individual red cell ChE falls below 70% of normal levels (Gallo & Lawryk, 1991).

It should, however, be noted that animal studies have clearly proven that the threshold for and severity of cholinergic signs are determined by the balance between AChE inhibition on the one hand, and the reactive down-regulation of the cholinergic system which is seen in response to AChE inhibition on the other. The mechanism of this down-regulation appears to be complex, as was illustrated by the finding of Chaudhuri *et al.* (1993) that chlorpyrifos produced fewer signs of poisoning in rats over the first 2 to 4 days than did parathion. This occurred despite both agents producing similar inhibition of brain AChE and similar reactive decreases in brain muscarinic receptor density. The authors hypothesised that chlorpyrifos and parathion produced their differential toxicity via a differential effect on presynaptic autoreceptors which regulate ACh release; they were able to support this by demonstration of up-regulation of some incompletely characterised receptors by chlorpyrifos and down-regulation by parathion. Hence there are good theoretical grounds to expect that, although AChE inhibition is the primary determinant of the severity of cholinergic signs (and probably the sole determinant of acute ones), the longer-term response is reduced by a number of secondary factors, possibly both presynaptic and postsynaptic.

Controlled dose studies

A well-controlled clinical study of 93 subjects with experimental sarin poisoning (Bowers *et al.,* 1964) showed subjective and objective slowing of mental ability, with tenseness and inability to maintain a train of thought once whole blood ChE fell by more than 60%. These effects preceded nausea and were followed by threatening dreams that night. Subjects were usually reported as being normal by the next morning. On the basis of data from rabbits (Bowers *et al.,* 1964) this peak blood ChE inhibition would correspond to roughly 50% inhibition of brain ChE.

Baker and Sedgwick (1996) conducted a controlled study of the effect of a 30-minute exposure to sarin as part of an electromyography study. Miosis and photophobia were shown by all eight subjects, and individual red cell AChE values fell by 35–49%. These effects resolved within 2 days, but AChE values were still depressed by 40% at 3 days, illustrating adaptation. No other gross signs of poisoning were seen.

Table 4.1 Esterase inhibition associated with acute signs and symptoms

Exposure	% Decrease in ChE			Main effects	Reference
	Red cell	Unspecified or whole blood	Plasma		
Short-term	92		84	unconsciousness, flaccid paralysis	Bobba *et al.* (1996)
	54		98	dizziness, nausea, vomiting, abdominal cramps	Aden-Abdi *et al.* (1990)
	43			miosis	Baker & Sedgwick (1996)
		60		mental slowing	Bowers *et al.* (1964)
	34		31	salivation, nausea, vomiting	Bobba *et al.* (1996)
	16		19	more complaints	Bellin & Chow (1974)
Long-term	27→43		83→93	transient abdominal cramps	Maxwell *et al.* (1981)
			50	miosis, sweating	Bhu *et al.* (1976)
		50		slowed reaction time	Durham *et al.* (1965)
	40–60			none	Becker *et al.* (1996)
		39		dizziness, muscle cramps	Bouma & Nesbit (1995)
			32	no effect	Brenner *et al.* (1989)
			23	more miosis & headache	Misra *et al.* (1988)
	20			more diarrhoea	Ciesielski *et al.* (1994)
	19		13	transient EMG changes	Verberk & Sallé (1977)
			7	more complaints	Richter *et al.* (1992a)

Metrifonate has been used as a treatment for schistosomiasis in millions of people since 1955. The active form of metrifonate is the insecticide dichlorvos, which is produced slowly from metrifonate by enzymic and spontaneous conversion. Reports of adverse reactions are rare (Cioli *et al.*, 1995) and usually involve acute gastrointestinal problems. In a prospective study 55 children were given oral metrifonate in three doses over six weeks for treatment of schistosomiasis (Maxwell *et al.,* 1981). A placebo was given to 26 controls. Red cell ChE fell by 27%, 35% and 43%, respectively, at 6 hours after each successive dose, and plasma ChE by 83%, 90% and 93%. This sustained fall was associated with abdominal cramps and discomfort in a few cases if the dose was taken without food, but no other signs. Electromyogram (EMG) measures were made just before and at 6 hours after the final dose, and showed normal conduction velocity but a small increase in EMG amplitude and repetitive activity in three of 55 individuals after the dose. This well-controlled study suggests a threshold for neuromuscular effects at between 35 and 43% inhibition of red cell ChE. In another trial of metrifonate (Onadeko, 1979), children were given the same dosage regimen as that used by Maxwell *et al.* (1981) of 1–3 doses each of 10 mg/kg at 2 week intervals, but followed up for longer periods of 3 months to 3 years. Of four treated once, nine treated twice, and seven treated three times, only one child showed transient colicky pain.

In another clinical study of metrifonate for control of schistosomiasis, 16 volunteers were each given a single oral dose at one of four levels (Aden-Abdi *et al.* 1990). The highest dose produced a mean peak plasma ChE inhibition of 98% and red cell ChE inhibition of 54%, this dose produced salivation, colic, nausea, vomiting and dizziness. The next highest dose only produced some salivation, with a 94% fall in plasma and a 34% fall in red cell ChE. Plasma levels of the agent fell with a half life of 2.2 hours. Both peak red cell ChE and peak plasma metrifonate levels provided a good correlation with all individual symptoms with the exception of salivation, which was only poorly correlated with dose.

Metrifonate has also been evaluated as a therapeutic treatment for Alzheimer's disease (Becker *et al.,* 1996). Weekly doses were used to maintain red cell AChE in the range of 40–60% inhibition in 50 patients for 3 months. The rate of cognitive deterioration in the treated patients was less than in those given a placebo, and no treatment-related adverse effects were seen.

A clinical study of 16 volunteers given oral mevinphos daily for one month recorded a fall in red cell ChE of 19% and plasma ChE of 13% at four weeks (Verberk & Sallé, 1977). The mevinphos caused no clinical signs but a small transient increase in Achilles tendon reflex force at the fourth week only ($p<0.05$), and no change in adductor pollicis EMG. Ulnar nerve fast fibre conduction velocity was unchanged, but the slow fibres showed a 7% fall through the entire study ($p<0.05$). However the experimental group started the study with a conduction velocity well above that of the control group and showed a downward trend even before dosing began, so this small change may well have been unrelated to the agent.

In rats, the spontaneous electroencephalogram (EEG) has been found to be a sensitive measure of exposure to a range of organophosphorus pesticides. In a series of elaborate studies Nagymajtényi *et al.* (1995) showed that continued treatment caused the spectral energy in the β frequency range of the EEG to increase both in absolute terms and, more significantly, relative to lower frequencies. Daily treatment with dimethoate, dichlorvos, or parathion-methyl at 4% of the acute LD_{50} caused a significant relative increase in this high-frequency energy (i.e. a decrease in the EEG index). There was a small but significant fall in brain ChE activity. The study was also continued over three generations (with daily dosing), and significant effects were seen at 1% of the LD_{50} in the second and third generation. These dose levels did not significantly depress brain ChE. The greater effect in the second and third generations may reflect their exposure as neonates, since individuals of the first generation were only exposed as adults. Earlier single high-dose studies had shown a single (ultimately lethal) dose of dimethoate to produce a similar change in EEG energy within 60 minutes of dosing at a level causing 30–35% inhibition of brain ChE (Dési *et al.,* 1991). Rats born to dams given dimethoate or parathion-methyl (at 3% of the acute LD_{50}) with continued dosing through to the first 8 weeks after weaning showed a significant change in EEG energy 4 weeks after the last dose (Laslo Nagymajtényi, personal communication, July 1997). There was a small, non-significant fall in brain ChE at this time. The effect was not significant if dosing was stopped at weaning, but it is not clear whether this reflected a specific sensitivity during the post-weaning period or resulted from the longer time available for recovery in the latter group. Unfortunately the degree of reversibility of these interesting and sensitive effects has not as yet been investigated by these workers. The nature of the EEG changes seen in these rats resembled that described by Burchfiel & Duffy, (1982) and Burchfiel *et al.,* (1976) in monkeys and humans exposed to sarin.

Uncontrolled dose studies

A study of 200 consecutive cases of suicide attempts with organophosphates, in which diazinon was the most common agent, showed miosis in 95%, fasciculation in 27%, muscle weakness in 26%, impaired consciousness in 10% and primary convulsions in 1% (Wadia *et al.,* 1974). The longest latency to death was 130 hours. A poor correlation between these signs and depression of red cell ChE was reported. In three of 11 cases where EMG measures were made there was a transient EMG decrement, but nerve conduction velocity was unchanged even a few hours before death. Of a larger series of cases reported in the same paper, 140 of 1500 cases were fatal.

In a study of 37 patients acutely poisoned with either ethyl parathion or fenitrothion, signs of intoxication were generally well correlated with ChE inhibition (Bobba *et al.,* 1996). A subgroup with severe poisoning (unconsciousness and flaccid paralysis) had a fall of 92% in red cell ChE and 84% in plasma ChE. For moderate poisoning (weakness and fasciculation) the equivalent falls were 81% and 60%, while for mild poisoning (salivation, nausea and vomiting) they were 34% and 31%.

In a study of 20 organophosphate pesticide spray handlers with several years' occupational exposure, signs and symptoms such as narrowed pupils, fatigue and inappropriate sweating were seen when plasma ChE fell by 50–75% (Bhu *et al.,* 1976).

A small study of 22 fenthion sprayers with a mean of 8.2 years' work experience showed a 30% fall in serum ChE one day after a typical 5–6 hour working shift with no protective clothing (Misra *et al.,* 1985). At this time headache was seen in 59% and giddiness in 50%, but these symptoms were reported in only 5% of 20 well-matched control hospital workers and in 9% of the sprayers after a three-week rest. Ocular signs were seen in

27% post-shift, and in 9% after the rest period, but in no hospital worker. No neurological abnormality was detected.

In a survey of eight cases of occupational intoxication with organophosphorus pesticides, Durham *et al.* (1965) measured complex reaction time and performance both during and on recovery from mild to moderate acute poisoning (three cases had 50% ChE decreases). Mean reaction time was 15% faster on recovery, while errors of omission decreased from 1.3 to 0.6, and errors of commission from 5.1 to 3.0. Although the improvements may have been aided by a learning effect on retest and recovery the authors stated that such learning effects were minimal.

In a chlorpyrifos manufacturing plant, monthly plasma ChE measures showed a decrease of 19% to 32% depending on degree of exposure. There was no increase in symptom reporting in 175 workers with 1 year or more exposure when compared with 335 well-matched non-exposed workers (Brenner *et al.*, 1989).

Workers engaged in the manufacture of dichlorvos and a carbamate pesticide were studied by Bellin & Chow (1974). Two groups of workers, 25 with 'high' exposure and 58 with 'low' exposure were compared with 56 university staff. Workers were tested 2 days after re-commencing work after a 2-week break, and therefore showed current exposure. No one was overtly poisoned, but plasma ChE levels fell by 18% and 19%, and red cell ChE by 16% and 14% in the high and low exposure groups of workers, respectively, suggesting that exposures (which had been determined by job classification) were in fact similar. There were complaints of a number of signs and symptoms, including blurred vision, sweating, nervousness and sore throat in the exposed workers, which were not seen in the controls. When analysed according to individual ChE inhibition the overall symptom score correlated with the fall in ChE ($p<0.05$), but no individual item did so. No data were collected relating to reversibility.

A survey of 202 farm workers and 42 non-farm workers (Ciesielski *et al.,* 1994) found the farm workers to have a 6.3% lower red cell ChE ($p<0.01$). The farm workers were randomly selected from those attending for mandatory ChE measurements. The participation rate was 96%. The nine workers who reported mixing or applying pesticides within 30 days prior to blood sampling showed an average red cell ChE 8.7% lower ($p<0.002$) than that of non-farm workers. Those farm workers with a fall in red cell ChE of more than 20% were 3.1 times more likely to report diarrhoea than the non-farm workers, but there were no significant increases in other signs.

Richter *et al*. (1992) reported that residents living near to sprayed fields who complained of ill-health effects had a mean plasma ChE decrease of only 7.3%, and that, in a sample of 18 residents, complaints of nausea and diarrhoea were significantly elevated in those whose urinary dimethylphosphate (DMP) plus DEP, combined, was above 0.2 mg/l (approximately 50 nmol/mmol creatinine). When compared with other values (see Table 3.1) this suggests low exposure, but the samples were obtained 'during the spraying season', and if spraying was intermittent and sampling not closely co-ordinated with individual exposure then the peak excretion may have been missed, leading to an under-estimate of exposure.

In a study of a family exposed to diazinon used for domestic pest control, Richter *et al.* (1992b) found evidence of fatigue and dizziness in two adults and vomiting in two infants that resolved after decontamination of the home. Urinary DEP levels were 0.45 to 1.7 mg/l before decontamination (equivalent to approximately 200 to 800 nmol/mole creatinine), which would suggest a high level of exposure (see Table 3.1), but serum ChE levels were depressed by only 6% to 22% relative to post-exposure values. It is not easy to explain this apparent dissociation between the relatively low level of ChE inhibition and the relatively high level of urinary metabolites. Nor is it clear whether these fairly non-specific health effects were related to the diazinon itself or to the associated solvent exposure.

In a single case report of a 12-week-old infant exposed to diazinon, Wagner & Orwick (1994) reported limb myotonia associated with normal serum ChE which reversed within 6 weeks of final exposure. Urine metabolites in this infant are given in Table 3.1. In another case of an infant poisoned by malathion, recovery occurred over 4 weeks (WHO, 1986).

4.3.2 Duration of acute effects

With some short-lived agents such as sarin, acute intoxication is relatively transient, whereas with other pesticides, particularly after dermal exposure, acute intoxication can be longer lasting, as is the ChE inhibition that causes it.

Premature re-entry into a field sprayed with mevinphos and phosphamidon caused illness in 19 of 22 harvesters, all of whom were examined (Whorton & Obrinsky, 1983). Plasma ChE fell by 40–90%, normalising by 21–91 days (although this time was disputed; Midtling *et al.*, 1984). All had weakness, nausea and blurred vision, and 24% showed anxiety. On follow-up at 1 month there were still complaints of headache, weakness and eyestrain. At 4 months only two complained of weakness, but 12 (63%) still complained of blurred vision. Anxiety levels remained constant during the study.

In 27 individuals using fenitrothion without adequate precautions over 13 to 15 days, falls of 39% (sprayers) and 56% (mixers) in blood ChE were associated with reversible dizziness, headache and abdominal cramps (Bouma & Nesbit, 1995). In another study of 24 fenthion sprayers with better personal protection, plasma ChE rose by 23% 3 weeks after exposure (Misra *et al.,* 1988). These workers had 8.5 years work experience, and reported more headache, paraesthesiae and ocular effects during the spraying season than 19 well-matched hospital workers. Motor and sensory nerve conduction velocity were unchanged, but seven of the 24 had repetitive EMG discharges which had disappeared three weeks after exposure.

In a study of the reversal of EEG effects in man, Grob *et al.* (1947) described persistence of the high voltage frontal δ bursts produced by experimental administration of DFP to last for 3–4 weeks. DFP is, however, atypical in that it is able to produce OPIDPN, and does not show spontaneous reactivation.

Extrapyramidal dysfunction lasting for 1 to 4 weeks and starting 4 to 40 days after poisoning was reported in six patients surviving severe fenthion poisoning as a result of suicide attempts (Senanayake & Sanmuganathan, 1995). This unusual effect probably reflected a direct action of the agent, as secondary hypoxic/ischaemic damage could be ruled out in four of the cases.

An interesting experimental investigation of the duration of DFP effects was carried out by Prendergast *et al.* (1997; see Section 4.4.2). Rats were given 14 daily doses of DFP sufficient to cause a 50% fall in brain AChE by day 17. Spontaneous motor activity was depressed over days 16–18, but recovered by day 20. The ability to learn a swim maze test showed a small but significant impairment ($p<0.05$) over days 16–18, but had returned to almost to normal by day 20. By day 21 brain ChE was depressed by only 8%. This rate of recovery of learning ability is consistent with the known rate of resynthesis of brain AChE.

The rate of recovery of a number of different indices in the rat after severe prolonged AChE inhibition was investigated by Bushnell *et al.* (1993). Chlorpyrifos was used at a range of doses sufficient to produce prolonged mild cholinergic signs (fine tremor) and 60–95% inhibition of brain AChE. This inhibition was sustained for at least 35 days, probably due to prolonged absorption from a subcutaneous depot. All dose levels resulted in a reactive down-regulation of muscarinic receptor density from day 7 onward. The duration of this down-regulation was assessed by measuring the hypothermia induced by challenge doses of the cholinergic agonist oxotremorine. This was reduced for between 32 and 52 days, indicating decreased functional cholinergic responsiveness extending over this period. Conditioned learning was impaired and motor response slowed but these two measures recovered within 21 days — a more rapid recovery than that of either AChE inhibition or receptor down-regulation. This early recovery was not seen, however, when a similar degree and time course of ChE inhibition was produced by repeated DFP injections. The impairment of learning produced by DFP persisted for as long as brain AChE was inhibited, a similar result to that obtained by Prendergast *et al.* (1997). These results suggest that rate of functional adaptive recovery was impaired by DFP, an action which could not be wholly explained by its action on AChE. A number of potential non-AChE related targets of organophosphorus esters are discussed in sections 2.6 and 6.4.

4.4 Persistent effects following acute poisoning

Acetylcholine is an excitatory transmitter. Hence increasing the duration and effective concentration of ACh at the synapse or neuromuscular junction by inhibition of AChE carries the additional possibility of excitotoxicity — the death of cells as a result of abnormally prolonged excitatory drive. This is normally considered to be a characteristic of prolonged glutamatergic activation, but can show itself in two cholinergically mediated forms: in muscle (Dettbarn, 1992) or in the brain (Olney *et al.,* 1991). The first of these is of only indirect relevance to this review, and relates to excitation at the neuromuscular junction. It is, however, better characterised than the brain effects as it is easier to study, since the brain effects are greatly complicated by secondary consequences of intoxication. Neuromuscular effects will therefore be briefly summarised in the next section and compared with effects on the central nervous system.

4.4.1 Effects on the neuromuscular system

Following inhibition by a variety of agents, including the insecticide parathion, and a mixture of diazinon and malathion (Dettbarn, 1992), muscle fibre necrosis can develop. Animal studies have shown that a minority (up to 15%) of muscle fibres develop a supercontraction, with mitochondrial swelling, an increase in intracellular calcium, followed by muscle fibre swelling, nuclear pyknosis and necrosis. These changes are initially confined to the end-plate region and, with organophosphates in the mouse, reverse over 5 to 14 days (Bright *et al.,* 1991; Dettbarn, 1992). Damage is proportional to AChE inhibition and to the severity of muscle fasciculation, is mediated by calcium influx and is use dependent. The end-plate necrosis can be prevented by a complete curare blockade during poisoning (Ariëns *et al.,* 1969). The high safety factor involved in neuromuscular transmission means that the ratio of ACh released to that needed to depolarise the post-synaptic membrane is high, which gives the end-plate very considerable potential to over-excite the local muscle fibre. Hence it is not surprising that prolonged activation leads to a calcium influx (and presumably entry of other ions and consequent water flux) beyond the capacity of the muscle fibre to withstand it. Necrosis was seen after 57% inhibition of AChE by sarin in the rat (Dettbarn, 1992), and seems invariably to be associated with dose levels causing fasciculation. Muscle necrosis is often associated with, but is not necessarily responsible for, the intermediate syndrome of transient proximal muscle weakness sometimes seen following severe acute intoxication (Section 2.3), since muscle necrosis has been seen in man after malathion and diazinon intoxication (Dettbarn, 1992).

In a controlled trial of sarin exposure in eight volunteers (Baker & Sedgwick, 1996) sufficient to produce a 53% fall in red cell ChE at 3 hours, the only clinical sign was miosis lasting up to 48 hours. No neuromuscular signs were seen, but single fibre EMG measures showed changes in jitter (variability of EMG latency originating in the terminal nerve branches); there was no change at 3 hours but a 14% increase at 3 days. Mean jitter was not significantly increased subsequently, but the incidence of abnormally long jitter intervals was increased in measures obtained over 4–15, but not 15–30 months. The study analysis appears to be flawed because data were collected as 10 to 30 multiple measures from each subject and these were apparently treated as independent measures. However, if the incidence of abnormally long intervals is normalised to one measure per subject, the incidence (mean ± SE) is 1.4 ± 1% (control), 12 ± 3.7% (3 days), 11 ± 4.5% (4–15 months) and 5.4 ± 3.6% (15–30 months). All of the increase at 4–15 months came from three of the subjects, which casts some doubt on the validity of a persisting effect with sarin. This is, however, an interesting and informative study.

The threshold for producing muscle damage may be lower for carbamates. Some muscle effects were seen in rats after a low-dose infusion with pyridostigmine, which produced no more than a 30% decrease in blood ChE which resolved over the first 7 days. A marked muscle effect was seen at a higher dose which produced 60–70% inhibition sustained over 14 days. Neither dose regimen produced muscle fasciculation (Hudson *et al.,* 1986). The ChE inhibition data in this study are not likely

to have been underestimated because of *in vitro* reactivation, since the authors took appropriate precautions to avoid artefacts. The nature of these changes was similar to those produced by organophosphates: disruption of the mitochondrial matrix was followed by a change in pre–post synaptic geometry, and invasion of Schwann cell processes over 7 to 14 days. These effects were not secondary to systemic toxicity, since rats showing damage remained asymptomatic during the fall in ChE.

Whilst not directly relevant to the central nervous system, such neuromuscular effects do show the potential of anticholinesterase agents, and carbamates in particular, to act at the neuromuscular analogue of the synapse. The effects can be produced at doses lower than those associated with severe toxicity. Were any analogous changes to be produced in the central nervous system, they would be expected to prove less readily reversible. The possibility of this occurring at such doses, however, must be smaller, since the synapse does not have the very high safety factor for transmission seen at the end-plate, and the presence of direct inhibitory systems to balance excitation would make the brain less vulnerable than muscle to excitotoxicity.

4.4.2 Effects on the nervous system

The best known lasting effect on the nervous system is OPIDPN. As the nature of OPIDPN and its possible contribution to chronic, low-level effects are relatively well understood, and have already been outlined in sections 2.4 and 2.5, the rest of this section will deal only with non-OPIDPN effects.

Experimental studies

Within the central nervous system excitotoxicity has been described following frank intoxication. Most studies have related to warfare agents. In rats, soman produces brain lesions of a severity proportional to the signs of acute poisoning (McDonough *et al.,* 1989). Damage is always seen after seizures, and often after tremors and muscle fasciculation (Petras, 1981). These brain lesions are associated with a lasting decrement in operant conditioning performance (McDonough *et al.,* 1986), as would be expected from their severity. The lesions involve diffuse cortical necrosis plus more focal neuronal loss within the limbic system (McLeod, 1985). The nature of this damage, plus the association with seizures, suggests that pathogenesis involves a combination of hypoxia secondary to breathing difficulties plus an additional direct cholinergically mediated excitotoxicity and secondary recruitment of glutamatergic excitotoxicity (Solberg & Belkin, 1997). Direct toxicity is obviously an important factor, since animals may still die even if provided with artificial ventilation, and some morphological damage can be reproduced even in tissue slices incubated *in vitro* (Lebeda *et al.,* 1988). At these high dose levels, a weak agonist action which has been described at the nicotinic receptor may become important as a mechanism additional to ChE inhibition (Millis *et al.,* 1988). This morphological damage is a high-dose effect which has never been described, and would not be anticipated following exposures too low to produce clear signs of poisoning. It might, however, be expected as a consequence of even a single severe poisoning episode.

More subtle changes were seen in a study of sarin poisoning in monkeys, which were either paralysed and ventilated to avoid hypoxia resulting from seizures following a single high dose, or were given 10 weekly doses at 20% of the level producing seizures (Burchfiel *et al.,* 1976). The latter animals showed ‘few if any’ overt signs of toxicity and no convulsions, but unfortunately ChE inhibition was not measured. Extensive depth and surface EEG monitoring with spectral analysis showed no subcortical changes, but increased fast (β) and slow (δ) cortical activity. The latter resolved within 24 hours, but the former remained elevated at 1 year after dosing. Data on the magnitude of the changes were not presented, but specimen records showed the β2 subfrequency to increase by 50% at 24 hours and 61% 1 year after the multiple low-level dose, or 38% and 23%, respectively, after the single high dose. Unfortunately numbers were too low for this interesting study to be considered reliable, there being only three animals in each experimental group, (six and four control animals, respectively). The time course of effects is particularly surprising, as almost any insult to the nervous system

would be expected to show some recovery between 24 hours and 1 year. Changes in β activity are difficult to detect, and the authors were unable reliably to identify abnormal records by visual inspection (Burchfiel & Duffy, 1982); it is likely that any such changes would have been missed in less thorough investigations. Such spectral changes are also non-specific and difficult to interpret.

A number of animal behavioural studies have investigated the longer-term effects of organophosphorus esters. Some of these have involved mid-range dose levels that would not have produced frank excitotoxicity. Prendergast *et al.* (1997) gave rats daily subcutaneous injections of DFP for 14 days, and followed their ability to learn a maze during this period. DFP delayed learning at a dose level which caused a 57% fall in neocortical AChE, and fasciculation in 5% of the rats. The peripheral ChE inhibitor, echothiapate, failed to produce these effects, indicating a central effect of DFP. Some other organophosphorus esters (such as dichlorvos) have, however, been shown to affect learning behaviour at rather lower doses, possibly by a non-cholinergic mechanism (see Section 2.6), although their effect is to enhance learning.

Clinical studies

Many potentially useful reports leave much to be desired in terms of adequacy of reporting, non-specific statements such as there being 'no sign of residual intoxication' being common (Klemmer *et al.,* 1978; Petros, 1990).

A clinical report of 639 cases of acute sarin poisoning (a result of a terrorist attack in Tokyo; Okumura *et al.,* 1996) described both the severity of acute poisoning and the time course of recovery. Of these cases 0.6% showed severe poisoning requiring ventilatory support, and had plasma ChE at 20% of the normal lower limit. Moderate poisoning was seen in 17% of patients, who showed fasciculation, weakness or convulsions and plasma ChE at 20% to 80% less than the normal lower limit. Mild poisoning, with ocular effects only, was seen in 83% of cases. In 33% of all cases an acute stress reaction requiring antidepressant therapy occurred. In four of 111 cases of severe or moderate poisoning this stress reaction was found to be succeeded by a post-traumatic stress disorder, identified at a 3-month follow-up examination. A single case report of acute sarin poisoning of sufficient severity to cause seizures and dyspnoea described an impairment of memory and learning ability 6 months after exposure (Hatta *et al.,* 1996).

In a report of 37 cases of mild to severe poisoning after three or more exposures to a variety of organophosphorus agents over an undefined period (Holmes & Gaon, 1956), one case showed persisting paroxysmal EEG spike discharges 1 year following severe intoxication. This may have resulted from secondary hypoxia. Rosenstock *et al.* (1990) reported a single case of memory deficit, persisting headache and fatigue, possibly associated with a moderate acute parathion poisoning episode (red cell ChE depressed by more than 50%) which had occurred 2 years previously.

In a large series of suicidal poisonings with organophosphorus pesticides (Wadia *et al.,* 1974), of 21 surviving cases with neurological deficits (mostly neck and limb weakness, facial palsy or eye spasms), none had signs that persisted beyond 3 days. It was difficult to evaluate anxiety in these patients because of the complicating effect of atropine therapy.

An account of eight cases of chlorpyrifos exposure from heavy domestic use over several weeks for pest control (Kaplan *et al.,* 1993) reported a number of persisting effects. In one case, acute exposure caused nausea and vomiting and development of paraesthesia resolving over 8–10 weeks, while in a second case an initially asymptomatic exposure was followed 4 weeks later by abnormal sensation, which had resolved at 6 months. In a third case fasciculation and tearing over 6 months of regular exposure, associated with a significant but unspecified fall in red cell ChE, was followed by some evidence of a mild peripheral neuropathy which was no longer present at 1 year. In four cases headache, nausea and cramps were followed by numbness, paraesthesia and mild memory loss at 1 month, resolving by 6 months. Unfortunately the electrophysiological investigations carried out on these patients were not sufficiently consistent to provide confidence in the clinical significance of the small deviations from the normal range. In a final case, where exposure was only associated with paraesthesia, a persisting memory loss

and intellectual deterioration developed; as with other cases little evidence was presented concerning the severity of the chlorpyriphos exposure, or to justify a causal link between exposure and ill-health.

Two cases of spray pilots continuously working under unsafe conditions were described by Dille & Smith (1964). Although the study gives no clear indication of severity of exposure, one pilot developed a psychotic depression. The other, with several episodes of moderate acute toxicity over 7 years, developed a (possibly well founded) anxiety and a fear of flying or driving.

In a single case of severe acute poisoning with demeton-S-methyl, where the individual survived only after intensive hospital care (Bartels & Friedel, 1979), synchronised slow (δ) activity was apparent for up to 20 days (at which time other signs had resolved), but the EEG was normal by visual inspection at day 56. Spectral analysis does not appear to have been used. In a second case of acute poisoning (with 'phosphorothiate' plus pyrethrins) initial salivation, lacrymation and bronchospasm occurred. At 1 month post exposure red cell ChE was 27% lower than at 2 months. At 28 months there was asymmetrical tremor, myoclonus and decreased somatosensory but normal cognitive function. Photon emission computerised tomography demonstrated an asymmetrical blood flow deficit in the temporal lobe and basal ganglia consistent with hypoxic or ischaemic damage (Callender *et al.* 1994).

An atypical case of protracted phosmet poisoning after five heavy dermal exposures at weekly intervals involved double vision, sweating, unsteady gait and change in voice tone 18 hours after the last exposure progressing to salivation and facial sweating with marked proximal weakness at 5 days, and paralysis and hallucinations at 9 days (Good *et al.,* 1993). Improvement was seen from 28 days, with spontaneous ventilation from 44 days, mild weakness at 64 days, and full recovery at 5 months. The red cell ChE was normal (both against the general population and against the patient's own 10-month value) on days five, 16 and 23, and there was apparently no complicating neurological disease. There was no fasciculation, but a decreased EMG amplitude over days 5–12, and decrement on repeated stimulation over days 9–47. A muscle biopsy at 2 months showed recovering end-plate damage. This may have been a case of cumulative exposure leading to adaptation of acute signs, but this would not explain the normal ChE values.

A number of other small or anecdotal studies have reported rather speculative putative long-term effects of poisoning. Thus, of five children who had recovered from very severe organophosphate poisoning, one became hyperactive (Harmon *et al.,* 1975), possibly as a consequence of organophosphate-induced coma. Two people with moderately severe occupational organophosphate pesticide poisoning subsequently committed homicide, and two developed aggressive behaviour after low-level exposure (Devinski *et al.,* 1992). In a collection of 16 cases selected from an undefined number, a period of depression or psychosis with memory or concentration problems was seen following one or more episodes of moderate to severe occupational poisoning (Gershon & Shaw, 1961). All individuals had recovered within 1 year of poisoning.

Delayed recovery was reported in a single study of an attempted suicide using impure malathion containing isomalathion (Dive *et al.,* 1994). Initial plasma and red cell ChE depressions were 100% and 90%, and the patient required ventilation for 2 days, developed depressed pulmonary and renal function over 5–13 days, and a distal weakness from day 10 with denervation potentials. The patient had recovered 3 months after exposure.

Epidemiological studies

Rosenstock *et al.* (1991) conducted a retrospective neuropsychological study of cases of severe accidental pesticide poisoning, 90% of which had shown nausea and vomiting during the acute poisoning phase. Of the identified population of 89 cases, 52 men, aged 15–44 years, who had had accidental occupational exposure to organophosphorus pesticides, but no other serious illness or neurological disorder, were selected for the study. Of these, 38 could be located and 36 agreed to participate. The voluntary participation rate was therefore 95% for the sample of subjects approached. Controls were 36 age-matched male volunteers, friends or brothers of the poisoned subjects from the same local

community. The groups were well matched for alcohol intake, but the controls had more formal education (88% compared with 83%). Measurements were made 10 to 34 months after intoxication, and against a background of normal occupational pesticide exposure in both the poisoned and the control groups; the incidence of minor poisonings not requiring hospitalisation was 42% in the poisoned group and 40% in the 25 individuals from the control group who were occupationally exposed. The mean duration of occupational exposure was 7 years in the poisoned and 9 years in the non-poisoned subjects. There had been no pesticide exposure within the three months prior to testing in 86% and 89% of the subjects in the poisoned and control groups respectively. A WHO core test battery showed the following significant ($p<0.01$) percentage differences between the poisoned and non-poisoned groups: pursuit aiming –20%, manual dexterity –11%, trails score +22%, visual retention –25%, digit-span test –27%, digit-symbol substitution –24% digit vigilance speed +19%, self-reported symptoms +53%. A non-significant increase was seen for simple reaction time and psychological symptom inventory (including anxiety), and non-significant decreases in vocabulary, spoken verbal learning and finger tapping speed. A small correction for mismatch in intelligence made little difference to the outcomes. There was no measure made of pre-morbid ability and, given that the study population was chosen on the basis that they had been involved in accidents, it is theoretically possible that the lower dexterity, attention, and visual motor skills pre-dated the organophosphate poisoning. However, the similar vocabulary scores and similar incidence of minor pesticide-related illnesses in those members of either group who worked with pesticides would tend to indicate otherwise.

Further evaluation (McConnell *et al.,* 1994) of these same poisoned and non-poisoned workers showed differences in vibrotactile threshold in finger and toe, a difference that could not be explained by variation in skin thickness. The largest difference was seen in those workers known to have been poisoned with methamidaphos, an agent known to cause OPIDPN in man after severe intoxication (WHO, 1986), and so may perhaps have represented threshold OPIDPN, although such a diagnosis cannot be confirmed on vibration threshold data alone.

Tabershaw & Cooper (1966) made a study of a population of 235 crop sprayers and farmers with occupational poisoning. The subjects were stratified according to the severity of poisoning (Group I had specific signs and depressed blood ChE, Group II signs only, and Group III no clear confirmatory evidence of organophosphorus pesticide poisoning). Within these groups 80%, 69% and 72%, respectively, were located and of these 67%, 67% and 68% agreed to participate. Assessment of symptom reporting for acute-type effects plus nervousness and insomnia was made in the winter when there was no pesticide use. Recovery from acute signs was subjectively assessed to be good; nearly all subjects reported rapid recovery, with some weakness lasting a few weeks. Within the whole sample, 11% reported cardiovascular or respiratory problems lasting at least 3 years, although no causal link with poisoning could be established. A further 18% reported that subsequent very low-level exposure to pesticides caused a distressing recurrence of the signs that they had experienced following an earlier severe poisoning episode; the authors attributed this recurrence to a psychogenic effect, and this seems a reasonable explanation.

A more recent study (Savage *et al.,* 1988) involved people accidentally poisoned, largely by occupational use, with a range of ten different organophosphate pesticides. All had been clinically diagnosed as having organophosphorus poisoning, and most had been hospitalised; 11% had experienced more than one episode of poisoning, but there was no measure made of severity of poisoning. The mean interval between poisoning and test was 9 years. Of a population of 303 poisoned subjects (cases), 188 were located and 141 met pre-determined criteria for alcohol use, age, lack of recent pesticide exposure and neurological disease. Of these 114 (81%) agreed to participate and 100 were assessed; 14 were lost owing to logistic problems. The control group was drawn from the same geographical area and matched for age, sex, years of education, social class, occupation and race. They were selected by recommendation from the cases, from lists of company employees or by the authors. A basic neurological examination and EEG test showed no abnormalities in either group. Based on personality tests designed to evaluate mood, 6% of the cases but none of

the controls were 'depressed'. Of 39 psychological tests, 22 showed statistically significant differences between the two groups, including a 6.4 point lower verbal IQ, and lower vocabulary, digit–symbol substitution and reading scores among the cases. Subjective assessment of speech comprehension, writing and problem solving showed similar differences, as did assessments obtained from subjects' relatives, which showed greater depression and irritability among the cases. There was no attempt to estimate or discuss the significance of pre-morbid intelligence in this study, which may well have differed from that of the control group and affect susceptibility to accidents. Although the groups were matched for school grade, this would only provide a crude match. Since relatives were interviewed, and moreover gave a reliable assessment of ability, it is unfortunate that no attempt to inquire about pre-morbid ability is reported. A further complication, the non-random selection of the control group (partly chosen by the poisoned group), may have resulted in controls with better social skills than their peers, which may have affected their IQ and neurobehavioural performance.

A further study (Steenland *et al.,* 1994) of neurological and neurobehavioural variables was carried out in a group of workers after occupational exposure; 28% of the poisonings were of sufficient severity to require overnight hospital treatment. All had either at least 20% ChE inhibition or unambiguous clinical signs of poisoning. The duration of exposure was undefined. The interval between poisoning and measurement, while not reported by the authors, did not correlate with any measures. Of the potential participants, 49% were located and, of these, 128 (70%) participated. A control group consisted of 90 friends of the subjects, on average 4.3 years older, but otherwise matched for years of schooling, race, smoking, drinking, hours of sleep, caffeine and solvent use. After correction for confounders the results indicated that there had been no changes in motor or sensory nerve conduction velocity or amplitude across the whole group, but within the group there was a significant decrease in vibration sensitivity ($p<0.05$) in those cases with a documented fall in erythrocyte or plasma ChE of more than 20%. The individuals who had been hospitalised showed a greater effect ($p<0.01$), and also had a significant increase in median nerve motor amplitude. When the seven neurobehavioural tests were corrected for confounders the continuous performance measure of sustained visual attention was reduced in all poisoned groups (p values ranged between <0.01 and 0.05), but symbol–digit substitution score was reduced only in the hospitalised group ($p<0.04$). Mood scales showed increased tension and confusion scores (p values ranged between <0.01 and 0.03) in all but the most severely poisoned (i.e. hospitalised) group, possibly due to the beneficial effect of medical attention. Loss of vibration sensitivity, visual attention and symbol–digit substitution score correlated with the number of days away from work as a result of the poisoning. The authors concluded that the study provided some evidence of deficits in CNS function, but pointed out that only a small proportion of the study population's variability could be accounted for by degree of poisoning.

In a complex paper, Richter *et al*. (1992a) reported that 11 ground crew workers involved in organophosphorus pesticide spraying of cotton, and having a significant but unspecified fall in blood ChE, showed a 28% lower sural nerve action potential amplitude than four less exposed co-workers ($p<0.05$). There was no change in peroneal nerve evoked response or conduction velocity. They further reported a group of 17 farmers and local residents complaining of fatigue, dizziness, weakness and other problems during the spraying season but who showed no significant changes in nerve conduction velocities, amplitudes or latencies when pre-spray and spray season measures were compared, although urinary metabolites confirmed low-level exposure. This study suggested that peripheral nerve changes are not a sensitive marker of recent exposure.

The results of mild but symptomatic exposure to a mixture of mevinphos, methomyl (a carbamate) and maneb (a manganese containing fungicide) in two acute incidents six months and two years before assessment was reported by Reidy *et al.* (1992). There was some additional long-term exposure continuing up to the time of testing. The acute exposures caused moderate symptoms, but were associated with falls of only 16% in plasma and 6% in red cell ChE. The study group consisted of 21 exposed farmers who were selected by their legal representative and were claiming compensation. A group of 11 local factory workers,

matched for age and alcohol consumption, served as controls. There were no differences between the groups in scores from cognitive, attention or memory measures, although there was considerable variability within the measures of each parameter, as would be expected in small groups. Digit–symbol substitution performance was not different, but finger tapping scores (not speed) were reduced in the exposed group ($p<0.01$); they also scored less well in a visual retention test ($p<0.03$). In a symptom questionnaire, the exposed group had higher scores for all 36 neurotoxic symptoms, including heart palpitations (often seen in over-reporting subjects); anxiety and depression scores were also significantly elevated. Similar scores were reported after successful settlement of their compensation claim. The non-random selection of the exposed study group must call into question the validity of these findings, since the size of the exposed population was not defined. In addition, the non-selective nature of the symptom reporting also suggests a non-specific cause. This study is therefore of limited value.

Summary of persisting effects on the nervous system

The main studies mentioned in the preceding sections are summarised in Table 4.2, and a summary of the indices used is given in Table 4.3. Clinical studies show a general lack of persisting effect, except for one report of stress disorder in a minority of those severely poisoned. In contrast, various long-term consequences are reported in the epidemiological studies. There are two likely reasons for this difference.

- ***Sensitivity: the methods used in the epidemiological studies were generally more sensitive than those applied in the clinical studies, and the sample size was generally larger.***

- ***Specificity: the clinical studies were usually of well-defined exposures in pre-defined groups. In the epidemiological studies the problems introduced by selective sampling, sample mismatching and the difficulties of estimating pre-morbid intelligence in cross-sectional study designs have been discussed for each study.***

It is difficult to decide which of these two factors is responsible for the differences observed in long-term consequences. Of the epidemiological studies, that of Rosenstock *et al.* (1991) provides the most convincing evidence, largely because the similar incidence of low-level poisoning in both poisoned and reference groups suggests good matching. As regards mechanism, it appears likely that most of the adverse effects described might be considered to be as much a result of the non-specific trauma of poisoning as of any agent-specific effect. Clearly, where poisoning is severe enough to cause direct excitotoxicity (as seen in animal studies and discussed earlier) lasting effects may be seen in man, as perhaps in the case reported by Holmes & Gaon (1956) already described. A mechanistic explanation of any persisting effects of exposures that were too low to cause excitotoxicity or hypoxia is not available at present, although a lasting down-regulation of receptors or an effect analogous to the hyper-stimulation that develops with repetitive stimulation (kindling) or long-term potentiation might be entertained. It is particularly unfortunate in this context that none of the clinical EEG studies were as thorough as those conducted in animals, since this would have provided very useful data.

A useful summary of the nature of the specific findings in the major epidemiological studies was provided by Steenland *et al.* (1994), to which that of Reidy *et al.* (1992) has been added (Table 4.3). It must, however, be borne in mind that within these studies, particularly in the case of Reidy *et al.* (1992), there are real difficulties in assigning causation to these statistically significant differences, and in no case can the differences be said to be unambiguously linked to poisoning. However this is not surprising given the highly individual and unpredictable nature of accidental poisonings, which makes prospective studies almost impossible. Even with this qualification it seems reasonable to conclude that various relatively subtle neuropsychological effects (see Table 4.3) are likely to follow acute poisoning of sufficient severity to require hospital treatment. However, it is curious that careful clinical studies of more severe individual intoxications show that no obvious lasting effects are produced in the absence of seizures or hypoxia.

Table 4.2 Reported consequences of acute poisoning in man

Sample size and study type	Outcome measures	Persisting effect	Reference
111, clinical	neurological, psychological	stress disorder in 4%	Okumura *et al.* (1996)
37, clinical	EEG	1 showed no recovery	Holmes & Gaon (1956)
21, clinical	neurological	none >3 days	Wadia *et al.* (1974)
8, clinical	neurological	none >1 year	Kaplan *et al.* (1993)
1, clinical	neurological	none >5 months	Good *et al.* (1993)
1, clinical	EEG	none >56 days	Bartels & Friedel (1979)
128, epidemiological	neurological	vibration sensitivity, attention, intellectual performance	Steenland *et al.* (1994)
114, epidemiological	symptoms	acquired intolerance in 18%	Tabershaw & Cooper (1966)
100, epidemiological	EEG, neuropsychological	intellectual performance	Savage *et al.* (1988)
52, epidemiological	neuropsychological	dexterity, attention, coordination	Rosenstock *et al.* (1991)
52, epidemiological	neurological	vibration threshold	McConnell *et al.* (1994)
21, epidemiological	neuropsychological	motor performance, symptoms	Reidy *et al.* (1992)

Table 4.3 Indices found to differ between samples of poisoned and control subjects in four epidemiological surveys

Test	Steenland *et al.* (1994)	Savage *et al.* (1988)	Rosenstock *et al.* (1991)	Reidy *et al.* (1992)
Sample size	110	100	36	21
Tapping	↓*	↓*	→	↓*
Simple reaction time	↓	–	↓	–
Sustained attention	↓*	–	↓*	→
Symbol–digit	↓*	↓*	↓*	–
Pursuit aiming	↓	–	↓*	–
Dexterity (Santa Ana)	↓	↓*	↓*	–

↓, worse performance; →, unchanged; – , not measured; *, statistically significant

5 Low-level exposures

5.1 Background

Low-level exposure to organophosphorus pesticides may be defined in various ways, but the definition used here is exposure at a level which is low enough not to produce any externally visible cholinergic signs.

Much of the concern about long-term and low-level effects of organophosphorus pesticides is a result of the known potential of these agents to cause acute toxicity, and of some to cause delayed neuropathy (OPIDPN). This has given cause for concern that longer-term effects may be found, and a number of authors (e.g. Mearns *et al.*, 1994) have remarked on similarities between chronic signs or symptoms seen in various groups and those signs or symptoms known to be caused by acute intoxication with organophosphates.

The extent of exposure to sheep dip organophosphates was evaluated by Rees (1996), in a prospective study over the dipping season. A population of 38 male dippers was identified, and 23 (63%) completed the study. They had previous exposure over a mean of 31 years. Only two had a significant (20%) fall in red cell ChE and the group as a whole showed no change in this or in plasma ChE. Total urinary metabolites (Table 3.1) showed a non-significant rise, the increase being mainly attributable to four cases, in which total metabolites rose to 40, 42, 70 and 106 nmol/mmol creatinine. The lack of change in urinary metabolites for the group as a whole may have been due to the limited urine collection period, but the lack of change in ChE suggests that there was little systemic absorption of the organophosphates in most dippers. However, 42% reported influenza-like symptoms after dipping which were consistent with, but not specific for, mild anticholinergic intoxication. Of the four with the largest increases in urinary metabolites only one reported symptoms after dipping. The use of protective clothing was described as inadequate. The studies reported by Gompertz & Verschoyle (1996) showed an overall increase of 80% in urinary metabolites after sheep dipping, with a few individual high values but many lying within the normal range. A survey by Stephens *et al.* (1995a) suggested a similar level of exposure, with a lower baseline value. There was no increased reporting of signs of acute toxicity in this group. It is clear from Table 3.1 that, even with inadequate protective clothing, systemic exposure through sheep dipping is less than that from manufacturing or from spraying in a hot climate. Hence it appears likely that, for the majority of workers, exposures associated with normal sheep dipping are 'low-dose' exposures.

5.2 Clinical studies

Sack *et al.* (1993) investigated neurological function in a group of 37 male pesticide lawn spray workers, selected on the basis of availability (all agreed to participate), having a mean of 11 years occupational exposure, primarily to organophosphorus pesticides and herbicides. The exposed group was compared with a group of 35 age- and height-matched male university staff. Data were normalised to account for the higher alcohol and caffeine intake of the exposed group. Neurological function was evaluated by postural sway, measured in the presence and absence of visual and proprioceptive cues. The latter were reduced, but not removed, by the subjects standing on a foam cushion.

Performance was similar in the two groups when cues were present, but deprivation of visual and proprioceptive cues led to a greater increase in the sway of the exposed group ($p = 0.03$). Whilst these were not ideal comparison populations (manual and non-manual workers), the results suggest that this novel test may be of value in future studies.

Duffy *et al*. (1979) conducted a clinical EEG follow up of 77 cases of accidental occupational exposure to the warfare agent sarin. Of these, 41 (the maximum exposure group) had three or more exposures over 6 years. The severity of the exposures was not reported in any detail, but was presumably not life-threatening, although associated with a fall in red cell ChE of at least 25% (i.e. one expected to be close to, but below, the threshold for producing acute effects). None of the subjects had significant exposure to sarin or pesticidal organophosphates over the year prior to investigation. Controls were 38 co-workers without exposure, and were matched for age, sex and socioeconomic class. Following spectral analysis of waking and sleeping EEG, 151 indices were evaluated. Eight of these indices showed significant differences between the exposed and control groups, six of them within the b frequency range. For the maximum exposure subgroup there were 33 significant differences, of which 31 were in the β range, mostly in temporal and occipital regions. The magnitude of the increase in overall spectral energy within the 24–27 Hz band was 14% in the whole group and 21% in the maximum exposure subgroup. There were no corresponding decreases in energy in the lower frequency bands, and the lack of this would have made detection by visual inspection difficult. Visual analysis showed some evidence of a fall in the a range, but this was not confirmed by Fourier analysis. Sleep EEG studies showed no significant changes in total sleep, but an increase in REM sleep from 11% in the control group to 15% in the exposure subgroup and 16% in the maximum exposure subgroup. ($n = 26; 67; 38$). However the attention applied to the detailed measurement and analysis of these indices was not applied to the reporting of severity of any intoxication.

Behan (1996) suggested that chronic fatigue symptoms may be caused by occupational exposure to organophosphates. The author reviewed ten cases selected on the basis of an association between occupational exposure and chronic fatigue syndrome. However, no evidence was presented to suggest that organophosphate exposure was over-represented in the population with chronic fatigue syndrome when compared with the normal population. Any evidence of association in time between exposure and development of the syndrome was unclear. Since the patients showed a significantly earlier release of growth hormone after pyridostigmine challenge than did normal controls, it was suggested that chronic fatigue syndrome shares a common pathogenesis with anticholinesterase agent poisoning. This suggestion appears to be entirely speculative.

In a single case report, a farm worker engaged in intensive spraying of demeton-S-methyl without protective clothing (but not recorded as having developed acute signs of intoxication) subsequently developed lethargy, paraesthesia, sweating, headache and an episodic psychosis (Bradwell, 1994). The only evidence to link these events is that the onset of the psychosis followed a pesticide exposure. No causal relationship was established.

5.3 Epidemiological studies

Several potentially useful epidemiological studies are of only limited value for the assessment of health effects arising from long-term low-level exposures as potential chronic effects were not investigated. For example, in their survey of acute and subacute symptoms in 202 farm workers — potentially a very informative group (see Section 4.3.1) — Ciesielski *et al.* (1994) only asked two questions pertaining to long-term effects. One of these related to the workers' subjective assessment of memory loss which, by its very nature, would hardly be expected to provide a reliable index. The second question was about anxiety and, whilst it is useful to know that this was not over represented in the exposed population (odds ratio 0.4–2.8), this does not provide the convincing evidence of lack of lasting effect that some have presumed it to give (Eyer, 1995). This study, together with the others summarised in Section 4.3.2, does, however, indicate that any lasting effects in the study population would have been subtle in nature.

5.3.1 A UK study of sheep dippers (Stephens *et al.,* 1995)

Study design

This major UK epidemiological study will be considered in some detail. The full results were reported in an HSE report (Stephens *et al.,* 1995b), and also in Stephens *et al.* (1995a), Stephens *et al.* (1996) and Beach *et al.* (1996). The study design had many excellent features not seen in other attempts to survey exposed populations. Two groups of outdoor manual workers, sheep dippers and quarry workers, were investigated in a cross-sectional study. The former had regular occupational exposure to sheep dip organophosphorus pesticides and the latter little or no exposure. The study had two main components:

- ***short-term effects — measurements (symptom questionnaire, mood test, urinary metabolites) made before, 24 hours after and 3 weeks after dipping; and***

- ***long-term effects — measurements (health and memory questionnaires, neuropsychological tests and urinary metabolites) made in a follow up study 1 year later (3 months after dipping).***

The initial study group consisted of 210 sheep dippers and 197 quarry workers. Participants were all male, and subjects were excluded from the study if illiterate, suffering from pre-existing neurological disease or exposed to other potentially neurotoxic chemicals. The control population of quarry workers was approached directly and offered the incentive of a prize draw; this gave a relatively high participation rate. The study population was drawn from a random selection of sheep farmers, approached either by post (the majority), or by telephone. The postal approach gave a very low participation rate, but the telephone approach a better one. Of those approached, 39% of the identified exposed population and 100% of the controls responded. Of those persons responding and also fulfilling the pre-determined selection criteria, 29% of the exposed and 32% of the control population participated in the component of the study related to short-term effects; of these some were lost before the follow up phase owing to logistic problems. The equivalent participation rates for the longer-term follow up study 1 year later were 23% and 26%. Hence although once contacted the response rates were similar, the overall positive response rate in the exposed group was only 13%, and 35% for the controls. The final numbers completing the follow up study were 158 exposed and 155 control subjects.

Although exposure could not be measured directly, cumulative individual exposure potential was estimated from the number of sheep dipped over the subject's lifetime. The outcome measures included standard automated and validated neuropsychological tests and a psychological and a general health questionnaire. Three small subgroups were given a basic clinical neurological examination.

Possible confounding of performance measurements at follow up by any immediate short-term organophosphorus pesticide exposure was discounted after very low levels of urinary metabolites were found at the time of testing.

Study results

The results of urine analyses confirmed that there was initial short-term low-level exposure, although not at the time of testing. The sheep dippers reported somewhat fewer generalised signs of ill-health than the quarry workers, which is important since it suggests that this was not a self-selected subpopulation atypical of the wider group in this aspect. Hence it can be concluded that the study population was recently, but not currently, exposed to subsymptomatic levels of organophosphates. None of the farmers had experienced acute poisoning (Spurgeon *et al.,* 1995).

The results of the follow up study involved seven neuropsychological tests and two health questionnaires. Four of these nine measures showed several significant differences between the exposed and control populations, and for one measure this difference was dose-related.

Simple reaction time was measured over eight consecutive trials and showed the exposed population to be 5% slower at the start of the test. This difference

increased to 8.6% on serial retesting. The all trials mean showed a decrease of 6.5% in the exposed group, which was reduced to 3.9% on adjustment ($p<0.051$; see below). There was a significant decrement for trials 6 and 7 ($p = 0.028$), but not for the final trial, 8. Reaction-time slowing could not be correlated with the estimated extent of exposure during dipping.

Symbol–digit substitution performance was also slower in the exposed group (–12% decreasing to $–6.8 \pm 3.2\%$ on correction). The mean difference across trials was significant ($p = 0.038$). Again, there was no correlation with dip exposure. Neither the visual component of this test nor the visual digit span test showed any differences between the two populations.

Syntactic reasoning showed a $48\% \pm 23\%$ slower reaction time in the exposed group after adjustment. This effect was more obvious with the more complex tasks, and reached an overall significance of $p = 0.037$. Accuracy was somewhat greater in the exposed group, suggesting a different performance strategy, but the effect on accuracy was no longer significant after adjustment. There was a greater effect on reaction time in the more highly-exposed subgroups, resulting in a significant dose–effect relationship ($p<0.0001$) which, however, only became apparent in the more complex tasks. A no-effect exposure level could not be determined. This test gave the most clearly positive results of the survey.

The category search and serial word learning tests showed no significant differences between groups. The exposed group showed slowed performance on the spatial recognition test, but this effect disappeared on correction for confounders (see below).

The subjective memory questionnaire showed no differences between the groups after correction for confounders, but the general health questionnaire showed a higher (worse) score in the exposed population (3.49 compared with 2.27, $p = 0.023$). The unadjusted proportions scoring four or more (a score commonly associated with clinical psychiatric disease) were 15% of the controls and 35% of the exposed sample. After adjustment this effect remained significant ($p = 0.035$), but did not show a dose–effect relationship.

Basic clinical neurological examinations were subsequently carried out on well matched samples of ten symptomatic farmers, ten asymptomatic farmers and ten controls. These showed little evidence of gross motor dysfunction, but some signs of sensory problems. Two-point sensory discrimination on the hand was significantly reduced by a factor of 2.3 in symptomatic and 1.3 in asymptomatic farmers. Discrimination on the foot was significantly reduced by 3.0 in the symptomatic group, but was essentially unchanged ($\times 0.91$) in the asymptomatic group; it remained unchanged on the finger or toe in both groups. This is unusual since the fingers and toes are generally the most sensitive areas in clinical studies.

Potential limitations of the study

This study was based on a cross-sectional design, and therefore had inherent limitations in terms of its ability to determine causality. These limitations were compounded by a number of probably unavoidable but undesirable problems with sample matching, as has already been pointed out by others (Watt, 1995; O'Brien *et al.* 1995). The most serious criticism concerns the response rate as only 13% of the exposed group responded versus 35% of the controls.

The factors leading to the lower participation rate amongst the sheep farmers in the study cannot be determined. The authors did not present an analysis of their outcome measures on the basis of the two recruitment methods, which might have shown whether the two groups differed in terms of outcome. It seems possible that the strong element of undetermined selection in the exposed population (essentially self-selection) could have influenced the outcome measures in at least two ways. Since the survey involved a free health check, the population may have been influenced to participate in the survey if they had some mental or physical health concern. This could have resulted in over representation of poorly performing individuals in the sampled population. Alternatively, since this 'official' survey involved questions about adequacy of personal protection and working practices, those individuals who had been careless about use of organophosphates might have been less willing to participate, either through lack of concern or fear of retribution. This could

have resulted in under representation of a subpopulation at greatest risk of exposure. The authors suggest that the major reason for lack of participation was the inclusion of a large proportion of 'hobby' sheep farmers in the postal survey who failed to respond due to an [undocumented] lack of exposure. This may well have accounted for many who failed to respond, but a survey based on a 13% response rate must be considered to be a survey representing a highly selected subpopulation and, what is more important, a subpopulation selected on the basis of largely unknown criteria.

A further problem related to sample matching within the subpopulations who did agree to participate. In the follow up study the controls were, on average, 6 years younger (23% in the 19–28 age group, compared with 3% of the exposed group). There were also differences in previous night's sleep and educational level, the latter being lower in controls. Controls were interviewed earlier in the day (only 3% of their third interviews were in the evening, compared with 30% for the exposed group). They were less likely to have English as a second language (12% compared with 23%), and were less likely to be unfamiliar with computers (43% compared with 67%). They were, however, less well educated (3% with further education, compared with 23% in the exposed group), and more likely to use tobacco (55% compared with 38%). Some of these factors, such as language, computer familiarity and time of day for testing, had a significant influence on outcome measures. Consequently the need to correct for these factors in the multivariate analysis resulted in marked modification of the results. For example, with the syntactic reasoning measure, although a 40% decrement was increased on adjustment to 48%, the introduction of uncertainty inherent in making this adjustment decreased the p value from <0.0001 to <0.037. In other cases this necessary adjustment decreased the differences between the groups, as for symbol–digit substitution, where the 'all trials' measure was adjusted from –12% ($p<0.0001$) to –6.8% ($p<0.038$). The need for such corrections evidently reduced the power of the investigation. One of the major correcting factors would have been that required to compensate for the time of day at which the measures were made. Heavy reliance on these corrections also means that any unrecognised (non-linear) interactions between these factors might have distorted the results in an unpredictable manner. A hypothetical example of this would be if the combination of fatigue associated with evening measures and of speaking English as a second language lead to a greater slowing of response than the effect of either of these factors alone would predict.

A third problem related to exposure measures. The responses to questions about dipping procedures indicated a wide range of experience in the use of organophosphorus pesticides. Workers in the follow up study had 2 to 45 years' experience, with a mean (± SD) of 15 ± 7 years, mostly at two dips per year of 1–3 days' duration. This variation was taken into account in the exposure assessment, and proved very useful as it enabled the exposed group to be subdivided in terms of exposure. However there was also a highly variable approach to personal protection between workers; 11% used hands or feet to ensure that sheep were immersed in dip, only 23% used gloves, 6% reported being soaked by dip and 32% used manual methods to bail out the dip after use. Additionally, not all individuals would have handled pesticide concentrates. Clearly some individuals would therefore have had much heavier exposure than others, even when normalised in terms of numbers of sheep dipped. Such uncertainty concerning exposure would have further reduced the power of the survey to detect positive effects. A further effect to note is the strong association between age and cumulative exposure. However the data show that flock size, not farming years, was the major determinant of the exposure index, so age would not have had a dominating effect on this analysis (Spurgeon *et al.,* 1995).

In summary, the low participation rate introduced a marked element of self-selection which may have led to either under- or over-representation of ill-health in the sample of the exposed population. Heavy reliance on internal correction for mismatch confounders may have introduced some distortion. The power of the study was, however, increased by taking account of exposure levels within the group, although uncertainty in quantification of this exposure limits the power to detect positive effects.

Synopsis

Interpretation of the results of this study must be undertaken at two levels. There is no doubt that the study clearly demonstrated statistically significant differences between these samples of sheep dippers and quarry workers in terms of simple reaction time, symbol–digit substitution and syntactic reasoning scores, and in the general health questionnaire responses. There was also some evidence of a selective sensory defect in the dippers. The extent to which these differences would have been typical of the whole exposed populations is less clear, as the population samples actually tested were highly selected (1 in 7.7 for the exposed, and 1 in 2.8 for the controls). Multivariate analysis showed that age, education, time of day, language, and computer familiarity were probably not responsible for all of the differences found.

Results of the initial tests and questionnaires suggested that individuals in the sample of the exposed population were not in a subjective state of physical ill-health in comparison with the controls, and had no subjective memory problems, although they did differ in psychological health. This suggests that physical ill-heath or poor memory are not likely to have biased the sample. The exposed sample were 50% more likely to have a general health questionnaire response consistent with 'psychiatric caseness', and this relationship cross-correlated positively with their attitude to sheep-dip hazard ($p = 0.026$). Hence the decision to participate may have been linked with concern regarding psychological ill-health, leading to an over-representation of psychological ill-health in the sample, by self selection. However, there was no corresponding relationship between the attitude survey and the cognitive test outcomes, suggesting that there was no conscious self-selection in this case. Indeed the relatively mild cognitive effects may well not have been subjectively apparent unless there had been a sudden deterioration.

The question of the role of pesticides in causation must be addressed. Since one group in this study consisted of farmers and the other of quarry workers they would differ in many ways (having chosen different occupations under different social pressures). Some of these differences could be allowed for as measured but uncontrolled variables. The fact that only one outcome measure could be correlated with the extent of potential organophosphorus dip exposure may suggest that other variables, such as responses to economic pressure or social isolation, may have had a causal role in those outcomes which did not correlate with exposure. It must be recognised, however, that the measure of dip exposure available was a very imprecise one, and that the level of exposure was (on an international scale for occupational exposure) relatively low. Furthermore, the magnitudes of the outcome effects were not large enough to give a great deal of power to the investigation. The index which did correlate with exposure (syntactic reasoning) only did so for the more challenging tasks, and it may be that simpler tasks (which showed smaller effects) were not challenging enough to show up the subtle differences that did occur. However the introduction of more difficult tasks might well have increased the drop out rate.

A large number of studies on the effects of current or previous low-level exposure have been reported (see below); generally these are less reliable than this UK study, but some do describe more highly-exposed populations.

5.3.2 Other studies of neuropsychological effects

In the following sections those studies where a time interval has been allowed for potential recovery from any acute effects before testing have been separated from those involving testing at the time of exposure.

Studies involving previous exposure only

An exploratory neuropsychological study of 57 fruit tree sprayers and 42 non-exposed farmers or shopkeepers was reported by Fiedler *et al.* (1997). The sprayers had used organophosphorus pesticides over a mean of 27 years. Levels of pesticide exposure were estimated [and will subsequently be reported], and were probably low to moderate. No subject had experienced any

previous episode of poisoning, and the tests were conducted in the non-spraying season to avoid any current exposure (as was confirmed by red cell ChE measurement). Exposed subjects were recruited from an identified population of licensed pesticide applicators, and controls were drawn from local farmers or shop keepers with little or no pesticide use. The positive response rate was 39% for the exposed and 11% for the control population, and of these positive responders the actual participation rates were 83% and 86% (excluding those rejected on grounds of age, lack of exposure, or prior illness). The groups were well-matched for age, but the control group had a mean of 1.7 years more education and a significantly better reading ability. Neuropsychological tests showed no difference in a number of tests of concentration, visual–motor function, verbal and visual memory and verbal function. However, simple reaction time was 11% slower in the exposed group ($p<0.01$). When the combined participants were ranked according to an index of cumulative organophosphorus pesticide exposure there was a significantly slower simple reaction time in those with higher exposure, but this was largely attributable to their greater age (4.7 years older), the pesticide exposure measure making a non-significant contribution to their slower reaction time. Overall this study showed no change in a wide range of neurobehavioural tests, but did show a significant increase in reaction time in the exposed group. This could not, however, be correlated to the level of exposure to pesticides. Given the considerable potential for selection bias introduced by the low positive response rate in this study, the results obtained (both positive and negative) should be interpreted with caution. It is clear, however, that no gross cognitive effect was seen in these moderately exposed workers.

A study by Ramos *et al.* (1986) compared 36 farm and public health workers exposed to organophosphorus and carbamate pesticides over an average of 5 years (and having normal whole blood ChE) with 36 non-exposed control farm and health workers. The basis for the group selection and the participation rate was not given, but groups were matched for age, although the exposed workers had two years less education than the controls. The exposed workers showed a reduced score on a test of manual dexterity (75% scored poorly compared with 28% of controls, $p<0.01$). This difference could be correlated with years of employment. The exposed workers reported more headaches and premature ejaculations, but no psychological problems, and had normal neurological examinations.

A neuropsychological and EEG study of volunteers with occupational pesticide exposure (Korsak & Sato, 1977) appeared to show significant differences in EEG spectrum, Bender visual/motor test and trail making test when 16 volunteers with low exposure were compared with 16 with high exposure. The dividing point between high and low exposure levels was defined as being equivalent to 4 days exposure per exposure year, but no indication of the likely degree or nature of exposure to organophosphates was given. Neither group showed decreased plasma ChE on the day of measurement. However, the study used a highly idiosyncratic index of exposure, which was really a distorted index of mean annual potential exposure. There was correction of data for age and concomitant DDT exposure, but not for social status or educational attainment and, importantly, the exposure index used was such as to artefactually place young people who had started work before the age of 18 in the high dose group. Spectral analysis of EEG records showed a number of differences which were significant at the 5% level in nine of 68 multiple comparisons. However the only major differences were a decrease at 13 Hz and 14 Hz in the occipital EEG. The statistical validity of these changes is unclear. There were no differences in the higher frequencies, which were, however, only examined up to 25 Hz. [It should be noted that the study of Duffy *et al.* (1979) showed effects at higher frequencies than this].

In a neuropsychological and EEG study (Metcalf & Holmes, 1969), 56 male workers engaged in the manufacture of a range of unspecified organophosphorus esters, with a mean of 6.5 years of occupational exposure, were compared with 22 controls. There was no description of the frequency or severity of exposure, or its relationship to the time of testing. There was a 2.7-fold increase in reported forgetfulness, a 7-fold increase in reported fatigue, a 2.4-fold increase in reported difficulty in thinking, as well as muscle aches, increased perspiration and visual problems among the

exposed population. The effects suggest that current exposure may have influenced the findings. An EEG investigation of 50 men, again of undefined exposure status, reported more prominent and episodic θ activity, and random slowing. The former effect would be consistent with organic damage and the latter with drowsiness. Unfortunately only general conclusions were reported in an anecdotal manner.

Studies involving current exposure

Durham *et al.* (1965) made two series of psychological tests of mental alertness in mixers, loaders and sprayers involved in aerial crop spraying, before and after the spray season. The extent of pesticide exposure and participant selection basis was not described, nor was the nature of the unexposed control group, and there was no mention of matching between the groups in terms of confounding variables. In the first series, with 53 exposed and 25 control subjects, the groups differed and there were fewer tests completed by the pesticide workers during the exposure period (but not the post-exposure period). However, on stratifying by exposure level no differences could be correlated with the degree of exposure. In the second series, in which additional tests were used with 68 and 22 subjects and a similar mix of occupational exposures, complex reaction time performance (error rates and reaction time) was not different between the exposed and control groups.

Rodnitzky *et al.* (1975) compared the responses of 12 farmers and 11 sprayers who predominantly used organophosphorus pesticides with 22 other farmers who either did not use organophosphates or who had been measured pre-season. The exposed groups had used pesticides within two weeks of testing, and had treated more than 500 acres. All individuals considered themselves asymptomatic at the time of testing. The response rates were not provided, but the groups were matched for age and educational background. The exposed farmers had a non-significant 15% lower plasma ChE, but the sprayers had a highly significant 28% fall. Overall the fall was 22%. Results of tests on verbal recall, vigilance, complex reaction time and proprioception were unchanged. Linguistic performance was assessed by the ability to repeat complex phrases (45% failure rate in controls) and was also unchanged. However this study used few subjects, involved current as well as cumulative exposure, and some of the controls had been exposed via long-term organophosphorus pesticide use.

Maizlish *et al.* (1987) investigated 46 pest-control workers who had used diazinon as a lawn treatment for an average of 39 days during the year of the study. These subjects were compared with 53 similar workers not exposed to organophosphorus pesticides. A neurobehavioural test battery was applied both before and after a working shift for each group. Exposure was estimated by urinary metabolites (see Table 3.1), and also by exposure assessment using dermal patches (0.1–10 mg diazinon per person per day) to be at a relatively low level (less than the current no observed effect level for plasma ChE inhibition). There were no reports of acute effects. The participation rate was 56% in both groups (payment was made to encourage participation). The groups were generally well matched, the control group being an average of 3 years older and having 2 years more education than the exposed group. Outcomes were adjusted to compensate for these differences. Of the measures evaluated, symbol–digit substitution was slower in the exposed workers, pre-shift measures were 4% slower (non-significant) and post-shift measures 9% slower ($p<0.02$), this reflecting a lesser improvement in the exposed workers than the controls between pre- and post-work shift performance. A pattern memory test showed a similar speed of recall and score in both groups, but the post-shift score was lower in the exposed workers when normalised to their pre-shift values ($p<0.03$). Complex reaction time showed a non-significant decrease after correction for confounders (a 5–10% change would have been detectable); hand–eye co-ordination showed a non-significant increase (a 10–20% change would have been detectable); pattern comparison showed a non-significant slowing (a 10–20% change would have been detectable); and finger tapping speed and vocabulary and memory tests gave unchanged results. The slowing in the symbol–digit test was greatest in a subset with more than 4.5 years spraying experience, but neither this test nor the pattern–memory test score could be associated with the level of urinary metabolites. These results suggest a small but significant decrease in performance after a working shift, but gave no evidence for a pre-shift (i.e.

cumulative) difference between the exposed and control groups. Since the control group was working at an approximately similar level of effort, the short-term decrease in performance may have been related to diazinon exposure.

A prospective longitudinal neuropsychological study of 49 pesticide applicators exposed predominantly to azinphos-methyl was reported by Daniell *et al.* (1992). The pesticide workers were recruited on a voluntary basis from participating orchards. The authors stated that although few workers refused to participate, the size of the potential population from which respondents were drawn was not clearly defined, and hence they were essentially a 'convenience sample'. The control population consisted of 40 beef slaughterhouse workers, recruited with a 65% voluntary participation rate. Of the subjects who volunteered, 86% of the pesticide and 78% of the slaughterhouse workers actually participated in the full study. Workers were tested both before and at least 4 weeks after the spraying season. The two populations could not be matched on the basis of first language (45% Spanish in the pesticide workers, and 12% Spanish in control workers), and so were divided into two subgroups and given tests in their preferred language. Within the language subgroups participants were reasonably well matched for age, education and alcohol use, but the Spanish speaking pesticide and control workers had 3.7 and 6.1 years less education respectively. The mean length of occupational exposure to pesticides was 7 years, mostly at a level resulting in less than 15% inhibition of red cell AChE, although three sprayers reported previous pesticide poisoning sufficient to need medical attention. Curiously these workers were not excluded from the main study. Some workers in the control group had experience of pesticide use prior to the study. When language-linked differences were compensated for there were no differences in pre-season performance between the pesticide and control groups in symbol-digit substitution, pattern memory, hand-eye coordination, finger tapping, or continuous performance. The pattern memory test showed less pre- or post-season improvement in both the Spanish speaking pesticide and Spanish speaking control workers. The symbol-digit test showed a smaller improvement in both groups of pesticide workers than controls, and this was significantly smaller in the case of the Spanish speaking workers, although the absolute changes were small, and the groups were not well matched for education. No differences could be attributed to occupation or correlated with pesticide exposure measures. In summary the study showed that low but measurable occupational exposure to azinphos-methyl did not result in significant neuropsychological impairment, although problems with sample size, selection, and matching would have limited the power of the study to detect small decrements.

Richter *et al.* (1992a) studied the neurobehavioural performance of 90 farmers and residents living close to fields sprayed with organophosphorus pesticides. Comparisons were made between peak season and post-season performance in various tests, and with an age, sex and education matched group of 35 residents not exposed to spray drift. The basis of selection and response rates were not reported. Post-season performance of the exposed group was similar to that of the non-exposed group in terms of reaction time, tapping, digit–symbol, digit span and Benton tests, and they showed significantly lower scores for anger and hostility. When this group was examined during the spraying season, scores in all but two neurobehavioural tests were significantly poorer. Simple reaction time showed a non-significant 3% rise, and dexterity (using the Santa Ana test) showed a significant 5% fall. All other tests showed decrements of 3–13%. Mood status showed increased tension, depression, fatigue and anger, as did symptom reports. The exposure levels of this group were not presented, but other similar groups showed in-season reductions of 6%, 2% and 13% in plasma, red cell, and whole blood ChE levels respectively. The authors drew attention to the inevitable association between pesticide exposure and heat stress and heavy work loads, which may also have influenced the test performance and mood scores.

A seven-country biomonitoring and cross-sectional epidemiological survey of low-dose occupational exposure to organophosphorus pesticides was reported by Richter (1993)*. The countries involved were

* The data from this study are presented as a series of separate study results, some of which may well have been reported elsewhere. The final report is supplemented by some selected data from the other participating countries, which are not described in sufficient detail to allow the critical evaluation which they may deserve. The original study data are available from WHO.

Bulgaria, the former Czechoslovakia, Hungary, Israel, Poland and the former Yugoslavia. The overall study involved collection of exposure and outcome data from 507 individuals. However, limitations in the conduct of the study meant that the individual studies could not be unified, although they shared some design features. A series of cross-sectional designs was used, with attempts at matching for age, sex, place of residence, alcohol intake and other neurotoxicant exposure. Plasma ChE and urinary metabolites were used as exposure measures, and the 1986 WHO neurobehavioural core test battery was used as a measure of effect. Complications were introduced by high alcohol intake in the Bulgarian, Hungarian, Polish and Czech study populations, with the Bulgarian study finding a significantly higher alcohol intake in the exposed population than in control populations. Alcohol intake was estimated both from self-reported intake and blood liver enzyme levels. Exposures to a wide range of organophosphorus agents in both manufacture and agricultural use produced plasma ChE depressions in the range of 14–53%. It was not clear if there had been previous acute intoxications in these subjects. Neurobehavioural test data from Israel showed post-season but not pre-season differences between exposed and control subjects similar to those described above. EEG data from Hungary showed elevated slow-wave activity post- but not pre-season, but increased β activity both pre and postseason. EEG data from Poland showed more θ activity. Without a cross correlation with alcohol intake and information on pesticide exposure at the time of EEG measurement it is not possible to evaluate the reports of EEG effects.

A follow-up study was made (Ames *et al.,* 1995) of agricultural workers shown by routine ChE monitoring to have had low-level organophosphorus pesticide exposure insufficient to cause overt signs of poisoning over the previous 5 years . Red cell ChE had fallen by more than 30% and plasma ChE by more than 40%, but there had been no report of acute effects. This study resembled that of Savage *et al.* (1988) described in Section 4.4.2, except that the acute consequences of the exposures had been less severe in this study population. A sample of 45 ChE-inhibited male workers was matched to 90 male controls drawn from the same community. The controls were, on average, 8.7 years younger, had one more year of schooling, but were well matched for race, first language, previous night's sleep, and coffee and alcohol consumption. Of ten measures, nerve conduction velocity, vibration sense, motor co-ordination, and mood (depression or tension) showed no differences between the two groups. Neurobehavioural tests showed a better serial digit test score in the exposed group ($p<0.05$), but no other differences. It is interesting to note that Ames and colleagues found that these measures were, however, changed by a higher degree of exposure, sufficient to cause acute poisoning (Steenland *et al.,* 1994).

In an abstract, Petrović *et al.* (1995) describe a comparison of 30 workers, occupationally exposed to pesticides over an average of 17 years, with a control group of 30 other workers matched for age, sex and socioeconomic status. None of the exposed workers had been acutely poisoned. The digit span test scores were not significantly different across the groups, but the exposed group scored less well on tests of spoken verbal learning ($p<0.05$) and textual memory ($p<0.01$). However it is not possible to make a proper analysis of this study in the absence of a full report.

5.3.3 Peripheral nervous system effects

Neuromuscular function was evaluated by Rayner *et al.* *(*1972) among 11 horticultural workers who used organophosphorus sprays for more than 4 hours per day, on at least 2 days per week over the year and for more than 5 years, and in a matched control group of 13 non-exposed workers. Some of the horticultural workers were also exposed to organochlorines and fungicides. Neither group had evidence of alcoholism or neurological disease. There was no difference in median nerve conduction velocity, motor latency or adductor pollicis EMG, but after data had been corrected for age, Achilles tendon reflex force was 62% lower in exposed workers than in controls ($p<0.005$) 3–4 days after spraying. Rather surprisingly the decrease was only 19% 1–4 hours post exposure ($p<0.05$). Pesticide exposure levels were not measured.

In a study of factory workers Jager *et al.* (1970) studied 36 workers exposed to organophosphorus and organochlorine pesticide, 24 exposed to organochlorine only and 28 workers not exposed to pesticides. The first group showed a decrement in EMG and abnormal after-discharges, despite a normal blood ChE, suggesting that EMG could provide a more sensitive measure than whole blood ChE for long-term continuing exposure. Although initial reports suggested that increased EMG amplitude might be a suitable index, this finding has not been confirmed; moreover there are obvious difficulties in making accurate comparisons of EMG amplitude between subjects, whereas after-discharges (an indication of hyper-excitability) are a much more robust effect.

Stålberg *et al.* (1978) studied 11 asymptomatic spraymen who had worked with organophosphorus, organochlorine and pyrethroid insecticides for 1–24 years with no history of acute poisoning. When pre-exposure and 1–24 hour post exposure values were compared, plasma and red cell ChE were lower by 8% ($p<0.01$) and 6% (not significant). Sensory, but not motor, nerve conduction velocity fell by 3% ($p = 0.05$), and there were no changes in EMG amplitude or decrement, or jitter.

Vibration sensitivity was measured (Stokes *et al.,* 1995) in orchard sprayers and non-exposed workers. A 16% and 15% participation rate provided 90 and 68 exposed and control subjects respectively. The groups were well matched for age, sex, education, neurological illness, alcohol use and area of residence. Five organophosphorus pesticides had been used by the sprayers over a mean of 20 years. The urinary metabolite levels measured are given in Table 3.1. Exposed workers reported an increase in headaches (OR = 6.0; 95%CI = 1–78) during the spraying season, but no increase in 21 other symptoms. Vibration thresholds were measured in the off-season, and showed a 75% increase in the finger ($p<0.01$), but not in the foot. There was no diabetes or carpal tunnel syndrome in either sample. The authors cite another study which reported increased vibration threshold in 229 organophosphorus pesticide production workers compared with 347 textile or fertiliser workers.

5.3.4 Psychological ill-health

A geographically-based study (Stoller *et al.,* 1965) compared schizophrenia incidence with local organophosphorus pesticide sales in Australian fruit growing areas. Schizophrenia was 1.9 times more common across two areas where sales were 7.9 times higher than in a reference area. However, on following up individual cases none could be linked to organophosphorus pesticide use. There was no correlation between schizophrenia and depression and pesticide sales over a larger population of 5034 growers. Such a survey could, however, only be expected to detect a very gross effect, since the exposure and effect measures were limited.

Symptoms of depression were reported in a survey of greenhouse workers whilst using organophosphates, carbamates, herbicides and fungicides (Parrón *et al.,* 1996b). A group of 25 sprayers, [selection procedure not defined] formed the high exposure group and a sample of 80 farmers, self-selected on the basis of responding to advertisements, formed the low exposure group. Most subjects wore no protective clothing due to the hot and humid working conditions. Tremor was reported in 33% of the high, and 7% of the low exposure group, and depression by 44% of the high, and 3% of the low exposure group. The greenhouse workers were exposed throughout the investigation; exposure was not assessed directly, but the workers had no sign of decreased serum or red cell ChE and there were no reports of acute intoxication.

A study of suicide rates (Parrón *et al.,* 1996a) used a study population drawn from the same area as that reported in Parrón *et al.,* 1996b. All 118 suicides in the study area (where 73% of the workforce is employed in indoor agriculture with intensive use of organophosphorus pesticides) were compared with all 133 suicides in either an outdoor agriculture area, or an industrial/service area, both with similar low pesticide use. The suicide rates in these three areas were 152, 104 and 90 per million inhabitants, while that over the whole district (Almeria, Spain) was 60 per million inhabitants. The areas did not differ in the number of migrant workers or pattern of marital status of the suicides, but the suicides in the study area were

younger. Suicides were less common in farmers in the low pesticide use area than in the high use area, and local health district reports of mood disorders were of a higher incidence (OR 1.4–2.5). Pesticide mediated suicide was 41% in the study area, but only 3% and 8% in the reference areas, while suicide by hanging was of similar incidence in all three areas. However, the suicide rates were not age adjusted, nor were they broken down by occupational group, limiting the value of the study considerably. Furthermore it is not clear if pesticide exposure, intensive work, more money for gambling or alcohol, or ready availability of pesticides as a means of suicide was the cause of the increased suicide rate. The increased rate of mood disorders argues against the latter, whereas the similar rates of suicide by non-pesticidal means argues in favour of it. If occupational use of pesticides were a causal factor, this could have been either due to an after-effect of acute pesticide poisonings (known to induce threatening dreams) or to a chronic effect.

A neuropsychological study of pesticide workers by Amr *et al.* (1997) focused particularly on psychological disorders. A sample of 208 mixed pesticide (organophosphate, carbamate, pyrethroid, and organochlorine) formulators was compared with 72 unexposed factory workers, and a sample of 172 mixed pesticide applicators was compared with 151 unexposed rural workers. The sample populations were matched for community type, age, socioeconomic status and educational level, and were randomly selected, although study participation rates were not provided. All exposed workers had used pesticides for at least 2 years, often with minimal safety precautions, and the level of exposure was probably greater for the formulators than the applicators. No exposure indices or information about the incidence of possible previous acute intoxications was provided. Both exposed groups showed a higher incidence of total psychiatric disorders when compared with their community control groups (50% compared with 32% for formulators, and 31% compared with 17% for applicators, both $p<0.01$). Amongst the formulators, 'depressive neurosis' was higher over the whole group (19% compared with 7%, $p<0.05$) and the incidence of depressive neurosis could also be related to duration of employment (with 10% incidence for employment up to 5 years and 32% incidence in the group with more than 20 years employment). However, there was no attempt at correction of these data for a possible age- rather than exposure-related effect. The formulators also reported increased irritability and erectile dysfunction. It is not clear whether the assessments were influenced by current exposure, hence acute low-level intoxication may have explained these reports. The authors commented on the lack of any markedly higher incidence of 'situational or reactive depression' (i.e. that linked to an obvious trigger such as marital problems) in either exposed group. This implies that a general over-reporting of depressive symptoms by the exposed groups was not likely. It is difficult to identify the role that organophosphorus pesticides might have played in the causation of the differences between the exposed and control groups. The large difference between the incidence of depression in the control rural and control factory workers illustrates the impact of occupation and associated conditions on psychological illness in the absence of pesticide exposure, and it is possible that factors such as work stress, fear of intoxication and exposure to solvents and other pesticides may have played a part in the exposed groups.

A psychological study (Levin *et al.,* 1976) evaluated 24 male farm workers and sprayers who had used organophosphorus pesticides for at least 2 weeks within less than 2 weeks of testing. They were compared with 24 farmers who either did not use pesticides or had been tested pre-season (i.e. prior to any exposure). The control group was well matched with the exposed group for age, years of education, drug use and smoking, but may have been exposed to organophosphorus pesticides in the previous season. The exposed group had a 22% lower plasma ChE, consistent with recent exposure, but did not differ from the control group in their red cell ChE. The exposed group showed significantly higher scores for work-related tension, sleep disturbance, restlessness and nervousness, but not depression. This difference originated entirely from the more highly exposed sprayer subgroup. As there were no individuals engaged in non-organophosphorus pesticide spraying in the control group it is not possible to conclude whether the stress effects were specifically organophosphorus pesticide related or generally work stress related.

5.4 Zero exposure studies

The effect of exposure stress in the absence of actual exposure was studied by Markowitz *et al.,* 1986. In a malathion manufacturing accident a port was transiently contaminated, and the authors were able to compare the reactions of a group of seamen who stayed sealed in their ship as a precaution during the incident with a well-matched control group who were in the port on another day. Of the 27 'exposed' seamen 79% participated, and 78% of the controls. When questioned 12 days later the 'exposed' group showed elevated subjective 'demoralisation' scores which were, however, only significantly increased amongst those who also reported 'little knowledge about toxic chemicals'. This apparently beneficial effect of toxicological knowledge may serve to encourage those involved in public education.

5.5 Summary of low-dose studies

When the many low exposure studies are summarised (see Table 5.1), a number of reservations noted by individual authors need to be borne in mind. In many cases worker exposure was inextricably associated with hard work, and in only a few studies could this factor be controlled for, as by Stephens *et al.* (1995a). Bystander exposure was sometimes associated with apprehension and fear (Maizlish *et al.,* 1987). In the case of the EEG study by Duffy *et al.* (1979), exposure may have been higher than 'low level'. Similarly it is not possible to be sure that the exposures described in the studies of Parrón *et al.* (1996a, 1996b) did not also include some relatively high exposures. The epidemiological studies of Stephens *et al.* (1995a), Maizlish *et al.* (1987), Richter *et al.* (1992a), Fiedler *et al.* (1997), Daniell *et al,* (1992) and Ames *et al.* (1995) appear to be the most reliable in terms of design; those by Maizlish *et al.* (1987) and Richter *et al.* (1992a) showed only acute (on exposure) effects, that of Stephens *et al.* (1995a) only a small (but significant) effect, that of Daniell *et al.* (1992) and Fiedler *et al.* (1997) mostly negative effects, and that of Ames *et al.* (1995) no effect. Where positive effects were seen, these were small enough not to be subjectively apparent.

Of peripheral signs, EMG decrement seems to be a sensitive and relatively anticholinesterase-specific measure of exposure. The measure of postural sway (Sack *et al.,* 1993) appears to be very sensitive to sensory–motor dysfunction, but is not specific. Of the neurobehavioural tests, speed seems to be more consistently affected than performance, and the most sensitive measures appear to be those that are the most difficult (i.e. take the longest time to answer).

Table 5.1 Summary of effects seen with low-level exposure[a] (ranked by group size)

Exposed sample size & study type	Measures	Effect	Reference
73 clinical[b]	EEG	increased β	Duffy *et al.* (1979)
37 clinical	postural sway	performance ↓*	Sack *et al.* (1993)
20 clinical	neurological	sensory ↓*	Stephens *et al.* (1995a)
158 epidemiological	neuropsychological	reaction time ↓ symbol–digit ↓* syntactic reason ↓* learning/memory →	Stephens *et al.* (1995a)
118 epidemiological[b]	demographic	suicide increased mood disorders ↑*	Parrón *et al.* (1996a)
105 epidemiological	symptom reports	tremor ↑*+	Parrón *et al.* (1996b)
90 epidemiological	neuropsychological.	reaction time ↓+ symbol–digit ↓+ dexterity ↓*+ anger ↓*+ anger ↑*	Richter *et al.* (1992a)
90 epidemiological	neuromuscular	vibration sense ↓*	Stokes *et al.* (1995)
57 epidemiological	neurobehavioural	reaction time ↓* symbol-digit → visual memory → verbal memory → verbal ability → dexterity ↓	Fiedler *et al.* (1997)
56 epidemiological[b]	EEG, psychological	EEG (more θ) subjective memory ↓* fatigue ↓*	Metcalf & Holmes (1969)
53 epidemiological	neuropsychological	slower performance	Durham *et al.* (1965)
49 epidemiological	neurobehavioural	pattern memory → symbol–digit → tapping → Coordination → Continuous performance →	Daniell *et al.* (1992)
45 epidemiological	neurological, neuropsychological	nerve conduction → vibration sense → mood → serial digit score ↑*	Ames *et al.* (1995)
36 epidemiological	neuromuscular	EMG decrement *	Jager *et al.* (1970)
36 epidemiological[b]	neurobehavioural	dexterity ↓*	Ramos *et al.* (1986)
24 epidemiological	psychological	tension ↓* sleep disturbance ↓* depression →	Levin *et al.* (1976)
23 epidemiological	neuropsychological	reaction time → vigilance → linguistic skill → proprioception →	Rodnitzky *et al.* (1975)
16 epidemiological	EEG, neuropsychological	EEG (decreased α) * visual/motor ↓*	Korsak & Sato (1977)
11 epidemiological	neuromuscular	EMG → nerve conduction → tendon reflex ↓*	Rayner *et al.* (1972)
11 epidemiological	neuromuscular	EMG → nerve cond. (motor) → nerve cond. (sens.) ↓* jitter →	Stålberg *et al.* (1978)

[a] Low-level exposure is here defined as a level insufficient to cause acute cholinergic signs
[b] Group may have included some people with acute intoxication

↓, worse performance; ↑, better performance; →, unchanged performance;
+, only during exposure; *, statistically significant

6 Studies in progress

6.1 Background

There are several relevant studies reported to be in progress;

- ***an epidemiological study funded by the Ministry of Agriculture, Fisheries and Food, Health and Safety Executive and Department of Health and being conducted by the Institute of Occupational Medicine in Edinburgh;***

- ***a neurological and epidemiological study being conducted by Dr Goran Jamal in Glasgow; and***

- ***an experimental biochemical study being conducted at the MRC Toxicology Unit in Leicester.***

Since the final results of none of these are available at present it is only possible to make brief mention of the objectives and comment on some data from these studies, and on a few others known to the author.

6.2 Institute of Occupational Medicine study

This information is based solely on HSE/MAFF press releases.

Commissioned in 1995, the study is due to be completed in April 1999. Its objectives are:

- ***to develop a method to determine the level of organophosphorus pesticide exposure during sheep dipping;***

- ***to survey a representative population of sheep dippers for nervous system effects which might be related to organophosphorus pesticide exposure, using a cross-sectional study design; and***

- ***to classify any effects in terms of clinical disease, and to make a risk assessment.***

It is further stated that any effects should be well defined, and that there should be a focus on peripheral neuropathy, neurophysiological and neuropsychological abnormalities and exposure–response relationships.

It is clear that the first objective is of great importance, but it will be difficult to find such a method that will be applicable to a cross-sectional study design, where retrospective assessment of exposure is an important factor. The second objective represents a reasonable compromise between an unachievably open-ended study and one which is too narrowly focused. It is interesting to note that this study will concentrate wholly on sheep-dip-related exposure, which does not lead to an exposure sufficient to inhibit blood ChE. The power of any such sheep dip study must be limited by the relatively low exposures in the UK compared with a study of, for example, WHO supervised spraymen working in a developing country. Although exposures associated with sheep dipping are the primary concern in the UK, such studies seem less appropriate when viewed in a world context. The interpretation of the results of the IOM study by international bodies may well also present problems, as it would be difficult to determine whether any positive results represented a purely local phenomenon specific to the spectrum of agents and conditions used for UK dipping, or a more general effect.

6.3 Glasgow study

This information is based on Jamal (1995) and on personal communications from Dr Goran Jamal.

Preliminary clinical neurological data derived from a largely self-selected group of patients with some degree of exposure to organophosphorus pesticides have been used to identify indices of ill-health which may be of value in a main study. These indices include measures of vibration sensitivity and autonomic control of skin blood flow.

A subsequent main study is now underway using three groups: an exposed group of 16 sheep farmers randomly selected from a group of 200 UK farmers claiming ill-health; a group of 16 individuals randomly selected from a register of sheep farmers near Glasgow; and a control group of 16 unexposed people. The second group of farmers reported no episodes of acute poisoning and no general ill-health. Red cell and plasma ChE were normal in all three groups. Indices [details not specified] were reported to indicate evidence of an axonopathy in both groups of farmers. It would appear that, unless the indices used are dramatically more sensitive than those used by other investigators, these sample sizes will prove to be too small to be useful. However a further collaborative study with the IOM is apparently planned.

6.4 MRC Toxicology Unit study

The data on which this information is based are from an MRC funded study carried out by the author (D Ray), of which a preliminary report has been published.

In order to provide essential mechanistic data about possible previously unrecognised effects of organophosphorus pesticides, their ability to interact with brain proteins has been experimentally surveyed. Pesticides or their oxons were incubated with rat brain homogenates and tested for their ability to prevent subsequent labelling by radioactive DFP. Present evidence shows that two novel brain proteins, of molecular weight 30 kDa and 90 kDa, show covalent binding with organophosphates at toxicologically relevant concentrations. These proteins, which account for the majority of DFP binding not associated with other known targets such as AChE, butyrylcholinesterase (BuChE) and NTE, have been partially characterised, and do not represent plasma contaminants. When compared with the known primary target in brain, AChE, the first group of proteins show a k_a 3.3 times higher using diazoxon, and five times higher using paraoxon. The second group show a k_a 3.7 times higher using diazoxon, and 6 times higher using dichlorvos. It is concluded that these brain proteins are likely to be significantly affected by subsymptomatic doses of these three commonly used organophosphates. However, in the case of chlorfenvinphos and demeton-S-methyl no significant interaction with the novel proteins could be measured, despite a clear effect of these agents on AChE. This indicates that the protein binding is both highly sensitive and target-compound specific, and not a broad class effect.

Interaction with protein does not necessarily indicate an adverse effect, as is well established in the case of plasma esterases. This same principle may also extend to these brain proteins. To address this point it will be necessary to establish whether these proteins have a biological function, and that is a future objective of the study. One of the proteins shows *N*-acyl peptide hydrolase activity, inhibitable *in vivo* by a dose of dichlorvos low enough to cause no cholinergic signs. It remains to be seen if this inhibition has functional consequences.

6.5 Other studies

A retrospective cohort study of exposed apple sprayers and non-exposed beef slaughterhouse workers has been proposed, but not yet reported, by Rosenstock *et al.* (1990).The epidemiological study of Petrović *et al.,* 1995 (reviewed in Section 5.3.2) has been reported only in abstract, and a full report is awaited.

Three relevant reports of as yet unpublished studies were presented at the 6th meeting of the *International Neurotoxicology Association* in Szeged, Hungary, in July 1997. These were published in abstract form, and are briefly summarised here.

L. Hinkova (Sofia, Bulgaria) reported on a prospective study of agricultural workers' exposure to organophosphorus pesticides in Bulgaria, using the WHO behavioural test battery (Hinkova & Vergieva, 1997). 45 Sprayers and loaders with an average of 4 years pesticide use were compared with 19 unexposed drivers, both before and after the spraying season. The groups were matched for age, alcohol consumption and disease. Exposure levels in the sprayers and loaders were high, with 70% reporting acute symptoms (sweating, ocular effects, fasciculation) during the previous season. Three cases of peripheral neuropathy were found in the exposed group, and individuals from this group also showed significantly poorer performance in the digit span and pursuit aiming tests at the end, but not before, the spraying season, suggesting an acute effect of moderate level exposure similar to that reported by others. Prior to a full report — and possibly a larger control group — it will not be possible to reach any conclusion regarding the absence of a chronic effect.

S. Gralewicz (Lodz, Poland) reported on the persistence of neurobehavioural effects produced by chlorfenvinphos in rats and rabbits (Gralewicz & Socko, 1997). After a single dose sufficient to cause a 35% fall in red cell ChE, behavioural measures took 21 days to return to normal levels. After ten daily doses sufficient to cause a 40% fall in ChE, an increase in stress-induced anti-nociception lasted for 40 days, which was 5 days longer than the depression in red cell ChE. On being given a scopolamine challenge 4 months after dosing, these rats also showed a significantly different performance in a radial arm maze test. These interesting studies suggest that sensitive measures can demonstrate a significant after-effect of moderate level poisoning, which outlasts any acute intoxication. Where reversibility was investigated, these effects were found to be ultimately reversible, but only a few such tests were repeated.

A review of the potential of organophosphorus pesticides to cause adverse effects on long-term health was presented by Brown *et al.* (1997). This review drew on both published reports and unpublished industrial data, with the stated aim that a final report would be published by ECETOC after ratification by the ECETOC Scientific Committee. The report presented essentially the manufacturers' view of the hazards, and drew attention to a number of problems (already discussed in detail here) such as confusion between acute and lasting effects due to continuing exposure, and the difficulties inherent in retrospective studies of poorly defined populations. The review concluded that studies conducted to current regulatory requirements do not include memory or neurobehavioural measures adequate to enable the detection of putative long-term effects; the authors felt that the positive evidence for the existence of such effects in man was inadequate.

A potentially useful survey of UK orchard sprayers exposed to chlorpyrifos is reported as being conducted by Dr R Stephens in a 1997 Health and Safety Executive research abstract. Cognitive function and psychiatric state were to be measured in workers with long-term previous, but no current exposure, and these are to be correlated with individual baseline measures of serum paraoxonase and other blood esterases.

7 Summary and conclusions

There is good evidence that organophosphorus pesticides cause three syndromes of poisoning in man and in experimental animals. These are acute pharmacological toxicity, the intermediate syndrome and delayed polyneuropathy. Acute poisoning is reasonably well defined in terms of nature and threshold, and, although low-level effects merge into the range of non-specific symptoms, its occurrence can be predicted by correctly conducted red cell ChE monitoring. In experimental animals severe acute poisoning leads to direct excitotoxicity with persisting consequences in the central nervous system, and to reversible effects at the neuromuscular junction. In man there is evidence, from epidemiological studies, for persisting after-effects of severe intoxication, but it is far from clear if this is an organophosphorus agent-specific effect or a general consequence of intoxication trauma, or (in the most severe cases) hypoxic–ischaemic damage. In these post-intoxication studies only a small proportion of the variability found could be accounted for by the severity of the original poisoning (Steenland *et al.,* 1994), but the worldwide incidence of severe poisoning is still unacceptably high. Intermediate syndrome only occurs as a complication of severe intoxication, and delayed neuropathy is only produced after severe intoxication with a limited range of agents.

There is limited evidence for short-term (up to a month or so) neurotoxic consequences of low-level exposure to organophosphorus pesticides, even in the absence of overt acute toxicity, serving to illustrate that modern neuropsychological tests are capable of detecting far more subtle effects than would a simple clinical examination. Longer lasting effects have been shown by some well designed studies and not by others. The UK study on sheep dippers by Stephens *et al.* (1995a), in particular, provides clear evidence of subtle, low-level cognitive differences between two study groups, which may be linked with one having an occupation that involved exposure to organophosphorus pesticides. However, difficulty in quantifying the low level of exposure in the exposed group lead to uncertainty about the causative role of pesticide exposure in this clearly occupation-related effect. The positive findings in this study are not incompatible with other studies which have failed to find more gross *subjectively apparent* cognitive changes, or with other studies with negative findings that could be explained by use of small groups or insufficiently sensitive indices. However, in studies where positive but reversible effects have been found, the lack of lasting effects in such subjects is the main reason for doubt concerning reports of long-term effects. Although there is at present insufficient evidence to eliminate the possibility that subtle, (i.e. not subjectively apparent) effects may be produced by low-level exposure to organophosphorus pesticides, based on the balance of published evidence it can be concluded that such exposures are not likely to be responsible, in themselves, for any adverse health effects large enough to be subjectively apparent.

When worldwide studies are compared it is evident that any class effect of organophosphorus pesticides should be much more apparent in those populations that have much higher exposure (e.g. tropical sprayers) or be easier to detect in populations with a better-quantified exposure (e.g. schistosomiasis patients) than is seen in the UK. Therefore the power of any UK study to detect

(and identify) possible effects must be limited, as is apparent from the work of Misra *et al.* (1985), Maxwell *et al.* (1981), Onadeko (1979) and Aden-Abdi *et al.* (1990). Attention in the UK could more usefully be directed to examining agent-specific effects in this country, and class effects could most usefully be evaluated in a prospective study of cases of higher exposure elsewhere. Were a clear effect identified under such conditions of high exposure, the information obtained could then be applied in the UK to study more subtle threshold effects. It should be recognised that a UK population with only low exposure is a poor choice for any investigation designed to identify a novel effect. Although UK populations do differ from others in the pattern of use and the specific spectrum of pesticides, it would be remarkable if the UK population with low exposure were to suffer an adverse effect not seen more clearly in more highly exposed populations elsewhere in the world.

The lack of definition of exposure groups in epidemiological studies, and the restricted use of the more sensitive neurobehavioural tests in clinical studies have given rise to problems. Had a number of otherwise excellent clinical studies used the same indices that were employed in the better neurobehavioural studies (e.g. the syntactic reasoning test of Stephens *et al.* 1995a), it should have been possible to draw a definitive conclusion on these matters several years ago. The cautionary points raised by Spurgeon *et al.* (1996) relating to the non-specific nature of low-level signs of organophosphorus agent intoxication are also important. These authors made the useful recommendation that any study of such symptom reporting should include an estimation of the known individual and societal factors that are liable to influence reporting rates.

A range of well known and validated tests is available for neurobehavioural studies. Slowing of performance appears generally to be a better index than does test score, and more difficult tasks may be required to show up subtle differences. It is particularly unfortunate that, after an interesting start (Burchfiel *et al.,* 1976) EEG measures have been applied only to cases with either poor exposure definition (Duffy *et al.,* 1979), inadequate reporting (Metcalf & Holmes, 1969), or technical limitations (Korsak & Sato, 1977). Recent EEG studies in animals have not addressed the question of reversibility in any detail. Of other indices, postural sway (Sack *et al.,* 1993) and vibration sensitivity (Stokes *et al.,* 1995) may be sensitive if non-specific, whereas EMG jitter and decrement are somewhat less sensitive but more specific.

In some cases (e.g. with carbamates), it is possible that biological effects which apparently were associated with very low ChE inhibition were in fact due to an underestimate resulting from an artefactual reactivation of ChE occurring between the time of sampling and completing the assay. This technical problem has been well described in the past, but has not been addressed by all investigators.

There are five further areas where it would be useful to follow up interesting observations.

- ***Suicide and depression. Previous studies of suicide incidence (see Section 5.3.4) suffered from poor data analysis, an association of an unusually high level of exposure with intensive workload and probable intermittent acute poisoning. However there are now sufficient data that suggest a possible effect to justify more thorough investigation of depression incidence in other high or medium exposure situations. A study of depression in low exposure groups, such as UK sheep dippers, would be more difficult to justify.***

- ***Adaptation to chronic exposure. The gradual adaptation that leads to diminution of acute signs with continuing exposure (see Section 2.2) is of interest because it could indicate that such adaptation might lead to increased occupational exposure due to lack of warning signs. Most occupational and other exposures would follow a steady or declining pattern with time (which would generate early warning signs of over exposure), but some may show a gradually increasing exposure pattern. The interesting question of whether such an incremental exposure in man might allow gradual development of tolerance with only minimal acute signs does not appear to have been adequately addressed in the published literature.***

- ***Cholinergic excitotoxicity (see Section 4.4). The dose-dependence and duration of some of the neuromuscular junction effects, particularly with carbamates, gives rise for concern that something similar may be seen in the central nervous system in cases of severe acute central intoxication. A thorough evaluation of this possibility at a realistic dose level would be valuable, if only as a reassurance.***

- ***The possibility of novel protein targets. Epidemiological data require the support of information on underlying mechanisms. The author is presently involved in work which seeks to identify more of the as yet uncharacterised protein targets known to be present in brain (see Section 6.4).***

- ***Individual predisposition. The role of individual differences in blood or liver metabolic capacity as determinants of actual (rather than theoretical) individual susceptibility*** in vivo ***is not yet clear (see Section 2.7), and would also benefit from further study.***

Finally, the contribution of other reviewers of this literature should be acknowledged. Neuropsychological literature has been reviewed by Davies (1990), behavioural effects by Levin & Rodnitzky (1976), psychological effects by Mearns *et al.* (1994) and general long-term effects by Jamal (1995), Eyer (1995) and Steenland, (1996).

The conclusions presented here are based on primary data sources but do, however, appear to agree with most other reviews. The spectrum of opinions offered by the reviewers mentioned above may be briefly summarised as follows: only acute intoxication leads to lasting effects (Eyer, 1995) or non-symptomatic exposures may lead to lasting effects (Levin & Rodnitzky 1976; Davies, 1995; Mearns *et al.,* 1994; Jamal, 1995; Steenland, 1996). Within this second group there is some disagreement regarding the possible or probable role of organophosphorus pesticides in the causation of these lasting effects. Almost all reviewers agree that further work is needed in order to clarify presently equivocal findings relating to the consequences of asymptomatic exposures, and that reported epidemiological studies have various shortcomings. These shortcomings are well summarised by Mearns *et al.* (1994), who noted that, across various studies, there was a lack of blind testing, a lack of sample matching, a lack of follow up, poor exposure assessment, low response rate, confounding of age with cumulative exposure, and poor interpretation of test results. Fortunately, few studies managed to combine all of these shortcomings. A number of authors, including Jamal (1995) and Richardson (1995) have drawn attention to the absence of any clear theoretical or mechanistic framework to explain long-term effects. This last issue should be recognised as an important research priority.

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Appendix

Structural classification of organophosphorus pesticides mentioned in this review

Di*methyl* phosphorothioates (P=S) or phosphates (P=O)

Bromophos, Chlorfenvinphos, Demeton-*S*-methyl, Dichlorvos, Fenthion, Fenitrothion, Iodofenphos, Methyl parathion, Mevinphos, Phosmet

Di*ethyl* phosphorothioates (P=S) or phosphates (P=O)

Chlorpyrifos, Coumaphos, Diazinon, Paraoxon

Phosphonothioates (P=S) and phosphonates (P=O)

Cyanophos, EPN (*O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate), Leptophos, Trichloronate, Metrifonate (Trichlorphon), Sarin

Phosphoro (mono- or di-) amidates

Isofenphos, Methamidophos, Mipafox, Propetamphos

Other structures

Amiprophos, DFP (di-isopropylfluorophosphate), DEF (S,S,S-tri-*n*-butyl phosphorotrithioate), dioxabenzofos (salithion), Isodiazinon, Merphos

Glossary of terms

Acute syndrome A somewhat variable set of cholinergic signs and symptoms seen after exposure to all acetylcholinesterase inhibitors (see sections 2.2 and 4.3). Unless acutely fatal the effects are fully reversible (by definition), but may be sustained during the course of chronic exposure to appropriate dose levels.

Intermediate syndrome A reversible syndrome sometimes seen as a later complication of severe acute poisoning, and characterised by proximal muscle weakness (see Section 2.3).

OPIDPN Organophosphorus ester-induced delayed polyneuropathy. Also known as delayed neuropathy (OPIDN). A specific syndrome produced by some organophosphates, phosphoramidates or phosphonates and characterised by a delayed onset of a mixed central and peripheral distal neuropathy with both motor and sensory components. The peripheral component is slowly reversible, but the central component is not (see Section 2.4). The target protein is neuropathy target esterase (NTE).

Esterases

Acetylcholinesterase (AChE) is responsible for the hydrolysis of acetylcholine in nervous tissue, but is also present in non-nervous tissues such as the red blood cell. It is both a target and a useful dose monitor.

Butyrylcholinesterase (BuChE, pseudocholinesterase, plasma ChE) has no known physiological substrate, but is often co-localised with AChE. Agents which inhibit BuChE will usually inhibit AChE, but sometimes with very different relative potencies. Plasma BuChE can be used as a dose monitor, although it is less useful as a predictor of toxicity than is red cell AChE.

A-esterases are tissue esterases capable of hydrolysing organophosphorus esters. They are important in detoxification but are also a target, as they are inhibited by organophosphorus esters.

Paraoxonase is an A-esterase found in blood, which has a fairly wide substrate affinity, but is usually assayed by its ability to hydrolyse paraoxon.

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