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Neurotoxic Pesticides and Behavioural Effects Upon Birds

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Abstract. Organochlorine, organophosphorus, carbamate, pyrethroid and neonicotinoid insecticides and organomercury fungicides are all neurotoxic and therefore have the potential to cause behavioural disturbances in birds. A number of studies have described behavioural effects caused to captive birds by neurotoxic pesticides, but it is very difficult to measure such effects in the field, which is a serous limitation given their potential to cause adverse effects at the population level. The mode of action, and the neurotoxic and behavioural effects of these compounds are briefly reviewed before considering evidence for their effects in the laboratory and field. Behavioural effects may cause adverse changes at the population level either directly or indirectly. Direct effects upon avian populations may be due to disturbances of reproduction, feeding, or avoidance of predation. Indirect effects on predators may be the consequence of direct action upon the prey population leading to either (1) reduction of numbers of the prey population, or (2) selective predation by the predator upon the most contaminated individuals within the prey population.

Attention is given to the historic evidence for neurotoxic and behavioural effects of persistent organochlorine insecticides, raising the question of retrospective analysis of existing data for this once important and intensively studied class of compounds. Less persistent pesticides currently in use may also have neurotoxic effects upon birds in the field. Sometimes, as with some OPs, their effects may outlast the persistence of their residues, and the ecotoxicity and persistence of some may be affected by interactions with other environmental chemicals. The development of new mechanistic biomarker assays could improve understanding of behavioural effects and possible associated effects at the population level caused by such compounds in the field.

Keywords: neurotoxicity; pesticides; birds; behavioural effects; organochlorine insecticides; organophosphorus insecticides; organomercury fungicides

Introduction

Since the early 1960s and the publication of 'Silent Spring' by Rachel Carson in 1962 much attention has been given to the possible effects of pesticides upon birds and other vertebrates in the field. Evidence of harmful effects of organochlorine insecticides such as DDT, aldrin and dieldrin upon wild populations of predatory birds contributed to the decision to impose wide ranging bans on the use of these compounds worldwide. Restrictions have also been placed on the use of organophosphorus, carbamate and pyrethroid insecticides and the organomercury fungicides because of established or perceived harmful effects upon animals in the field. It is noteworthy that the four main groups of insecticides which are, or have been, widely used are all neurotoxic (Walker, 2001); the recently developed neonicotinoids are also neurotoxic. It appears that receptors within the nervous system present especially vulnerable targets not only for man made pesticides, but also for plant toxins that have evolved during the course of 'plant-animal warfare'

(Harborne, 1993; Walker, 2001). Herbicides and fungicides, by contrast, rarely show high toxicity to animals.

The neurotoxic character of insecticides attracted the attention and interest of behavioural biologists working in the field of ecotoxicology at an early stage. Many kinds of physiological disturbances caused by exposure to pollutants could, in theory, lead to behavioural changes but neurological disturbances seemed to be paramount importance. Alterations in the function of the nervous system were very likely to be expressed as changes in behaviour. Behavioural effects of pesticides were looked at from two related points of view. On the one hand, toxicity tests working to behavioural end points might present a sensitive means of testing for toxicity of environmental chemicals in an integrative way; that is, behavioural responses could represent an integrative measurement of lower level effects caused by one or several chemicals. The other point of view was that behavioural effects in the field might lead to population declines as a consequence of, for example, reduced ability to find food or to reproduce. The issue to emphasise here is that the usefulness of the former approach is dependant on the significance of the latter. The case for developing such tests becomes a strong one if it can be shown that pesticides cause population declines by behavioural mechanisms following realistic levels of exposure in the field.

An early contribution to this subject came from Warner et al. (1966), who developed a test system for fish which utilised several behavioural parameters. They were able to demonstrate changes in the behaviour of goldfish caused by sublethal concentrations of toxaphene (severe changes at 1.8 μg/l, more mild effects at 0.44 μg/l). Toxaphene exerts neurotoxicity by acting upon GABA receptors. More recently, Beauvais et al. (2000) have demonstrated behavioural changes in larval rainbow trout exposed to sublethal doses of the organophosphorus (OP) insecticides diazinon and malathion, even down to doses that only caused 20% inhibition of brain cholinesterase. These and other studies have described assays for fish, which can demonstrate behavioural changes at levels of exposure well below lethal concentrations. A critical and detailed review on the question of behavioural responses of birds to pesticides and other environmental contaminants was published by David Peakall (1985). He concluded that a wide variety of behavioural tests had been tried in birds, varying considerably in their complexity and efficacy. Unfortunately some of the tests which seemed most relevant from an ecological point of view—for example, on breeding behaviour, and capture of prey—were the most difficult to perform. In field studies it was particularly difficult to identify behavioural effects of environmental chemicals. Finally, he concluded that, although behavioural effects had been demonstrated down to doses one order of magnitude below those causing lethality, they were less sensitive than biochemical changes—biochemical changes that could be incorporated into biomarker assays.

The present short review focuses on the possible ecological significance of behavioural effects caused by pesticides. It draws on material from the above review, and on discussions with David Peakall—although interpretations are the responsibility of the author alone.

Ecological consequences of behavioural effects

Behaviour is here defined as the overt action an organism takes to adjust to environmental circumstance so as to ensure its survival (Odum, 1971). The implementation of overt actions is a function of the nervous system, and the disruption of nervous function by neurotoxic compounds may be expected to cause changes in behaviour. In the living environment, pollutants may cause changes at the population level because of DIRECT or INDIRECT behavioural effects. Direct effects may be upon feeding, avoidance of predation or reproduction or any combination of these. Adverse effects upon any of these functions may lead to population declines. Indirect effects upon predatory birds may be due to pollutants having behavioural or other effects upon their prey. Thus, the decline of a prey species caused by behavioural effects may lead to a decline of the predator consequent upon a reduction of food supply. An important consideration with putative behavioural changes caused by pollutants in the field is the duration of the EFFECT. In theory persistent neurotoxic compounds like the organochlorine insecticides dieldrin and DDT generally present a greater hazard than more biodegradable compounds such as organophosphorus, carbamate or pyrethroid insecticides, because neurotoxic action is likely to be much more prolonged. That said, there are some non-persistent compounds which

produce effects that outlast the compounds originally causing them, a point that will be returned to shortly.

Considering direct effects upon feeding, these may be upon one or several different components of this activity (Atchison et al., 1996). Included here are searching for, encountering, choosing, capturing and handling of prey. Learning and the functioning of the sensory system are important in searching, encountering and choosing. Capturing prey may sometimes be very difficult and involve highly skilled hunting techniques and sophisticated coordination. Indeed, adverse effects of pollutants upon capturing and handling prey have been demonstrated in a number of aquatic species (Atchison et al., 1996). Direct effects may also be upon the ability of birds to avoid predation, as has been well demonstrated in a number of studies with fish (Beitinger, 1990). Although it seems very likely that the same principle may apply in the case of birds, hard evidence is very scanty. In one study, Buerger et al. (1991) dosed bobwhite quail with methyl parathion before releasing them and observing subsequent events with the aid of radiotelemetry. Over a study period of 3 years, it was found that survival time was significantly less in birds receiving 6 mg/kg of the OP than in control birds. This was attributed to greater susceptibility to predation. Direct behavioural effects upon reproduction can, in theory, involve disruption of pairing or mating, failure to incubate eggs, desertion of nest, and failure to protect nest or to feed young. One study with four different species of ducks in the field gave evidence of reduced survival of ducklings following the application of methyl parathion (1.4 kg a.i./hectare) which may have been the consequence of brood abandonment (Brewer et al., 1988).

Indirect effects may be the outcome of behavioural changes caused to prey species. Apart from the question of reductions in prey populations, behavioural changes of prey may foreshadow selective predation. Neurotoxic compounds can impair the ability of the prey species to avoid predation; they may also cause aberrant behaviour which draws the attention of the predator. For example, prey species may show unusual, poorly coordinated movements as well as tremors and twitches in the early stages of poisoning by organochlorine or organophosphorus insecticides. The potential significance of this—especially with persistent insecticides—is that predators will ingest relatively large amounts of toxic compounds, because they feed preferentially on the most contaminated members of the prey population.

Mechanisms of neurotoxic action

Several different receptors within the nervous system are targets for the action of neurotoxic insecticides. They have contrasting functions and locations within the peripheral and central nervous systems of vertebrates and invertebrates (Eldefrawi and Eldefrawi, 1990; Krieger et al., 2001). There are marked differences between species and groups in the form and distribution of these targets. In principle, the nature of behavioural effects caused by neurotoxic compounds should depend on the type of receptor, and consequently the mode(s) of action involved. The situation is complicated, however, by the possibility of differences between species/groups in their response to any particular insecticide. Some of the more important modes of action of insecticides will now be briefly reviewed.

Organophosphorus and carbamate insecticides act principally through inhibition of acetylcholinesterase which is located in the postsynaptic membrane of cholinergic synapses. Cholinergic synapses occur in both the central and peripheral nervous systems of vertebrates. Early symptoms of anticholinesterase poisoning in vertebrates are many and varied, but death is often due to respiratory failure. A few organophosphorus compounds, for example, DFP, mipafox and methamidophos can also cause delayed neuropathy, referred to as ‘organophosphate induced-delayed-neuropathy (OPIDN)’ (Johnson, 1992). Here, deterioration of peripheral nerves occurs some time after exposure and the disappearance of OP from the body. The effect is the long term consequence of the combination of the OP with an esterase of the nervous system termed ‘neuropathy target esterase’; usually the symptoms only appear with the ageing of the bound OP moiety attached to the enzyme (Johnson, 1992). There is evidence that birds are particularly sensitive to this type of neurotoxicity. Indeed, the domestic fowl is the preferred laboratory species when testing for OPIDN. Recently it has been shown that OPs can bind very strongly to sites other than cholinesterase or neuropathy target esterase in rat brain (Richards et al., 1999). Evidence for the existence of OP targets other than acetylcholinesterase has contributed to the ongoing controversy about possible long term neurological effects of OPs upon sheep farmers, and gulf war veterans. It remains a possibility that birds exposed to OPs in the field may have experienced long term neurological and behavioural effects unrelated to the

inhibition of acetylcholinesterase, although this has not been demonstrated in field studies.

Pyrethroid insecticides and DDT act upon the voltage-sensitive Na^+ channels of axonal membranes. They slow down the inactivation of Na^+ channels when an action potential is developed. Consequently they can cause repetitive discharge from nerves, thereby disrupting the transmission of the action potential. Typical symptoms of poisoning of vertebrates include tremors, twitches and prostration. Type II pyrethroids, which contain a cyano-group, can also cause convulsions—a symptom that is characteristic of action upon gamma-amino butyric acid (GABA) receptors rather than Na^+ channels (see next para.). Cypermethrin and decamethrin are examples of Type-II pyrethroids.

Cyclodiene insecticides such as aldrin, dieldrin, heptachlor and endrin and gamma HCH (lindane) act as inhibitors of GABA receptors (Krieger, 2001). The GABA receptor is the major type of inhibitory receptor in the vertebrate brain, and the cyclodienes act upon the GABA A form of it. This interaction in the central nervous system leads to a number of neurological disturbances, including characteristic tonic convulsions. Predatory birds poisoned by cyclodienes often show clenched claws.

Organomercury fungicides are also neurotoxic (Environmental Health Criteria 86, 1989; Wolfe et al., 1998; Walker, 2001). Methyl mercury, for example, can cause brain damage accompanied by a variety of neurological and behavioural disturbances, including difficulties with walking and flying (Borg et al., 1970). The toxicity of organic mercury is related to its tendency to bind to the -SH groups of proteins within the brain.

Effects of neurotoxic pesticides

DDT and metabolites

The toxic effects of DDT have frequently been related to the levels of persistent residues of the parent compound and its stable metabolites. Thus, by the analysis of carcasses (found dead or shot) and of eggs, it has sometimes been possible to attribute harmful effects in the field to these compounds (Walker, 2001). However, the issue is complicated by the question of the differential toxicity of the residues. p,p′ DDT generally has higher acute toxicity towards vertebrates than its principal stable metabolite, p,p′ DDE; it is some 3-fold more toxic over a range of species (Environmental Health Criteria 9, 1979). In one study with the feral pigeon (*Columba livia*), birds were dosed with feed containing p,p′ DDE at the level of 1,000 ppm over a period of 28 days (Bailey et al., 1969a). Twelve out of thirty-six birds died during this period, but none of them showed symptoms associated with p,p′ DDT poisoning—in contrast with birds receiving the latter compound in the same experiment, many of which showed marked and sustained tremoring (Bailey et al., 1969b). p,p′ DDE has been shown to cause eggshell thinning in birds, probably because of its ability to inhibit the calcium pump operated by calcium ATP-ase in the shell gland (Lundholm, 1987). In sensitive species such as the American kestrel (*Falco sparverius*), p,p′ DDE levels as low as 3 ppm in the feed can cause eggshell thinning (Wiemeyer and Porter, 1970). Thus, there is evidence not only of differential toxicity but also of a different mode of action when comparing p,p′ DDT with its principal persistent metabolite.

With these points in mind, it is a little surprising that most of the behavioural studies on birds for these compounds have been conducted with p,p′ DDE rather than the markedly neurotoxic parent compound (Peakall, 1985). One or two studies have demonstrated behavioural effects of p,p′ DDE, notably in a study of ring doves (*Streptopelia risoria*) by Haegele and Hudson (1977). Courtship behaviour was shown to be affected by 10 ppm p,p′ DDE in the diet administered over a period of 63 days, which yielded a brain residue of 2.9 ppm. In another study, disturbance of nocturnal activity of white-throated sparrows (*Zonotrichia albicollis*) was caused by both p,p′ DDT and p,p′ DDE, at dietary levels of 5 ppm or 25 ppm (Mahoney, 1975).

With DDT and other persistent neurotoxic compounds there may be selective predation upon intoxicated individuals of the prey population. Such selective predation has been demonstrated experimentally in the case of newts (*Triturus cristatus*) preying upon tadpoles of the frog (*Rana temporaria*) (Cooke, 1971). Tadpoles dosed with DDT showed hyperactivity when they contained 0.5–0.6 ppm of p,p′ DDT, and became relatively inactive (‘resigned’) at somewhat higher tissue levels. The number of succesful attacks by the newts was 8–9 fold greater upon dosed tadpoles than upon controls. When DDT was widely used there were examples of birds being poisoned by this

insecticide in the field (see for example, Stickel et al., 1966; Bailey et al., 1970). It is tempting to speculate that there may have been selective predation—and therefore enhanced uptake of DDT residues—by raptorial birds.

The cyclodienes

The following account refers mainly to aldrin, dieldrin and heptachlor, the use of which was largely discontinued by the 1980s because of harmful effects upon the environment and perceived human health hazards. Other cyclodienes include endrin, endosulfan, chlordane and telodrin. The toxicity of aldrin appears to be due to its stable metabolite dieldrin.

With vertebrates, a characteristic symptom of severe cyclodiene poisoning is convulsions, in keeping with action upon GABA receptors associated with inhibitory synapses of the central nervous system. However, with a continuing low level of exposure a number of other milder symptoms are often shown before tissue concentrations are high enough to cause convulsions. This has been observed both in occupationally exposed workers and experimental animals dosed with cyclodienes in the laboratory (Jaeger, 1970; Environmental Health Criteria 91, 1989). Workers suffering from sublethal dieldrin intoxication have experienced a variety of symptoms including headache, dizziness, drowsiness, hyperirritability, nausea, anorexia and, in more severe cases, convulsions. Broadly speaking, these symptoms were shown with blood levels of the order 200–600 μg/l dieldrin; above this came severe convulsions and death. In a recent publication (Krieger, 2001), the effects of long term chronic exposure to cyclodienes have been summarised. After exposure over a period of months (most frequently 8 months or more), mild symptoms of poisoning began to be manifest, which progressed to more severe symptoms. Initially these symptoms included mild, persistent headache, dizziness, insomnia, nystagmus, diplopia, tinnitus, slight involuntary movement and blurred vision. More severe symptoms were myoclonic jerkings of one or more limbs, sometimes progressing to convulsions.

Experimental animals exposed to sublethal levels of cyclodienes have shown changes in electroencephalogram patterns, disorientation, loss of muscular coordination, vomiting and convulsions (Environmental Health Perspectives 91, 1989). In the human studies overt symptoms of intoxication were shown at or below blood concentrations 30% of those associated with lethal intoxication.

Peakall (1985) cites a number of laboratory studies where sublethal doses of dieldrin were shown to cause behavioural effects in sharp-tailed grouse (*Tympanuchus phasianellus*), bobwhite quail (*Colinus virginianus*), Japanese quail (*Coturnix japonica*), pheasant (*Phasianus colchicus*) and mallard duck (*Anas platyrhynchos*). Effects reported included a decrease of aggressive behaviour in mallard, effects on social and breeding behaviour in sharp-tailed grouse, in operant behaviour of bobwhite quail, of avoidance behaviour of Japanese quail and a variety of effects in the pheasant. Behavioural disturbances were observed in bobwhite quail with brain levels of 4 ppm dieldrin.

In a study conducted upon the mallard duck (Sharma et al., 1976), behavioural changes were observed when birds received dietary levels of 4, 10 or 30 ppm dieldrin in their feed, and these were related to reductions in the levels of the biogenic amines serotonin, dopamine and noradrenaline. Increased avoidance and decreased encounters were observed at the lowest dose (4 ppm), where the concentrations of dieldrin in brain and liver were 0.12 and 2.3 ppm, respectively. At a dose of 10 ppm, the effects on behaviour were more pronounced and the brain and blood concentrations of dieldrin were 0.26 and 0.42 ppm, respectively. Heinz et al. (1980) observed decreases in dopamine and norepinephrine in ring doves after receiving 4 or 6 ppm dieldrin in their food.

During the late 1950s and the 1960s there were many reports of granivorous and predatory birds dying of cyclodiene poisoning in the field (see Walker, 2001). In Great Britain long term studies were initiated to monitor residues in birds found dead in the field and in eggs, and to relate these, where possible, to population trends of certain raptors locally and nationally. Particular attention was given to the sparrowhawk (*Accipiter nisus*) (Newton, 1986; Newton and Wyllie, 1992) and the peregrine (*Falco peregrinus*) (Ratcliffe, 1993). With both species population crashes in Britain during the late 1950s/early 1960s were related to the effects of cyclodienes. This interpretation was strengthened by the subsequent recovery of both populations with declining residues of dieldrin and heptachlor in the birds following largescale bans and restrictions on the insecticides. One puzzling observation was that sparrowhawk and kestrel (*Falco tinnunculus*) populations began to recover in the worst affected areas only after dieldrin residues (geometric

means) in the livers of birds found dead in the field fell to 1 ppm and below (Newton and Wyllie, 1992). Laboratory studies with Japanese quail and domestic pigeons had suggested that individuals dying from acute dieldrin poisoning normally contained 10 ppm or more of the chemical in liver (Robinson et al., 1967). Thus, very few individuals (no more than 5%) of the birds found dead had high enough residues to suggest death by dieldrin poisoning. Was this sufficient to explain the failure of the raptors to recover? Residue data for sparrowhawks and kestrels for the period 1963–86 were reexamined in an attempt to throw further light on this question (Walker and Newton, 1999). In the area most highly contaminated by dieldrin, both species showed a wide range of residues, and frequency diagrams showed peaks centring on c 20 ppm which evidently represented individuals dying from acute dieldrin toxicity. There was also, however, a substantial number of individuals containing residues of 3–9 ppm, these amounting to some 20–35% of the entire samples collected when numbers of raptors were very low. It seems clear that most of the individuals with these residues would have experienced sublethal effects of the kind described earlier, and that effects such as loss of coordination, disorientation and convulsions would very probably have impaired hunting skills. (As noted above, Sharma et al., 1976 demonstrated behavioural effects and depressed levels of biogenic amines caused by dieldrin in mallards at a concentration of 2.3 ppm in the liver.) Highly specialised predators such as the sparrowhawk which feed upon highly mobile prey (other birds in this case) are dependant upon sophisticated hunting skills (Newton, 1979).

To explore these ideas further, data for the sparrowhawk was incorporated into population models (Sibly et al., 2000). Two postulates were compared:

(1) that deaths attributable to dieldrin were limited to individuals with 9 ppm or more of the insecticide in liver (29% of total sample);
(2) that deaths attributable to dieldrin included all individuals with 3 ppm or more in the liver (65% of total sample).

Only in model 2 was the percentage mortality attributed to dieldrin sufficiently high to explain the population decline of the sparrowhawk in Eastern England in terms of the adverse effects of this chemical. Overall, these results strongly suggest that sublethal effects were important not only in the initial decline of the sparrowhawk but also in the delay in recovery after the critical bans on the use of cyclodienes. It also seems highly probable that starvation consequent upon a loss of hunting skills contributed to these population changes.

Organophosphorus (OP) and carbamate insecticides

With the withdrawal of organochlorine insecticides from the market, the attention of environmentalists moved increasingly to the less persistent insecticides such as OPs and carbamates which superceded them and whose toxic effects were likely to be more transitory. This did not, however, resolve all concerns about possible behavioural effects. Although the original compounds and their active metabolites have short half lives, the biochemical lesions that they cause may be more prolonged. In the first place inhibited acetylcholinesterase can be slow to reactivate, especially where ageing has occurred, and restoration of activity may depend largely upon the synthesis of new enzyme. There is also the question of OPs operating through other sites of action. As described earlier, a few OPs have been shown to interact with neuropathy target esterase (NTE) causing delayed neuropathy (Johnson, 1992). There is also evidence of other sites in the central nervous system which are more sensitive to attack by OPs than acetylcholinesterase itself (Richards et al., 1999). Thus, it is not safe to assume that all neurotoxic effects produced in the field will be transitory. Finally, even transitory behavioural effects may be of ecological significance, notwithstanding the difficulty of detecting them in the field.

In the investigation of OP effects upon birds and other vertebrates the inhibition of brain cholinesterase can be used as a biomarker assay for both exposure and, more importantly, toxic effect. This topic has been reviewed by Mineau, 1991. Many studies have shown that sublethal doses of OPs can cause behavioural effects in birds, and frequently these effects have been related to levels of cholinesterase inhibition (Peakall, 1985; Mineau, 1991). In many cases behavioural effects were apparent when depression of brain acetylcholinesterase exceeded 40% (Grue et al. in Mineau, 1991). There was, however, considerable variation between compounds and species, and in some cases such effects were not

apparent until there was a greater reduction of activity than 40%.

Grue et al. (1991) observed that 'the general picture of acute anticholinesterase intoxication is mainly one of "depression", as shown by a reduction of a wide variety of behavioural outputs, both innate and learned'. However, at an early stage of intoxication, reduced activity may alternate with periods of hyperactivity. Grue and Shipley (1981) dosed male starlings (*Sturnus vulgaris*) with dicrotophos to cause around 50% inhibition of brain acetylcholinesterase activity. The dosed birds were less active than controls, showing significantly more time perching and significantly less time foraging, singing, and displaying. Hart (1993) dosed starlings with chlorfenvinphos and observed that inhibition of brain cholinesterase by around 40% reduced flying and singing and increased resting. Fryday et al. (1995) showed that a dose of 5 mg/kg chlorfenvinphos reduced the flying activity of starlings over a 2 h period. An important consequence of the depressive effect of OPs on birds is to reduce counting efficiency in field studies of pesticide effects, because affected birds may be overlooked on account of their immobility (Grue et al., 1991). Fenitrothion fed to black-capped chickadees (*Parus atricapillus*) caused inhibition of brain cholinesterase by 50–60% but did not cause impairment of memory in a food caching experiment (Mineau et al., 1994).

It is much more difficult to establish behavioural effects in the field than in the laboratory (Peakall, 1985). In one study, however (Busby et al., 1990), evidence was produced of both behavioural effects, and associated effects at the population level after forest spraying with fenitrothion. Spraying of spruce forest in New Brunswick, Canada, led to estimated reductions of mean brain cholinesterase activity of white-throated sparrows of 42% and 30%, respectively on two consecutive spray operations. There was a wide range of individual responses, ranging from deaths due to acute poisoning to a variety of sublethal effects. Reported behavioural effects included inability to defend territories, disruption of incubation patterns and clutch desertion. The reproductive successs of birds in the sprayed area was about 1/4 of that in the control area. In a field study red-winged blackbirds (*Agelaius phoeniceus*) exposed to prey contaminated with parathion developed taste aversion to the prey species in question even where uncontaminated by the chemical (Nicolaus and Lee, 1999).

Organomercury fungicides

Like aldrin and dieldrin, cases of secondary poisoning by organomercury compounds were reported during the 1950s, and were particularly associated with the use of methyl mercury as a fungicidal seed dressing (Borg et al., 1970). Early symptoms of organomercury poisoning in birds are similar to those observed in mammals (Borg et al., 1970; Wolfe et al., 1998). Reduced food consumption and weakness of the extremities leads to poor muscle coordination and ataxia. In the later stages birds can neither walk nor fly. Such effects in the field would certainly affect the ability of birds to feed; as with cyclodienes, early effects of organomercury poisoning would be likely to affect the hunting skills of predatory birds. Although lethal and sublethal effects of organomercury were reported from the field in Scandinavia at the time that organomercury seed dressings were in use, no studies from that time established effects at the population level.

Discussion

The connection between the sublethal neurotoxic action of some pesticides and various behavioural disturbances in birds and other vertebrates has been demonstrated in a number experimental studies and this inevitably raises questions about the significance or otherwise of such effects in the field. There have been many reports of both lethal and sublethal effects of such compounds from field studies, and it seems clear that behavioural disturbances must have been caused as well (see, for example, Busby et al., 1990). However, behavioural effects are notoriously difficult to quantify in the field, and there is a lack of hard evidence linking them to population declines (Heinz, 1989; Peakall, 1996). In the foregoing account a number of examples were given of the quantification of behavioural effects in field studies. These and some others are mentioned by Peakall (1996). However, he also points out that it can be very difficult to prove that changes have actually been caused by the environmental chemicals with which they are associated. In concept, biomarkers for toxic effect can resolve this problem by establishing (1) that there is a dose–response relationship between the level of an environmental chemical and a biomarker response which provides clear evidence of causality in the field

and (2) that this biomarker response is causally related to a quantifiable behavioural change (Walker, 2001). This approach has already been successfully followed when using brain cholinesterase inhibition as a biomarker for OP toxicity in the field, as discussed earlier in this review. There is, however, the further question alluded to by Peakall (1996)—even where behavioural changes can be related to the action of environmental chemicals, do these cause population declines? There are two well documented cases where the use of biomarker technology has facilitated the identification of such causal relationships. One is eggshell thinning caused by DDE bringing the decline of the Peregrine falcon and other raptors in North America (Peakall, 1993); the other is imposex caused by tributyl tin in the dogwhelk (*Nucella lapillus*) leading to reproductive failure and population decline in the United Kingdom (Gibbs and Bryan, 1986). Progress in this direction depends on utilising appropriate mechanistic biomarker assays in the context of properly designed—usually long term—population studies. Unfortunately such studies are expensive and time consuming—quick and easy fixes do not provide the answer! Understanding the long term ecological significance of behavioural effects of environmental chemicals will depend on long term commitments to funding of environmental research programmes by agencies and governments.

It may be argued that, with the extensive bans that have been placed on the use of organochlorine insecticides and methyl mercury fungicides, there is now little reason for concern about possible behavioural effects of neurotoxic pesticides. Whilst it is true that these markedly persistent neurotoxins have presented a more obvious threat to birds than the more biodegradable organophosphorus, carbamate, pyrethroid or neonicotinoid insecticides, there are other important considerations. In the first place, even the readily biodegradable compounds still in use may cause transitory behavioural disturbances which could be of ecological significance (Mineau, 1991; Grue and Shipley, 1981). Also, it should be born in mind that neurotoxic effects of degradable compounds may be potentiated/prolonged due to interaction with other pesticides (see Walker et al., 2001, 153–62). Further, there is increasing evidence that organophosphorus compounds can produce neurotoxic effects which persist for longer than the active form of the insecticides (see discussion above).

Although the persistent neurotoxic pesticides are now little used, it should be remembered that we know more about their environmental fate and long term effects than we do for most other pollutants and they now serve as valuable MODELS for other compounds with similar properties. Our understanding of their behaviour gives insight into the ecological risks presented by other persistent neurotoxic pollutants. The marked persistence of some organochlorine insecticides and organomercurial fungicides, once so troublesome to environmentalists, carried a hidden benefit; their residues could be determined in tissues of vertebrates recently dying in the field, and used as evidence of toxic effect. The magnitude of residues found in the tissues of dead birds provides an index of sublethal as well as lethal effects. A recent retrospective analysis of residue data for dieldrin in predatory birds gave evidence suggesting that a considerable proportion of birds found dead in the field would have experienced sublethal neurotoxic effects (Walker and Newton, 1999). When data from this study were incorporated into a population model for the sparrowhawk, there was support for the view that sublethal as well as lethal effects of cyclodienes were implicated in the decline of this species in Britain during the 1950s and early 1960s (Sibly et al., 2000). Sublethal neurotoxic effects would have included behavioural disturbances. It would be interesting to carry out retrospective analyses of other population studies which relate decline to cyclodiene residues to see whether they yield similar results.

Returning to less persistent neurotoxic compounds, there is seldom residue data that can be used to identify behavioural effects which may be relevant to population declines. On the other hand, biomarker assays can, in principle, provide a measure for neurotoxic and behavioural effects of non-persistent chemicals in the field (Peakall, 1992). Inhibition of cholinesterase has already been used as a biomarker of neurotoxic effects which can be related to consequent behavioural disturbances both in the laboratory and the field (Peakall, 1985, 1992; Busby et al., 1990; Mineau, 1991). With the widespread use of non-persistent neurotoxic insecticides, the development of biomarker assays for pyrethroids, carbamates and neonicotinoids could provide the technology needed to investigate possible behavioural effects in the field. The need is for sensitive, specific, relatively inexpensive mechanistic biomarker assays that can be used by

non specialists, in the field as well as in the laboratory, which can provide evidence of links between behavioural effects of neurotoxic pollutants and population changes in the field (Peakall and Shugart, 1993).

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