CHAPTER 15

NEURODEGENERATIONS INDUCED BY ORGANOPHOSPHOROUS COMPOUNDS

Alan J. Hargreaves
School of Science and Technology, Nottingham Trent University, Nottingham, UK
Email: alan.hargreaves@ntu.ac.uk

Abstract: Organophosphorous compounds (OPs) are widely used in agriculture, industry and the home. Though best known for their acute effects when used as pesticides, which target acetylcholinesterase (AChE) activity in neuromuscular junctions and the central nervous system, not all OPs are potent inhibitors of this enzyme. The widespread use of OPs has heightened concern regarding their toxicity in man, with numerous reports linking OPs to various forms of delayed neuropathy encompassing a range of neurodegenerative, psychological and neurobehavioral effects. There is mounting evidence to suggest that sub-acute levels of OPs have the ability to interact directly with a range of target proteins in addition to AChE (i.e., noncholinergic targets), causing major disruption of membrane and protein turnover, protein phosphorylation, mitochondrial dysfunction, oxidative stress and cytoskeletal re-organisation, although the mechanisms involved are not fully understood. However, major advances have been made in the study of one OP binding protein neuropathy target esterase (NTE) in terms of its true physiological role. Additionally, there is increasing evidence for the ability of OPs to cause disruption in a number of metabolic and cell signalling pathways that affect neuronal cell proliferation, differentiation and survival and to interact direct with non-esterase proteins such as tubulin. The aim of this chapter is to review our current understanding of delayed neurotoxicity, to discuss how these molecular events may relate to each other and to suggest possible future directions in mechanistic studies of OP toxicity.

INTRODUCTION

Organophosphorous compounds (OPs) have been widely used in agriculture (e.g., as pesticides) and industry (e.g., as flame retardants and lubricants) over the past half century.[1-3]

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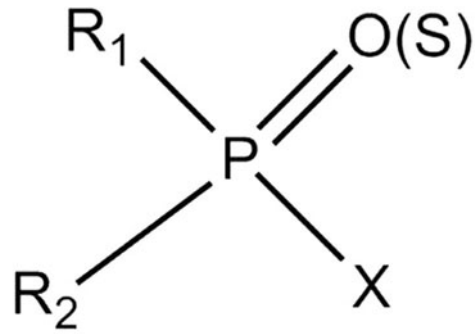

Figure 1. Generalised structure of commonly used organophosphorous compounds. Many of the commercially used OPs comprise a pentavalent phosphorous atom linked via a double bond to an oxygen atom (in organophosphates) or sulphur (organophosphorothioates). Groups R1 and R2 are typically ethoxy or methoxy groups although other substituents are possible. The group labelled 'X' is an ester linked aliphatic, homocyclic or heterocyclic arrangement and in some compounds (e.g., trichlorfon) contains halogen groups. This is the most easily hydrolysed group and, as such, becomes the 'leaving group' that is displaced when an OP binds covalently to the active site of AChE.

Figure 1 shows the general structure of commonly used OPs. A typical structure comprises a pentavalent phosphorous atom linked by a double bond to either oxygen (organophosphate) or a sulphur (organophosphorothioate) atom, with various possible combinations of aryl and alkyl substituent groups ester-linked to phosphorous via the other 3 bonds.

Organophosphorous compounds are the most widely used group of pesticides, exerting their acute toxicity via inhibition of acetylcholinesterase (AChE) in target organisms, although OPs used as industrial lubricants, flame retardants, etc., are not all classed as strong AChE inhibitors.[1-4] As exposure to many OPs can result in various forms of neurotoxicity, their widespread use has fuelled concern over their potential toxicity in humans, leading to the imposition of bans or restrictions on the use of some OPs in a number of western countries over recent years.

One example is diazinon (Fig. 2), which is used in many countries as an agricultural or domestic pesticide and is currently the only licensed sheep dip pesticide in the UK.[5] Although diazinon was considered to be only moderately toxic based on LD_{50} tests in the rat,[6] concern has been raised regarding the hazards posed by accidental exposure, particularly in pesticide handlers and workers.[7] As a result of these concerns, diazinon is now restricted or banned altogether in domestic pesticides in the USA, as an agricultural insecticide in the European Union 9 (EC 2007) and for sheep dipping in Australia.[8-12]

A well studied form of delayed neurotoxicity is OP-induced delayed neuropathy (OPIDN), the clinical symptoms of which occur 1-3 weeks following exposure to OPs such as tricresyl phosphate.[13-15] A major outbreak in the last century, referred to as 'Ginger Jake poisoning', occurred in the USA during the 1930s when alcohol was prohibited. This was found to have been caused by an alcohol-containing 'remedy' called Ginger Jake, which had been adulterated with tricresyl phosphate (TCP), causing partial paralysis in thousands of individuals.[16,17] Some OPs used as pesticides have also been linked to the induction of OPIDN, although in the case of chlorpyrifos and leptophos this required exposure to very high acutely toxic doses and diazinon has been deemed as unlikely to pose a significant risk of inducing OPIDN.[2,3,18-20] Pesticides deemed to pose a significant risk of inducing OPIDN have either been banned or restricted in use.[1-3] Although food

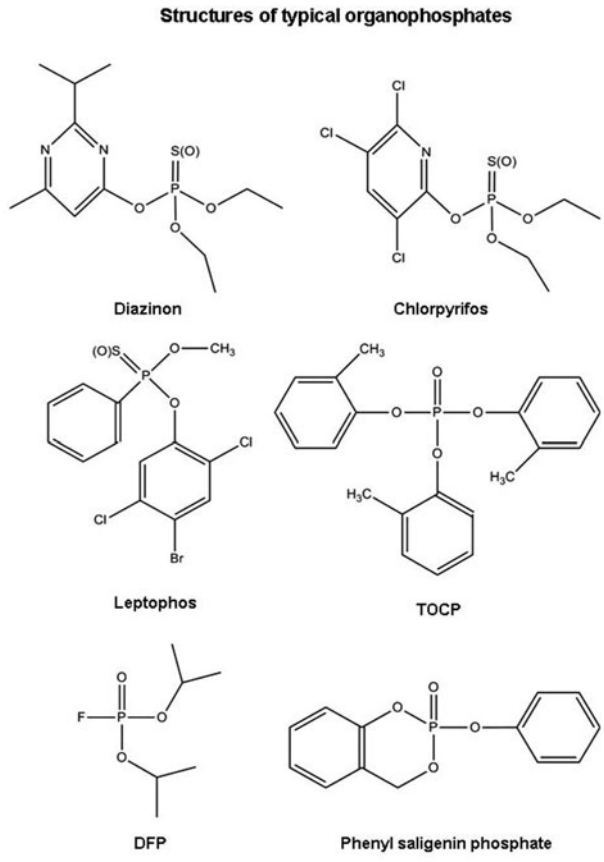

Figure 2. Structures of typical OPs. Shown are the chemical structures of some typical OPs discussed in this chapter. In order to become potent inhibitors of AChE, the organophosphorothioates (e.g., diazinon, chlorpyrifos and leptophos) require bioactivation to their 'oxon' derivatives (e.g., chlorpyrifosoxon) by specific microsomal cytochrome P450s, as a result of which the sulphur atom is replaced by an oxygen atom.

adulteration with OPs such as TCP and cases of accidentally induced OPIDN by pesticides are now thankfully very rare, OP pesticides are still in widespread use.

Furthermore, TCP is widely used in the aviation industry due to its flame retardant and lubricant properties, with the ability to function as a lubricant at very high temperatures. Though not considered a strong AChE inhibitor, this OP is a potent inhibitor of neuropathy target esterase (NTE) which, like AChE, is a member of the serine hydrolase family of enzymes. This observation, together with the fact that strong inhibitors of AChE are often weak inhibitors of NTE is consistent with the idea that individual OPs can interact with a variety of serine hydrolases with different levels of efficacy.[21-23]

TCP is, in fact, the compound of choice for studying OPIDN in animal models.[13-15] It is used in the aviation industry as a fuel and hydraulic fluid additive but is also used as a plasticiser, lubricant and flame retardant.[24-26] Isomers of TCP (such as tri-ortho-cresyl phosphate or TOCP) along with other components of aviation hydraulic fluids have been detected in air cabins and cockpits on commercial and military aircraft.[24-27] As a result,

OP poisoning has been tentatively implicated in the phenomenon of air cabin sickness.[26] Although direct involvement of OPs in air cabin sickness is not yet proven, the symptoms of air cabin sickness are similar to those caused by OP exposure.[25] Furthermore, concern has been raised about the validity of the methods of recording cockpit air quality and performing laboratory experiments on hydraulic fuel OP toxicity.[26]

Commercial preparations of TCP contain a mixture of *ortho*-, *para*- and *meta*-isomers. In response to the scientific evidence that the *ortho*- isomer of TOCP was the most potent inducer of OPIDN, it was stipulated that the level of this isomer in aviation fluids should be reduced to <1% of total TCP and the total TCP concentration to less than 3% of the total volume.[13,14,28] While the level of TCP in most cases falls within this range, it is possible that toxicity of the TOCP component may be under estimated due to the presence of more highly toxic mono- and di-*ortho*cresyl variants.[25,26] Furthermore, although recorded levels of TOCP in cabin air are lower than the recommended maximum under *normal* flying conditions, they are often within an order of magnitude of these limits.[26,29] Moreover, long term repeated exposure may be a problem for frequent fliers, predisposed individuals and/or when serious leaks enter the air cabin environment.

In addition, exposure to other TCP isomers in hydraulic fluids and their potential to interact with the *ortho* isomer in exerting a toxic effect may have been overlooked.[25,26] Indeed, little is known about the toxicity of this compound in combination with other isomers of TCP (TPCP and TMCP) and other OPs (e.g., triphenyl phosphate) present in some aviation fluids.

Thus, despite restrictions on the use of a range of OPs, there remains a significant public health concern since exposure to several of those still in use have been linked with various forms of delayed neurotoxicity.[3,13,14,30-34]

MECHANISMS OF ACUTE AND DELAYED NEUROTOXICITY OF OPs

The main neurotoxic effects reported from OP exposure in humans are:

- Acute toxicity, which occurs within hours of exposure.
- Organophosphate induced delayed neuropathy (OPIDN), which can occur up to several weeks following exposure.
- Chronic neurotoxicity including neurobehavioral and neurological deficits.

In addition, a number of OPs have been proposed to be developmental neurotoxicants but time constraints limit this chapter mainly to a discussion of effects on the mature nervous system. The first two phenomena listed above are the best understood and, together with chronic neurotoxicity, will form the main focus of the rest of this chapter. Although it is known that OPs can be used as nerve agents[2,3] in chemical warfare the review will focus on selected OPs used for beneficial purposes.

Acute Toxicity of OPs

Figure 1 shows the general structure of commonly used OPs. A typical structure comprises a pentavalent phosphorous atom linked by a double bond to either oxygen (organophosphate) or a sulphur (organophosphorothioate) atom, with various possible combinations of aryl and alkyl substituent groups attached to phosphorous via the other 3 bonds.

Table 1. Effects of receptor over-stimulation following acute exposure to OPs

Receptor System Affected	Clinical Signs
Muscarinic receptors	Bradycardia, bronchoconstriction, bronchorrhoea, hypotension, increased gastrointestinal motility, abdominal cramps, miosis and hypersalivation
Nicotinic receptors	Hypertension, tachycardia, fibrillation, fasciculation, striated muscle necrosis
Both central muscarinic and nicotinic receptors	Tremor, loss of movement co-ordination, seizures, central depression of respiration, coma, death

Organophosphorothioates such as chlorpyrifos and diazinon (Fig. 2) are commonly used as insecticides, capitalising on their ability to potently inhibit acetylcholinesterase (AChE) in target organisms. However, in order to become potent AChE inhibitors, such OPs require bioactivation, which involves the replacement of sulphur by oxygen resulting in the formation of an oxon derivative (Figs. 1 and 2). Over exposure to OPs in humans can result in the accumulation of acetylcholine (ACh) at neuromuscular junctions, causing neuromuscular block and respiratory failure in extreme cases.[3,32] Although acute exposure is relatively rare in humans, it can be followed by the development of various forms of delayed neuropathology (discussed later).

The main consequence of acute exposure to OP pesticides and nerve gases is the inhibition of AChE. This can lead to cholinergic crisis which is associated with hyperstimulation of muscarinic and nicotinic ACh receptors and related clinical effects (Table 1). If death is the result, this is usually caused by respiratory failure and/or cardiac arrest. Figure 3 shows a schematic representation of the mechanism of interaction of OPs with AChE. The OP binds irreversibly with the hydroxyl group in the side chain of the serine residue at the active site of AChE. Levels of nerve AChE

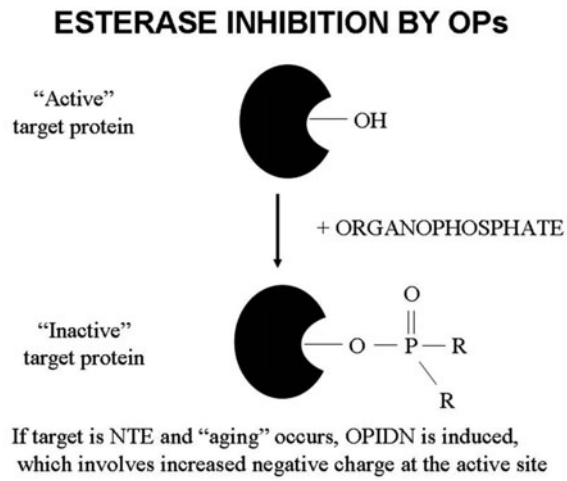

Figure 3. Mechanism of esterase inhibition by OPs. OPs bind covalently to an active site serine with the loss of the group labelled 'X' in Figure 1.

inhibition greater than approximately 70% will lead to the accumulation of ACh in synaptic clefts at neuromuscular junctions, causing neuromuscular block and respiratory failure in severe cases.

Current strategies for the treatment of acute OP toxicity include early interventions using the anticholinergic agent atropine (to block muscarinic AChE receptor activation), cholinergic re-activators (e.g., oximes) and anticonvulsant drugs such as benzodiazepines.[36,37] As organophosphorothioates can be degraded by serum paraoxonases such as PON1, individuals lacking or carrying reduced levels this enzyme are more susceptible to the delayed toxic effects of this group of agents.[2,3] Thus, another potential treatment is the injection of recombinant paraoxonase PON1, which is inactive or deficient in some individuals.[38] However, these treatments do not prevent the development of delayed neurotoxicity, consistent with the possibility that delayed effects may be triggered by the interaction of OPs with molecular targets other than AChE. Progress towards recovery following acute exposure of humans to OPs can be followed by measuring AChE, butyryl cholinesterase and neuropathy target esterase (NTE) activities in blood cells.[18,39,40]

OP-Induced Delayed Neuropathies (OPIDN)

OPIDN is a neurodegenerative condition that particularly affects nerves with long fibre tracts in both the central and peripheral nervous systems. Some but not all OPs have been found to induce OPIDN, which is characterised by delayed onset of an extended period of ataxia and upper motor neurone spasticity arising from single or repeated exposure to OPs.[1-3,21-23,41]

After exposure to OPs, apart from recognised cholinergic effects, no obvious OPIDN-related clinical changes are observed for a period of at least a few days up to several weeks. This is followed by the progressive development of symptoms outlined in Table 2, though not all of these are seen or reach the same level of severity in all patients due to a variety of reasons such as the chemical involved, the level, route and frequency of exposure, inter-individual differences in susceptibly and whether co-exposure to other toxins is involved.[15,41] After disease development there is normally

Table 2. Clinical effects observed in OPIDN

Early symptoms include cramp, burning and/or stinging sensation in the calves and possibly the ankles or feet.
This may be followed by numbness and paraesthesia in the legs and feet.
Weakness of the limbs develops and may later progress to the hands and arms.
When walking, the toes of both feet may drag on the floor leading to a neuropathic 'steppage gait'.
Symptoms may progress to abnormal balance and deterioration of certain limb reflexes.
In severe cases a flaccid paralysis will eventually develop and subjects may suffer bladder and bowel problems.
Any permanent CNS damage will become evident during recovery, in the form of hyper reflexia, spastic gait and increased motor tone.

a period of stabilisation during which the acquired level of paralysis persists; provided that significant damage to the CNS has not occurred, this is followed by a gradual recovery starting in the hands and arms. However, as the CNS is incapable of nerve regeneration, if there has been significant CNS damage, neurological deficits may persist for many years and in the most severe cases may be irreversible.[15,41] Damage to the CNS would be masked in the early stages of OPIDN due to the predominantly peripheral neuropathic symptoms but would become apparent during recovery of peripheral nerve function as hyper reflexia, abnormal motor tone and the development of a spastic gait, in which the legs are kept close together and move rigidly with toes dragging along the floor.[41]

The risk of developing OPIDN following acute or repeated exposure to OPs can be monitored by measuring NTE activity in the blood cell of pesticide workers and handlers, or from individuals exposed to pesticides deliberately or accidentally.[39] A similar approach in studies of NTE in animal models of OPIDN revealed the hen to be the most sensitive species.[42] The hen has therefore become the preferred model for screening purposes and its use has led to the imposition of restrictions on the use of a number of OPs. Studies on the hen confirmed that TOCP was a potent inducer of OPIDN and that OP pesticides such as chlorpyrifos, leptophos and trichlorfon were also able to induce the condition but only following exposure to much higher often acutely toxic doses.[18,42,44]

Chronic Neurobehavioral, Cognitive and Developmental Neurotoxicity of OPs

A number of epidemiological studies have suggested that exposure to of adults to acute or repeated sub acute levels of OPs can result in long term neurological and neurobehavioral effects, affecting the CNS to a greater degree than the PNS. For example, a study on pesticide workers occupationally exposed to acute organophosphate pesticide poisoning were found to show significant impairment in a range of WHO-approved tests of behaviour and cognitive function.[45] A recent study on the effects of low level exposure to diazinon in both retired and working sheep dippers showed that sub-acute exposure was associated with increased risk of neurobehavioral dysfunction.[46] Sub acute levels of chlorpyrifos were also found to affect cognitive skills of rats exposed during weaning, raising concern about the risk of developmental effects of this pesticide.[47] This concern was further strengthened by epidemiological studies on children applying pesticides to crops showing significant impairment in tests of neurobehavioral and cognitive skills, the latter becoming worse at longer exposure times.[48]

As these types of lesion differ from both acute toxicity and OPIDN in that they can last for many years during which central effects predominate, they have been collectively referred to as OP-induced chronic neurotoxicity.[3,15] However, it should be noted that the term encompasses a wide range of neurological and neurobehavioral deficits resulting from the neurodegenerative effects of single acutely toxic or repeated sub-acute doses of OP. Many of these conditions may reflect the interaction of OPs with distinct molecular targets and/or the extent of cell death in specific populations of neurons in the CNS and the level of maturity of the nervous system at the point of exposure.

Typical symptoms and pathological lesions presented by affected individuals are shown in Table 3. These effects have been observed to varying degrees in the aforementioned studies on human and animal models of OP exposure. It is interesting to note that many of these symptoms are also exhibited by individuals suffering

Table 3. Clinical signs and pathological lesion observed in chromic OP-induced neurotoxicity

Physical	Headache, drowsiness, dizziness, fatigue, generalised weakness and tremors
Cognitive	Deficits in concentration, memory and cognitive ability
Behavioural	Anxiety, apathy, confusion, restlessness, labile emotion, lethargy, anorexia, insomnia, depression, irritability

from air cabin sickness, consistent with the possibility that the latter is due at least in part to OP exposure. As indicated earlier, the neuropathic OP of greatest concern has been the ortho isomer of tricresyl phosphate due its potent ability to induce OPIDN.[13,14] Although this isomer is now kept to a minimum level in aviation oils,[28] there are several other OPs in aviation fluids.[25,26] It is therefore clear that the ability of all aviation fluid OPs to individually or collectively induce chronic neurotoxicity needs to be investigated.

MOLECULAR TARGETS OF OPs

The fact that exposure to sub-acute levels of OPs can result in neurotoxicity supports the view that the a range of delayed neuropathic effects are due at least in part to the interaction of OPs with molecular targets other than AChE, such as other serine hydrolases.[49] In this section of the chapter we will discuss evidence to support the view that OPs have noncholinergic targets, linking these conditions to OP exposure and outline some potential future directions for research.

NEUROPATHY TARGET ESTERASE (NTE)

The primary target of OPs that induce OPIDN is considered to be NTE.[21-23,50,51] OPIDN inducers are far more potent inhibitors of NTE than they are of AChE,[52] which has formed the basis of OP screening methods using the hen model. Returning to Figure 3, if the esterase is NTE and aging occurs after binding to the active site serine residue the inhibition becomes irreversible and OPIDN will occur. Only those OPs able to induce aging of NTE, which involves increased negative charge at the active site,[21-23,50] can induce OPIDN. The fact that NTE can be inhibited significantly by non-aging OPs with no apparent effect on neural function implies that the esterase activity per se may not be essential for neural function.

Major advances in recent years led to the cloning of *NTE* gene and sequencing of protein and the use of recombinant NTE in a number of biochemical studies.[50,51,53] Such studies have shown that NTE is a membrane associated protein located mainly in the SER capable of forming an ion channel when reconstituted in artificial phospholipid vesicles.[54] Of particular interest is the fact that only neuropathic OPs (i.e., those that induce aging of NTE) were able to block ion conductance in vitro suggesting that this potential role of NTE may be impaired in OPIDN.[54] The catalytic domain resides in

the C terminal region and the amino terminal region contains regulatory domains that, coupled with membrane association, are required for optimal esterase activity.[55,56] In addition to its well established OP-sensitive esterase activity,[21-23] NTE has also been found to act as a lipid hydrolase acting on phosphatidylcholine as a substrate,[56,57] suggesting that it may play a role in the regulation of phospholipid turnover in cell membranes.

The *NTE* gene sequence was found to be similar to that of Swiss Cheese (SWS) protein in *Drosophila melanogaster*; when the *SWS* gene was mutated it resulted in the development of a central neuropathy.[58] Deletion of the same gene in the mouse resulted in a similar neuropathy to that observed in Drosophila.[59] This neurodegenerative condition involved glial hyper wrapping and neuronal apoptotic cell death in large areas of the brain, indicating an essential role for NTE and similar proteins in neuronal glial interactions important in nervous system function.[51,58] Further studies on Drosophila suggested that the neuropathy was accompanied by significant inhibition of the lipid hydrolase activity exhibited by NTE,[60] suggesting that disruption of phospholipid homeostasis was a major factor in the development of the neuropathy. It remains to be established whether the same occurs in OPIDN which, as discussed earlier, affects primarily the peripheral nervous system.

Interestingly, it was found that NTE and SWS were more closely related than NTE was to the serine hydrolase family, containing a common highly conserved domain present throughout phylogeny.[51,61] As indicated earlier, doubt has been cast as to the importance of the classical esterase activity of NTE in adults as non-NTE aging OPs can inhibit NTE significantly without inducing OPIDN.[21-23] Glynn proposed that inhibition and aging of NTE might have an effect on another NTE related function such as lipid hydrolysis or ion channel activity NTE or by causing a change in function of NTE by affecting its interaction with other macromolecules,[51] which would seem to be borne out by the SWS studies outlined above. The importance of NTE per se in nerve function was further emphasized by the discovery of *NTE* mutants with disrupted esterase activity associated with motor neurone disease,[62,63] consistent with mounting evidence that NTE plays a vital role in the maintenance of neurons in adult mammals.[64,65]

A number of cellular studies have been performed in differentiating cell lines to determine the role of NTE in neuronal cell differentiation. Exposure of differentiating mouse N2a neuroblastoma cells to the OPIDN inducing OP phenyl saligenin phosphate (PSP: An analogue of the neuropathic metabolite of TOCP), resulted in reduced outgrowth of axon-like processes in association with complete inhibition of NTE.[66] A weak inducer of OPIDN chlorpyrifos was also found to inhibit neurite outgrowth and NTE in the same cellular system.[67] The observation that knock down of *NTE* in differentiating SH-SY5Y cells had no effect on the extent of neurite outgrowth but that moderately increased expression could increase the rate of outgrowth suggested that the enzyme may play a subtle regulatory role but also that other targets may be involved in the ability of OPs to inhibit neurite outgrowth.[68,69] Furthermore the increased expression of NTE caused mitotic arrest in a kidney cell line but had no effect on proliferation in human SH-SY5Y neuroblastoma cells suggesting that it may be the ability to regulate cell proliferation in certain cell types.[70] Further work is warranted in coculture systems to reflect the neuronal-glial interactions that occur in vivo more closely. Nevertheless, one of the implications of the results from manipulated NTE expression studies is that other targets may be involved in the neuropathic effects of NTE, some of which are discussed in the following section.

OTHER POTENTIAL MOLECULAR TARGETS OF ORGANOPHOSPHATES

Cytoskeletal Proteins

In early ex vivo biochemical studies of cytoskeletal enriched extracts from hens induced to exhibit OPIDN, it was found that TOCP exposure was associated with hyperphosphorylation of cytoskeletal proteins.[13,71] Histopathological studies in TOCP treated hens confirmed the presence of abnormal aggregations of highly phosphorylated neurofilament heavy chain (NFH) prior to the onset for clinical signs of OPIDN.[72,73] Inhibition of neurite outgrowth in differentiating N2a cells by phenyl saligerim phosphate (PSP) was also found to be associated with a transient increase in neurofilament phosphorylation again suggesting that disruption of cytoskeletal protein phosphorylation is a key event in OP-induced neurotoxicity.[66] However, the relationship (if any) between these changes and NTE inhibition remains to be established.

One explanation for the altered phosphorylation status of neurofilaments could be the activation of calmodulin kinase as suggested in one study.[71] However, PSP exposure in differentiating N2a cells was also found to cause increased activity of the mitogen activated protein (MEP) kinase MAP kinase ERK1/2 for which NFH is a substrate,[74] suggesting that this agent was able to affect cytoskeletal integrity by disruption of signalling pathways associated with neuronal cell differentiation and survival.[66,75] However, not all neurite inhibitory OPs appear to induce NFH hyperphosphorylation as this effect was not observed in chlorpyrifos- or leptophos-treated differentiating N2a cells.[67,76] Further work would help to determine whether increased ERK activation is an effect limited to strong inducers of OPIDN. Further evidence that OPs can disturb the phosphorylation status and/or organisation of cytoskeletal proteins was obtained in a cellular study of the effects of diazinon on neurite outgrowth.[77] Neurite inhibition was associated with increased levels and phosphorylation of the actin binding protein cofilin which regulates the dynamic properties of the microfilament network and decreased staining of neurites with anti actin antibodies suggesting a reduction in the level of actin polymer in OP treated cells. Microfilament disruption was also observed in a study on human neuroblastoma cells though distinct OPs were found to affect microfilament levels differently, again suggesting that not all OPs act in exactly the same way.[78]

Proteolytic Enzymes

One consequence of Wallerian degeneration is the influx of extracellular Ca^{2+} leading to the activation of calcium dependent enzymes such as the protease calpain. The finding that symptoms of OPIDN could be blocked by Ca^{2+} channel blockers,[79] and that calpain is activated at an early stage in TOCP treated hens,[80] supports the view that calpain-mediated proteolysis of cytoskeletal proteins such as neurofilaments is an early event in OPIDN and may explain the consistent pattern of reduced levels of neurofilament heavy chain observed in cellular and animal models exposed to a range of OPs.[66,67,80,81] However one study observed reduced calpain activity concomitant with cytoskeletal protein degradation in hens treated with the OPIDN inducer diisopropylphosphorofluoridate (DFP) suggesting that other proteases may be activated by some OPs to induce axonal degeneration.[82] One should not lose site of the fact that one of the major pathways of proteolytic processing known to be essential for neuronal form and function, the ubiquitin dependent pathway, includes a number of

serine hydrolase activities that could be targetted by OPs.[83] The fact that this system is disrupted in a number of neurodegenerative disorders involving abnormal protein aggregation,[83] and the presence of abnormal NFH aggregation in cellular and animal model of OP exposure,[66,72,73,81] suggests that proteasomes might be a potential target of OPs. Further work to determine the effect of OPs on the mechanisms of protein turnover in axons would therefore be of value.

Mitochondria

Several studies have suggested that OP exposure can result in structural changes and impairment in the activity of a range of key mitochondrial enzyme activities such as succinate dehydrogenase, NADH dehydrogenase and cytochrome oxidase.[84] Other studies have suggested that OPs can disrupt energy metabolism, mitochondrial membrane organisation and respiratory activity, leading to apoptotic cell death in cultured neurons.[85] Some OPs also showed the ability to cause major disruption to the mitochondrial transmembrane potential, a good indicator of mitochondrial well being.[86] Major disruption of mitochondrial energy metabolism could lead to the reduction in cellular ATP levels that might be the trigger for many OP related effects in the rest of the cell including apoptosis and oxidative stress.[87] OPs also induced major changes in mitochondrial ultrastructure prior to cell death in lumbar spinal neurons, again suggesting that major disruption of mitochondria can occur at an early stage of OPIDN.[88] Taken together these and other studies indicate that mitochondria are targetted at least by some OPs. Further analysis of the mitochondrial proteome might help to further establish the nature of OP effects on mitochondrial function and how this relates to the various types of OP related toxicity.

OTHER POTENTIAL 'NON-ESTERASE' OP BINDING PROTEINS

Despite the well held view that OPs bind selectively to a motif in the active site of AChE, NTE and/or other serine hydrolases, there is increasing evidence to suggest that other proteins may interact directly with some OPs. It has been suggested that tyrosine and lysine residues on such proteins may provide alternative OP binding sites and that this may be relevant to the numerous proteins (e.g., of the neuronal cytoskeleton) that exhibit altered phosphorylation status.[89] A mass spectrometry approach was used to detect binding of chlorpyrifosoxon, dichlorvos, diisopropylfluorophosphate (DFP) and sarin to human serum albumin, suggesting that this approach could form the basis of a prognostic test of the response to exposure.[90] A similar outcome was found for human transferrin.[91]

Chlorpyrifosoxon disrupts microtubules in vivo and was shown to have a direct effect on microtubule proteins by preventing their ability to polymerise in vitro. Further analysis by mass spectrometry demonstrated the covalent interaction of chlorpyrifos with tyrosine residues in tubulin, the core protein of microtubules.[94,95] This may represent one of the ways in which OPs differ in the specific lesions they produce and could indicate a role for such interactions in the chronic or developmental effects of this particular OP. Further proteomic analysis of OP treated cells and organisms will no doubt reveal other potential targets of OPs and help to establish the way in which different OPs affect neural form and function.

CONCLUSION

Organophosphorus esters are capable of inducing acute toxicity by inhibition of AChE and cause various forms of delayed toxicity by interacting with a variety of noncholinergic targets which have not yet been fully characterised. Exactly what molecular changes underlie these various forms of neurotoxicity is not fully understood but a proposed scheme for the mechanism(s) of toxicity is presented in Figure 4, bearing in mind that the exact

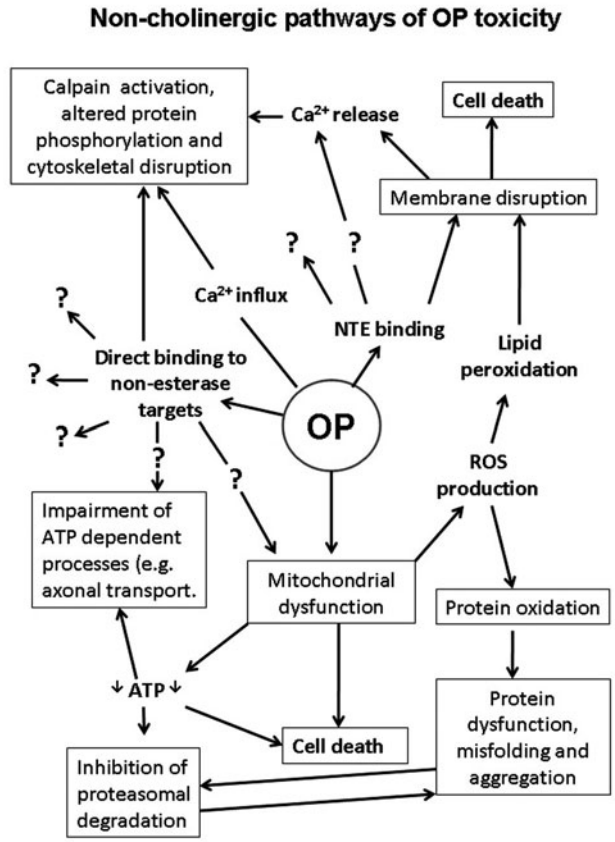

Figure 4. Noncholinergic pathways of OP toxicity. Shown is a schematic representation of the possible inter relationships between the different primary targets and pathways affected by sub-acute exposure to OPs. Binding to NTE may affect both its putative channel role in the endoplasmic reticulum and its lipid hydrolase activity, causing disruption of membranes with potential Ca^{2+} release, membrane cleavage and/or cell death, in addition to as yet unidentified roles. OPs may also cause ATP depletion, oxidative stress and/or induce apoptosis via mitochondrial impairment. If lipid peroxidation is significant this could contribute to the membrane disruption mentioned earlier while protein oxidation may result in altered protein function and/or turnover by causing mis-folding, which could in turn affect the ability of the ubiquitin-dependent proteasomal pathway of protein degradation, which may also be impaired by ATP depletion. The latter would also start to affect metabolic pathways and a range of ATP dependent processes such as axonal transport, which is essential for nerve maintenance and regeneration. Binding of OPs to non-esterase targets may induce cytoskeletal disruption (e.g., in the case of chlorpyrifosoxon binding to tubulin and a range of other effects yet to be determined. The exact mechanism will always depend on the specific OP(s), the level and duration of exposure.

effects will depend OP structure, targets affected, route, level and duration of exposure and inter individual variation. The neurodegenerative condition OPIDN is induced up to several weeks after exposure and is preceded by a number of molecular lesions including inhibition (and aging) of NTE and disruption of the organisation and/or phosphorylation status of cytoskeletal proteins important in the growth and maintenance of axons. NTE is now known to have a range of important esterase and non-esterase functions and may play a regulatory role in axon maintenance. Recent developments suggest that a number of other proteins such as transferrin, albumin and tubulin are capable of binding certain OPs in tyrosine residues, breaking the myth that an active site serine was the only motif available. Further work on NTE functions and the characterisation of novel OP targets and their role in toxicity will help to establish the molecular basis of OP-induced neurodegeneration more fully.

REFERENCES

1. Chambers JE. Organophosphate compounds an overview. In: Chambers JE, Levi P, eds. Organophosphates: Chemistry, fate and effects. San Diego: Academic Press, 1992:3-17.
2. Krieger RI, ed. Handbook of pesticide toxicology: Principles, 2nd edition. San Diego: Academic Press, 2001.
3. Gupta RC, ed. Toxicology of organophosphate and carbamate compounds. San Diego: Elsevier, 2006.
4. Aldridge WN. Tricresyl phosphates and cholinesterase. Biochem J 1954; 56:185-189.
5. Olsen KR, ed. Poisoning and drug overdose. Stamford, Connecticut: Appleton and Lange, 1998.
6. Olsen KR, ed. Poisoning and drug overdose. Stamford Connecticut; Appleton and Lange, 1998.
7. Committee on Toxicology (COT) of Chemicals in food, consumer products and he environment organophosphates. Department of Health, 2007.
8. Environmental Protection Agency. Annual Report, 2000. Office of Pesticide Programs. http://www.epa.gov/oppfead1/annual/2000/2000annual.pdf
9. Environmental Protection Agency. Reregistration Eligibility Decision for Diazinon, 2006. http://www.sps.gov/pesticides/reregistration/REDS/diazinon_red.pdf
10. European Commission (EC) decision of 6 June 2007 concerning the non inclusion of diazinon in Annex I to the Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection agents containing that substance. Official Journal of the European Union 2007; 140:9-10.
11. Australian Pesticides and Veterinary Medicines Authority (APVMA). APVMA suspends the use of diazinon for sheep dipping and jetting. Media Release, 2007.
12. Department of Primary Industries (DPI) Sheep body lice: Control and eradication. Agriculture Notes of the DPI State of Victoria, 2007; AG1110.
13. AbouDonia MB, Lapadula DM. Mechanisms of organophosphorusester induced delayed neurotoxicity: Type I and II. Ann Rev Pharmacol Toxicol 1990; 30:405-440.
14. Lotti M. The pathogenesis of organophosphate polyneuropathy. Crit Rev Toxicol 1992; 21:465-487.
15. AbouDonia MB. Organophosphorus ester induced chronic neurotoxicity. Arch Env Health 2003; 58:484-497.
16. Bishop EL, Stewart HC. Incidence of partial paralysis. Am J Public Health 1930; 20:1307-1312.
17. Zeligs MA. Upper motor neuron sequelae in "Jake" paralysis: a clinical follow up study. J Nerv Ment Dis 1938; 87:464-470.
18. Richardson RJ, Moore TB, Kayyali US et al. Chlorpyrifos: Assessment of potential or delayed neurotoxicity by repeated dosing in adult hens with monitoring of brain acetylcholinesterase, brain and lymphocyte neurotoxic esterase and plasma butyrylcholinesterase activities. Toxicol Sci 1993; 21:89-96.
19. Chow E, Seiber JN, Wilson BW. Isophenos and an in vitro assay for delayed neuropathic potential. Toxicol Appl Pharmacol 1986; 83:178-183.
20. de Blaquiere GE, Waters L, Blain PG et al. Electrophysiological and biochemical effects of single and multiple doses of the organophosphate diazinon in the mouse. Toxicol Appl Pharmacol 2000; 166:81-91.
21. Johnson MK. The primary biochemical lesion leading to the delayed neurotoxic effects of some organophosphorus esters. J Neurochem 1974; 23:785-789.
22. Johnson MK. The target for initiation of delayed neurotoxicity by organophosphorus esters: biochemical studies and toxicological applications. In: Hodgson E, Bend JR, Philpot RM, Eds. Reviews in Biochemical Toxicology. New York: Elsevier, 1982; 4:141-212.
23. Johnson MK. Organophosphates and delayed neuropathy- is NTE alive and well? Toxicol Appl Pharmacol 1990; 102:385-389.

24. Committee on Air Quality in Passenger Cabins of Commercial Aircraft, Board on Environmental Studies and Toxicology, National Research Council, National Academy of Sciences. The Airliner Cabin Environment and Health of Passengers and Crew. Washington DC; National Academic Press, 2002.
25. Winder C, ed. Contaminated Air Protection: Proceedings of the Air Safety and Cabin Air Quality International Aero Industry Conference. British Airline Pilots Association (BALPA) and the University of New South Wales, Australia, 2005.
26. Winder C. Air monitoring studies for air cabin contamination. Curr Top Toxicol 2006; 3:33-48.
27. Civil Aviation Authority. Cabin air quality, CAA paper, 2004:04.
28. Harris MO, McLure P, Chessin RL et al. Toxicological profile for hydraulic fluids. ATDSR, USA; 1997.
29. Hanhela PJ, Kibby J, DeNola G et al. Organophosphate and amine contamination of cockpit air in the Hawk, F-111 and Hercules C-130 aircraft. DSTO Defence Science and Technology Organisation, Victoria, Australia; 2005.
30. Nutley BP, Crocker J. Biological monitoring of workers occupationally exposed to organophosphorous pesticides. Pesticide Science 1993; 39:315-322.
31. Pilkington A, Buchanan D, Jamal GA et al. An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers. Occup Environ Med 2001; 58:702-710.
32. Costa LG. Current issues in organophosphate toxicology. Clin Chim Acta 2006; 366:1-13.
33. Karalliedde L, Baker D, Marrs TC. Organophosphate-induced intermediate syndrome: aetiology and relationships with myopathy. Toxicol Rev 2006; 25:1-14.
34. Slotkin TA, Ryde TI, Levin ED et al. Developmental neurotoxicity of low dose diazinon exposure of neonatal rats: Effects on serotonin systems in adolescence and adulthood. Brain Res Bull 2008; 75:640-647.
35. Slotkin T, Seidler FJ. Developmental neurotoxicants target differentiation into the serotonin phenotype: Chlorpyrifos, diazinon, dieldrin and divalent nickel. Toxicol Appl Pharmacol 2008; 233:211-219.
36. Thiermann H, Szinicz L, Eyer F et al. Modern strategies in therapy of organophosphate poisoning. Toxicol Lett 1999; 107:233-239.
37. Eddleston M, Buckley NA, Eyer P et al. Management of acute organophosphorus pesticide poisoning. The Lancet 2008; 371:597-607.
38. Akhmedova SN, Yakimovsky AK, Schwartz EI. Paraoxonase 1 Met-Leu polymorphism is associated with Parkinson's disease. J Neurol Sci 2001; 184:179-182.
39. Lotti M, Moretto R, Zoppellari R et al. Inhibition of neuropathy target esterase predicts the development of OPIDN. Arch Toxicol 1986; 59:176-179.
40. Bissbort SH, Vermaak WJH, Elias J et al. Novel test and its automation for the determination of erythrocyte acetylcholinesterase and its application to organophosphate poisoning. Clin Chim Acta 2001; 303:139-145.
41. Jokanovic M, Kosanovic M, Brkic D et al. Organophosphate induced delayed polyneuropathy in man: an overview. Clin Neurol Neurosurg 2010; in press.doi:10.1016/j.clineuro.2010.08.015
42. Lotti M, Johnson MK. Neurotoxicity of organophosphorus pesticides: predictions can be based on in vitro studies with hen and human enzymes. Arch Toxicol 1978; 41:215-221.
43. El Sebae AH, Soliman SA, AboElamayem M et al. Neurotoxicity of organophosphorus insecticides leptophos and EPN. J Env Sci Health 1977; B12:269-288.
44. Abou-Donia MB, Graham DG. Delayed neurotoxicity of sub chronic oral administration of leptophos to hens: recovery during four months after exposure. J Toxicol Env Health 1979; 5:1133-1147.
45. Rosenstock L, Keifer M, Daniell WE et al. Chronic central nervous system effects of acute organophosphate pesticide intoxication. The Lancet 1991; 338:223-227.
46. Mackenzie Ross SJ, Brewin CR, Curran HV et al. Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. Neuro Toxicol Teratol 2010; 32:452-459.
47. Jett DA, Navoa RV, Beckles RA et al. Cognitive function and cholinergic neurochemistry in weanling rats exposed to chlorpyrifos. Toxicol Appl Pharmacol 2001; 174:89-98.
48. Abdel Rasoul GM, Abou Salem ME, Mechael AA et al. Effects of occupational pesticide exposure on children applying pesticides. Neuro Toxicology 2008; 29:833-888.
49. Casida JE, Quistad GB. Organophosphate toxicology: Safety aspects of nonacetylcholinesterase secondary targets. Toxicology 2004; 17:983-998.
50. Glynn P. Neuropathy target esterase. Biochem J 1999; 344:625-631.
51. Glynn P. Neural development and neuropathy target esterase: two faces of neuropathy target esterase. Prog Neurobiol 2000; 61:61-74.
52. Kropp TJ, Richardson RJ. Relative inhibitory potencies of chlorpyrifosoxon, chlorpyrifos methyl oxon and mipafox for acetylcholinesterase versus neuropathy target esterase. J Toxicol Environ Health A 2003; 278:8820-8825.
53. Glynn P. Molecular cloning of neuropathy target esterase. Chem Biol Interact 1999; 119-120:513-517.
54. Forshaw PJ, Atkins J, Ray DE et al. The catalytic domain of human neuropathy target esterase mediates an organophosphate sensitive ion conductance across liposome membranes. J Neurochem 2001:400-406.

55. Atkins J, Luthjens LH, Hom ML et al. Monomers of the catalytic domain of human neuropathy target esterase are active in the presence of phospholipid. Biochem J 2002; 361:119-123.
56. Li Y, Dinsdale D, Glynn P. Protein domains, catalytic activity and subcellular distribution of neuropathy target esterase in mammalian cells. J Biol Chem 2003; 278:8820-8825.
57. Glynn P. Neuropathy target esterase and phospholipiddeacylation. Biochim Biophys Acta 2005; 1736:87-93.
58. Kretzschmar D, Hasan G, Sharma S et al. The Swiss cheese mutant causes glial hyper wrapping and brain degeneration in Drosophila. J Neurosci 1997; 17:7425-7432.
59. Akassoglou K, Malester B, Xu J et al. Brain-specific degeneration of neuropathy target esterase/swiss cheese results in neurodegeneration. Proc Natl Acad Sci USA 2004; 101:5075-5780.
60. Mühlig-Versen M, Bettencourt de Cruz A, Tschäpe JA et al. Loss of swiss cheese/neuropathy target esterase activity causes disruption of phosphatidylcholine homeostasis and neuronal and glial cell death in Drosophila. J Neurosci 2005; 25:2865-2873.
61. Lush MJ, Li Y, Read DJ et al. Neuropathy target esterase and a homologous Drosophila neurodegeneration mutant protein contain a domain conserved from bacteria to man. Biochem J 1998; 332:1-4.
62. Rainier S, Bui M, Mark E et al. Neuropathy target esterase gene mutations cause motor neuron disease. Am J Human Genet 2008; 82:780-785.
63. Hein ND, Stuckley JA, Rainier SR et al. Constructs of human neuropathy target esterase catalytic domain containing mutations related to motor neuron disease have altered enzymatic properties. Toxicol Lett 2010; 196:67-73.
64. Read DJ, Li Y, Chao MV et al. Neuropathy target esterase is required for adult vertebrate axon maintenance. J Neurosci 2009; 29:11594-11600.
65. Chang PA, Wu YJ. Neuropathy target esterase: an essential enzyme for neural development and axonal maintenance. Int J Biochem Cell Biol 2010; 42:573-575.
66. Hargreaves AJ, Fowler, MJ, Sachana, M et al. Inhibition of neurite outgrowth in differentiating mouse N2a neuroblastoma cells by phenyl saligenin phosphate: Effects on neurofilament heavy chain phosphorylation, MAP kinase (ERK 1/2) activation and neuropathy target esterase activity. Biochemical Pharmacology 2006; 71:1240-1247.
67. Sachana M, Flaskos J, Alexaki E et al. The toxicity of chlorpyrifos towards differentiating mouse N2a neuroblastoma cells. Toxicol In Vitro 2001; 15:369-372.
68. Chang PA, Wu YJ, Chen R et al. Inhibition of neuropathy target esterase expressing by antisense RNA does not affect neural differentiation in human neuroblastoma (SK-N-SH) cell line. Mol Cell Biochem 2005; 272:47-54.
69. Chang PA, Chen R, Wu YJ. Reduction of neuropathy target esterase does not affect neuronal differentiation but moderate expression induces neuronal differentiation in human neuroblastoma (SK-N-SH) cell line. Mol Brain Res 2005; 141:30-38.
70. Chang PA, Liu CY, Chen R et al. Effect of over-expression of neuropathy target esterase on mammalian cell proliferation. Cell Prolif 2006; 39:429-440.
71. Suwita E, Lapadula DM, Abou-Donia MB. Calcium and calmodulin enhanced in vitro phosphorylation of hen brain cold stable microtubules and spinal cord neurofilament proteins following a single oral dose of tri-o-cresyl phosphate. Proc Natl Acad Sci USA 1986; 83:6174-6178.
72. Jensen KF, Lapadula DM, Knoth Anderson J et al. Anomalous phosphorylatedneurofilament aggregations in central and peripheral axons of hens treated with tri-ortho-cresyl phosphate (TOCP). J Neurosci Res 1992; 33:455-460.
73. Jortner BS, Perkins SK, Ehrich M. Immunohistochemical study of phosphorylated neurofilaments during the evolution of organophosphorus ester-induced delayed neuropathy. Neuro Toxicology 1999; 20:971-976.
74. Veeranna, Amin ND, Ahn NG et al. Mitogen-activated protein kinases (ERK 1/2) phosphorylate Lys-Ser-Pro (KSP) repeats in neurofilament proteins NFH and NFM. J Neurosci 1998; 18:4008-4021.
75. Perron JC, Bixby JL. Distinct neurite outgrowth signalling pathways converge on ERK activation. Mol Cell Neurosci 1999; 13:362-378.
76. Sachana M, Flaskos J, Alexaki E et al. Inhibition of neurite outgrowth in N2a cells by leptophos and carbaryl: effects on neurofilament heavy chain, GAP-43, HSP-70. Toxicol In Vitro 2003; 17:115-120.
77. Harris W, Sachana M, Flaskos J et al. Proteomic analysis of differentiating neuroblastoma cells treated with sub-lethal neurite inhibitory concentrations of diazinon: Identification of novel biomarkers of effect. Toxicol Appl Pharmacol 2009; 240:159-165.
78. Carlson K, Ehrich M. Organophosphorus compounds alter F-actin content in SH-SY5Y human neuroblastoma cells. Neuro Toxicology 2001; 22:819-827.
79. El-Fawal HA, Ehrich MF. Calpain activity in organophosphorus-induced delayed neuropathy (OPIDN): Effects of a phenylalkylamine calcium channel blocker. Ann NY Acad Sci 1993; 679:325-329.
80. Song F, Yan Y, Zhao X et al. Neurofilament degradation as an early molecular event in tri-ortho-cresyl phosphate (TOCP) induced delayed neuropathy. Toxicology 2009; 258:94-100.

81. Flaskos J, Harris W, Sachana M et al. The effects of diazinon and cypermethrin on the differentiation of neuronal and glial cell lines. Toxicol Appl Pharmacol 2007; 219:172-180.
82. Gupta RP, Abou-Donia MB. Diisopropylphosphorofluoridate (DFP) treatment alters calcium activated protease activity and cytoskeletal proteins of the sciatic nerve. Brain Res 1995; 677:162-166.
83. Layfield R, Lowe J, Bedford L. The ubiquitin-proteasome system and neurodegenerative disorders. Essays Biochem 2005; 41:157-171.
84. Masoud A, Kiran R, Sandhir R. Impaired mitochondrial functions in organophosphorus induced delayed neuropathy in rats. Cell Mol Neurobiol 2009; 29:1245-1255.
85. Kaur P, Radotra B, Minz RZ et al. Impaired mitochondrial metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure. Neuro Toxicology 2007; 28:1208-1219.
86. Carlson K, Ehrich M. Organophosphorus compound induced modification of SH-SY5Y human neuroblastoma mitochondrial transmembrane potential. Toxicol Appl Pharmacol 1999; 160:33-42.
87. Milatovic D, Gupta RC, Aschner M. Anticholinesterase activity and oxidative stress. Scientific World J 2006; 6:295-310.
88. Mou DL, Wang YP, Song JF et al. Triorthocresyl phosphate-induced neuronal losses in lumbar spinal cord of hens—an immunohistochemistry and ultrastructure study. Int J Neurosci 2006; 116: 1303-1316.
89. Lockridge O, Schopfer LM. Review of tyrosine and lysine as new motifs for organophosphate binding to proteins that have no active site serine. Chem Biol Interact 2010; 187:344-388.
90. Li B, Schopfer LM, Hinrichs SH et al. Matrix-assisted laser desorption/ionization time of flight mass spectrometry assay for organophosphorus toxicants bound to human albumin at Tyr411. Anal Biochem 2007; 361:263-272.
91. Li B, Schopfer LM, Grigoryan H et al. Tyrosines of human and mouse transferrin covalently labelled by organophosphorus agents: a new motif for binding to proteins that have no active site serine. Toxicol Sci 2009; 107:144-155.
92. Grigoryan H, Lockridge O. Nanoimages show disruption of tubulin polymerisation by chlorpyrifosoxon: implications for neurotoxicity. Toxicol Appl Pharmacol 2009; 240:143-148.
93. Jiang W, Duysen EG, Hansen H et al. Mice treated with chlorpyrifos or chlorpyrifosoxon have organophosphorylated tubulin in the brain and disrupted microtubule structures, suggesting a role for tubulin in neurotoxicity associated with exposure to organophosphorus agents. Toxicol Sci 2010; 115: 183-193.
94. Grigoryan H, Schopfer LM, Thompson CM et al. Mass spectrometry identifies covalent binding of soman, sarin, chlorpyrifosoxon, diisopropylfluorophosphate and FP-biotin to tyrosines on tubulin: a potential mechanism of long term toxicity by organophosphorus agents. Chem Biol Interact 2008; 175: 180-186.
95. Grigoryan H, Schopfer LM, Peeples ES et al. Mass spectrometry identifies multiple organophosphorylated sites on tubulin. Toxicol Appl Pharmacol 2009; 240:149-158.