SOME NEUROLOGICAL EFFECTS OF PESTICIDES

DAVID RAY

Medical Research Council
Toxicologic Unit,
Carshalton, U.K.

I have been asked to speak about the toxic actions of pesticides in man from the theoretical viewpoint. The wide range of pesticides used today makes it impossible to give a comprehensive survey, and so after a brief general introduction I will deal with just two mayor classes, the pyrethroid and the organophosphate insecticides. As my initial training was in physiology and most of my colleagues at the U.K. Medical Research Council Toxicology Unit are chemists, I must appologise if this talk concentrates largely on the physiology of the nervous system and on chemistry. It may also be a little biased towards experimental animal studies. These are however key areas in the understanding of pesticide toxicology.

The reason for this is that most insecticides are designed to attack the insect nervous system. Although our own nervous system is far more elaborate than that of insects, it shares many fundamental processes with that of insects. Sometimes even common proteins are involved. This means that insect neurotoxins are often potential mammalian neurotoxins. Our nervous systems is characterised by its highly specialised nature, with little duplication at the lower levels; its complex, interdepentent nature; the lack of capacity for replacement of central neurones; and the unique anatomy of neurones with long axonal processes which may extend half a metre from a cell body only one hundred thousandth of a metre in diameter. The blood-brain barrier normally protects the brain against toxic agents, but presents only a minor barrier to most insecticides. This is because they are deliberately made lipophylie to enable rapid penetration of the waxy insect cuticle and to wet the protective hairs of larvae. Consequently most insecticides are free to penetrate the mammalian central nervous system once absorbed. An important method of overcoming this problem is to exploit differential rates of metabolic detoxification. Mammals have powerful broad spectrum metabolic systems for breaking down foreign compounds, and the additional advantage of high body temperature and metabolic rate. The pyrethroids and organophosphates are both detoxified by hydrolysis, which gives rats a 4 to 400 times lower susceptibility than houseflies to or-

ganophosphates, and a 50 to 5000 times lower susceptibility to pyrethroids.

Although such resistence can be dramatic, it is not an ideal mechanism since it can be overcome by sudden rapid absorption of pesticide, or by suppression of metabolic capacity by disease or exposure to metabolism inhibiting agents. It would clearly be better to attack other aspects of insect physiology such as the hormonal control of moulting, reproduction or feeding which are not shared by mammals. However the great diversity of pest species, and the current demand for rapid acting broad spectrum pesticides means that neurotoxic pesticides continue to predominate.

This common mechanism of mammalian resistance leads to certain similarities in the toxicology of pyrethroids and organophosphates for since both are higly toxic, yet rapidly broken down in the body, route (and hence speed) of absorption are critical. Typically intravenous administration is an order of magnitude more toxic than oral administration, which is itself an order of magnitude more toxic than dermal administration. Whilst some exceptions can occur - usually as a result of unexpectedly long persistance of highly lipophylic agents in body fat - these are rare. Chemical degradation in the wider environment is also relatively rapid, in contrast to the organochlorines. In the case of the pyrethroids, reducing their rapid photodecomposition sufficiently to enable their outdoor use was a major chemical problem. This relatively short persistence means that, given a suitable delay between crop treatment and harvest, accumulation of toxic residues in plant products represents only a minor for both pyrethroids and organophosphates compared to the risk of acute intoxication of workers by exposure to concentrates. A further similarity between organophosphates and pyrethroids is that their high affinities for their primary targets in the nervous system make secondary actions such as potential carcinogenicity or teratogenicity unimportat for practical sub-lethal exposures.

The pyrethroids are complex esters of derivatives of chrysanthemic acid (with its characteristic cyclopropane ring) and aromatic

alcohols. The increased photostability of the synthetic pyrethroids has been obtained largely through halogenation of the acid and addition of a cyano group and this also alters their toxic properties. Metabolic inactivation is by ester hydrolysis catalysed by plasma and liver esterases and oxidases, followed by hydroxylation, conjugation and urinary excretion. The pyrethroids have a very high affinity for the sodium channel complex found in the membrane of all excitable cells and bind it with affinity constants of the order of 10^{-10}M. This gives them a very high toxic potential, limited however by rapid metabolic disposal and extensive non-specific binding to non-target tissues. Once pound to the active sodium channel complex, pyrethroids exert a very subtle effect, markedly slowing the kinetics of the trans-membrane sodium current, but not altering its voltage threshold or other characteristics. A consequence of this is that the call does not normally die or depolarise, but functions in a new and abnormally hyperexcitable state, the sodium current extending beyond its normal duration and causing an after depolarisation which may lead to repetitive action potentials. Only a small proportion of sodium channels need to be modified to produce this effect. The much shorter after potential produced by cismethrin in rat muscle is contrasted with the longer one produced by deltamethrin. These records were carefully selected to avoid provoking repetitive firing, but even cismethrin is capable of producing abnormal double motor and plate potentials under normal conditions. As might be expected, the situation is much more complex in the CNS, and some workers have postulated additional primary sites of action on GABA or noradrenaline mediated neurotransmission, but I believe that most actions can be explained in terms of sodium channel effects.

The primary actions of the pyrethroids can be broken into three divisions. Firstly local actions. These are a paraesthesia of locally exposed skin, especially of the thinner skin of the face, characterised by a spontaneous tingling sensation not accompanied by inflammation or hyperaemia or impaired sensitivity to touch or heat. This paraesthesia has been described in man after topical application of a

number of pyrethroids at thresholds of 10-100 g/cm^2 of skin. It is an unpleasant but apparently harmless effect, becoming noticeable about an hour after application, and lasting for up to 24 hours. It can be treated by lavage of the skin with a solvent oil or cream to remove the pyrethroid. In experimental applications to rat skin we have found that nearly all the pyrethroid remains pound to the outer epidermis and would thus be susceptible to solvents for several hours after application. Because of the lipophylic nature of the pyrethroids, washing with soap and water has little effect compared to washing with all, in the apsence or a ready clinical measure or exposure to pyrethroids, this paraesthesia may well serve as a good indicator of dermal exposure by faulty working practices such as inadequate protective clothing, and thus provide an useful warning sign. It should be distinguished from the classic allergic skin reaction in responsa to the plant protein present in natural pyrethrum extracts.

Systemic poisoning is of two distinct kinds. There is very little human data available but in experimental work with rats and dogs two types of intoxication can be distinguished. Type I is produced by pyrethroids giving a 2 - 30 msec prolongation of the sodium current and is characterised by severa fine tremor, reflex superexcitability and incoordinated muscle twitches, closely resembling those produced by DDT which acts in much the same way. No convulsions are seen and consciousness may be retained until death occurs as a result of hyperthermia. The type II intoxication produced by pyrethroids which prolong sodium current by 20 - 200 msec is characterised by profuse watery salivation and apparent irritation of the mouth; increased postural tone leading to abnormal gait; adrenal activation; chorecathetotic spasms of spinal origin, initially evoked by stimuli and then becoming spontaneous; tonic seizures and death. There is some evidence that the pyrethroids are secreted in the saliva, and this, rather than a primary cholinergic effect appears to cause the salivation via local irritation. Oral administration in dogs and man is also associated with severe gastro.intestinal irritation. The other signs, are attributable to direct actions on the nervous system. They are

more co-ordinated and complex than the type I. Some pyrethroids with intermediate prolongation of sodium currents produce a complex mixed syndrome of both type I and type II simultaneously.

I will now allow myself a brief electrophysiological digression, and apologise in advance for the specialist illustrations. There are few EEG abnormalities associated with even severe type I intoxication, but sensory evoked potentials and EMG responses are markedly enhanded especially in the cerebellum. These are late in latency, but in the brainstem clear reflex enhancement as well as repetitive sensory discharges can be demonstrated. This, and the tremor lead to large increases in neuronal activity and metabolic rate, most notably in the cerebellum as may be seen in deoxyglucose autoradiograms. Type II intoxication does however lead to clear EEG changes: frontal cortical spike trains can be evoked by auditory stimuli before overt motor signs are seen, and chorecathetcsis is associated with generalised spike and slow wave activity.

An important feature of both classes of intoxication is that they are fully reversible, even at late stages, and are only seen whilst elevated brain levels of pyrethroid persist. Prolonged and repeated severe type II intoxication has produced patchy axonal swelling in rats, but this also was reversible over a few days and probably secondary to a massive increase in activity. The pyrethroids may thus be described as functional toxins in that they disturb normal function, and are cytotoxic only indirectly via the systemic consequences of this disturbance.

The pyrethroids also produce a number of additional effects on the adrenal and cardiovascular systems. Both classes cause marked adrenal hyperactivity at low doses, probably by a diraect mechanism. Also in the brain some neocortical areas show blood flow increases mardedly in excess of those needed to supply the increased metabolic demand. These are amongst the highest blood flow increases ever recorded. In addition to this specific local effect, which is produced by both classes, the type II pirethroids only also cause a veratridine like increase in myocardial contractility, resulting in increased

cardiac output. This combined with the adrenal stimulation also produced can lead to cardiac arrythmias in type II, but not type I poisoning.

A consequence of the essentially functional and transient nature of pyrethroid poisioning is that therapy need only be symptomatic. Thorough washing of the skin or gastric lavage followed by careful observation is indicated. Animal studies and a single human (fatal) case indicate that signs of poisoning should be seen within a few hours of oral administration, wheras in man and animals 12-24 hours may elapse before signs of systemic poisoning are seen after dermal exposure. Studies of neuronal function in the rat hippocampus show that both classes of pyrethroid selectively prolong inhibition, and it appears that normal inhibitory processes continue to work well even during intoxication. Hence therapy with diazapam is of only limited value in animals and man, and even general anaesthesia results in only a twofold reduction in dose threshold for motor signs. The centrally acting muscle relaxant mephenesin in very effective at controlling choreoathetosis however, if given as an intravenous infusion and methocarbamol has also been used. Atropine may be useful in controlling the gastrointestinal and salivary secretions evoked by type II pyrethroids. The biological half life of most pyrethroids is of the order of a few hours, so intoxication should repidly diminish once the source of pyrethroid has been removed, although intoxication lasting as long as 10 days has been described after dermal exposure, probably due to skin or body fat acting as a reservoir for pyrethroid.

Organophosphates are potent inhibitors of esterases. They are all esters of phosphoric, phosphicnic, phosphonic or phosphoramic acid, and are closely related to esters of carbamic acid, the carbamates. Synthetic chemists have exercised their ingenuity for many years, and large numbers of insecticidal organophosphates have been produced. The organophosphate esters act as substrates for esterase enzymes, and bind with very high affinity, but, unlike the association of pyrethroids with their target, the sodium channel

complex; this reaction is covalent, involving loss of the leaving group x, and essentially irreversible over the short term. The apparent instantaneous affinities are of the order of 10^{-7} M, but enzyme inhibition can be produced by 10^{-6} M or less if sufficient time is allowed for the reaction to take place. Once formed, the organophosphate-enzyme bond can be broken after hydrolysis of the organophospate ester, but this is a slow process with rate constants of 10^{-1} to 10^{-6} per minute for acetylcholinesterase (AChE), which compares with $3x10^{6}$ per minute for the normal substrate acetyl choline. This hydrolysis leads to spontaneous reactivation of the enzyme, but can be prevented by a slow side reaction called "againg", whereby the organophosphate is dealkylated, which has the effect of completely stabilising the organophosphate-enzyme complex and stopping any further hydrolysis. The only way that enzyme activity can return after the againg reaction, is by re-synthesis of new enzyme, a process taking weeks. The major target esterase is neural acetylcholinesterase (AChE), but this is not the only target. Other esterases such as chymotrypsin are inhibited and also plasma cholinesterase. It is noted that inhibition of brain and plasma enzymes does not correlate well for various reasons guthion having little affinity for the plasma enzyme, whilst the active metabolite of OMPA is too unstable to reach the brain. An important consequence of this is that plasm a cholinesterase should not be used as an index or risk to the CNS: erythrocyte or whole blood cholinesterase are much better indexes. Other important esterases are those carboxyesterases which break down organophosphates and pyrethroids.

These can be inhibited as well which can slow degradation and markedly increase toxicity. It is also interesting to note how feeding Fenchlorphos which, although producing only moderate AChE inhibition itself, markedly potentiates the action of acutely dosed malathion. Similarly non-toxic doses of the organophosphate defoliant DEF increase the toxicity of pyrethroids up to 20 or 30 fold since it is a poor AChE, but good carboxyesterase inhibitor.

Once this is understood, we can return to the general chemical

structure of the organophosphates and it will be seen that although the nature of the leaving group often determines the initial affinity of the organophosphate for the enzyme, its cleavage and loss during binding means that it plays no further role and cannot influence the crucial rate of reactivation of the inhibited enzyme. This is determined by the two "ester" residues, most frequently either methyl or ethyl groups. Ethyl groups slow reactivation to about 60 hours, whereas methyl gropus allow more rapid reactivation over 1-2 hours. If the linking atom is a sulphur then reactivation is more rapid still, but if a nitrogen (in phosphoramidates) it is very slow. The double bond atom has a vital influence since only phosphates can inhibit esterases., phosphorothioates only act indirectly after conversion to phosphates. This does not only occur in the body but also spontaneously, especially on exposure to ultraviolet light. Under conditions of poorly controlled synthesis, storage or even as residues on crops (provided the weather is dry enough to prevent hydrolysis and sunny enough to provide intense ultraviolet light) accumulation of toxic " 0xon" impurity can greatly increase the toxicity of some "-thion" organophosphates. An example of this is the isomerisation of malathion which can markedly increase its toxicity. An important consequence of this is to realise that impurities may dramatically alter the toxic properties of some organophosphates, especially phosphorothioates, and to analyse samples wherever possible. Phosphorothioates are very lipophylic and, as with the pyrethroids, body fat can act as a reservoir leading to persistence in the body. Again as with pyrethroids, rapid metabolic destruction and lipophylicity dominate the toxico-kinetics of all organophosphates. Formulation is also important; lipophylic solvents speeding skin absorption for dichlorvos.

There is fortunately a large excess of AChE activity at both the peripheral motor end plate, ganglion, and central synapse; but if inhibition exceeds about 90% signs of acuts poisoning are seen. This involves classic nicotinic and muscarinic signs of fascicular twitching of the muscles and eventual loss of contractility, change in

heart rate, increased saliva, sweat and tear secretion, gastrointestinal disturbances, confusion, nightmares, anxiety, convulsions and coma. Some non-cholinergic effects are seen, -largely limited to rodents- notably an inhibition of whole body metabolic rate produced by the defoliant DEF. This causes large falls in body temperature of rodents, which depend heavilly on this cold induced metabolism to maintain their body temperature. A further effect is a lung oedema with type II lung cell damage produced by certain malathion breakdown impurities. It is not known what the significance of these might be in man.

As with the pyrethroids these acute effects are essentially functional toxicity without direct cytotoxicity, and symptomatic treatment with removal of the toxic agent is all that is necessary. Atropins in large doses and artifical ventilation when needed control the peripheral signs, whist diazepam gives good control of central effects. Spontaneous metabolic detoxification is not as rapid as for the pyrethroids however, and may be inadequate after severe organophosphate poisoning. In this case natural reactivation of the enzyme may be supplimented by use of oximes such as pralidoxims. Oximes have a high affinity for the inactive organophosphate-enzyme complex, and donate what is efectively a new leaving group to replace that lost from the organophosphate when it first bound to the enzyme. This destabilises the complex with breaks down, liberating normal uninhibited enzyme. It might be feared that the new oxime-derived forms of the organophosphates also generated would be hazardous, but fortunately they are all very unstable in the free form, and rapidly hydrolise. Oxime therapy is not just of value during the first few days of therapy, but may also be important during the later stages. If continued for up to 14 days it can trap the organophosphates slowly but continuously released from resevoirs of reactivated enzymas, and also those slowly released from body fat. Such late release can cause apparent relapses into acute intoxication if oxime therapy is discontinued early, as has been seen in man with fenitrothion.

There is however an important difference between the organophosphates and the pyrethroids, in that some organophosphates have the additional potential to produce direct cytotoxicity as well as the acute effects already described. This is the well known organophosphate induced delayed neuropathy (OPIDN). It is characterised by a patchy but predominantly distal degeneration of the longer, largely motor, nervas with secondary demyelination. In man and hens this causes a severe ataxis which develops after a latent period of about three weeks. If sufficiently intense to cause muscle wasting, the paralysis is then irreversible. In rats mild histological changes are seen, but no motor signs develop which makes them less useful as a model. The development of OPIDN is not well understood, although local injection experiments by Villanova have clearly shown that it is the nerve fibre rather than the nerve cell body that is the primary target. The early events of OPIDN are, however better understood largely due to the biochemical work of my colleague Dr. Martin Johnson. He has shown that the phosphorylation of a target protein early in poisoning is a critical process for initiation of OPIDN. This protein is called Neuropathy Target Esterase (NTE) as, although its esterase activity plays no direct role in OPIDN, it provides a convenient measure of the interaction of NTE with organophosphates, NTE is a large membrane bound protein with a molecular weight of about 800.000 - 1.000.000 which has not been isolated in pure form, but is characterised in terms of esterase activity or DFP binding. The difference between the activity of a tissue remaining after incubation with non-neuropathic paroxon and that remaining after incubation with a neuropathic agent such as mipafox can be considered as the NTE activity. This is a small fraction of the total esterase or binding capacity of the uninhibited tissue, but is well correlated with neuropathic potential. For initation of neuropathy, NTE must be converted to the "aged" form, i.e. the organophosphate-enzyme complex must be de-alkylated. More reversible inhibition is not enough suggesting that is not the loss of catalytic activity that is critical, as with AChE, but rather the formation of the

reactive residue that initiates the neuropathy.

When tested with a large number of agents, neuropathic potential correlates well with the capacity to irreversibly inhibit NTE activity to more than 70% (or 50% for chronic dosing), but it can be seen that only those with the capacity to "age" as well as inhibit are neuropathic. This is summarised taking into account the fact which shows that phosphinates, carbamates and sulphonates have no neuropathic potential. As they inhibit but do not "age" they are even protective under some circumstances. The NTE assey is capable of prediction of the quantitative risk from different doses, but the results must be interpreted with some care. The first complication is that peak NTE inhibition at day one is the relevant factor, even though the neuropathy may take some weeks to develop. Consequently measurements taken after several weeks, when damage is first suspected, may be too late. As an example this takes place for hens dosed with DFP. Species differences must also be allowed for, as human NTE (conveniently measured in leucocytes) may not exactly parallel hen NTE, and metabolism and kinetics differ also.

Fortunately AChE and NTE do not show parallel affinities for organophosphates, and this has been exploited to enable selection of safer pesticides which have NTE inhibition ED 50's well above the lethal level. This means that OPIDN should only be seen after severe acute intoxication which would have proved fatal without acute anticholinesterase therapy. A further advantage of a quantitative test such NTE inhibition or binding is that instead of a yes/no result a real estimate of risk is obtained, extending even into the hen neuropathy negative range.

A final complication is introduced by the occurance of an "intermediate" syndrome of poisoning after severe fenthion, monocrotophos, dimethoate and methamidophos intoxication in man. This has been described by Senanayake as a severe muscle weakness developing after 1 - 4 days, and lasting for up to 18 days, ie. after the acute cholinergic crisis, but before signs of OPIDN. An interesting characteristic is that only the respiratory and proximal limb muscles are

weekened, and whilst grip strength may be normal, spontaneous respiration may become impossible. This condition is reversible with time, provided artificial ventilation is provided, but illustrates the importance of careful patient monitoring. It is possible that the mechanism is linked to prolonged muscle and plate depolarisation, but little work has been done as yet lastly the various toxic and detoxifying pathways followed by organophosphates is sumarised: AChE inhibition, NTE inhibition, alkylation of macromolecules, and lung damage; oxidative attack, conjugation and hydrolysis. In conclusion I hope I have demonstrated that our present theoretical knowledge has equipped us to make some very useful predictions about insecticide toxicology, therapy and safe design of pesticides. There are clear gaps in our understanding which need to be filled, and finding answers to these questions should provide an interesting challenge for the future.