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THE ROLE OF POND STUDIES IN ASSESSING THE HAZARD OF TOXIC CHEMICALS TO FRESHWATER ECOSYSTEMS

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Summary Pond studies provide a validation of predictions of fate and effects of chemicals in freshwater and a means of observing toxic effects on a much wider range of organisms than is possible in simple, laboratory systems. It is also possible to study indirect effects of pesticide treatments and the effects of dispersion on toxicity under realistic field conditions.

Replication of pond systems is necessary to estimate the magnitude of particular biological effects and to study indirect effects such as predator/prey interactions and algal blooms. These points are illustrated by reference to studies with chlorpyrifos carried out by Hurlbert *et al*.

On the other hand it is not necessary to replicate treatments to obtain valuable qualitative information on fate and effects of new compounds. This is illustrated by reference to a pond study carried out with cypermethrin.

INTRODUCTION

During the development stages of a new pesticide a great deal of information is obtained concerning its chemical and physical properties, its rate of biodegradation and its toxicity towards aquatic organisms such as fish and *Daphnia*. If the pesticide is highly toxic to aquatic organisms, if it is not easily biodegraded or if it is being developed for control of aquatic plants or insects a second tier of laboratory studies might be carried out. These may include acute and chronic toxicity studies using a wider range of aquatic organisms, studies of possible sub-lethal effects on growth, reproduction and behaviour and studies of bioaccumulation in single organisms or in model ecosystems.

However extensive the laboratory data, they cannot be used to predict toxicity to all of the many organisms that occur in natural environments and they cannot be used to predict the occurrence or magnitude of secondary effects such as deoxygenation following the use of aquatic herbicides or algal blooms following the use of insecticides. In order to overcome this problem several groups of workers, including ourselves, have carried out experimental work in ponds to investigate the fate and effects of pesticides under more realistic conditions than can be achieved in laboratory systems.

The organochlorine insecticides have been studied in various kinds of freshwater systems but most of these field investigations have lacked controls or adequate replication. On the other hand several groups of workers have carried out replicated pond studies with chlorpyrifos, an organophosphorus insecticide and we have some data for a synthetic pyrethroid, cypermethrin, obtained in an unreplicated pond study. These two sets of data will be used to illustrate the advantages and limitations of replicated and unreplicated pond studies.

Replicated pond studies with chlorpyrifos

Hurlbert et al (1970) carried out detailed studies of the distribution, fate and biological effects of the insecticide chlorpyrifos (0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate) in experimental ponds near Bakersfield, California. The ponds consisted of shallow, unlined excavations measuring 8 m x 17 m and were maintained at a depth of about 24 cm. Insecticide was applied four times at intervals of two weeks to the surfaces of the ponds at dosages of 0, 0.011, 0.056, 0.11 and 1.12 kg ha^{-1}. Residues of chlorpyrifos in water, mud and vegetation were determined for the ponds treated with 0.056 kg ha^{-1} (a practical rate used for mosquito and midge control) and 1.12 kg ha^{-1}. The initial (4 h after treatment) residue of chlorpyrifos in the pond water treated at the higher rate was 0.22 mg l^{-1} (cf a nominal concentration of 0.288 mg l^{-1}) but approximately half of this material was associated with a small amount of suspended particulate matter in the water column. After 24 h the residue in the water had declined to 0.10 mg l^{-1} and after 7 days had declined to the limit of detection of the analytical technique. Residues in the bottom sediment were negligible 4 h after the insecticide treatment but gradually increased to attain a maximum of 0.52 mg kg^{-1} 7 days after treatment. Residues in aquatic vegetation were initially much higher than in other compartments of the system but declined rapidly (26 mg kg^{-1} at 4 h, 1.1 mg kg^{-1} at 7 days). Caged mosquito fish, *Gambusia affinis*, exposed to the 0.056 kg ha^{-1} treatment had residues of 2.8 mg kg^{-1} at 4 h, 1.7 mg kg^{-1} at 24 h and 0.1 mg kg^{-1} at two weeks after treatment. At all except the highest treatment rate chlorpyrifos caused only slight (<10%) mortality of mosquito fish and did not appear to inhibit their reproduction.

Corixids constituted the major portion of the aquatic insect fauna, sampled with the aid of a nekton tow net. Populations were drastically reduced in ponds receiving 0.056 kg ha^{-1} or higher rates. Population recovery was gradual in all ponds except those treated at 1.12 kg ha^{-1}.

The cladoceran, *Moina micrura*, was particularly susceptible to chlorpyrifos and was virtually eliminated from all ponds receiving chlorpyrifos treatments, even at low dosages. The cyclopoid copepod, *Cyclops vernalis*, was less susceptible than the cladoceran, being eliminated by treatments of 0.056 kg ha^{-1} or higher rates. On the other hand populations of the calanoid copepod, *Diaptomus pallidus*, and the relatively large, predaceous rotifer, *Asplanchna brightwelli*, increased rapidly in those ponds where populations of *M. micrura* and *C. vernalis* were reduced by chlorpyrifos treatments.

In a second series of experiments Hurlbert et al (1972) carried out more detailed studies to investigate the reasons for the observed increases in *Diaptomus* and *Asplancha* populations. Chlorpyrifos was applied three times, at intervals of approximately two weeks, to eight shallow ponds. Four were treated at 0.028 kg ha^{-1} and four at 0.28 kg ha^{-1}, while four were kept as controls. Aquatic insects, planktonic crustaceans, phytoplankton, small herbivorous rotifers and *Asplancha* populations were sampled and enumerated. As a result of these more detailed studies Hurlbert et al attributed increases in *Asplancha* populations to an increase in their food supply (small herbivorous rotifers) and a decrease in populations of their predators (aquatic insects). Increases in *Diaptomus* populations were attributed to decreases in populations of their major predator, *C. vernalis*, which was much more susceptible to chlorpyrifos than was *Diaptomus*.

It was also noted that the reduction of herbivorous planktonic crustaceans by chlorpyrifos and the restraint of herbivorous rotifers by Asplancha predation permitted the rapid increase of phytoplankton populations in treated ponds. The key role of predatory insects and other top predators, e.g. mosquito fish, in controlling all populations at lower tropic levels was discussed.

Macek et al (1972) studied the effects of chlorpyrifos applied at rates of 0.011 and 0.056 kg ha^{-1} to 0.04 ha fish ponds containing bluegills (Lepomis macrochirus) and largemough bass (Micropterus salmoides). Each of the rectangular ponds had a mean depth of 0.25 m at the shallow end and 2.0 m at the deep end. Each was stocked with 275 bluegills and 275 bass. Residues of chlorpyrifos in pond water one day after treatment ranged from 0.97 μg l^{-1} in a pond treated at 0.011 kg ha^{-1} to 2.4 μg l^{-1} in a pond treated at 0.056 kg ha^{-1}. Cumulative mortality of fish in ponds treated with 0.011 kg ha^{-1} was 3% for bluegills and 10% for bass. In ponds treated at 0.056 kg ha^{-1} cumulative mortality was 55% for bluegills and 46% for bass. These results were not significantly different from predictions based on laboratory toxicity data (96 h LC_{50} for bluegills: 3.6 (1.6-4.1) μg l^{-1}). Aquatic insects colonising plate samplers were represented by the larvae of midges (Chironomidae), mayflies (Ephemeridae, Heptageniidae, Baetidae) and caddisflies (Leptoceridae and Hydroptilidae). Treatment of ponds at 0.056 kg ha^{-1} eliminated the larvae of caddisflies and had a marked effect on numbers of the larvae of chironomids and mayflies. Treatments at 0.011 kg ha^{-1} also had a pronounced effect on caddisfly larvae but did not affect the numbers of chironomid or mayfly larvae.

Butcher et al (1975, 1977) have investigated the secondary effects of chlorpyrifos in promoting algal blooms. For this purpose they used artificial ponds 2.5 x 1.8 x 0.6 m in depth lined with polyethylene. Photosynthetic productivity was estimated by following changes in total carbon dioxide. These workers supported the idea that release of grazing pressure by zooplankton is a primary underlying cause of algal blooms following insecticide treatments.

An unreplicated pond study with cypermethrin

During 1977 the dispersion and toxic effects of cypermethrin [S,R]-α-cyano-3-phenoxybenzyl (IR,IS,cis,tr)-2,2-dimethyl-3-(2,2 dichlorovinyl) cyclopropane carboxylate were studied in a pond situated at Headcorn, Kent. A mature, rectangular pond 20 m long, 5 m wide and 0.7 m deep was divided into two similar ponds each 10 m long, by constructing a concrete wall across it. About two months later, i.e. on 1st June, 1977, one of the ponds was treated with a 40% EC formulation of cypermethrin at a dosage of 100 g a.i. ha^{-1}, i.e. somewhat in excess of dosages recommended for most agricultural purposes, by spraying over the surface of the pond. Treatment was carried out with a handheld, 2 m boom fed from a knapsack sprayer. The second pond served as a control. Samples of surface water, sub-surface water, pond sediment, aquatic vegetation and fish were removed at various times from 0-16 weeks after treatment for residue analysis. Using the data obtained from these analyses plus estimates of amounts of free surface water, surface vegetation, subsurface water, sediment and fish we have calculated the mass of cypermethrin in various compartments of the ecosystem (Table 1).

These calculations indicate that one hour after application the total mass of cypermethrin in all compartments was equivalent to only 30-50% of the applied insecticide. It is possible that much of the cypermethrin not accounted for was present in 'pockets' containing high surface concentrations that were missed by the sampling procedure. Aquatic vegetation beneath the surface of the pond was not sampled and it is possible that a considerable amount of cypermethrin was present in the submerged vegetation.

Twelve days before treatment each of the ponds was stocked with 75 small rudd, *Scardinius erythrophthalmus*, mean weight 27 g. There were no deaths among these fish after treatment even though concentrations of cypermethrin in pond water were considerably higher than concentrations that were toxic to rudd in laboratory tests (96 h LC_{50} for rudd = 0.4-0.5 $\mu g\ l^{-1}$). However, it is likely that much of the insecticide present in the water column was bound onto small amounts of suspended matter in the pond water and therefore could not be absorbed via the gill membranes. Residues of cypermethrin in fish were initially in the range 41-65 $\mu g\ kg^{-1}$ and there was a gradual decline to <10 $\mu g\ kg^{-1}$ from 2-16 weeks after treatment.

The cypermethrin treatment had a marked effect on zooplankton populations. In the treated pond there was a very high mortality of both daphnids and copepods during the week after treatment. There was no indication of repopulation until eight weeks later but thereafter the number of zooplankton increased exponentially and at 15 weeks after treatment the number present far exceeded the number in the untreated pond. Sweep net samples of macroinvertebrates showed that there was also a marked effect on all families of aquatic insects although other groups of invertebrates, e.g. molluscs and turbellarians, were apparently not affected. Data on repopulation by aquatic invertebrates were rather limited but there was evidence of repopulation by most of the major taxonomic groups from 11-15 weeks after treatment.

In this study 42 different taxa, i.e. species, genus or family, were identified belonging to 34 different families of invertebrates. The numbers of individuals in each invertebrate family were used to calculate evenness and diversity indices for the macroinvertebrate communities in the experimental ponds (Figure 1). The main effect of the insecticide treatment was on the species richness component of diversity which was severely depressed during the two weeks immediately after treatment of the pond. The evenness component of diversity was affected to a lesser degree and recovered more quickly than species richness. The values for diversity, $\overline{H}$, reflect the sum total of interactions between species richness and evenness.

There was an increase in the amount of filamentous algae in the treated pond a few weeks after treatment, an effect probably caused by elimination of planktonic crustacea and larvae of the mayfly, *Cloeon dipterum.*

DISCUSSION

Hurlbert (1975) has pointed out that replication of ponds is essential if the objective of the study is to obtain new basic information concerning pesticide-induced stress on ecosystems. Variability between water quality and communities of animals and plants in different ponds is likely to be considerable. Without adequate system replication it may be possible to attribute only gross biological effects to an applied stress and impossible to estimate the magnitude of observed effects. Effects of a minor nature may not be detected at all.

Nevertheless, replication of treatments may not be the best strategy, especially if little is known about the dispersion characteristics and biological effects of the chemical in freshwater. If laboratory studies indicate that a new pesticide is toxic to aquatic organisms it may be desirable first to evaluate its fate and effects in an unreplicated pond study. Samples should be taken from a wide variety of different components of the ecosystem, e.g. zooplankton, fish and macroinvertebrates. This approach is likely to detect any important biological effects, whether directly or indirectly attributable to pesticide treatment. If there is any doubt that a given effect is attributable to the treatment, the problem may be investigated in the laboratory or, if this is not possible, through replicated pond studies.

The effort involved in collecting data for the many different organisms, or groups of organisms, present in freshwater can be disproportionate to the rewards. For example, identification and counting of invertebrates can be time-consuming and demanding in terms of skilled manpower, especially if each animal is identified to the specific, or even the generic, level. In common with other groups of workers (e.g. Nuttal and Purves, 1974) we have therefore concluded that the family level of taxonomy is an adequate basis for analysis of effects of pollution on communities of freshwater invertebrates.

There are a wide variety of analyses designed to reduce species-abundance data to some more usable form that can be related to environmental data. Hellawell (1978) discusses a variety of these in relation to biological surveillance of rivers. We have found that diversity calculated by the Shannon-Weiner index is a convenient and relatively sensitive measure of change in macroinvertebrate community structure in small bodies of water and would recommend its use for studies involving effects of toxic chemicals (Crossland, 1979).

In summary, pond studies provide validation of predictions of fate and effects, based on laboratory data and a means of observing toxic effects on a much wider range of organisms than is possible in simple, laboratory systems. They also allow the study of indirect effects of pesticide treatments and of the effects of dispersion on toxicity under realistic field conditions.

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References

BUTCHER, J.; BOYER, N.; FOWLER, C. D. (1975) Impact of Dursban and Abate on microbial numbers and some chemical properties of standing ponds. Proc. 10th Canadian Symp. Water Poll. Res. Canada, 33-41.

BUTCHER, J.; BOYER, N.; FOWLER, C. D. (1977) Some changes in pond chemistry and photosynthetic activity following treatment with increasing concentrations of chlorpyrifos. Bull. Env. Contam. and Toxicol. 17 (6), 752-758.

CROSSLAND, N.O. (1979) Use of biological indices for monitoring effects of pollution in freshwater. Environmental Pollution Management 9 (3), 76-80.

HELLAWELL, J. N. (1978) Biological surveillance of rivers. N.E.R.C. Publication.

HURLBERT, S. H.; MULLA, M. S.; KEITH, J. O.; WESTLAKE, W. E.; DUSH, M. E. (1970) Biological effects and persistence of Dursban in a freshwater pond. J. Economic Entomology 63 (1), 43-52.

HURLBERT, S. H.; MULLA, M. S.; WILLSON, H. R. (1972) Effects of an organophosphorus compound on the phytoplankton, zooplankton and insect populations of freshwater ponds. Ecological Monographs 42 (3), 269-299.

HURLBERT, S. H. (1975) Secondary effects of pesticides on aquatic systems. Residue Reviews 57, 81-148.

MACEK, K. J.; WALSH, D. F.; HOGAN, J. W.; HOLZ, D. D. (1972) Toxicity of the insecticide Dursban to fish and aquatic invertebrates in ponds. Trans. Am. Fish Soc. 101, 420-427.

NUTTAL, P. M.; PURVES, J. B. (1974) Numerical indices applied to the results of a survey of the macroinvertebrate fauna of the Tamar catchment (Southwest England). Freshwater Biol. 4, 213-222.

SHANNON, C. E.; WEAVER, W. (1949) The Mathematical Theory of Communication. Urbana; University of Illinois Press.

Table 1

Calculated mass (mg) of cypermethrin in various compartments of the treated pond

Compartment	Time after treatment			
	1 Hour	4 Hours	1 Week	4 Weeks
Surface vegetation				
P. natans	38 - 76	50.5 - 101	0.7 - 1.4	0.2 - 0.4
C. stagnalis	6.6	8.4	0.08	0.02
Miscellaneous	10.5 - 52	9 - 45		
Water				
Surface	14 - 64	5.9 - 27	0.02 - 0.04	<0.001
Sub-surface	43	31	6.7	<0.3
Sediment	25	16	<6	21
Fish	0.1	0.1	ca 0.1	ca 0.05
Total	150 - 236	127 - 216	<15	<22
Total as % of nominal dosage	30 - 50	26 - 42	<3	<5

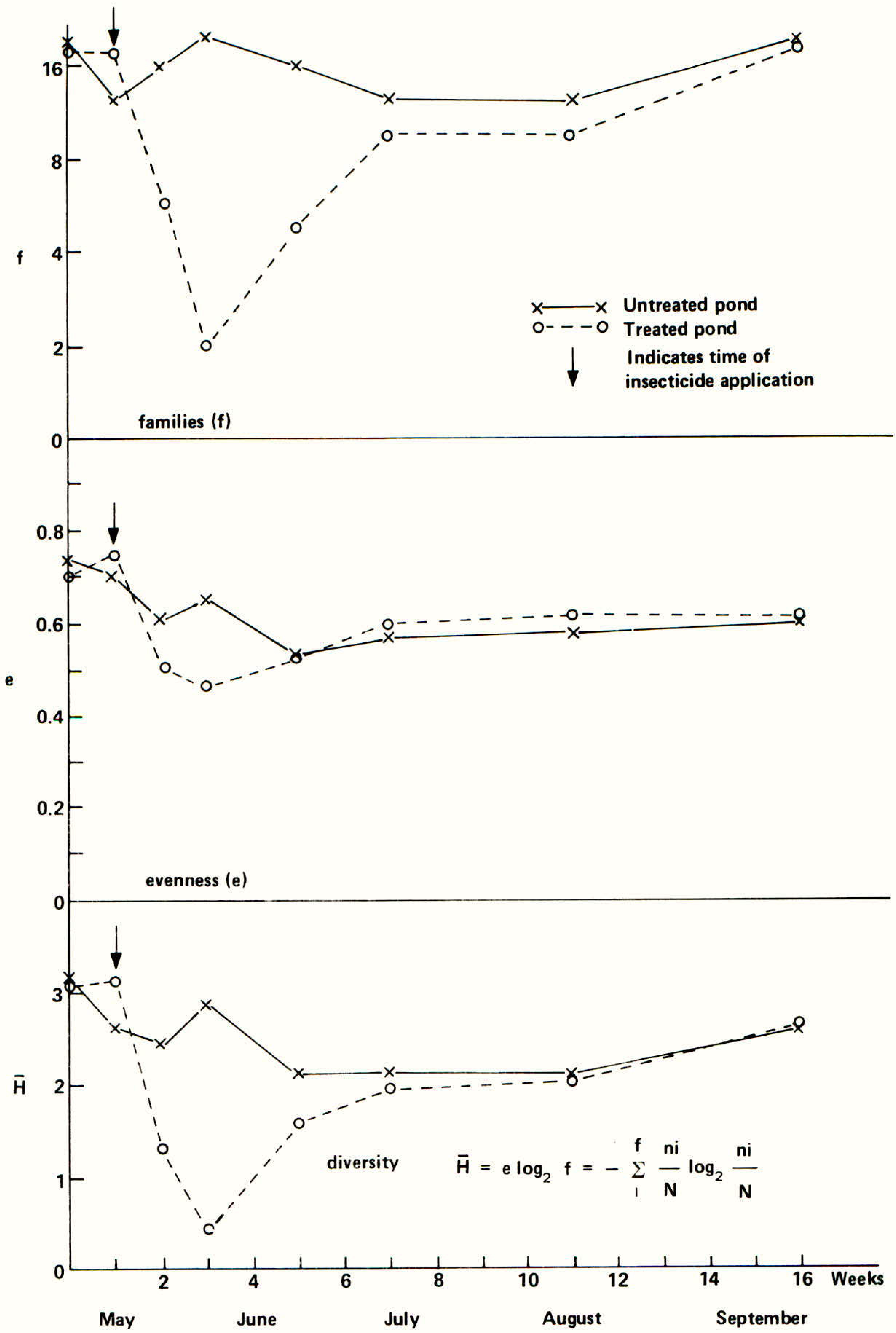

Fig 1 Effect of cypermethrin on diversity of aquatic invertebrates in a pond

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THE EVOLUTION OF AN ASSESSMENT PROGRAMME TO DETERMINE THE SIDE EFFECTS OF PESTICIDES ON TERRESTRIAL ORGANISMS

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Summary An outline of the development of the Ecology Section at Jealotts Hill is given, showing changes in response to current thought on potential hazards, and to requests of Registration Authorities. The importance of realistic field testing is stressed.

Résumé Le développment de la Section Ecologie à Jealotts Hill est tracé, en démontrant les changements aux idées courantes sur les risques potentiels et aux demandes des autorités d'homologation. L'importance d'essais réalistes en plein champ est soulignée.

INTRODUCTION

The establishment of a separate Ecology Section at Jealotts Hill occurred in 1963, the year before the replacement of the old Notifications Scheme by the Pesticides Safety Precautions Scheme. At that time the requirements of Registration Authorities both here and abroad were almost entirely concerned with toxicity of pesticides to man and his domestic animals. The Section began as a team of 2, with a remit to examine side effects in both terrestrial and aquatic eco-systems. Its initial work was concerned mainly with macro-invertebrates, but it has since widened its scope by taking on new work and by absorbing units from other Sections. Currently it consists of some 30 workers, of whom half are graduates, and is divided into 3 areas of study. One part of the Section is concerned with the chemical and physical fate of the pesticide, especially its degradation in the soil. Another part specialises in micro-organisms, in particular the effects of the pesticide on the degradation of organic matter, nitrogen transformations and biomass. The remainder of the Ecology Section deals with larger organisms, both terrestrial and aquatic, studying the effects of a compound on non-target organisms and its movement in food chains.

The perennial problems of ecological testing are:

1. Which organisms should be tested?
2. In laboratory or field?
3. By what method?
4. In what detail?

There may well be limitations on the time or expertise available,and how to use it to best advantage.

This paper is concerned only with the development of our methods for assessing the effects of a pesticide on terrestrial animals. Laboratory toxicity testing on mammals and birds is excluded, since this is not done at Jealotts Hill. Emphasis throughout the Ecology Section's existence has been on changes in populations in the field. It has always been considered desirable to observe effects under conditions as near normal as possible consistent with obtaining usable results, thus avoiding some of the difficulties of extrapolation of laboratory work. An exception to this philosophy has been the standard tests on honeybees, and latterly on more of the beneficial arthropod parasites and predators. Such tests give useful comparisons with other compounds with longer histories of use, but whenever any doubt exists it is considered essential to see what really happens in the field.

Pesticide residues in animals during field trials have been mainly restricted to earthworms and birds. In the laboratory metabolism and bioaccumulation have been done, including an adaptation of the model ecosystems devised by Metcalf (1971).

The following discussion deals in turn with direct or indirect effects on different organisms.

Honeybees

Bees have long been recognised as important both as honey producers and pollinators, and in a number of countries the results of toxicity tests are required at an early stage in the registration of a pesticide. Laboratory tests were done originally by the Entomology Section, from which the Ecology Section was derived. It follows therefore that the methods evolved from those used in the insecticide screen at that time.

Contact tests were done in a Potter Tower ,in which groups of worker bees were sprayed with a series of concentrations of the test compound in water. The bees were then confined in jars with food and water, and survival was monitored at 24 hours. The result was expressed as an LC50. This method had the disadvantage that the actual dose per bee was not known. Therefore in 1973 the decision was made to change the method by applying a measured droplet in acetone to individual anaesthetised bees. This was not done without some misgivings, as the more 'natural' spray was replaced by a single large drop in solvent. The acetone was, of course, used on control bees, and shown to be harmless in itself at the volume applied. Some years after this change a study was done at Jealotts Hill by T.Holt comparing the effects of different droplet sizes of 2 compounds. A carousel to which flying bees were attached passed through sprays produced by a spinning disc. There was little difference between the same dose administered as a single 1μl drop and as a spray with a droplet diameter of 150μm.

A further development took place in 1975, when wire-mesh cylinders were substituted for solid containers with netting tops, to avoid excessive fumigant action. This brought the method to follow that developed by Stevenson at Rothamsted, and recommended by the Pesticide Safety Precautions Scheme in Working Document 13 (1971). In addition to the specified details the bees under test are kept at a R.H. of 60-65%,as this is considered important for both survival and standardisation. Observations are usually made at intervals of 1,2,4,24 and 48 hours after pesticide application, but can be extended to 5 days if considered necessary. The shorter intervals give an indication of speed of action and the effect on behaviour. This is useful knowledge if field work is to be considered. Contact test methods in which bees are made to walk on treated surfaces as practiced in some countries (Cairaschi, 1975; Drescher et al, 1975) are not yet part of our testing programme.

Oral tests have undergone fewer fundamental changes. After early work by J.Newman at Jealotts Hill in which the bees were fed individually from a syringe (as currently practiced in Germany and Holland (Drescher et al,1975)) the system was adopted whereby a group of 10 bees were allowed to drink a measured amount of pesticide suspended in dilute honey. Over the years the honey was replaced by 20% sucrose solution; the plastic feeding cup by a glass tube with a small drinking hole, and the net-top jars by wire-mesh cages. The method now also conforms closely with the PSPS guidelines (1971), with the additions previously noted for contact testing.

Field testing is carried out only when the proposed use of a compound indicates a possible hazard to bees, or to provide evidence of the practical safety of a compound of low toxicity. Newman did some work in the early 1960s on observing bees sprayed with fluorescent dyes, but the main programme at Jealotts Hill dates from 1975, and follows in all essentials the method devised by Stevenson and described in PSPS Working Document 15 (1974). Bees are allowed access to 3 attractive, separated fields sprayed either with the test compound, a toxic standard, or left untreated. Deaths at the hive are monitored, and foraging activity and colony state is also recorded, with pollen collection on some occasions. The method has been found useful, but a trial can be ruined by factors such as bad weather, crop variation, alternative bee attractants and unplanned additional sprays.

Soil Microarthropods

Studies in this group of animals have been continuous since the inception of the Ecology Section, since they are considered to be an indicator community with diverse habits. Some of the organochlorine insecticides were known to cause an imbalance between predators and prey (Brown,1978), and it was considered desirable to watch for this aspect in the newer compounds. Some trials have been done under normal cropping regimes, but populations tend to be lower and less diverse in cultivated land. The standard practice has been to apply pesticide to replicated 6 m square plots under grass at normal and 10 x normal rates, and monitor changes in microarthropod abundance and species diversity at intervals after treatment. Observations usually continue for at least 3 years, with annual application of the pesticide, sometimes on several sites. The two large cores per plot taken in 1964 were changed the following year to 3 samples, each of 4 small bulked cores, to reduce sample variation caused by aggregations. Recently, following a statistical review of the previous results, the number of samples has been reduced to 2 per plot with a consequent saving in time. At an early stage the decision was taken to separate the animals from the soil using a flotation and differential wetting technique (Newman, 1970) rather than the more common dynamic methods (Murphy, 1962; Southwood, 1966). Funnel separation was preferred to flotation for soil microarthropods by Edwards and Fletcher (1971) in their method review. We have, however, found the flotation method very satisfactory for most agricultural soils, and especially on clays, where it is more efficient at extracting the soft-bodied euedaphic forms such as Rhodacaridae and Onychiuridae. This was shown by S.Richardson at Jealotts Hill in a comparison with the Reading University Macfadyen apparatus.

Initially animals were identified to major groups only, but some of these were heterogeneous both in vertical distribution and reputed habits, so that between 1966 and 1969 indentification was extended to species level in the Mesostigmata, Cryptostigmata and Collembola. Overall the soil microarthropods have been found to be a resilient group. Ground surface sprays of insecticides at excessively high rates have caused temporary reductions of some hemiedaphic species, but no more than that by the physical disturbance of ploughing, while euedaphic species have been little affected. Consequently the amount of work in this area is being reduced to allow more input on predators and parasites of pests on crops.

Earthworms

Earthworms are important not only in relation to soil structure and organic matter turnover, but also as food for birds and mammals. Two aspects have therefore been considered,; the effect of a pesticide on earthworm numbers and biomass, and its presence as residue in the bodies.

Except for the purpose of method comparison, sampling has always been done by expelling the worms from soil by an irritant solution of 0.2% formaldehyde (Raw, 1959). The work has usually been done on the same field trials or long-term plots as used for the microarthropods. During 16 years the main changes have been to increase the number of samples, to include a toxic standard, and to identify the species of worm. Identification of the worm was introduced in order to detect changes in species of different habits, which might not be evident if only total worm populations were assessed. Currently sampling takes place in the spring shortly after pesticide application, and in the autumn. Two $0.36m^2$ quadrats are sampled on a central area in each plot, using 9 l of formaldehyde per quadrat. The position of the quadrats is defined, and a rotation in a 2-year cycle ensures that worms have ample time to re-colonise quadrats, and that microarthropod samples are not taken from the worm areas. In the course of a study at Jealotts Hill to test the efficiency of formaldehyde expulsion against digging and hand-sorting P. Edwards showed that populations in a formaldehyde-treated quadrat returned to normal within the year.

Analysis of earthworm bodies for pesticide residues used to be done on the samples expelled during standard population checks. Recently, however, this work has been restricted to compounds where an environmental hazard is suspected. On such occasions a trial is set up appropriate to the use of the pesticide, and samples taken at frequent intervals, beginning shortly after application.

Arthropod Predators and Parasites

The evolution of Jealotts Hill studies on predators and parasites of pests shows three distinct stages.

Occasional laboratory tests on the toxicity of a new compound to well-recognised predators such as *Coccinella* and *Chrysopa* had been done by the Entomology Section for some years. This work was eventually handed on to the Ecology Section, which did additional tests with *Phytoseiulus*, *Encarsia*, and *Aphidius*.

Our belief in the value of field experimentation led to extended studies in 1967 on the larger arthropods of the soil surface. An effective pitfall trap system was chosen after preliminary experiments. These included mark-recapture tests which indicated a long range of movement of carabid beetles, and hence the necessity for large plots. The pitfalls gave convincing results in subsequent trials in fields of barley and sugar beet sprayed with ethirimol and pirimicarb. Sticky traps tried at the same time failed to assess flying insects satisfactorily, and their use was not repeated.

In recent years the potential importance of predators and parasites of pests the so-called"beneficials", has become more widely recognised. Although instances of successful biological control by introduction in the field are few, there have been a number of occasions where natural limitation of pest numbers has only been discovered after the balance has been upset by pesticide use. Selective action against the pest is a good principle, in that remaining enemies can reduce any surviving pests, which might be the progenitors of a resistant strain or just provide a nucleus for resurgence. The beneficials may also be available to supress reinvasion or deal with other pests on following crops.

Since 1977 the Jealotts Hill programme on beneficials has been expanded. *Trichogramma* was added to the test insects, using Hassan's method (1977), but the main effort has been in field studies. It was not considered justified to confine observations to the few species recorded as attacking pests, for there may well be others equally important but, as yet, unrecorded through lack of observation. The practical difficulties in attempting to study the whole arthropod ecosystem are the large numbers involved, problems of identification, impossibility of completeness, and the interpretation of results. Pitfall traps continue to be used, and the D-Vac suction sampler for animals in the foliage. In orchard trials on resurgence of spider-mites following insecticide applications it was also considered necessry to pick leaf clusters and brush off those mites which were not satisfactorily removed by suction. Tetranychid egg clusters in defined areas on twigs were counted to assess potential mite populations in the following season. In 1979 trials are being done to compare the relative effects of an aphicide and broad-spectrum insecticides. The arthropod population is here being considered not only for internal balance of predators and pests, but also as a source of food for partridge chicks (Potts; Vickerman, 1974 et. seq.).

Birds

Field observations on wild bird populations have been restricted to situations where a hazard might be expected. Where a compound has been shown to be somewhat toxic to the few species of domestic birds used in routine laboratory feeding tests, and its proposed use indicates a hazard (e.g. as a seed dressing), then field tests are necessary. Extrapolation from the cage tests alone would be inadmissible due to species variation in toxicity (Stanley; Bunyan, 1979), the different factors of behaviour, alternative food choices and other pressures in the wild.

Up to 1972 visual counts were made on the numbers of species and individuals present in the vicinity of treated fields before and after pesticide application. These observations were combined with the results of searches for dead birds pre- and post-treatment. This method suffers, however, from two main defects. Firstly it does not take account of birds in transit. Secondly it does not distinguish between birds which have disappeared after pesticide poisoning, and those which have gone to feed elsewhere because the pesticide has removed their food source from the test fields.

It was decided in 1973 to introduce an assessment programme based on resident birds which has established territories in and around the test fields. It was argued that if these were unharmed by a pesticide treatment they would still be found defending their territories even if the attraction to the treated field ceased. A modified territory mapping method was used, based on the recommendations of the International Bird Census Committee (1970). Greater sensitivity over short periods was introduced by including single records of contemporary song. The method gave promising results with drazoxolon-treated grass seed in 1973 and with other compounds subsequently.

One criticism of this method was that reinvasion by other birds following mortality of territory holders could mark any changes. Consequently in 1976 a trial was arranged in collaboration with the M.A.A.F. Pest Infestation Control Laboratory and with the technical support of the British Trust for Ornithology (Edwards et al, 1979). After initial territory assessment birds were caught by mist netting on contrasting farmland sites and removed from the area to simulate pesticide poisoning. Further territory assessments then took place at intervals. Estimates of territories before and after removal on the treated and on two untreated control sites were then compared with the number of birds known to have been removed. Significant reinvasion was limited to a few species, for example the blackbird, but was always incomplete. It was concluded that the method is suitable for detecting short-term pesticide effects.

Territory studies have been extended to examine the success of hatching of the eggs and fledging of the young. Nests in natural situations have been used, but in some circumstances these have been augmented by nest boxes. For instance in an orchard, where it was suggested that a new acaricide might contaminate the insect food offered to pulli, nest boxes were placed in the orchard to allow more precise observations.

Mammals

Most compounds with a serious effect on mammals when applied at field rates are likely to have been previously rejected in toxicological tests because of hazard to man and his domestic animals. It follows that comparatively few field studies have been carried out. Some monitoring has, however, taken place in response to requests for information about pesticide effects on game animals.

More recently trials have been done on the effects of the rodenticide 'Ratak' on the woodmouse (*Apodemus sylvaticus*) (Edwards,1977). The objective was twofold; to test the effectiveness of the rodenticide, and to investigate a method of assessing side effects of pesticides on small mammals. A mark-release-recapture technique was employed, using pairs of live traps in a 6 x 6 grid. Pre-treatment baiting periods identified the frequent visitors, which were classified as home-range animals. It was the disappearance of all these individuals from the treated plots which carried most weight in the post-treatment assessments. As very few of the less frequent visitors reappeared following treatment it was suggested that these mice were also poisoned. The method was concluded to have potential for use with animals susceptible to this form of trapping.

DISCUSSION

In addition to the studies described, an important aspect is the follow-up of reports of incidents in the field during normal use, or investigation of adverse comments by other workers. These investigations may be very time-consuming, but whether they are worthwhile or a waste of time may depend largely on the quality of the original observations.

With increasing requirements from Registration Authorities worldwide it is less easy now to offer voluntary work on the ecological effects of pesticides. It is hoped that the background given, with the illustrations to be shown in speaking to the paper, will encourage discussion on future strategies for assessing the impact of pesticides on non-target animals and evaluating the results.

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References

BROWN, A.W.A. (1978) Ecology of Pesticides. New York; Wiley. 525pp. ISBN 0 471 10790 5

CAIRASCHI, E.A. (1975) Méthode d'essais d'efficacité pratique (toxicité) de produits insecticides vis-à-vis des abeilles domestiques. Societé Française de Phytiatrie et Phytopharmacie, Commission des Essais biologiques.

DRESCHER, W. *et al* (1975) Vorlaufige Richtlinien für die Prufung von Pflanzen behandlungsmitteln auf Bienengefärlichkeit..

EDWARDS, C.A.; FLETCHER, K.E. (1971) A comparison of extraction methods for terrestrial arthropods. In: Phillipson, J. (Ed.) Methods of study in quantitative soil ecology: population, production and energy flow. London; Blackwell, p. 150-185. ISBN 632 05680 0.

EDWARDS, P.J. (1977) Control of the woodmouse, Apodemus sylvaticus on arable farmland by 'Ratak'. Proceedings 1977 British Crop Protection Conference. 461-467.

EDWARDS, P.J. et al (1979) The use of a bird territory mapping method for detecting mortality following pesticide application. Agro-Ecosystems 5, 271-282

HASSAN, S.A. (1977) Standardised techniques for testing side-effects of pesticides on beneficial arthropods in the laboratory. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. 84(3), 158-163.

INTERNATIONAL BIRD CENSUS COMMITTEE (1970) Recommendations for an international standard for a mapping method in bird census work. Bulletin Ecological Research Committee 9, 49-52.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD. PESTICIDES SAFETY PRECAUTIONS SCHEME (1971). Working Document 13. Laboratory testing of pesticide products for toxicity to honeybees.

IBID (1974) Working Document 15. Testing pesticide products for toxicity to foraging bees under field conditions.

METCALF, R.L. et al (1971) Model ecosystems for the evaluation of pesticide biodegradability and ecological magnification. Environmental Science and Technology 5(8), 709-713.

MURPHY, P.W. (Ed.) (1962) Progress in Soil Zoology. London; Butterworths. 398 pp.

NEWMAN, J.F. (1970) The extraction of arthropods from soil by flotation and differential wetting. In: FAO Manual, Crop loss assessment methods. Rome;Food and Agriculture Organisation of the United Nations. 3.1.2/1 - 3.1.2/10.

POTTS, G.R; VICKERMAN, G.P. (1974) Studies on the cereal ecosystem. In: MACFADYEN,E. (Ed.) Advances in Ecological Research 8. London; Academic Press. p.107-197. ISBN 0-12-013908-1.

RAW,F. (1959) Estimating earthworm populations by using formalin. Nature 184, 1661-1662.

SOUTHWOOD, T.R.E. (1966) Ecological Methods. London, Methuen, 391pp.

STANLEY, P.I; BUNYAN, P.J. (1979) Hazards to wintering geese and other wildlife from the use of dieldrin, chlorfenvinphos and carbophenothion as wheat seed treatments. Philosophical Transactions Royal Society of London, Series B. (in press).