1.
Effects on Birds

Chairman: Dr A. R. HARDY

AVIAN MORTALITY IN AGRO-ECOSYSTEMS
1. THE CASE AGAINST GRANULAR INSECTICIDES IN CANADA

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ABSTRACT

Voluntary notification of kills provides a very biased view of the direct lethal effects of pesticides on birds, under-representing kills of small, widely dispersed birds such as breeding songbirds. Unreported avian mortality may be extensive under some pesticide use conditions. The problem of granular insecticides in Canada is used as an example.

INTRODUCTION

The current view of the impact of farming on birds is that direct lethal effects have been largely brought under control since the removal of notorious products such as cyclodiene seed dressings, and as a result of on-going monitoring schemes such as the U.K. Wildlife Incident Investigation Scheme. A key part of such voluntary notification systems is the cooperation of pesticide users and bystanders. However, even in Britain where the interest in birdlife appears to be high, there is some doubt as to the willingness of farmers to report kills to the authorities (O'Connor & Shrubb 1986 pp. 212-213) and thus, the onus of such reporting may often fall on bystanders. Even if a farmer is willing to report a kill, he has to be aware that it has occurred. Kills are, therefore, much more likely to be reported when they involve large numbers of large birds in heavily frequented areas. These criteria immediately bias the likelihood of discovery in favour of situations involving staging or migrating flocks and relatively large birds such as waterfowl or game birds.

DISCUSSION

Kills reported following the use of granular insecticides

The Canadian experience with granular insecticides illustrates the reporting bias just mentioned. Table 1 summarizes the recorded bird kills ascribed to the use of granular insecticides in Canada. There is no comprehensive Canadian kill reporting network; a few veterinary schools are routinely sent wildlife samples for diagnosis and there is a toll-free line in British Columbia which was set up following the carbofuran incidents reported in Table 1 (this has not yet yielded any knowledge of new kills). The Canadian Wildlife Service and provincial wildlife departments, as well as provincial and federal agriculture departments, are also notified on occasion.

Most of the recorded kills involved ducks feeding in partly flooded turnip fields (carbofuran kills) or in cauliflower fields (fensulfothion kills) in British Columbia. The carbofuran kills elicited the most interest because of the large numbers of birds involved. Factors which were considered to have resulted in these kills were the extreme toxicity of carbofuran to waterfowl, shallow incorporation of the granules common in this type of culture, acidic soils which allowed the granules to persist from the time of spring application to the next fall/winter period and, in one case at least, application by hand which resulted in over-application of the product. (In that one case, the grower was successfully prosecuted.) In January 1976, the manufacturer voluntarily withdrew granular carbofuran from Lower British Columbia because of the acidic soils and the presence of staging waterfowl. A kill in 1977 probably resulted from the use of previously purchased stock. The re-introduction of the product in 1986 resulted, that same year, in the kill of an estimated 500-1200 Savannah and Lincoln's Sparrows and an unknown number of ducks over the course of the 1986-87 winter (P.E. Whitehead, Canadian Wildlife Service *pers. comm.*). Presumably, it is only a matter of time before the next major kill of waterfowl is reported.

TABLE 1

Summary of kills recorded following the use of granular insecticides in Canada (1973 - 1988).

Pesticide	No. Incidents	No. Birds involved		
		Waterfowl	Passerines	Other
carbofuran	6	1290+	3200+	2
fensulfothion	4	213	40+	-
phorate	1	5+	-	-

Sources: Edwards 1986, A. Godfrey *pers. comm.*, NRCC 1979, Saskatchewan College of Veterinary Medicine 1984, P.E. Whitehead *pers.comm.*

Two kills were recorded outside British Columbia. One small kill of Canada Geese involved granular phorate in the Maritimes (A. Godfrey, P.E.I. Wildlife *pers.comm.*). In the second incident, a flock of migrating Lapland Longspurs alighted in a freshly seeded rape field in Saskatchewan. The farmer reported the kill. I estimated a minimum kill size of 2,000 based on reports by the farmer and the investigating pathologist, but this might be a gross underestimate because the fields were harrowed twice and much scavenging had taken place before any carcass count was made. The exact size of the kill will never be known, but Lapland Longspurs and other northern-nesting songbirds migrate in flocks which can number tens of thousands. We do not know whether this kill represents an unusual situation or a common occurrence. No information exists on the availability of granular insecticides to birds following this specific use pattern. Given that an estimated 425,000 ha hectares of rape are treated annually with carbofuran alone (Madder & Stemeroff 1986), that much of the planting takes place at a time when migrant songbirds are arriving to or crossing the prairies and that many of those species like to roost and forage in bare fields, there is an urgent need to assess the impact of this agricultural practice.

The second largest granular insecticide market in Canada, corn (maize), has not been associated with any bird kills. Granular insecticides are used as a prophylactic control measure for two species of corn rootworms which are especially prevalent in the south and west of the province of Ontario. I estimate, based on pesticide sales information (Table 2), that 70% of the total area treated with granular insecticides in Canada (excluding the canola market) lies in Ontario; most of this Ontario use is for corn. Estimates of areas of corn treated with registered granular products in Ontario are available for 1980 - 1985 (Table 3). The absence of reported kills can be interpreted in two ways: 1) the use of granular insecticides in corn does not give rise to significant wildlife mortality; or 2) wildlife mortality does occur but is not represented in the kill record. South-western Ontario boasts one of the main veterinary centres (Univ. of Guelph) specializing in wildlife pathological investigations so that a lack of a reporting structure is not a factor.

TABLE 2

Surface area estimated to be treated yearly with granular insecticides in Canada excluding their use on rape. Averaged from 1985 and 1986 data (in ha).

Province	No. ha	Province	No. ha
Prince Edward Island	15,212	Manitoba	30,172
Nova Scotia	2,084	Saskatchewan	8,924
New Brunswick	15,236	Alberta	19,312
Quebec	39,179	British Columbia	5,412
Ontario	317,318		
		GRAND TOTAL	452,848

Calculations based on Environment Canada 1986, 1987.

TABLE 3

Cumulative (1980-85) area of corn treated with granular insecticides in Ontario.

Chemical name	Trade name	Area (ha)
terbufos	Counter 15G	765,645
fonofos	Dyfonate 20G	392,027
phorate	Thimet 15G	222,939
carbofuran	Furadan 10G	218,443
chlorpyrifos	Lorsban 15G	85,507
fensulfothion	Dasanit 15G	68,472
disulfoton	Di-syston 15G	46,582
isofenphos	Amaze 20G	8,475
chlorfenvinphos	Birlane 10G	3,080
GRAND TOTAL		1,811,170

Calculated from Madder and Stemeroff 1986.

A number of studies have looked at the impact of granular insecticides under corn-growing conditions typical of those found in Ontario, *i.e.* spring planting with similar rates and methods of application. All studies uncovered avian mortality, primarily of granivorous songbirds. Table 4 summarizes the results from these studies. This table reports only uncorrected mortality rates and there are several reasons why these are gross underestimates of the true mortality rates sustained in these fields: 1) they assume that carcass search efficiency is 100%, though placement of 'dummy' birds in cornfields has shown rates of recovery that are often in the 50% range (Mineau & Collins 1988); 2) they make no allowance for birds dying off-field; 3) they assume no removal by scavengers even though scavenging pressure has been found to result in the overnight disappearance of 62% to 92% of fresh songbird carcasses placed in freshly planted cornfields (Balcomb 1986); 4) application in one of the studies (Iowa) was made so as to reduce the avian hazard: some field personnel following the planting equipment on foot and kicking soil over any spills observed; and 5) the Iowa sites were chosen to represent areas with no or little woody vegetation, *i.e.* a 'corn desert' with low bird densities. Another study in Utah (Booth *et al.* 1983) reported an uncorrected kill rate of 8.9 birds per ha. The application rate was higher than for studies reported in Table 4 and, more importantly, there was productive bird habitat nearby.

TABLE 4

Summary of field studies which investigated avian mortality under conditions typical* of corn-growing in Ontario.

Location, Year	Application rate (kg ai/ha)	Application method	Field area treated & searched (ha)	Carcasses recovered	Uncorrected mortality rate/ha
Maryland 1980 (*1*)	1.1	F	25 - 38	10	0.26 - 0.40
Iowa 1986 (*2*)	1.5	B	124	29	0.23
Illinois 1986 (*3*)	1.5	B	69.2	103	1.5

* With the only exception that both the 10G and 15G formulations were tested whereas only the 10G formulation is registered in Canada. None of the studies have found any significant difference between the mortality rates sustained with the two formulations.

F = in furrow without diffuser boot B = banded in furrow

1. Balcomb *et al.* 1984a - very low search effort.
2. Booth *et al.* 1986 - spills covered by investigators; poor bird habitat (see text).
3. Booth *et al.* 1986.

Using the uncorrected mortality rates summarized in Table 4 and extrapolating to the similar conditions in Ontario, we can estimate what the likely Ontario kill should have been. In doing so, we assume that bird breeding densities in those studies were representative of those found in Ontario. This is likely given the wide range of habitat suitability represented, spanning the 'corn deserts' of Iowa to the small-field mosaics of Illinois.

Between 1980 and 1985, from 50,000 to over 300,000 dead birds (primarily songbirds) should have been recovered from the use of carbofuran alone. (Fortunately, the popularity of carbofuran, one of the most toxic granular insecticides presently available, did decrease dramatically during that period.) However, carbofuran is not the only granular insecticide capable of killing birds. When relative risk factors for some of the major alternatives are compared, individual granules of three other products, (fensulfothion, fonofos and phorate) are found to be as hazardous as carbofuran to some of the available test species (Table 5). Using the same mortality rates as for carbofuran, fensulfothion could have accounted for 16,000 to 103,000 dead birds, fonofos for 90,000 to 588,000, and phorate for 51,000 to 334,000 over the same 6 year period; in all, from 200,000 to over 1 million birds may have died. These figures exclude secondary kills, which have been well documented, at least with carbofuran (Balcomb 1983). Other granular products appear to be somewhat less toxic and may give rise to lesser impacts.

The inevitability of kills under current agronomic conditions

Granular insecticides have long been known to be attractive to birds although it is not known whether they are mistaken for food or grit or both (Balcomb 1984a). The main granule bases in Canada are clay, sand and corn cob which are all attractive. In a sample of 105 Horned Larks which died following ingestion of the sand core carbofuran granules, the number of granules retrieved from their gastro-intestinal tracts ranged from 0 to 53, the median and mode of the distribution were 2, and 30% of the birds were found with 5 or more granules (Booth *et al*. 1983). In the case of carbofuran, the rapid onset of toxic symptoms undoubtedly resulted in relatively few granules being ingested. Kenaga (1974) reported that, when available *at libitum*, large quantities *i.e.* up to 4.9 g./bird/week, of clay or corn cob granules were consumed by caged Bobwhite Quail.

The problem of soil-applied granular insecticides is primarily one of agricultural engineering. Some incorporation of the granules is required in order to obtain the maximum benefit. Unfortunately, the level of incorporation required for an efficacious treatment falls short of what is required for wildlife protection. The efficiency of granule incorporation in the course of corn planting has been investigated. Erbach & Tollefson (1983) tested three common brands of planters. When granules were banded into the seed furrow in front of the press wheel or seed-firming disks (the recommended method of application in Ontario - OMAF 1985) an estimated 14.7% of granules were left exposed. No differences were noted between the three models tested. Hummel (1983) looked at one brand of planter run at three different speeds. He reported a proportion of exposed granules ranging between 18.1 % and 69.0 % given the same instrument configuration as in the previously mentioned study. The best incorporation of granulars using commercially available equipment was found to be 99.6 % when granules were deposited into the seed furrow by means of a drop tube without the benefit of a diffuser boot (*op. cit.*). Unfortunately, this is not the recommended method of application in Ontario and, even if it was, there is some doubt that even this level of incorporation would be adequate to

TABLE 5

Relative hazard[a] of some granular insecticides used on corn.

Chemical name	Based on toxicity of granules			Based on toxicity of technical pesticide							
	Bobwhite[b]	Ringed Turtle Dove[b]	Red-winged Blackbird[c]	Bobwhite[b]	Pheasant[d]	Mallard[d]	Rock Dove[e]	Red-winged[e] Blackbird	Common[e] Grackle	Starling[e]	House Sparrow[e]
terbufos	0.14	–	–	0.25	–	–	–	–	–	–	–
fonophos	1.0	–	0.39	1.2	–	0.029	–	–	–	–	–
phorate	0.22	0.16	0.91	0.69	0.23	0.26	–	0.17	0.67	0.30	–
carbofuran	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
chlorpyrifos	0.033	0.013	–	0.11	0.070	0.002	0.040	0.010	0.071	0.022	0.040
fensulfothion	3.0	2.2	–	6.7	2.0	0.35	1.6	1.0	3.5	6.6	2.8
disulfoton	0.18	–	–	0.45	0.15	0.027	–	0.059	0.42	–	–

a) Based on granule weights given in Hill & Camardese 1984. Carbofuran hazard arbitrarily set at 1. A hazard ratio of 0.5 indicates that each granule is half as toxic as a carbofuran granule to the test species.

b) Toxicity data from Hill & Camardese 1984.

c) Toxicity data from Balcomb 1984.

d) Toxicity data from Hudson *et. al.* 1984.

e) Toxicity data from Schafer *et. al.* 1983.

protect wildlife given the results of Balcomb *et al.* (1984a) with this application technique. All of these incorporation rates are idealized because all tests were performed on precisely calibrated machinery under carefully controlled field or laboratory conditions by trained agricultural engineers. The tests did not estimate the spillage which occurs at the ends of the rows or because of uneven terrain or trash on the soil surface. Furthermore, both of these studies made use of fluorescent dyes but did not measure the efficiency of incorporation of the dye onto the granules or the ability of the overhead camera systems to record all unincorporated granules. Unincorporated granules were expressed as a proportion of the nominal rate of application. The calculated rates of incorporation, therefore, assume that all granules would be visible if unincorporated, which is unlikely to be the case.

Given the individual granule weights provided by Hill and Camardese (1984) for the corn granulars under consideration, and recommended application rates, we can estimate the number of granules deposited at planting. The standard application rates would be 34 granules per cm of row for a relatively heavy granule such as carbofuran (.320 mg) to 114 granules per cm of row for lighter granules such as terbufos or chlorpyrifos (.065 mg). Application rates for all corn granulars are only a function of the individual granule strength (i.e. whether it is a 10G, 15G or 20G granule). Variations in the toxicity of the various active ingredients to the corn rootworm are, therefore, assumed to be negligible despite the huge variation in their toxicity to non-target organisms. Given the best incorporation reported above for planting conditions typical of those found in Ontario (85.3%), we can estimate that the number of exposed granules available to surface-feeding wildlife ranges from 5 to 17 per cm of row or from 7 million to 23 million per hectare of planted corn at the usual row spacing of 75 cm. This excludes spills resulting from turns and uneven terrain which are reportedly frequent during normal planting operations (Balcomb *et al.* 1984a, Booth *et al.* 1986).

Given the sheer surplus of granules available to birds, their attractiveness and the fact that from 1 to 5 of granules of several of the products exceed the lethal dose for a small songbird (Balcomb *et al.* (1984b), it would not be surprising if mortality was widespread.

<u>The estimated Ontario kill in perspective</u>

Clearly, the magnitude of the recorded kills in Canada (Table 1) are insignificant in comparison to even a conservatively estimated total kill of songbirds for Ontario alone. From a management or regulatory perspective, it makes little sense to expend a great deal of effort on the investigation of, and enforcement measures related to the occasional reported kill of waterfowl if the steady drain on breeding populations caused by the same toxic agents is ignored. I believe that granular insecticides have the potential to significantly alter the population levels of species which nest in close association with corn and, possibly, with other cultures which make intensive use of granular insecticides.

One worrying aspect is that the reported kill rates, even when uncorrected, constitute a large proportion of the total songbird breeding density reported for those types of habitat. Erskine (1971,1972,1976) compiled breeding pair densities for 10 ha plots of farmland in southern Ontario and Quebec. Plots in grazed pastures ranged between 1 and 2 pairs/ha whereas plots with a field crop component such as hay generally supported densities between 2 and 4.5 pairs/ha. The two plots which listed corn as a component supported 2.3 and 2.8 pairs/ha. The most abundant species seldom exceeded one pair/ha on any of the plot types. These values are remarkably similar to those obtained from the Common Bird Census in Britain which censuses agricultural land (O'Connor & Shrub 1986 p. 114). Total breeding densities ranged from 1 pair/ ha for very open habitats to about 4/ha for habitats with a good complement of three-dimensional structure such as hedges and trees. Much lower values (between 0.3-0.4 pairs/ha) were obtained by Oelke (1981) for birds in agricultural land in the German Federal Republic.

I do not believe that the situation postulated for Southern Ontario is any different to that in other areas of granular insecticide use. The U.S. kill record shows a bias similar to ours in that few, if any, agricultural songbird kills were ever reported before the aforementioned field studies (J. Bascietto, U.S.E.P.A *pers. comm.*). In the U.K., reported granular kills have primarily dealt with flocks of Black-headed Gulls and Lapwings feeding on contaminated earthworms (Hardy *et al.* 1986), despite evidence that songbirds are exposed to unincorporated granules and are insufficiently considered or missed altogether in surveillance exercises (Bunyan *et al.* 1981).

CONCLUSION

Though major improvements to the avian component of the agricultural landscape were achieved through the banning of such damaging products as organochlorine insecticides, some current pesticide use patterns such as the use of toxic granular insecticides are still reaping an unacceptably heavy toll of wildlife from agricultural land. That this problem has not yet come to light in areas of intensive granular use such as Southern Ontario, may be a function of the difficulty in detecting such 'diffuse' mortality. I believe that there should be a concerted effort on the part of all stakeholders to measure this problem accurately and to investigate ways of eliminating it or at least reducing it to a more acceptable level. We should explore ways to replace granular formulations of highly toxic products with other types of controlled release formulations (such as polymer microcapsules) less injurious to wildlife.

ACKNOWLEDGMENTS

Over the years, I have greatly benefitted from discussions with two ex-members of the Ecological Effects Branch of the U.S.E.P.A., R. Balcomb and J. Bascietto. It is mainly through the perseverance of these individuals that many of the problems discussed here have come to light. I am thankful to H. Boyd, K.E. Freemark, G.A. Fox, A. Harfenist and D.B. Peakall for reviewing earlier drafts of this paper.

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AVIAN MORTALITY IN AGRO-ECOSYSTEMS
2. METHODS OF DETECTION

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ABSTRACT

As a result of recent efforts in the U.S. to standardize field impact methodology in pesticide evaluations, there has been a renewed interest in defining the sensitivity of available methods. Here, we consider the methods available to detect avian mortality, with emphasis on the most straightforward: searching for carcasses. A simple model for carcass searching is presented.

INTRODUCTION

Extensive direct avian mortality is a likely impact of some of today's agronomic practices (Mineau 1988). It is relevant to ask whether we have the tools to detect such mortality should it occur. Once mortality is detected, then it must be quantified. In this presentation, we wish to address the aspect of detection.

DISCUSSION

Carcass searching

Finding carcasses is obviously the most direct way to determine that mortality has occurred. However, even such a seemingly simple procedure is fraught with problems. We briefly discuss some of the relevant aspects of carcass searching by presenting a simple model which defines the likelihood of success as a function of a number of search parameters or fixed environmental attributes.

A Simple Model of Carcass Searching

The mathematical description for the model is given in Appendix 1. Basically, the model assumes pesticide treatment on day 0 and needs the following variables as input: plot population size, background and pesticide-induced mortality rates (which can vary over time), carcass removal rates through scavenging, the type of search pattern (beginning on day 1 at the earliest), the efficiency of finding carcasses, the assay sensitivity and assay specificity. To keep the model simple, we assume a closed system with no bird immigration or emigration. The result from the search does not estimate the extent of mortality but rather the probability that pesticide-induced mortality will be detected if it has occured. This model is not presented here as the best or final effort but merely as an example of the way in which the success of a carcass searching strategy can be assessed even before any effort has been expended.

The approach taken here differs substantially from, but in some ways is complementary to, that presently being developed by the U.S.E.P.A for their field testing guidelines (U.S.E.P.A. 1987). Inherent in our model is the assumption that our single field or plot is representative of all fields treated with the same pesticide. We ask whether we have a reasonable chance of detecting pesticide mortality. The U.S.E.P.A. (*op. cit.*) begins with the assumption that the impact may not manifest itself on every site, and their intent is to identify any pesticide which causes this impact (however defined) a certain proportion (*eg.* 20%) of the time . Our model should be able to aid in the determination of the number of plots required once a given 'level of concern' is set.

We now wish to consider the various parameters which will influence the success of a carcass searching strategy.

Time to Death

In theory, any carcass searching exercise is only defensible as a viable monitoring strategy if the pesticide under study has a relatively rapid toxic action. In practice, it is difficult to know what constitutes a suitably rapid effect. Even slightly different rates of 'knockdown' and death have the potential to give rise to a biased view of which compounds are acutely hazardous and which are not. Unfortunately, accurate times to death at various acute dosages are seldom available, from the literature or from current data submissions, to help with the interpretation of the field results. Hill and Camardese (1984) remarked that the onset of symptoms was more rapid with the carbamates than with the organophosphates they tested.

Consideration of the 'time to death' is also crucial when dealing with 'indirect' mortality *i.e.* mortality that is a result of a given pesticide treatment but which is not related to the direct toxic action of the compound. For example, it has been argued that starvation may play a very important role where exposure to organophosphate insecticides leads to anorexia and where the energetic needs of a bird are such that it cannot withstand a very long period of starvation (Pope & Ward 1972, Mineau & Peakall 1987). Time to death then becomes a function of the size of the bird and of its metabolic needs rather than a function of the speed of action of the pesticide. Other examples of delayed mortality are discussed below.

We did not enter 'time to death' as a separate parameter, but it is an important component of the 'carcass search efficiency' which is discussed below. Search efficiency is likely to be minimal or nil if most or all of the exposed individuals die off the field site.

Assay Sensitivity and Specificity

Under ideal conditions, one should be able to ascertain a cause-effect relationship between any observed mortality and the pesticide being investigated. The assay specificity is defined as 1 minus the probability of a false positive while the assay sensitivity is 1 minus the probability of a false negative. Under ideal conditions, both are equal to unity. One example of an assay specificity of less than 1 is the use of a non-specific method of diagnosis such as measurements of cholinesterase inhibition when nearby areas have been treated with cholinesterase-inhibiting agents other than the one under study. Under experimental conditions, the assay specificity is usually under the control of the investigator, but this is not necessarily so in operational surveillance programs. Assay sensitivity is more likely to fall short of unity: even though the pesticide under study caused mortality, a clear diagnosis is not always possible. This may be due to an inability to detect a relatively labile residue, to a spontaneous recovery of the assay used (eg. cholinesterase recovery following carbamate intoxication) or, more simply, to a lack of material to analyse if the carcass has greatly deteriorated.

In the examples given below, we have set the assay sensitivity and specificity equal to 1 and the background mortality rate to 0. The probability of detecting an impact is, therefore, the probability of detecting a single carcass.

Population Size

Typical avian population densities in agricultural landscapes have been found to vary between 0.3 pairs/ha to 4.5 pairs/ha, one of the important factors being the degree of three-dimensional relief available (Erskine 1971, 1972, 1976, Oelke 1981, O'Connor and Shrubb 1986). The most numerous species seldom exceeds 1 pair/ha. Species present are not all equally likely to be exposed to a given pesticide application, thus it is important to define a likely indicator population for which an exposure route can be identified. It has been our experience with the North American literature that information on bird use of agricultural fields is scant. It is usually necessary to define an indicator population following on-site investigation such as was done by Bunyan *et al.* (1981).

Figure 1 explores the influence of population size on the likelihood of detecting mortality given a single search on day 1 and a search efficiency of 50%, a likely figure on the basis of the information presented below. Figure 1c looks at changing scavenging rates and Fig. 1d examines various rates of pesticide-induced mortality. For example, Fig. 1d tells us that, when scavenging rates are set at 50%, the total indicator population size needs to be in the range of 50 for 90% probability of detecting a 20% kill given a single but timely search. However, the same set of curves indicates that a population of 20 would be adequate for kill rates of 50% or more. Based on a workable field or plot size of 10-20 ha, a population of 20 birds or more is likely under good conditions and this number will be used in a number of simulations which follow. On occasion, one may be forced to work with a smaller number of birds in unproductive habitats or where some of the less common species demonstrate an inherent vulnerability to a given pesticide treatment. Some of our simulations will also be run on a population size of 5 as a worst-case situation.

Search Efficiency and Scavenging Losses

Assuming that the pattern of mortality is such that carcasses are likely to be recovered on or very near the treatment area, then the success of a carcass searching strategy hinges on two important variables: the search efficiency and the expected survival rates of the carcasses. Both have been examined under actual field conditions and some of the relevant studies are summarized in Table 1. Much of the information contained in this table comes from field studies performed for pesticide review purposes in the U.S. Such information is not considered by the U.S.E.P.A. to be confidential and is available upon request.

In the only test performed in a field crop proper (wheat 25 cm tall), 30 individuals searching for an entire day only found 50% of the planted birds. Two habitat types have been examined in more detail than others: corn fields and turfed sites, principally golf courses. In both cases, search efficiency measurements were conducted assuming that birds died very suddenly without the possibility of seeking shelter. No effort was ever made to hide carcasses from view whether planted mid-field or along the edges.

TABLE 1

Scavenging rates expressed as the proportion of fresh planted carcasses completely removed by scavengers during the first 24 hour period after placement and search efficiency rates expressed as the proportion of carcasses retrieved by searchers for different habitats.

Habitat and conditions	Scavenging[a]	Search efficiency[b]	Refs.
Bare cornfield - whole field	4.0% - 92%	45% - 100%	1-4
Bare cornfield - edge	-	37% - 89%	3,4
Growing corn - whole field	5.6% - 48%	10% - 81%	1,3,4
Growing corn - edge	-	32% - 68%	3,4
Golf course - fairway	0% - 62%	80% - 100%	5,6
Golf course - rough and edge	41% - 86%	0% - 93%	6,7
Urban lawns	0%	70%	8
Base of TV tower	50% - 93%	-	9,10
Pine seed orchard	-	74% - 100%	11
Rangeland	6.7% - 13%	-	12
Along brushy wood's edge	10% - 33%	56% - 63%	13
Marsh - carcasses in the open	24%	12%	14
" - carc. in the vegetation	50%	0%	14
Wheat - 25cm tall	-	50%	15
Spruce-fir forest	0%	-	16

a. Range of individual trials or fields.
b. Range of individual search efforts and plot means combined.
(1)Booth *et al.* 1986; (2)Balcomb 1986; (3)Dingledine & Jaber 1987; (4)Dingledine 1985; (5)Kendall *et al.* 1987; (6)Palmer *et al.* 1987; (7)Fletcher 1987; (8)Mellott *et al.* 1987; (9)Crawford 1971; (10)Crawford 1974; (11)Overgaard *et al.* 1983; (12)Rosene & Lay 1963; (13)Stickel & Chura 1964; (14)Stutzenbaker *et al.* 1984; (15)Butcher *in* Heinz *et al.* 1979; (16)Fowle 1965.

Of all the studies reported in table 1, only one (Stutzenbaker *et al.* 1984) tried to measure a search efficiency rate for a toxicant which allows birds to seek shelter in their preferred habitat, in this case a shallow marsh with good overhead cover. A search team of 8 experienced individuals with no time limitation found none of the 50 duck carcasses planted over the 40 ha area. Table 1 shows that carcass searching efficiencies are extremely variable. This is the case even within any given study where the search pattern is well established and performed regularly by the same trained individuals. This indicates that little confidence should be put in surveillance exercises which rely on a small number of searches conducted by different individuals who are given vague instructions to search the sites for casualties.

In practice, there is a limit to the intensity of the search effort that should be deployed because the presence of observers may, in itself, deter some species from using the fields and coming into contact with the pesticide. Presumably, searches should immediately follow, but not coincide with, periods of high bird activity.

Scavenging rates also appear to be variable. For example, the overnight disappearance rate of small to medium sized birds (passerines to Bobwhite Quail) from freshly-planted corn fields in the State of Maryland ranged from 4.0% to 92%. The higher rates, from Balcomb (1986), were obtained using passerines. The extent to which some of the other studies were biased by using larger carcasses should be examined.

On the basis of Table 1, we have selected 50% as being a realistic value for both search efficiency and scavenging loss. Figure 1a explores the influence of a variable removal rate by scavengers on the probability of discovering a carcass and the relative success of searching at various times after application. Given that we have set mortality to be instantaneous following the pesticide application, it is not surprising that a heavy price is paid for any delay in searching. Such delays will be further considered below. Figure 1b looks at the problems encountered when scavenging rates are high.

Time to first search and number of searches

There are a number of reasons for delaying the search of an area. Often, the application itself will occupy the experimenters for one or more days. Alternatively, it may be unsafe for searchers to enter an area immediately after application. Following the initial search, the number of subsequent searches is theoretically unlimited, but is practically subject to manpower and cost requirements. Also, as mentioned above, too much time spent on the field site may influence the outcome. In theory at least, the timing and number of searches should be matched to the expected mortality. We examine three types of mortality functions in our model. In the first and most simple case, mortality is instantaneous following the pesticide application and there is no residual effect. In the second case, the mortality rate is constant over time, at least on a short term basis, as might be the case with slow residue breakdown or prolonged granule availability. The third possibility is for pesticide-induced mortality to follow a declining function with time. This would be the case for a sprayed pesticide where residues decline with a predictable half-life, or even a granular product where granule availability decreases rapidly with time. We have arbitrarily set the mortality rate to decline by 50% every day.

Figure 2 explores the influence of the pesticide-induced mortality rate on the probability of carcass detection for single searches conducted on various days following application and for plot population sizes of 5 and 20 birds subject to the three types of mortality functions just outlined. These simulations are very relevant to surveillance exercises such as the one described by Bunyan *et al.* (1981). In that case, observers were requested to limit observations to 10 ha and to visit the site 2 or 3 days after treatment and then 25-30 days after treatment. Given an indicator population of 20 (a reasonably generous estimate for 10 ha), search and removal rates set as above, and a constant hazard from the granules over the short term (i.e. no heavy rains or other factors which may affect granule availability), we can analyse the success of the search strategy. Figure 2e tells us that waiting till day 2 would have been ideal with mortality rates up to 50%; for any higher mortality rate, searching on day 1 would have been most likely to uncover the problem. The general decline in the probability of detection at very high mortality rates over time results from there not being any more birds to die and to be found. This raises the interesting possibility that mortality may sometimes be easier to detect when it is low than when it is high. Under most situations, but especially where the mortality is extensive and the population size is small, there is a very heavy penalty associated with any delay in implementing searches.

In all the simulations, we have kept the scavenging rate the same over the entire period. Whereas this is expected to be true for new carcasses being generated over time, there is evidence that complete removal of carcasses by scavengers actually declines over the life of the carcasses as they lose some of their attractiveness due to partial scavenging or dessication. Although we have kept the removal rate constant, the bias is partially offset by also keeping the assay sensitivity equal to 1. As carcasses become 'feather spots', we also loose the ability to ascribe a cause-effect relationship between the death and pesticide exposure.

Whereas Figure 2 primarily addresses surveillance exercises, Figure 3 looks at the situation for fields or plots under intensive study where regular searching is possible. Again, we define three types of mortality curves, but this time, we consider two search strategies. In Figures 3a, 3c and 3e, we look at the cost-effectiveness of multiple searches, given that they can begin in earnest on day 1. A population size of 5 was used for this simulation because our previous simulations clearly demonstrated that our power of detection was quite low under single search conditions. As expected, returns are minimal beyond the second or third search. A different problem arises when searching cannot begin immediately for reasons previously given but when searches can be frequent and regular following the first one (Figs. 3b, 3d and 3f). We conclude from these simulations that no amount of effort is likely to offset the lost time, especially in cases where the mortality rate was high.

Population Surveys

In the absence of bodies, it ought to be possible to deduce that mortality has taken place through a survey of the live population. In practice, this method has limitations. It has been more commonly used in forestry situations where the ability to recover carcasses is generally low. Also, most survey methods are best performed in homogeneous habitats and not well suited to habitat mosaics, which are the norm for farmland (Oelke, 1981).

Replacement of dead breeding birds by 'floaters' (*i.e.* individuals not occupying a territory) is probably the single most important problem to contend with if one is relying on surveys of singing males to infer mortality of resident breeders. Replacement rates are rapid in boreal forests, replacement taking place overnight or on the morning following removal (Mineau & Peakall 1987). An experiment designed to look at replacement rates in the British agricultural landscape yielded less dramatic results (Edwards *et al.* 1979) but nevertheless indicated that replacement could be rapid for some species. When replacement is expected to be a problem, one must turn to marked populations or to observations of nests to be assured of the continued presence of the local breeders. Both of these approaches are much more time- and effort-intensive and may be impracticable where nests are difficult to locate. [Use of radio-transmitter technology may also yield valuable information on the true efficiency of carcass searching.] Alternatively, replacement rates can be measured and data from removal plots (*op. cit.*) used to set upper bounds on population changes that could be observed in the event of mortality.

One problem which is difficult to overcome is a pesticide-induced alteration in behaviour which effectively removes individuals from the surveyable population without killing them. Cholinesterase inhibitors are a well known example of such compounds (Grue and Shipley 1981). It could be argued, however, that in an ecological context, removal of a breeding individual even for a short duration is equivalent to death.

Chemical or Biochemical Markers

Given a thorough knowledge of the dose-response characteristics of a pesticide, the case could be made that a defined residue load or biochemical response such as enzyme inhibition is indicative of that individual's probability of dying. That determination must remain a probabilistic one because, death may not be observed especially if the organism is sampled destructively. Exposure to a pesticide may render an organism more vulnerable to the stresses of everyday life and greatly shorten its life span. For example, a neurotoxic agent such as a cholinesterase-inhibiting insecticide may render an organism more susceptible to predation (Galindo *et al.* 1985) or to adverse climatic conditions (Rattner *et al.* 1982). An anti-coagulant may not affect an individual's survival until it injures itself. All of these situations (predation, climate, injury) have a strong stochastic component and they may or may not coincide with the period during which the organisms are under observation.

In some cases, mortality of part of the population can be inferred from a skewed distribution of a marker such as cholinesterase depression (Mineau and Peakall 1987); preliminary evidence shows that collections of live birds following forest spraying are biased toward the least affected individuals. In some cases, it could be argued that for every individual collected, there are others which cannot be found and collected because they are either dead or very severely impaired (ecologically dead). This bias need not be restricted to forest habitats although the difficulty of finding carcasses in forests obviously makes the collection bias worse.

CONCLUSION

If an overall conclusion can be drawn from the above, it is that nothing is ever as simple and straightforward as it looks. Many field studies have purported to show that a given product or formulation is safe on the basis of a lack of bodies. Very seldom has the true power of these studies been investigated. A surveillance exercise is unlikely to be useful except under conditions of catastrophic impact on a large segment of the population. If there is a universal approach to testing field impacts during the breeding season, then surely it must involve monitoring the continued presence and success of local nesters. Unfortunately, it is often difficult to find a sufficient number of the desired species to give a meaningful result. Carcass searching does have a role to play but its true power must be carefully considered. We have not yet seen any studies which have satisfactorily dealt with the real probability of detection given the problematic aspects of 'time to death' and the behaviour of sick birds. Whereas field studies can be helpful in pointing to a problem, they have a very low power when it comes to exonerating any given pesticide.

ACKNOWLEDGMENTS

We thank H. Boyd and G.A. Fox and A. Harfenist for reviewing earlier drafts.

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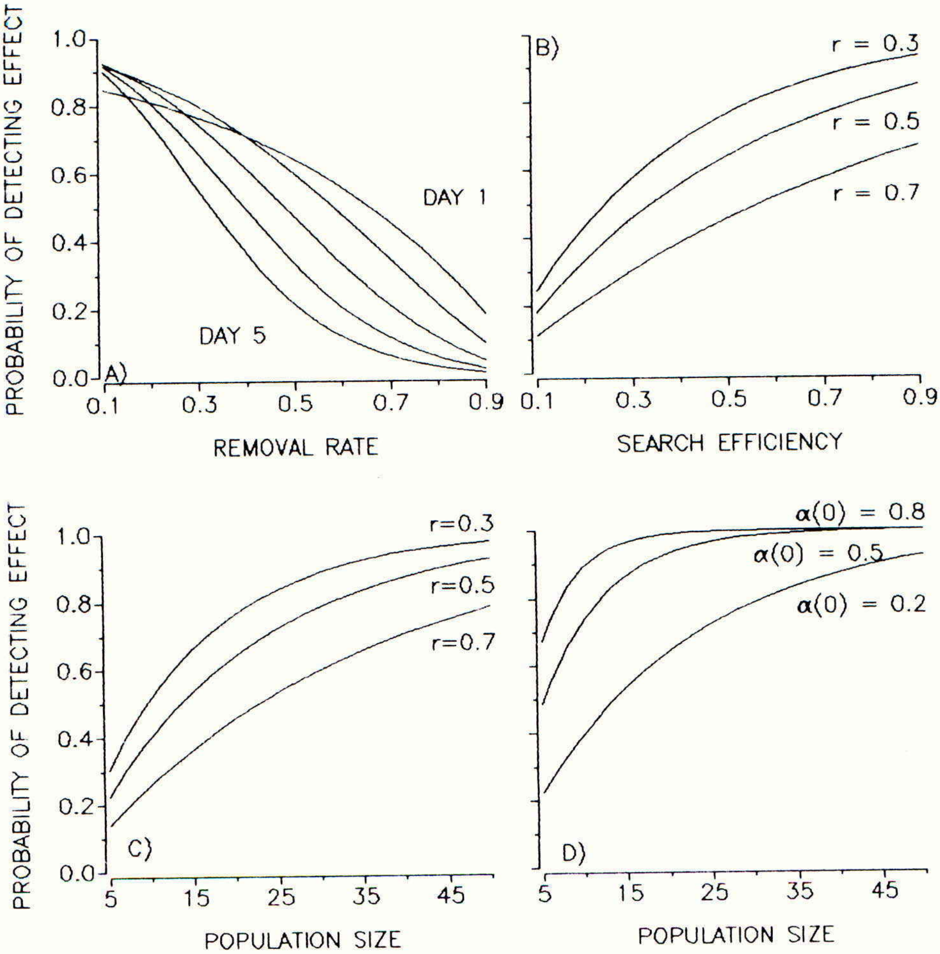

FIGURE 1 FACTORS AFFECTING PROBABILITY OF DETECTING EFFECT: POPULATION SIZE = 20, REMOVAL RATE $r=0.5$, SINGLE SEARCH ON DAY 1, MORTALITY RATE $\alpha(0)=0.2$, SEARCH EFFICIENCY = 0.5 AND ASSAY SENSITIVITY = 1.0 UNLESS OTHERWISE NOTED

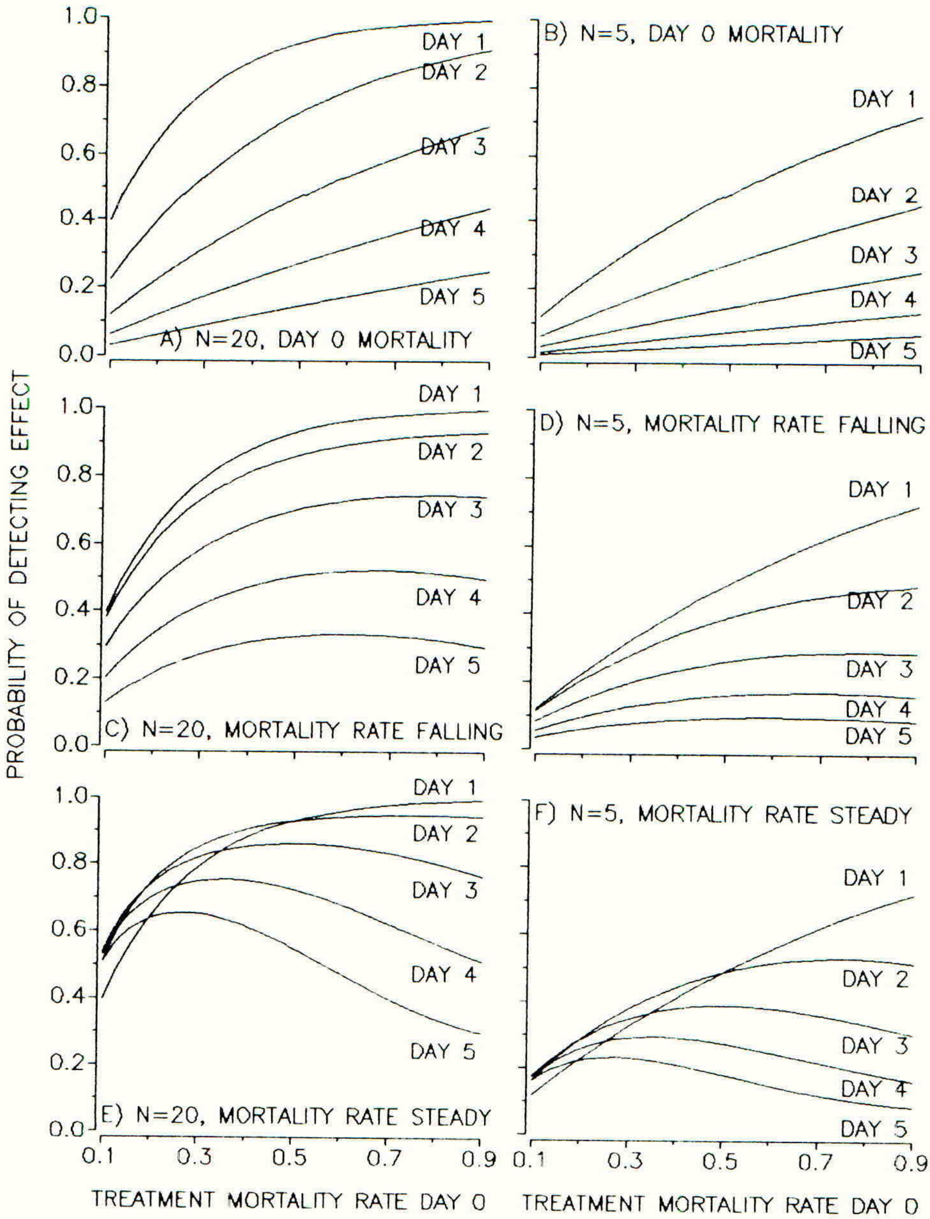

FIGURE 2: ONE DAY CARCASS SEARCH WITH REMOVAL RATE 0.5, SEARCH EFFICIENCY 0.5 AND ASSAY SENSITIVITY 1.00

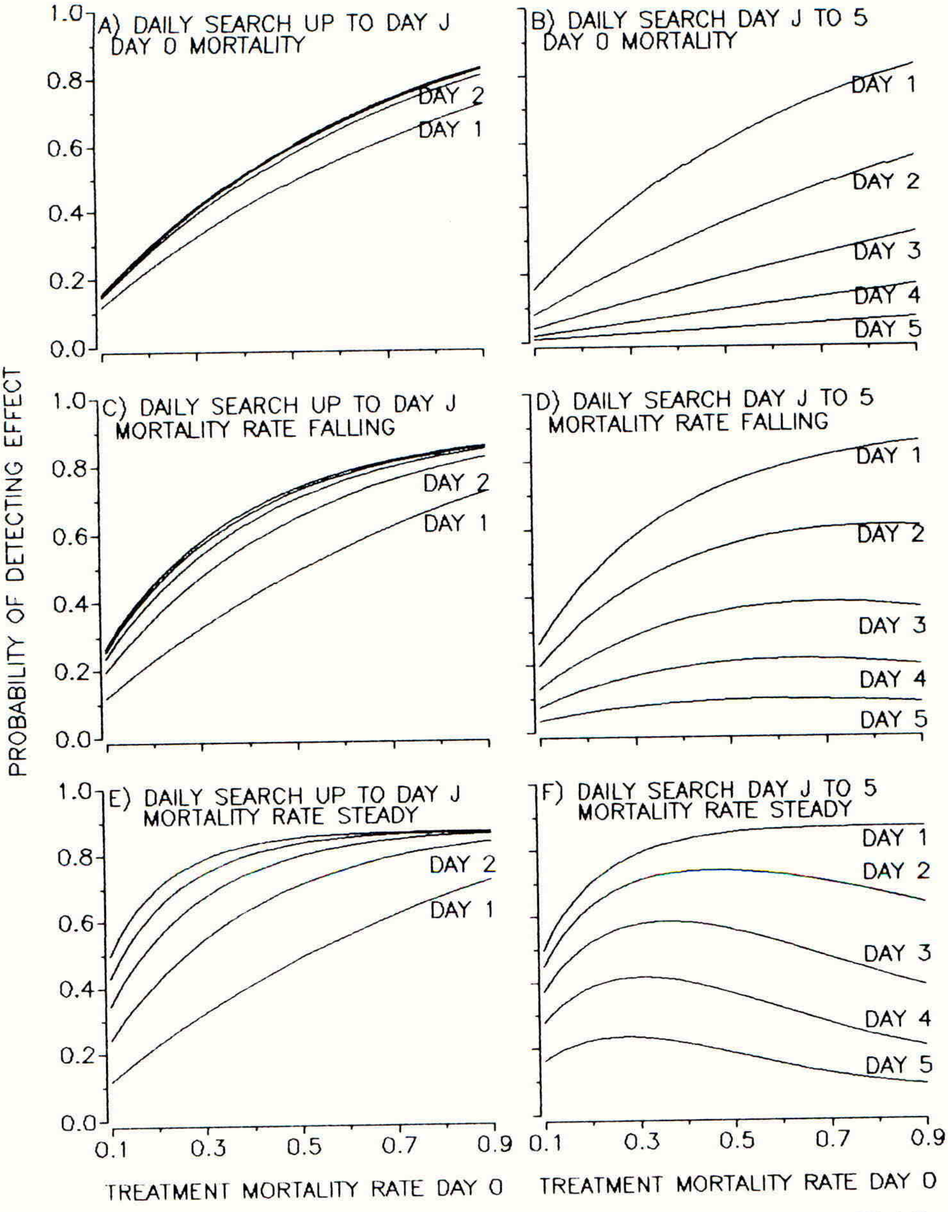

FIGURE 3: MULTIPLE CARCASS SEARCHES WITH N=5, REMOVAL RATE 0.5, SEARCH EFFICIENCY 0.5 AND ASSAY SENSITIVITY 1.00

APPENDIX 1: CALCULATION OF PROBABILITY OF DETECTING A TREATMENT EFFECT FOR AN INDIVIDUAL PLOT.

The probability of detecting an effect is calculated as the probability of recovering dead birds and having an assay identify a critical number of carcasses as treatment-related. The background mortality rate is assumed to be negligible but the number of carcasses recovered is not the critical event. The model describing the chance of recovering a positive carcass is built up from several simple probability models describing: treatment mortality, scavenging, search efficiency and assay sensitivity.

TREATMENT MORTALITY

Consider a treatment which has a daily mortality rate of $\alpha(j)$ on day j ($j=0,...J$) i.e. the probability that an individual alive on day j is killed that day is $\alpha(j)$. Given an initial population of N individuals and assuming the treatment affects individuals independently, the probability that n_j birds would be killed on day j would be given by a binomial distribution with parameters $\alpha(j)$ and $N-\sum_{i=0}^{j-1} n_i$.

SCAVENGING

Scavengers are assumed to remove a portion of the carcasses before the search is undertaken. The probability that a carcass present on day i is removed that day is r. The removal rate is assumed to be independent of age of carcass. This implies that the probability a carcass is present on day j after the bird was killed is $(1-r)^j$.

SEARCH EFFICIENCY

The carcass search is assumed to have an efficiency e, that is the probability that a carcass present at the time of search is found is e. Thus the number of carcasses recovered by the search on a given day may be treated as a binomial random variable. When multiple searches are done it is assumed that the probabilty of recovering the carcasses is independent between searches. This may not be a realistic assumption since carcasses which are not recovered on one day would be those which are well hidden and hence would remain difficult to locate.

ASSAY SENSITIVITY

Each carcass is assayed to determine if the mortality could be attributed to the treatment. This assay is assumed to detect the treatment with probability s, when the death was treatment related. The age of the carcass is assumed not to affect the assay.

DISTRIBUTION OF NUMBER OF POSITIVE RECOVERED CARCASSES

The above distributions can be combined to give the probability of recovering k positive carcasses. It can be shown that the number of positive recovered carcasses would have a binomial distribution with probability parameter varying according to the pattern of search and the population parameter equal to the number of birds.

If there is a single search on day J then the probability parameter becomes

$$p = s\ e \left\{ \sum_{j=0}^{J-1} \alpha(j) \left[\prod_{i=0}^{j-1} (1-\alpha(i)) \right] (1-r)^{(J-j)} \right\}$$

If the search is repeated daily from day 1 to day J then the probability parameter becomes

$$p = s\ e \left\{ \sum_{j=0}^{J-1} \alpha(j) \left[\prod_{i=0}^{j-1} (1-\alpha(i)) \right] \left[\sum_{i=0}^{J-j-1} (1-e)^{i} (1-r)^{(i+1)} \right] \right\}$$

If the search starts at day J and continues to day 5 then the probability parameter becomes

$$p = s\ e \left\{ \sum_{j=0}^{J-1} \alpha(j) \left[\prod_{i=0}^{j-1} (1-\alpha(i)) \right] A_j \right\}$$

$$\text{where } A_j = \sum_{i=J-j-1}^{4-j} (1-e)^{(i-J+j+1)} (1-r)^{(i+1)} \text{ if } j<J-1$$

$$\text{and } A_j = \sum_{i=J-j-1}^{4-j} (1-e)^{i} (1-r)^{(i+1)} \text{ if } j \geq J-1$$

STRATEGIES FOR ASSESSING PESTICIDE HAZARDS TO BIRDS

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ABSTRACT

This paper discusses methods suitable for acquiring the information needed to anticipate and prevent pesticide hazards to birds. An exploratory approach is advocated, beginning with a review of available information to identify potential hazards, and the species likely to be affected. Suitable tests of these predictions should be included in field trials. Where possible, the tests should include measures of residues, metabolites or physiological effects to establish that the subjects are being exposed to the chemical. Measures of behaviour may be used to indicate whether the functioning of the animal is affected. Potentially serious effects may be reflected in measures of reproductive performance, energy reserves and mortality. Where significant effects are found, a more comprehensive study should be carried out to predict long-term consequences, and to identify **appropriate** restrictions or modifications to the proposed use of the pesticide.

INTRODUCTION

Experience gained from the use of pesticides such as dieldrin, chlorfenvinphos and carbophenothion in the United Kingdom (Stanley & Bunyan 1979), shows that an exploratory approach is required if pesticide hazards to wild birds are to be identified in advance of introduction to commercial use. Assessment should begin with a review of available information on the properties and intended use of the chemical, and the ecology of the habitats in which it will be used. This should identify the causal pathways likely to result in unacceptable consequences, such as widespread mortality or reproductive failure, or long-term decline of bird populations. Tests should then be carried out to confirm or negate the existence and significance of suspected hazards. Ideally, mortality (see Mineau & Collins 1988) and effects on reproduction should be measured directly. This may be difficult, however, and within the development period of a new chemical, may not provide adequate information to assess long-term effects. We therefore need tests which can be employed after a single application of pesticide, to detect short-term effects which could lead to unacceptable consequences. Where short-term effects are found, further investigation of the routes by which they are caused may help to identify appropriate restrictions on the use of the pesticide, or suggest ways of circumventing the problem.

MEASURES OF EXPOSURE

The assessment of short-term effects should generally include tests for residues of the pesticide and its metabolites, or for its physiological effects (e.g. Thompson, Walker & Hardy 1988). These will confirm whether species thought to be vulnerable are actually being exposed, and will generally be simpler than attempting to investigate the routes by which exposure occurs. Mineau and Peakall (1987) have drawn attention to the potential for severe sampling bias in favour of less-affected individuals, when collecting birds for exposure assessment after spraying. This difficulty can be completely overcome only by the use of radio-tagging techniques to locate subjects. Ideally, sampling should be non-destructive, if only to avoid the risk that subsequent samples will be distorted by immigration from outside the study area to replace birds that are removed (Niethammer & Baskett 1983). Subjects should be caught alive (Spencer 1984), and assays based on blood sampling (e.g. from the brachial vein) or tissue biopsy (Clark *et al.*, 1982). Histological techniques (Tarrant 1988) may also be useful. Repeated sampling of marked individuals over a suitable period before and after pesticide application is particularly valuable, both in increasing sensitivity and in revealing the time-course of exposure (Hardy *et al.* 1987). Appropriate licences should, of course, be obtained for all sampling activities.

MEASURES OF BEHAVIOURAL CHANGE

Studies of the behavioural responses of birds to pesticides have been reviewed by Peakall (1985). He concluded that behavioural measures are generally no more sensitive to pesticide effects than biochemical and physiological measures, which are often easier to obtain. As Peakall states, however, the implications of behavioural effects may be easier to interpret. If exposure is demonstrated, but is less than that known to be associated with mortality, or if the lethal levels are unknown for the species concerned, then behavioural measures can be used to indicate whether the functioning of the animal is affected. Also, behavioural measures could detect indirect effects of pesticides, for instance through depletion of insect prey, which may affect birds even when they suffer no direct exposure. Finally, behavioural observations may indicate how the animal is coming into contact with a pesticide, and suggest ways of modifying the formulation or application method to prevent exposure occurring.

Peakall (1985) found that, in the studies which he reviewed, the degree of pesticide exposure producing behavioural change was usually within one order of magnitude of the lethal exposure. Thus the detection of an effect on any behavioural measure would imply that the margin of safety is small for the pesticide concerned. This suggests that in the initial assessment of potential hazards, emphasis could be placed on behavioural measures which are easy to obtain, or if possible, on types of behaviour which are thought to be particularly sensitive to pesticide effect. If significant effects are found, it will be necessary to assess their long-term implications. The emphasis would therefore shift to the types of behaviour which are most likely to affect reproduction and survival, and on by how much and for how long they are affected.

Methodology

Martin and Bateson (1986) have provided an excellent introduction to methodological and technical aspects of measuring behaviour, from the definition of behavioural units and the design of sampling schedules, to the statistical treatment of data. Fletcher and Greig-Smith (1988) discuss some of the issues involved, in relation to field trials of pesticides. Recent papers have stressed the importance of precision in the definition of behavioural measures (Fraser & Rushen 1987), and the implications of individual differences for experimental design and analysis (Martin & Kraemer 1987).

Ideally, behaviour should be measured for control and treated subjects, both before and after treatment. This allows individual differences to be accounted for, and controls for changes over time which are unrelated to the pesticide treatment. This type of experimental design does present difficulties in field trials. Some measures, for instance of behaviours associated with reproduction, are available at different times for different individuals, so that only a proportion of subjects will provide data both before and after treatment. Also, repeated sampling of the same individual necessitates marking subjects so that they can be recognised, unless they can be reliably identified by location, for instance in relation to nest or territory. Marking techniques are reviewed by Marion and Shamis (1977). It is always important to be able to identify repeated measures from the same subject, in order to avoid treating them as if they referred to different subjects (the 'pooling fallacy', see Martin & Bateson 1986). Marking of subjects is also required to assess whether pesticide effects are general or affect only a proportion of the population, and to test whether individuals affected by pesticides are under-represented in post-treatment samples as a result of becoming less active (Grue & Shipley 1981).

Field assessments of pesticide hazards should ideally be carried out so as to be representative of the full range of conditions in which commercial use is envisaged. In practice, however, the range of conditions in field trials will be restricted. It may also be necessary to introduce some artificial procedures in order to obtain the measurements required. In bird studies, perhaps the most common will be the provision of suitable nesting boxes (du Feu 1985), to facilitate the monitoring of reproductive performance and breeding behaviour. Other examples are the application of pesticide in the absence of a pest outbreak (Spray *et al.* 1987), enclosure of nests to prevent predation (Powell 1984), direct dosing of subjects, the removal of one parent from each breeding pair, and adjustment of broods to a uniform number of chicks (Grue *et al.* 1982). In assessing the implications of results from field trials, the effects of all constraints on the realism of trial conditions must be taken into account. Also necessary, and more difficult, is assessment of contingencies which do not arise during the field trial, but which could present an occasional hazard in commercial use.

Technical aids

A range of technical aids may be employed in the observation and recording of behavioural data. Direct observation may be assisted by the use of binoculars, telescope and several types of night-vision equipment. Behaviour may be recorded on video or cine-film and subsequently observed at a conveniently slower speed. Observations can be recorded on paper, spoken into a tape-recorder or keyed directly into a computer or similar device (Martin & Bateson 1986). The use of technical aids should be designed to minimise bias, which they can introduce by distracting the observer's attention from the continuing behaviour of the subject.

Observers should be concealed, if necessary, to ensure that their presence and activities do not directly influence the behaviour of subjects. Behaviour may be monitored remotely, and without the need for human observers, using various types of detector. Some direct observations should also be made, however, to confirm that the automatically-recorded data represents behaviour in the manner intended. Animal movement in an enclosed space, or past some fixed point, can be monitored using infra-red or ultra-sonic detectors. Radio-transmitters or transponders attached to the subject can be very useful, but may involve considerable practical and technical difficulties (Kenward 1987, 1988). In some habitats, radio-tracking may provide the only practicable means of locating marked individuals for repeated observations. Radio-tracking can provide information on ranging behaviour and habitat use, and transmitters can be designed to vary their signals according to the posture or activity of subjects. Signals can be monitored continuously using automatic scanning equipment.

Behaviour of captive animals

Studies of captive animals are cheaper and easier to control than field studies, and might provide a more convenient means of testing whether the functioning of the animal is likely to be affected at pesticide exposure levels occurring in the field. Most of the behavioural tests listed by Peakall (1985) are for captive studies. However, behaviour is usually very dependent on environment, and it is impossible to provide truly naturalistic conditions in captivity. Thus, while a behavioural effect in a captive study would indicate a definite need for investigation in the field, the lack of an effect may not necessarily imply that the pesticide is safe. A more serious difficulty, in view of the potential for significant differences in the toxicity of the same compound to different species (e.g. Stanley & Bunyan 1979; Niethammer & Baskett 1983), is that it may often be impracticable to conduct captive studies with the species which are thought to be at risk in the wild.

Results of studies with captive birds do have some general implications, however, for the design and interpretation of field trials. Grue and Shipley (1981) demonstrated reduced singing, flying and feeding activity in captive starlings (*Sturnus vulgaris*) sub-lethally dosed with an organophosphate pesticide, and concluded that counts of birds in the field after spraying might show decreases due to reduced activity, rather than mortality or emigration. Affected individuals might also tend to be under-represented in post-treatment assessments of exposure (Mineau & Peakall 1987) and behaviour, as mentioned earlier. In similar experiments with a different organophosphate Hart (unpublished) has shown that feeding activity is progressively inhibited by increasing exposure, while the effect on singing is more complex, and may include a stimulatory effect at lower exposures (Figure 1). The variety of effects reported for organophosphates is remarked by Grue *et al.* (1983), and implies difficulties in interpreting the results of field studies.

Field studies of behaviour

It was suggested above that potential hazards should first be screened using the most convenient behavioural measures, and that when positive results are being followed up, the emphasis should shift to measures which are more directly related to reproduction and survival. In practice, the choice of behavioural measures is often severely constrained by practical considerations. In particular, the assessment of pesticide effects is more difficult outside the breeding season. Firstly, the birds tend to range more widely, and may form flocks. Thus we lose the benefit of the nest as a fixed focal point, at which behaviour can be monitored relatively easily. Increased mobility also makes it difficult to identify which individuals are most likely to be exposed to the chemical, and to identify unexposed birds to act as controls. Secondly, it is more difficult to assess the significance of behavioural effects outside the breeding season, because the causal link to effects on reproduction is less immediate.

The following sections discuss techniques applicable to the measurement of five broad categories of behaviour, distinguished by the practical and interpretational difficulties which they present. Methods from the different sections can of course be combined, as in the different approaches to mapping breeding territories described by Powell (1984) and Spray *et al.* (1987).

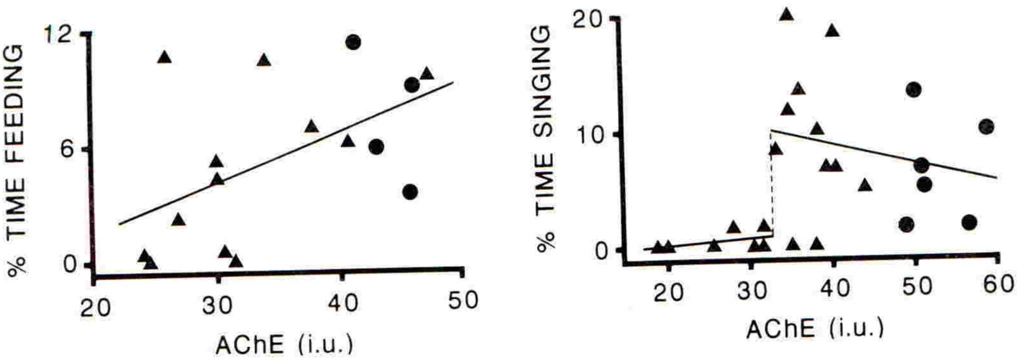

FIGURE 1. Feeding by female starlings and singing by male starlings during 8 hours after treatment with the organophosphate chlorfenvinphos, in relation to brain acetyl-cholinesterase activity (AChE) which is inhibited by the pesticide. Circles refer to control subjects, dosed with corn oil. The slope of the regression line for feeding is significantly different from zero. A step function fits the singing data better than a smooth curve. The step is significant but the fit is still poor, possibly due to differences between subjects in the position of the threshold. The data suggest an inverse relationship to the right of the threshold, but neither slope is significantly different from zero.

Parental visits to nests

The frequency of parental visits to eggs or young is a measure which is relatively easy to record, and is of obvious significance to reproductive performance. Grue *et al.* (1982) and Powell (1984) used direct visual observation to record the frequency with which birds visited their nests while feeding chicks. Nest visits can be monitored automatically using cameras, a technique which has been applied to studies of pesticide effects by Spray *et al.* (1987). Several variations of the technique have been developed, but in general a cine camera with facilities for remote triggering of single frame exposures is mounted so as to obtain a picture of the bird arriving at the nest. Food brought in the bird's bill may be identifiable if resolution on the film is adequate, and the time of each visit is recorded by placing a suitable timepiece within the field of view. Automatic recording by cameras precludes the need for human observers, and makes it practicable to record from many nests concurrently, though visual inspection of the resulting film is a laborious task. The technique is unsuitable for species which are intolerant of disturbance at the nest, because the installation and operation of a camera may result in nest desertion.

An alternative technique for monitoring visits to nests is to use microprocessor-based equipment, to record the frequency with which arriving and departing birds trigger suitable electronic detectors. A system of this type is being used in the ADAS Boxworth Project (Hardy 1986) to monitor nest-visits by tree sparrows (*Passer montanus*) during periods when an organophosphate insecticide is applied to nearby cereal crops. Birds visiting a nest-box pass through two infra-red beams, which are placed so that the order in which the beams are broken indicates whether the bird is entering or leaving. The times of beam-breaks at a number of nests are recorded by a central data-logger, and are later transferred to computer for analysis. In practice a proportion of beam-break sequences cannot be resolved as entries or exits, and visits by parents cannot be distinguished from visits by animals other than the parents, nor from beam-breaks caused by large chicks able to reach the nest entrance. Therefore, the system does not provide the precise record of nest-visits available from cameras or direct observation, nor does it provide any information about the food collected. Instead, it provides a measure of activity at the nest entrance. The relation of this measure to actual visit rates can be calibrated by means of short periods of direct observation. The advantages of the system are that it requires still less maintenance, less disturbance to the birds, and much less manual data processing than the use of cameras.

Diet and foraging behaviour

Food-gathering and the composition of the diet are clearly important to reproductive performance and adult survival. They also play a major role in determining how, and to what extent, birds are exposed to chemicals in their environment. Furthermore, studies of captive birds, such as those mentioned above, suggest that feeding will be affected by exposure to at least some pesticides. It is also the type of behaviour most likely to show any indirect pesticide effects which act through depletion of prey species.

During the breeding season, monitoring of nest-visits provides some information about nestling diet. The food brought to chicks can be identified by analysing their faeces, though the analysis is laborious, and may be complicated by the varying degrees of digestion suffered by different foodstuffs (Ralph *et al.* 1985). Food brought to young can be collected by fitting them with collars which prevent swallowing (Powell 1984), but this technique can have adverse effects which must be taken into account.

A variety of methods may be employed to record the activities of birds away from the nest, depending on the nature of the habitat and the behaviour of the subject species. Ideally the rate of prey ingestion should be determined, since this is directly related to the energy gained and hence to consequences for reproduction and survival, as well as to exposure to any pesticide residue in the prey. More detailed measures can be obtained, for instance the average time spent searching for and handling prey items, and the proportions rejected or lost to other foragers. In practice recording detailed foraging behaviour is likely to be difficult, and virtually impossible for birds foraging in dense standing crops. In such circumstances the best that can be done is to monitor the proportions of time spent in different parts of the habitat, either by direct observation of movements between areas (Fletcher & Greig-Smith 1988) or by radio-tracking.

Anti-predator behaviour

In the presence of predators, birds display a variety of behavioural responses which appear to reduce the risk of predation, including alarm calls, mobbing and flight. One would expect that any disruption of anti-predator behaviour as a result of pesticide exposure would have significant consequences for the survival of adults and their young, and also for the predator if secondary poisoning is possible. Some anti-predator responses may be assessed by a human observer simply approaching subjects, and recording the intensity of responses and the distance at which they are elicited. Measures of responses at the nest, where they are easiest to obtain, have been used in several studies of pesticide effects (Peakall 1985). Similar techniques could be applied at other sites such as feeding or roosting areas, though the results will probably be much more variable. Care is required to control for the possibility that subjects could habituate to the human stimulus, and it would probably be a major task to obtain the information necessary to understand the implications of any observed change in responses. Nevertheless, in some situations these measures are easy to record and may be the most convenient behavioural indicator of pesticide effect.

Song and display behaviour

Behaviours which birds use for communication tend, by their nature, to be conspicuous, and consequently are often relatively simple to observe and record. Results obtained with captive starlings, mentioned above, suggest that singing is likely to be affected by organophosphate pesticides, though probably not in direct proportion to degree of exposure. Again, if changes in these behaviours occurred in field trials, it would probably be difficult to predict the consequences for the population. Nevertheless, where song and display behaviours are easy to monitor they may provide convenient indicators of pesticide effect. A simple measure of the numbers of birds singing was used by Spray *et al.* (1987), to assess effects of fenitrothion spraying in a Scottish forestry plantation. We are currently investigating the use of counts of the conspicuous song-flights of skylarks (*Alauda arvensis*), which commonly nest in growing crops. Sonagraphic techniques (e.g. Dabelsteen 1984) could be used to investigate effects of pesticides on the detailed structure of birdsong.

General measures of activity

Lethargy is a common symptom of poisoning, and at higher exposures, may be accompanied by apparent loss of coordination. If general reductions in activity occurred in the wild it would be difficult to assess the consequences for reproduction and survival: for instance, the loss of time normally spent on activities such as foraging and territory maintenance might be partly offset by energy savings and reduction in exposure to predators. General measures of activity are therefore likely to be useful in the initial phase of assessment, as non-specific indicators of pesticide effect. Any conveniently observed, consistently quantifiable aspect of bird activity could be used, for instance the frequency with which birds pass over a field boundary or through clearings. The use of radio-tags which change their signal when the subject is active, offers a possible means of monitoring general activity even in habitats where the subject is rarely observed.

MEASURES OF REPRODUCTIVE PERFORMANCE

Measures of reproductive performance are valuable in assessing pesticide hazards, because they are often relatively simple to obtain, and because of the direct influence of reproductive output on population stability. Quality of offspring, for instance in terms of individual size or weight at fledging, may be as important as the number produced, since it may affect the probability that the individual survives to breed. Growth rates are likely to be more sensitive to short-term pesticide effects on, for example, parents' feeding rates. Though transient, such effects may be significant. Delayed growth and maturity imply prolonged exposure to risks of chick mortality, though this would not result in a significant number of additional deaths in a small study sample unless the risks were relatively high. Furthermore, what appears as a brief showing of growth might, under less favourable conditions, result in starvation.

Techniques for the measurement and analysis of reproductive performance are illustrated by the studies of Powell (1984) and Spray *et al.* (1987). Care must be taken to avoid excessive disturbance at the nest, especially during the egg-laying period, and when the young are close to fledging.

Nestlings may be weighed together or separately, and various linear measurements may be made: most commonly wing, tarsus or bill length (Spencer 1984). Brisbin *et al.* (1987) describe statistical approaches which open up new approaches for investigating growth, and cite an example of their application to assessing pesticide effects.

MEASURING ENERGY RESERVES

One way in which pesticide effects on bird behaviour could cause increased mortality is through reductions in energy reserves. This could result, for instance, from reduced food intake or increased energy expenditure, or if adult birds responded to pesticide effects by maintaining reproductive performance at the expense of their own survival prospects. Even when reductions in energy reserves cause no detectable mortality in a field trial, the possibility that they could cause starvation under less favourable conditions should be considered.

Changes in energy reserves could be detected simply by catching and weighing the same birds before and after pesticide treatment. Pesticide effects may be transient, however, and are more likely to be detected if a series of weighings is obtained for each subject. A variety of devices have been developed for remote weighing of birds on their nests (e.g. Sibly & McCleery 1980) and on perches (e.g. Crick & Fry 1986).

INTEGRATED STUDIES

Powell's (1984) study provides a good example of the way in which the variety of measures, which are required to provide a comprehensive assessment of pesticide hazard to a single species, can be brought together in an integrated programme. A similarly integrated approach has been adopted in the ADAS Boxworth Study (Hardy 1986), though in this case the full range of ecological effects are being examined in a comparison of three contrasting pesticide regimes. No amount of investigation will rule out the possibility that a chemical will eventually cause a hazard by acting in some unexpected fashion, but a comprehensive assessment of this type must increase the chance that any problems will be detected. Accordingly, there can be reasonable confidence that unacceptable consequences will not occur, when, as in Powell's (1984) study, no substantial short-term effects are detected. If effects on the various measures are found, then their attribution to pesticide effect will be better substantiated if they were recorded for the same subjects, and can be correlated with one another. A comprehensive study should also provide much of the information required for the detailed analysis of long-term consequences, which will be necessary if the effects found are neither obviously negligible, nor obviously unacceptable. It is likely, however, that in this situation it will be necessary to conduct further field trials, focussing more closely on the revealed hazard and its impact on reproduction and survival. This will be costly, but is preferable to the risk that considerable environmental damage could occur before an unanticipated hazard is detected by post-registration monitoring.

ACKNOWLEDGEMENTS

I am grateful to several colleagues for helpful criticism of earlier drafts, especially Peter Greig-Smith, Ian Inglis and Humphrey Crick. Also to Andy Cunningham, Angela Lewis and others for their collaboration in obtaining the data shown in Figure 1, and to Richard Fairall for designing the automatic monitoring equipment described in the text.

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ESTERASES AS INDICATORS OF AVIAN EXPOSURE TO INSECTICIDES

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ABSTRACT

Following a field application of the organophosphorus insecticide demeton-S-methyl a significant increase in the serum carboxylesterase activity of the house sparrow (Passer domesticus) was observed. At the same time a slight decrease in serum cholinesterase activity and a significant decrease in brain acetylcholinesterase activity occurred. This paper reviews the limitations of existing methods of assaying esterase activity and proposals are made for the development of sensitive and specific ELISA methods.

INTRODUCTION

"B" esterases, such as brain acetylcholinesterase, are inhibited by the "active" oxon forms of organophosphorus and by carbamate pesticides (Busby et al 1987, Ludke et al 1975).

To date, the use of avian serum "B" esterases as bioindicators in the monitoring of exposure to pesticides, has been limited to the measurement of their activities. However, there are indications that an increase in serum carboxylesterase activity, presumably due to enzyme release, occurs at low levels of exposure to pesticides (Bunyan and Taylor 1966) and this increase can be masked by inhibition of the enzyme as levels of exposure increase. It should, therefore, be possible to monitor exposure to low levels of pesticides by developing a method for the sensitive and specific assay of esterase protein. This would enable an accurate determination of the specific activity of the esterase.

This paper will discuss the development of this approach for assessing exposure of birds to organophosphorus and carbamate pesticides with particular reference to data collected during the Boxworth Project. (The Boxworth Project is a seven year field study by the Ministry of Agriculture, Fisheries and Food to evaluate the economic and ecological effects of different pesticide regimes in intensive winter wheat production (Hardy 1986)).

MATERIALS AND METHODS

Materials

All chemicals were AR grade unless otherwise stated and were purchased from Sigma Chemical Company Limited.

Sample Collection

House sparrows (Passer domesticus) were caught in mist nets at the edges of fields before and after application of demeton-S-methyl (S-2-ethylthioethyl O,O-dimethyl phosphorothioate). Blood samples were collected by puncture of the brachial vein of the birds and serum was separated by centrifugation and assayed immediately for cholinesterase activity. Carboxylesterase activity was assayed after storage at -20°C for up to three days. Previous experiments had shown that the activity of carboxylesterases inhibited by demeton-S-methyl was not affected by storage in this manner. Brain samples were taken from a limited number of birds, homogenised whole in 0.1% Triton X-100 in 25 mM tris-HCl (pH 7.6) and assayed immediately for acetylcholinesterase activity.

Esterase assays

Serum carboxylesterase activity towards α-naphthyl acetate was measured by the method of Gomori (1953) as adapted by Bunyan *et al* (1968).

Serum cholinesterase activity towards butyrylthiocholine was measured by the method of Ellman *et al* (1961) as adapted by Westlake *et al* (1980). Brain acetylcholinesterase activity toward acetylthiocholine was assayed according to the method of Ellman *et al* (1961).

RESULTS

The inhibition of esterases of house sparrows one to three days after the field application of demeton-S-methyl is shown in figures 1 and 2. Serum cholinesterase activity was depressed 24 hours after spraying, when compared to pre-spray controls, but returned to within pre-spray levels after 48 hours. When data from all three days post-spray were combined no significant inhibition was observed, therefore, it is necessary to take samples within the first 24 hours in order to show inhibition.

Brain acetylcholinesterase activity was significantly inhibited on all three days after spraying compared to pre-spray controls.

Serum carboxylesterase activity showed a significant increase in activity one and two days after spraying, compared to pre-spray levels, but returned to control levels by day 3.

The combined post-spray data in figures 1 and 2 gives considerably less information, which may lead to misinterpretation, when compared to data taken at 24 hour intervals over the three day period.

DISCUSSION

These results illustrate the limitations of existing non-destructive methods of monitoring exposure of birds to pesticides, which rely upon the inhibition of "B" esterases.

Brain acetylcholinesterase, the inhibition of which has been shown to have biological consequences such as reduced nestling care and decreased territorial display (Grue *et al* 1982), showed significant inhibition at all 3 sampling times. The inhibition was in excess of the 20% suggested by Ludke *et al* (1975) to provide a reliable indication of exposure to an anticholinesterase compound. However, this cannot provide the basis for a non-destructive sampling procedure. Serum cholinesterase showed slight inhibition (19%) at 24 hours and recovered to within control levels after 48 hours. This level of inhibition was insufficient in itself to give a reliable index of exposure. Laboratory data have shown that serum cholinesterase reaches maximum inhibition within 3 - 6 hours after exposure to organophosphates; for example, in mallard ducklings (*Anas platyrhynchos*) exposed to dicrotophos and fenthion (Fleming 1981) and starlings (*Sturnus vulgaris*) to demeton-S-methyl (Thompson unpublished data). Its rapid

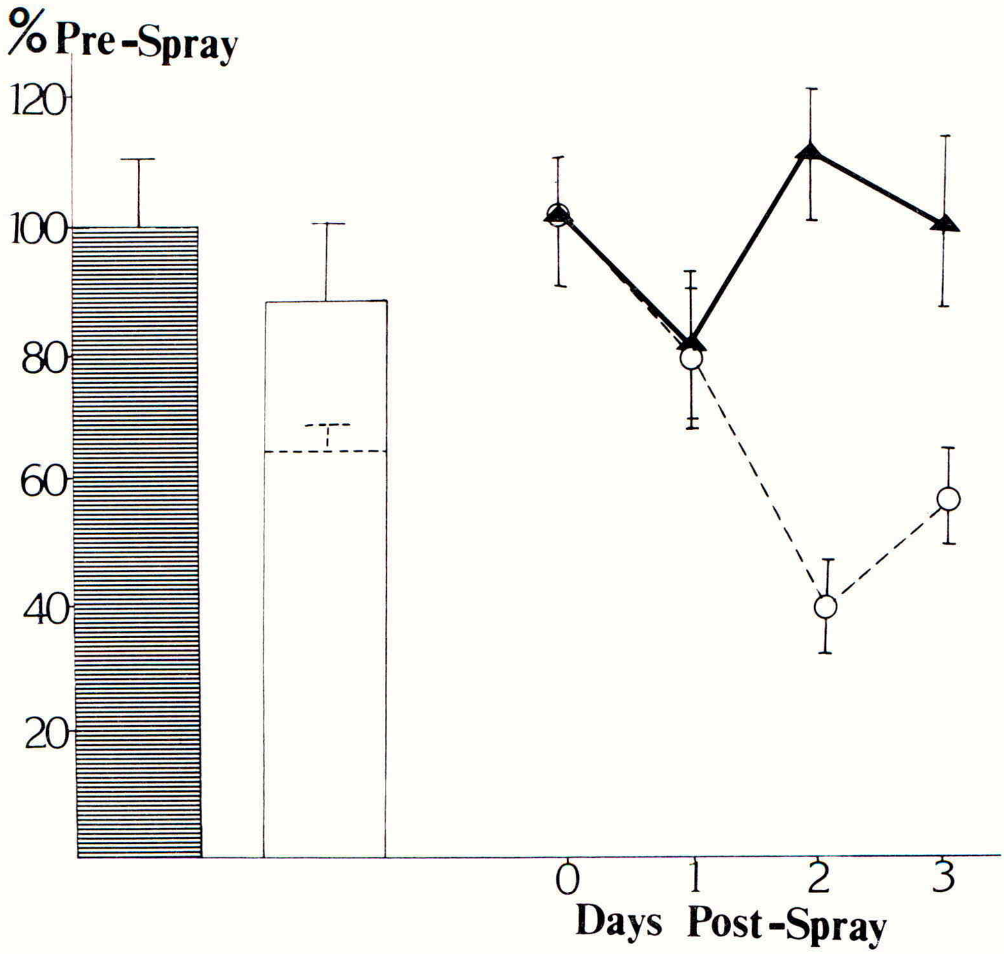

Figure 1 Inhibition of house sparrow brain acetylcholinesterase activity and serum cholinesterase activity after application of demeton-S-methyl. Histograms represent data for pre-spray (shaded n=8) and all post-spray (unshaded n=14) samples (AChE broken line $p<0.01$, ChE solid line). Points represent brain AChE (○) and serum ChE (▲) activity of samples pre-spray (day 0 n=8), one (n=6) (ChE $p<0.1$), two (n=5) (AChE $p<0.001$) and three (n=3) (AChE $p<0.01$) days post spray. All activities are expressed as a percentage of pre-spray levels (day 0 and unshaded area) (AChE=0.78±0.07 μmol/min/mg protein, ChE=1.88±0.18 μmol/min/ml).

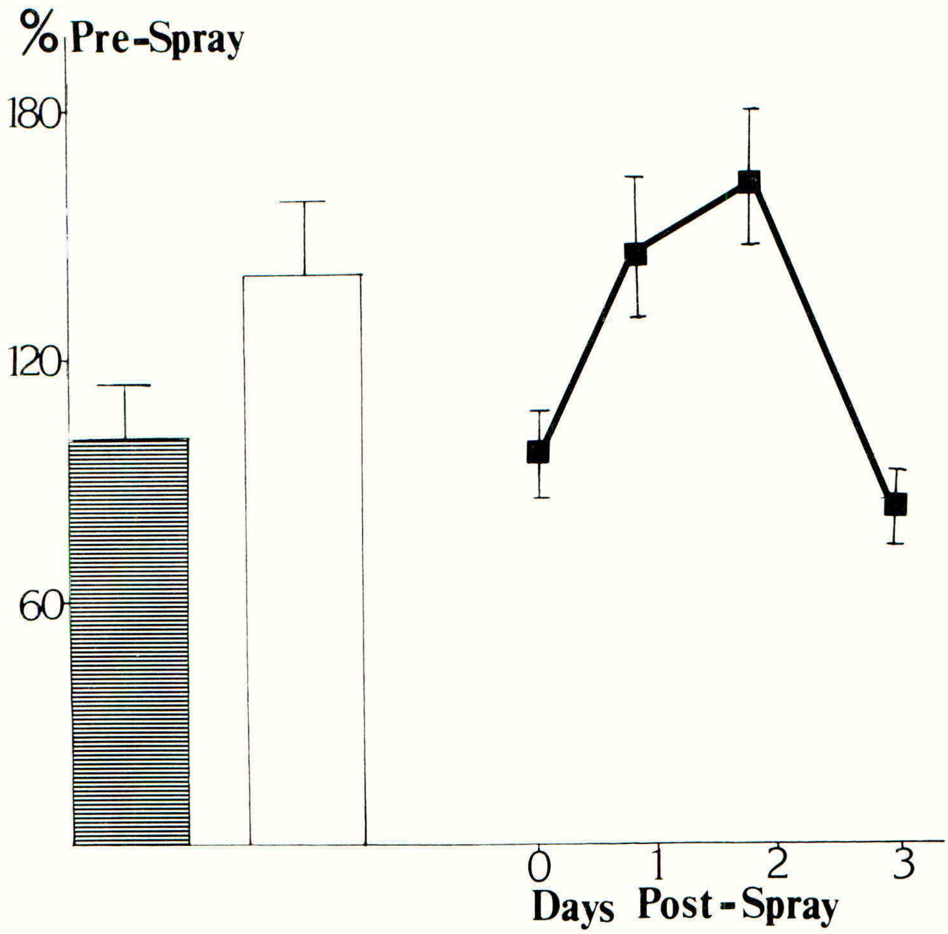

Figure 2 Effect of demeton-S-methyl application on house sparrow serum carboxylesterase activity. Activities are expressed as a percentage of pre-spray controls (n=8 0.82±0.12 μmol/min/ml) (day 0 and shaded area of histogram). Data are shown for all post-spray samples (unshaded area of histogram n=14) and points represent samples taken one (n=6) ($p<0.1$), two (n=5) ($p<0.05$) and three (n=3) days post-spray.

return to control levels suggests that recovery is due to enzyme release rather than reactivation (Fleming 1981, Thompson unpublished data). Such rapid recovery of activity to control levels, also shown by this study, limits the usefulness of this method for monitoring exposure to low levels of pesticides to within the first 24 hours post-spray.

The increase in carboxylesterase activity post-spray suggests that this enzyme could provide a useful additional approach to monitoring pesticide exposure even though it is less sensitive to inhibition than serum cholinesterase. It is interesting to note that the maximum effect on both brain acetylcholinesterase and serum carboxylesterase activity occurs between 24 and 48 hours after demeton-S-methyl application whereas maximal cholinesterase inhibition occurs within 24 hours.

The increase in carboxylesterase activity seen after demeton-S-methyl application in the field has also been observed in the laboratory. Starlings dosed with a single oral dose of 0.2 mg/kg (approximately 1% LD50) showed an increase in carboxylesterase activity, whereas dosing at the rate of 2.0 mg/kg (approximately 10% LD50) led to an inhibition of activity (Thompson unpublished data).

The increase in carboxylesterase activity suggests that release of the esterase occurs as a response to exposure to a xenobiotic. There is evidence for a similar increase in serum cholinesterase activity of 20% between days 1 and 2 post-spray which may also be attributable to a similar response. This may be a defence mechanism by which the bird responds to the challenge of a lipophilic foreign compound. These increases in activity can obscure the inhibition of the esterases which is proceeding at the same time. For example, if the quantity of enzyme present were doubled, but 50% of the activity were inhibited, the net result would be no change in total activity. Much could be gained if both the activity and the amount of enzyme present could be determined and hence the specific activity of the enzyme calculated. This would enable the detection of increases in esterase protein at low levels of exposure, increases that would be masked by inhibition if only enzyme activity was being measured. Such an approach should be possible by raising antibodies to a purified serum esterase and using these to establish the activity of the particular esterase, and the amount of protein associated with it.

In the case of the starling there is one major serum carboxylesterase which appears to be a suitable enzyme for further study (Martin et al 1983, Thompson et al 1988). Antibodies raised to the purified enzyme could be used to establish the activity of the antigenic esterase in a serum sample, by immunoprecipitation. In this method the antigenic esterase is removed by precipitation, using the antibody, and the remaining activity determined. Also in an enzyme - linked immunoabsorbant assay (ELISA), the amount of antigenic esterase present in a serum sample could be measured. Such ELISA techniques are powerful analytical tools and are typically used in the range of 1-100 ng antigenic protein present and an antibody dilution factor of 10,000 - 20,000 (Johnstone and Thorpe 1982).

Using these methods the activity of a specific esterase can be measured, rather than the activity of a group of esterases. Further, the amount of the protein associated with the activity can also be determined and the specific activity of an individual enzyme estimated. Thus an increase in enzymic protein occurring simultaneously with an inhibition of esterase activity would lead to a decrease in specific activity of the esterase being monitored. This method should also eliminate the problem of diurnal variation in carboxylesterase activity (Thompson et al 1988) since the increase observed is thought to be due to enzyme release rather than an increase in the activity of individual enzymes. Therefore, diurnal variation would bring no detectable change in specific activity.

This approach to monitoring exposure should reduce the problems of interpretation related to the use of serum "B" esterases and increase the sensitivity of the method, especially if antibodies can be raised to both carboxylesterases and cholinesterases. Thus, the greater sensitivity of the cholinesterases to inhibition could be used in conjunction with the less sensitive carboxylesterases leading to monitoring of exposure to a wide range of dosing levels, probably as low as 1% of the LD50. In this way it may be possible to relate changes in serum "B" esterase activity more closely to brain acetylcholinesterase inhibition.

ACKNOWLEDGEMENTS

The authors would like to thank M.R. Fletcher and K.A. Tarrant for their help in the collection of field data and Dr. M.I. Mackness for his helpful discussions.

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THE USE OF DIRECT OBSERVATIONS IN ASSESSING PESTICIDE HAZARDS TO BIRDS

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ABSTRACT

In field tests of the hazards to birds from pesticide use, emphasis is usually placed on searches for carcases. However, direct observation of birds' activities before and after applications is also a valuable tool to address three aspects : (1) providing evidence that the test site is representative of typical usage conditions; (2) predicting the likely exposure of species to the pesticide; (3) measuring actual exposure; and (4) assessing the effects of the application on the numbers and activity of local birds. Previous studies have relied on simple counts of the numbers of birds present and their frequency of occurrence. More detailed records can provide additional information on which to base stronger predictions and interpretations. This paper draws on data gathered in two field trials, illustrating the value of detailed, quantified observations in this type of study.

INTRODUCTION

Of the many forms of wildlife that are potentially at risk from pesticide applications, birds are among the most obvious, the strongest objects of public concern, and the most readily assessed. Consequently, they have figured heavily in programmes of hazard assessment in the field. However, emphasis is usually placed on the search for carcases, which can be examined for evidence to suggest whether or not pesticides were responsible for death. Carcase searching is fraught with bias and uncertainty (Balcomb 1986, Rosene & Lay 1963), and alternative methods are required to strengthen the interpretation of field trial results. Among the possibilities are capture-recapture studies, territory mapping (Edwards *et al.* 1979), monitoring of nest success (Spray, Crick & Hart 1987) and radio-tracking (Hegdal & Blaskiewicz 1984). Direct observations of birds' behaviour can also provide valuable information, and this paper examines how observations can be designed and interpreted to aid the assessment of hazards to birds. We argue that studies should be tailored specifically to the purpose of the observations, the nature of the pesticide application, and the bird species involved.

PURPOSES OF OBSERVATIONAL STUDIES

It is helpful to distinguish four objectives which can be addressed through observation :

(a) assessment of how typical is the test site as an example of conditions in which a pesticide is recommended for use - this involves the number and range of species present, their densities, and behaviour on the treated field;

(b) prediction of likely exposure to a pesticide - identifying the species most at risk, and estimating their likely contact with the chemical;

(c) measurement of actual exposure after application;

(d) assessment of changes in the size and activity of bird populations following an application.

Clearly, (a) and (b) require observations before treatment, whereas (c) and (d) are concerned chiefly with events afterwards. There is also scope for varying the approaches taken within observation sessions, focussing on three aspects - the numbers, frequency of occurrence, and behaviour of birds on the trial site.

DATA RECORDING

The customary approach, suitable for most pesticide studies on field crops, is to position one or more observers at suitable vantage points from which birds can be observed (by eye, binoculars, telescope or video camera), and information recorded on paper, tape-recorder, portable data-logger or other devices. The data record may contain a range of parameters (Table 1).

TABLE 1

Measures of bird activity obtained directly or indirectly during observational studies

S	Number of species
N	Number of identified individual birds of a species
N_{min}	Minimum numbers of individuals (= maximum seen simultaneously)
$\bar{n}$	Average number present on regular scans
P	Proportion of scans on which a species was present
T	Duration of bird's visit to site (average $\bar{T}$)
B_1)	
B_2)	Rates of performance of behaviour (eg. feeding B_{feed})
.)	
.)	
.)	
B_i)	
V	Total number of visits
h	Duration of observation sessions
Q	Percentage of sessions in which species was present

One of the simplest pieces of information is a species list accumulated over a standard time period, yielding S, the number of species seen. For each species, this can be expanded to distinguish age- and sex-classes, and to include numbers of individuals. If these are individually identifiable (by coloured rings, wing-tags, or plumage peculiarities) the measure N reflects the actual number of birds involved; otherwise the minimum number of birds, N_{min} can be estimated as the maximum number seen simultaneously. When applied to species in which family groups occupy distinct home ranges (eg. partridges; Green 1984) N_{min} may be a true measure of N, but in some cases the correspondence is likely to be poor.

It is also important to know how often birds visit a field, and for how long. In principle, all arrivals and departures might be timed, providing a full record of the frequency and duration of visits. However, the size and topography of the site, weather conditions, and the amount of activity among birds will often preclude this ideal, even when teams of observers share the work. It is generally preferable to schedule observations into a series of standard 'samples' of activity (Altmann 1974). Birds can be counted at regular times during a session (eg. every 5 minutes), the interval chosen to balance the advantages of a large number of counts against the need for statistical independence of successive counts, and the time required to make the assessment on each occasion. (This might involve systematic scanning from one side of a field to the other). The resulting scores yield a frequency distribution from which the average number of birds present ($\overline{n}$), the proportion of counts in which the species was seen (P) and other measures can be obtained.

The behaviour of birds can be examined further in the course of regular scans of a field, by scoring the numbers feeding, preening, displaying, etc. (see Inglis and Lazarus 1981). In addition, 'focal animal' watches (Altmann 1974) offer an opportunity for gathering detailed information on individuals, by concentrating attention onto a single bird, observed continuously for a predetermined period. Relevant activities such as pecking rate, rate of movement, number of interactions with others, etc. can be recorded (behaviours B_1, B_2.....B_i) and averaged for several individuals if desired.

All these aspects can be further refined by subdividing the data into different time periods (eg. first, second, third hours after dawn) or different parts of the study area. Also, organisation into standard observation schedules does not preclude opportunistic records of occasional behaviour (eg. vomiting, lack of co-ordination) that may provide valuable indications of direct toxic effects.

Data gathered within each observation session can be used in comparisons of sessions. This might include assessing consistency of behaviour from day to day, variations in bird numbers and activity in relation to weather conditions, or changes after a pesticide application.

DESIGN OF FIELD PROTOCOLS

It is rarely possible to record all the information listed above for all species. The choice of which parameters to measure depends on the purposes of the study (see above), on logistic constraints, and on prior judgements of the probable nature and scale of hazards, suggested by the toxicity and proposed use of the chemical.

Objective (a)

Almost any of the features listed in Table 1 could be used in assessing the representativeness of the chosen site, if similar observations were made elsewhere. More usually, however, the overall density and species-composition of birds at the site (eg. S, N, $\overline{n}$) can be compared to previous knowledge of the birds typically present in farmland habitats (eg. reviews of information from Common Bird Censuses and other long-term surveys (Williamson 1967; O'Connor & Shrubb 1986). It is thus important to schedule an adequate number of observation sessions before pesticide application to permit a judgement on the validity of extrapolation of results from the trial site.

Objective (b)

Predicting which species are most likely to be exposed, and estimating their probable contact with a pesticide requires data of several kinds. For an individual bird, the frequency of visits, their duration, the intensity of foraging activity, and selectivity of feeding are all important.

These features should be quantified in order to estimate the probability of the bird ingesting enough food to acquire a lethal or debilitating dose of pesticide; knowledge of LD_{50} values and bodyweights is also valuable.

The ease with which this information can be obtained varies among species (for example, it is much easier to count pecking rates for pheasants than for finches), and interspecies comparisons must rely on the highest common denominator. Ranking species on a 'risk index' allows selection of particularly vulnerable species, or suitable representative 'indicator' species for more detailed study. Bunyan *et al.* (1981) did this by calculating the geometric mean of the total numbers of birds seen and the total number of sightings of the species. It is difficult to assign biological significance to this measure, and alternative indices are preferable.

On the grounds that hazard is related to the total amount of pesticide taken up by birds of a species, overall exposure could be measured by the average number present at standard intervals ($\overline{n}$) multiplied by the proportion of time spent feeding (B_{feed}). However, this fails to distinguish between a large number of birds each making a few visits and a small number of residents making repeated visits, nor does it take account of the duration of visits. One alternative is

$$\frac{V}{h} \cdot \overline{T} \cdot B_{feed} \cdot \frac{1}{N_{min}}$$

In practice, even this level of detail is hard to obtain for all species, and an impression of relative risk may have to be derived from simpler measures such as

$$\frac{\overline{n}}{N_{min}}$$

Objective (c)

Once key species have been chosen (in advance of the study, or based on pre-treatment data) it is more straightforward to design specific observations to quantify behaviour. These will depend on the species, but should aim to provide a measure of the quantity of pesticide consumed by individual birds. A major difficulty is bias due to effects of poisoning. If birds avoid the area - owing to a learned aversion to the pesticide, or because of debilitating effects on behaviour - disproportionate observations may be made of birds foraging before they accumulate a heavy dose. If birds are individually recognisable, this can be identified and taken into account.

Objective (d)

Changes attributable to the effects of a pesticide can be sought at several levels, from the details of a bird's foraging behaviour (slower feeding rate, signs of distress or distaste) to changes in the numbers of birds visiting the field. Almost any of the parameters mentioned can be used to examine this issue. One important question is whether to base interpretation on comparisons with matched 'control' sites, or to regard each site as its own control, by making pre- and post-application comparisons. Neither alternative is totally satisfactory, since it is usually difficult to exclude the possibility of site-to-site differences, and the effects of unmeasured confounding variables (eg. changing weather conditions or local food supplies). Additional evidence may sometimes be gleaned from the detailed timing of changes in relation to application date, and the replication of changes on a number of treated sites. In comparisons of this kind, the likelihood of differences between observers in the reliability of recording detailed behavioural data must be borne in mind.

TWO CASE STUDIES

To illustrate the practical application of these considerations, we will draw on data gathered in two recent field trials. At site A, molluscicide pellets were twice broadcast in October on a newly-sown field of winter wheat, and birds were observed in a total of 12 sessions near dawn and 11 near dusk (total 67 hours), ranging from 6 days before to 10 days after the applications. At site B, birds were observed in April during 6 dawn and 7 dusk sessions (50 hours) after sowing of peas with an insecticidal seed treatment. In both cases, similar methods were employed: a single observer (MRF) remained at one vantage point and recorded in a notebook all arrivals and departures of birds from the field. together with notes on their location and behaviour. The resulting data have been analysed to provide indices of the kinds described above, to meet a variety of objectives.

TABLE 2

Summary of occurrence of the seven most frequent bird species in dawn counts during two field trials. Symbols are defined in Table 1.

	Site A			Site B		
	Q	N_{min}	V	Q	N_{min}	V
Woodpigeon *Columba palumbus*	75	30	151	100	32	188
Pheasant *Phasianus colchicus*	83	10	109	100	9	120
Red-legged partridge *Alectoris rufa*	17	6	8	100	8	54
Magpie *Pica pica*	17	3	4	100	2	26
Rook *Corvus frugilegus*	-	-	-	83	10	32
Blackbird *Turdus merula*	42	3	14	100	2	30
Yellowhammer *Emberiza citrinella*	-	-	-	67	3	19
Jay *Garrulus glandarius*	75	3	25	-	-	-
Meadow Pipit *Anthus pratensis*	50	16	80	-	-	-
Total no. of spp.		19			20	

Table 2 lists the seven species seen most frequently at each site, with several measures of activity in dawn watches. There is considerable similarity in species composition, in the total number of species recorded, and in the identity of those that occurred most often (pheasant and woodpigeon). These two species were selected for more detailed examination, together with others (rook and red-legged partridge) whose habits suggested that they might be at risk from eating treated seed at site B.

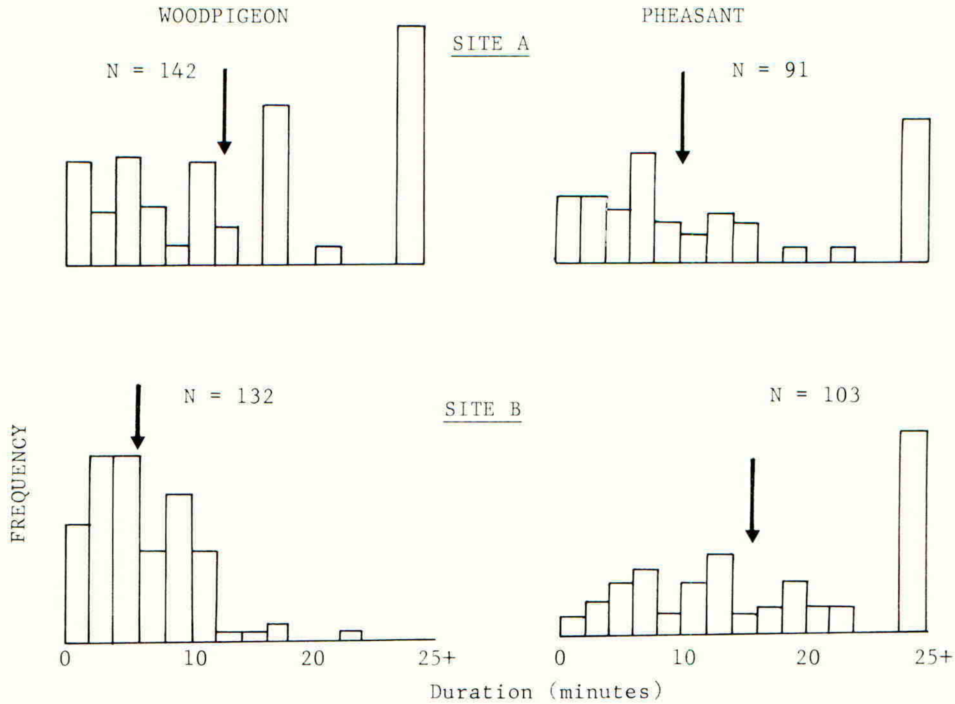

FIG.1 Durations of visits to treated field by woodpigeons and pheasants at both field trial sites. Arrows indicate median visits.

Behaviour differed between the sites; woodpigeons at site A remained much longer on the field than those at site B (Fig.1). This was partly due to disturbance; 23% of visits at site B were ended by birds flying off as people or vehicles passed along a nearby road, whereas only 2% ended in this way at site A. From Fig.1, the median duration of a visit can be used as a measure of T, although there was clearly wide variation, indicating the need to employ a sensitivity analysis in calculations of risk indices.

Different species at the same site are compared in Table 3, based on the amount of time spent on the field, estimated for an 'average bird' (indices X_2 and X_5) and for all birds of the species combined (indices X_1 and X_4). Because of the uncertainty in estimates of T and N_{min}, numerical differences in these measures cannot be interpreted in detail. However it is evident that the longer visits by red-legged partridges more than outweighed the higher visiting rate and greater numbers of woodpigeons, resulting in a five times greater period of potential exposure for each bird.

TABLE 3

Indices of exposure of four bird species at site B, during dawn watches. Symbols are defined in Table 1.

	Woodpigeon	Pheasant	Red-legged Partridge	Rook
X_1 Total visits per hour (V/h)	7.37	4.71	2.12	1.26
X_2 Visits per bird per hour ($V/h.N_{min}$)	0.23	0.52	0.26	0.13
X_3 Median visit duration, mins ($\overline{T}$)	6	16	33	4.5
X_4 Total exposure ($V.\overline{T}/h$)	44.24	75.29	69.88	5.65
X_5 Total exposure per bird ($V.\overline{T}/h.N_{min}$)	1.38	8.37	8.74	0.56

Further analyses revealed that at site A, exposure was similar at dawn and dusk for woodpigeons (X_5 = 1.95 and 1.94) and pheasants (2.71 and 2.86); at site B, however, exposure was much lower in evening sessions (woodpigeon, 1.84 and 0.90; pheasant, 8.37 and 3.06). This and other differences between the studies are attributable to seasonal changes in activity (eg. winter flocks of woodpigeons giving way to a more dispersed breeding population), and to local features such as the proximity of roost sites.

In these studies, no detailed measures of feeding behaviour were recorded, so that it is not possible to extend the analysis of exposure further. To do so would require data on pecking and pacing rates (Holyoak 1974; Murton 1971), to represent the rate of contact with treated seeds or pellets. At this level, species differences in food selection behaviour may override the relative periods for which species are at risk.

These examples illustrate how a number of simple measures of observed behaviour can be combined in a logical way that indicates birds' levels of exposure. It is not necessary to obtain a comprehensive record - appropriate periodic sampling of behaviour can provide equally useful information. For example n, the average number of birds present at 5-min intervals, was clearly correlated with a more complex measure of exposure in these studies (Fig.2). Generally, it is advisable to programme a series of more detailed behavioural records inbetween regular scans of the numbers of birds present.

CONCLUSION

We have tried to outline the potential and the limitations of direct observation as a tool in the assessment of pesticide hazards to birds. Previous studies have largely used casual observation to record symptoms of poisoning (eg. Balcomb *et al.* 1984), but we suggest that a carefully structured programme of standard counts and behavioural scores can provide valuable additional information. The broad range of bird species and contexts in which studies are carried out suggest that there is no single suitable general protocol. Rather, it is necessary to design fieldwork procedures for particular cases, with the aid of expert knowledge of bird behaviour and experience of pesticide poisoning effects.

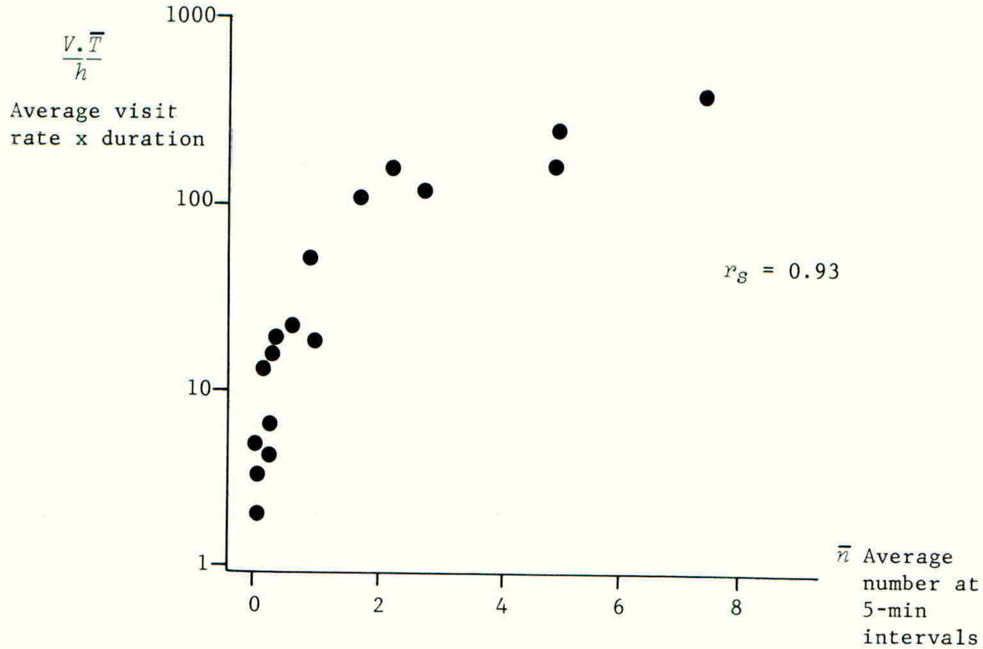

FIG.2 Correlation between two measures of the potential exposure of wood pigeons at site B. Each point represents an independent observation session.

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AN APPRAISAL OF METHODS USED TO ASSESS THE EFFECT ON BIRDS AND MAMMALS OF CHLORPYRIFOS APPLIED TO GRASSLAND

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ABSTRACT

This paper assesses critically two experiments done to determine the impact on birds and mammals of an insecticide applied to grassland. Two experiments are outlined in which chlorpyrifos was applied for leatherjacket control and its effects on the resident fauna were assessed by making observations for 2 h at dawn and dusk on the birds and mammals visiting the field. No untoward effects were found. However, key points to emerge from a consideration of the procedures used were: the care and thoroughness required in searches for dead birds and animals; the desirability of long-term studies; the benefits of using observers with a good local knowledge; the need to do analyses for pesticide residues in samples of the materials consumed by birds and mammals in treated fields; and the desirability of capturing animals which have fed in treated fields to assess residue levels in their tissues.

INTRODUCTION

Permanent grassland, which covers some 5 million ha of the UK together with the hedgerows, ditches and woodland that are often associated with it provides a habitat for a wide and diverse spectrum of birds and mammals. Many of them are dependent on grass for their existence, feeding either directly on the crop e.g. rabbits (*Oryctolagus cuniculus*) and hares (*Lepus capensis*) or on the insects that inhabit it blackbirds (*Turdus merula*), starlings (*Sturnus vulgaris*). Destruction of this habitat by removing woodland or hedgerows, reseeding with a ryegrass monoculture, or simply intensive grassland management all have a major effect on the suitability of the crop for its fauna. For various reasons there may also be an increase in the use of insecticides in grassland. The present work set out to test a method for assessing the impact of an insecticide, chlorpyrifos (Dursban 4), on the birds and mammals in two permanent pastures.

Treatment with chlorpyrifos is a very effective means of controlling leatherjackets (Tipula spp). The insect larvae come into contact with the chemical when they move to or near the soil surface (usually at night). Many of the larvae die on the soil surface where they are easily accessible to the birds that feed on them. Consequently, as well as making direct observations on the birds and mammals that visited the treated fields, to assess the quantity of chlorpyrifos likely to be consumed by them, samples of leatherjackets were taken at intervals and analysed for residues of chlorpyrifos.

MATERIALS AND METHODS

A permanent pasture in Devon and one in South Yorkshire, each with a substantial leatherjacket population that warranted treatment with insecticide, and known to have a diverse and abundant bird and mammal population were selected. The field in Devon (at North Wyke, Near Okehampton) was a re-seeded permanent pasture of 3.5 ha, surrounded on three sides by tall hedges. A woodland bordered the fourth side. The field in South Yorkshire (at Bentley, Near Doncaster) was a permanent pasture of 2.2 ha.

Chlorpyrifos (Dursban 4) was sprayed on to each field for leatherjacket control at the recommended rate, of 0.72 kg ai/ha in 275 l water/ha (Devon site) or 400 l water/ha (South Yorkshire site) in mid April 1985. Observations were made for two hours or more at dawn and dusk 6 and 4 days before spraying and 1, 3, 7, 10 and 14 days after spraying for the field in Devon and 1 and 2 days before spraying and 1, 2, 3, 7, 10 and 14 days after spraying for the field in South Yorkshire. In each field, the observers were stationed in a hide where they had a clear view of the whole area through binoculars. The number and where feasible sex of each species of bird and mammal present was recorded, as was the length of time they spent in the field.

Thorough searches were made by one person for cadavers or ailing animals. Searching lasted for at least one hour, in each field and the surrounding hedges and ditches on the same days that wildlife observations were made.

Samples of leatherjackets of 10 g or more were collected from each of three sampling sites in the treated fields 2h before treatment and 1h, 1, 2, 7, 10 and 14, days after treatment. Three samples of leatherjackets of 10g or more were also obtained from a nearby untreated field 7 and 10 days after the date of spraying the treated field. The samples were kept in a freezer at -25°C or colder until residue analyses were done, using standard techniques. (A 2.5 g sample of leatherjacket material was homogenised in a 2:1 acetone:hexane solution in a centrifuge tube. A small volume of water was added and the tube was centrifuged after shaking, and then re-extracted with hexane. The extracts were partitioned with acetonitrile and an extract in hexane evaporated to an oily residue with one drop of liquid paraffin in a stream of nitrogen. The extract was then purified in a Florisil column. Pure extracts were analysed by gas-liquid chomatography using electron capture detection).

RESULTS

Detailed results are given in Clements & Bale (1988).

In Devon the most frequently occurring birds were carrion crow (Corvus corone corone), pheasant (Phasianus colchicus), magpie (Pica pica) and blackbird. Their numbers, remained more or less constant throughout the study period (Table 1). One, two or three rabbits were also seen on every occasion except one.

TABLE 1.

Species and numbers of birds and mammals observed to feed at dusk in the treated field in Devon at intervals of 6 and 4 days before and 1, 2, 3, 7, 10 and 14 days after spraying with chlorpyrifos.

	Days before or after treatment							
Species	-6	-4	+1	+2	+3	+7	+10	+14
Carrion crow (Corvus corone corone)	-	4	2	4	4	6	6	6
Pheasant (Phasianus colchicus)	3	7	4	5	6	5	5	5
Magpie (Pica pica)	1	2	1	2	1	2	2	2
Blackbird (Turdus merula)	1	1	-	1	2	1	1	2

The community of birds and mammals using the field in the pre-spraying period continued to forage for food throughout the post spraying period. Three pairs of carrion crows had nests and were incubating eggs. A pair of magpies was nest building during the period of the observations. One pair of mistle thrushes (Turdus viscivorus) was collecting food for young and a second pair began to feed newly-hatched young at a nearby site. A cock pheasant with five hens and an unattached male used the area to feed throughout. A pair of buzzards (Buteo buteo) fed on dead and dying leatherjackets lying on the soil surface. Rabbits continued to graze at the field edge, as did a pair of roe deer (Capreolus capreolus).

At the site in South Yorkshire 14 species of birds and mammals were resident in and around the treated area. Those occurring most commonly were magpie, carrion crow, moorhen (Gallinula chloropus), mallard (Anas platyrhyncos), starling and fieldfare (Terdus pilaris).

The detailed observations made at these sites indicate that birds of some species, notably magpies, crows and moorhens, frequently left the field during a dawn or dusk period, then individuals of the same species were observed to 'return'. In view of the territorial behaviour of some of these species, it is probable that it was the same birds returning to feed on several occasions.

Estimates of chlorpyrifos residues found at both sites are given in Table 2.

TABLE 2

Chlorpyrifos residues in leatherjacket samples recovered from two pastures at 1 h - 14 d intervals after treatment

Interval after treatment	Site Devon (mean ppm ± s.e)	Yorkshire (mean ppm ± s.e)
1 hour	0.07 ± 0.065	0.84 ± 0.152
1 day	0.62 ± 0.057	0.94 ± 0.142
3 days	0.99 ± 0.088	0.99 ± 0.089
7 days	1.09 ± 0.068	0.92 ± 0.198
10 days	1.17 ± 0.139	1.02 ± 0.114
14 days	0.64 ± 0.125	0.79 ± 0.056
Untreated	0.02 ± 0.002	0.02 ± 0.003

No dead birds or mammals were found at either site nor was any abnormal behaviour of the resident wildlife noted. Subsequent to the conclusion of the present study further casual searches and observations were made for a period of 6 months, but no dead bird or other vertebrate animals have been found.

DISCUSSION AND CONCLUSIONS

The major aim of the experiment was achieved in that careful and detailed observations of the birds and animals visiting treated fields were made. No dead or dying specimens were found. However, the method used highlighted the need for particular care with some aspects of the procedures used and in interpreting some of the data produced. Five areas identified for special comment are:

1) Searches for dead birds and animals

Considerable care is required to find cadavers in grassland, as they are easily concealed. More to the point, because of the difficulty in collecting 'negative' data, is the need to search even more thoroughly if none are found initially. It is essential to make it clear how thorough any searches were, by stipulating the length of time taken. Searching on six occasions for a period of 1h seemed adequate for fields of 2-3.5 ha. If larger fields are involved it would probably be necessary to use a team of searchers rather than one individual.

There is probably a need to make searches on several occasions as cadavers would probably be quickly removed by various carrion feeders. Consequently making just one, albeit thorough search, at the end of an experiment such as the present one, would not have been adequate.

It is conceivable, even likely, that had any birds or animals been poisoned, they would have died elsewhere and their cadavers not have been found in the searches. However, the regular feeding and frequent return to the area of territorial species suggests that they at least were unaffected. There may be little point in studying or recording non-territorial species unless cadavers are found since it is difficult to know whether the same individuals are being observed on separate occasions.

2) Long term studies
It is desirable in studies of this nature to make repeated observations subsequent to an initial study, as with the admittedly casual observations made in the present work. Otherwise the effects of birds and mammals feeding persistently in a treated area will be underestimated. However, in the present work the contaminated food i.e. leatherjackets killed by chlorpyrifos, had rotted-away within 2-3 weeks and this hazard was thus extinguished.

3) Observers with good local knowledge
Clearly, using competent observers is pivotal to studies of this nature, so that they can identify birds and mammals correctly and be aware of their normal behaviour patterns so that deviations from it will be noted. Probably, and as in this case, an observer with detailed local knowledge is likely to recognise individual birds (or mammals) and to know of their nest sites and to be able to visit them too. This makes for a far more complete study.

4) Residue analyses of material consumed
Determining pesticide residue levels in material consumed by birds and mammals visiting a treated area allows calculations to be made, based on data obtained in laboratory toxicity tests, of the likelihood of effects occurring. For example, in the present study, the toxicity of chlorpyrifos to most vertebrates is known to be very low; the acute oral LD50 value for rabbits is 1000-2000 mg/kg liveweight and for chickens the relevant figure is 32 mg/kg (Worthing & Walker 1983). Of the birds observed in the present study, a starling for example, typically weighs 80g (Brough 1983) and would eat around 9g of invertebrates per day (Feare 1984). Consequently, even if it fed on leatherjackets alone it would ingest probably only 9 x 1.17 ppm or 0.0105 mg of chlorpyrifos per 80g of bodyweight at the most, or about 1/240 of the LD50 value for chickens.

Cognisance was taken of the cautions listed above in the present work and no untoward effect of chlorpyrifos on birds or mammals found.

5. Assess residues in feeding birds and mammals
If any cadavers had been found, residue analyses would have been done on various tissues as a matter of course. Since none died it may have been desirable to have captured feeding birds and animals for analysis. However, it is difficult to capture them or do some of the possible analyses without killing them. This is hardly likely to be a move that is welcomed by those most concerned with protecting wildlife.

ACKNOWLEDGEMENTS

The Institute for Grassland and Animal Production is funded through the Agricultural and Food Research Council. The above work was funded by The Dow Chemical Company and the residue analayses carried out by the Company at their Company Letcombe Laboratories. Dursban is a trade mark of The Dow Chemical Company. We gratefully acknowledge the cooperation of the farmers involved and the field assistance of Mr P Singleton and Mr K C Tyson.

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DISCUSSION

P. Greig-Smith, ADAS Tolworth Laboratory: Territorial birds may die and are likely to be replaced. I wonder how sure one is that the birds present at the end are the same as those at the beginning.

R. Clements , Inst. Grassland and Animal Production, Hurley: We can't be sure, but it does seem likely that they will be the same. The birds were territorial and our local observers had detailed knowledge of the nest sites.

A. Hardy, ADAS Worplesdon Laboratory: That is a very good point. ADAS has published, jointly with industry, work which has identified territorial species. With these species we can measure a population difference if birds are removed. This contrasts with species in which a non-breeding surplus in the population automatically fills up the territories of birds which disappear.

P. Mineau, Canadian Wildlife Service: I would point out that, from the model that we have developed, if you have anything like the normal scavenging pressure on the sites it is not realistic to think that you will still find corpses fourteen days after pesticide application. Also, at both sites you seem to have peak residue levels 10 days after application. This seems surprisingly late.

R. Clements: I have no explanation for that delay and am equally surprised by it. However, by 14 days after spraying, though we were still able to pick up a few leatherjackets from the soil surface for residue analysis, I would suspect that very little of that material would be consumed by birds.

A. Burn, Cambridge University: I suggest that the main threat to these species is the loss of their food supply, rather than any direct or indirect exposure to the pesticide.

R. Clements: There may be a risk there, but I would question what happens in those years when leatherjackets are not present for other reasons, - that is, as part of the natural cycle. Also, I would stress that it is unlikely that all of those areas which would be cost effective to spray for leatherjacket control will be treated.

A. Cooke, Nature Conservancy Council: The real problem could come when material like chlorpyrifos is used in the wild countryside, perhaps on moorland areas where no insecticides are used at the moment. In that situation it would presumably have a devastating effect on the invertebrate fauna.

R. Clements: I could not imagine anyone justifying the use of chlorpyrifos on moorland, which has a very low leatherjacket population. Chlorpyrifos is only likely to be used on pastures which are managed intensively.

A. Cooke: I am not talking about the control of leatherjackets on moorland. However, for other invertebrates, there are trials which have been undertaken in areas that have certainly have had no previous insecticide applications.

A. Hardy: How far have the speakers addressed the problem of long-term implications as opposed to short-term assessment?

M. Greaves, Inst. Arable Crops Research, Long Ashton: As the Symposium Organiser I felt obliged to ask that question myself, perhaps for slightly different reasons. I am puzzled to know what is long-term? How do you define it? Do you define it for each species, for groups or do you make generalisations about whether long-term means for one season or more?

A. Hardy: I think that really is the crux of the matter. Do you define it in relation to the biology of the species likely to be exposed around the site or do you define it in terms of the biological life of the chemical itself and, therefore, define your parameters and system around that?

H. Crick, Aberdeen University: Surely one would have to try to integrate the results into population dynamics models to assess the long-term effects. Once we have got effects on reproductive rates or on fatalities we can see, or hopefully predict, what the effects on long-term populations will be.

A. Hardy: We do have the advantage of a number of extremely good databases in the UK. For example, all the monitoring work done through the British Trust for Ornithology has allowed prediction and assessment of effects on populations of some birds over a period of years. Does this information exist for other groups?

P. Mineau: The problem we have encountered is that, even if information is available, it is for very common species that are well studied with good life history tables. These are generally not the species which one should be concerned with. We have some recent experience in looking at endangered species.

G. Tucker, British Trust for Ornithology: For many years the Common Bird Census has been monitoring breeding population trends. There is a possibility that many of our wintering birds might be affected greatly by the effects of pesticides on their food supply. We have no information regarding recent or long-term population changes for these birds.

H. Crick: Could I ask P. Mineau whether he believes that, in the vast area of Ontario, a few thousand dead birds really matter in terms of population stability?

P. Mineau: Firstly, it wasn't a few thousand. The lower estimate was about four hundred thousand and the higher about a million. We are now trying to assess the significance of this with the aid of Breeding Bird Surveys using a transect count method. This has been going on for many years in North America. The method is not very specific to habitat but, nevertheless, it does tend to show that most species of ground sparrows in that part of Canada are decreasing.