Chapter 7

Pesticides

Introduction

Pesticides are substances which have been designed or chosen for selective toxicity to certain organisms. Although their toxicity *is* selective, they are often also toxic to other species although usually to a lesser degree. As well as being of interest in terms of their mode of action they are of concern to toxicologists for two reasons: (1) they may be toxic to man either in acute poisonings or after chronic exposure; and (2) they have toxic effects on some non-target organisms in the environment. This latter point was highlighted in 1963 by Rachel Carson in her book *Silent Spring*.

Human poisonings from accidental exposure to pesticides have occurred since they were first used and in some cases many people have been poisoned, sometimes fatally in single incidents. Many of these cases have been due to accidental contamination of food with pesticides or their inappropriate use (Table 7.1). For example, the use of organic mercury fungicides to treat seed grain which is then used to feed animals has resulted in several mass poisonings of humans. Occupational poisoning has also occurred in agricultural workers through accidental contamination or inappropriate use. The careless use of pesticides, such as spraying without adequate protection, may also lead to exposure of the operator.

Chronic toxicity due to the pesticides present in our environment is more difficult to identify although with the development of improved analytical techniques the detection of residues has become easier. Such techniques have shown that most people in the Western World are indeed exposed to and in many cases contaminated with, certain pesticides. However, pesticides have become a very important part of our society especially in terms of agricultural economics and, although their use may be curtailed in some instances, it is unlikely to be completely halted when risk/benefit considerations are made.

Pesticides can be divided into several groups, such as insecticides, fungicides, herbicides and rodenticides, depending on the target organism. Those that have been specifically designed for a purpose often utilize a particular biological, metabolic or other feature of the target species, but unfortunately such features are rarely entirely unique to that species so other similar species may also be affected. A simple example of selective toxicity in a pesticide is

Table 7.1. Mass poisonings due to pesticides.

Pesticide involved	Material contaminated	Number affected	(died)	Location
Endrin	Flour	159	(0)	Wales
Endrin	Four	691	(24)	Qatar
Parathion	Flour	600	(88)	Colombia
Parathion	Sugar	300	(17)	Mexico
Hexachlorobenzene	Seed grain	>3000	(3–11%)	Turkey
Organic mercury	Seed grain	321	(35)	Iraq
Pentachlorophenol	Nursery linens	20	(2)	USA

Source: *Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health,* (Washington, DC: US Governmental Printing Office, 1969).

the use of warfarin as a rodenticide. This depends on the lack of the vomit reflex in rats so that they are unable to vomit after ingesting the poison.

Other pesticides depend on more sophisticated biochemical differences. For example, the insecticide malathion is metabolized by hydrolysis in mammals to yield the acidic metabolite, which is readily excreted (Figure 2.20). In insects, however, the preferred metabolic route is oxidation to yield malaoxon which is toxic by inhibition of cholinesterase (see below). Although pesticides may all be perceived by the general public as equally hazardous to man, they vary in their toxicity to mammals, and other non target wildlife, and in their effects on the environment.

Some examples of the major pesticide types are as follows:

Insecticides: Organophosphorus compounds, carbamate and organochlorine compounds. Natural products such as pyrethrins.

Herbicides: Chlorophenoxy compounds, dinitrophenols, bipyridyls, carbamates, triazines, substituted ureas, aromatic amides.

Fungicides: alkyl mercury compounds, chlorinated hydrocarbons, dialkyldithiocarbamates, organotin compounds.

Rodenticides: inorganic agents, natural products, fluorinated aliphatics, α-napht hylthiourea.

It is clear from this list that pesticides comprise a wide range of chemical types and their modes of action will be very different. However, their toxicity to man and other mammals may be due to a different mechanism from their pesticidal action.

We will now consider some toxicologically important examples of pesticides.

DDT

Perhaps the best known organochlorine insecticide is DDT, (dichlorodiphenyl-trichloroethane; Figure 7.1). It was introduced in 1945 for the control of malarial mosquitoes and was extremely successful, being a major factor in the

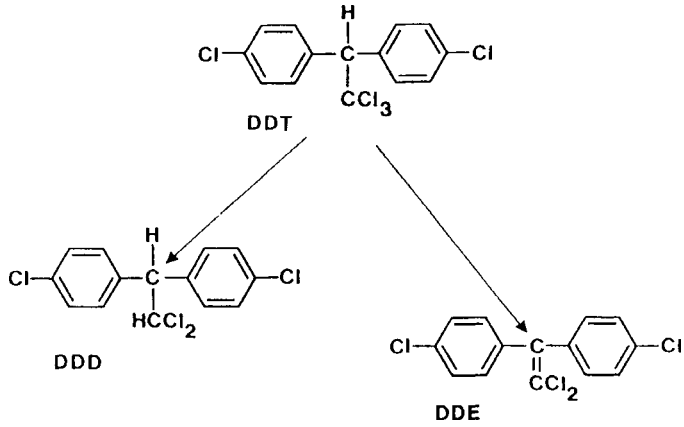

Figure 7.1. Two of the pathways of metabolism of the insecticide dichloro-diphenyl-trichloroethane (DDT).

reduction in malaria after the Second World War. DDT is a contact poison which is highly potent against the insect nervous system but is relatively non-toxic to man. A dose of at least 10 mg kg^{-1} is required for toxic effects to occur in man and no human fatalities have been reported. Indeed human volunteers were induced to take 0.5 mg kg^{-1} (35 mg) daily for over a year and there was no demonstrable toxicity. Although some reports have suggested association between chronic disease and DDT, no causal relationship has been found and other reports have not found such associations. Large doses cause tremors, hyperexcitability and convulsions, paresthesias, irritability and dizziness. In experimental animals liver damage occurs after single large doses and hypertrophy and other histological changes in liver have been reported after chronic exposure. Toxic effects seem mainly to involve the nervous system in mammals as in insects. The mechanism of action is unknown but the primary site of action is thought to be sensory; motor nerve fibres and the motor cortex are possible targets. DDT may alter transport of Na^+ and K^+ across nerve membranes perhaps by interfering with the energy metabolism required for this transport.

DDT is chemically stable, highly insoluble in water, but soluble in body fat and is consequently very persistent in biological systems and the environment (Table 7.2). It is poorly absorbed through the skin and is metabolized in animals by a number of routes (Figure 7.1) but the metabolite DDE is more persistent than the parent compound (Table 7.2). There are other metabolites

Table 7.2. Persistence of the insecticide DDT and its metabolites.

Compound	Half-life in pigeon (days)	Half-life in soil (yrs)
DDT	28	2.5–5
DDD	23	
DDE	250	

such as an acidic derivative which is more water-soluble but the conversion to these is slow and does not involve major routes. There are also microbial and environmental degradation to other metabolites.

It is because of this persistence, that DDT levels in the environment have been increasing ever since it was first used. Furthermore, the DDT concentration in some of the exposed organisms increases at each higher trophic level of the food chain (see Chapter 8). For example, small organisms such as plankton or daphnia absorb DDT passively or via filter feeding from river or lake water and this enters their body fat. The concentration in the tissues of these organisms may be several hundred or thousand fold greater than the concentration in the surrounding water. Then, either insects or small fish eat these small organisms and the DDT is transferred to their fat tissue (Table 7.3). These small organisms are in turn eaten by still larger organisms and so on up the food chain. As DDT is fat soluble it remains in the organism and is then transferred into the fat of the predator or animal at the top of the food chain which may be man. The result is that relatively high concentrations of DDT can occur in those animals at the top of the food chain by a continuous process of amplification or biomagnification despite the fact that the initial concentration of DDT in the water is low. This is illustrated by the following example: in one area of California plankton were found to contain 4 ppm of DDT, while the bass found in the same area contained 138 ppm and the grebes feeding on them 1500 ppm. So what seems to be a negligible concentration of DDT in the river or lake water or at the bottom of a food chain may be biologically very significant at the top. Toxic concentrations of DDT appear to affect birds and fish particularly in the production of eggs. It can be shown, for example, that there is a relationship between shell thickness and DDE concentration in birds of prey such as the kestrel (Figure 7.2).

In man, as in other animals exposed to DDT, most is located in the body fat. The concentration in fat is proportional to the intake, reaching a plateau with a half-life of around six months. The estimated intake for humans in USA was around 35 mg $year^{-1}$ in 1969 but the level in food is declining as is the amount in human fat. The acceptable yearly intake for humans as given by the FAO/WHO guidelines is 255 mg $year^{-1}$. The DDT either comes from eating food of animal origin where the animal itself or another lower in the food chain has been exposed, or from vegetables or fruit which have been sprayed or otherwise contaminated.

The DDT in fat does not appear to be harmful to animals, however, and there is no correlation between adipose tissue levels and signs of poisoning.

Table 7.3. Example of a food chain.

Organism	Tropic Level
Pine trees	1st Producers
Aphids	2nd Herbivores
Spiders	3rd Insectivores
Tits and Warblers	4th Insectivores
Hawks	5th Carnivores

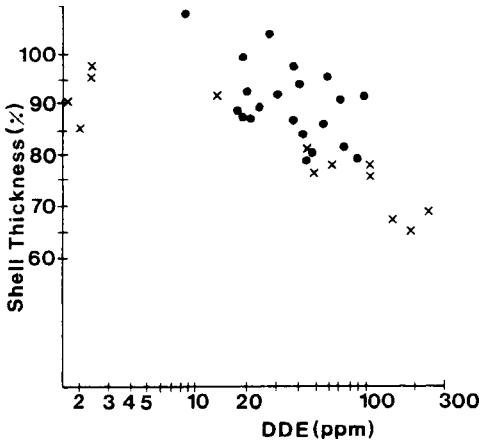

Figure 7.2. The relationship between shell thickness and residues of the DDT metabolite DDE.The data is from kestrel eggs collected in Ithaca, New York in 1970 (●) or experimentally induced with dietary DDE (X). The data is the mean clutch thickness expressed as percent of control egg thickness collected before DDT use. Data from Lincer, J.L. (1975), *Journal of Applied Ecology,* **12**, 781.

It is the concentration circulating in the blood which is more relevant to the toxic effects, and more particularly the level in the brain. However, if there is a reduction of the fat content of the body, the blood level will rise. Experiments with rats have shown that this increase in blood level can lead to toxicity. It has been found recently that bats in the southern USA have high levels of DDT even though it is no longer used. This is probably because the bats eat large quantities of insects and there is sufficient residual DDT in the environment for it to appear in food chains. In a particular species of bat this has been a problem because the DDT is passed via the milk to young bats and this then enters their fat tissues. When the bats go on mass long distance-migration, they start to mobilize this fat and so their blood levels of DDT increase until they become sufficient to cause toxicity and death.

Human milk may also contain DDT and as with other food chains there is a concentration effect. For example, lactating mothers exposed to 0.0005 mg kg^{-1} day^{-1} were found to produce milk containing 0.08 ppm DDT, hence their infants were exposed to 0.0112 mg kg^{-1} day^{-1}, an exposure some twenty times greater than the mothers.

There is no real evidence that DDT under such chronic exposure conditions is overtly toxic in man although there is some evidence that it is carcinogenic in mice. Consequently continuous exposure to low levels of DDT may constitute a long-term hazard. Chronic exposure to DDT does lead to induction of the microsomal enzymes involved in the metabolism of foreign compounds. It may be this effect that causes the hormonal imbalance seen in birds, as some hormones are also metabolized by the microsomal enzymes.

Most other organochlorine insecticides such as heptachlor, gamma-HCH, dieldrin and aldrin, have similar problems of persistence to DDT.

Organophosphorus Compounds

The use of organochlorine insecticides has decreased recently because of their persistence and because of fears about their long-term effects. The case against DDT is mainly due to its environmental impact on wildlife rather than its toxicity to man, which seems to be low. However, the organophosphorus compounds which have replaced the organochlorine type of insecticide are often more toxic to mammals (maybe as much as one hundred times more toxic) if less persistent. For example, organophosphorus compounds are the major cause of poisoning in agricultural workers in California.

There are many organophosphorus compounds now used as insecticides and their mode of action and toxicity is similar. As already indicated organophosphorus compounds are more toxic and have been responsible for more human deaths and illness than the organochlorine type of pesticide. Parathion, first synthesized in 1944 (Figure 7.3), is one widely used organophosphorus insecticide which has featured in a number of documented mass human poisonings (Table 7.1) and probably in many isolated incidents. Parathion has high mammalian toxicity and consequently it has been superseded by other less toxic organophosphorus compounds for certain uses. One such insecticide is malathion (Figure 2.20) which is more selective in its toxicity mainly because of differences in its metabolism between mammals and insects. However, the effects of organophosphorus compounds are qualitatively similar and can be considered collectively.

Poisoning with organophosphorus compounds is an example of an exaggerated pharmacological effect rather than of direct toxic action and the toxicity may be either cumulative following chronic exposure or acute after a single exposure. The toxic effects are due to the inhibition of cholinesterase enzymes, in particular acetylcholinesterase, by the organophosphate. This enzyme is responsible for the hydrolysis of acetylcholine to choline and acetate (Figure 7.4) which effectively terminates the action of acetylcholine as a chemical transmitter of nerve impulses at synaptic nerve endings. The result of the inhibition by organophosphorus compounds is a build up of acetylcholine and so excessive stimulation of the nerve. Depending on the particular organophosphorus compound the inhibition may be reversible or irreversible. The acetylcholinesterases in different tissues such as plasma and nerves are different and so are not equally inhibited by organophosphorus compounds. There are degrees of inhibition of the total body acetylcholinesterase; in mammals a level of 50 per cent inhibition leads to toxic effects and 80–90 per

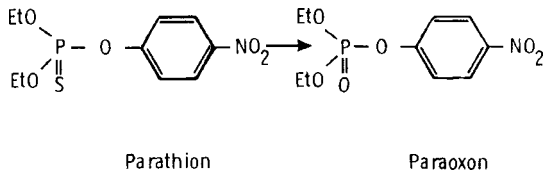

Figure 7.3. The oxidative metabolism of the insecticide parathion.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

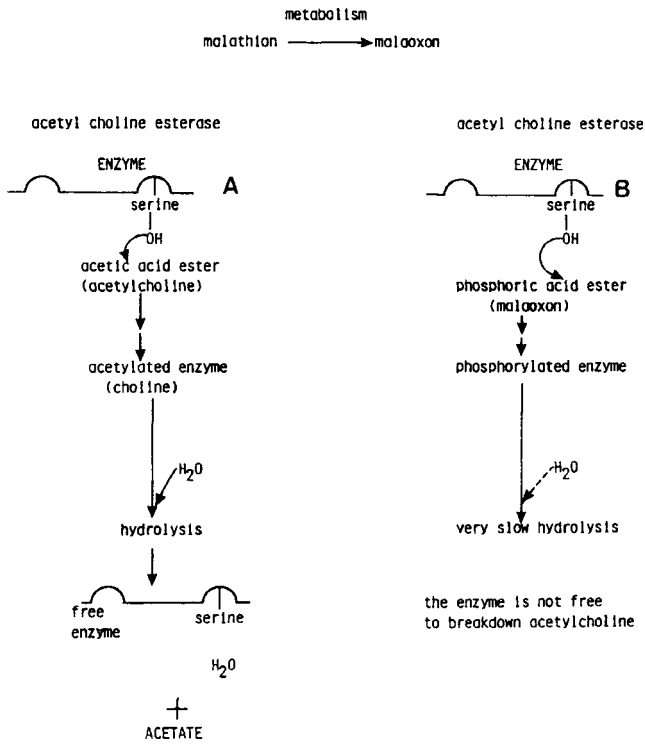

Figure 7.4. The mechanisms of hydrolysis of acetylcholine by acetylcholinesterase (A) and the interaction of the malathion metabolite malaoxon with the enzyme (B).

cent inhibition will be lethal. The mechanism of toxicity of organophosphorus compounds relies on their similarity to the normal substrate acetylcholine (Figure 7.4). Thus, the organophosphorus compound is also a substrate for the enzyme but unlike acetylcholine, the product remains bound to the active site and the resulting complex may be only slowly hydrolyzed, if at all. With those organophosphorus compounds causing irreversible inhibition, resynthesis of the enzyme is necessary.

Malathion itself is not a substrate for cholinesterases but requires metabolism to malaoxon. This takes place readily in insects but in mammals hydrolysis is the preferred route and this leads to a readily excreted diacid (Figure 2.20). This is the basis of the selective toxicity.

The toxic effects of organophosphorus compounds centre around the excessive cholinergic stimulation with death occurring a a result of neuromuscular paralysis and central depression. Some organophosphorus compounds may also cause damage to the peripheral nerves but this delayed neurotoxic effect is believed to be caused by a different mechanism.

Paraquat

The examples mentioned so far have been insecticides which, as a group, are

probably more important than other pesticides in terms of human and environmental toxicity. However, one particular herbicide is of particular importance and notoriety in terms of human toxicology. This is paraquat (Figure 7.5) which, during the twenty years of its use, has featured in several hundred cases of fatal human poisoning. Unlike the organophosphorus compounds, however, this has not been the result of accidental contamination of food and unlike the organochlorines there has been no particular environmental impact. Paraquat poisoning has mainly been the result of deliberate ingestion, usually orally, for suicide or murder with a few cases of accidental direct ingestion. Paraquat is a contact herbicide which binds very strongly to soil. Consequently it does not leach out of soil after being sprayed onto plants and does not have an environmental effect either on other plants or animals. Paraquat kills the plant by interfering with photosynthesis and its toxicity to animals may have some similarities at the biochemical level. When ingested by humans paraquat is usually fatal but even if it is not it may cause serious lung and kidney damage. The lung is the target organ because it *selectively* accumulates paraquat and consequently the concentration in the alveolar type I and II lung cells reaches sufficient levels to cause toxic effects in those cells. The concentration in the lungs reaches a level several times that in the plasma and the paraquat is retained in the lung even when the plasma concentration is falling. Paraquat is taken up by the lung because of a structural similarity with diamines and polyamines, such as putrescine, spermine and spermidine (Figure 7.5). The presence of two nitrogens in paraquat, with a particular intramolecular distance, enables paraquat, but not the herbicide diquat, to be taken up by a selective active transport system in the lung for which polyamines are the normal substrate. The only other organ with an uptake system for polyamines is the brain which does not seem to accumulate paraquat.

Paraquat is believed to cause toxicity via its free radical form which is stable and results from an enzyme-mediated, one electron reduction which requires NADPH (Figure 7.6). In the presence of oxygen this generates superoxide anion and the paraquat cation reforms. This redox cycling continues to produce superoxide and deplete NADPH. The superoxide can lead to the production of hydrogen peroxide and hydroxyl radicals. Hydroxyl radicals are highly reactive and can cause lipid peroxidation which in turn causes further metabolic disruption. The presence of oxygen in the lungs is clearly an important factor in the pathogenesis of the lung lesion. The toxicity to the lungs is a direct

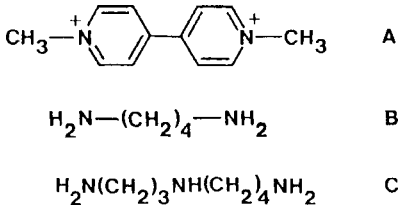

Figure 7.5. The structure of the herbicide paraquat (A), and the polyamines putrescine (B) and spermine (C)

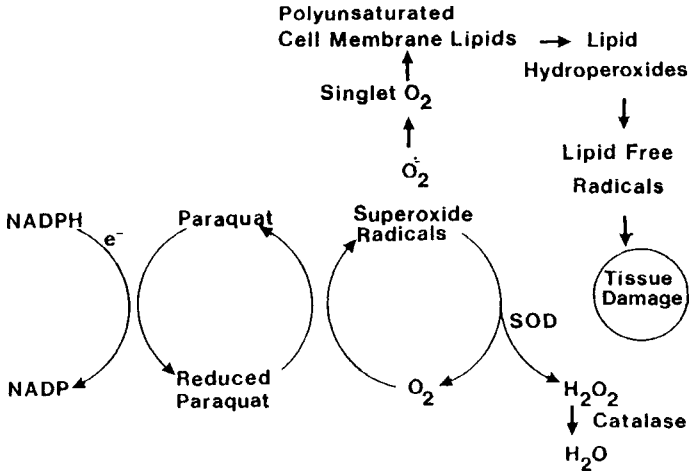

Figure 7.6. The proposed mechanism for the lung toxicity of paraquat. SOD is the enzyme superoxide dismutase.
Adapted from Data from Vale, J.A. and Meredith, T.J., Paraquat Poisoning, pp. 135–141, Figure 21.2 in *Poisoning-Diagnosis and Treatment,* Vale, J.A. and Meredith, T.J., (Eds) Update Books, London, 1981.

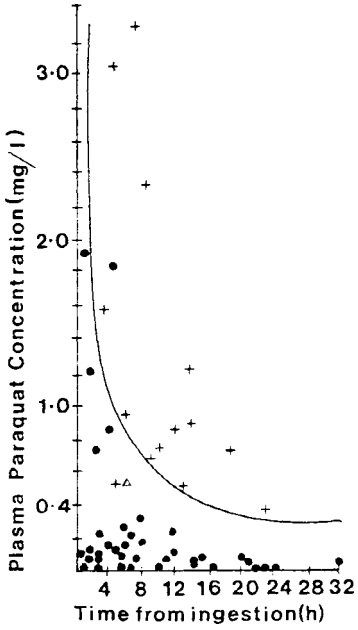

Figure 7.7. The relationship between plasma paraquat concentration and the outcome of the poisoning, death (+) or survival (●). Δ is an aspiration death.
Data from Vale, J.A. and Meredith, T.J., Paraquat Poisoning, pp. 135–141, Figure 21.4 in *Poisoning-Diagnosis and Treatment,* Vale, J.A. and Meredith, T.J., (Eds) Update Books, London. 1981.

result of the distribution of paraquat as the active uptake into lung cells gives rise to the relatively high and toxic concentration.

Paraquat causes a progressive fibrosis of the lungs and also damages the kidneys; once absorbed there is no antidote. The only treatments available are either an attempt to limit absorption by oral administration of substances such as Fullers Earth which adsorb paraquat or the use of haemodialysis or haemoperfusion to rid the blood of the paraquat. After the paraquat has accumulated in the lungs, however, there is no effective treatment currently available.

Paraquat has been used on many occasions for suicide and parasuicide attempts but unfortunately for the victim death is slow and painful, occurring over a period of a several days to a week or more with the progressive fibrosis of the lung leading to eventual suffocation. The prognosis is usually bad and the plasma level of paraquat indicates the likely outcome (Figure 7.7).

Fluoroacetate

Monofluoroacetate is an interesting example of a pesticide which is also a natural product. This compound is highly toxic by virtue of its very specific blockade of Krebs (tricarboxylic acid) cycle. Fluoroacetate is a pseudosubstrate and is successfully incorporated into Krebs cycle as fluoroacetyl CoA. The fluorocitrate produced will bind to the enzyme aconitase, but after binding the pseudosubstrate, the enzyme cannot remove the fluorine atom and so the enzyme is blocked. Therefore, Krebs cycle is unable to function and the cell and organism dies through lack of metabolic intermediates and energy.

Fluoroacetate is found naturally in some plants in Australia, Africa and South America. Some indigenous animals in Australia, especially the skink and emu have developed tolerance. However, introduced and unadapted animals, such as rats, mice, cats and dogs and those living outside the areas where fluoroacetate producing plants grow, are more susceptible to fluoroacetate toxicity (see Twigg and King, 1991). This is a example of what has been termed ‘chemical warfare’ between plants and animals. The plants produce such toxic substances to stop animals eating them. However, fluoroacetate is also used as a pesticide for example in New Zealand, where it is known as 1080 and is used to kill possums which have become pests.

Questions

1. Contrast the toxicology of organochlorine insecticides such as DDT and the organophosphorus type such as parathion.
2. Discuss the toxicology of paraquat and in particular explain the mechanism underlying the specific organ damage.
3. Explain what is meant by the term ‘selective toxicity’. Illustrate your answer with specific examples.

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