SESSION 8C

EFFECTS AND FATE OF PESTICIDES: NEW METHODS AND RESULTS

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A NON-INVASIVE APPROACH FOR MONITORING THE EXPOSURE OF BARN OWLS TO RODENTICIDES

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ABSTRACT

The potential environmental hazards of rodenticide use depend not only on the toxicity of the active ingredient but also on the exposure of the non-target animal. Two types of hazard may arise from rodenticide use; primary hazard resulting from the direct consumption of bait and secondary hazard through the consumption of poisoned rodents. Studies have been carried out to develop a non-invasive approach to monitoring the exposure of a night hunting predator, the barn owl, to residues of an anticoagulant rodenticide flocoumafen (Storm) in its prey based on pellet analysis. Laboratory studies confirmed that caged owls fed mice containing residues of flocoumafen eliminate a consistent percentage of these residues in regurgitated pellets. These pellet residues are well in excess of the limit of determination of the analytical method. Field studies showed that location of owl roost and nest sites and the collection and storage of pellets for subsequent residue analysis and identification of prey were feasible. In addition interviews with farmers were used to provide information on rodenticide usage in the locality of the barn owl roost/nest sites.

INTRODUCTION

Rodents are important pests in and around farms in the U.K. Many different methods have been devised to control rodent pests but, in practice, effective control largely relies on the use of anticoagulant rodenticide baits. Second generation anticoagulants such as flocoumafen are highly active rodenticides, distinguished from the first generation poisons by their effectiveness against resistant rodent strains and their ability to cause death after a single feed. (Johnson and Scott, 1986).

The potential environmental hazards of rodenticide use depend not only on the toxicity of the active ingredients but also on the exposure of the non-target animal. In recent years there has been concern about the potential for secondary poisoning of predators and scavengers from the use of anticoagulant baits used for rodent control on farms. The barn owl, a night hunting predator that feeds on live rodents and birds (Mikkola, 1983), may therefore be at risk from the use of such baits.

This paper describes a non-invasive method based on pellet analysis for monitoring exposure of barn owls to an anticoagulant rodenticide, such as flocoumafen. The validity of the method has been confirmed using a laboratory feeding study with captive barn owls. In addition, field studies, which have involved locating barn owl roost and nest sites and the collection, storage and subsequent analysis of pellets, have supported the feasibility of the approach.

BARN OWL FEEDING STUDY

The secondary hazard a rodenticide poses depends on the quantity of active ingredient present in live and dead rodents and their availability to non-target species. Dogs, cats, scavenging birds and birds of prey are all potentially at risk. Residue analysis of the rodents (live, trapped and dead; see Table 1) gives an indication of the total body burdens of flocoumafen. From this information an estimate of the **potential** secondary hazard of the compound can be made. However, the **actual** exposure of predators to residues of flocoumafen in their prey is particularly difficult to assess since it is dependent on factors such as hunting behaviour, food availability and other physical and environmental factors unique to the local vicinity. The validity of monitoring for exposure to flocoumafen (and possibly other rodenticides) by analysis of residues in regurgitated pellets was assessed in a feeding study with barn owls.

Table 1 shows the average flocoumafen residues found in rodents killed on trials with different bait formulations and baiting strategies. The recommended strategy for flocoumafen baiting, i.e. using the wax block formulation and a pulse baiting regime, results in the rodent carcasses with concentrations of flocoumafen less than 50% of that seen in rodent carcasses from trials using a loose grain bait and a surplus baiting method.

TABLE 1. Mean flocoumafen residues in rodents found during carcass searches

Bait formulation	Baiting strategy	Flocoumafen conc. (mg/kg)	
		Rats	Mice
Loose grain	Surplus	2.1	2.1
Wax block	Surplus	1.7	2.4
Wax block	Surplus	1.2	2.6
Wax block	Restricted*	0.87	2.3
Wax block	Pulse	0.79	1.2

* Bait topped up twice per week

Four barn owls (*Tyto alba*), weighing 321 – 359 g, were held in outdoor flight pens (4.5 m × 2 m × 2 m) constructed of wire netting on wooden framing with a base of concrete slabs and fitted with partial shelter, nest box, perching and a water bowl. They were acclimatized to a daily ration of four mice (approximately 80 g mouse per day per bird). Each bird was then offered a single mouse containing 3.3 – 4.1 mg/kg flocoumafen on a single occasion, followed by reversion to the standard ration. The test mice had been previously fed on a diet containing [^{14}C]-flocoumafen and sacrificed 24 hours later. All the birds survived the flocoumafen dose, which ranged from 0.11 – 0.23 mg/kg per bird. There were no symptoms of anticoagulant poisoning (e.g. sublethal haemorrhaging) in any of the birds. Two of the birds from the study were paired and bred successfully in captivity the next season.

Regurgitated pellets were collected from each bird throughout an eight day period following dosing with [^{14}C]-flocoumafen treated mice. The elimination of radioactivity in pellets was monitored and in total constituted 44% (range 35 – 55%) over the collection period, with the highest residues being observed in the first 24 hour period after consumption of the [^{14}C]-flocoumafen treated mice. Further detailed analysis confirmed that the flocoumafen residues in pellets from the first 24 hours represented a significant proportion (15%, range 8 – 26%) of the original flocoumafen residue in the mice.

Calculations based on these data confirm that if a barn owl were to consume a wild rodent that had fed on a flocoumafen bait this non-invasive monitoring method would detect residues of the rodenticide in subsequently regurgitated pellets.

Assuming a mouse weighing 20 g containing a flocoumafen residue of 1 mg/kg (a typical value found in live trapped mice during field trials) is consumed by a barn owl, the subsequent pellet (typical weight 5 g) would contain a flocoumafen residue of:

$$1 \text{ mg/kg} \times 0.15 \times \frac{20 \text{ g}}{5 \text{ g}} = 0.6 \text{ mg/kg}$$

Since the limit of determination for flocoumafen in pellets (0.02 mg/kg) is significantly lower than the anticipated residues in pellets following consumption of a single mouse, the use of pellet analysis is a sufficiently sensitive approach for monitoring the exposure of barn owls to flocoumafen residues in their prey.

FIELD STUDIES

Field studies were set up in Eire in order to locate suitable barn owl roost/nest sites which would yield a supply of regurgitated pellets for subsequent analysis. Eire was an appropriate location for the studies, since from 1986 onwards farmers have been buying and using flocoumafen for rat control where barn owls might be living and hunting. In addition, most of the preferred prey of the barn owl are absent from Eire. Only four of the small mammals commonly found in the U.K. are widespread, namely the wood mouse, house mouse, pygmy shrew and brown rat. This limited range of prey in Eire made the house mouse and brown rat more important prey items for the barn owl. The work was carried out during the winter season when rats and mice faced with a shortage of food move from the fields, ditches and hedgerows into farms for a more reliable food supply. Farmers use rodenticide baits particularly during this period to control these pests.

Nineteen barn owl sites were located in Southern Eire (Fig 1). Since the barn owl hunts within a close range of the nest/roost site, farmers and land users within a 1 mile radius of each site were interviewed to identify the type and quantities of rodenticides used (Fig 2). A typical trial site map is shown (Fig 3).

During the first visit to a roost/nest site any pellets found were removed so that any subsequent pellets collected were of a known age. Sites were then visited at regular intervals (i.e. every few days) and 'fresh' pellets collected for prey identification and residue analysis. Pellets were individually packaged in paper envelopes, labelled and stored in a portable deep freeze until transportation to the laboratory for analysis.

Pellets were analysed for prey content. Each pellet was broken up individually, dissected and the bones within it removed and identified (Yalden, 1977). The average weights for small mammals, frogs and birds (Fairley and Smal, 1988) were used in conjunction with the number of each species found in order to estimate the percentage biomass for each of the prey species in the diet of the barn owls. Prey analysis revealed that over all the sites the brown rat was the most important prey item comprising 35% of the owls' diet by weight, followed by wood mouse, bank vole and house mouse. Frogs, pygmy shrew and rabbit were also present in the diet but these represented only a small percentage

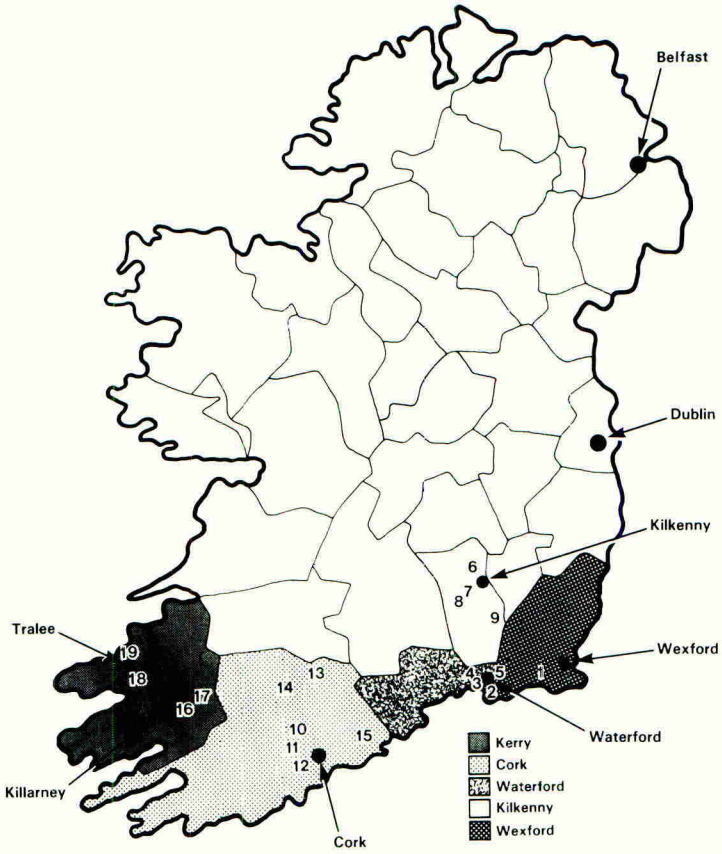

FIG 1. A map of Ireland showing the counties visited and the approximate positions of the barn owl roost/nest sites

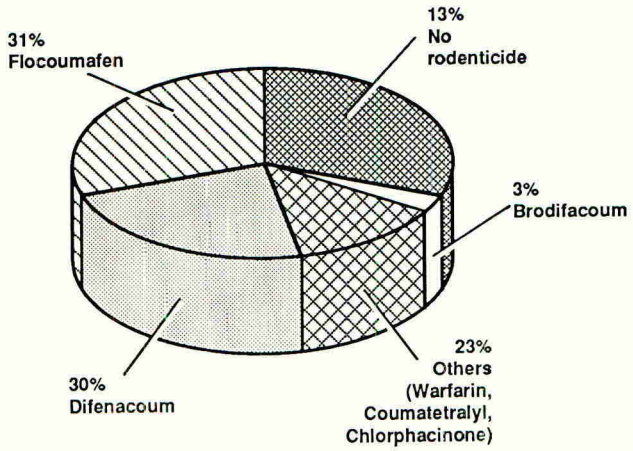

FIG 2. Use of rodenticides in owl site areas in Southern Eire

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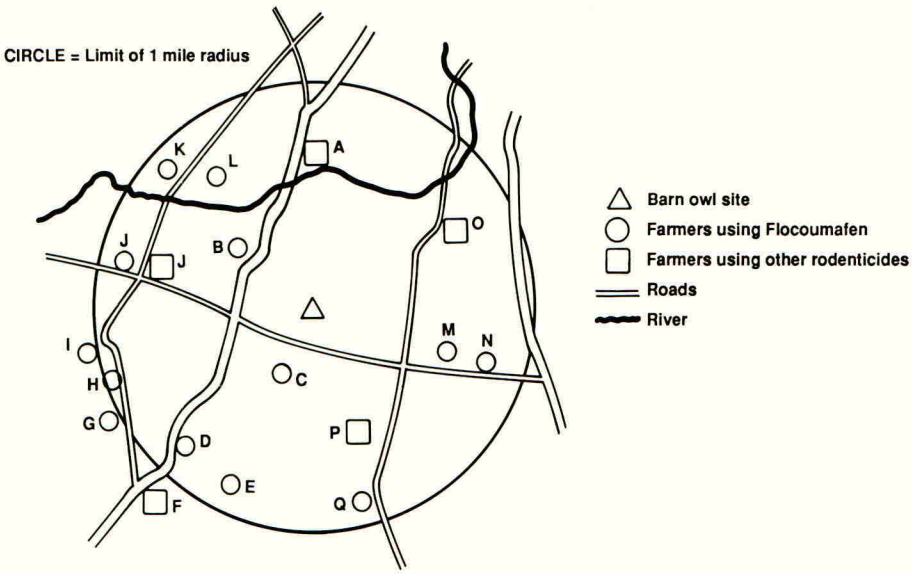

FIG 3. Map showing a typical barn owl site in Co. Kilkenny and surrounding areas over which farmers were interviewed

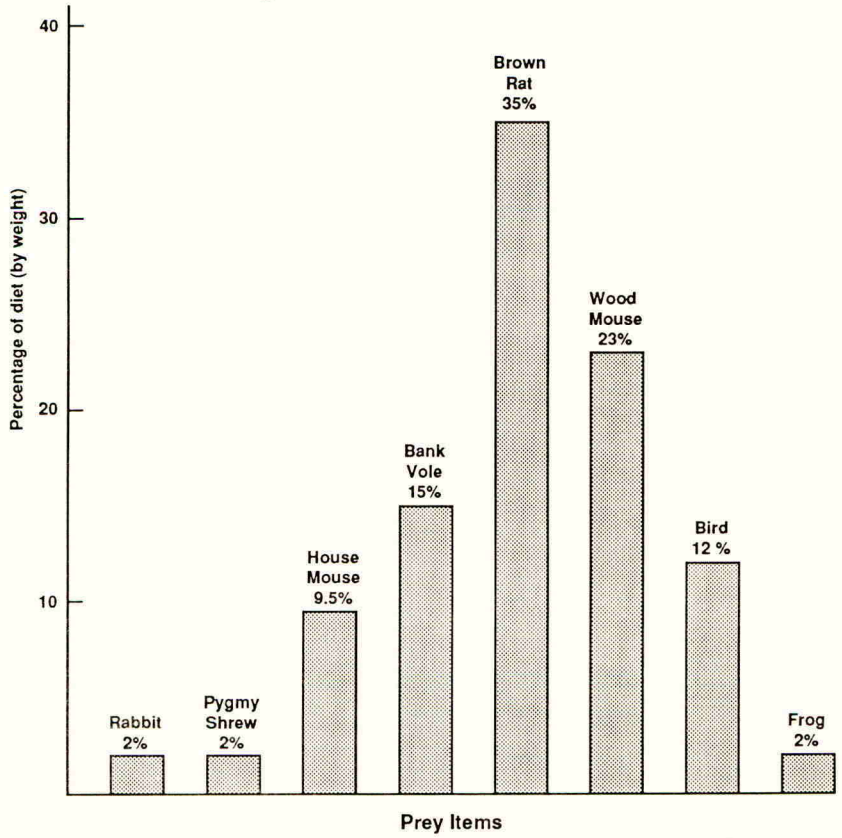

FIG 4. Identification of the percentage of diet by weight for all prey species found in barn owl pellets collected during field studies in Eire

(Fig 4). The bank vole has a limited distribution in Eire and no remains were found in pellets collected from sites in the eastern counties of Waterford, Wexford and Kilkenny. At these sites the brown rat was the most important prey item. At sites where bank vole remains were found in the pellets (Co. Cork and Co. Kerry) it was the single most important component of the diet.

Analysis of residues of flocoumafen was carried out on a selection (n = 89) of previously dissected fresh pellets; selection was based on the presence of brown rat or house mouse remains and whether flocoumafen was in regular use by local farmers. No residues were found (limit of determination of 0.02 mg/kg), suggesting that during the study period barn owls were not exposed to flocoumafen residues in their prey.

CONCLUSIONS

In the past it has only been possible to make a rough assessment of the likely potential for secondary hazard by determining the total body burden of rodenticides in live or trapped rodents in baited areas and combining this data with data on toxicity and feeding habits. Approaches used to obtain data on feeding have been used that include faunal analysis of pellets, the use of chemical bone markers in baits (Crier, 1970) and the use of radiotelemetry techniques to study foraging behaviour (Hegdal and Baskiewicz, 1984).

The laboratory and field studies reported here confirm that chemical analysis of regurgitated pellets for flocoumafen residues is both a sensitive and feasible approach to non-invasive monitoring of the exposure of barn owls to residues of flocoumafen (and most likely other rodenticides) in their prey. Now that the principles behind the method have been successfully established, it should be possible to conduct monitoring studies of barn owls in areas of regular rodenticide use to obtain an accurate assessment of exposure.

ACKNOWLEDGEMENTS

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PREDICTING THE SIDE-EFFECTS OF PESTICIDES ON BENEFICIAL INVERTEBRATES.

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ABSTRACT

Pesticide side-effects are notoriously difficult to predict, given the large number of interacting factors that mediate the toxic effects of the chemical and the subsequent recovery by the insect population. This review presents a series of novel approaches to this problem which may improve the accuracy of predictions in the future. Using modelling techniques, supported by new experimental methods it should be possible to determine optimum dose-rates for hazardous compounds, identify the species most at risk in the beneficial insect community and to rank pesticides in terms of the hazard that they pose. We also review the prospects for predicting rates of recovery by beneficial invertebrate populations.

INTRODUCTION

Tests of plant protection products are now carried out against beneficial invertebrates as part of the pesticide clearance procedure in several european countries (eg. Bode, Brasse & Kokta, 1988). These tests may be laboratory-based (eg. Hassan, 1989) or field-based, providing data which are measured against criteria of safety. Prediction of the hazard posed by a particular product is complex given the combination of chemical, toxicological, ecological and operational factors that mediate pesticide side-effects (Jepson, 1989). For this reason, the ability of most current assessment techniques to give an accurate index of hazard remains untested. We attempt here to review the prospect for improving the quality and precision of hazard predictions, emphasising some of the more recent and novel research findings.

RISK AS A FUNCTION OF EXPOSURE AND SUSCEPTIBILITY

Short-term, micro-scale effects are a function of exposure and susceptibility (Jepson, 1989). At the time of spraying, the probability that an insect will suffer a lethal dose will be a function of its position in the canopy, the number and volume of spray droplets that impinge upon it and its topical tolerance distribution to the pesticide formulation.

We have measured the exposure of beneficial invertebrates, occupying different canopy positions in cereals, to direct spraying (Cilgi et al., 1988; Cilgi and Jepson, 1990). This has enabled

predictions of effects for hypothetical populations which have a known distribution through the crop canopy. Thus with an adult Coccinella septempunctata (Coleoptera: Coccinellidae) population distributed with 50% of the insects on the ear, 25% on flag leaves and 25% on first leaves, we predict high levels of mortality at the application rate of 340 g.AI/ha (Fig. 1). We also estimate that application at rates as low as 10% of field rate would give 70% mortality. Following spraying, side-effects will be a function of pesticide uptake from the substrate, which is itself a function of availability and the activity and distribution of the insect concerned. A model developed from in-situ bioassay results (see below) has shown that at 10% of field rate, the coccinellid population above would suffer a further 24.5% mortality over the next 72h as a result of residual uptake. This gives a total estimated mortality of approximately 95% from these two routes of exposure at 34 g.Ai/ha. In order to keep mortality below 20%, application at less than 2% of field rate would be required.

For less toxic products, this methodology should permit, for the first time, calculation of optimum dose-rates which minimise effects on important beneficial species. It could also be used as a tool within product development and clearance where simple computer models, integrating laboratory toxicology with ecological, behavioural and operational parameters, could replace certain types of field tests.

HAZARD INDICES BASED ON INTRINSIC SPECIES CHARACTERISTICS

The time required to collect the data for, and validate the above models may delay their implementation. Alternative techniques may however be used to determine which species are most at risk in a given situation. We have developed a simple predictive model which ranks invertebrates in order of potential risk (Jepson et al., 1990). The index is the ratio between susceptibility (expressed as the species' topical tolerance distribution at end-point) and an exposure function (based on walking track width, walking speed and the proportion of the area, covered by the insect, that is contacted). This index has been shown to correctly predict the ranking of residual susceptibility for some coleopteran predators and could be used, in the future, to select organisms for clearance testing purposes against specific classes of pesticides or to aid interpretation of field tests. A further example of this procedure for seven coleopteran predators is given in Wiles et al., (1990) where Tachyporus hypnorum (Coleoptera: Staphylinidae) was shown to have the highest index level amongst cereal aphid predators, by virtue of its high susceptibility and high level of surface contact relative to body size.

RANKING PESTICIDE HAZARD USING IN-SITU BIOASSAYS

We have used simple leaf and soil-cage bioassays to measure the toxicity and persistence of dimethoate, deltamethrin and pirimicarb to carabid and coccinellid predators of cereal aphids (Cilgi et al., 1988; Jepson, 1989). These assays have the advantage that they expose insects to realistic concentrations of pesticide under natural conditions. As part of this study, we exposed C. septempunctata

adults to treated soil following 24h exposure in cages on treated flag leaves (Fig. 2). Deltamethrin was slightly less toxic than dimethoate following this transfer. We found this product to be less toxic to other species at soil level and thus, despite its high intrinsic toxicity compared with dimethoate, we concluded that epigeal invertebrates might be less affected by deltamethrin than by dimethoate. Recent large-scale, field investigations (Vickerman et al., 1987 a & b) have confirmed the ranking of toxicity of these products to epigeal groups such as Carabidae. Comparative, in-situ bioassays may therefore offer an alternative method of evaluating the relative harmfulness of products in a cost-effective way. The findings of these studies may then be followed up by semi-field, cage tests (Sotherton et al., 1988), avoiding the need for expensive, high risk, field experiments.

LONG-TERM EFFECTS AND THE IMPORTANCE OF SPATIAL SCALE

The above models cannot be used to predict effects beyond a few days because they fail to take into account the activity and redistribution of the non-target species concerned. We have demonstrated elsewhere that insects and spiders diffuse into treated areas, leading to local recovery effects (Duffield & Baker, 1990; Jepson and Thacker, 1990; Thomas et al., 1990). The extent and duration of population depletion will therefore be a function of the area treated, with large fields or groups of fields suffering effects into subsequent seasons, even after a single pesticide application. These estimates of recovery rate, once verified, may provide a method of evaluating the duration of reduced natural enemy population density. In some circumstances, this period of reduced biological control may lead to pest resurgence. Duffield and Baker (1990) have demonstrated that the rate of increase in cereal aphid populations was higher at the centre of dimethoate treated areas, compared with the edge where predators had reinvaded (Fig. 3). This data could be used to calculate the economic consequence of pesticide side-effects on beneficial arthropods and thus provide a more rational basis for the development of fully integrated pest-management programmes.

THE FUTURE OF BENEFICIAL INVERTEBRATE TESTING

Rather than relying upon the rapid stepwise progression from laboratory tests to field trials (Hassan, 1989), we propose that hazard index rankings should be used to select beneficial invertebrate species for in-situ assays within the selected target crop. This data-set would feed into simple models predicting hazard, which could be verified by semi-field tests, if necessary. The models could also be used to determine optimum dose-rates for compounds that fail to meet safety criteria. More sophisticated models of exposure, uptake and recovery, could eventually replace a large proportion of field trials given their ability to predict the outcome of experiments with a wide range of treatment, crop, scale and invertebrate population variables.

Fig 1. Lines of predicted percentage mortality for Coccinella septempunctata adults, inhabiting different winter-wheat crop strata, for different dimethoate concentrations. (Crop GS 82: pesticide applied in 200l water/ha via standard hydraulic sprayer). Data from Cilgi and Jepson (1990).

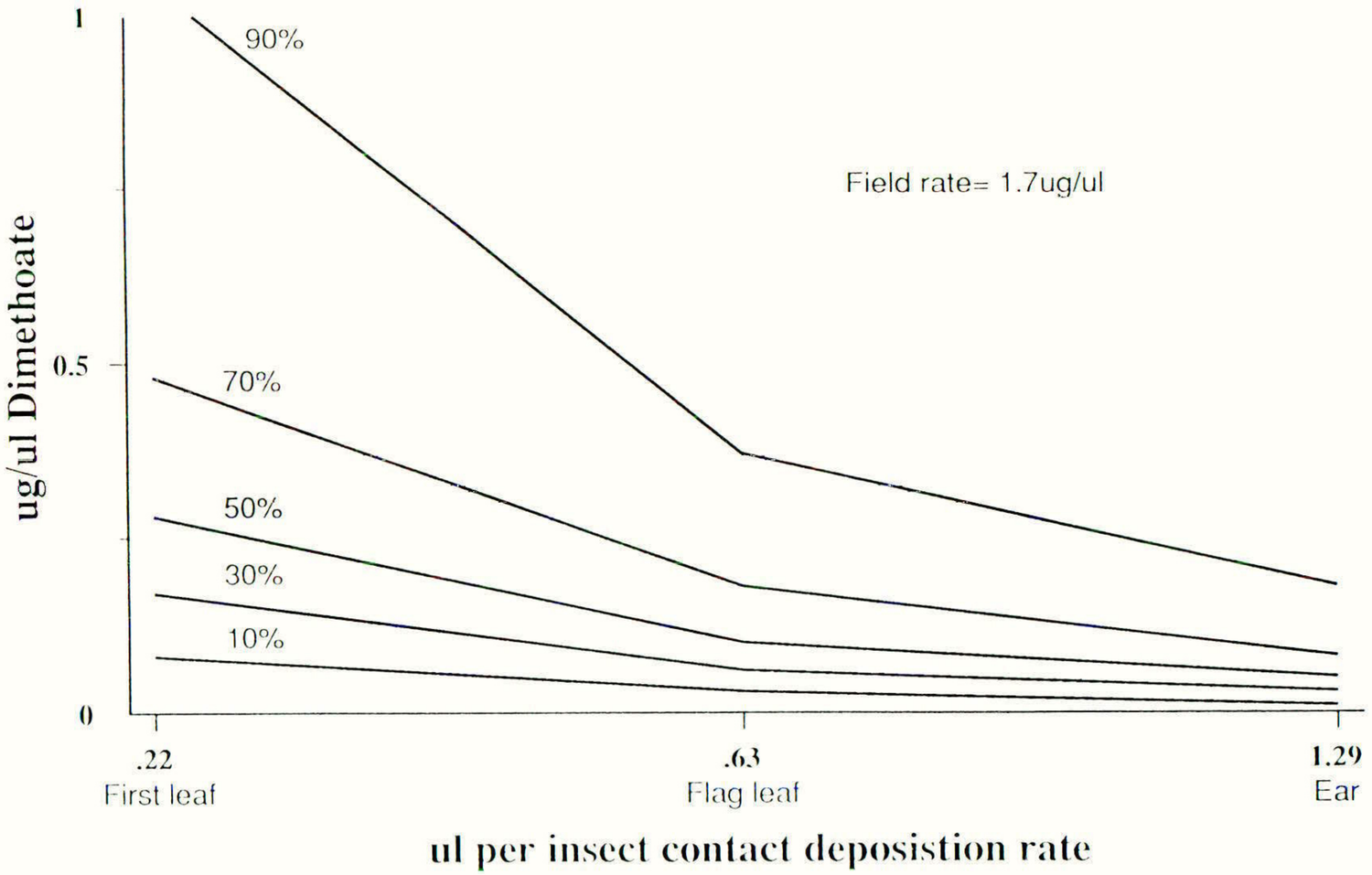


Fig 2. Cumulative mortality of Coccinella septempunctata adults placed on treated soil following 24h exposure to dimethoate residues on winter-wheat flag leaves. Application at 340g.AI/ha in 200l water: test soil under crop canopy at the time of spraying.

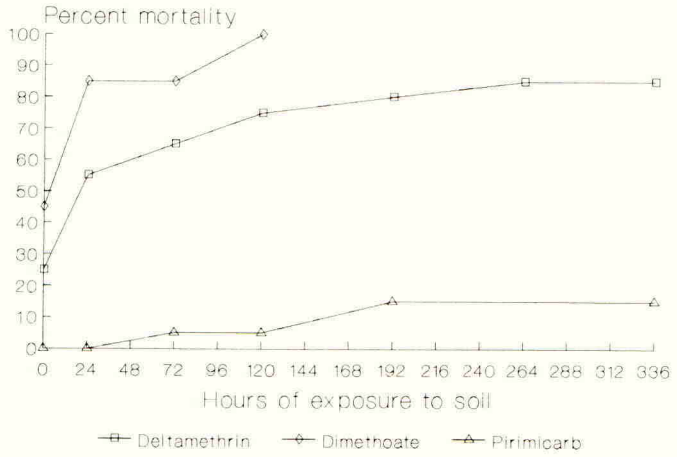
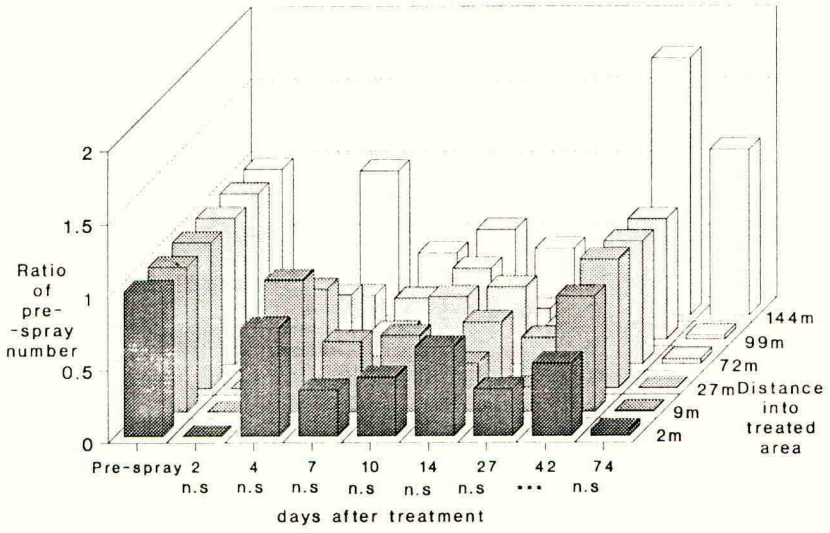


Fig 3. Proportional increase in cereal aphid capture rates in pitfall traps placed within a dimethoate treated plot. Data given for trap collections on a range of dates after treatment at successively greater distances into the sprayed area. (From Duffield and Baker (1990).



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AN EVALUATION OF LABORATORY AND FIELD STUDIES FOR THE ASSESSMENT OF THE ENVIRONMENTAL EFFECTS OF PESTICIDES

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ABSTRACT

Laboratory assays of the effects of pesticides on soil-inhabiting invertebrates involve: injection, topical application, contact, or exposure in natural or artificial soil. They also test effects on soil microorganism populations or biomass. They screen chemicals for toxicity, with a range of doses, under controlled environmental conditions, against uniform test organisms. Similarly the effects of pesticides on nutrient transformations and respiration demonstrates possible adverse effects. However, although laboratory tests are reproducible, they rarely predict the overall field effects of chemicals reaching various soil types with variable climatic conditions, accurately. Field tests can demonstrate "real world" effects in particular locations but provide extremely variable results, dependent upon: dosage and method of application, soil type and structure, degree of cultivation, soil organic matter status, and climatic conditions. Accurate assessment of environmental hazards due to agrochemicals requires an integrated combination of data on their physiochemical characteristics and biological toxicity, together with rigidly controlled laboratory assays, combined with field tests concentrating on the more dominant key organisms and on soil processes such as organic matter breakdown, nutrient turnover and soil respiration, to assess the overall potential environmental hazards. Examples of how environmental hazards can be predicted successfully in these ways are given.

INTRODUCTION

Agricultural production of crops depends upon maintenance of soil structure and fertility, which in turn is dependent upon the biological functioning of complex soil ecosystems. A large proportion of pesticides is applied directly to soil or reaches soil through spray fall out. Hence a knowledge of the impact of any particular pesticides, that is likely to reach soil is essential before the widespread use of the pesticide is permitted.
There is considerable diversity in the ways in which such impacts can be forecast and tests which will enable potential hazards to be predicted successfully. There is an enormous literature on the effects of diverse agrochemicals on soil-inhabiting organisms and dynamic soil processes. The techniques used involve both laboratory and field methods and there is virtually no standardization of methods, of testing, application of pesticides, doses or assessment of effects. Many decisions on pesticide registration have been based on limited data concerning specific groups of organisms or one or two studies. There are few holistic testing methodologies which take account of the effects of pesticides on soil-inhabiting invertebrates, microorganisms and dynamic soil processes

(Edwards, 1988a and 1988b). The aim of this paper is to review briefly, the available methodologies, compare the benefits and shortcomings of field and laboratory testing and discuss how a range of data can be coordinated in a decision-making process.

LABORATORY ASSAYS

Laboratory tests can be used to assess the effects of pesticides on both individual organisms and on soil processes. They are most effective in screening pesticides for potential environmental impacts.

1. Soil Invertebrates

Soil-inhabiting invertebrates are extremely diverse in form, size, numbers and function. They can be pests of plants, predators or parasites of plant pests, or play an important role in the breakdown of organic matter in soil and the recycling of nutrients. It is possible to test the effects of pesticides on populations of invertebrates in intact soil sample microcosms collected from the field and containing a representative range of species of invertebrates. However, there are few advantages of such assays over field tests. Invertebrates can be exposed to pesticides in the laboratory by a range of methods:

Injection of test chemical - The effects of pesticides on soil-inhabiting invertebrates can be tested by injecting chemicals into their bodies or guts. Such methods result in unrealistic exposure, and results are impossible to assess in field environmental terms.
Topical application of chemical - This is often used to screen the effects of chemicals on test animals during the development of new pesticides. It has been adapted for use on carabid beetles and larger invertebrates.
Immersion - The exposure of invertebrates to chemicals by immersion in dilute solutions of pesticides has been used mainly for animals which can survive immersion in liquids for extended periods Many soil-inhabiting invertebrates are exposed to pesticides in the water films, so this test has some relevance to exposure under normal conditions, but is still only useful as a screening technique and results of immersion tests cannot be expressed in terms of environmental hazards.
Exposure to treated surfaces - The pesticide is applied to filter paper, or to a glass surface, and the test animals are exposed to the residue. It is suitable for ranking the toxicity of a range of pesticides but difficult to correlate results with those from exposure in the field.
Exposure in treated soil - Pesticides can be applied to soil and then test animals kept in it for a defined period before assessing mortality. Chemicals become absorbed onto organic matter and clay fractions in soil, and are often bound in a nontoxic form, so the toxicity of any chemical to the test species differs greatly with the soil type to which the chemical is applied. One solution was proposed by Edwards (1983) who prepared a standardized artificial soil consisting of 69% sand, 20% kaolinite clay, 10% sphagnum peat, and 1% calcium carbonate and used this for test. This enabled different pesticides to be tested for toxicity to soil organisms in a much more standardized manner.

Feeding experiments - The effects of pesticides on soil-inhabiting invertebrates can be assessed by treating plant material or artificial diets with the chemical and then feeding it to the test animals. Such experiments produce very variable results and are difficult to relate to field exposure.
Model ecosystem or microcosms - Model ecosystems or microcosms which contain several soil-inhabiting species of invertebrates, belonging to different trophic levels and maintained under relatively natural conditions, have been used extensively to predict the environmental effects of pesticides.

2. Microorganisms

Microbial populations in soil are extremely large with 10^6 to 10^9 bacteria, 10^5 to 10^8 actinomycetes, and 10^4 to 10^7 fungi per gram of soil. Microbes differ greatly in function, being involved in functions such as organic matter breakdown, degradation of pesticides, nutrient cycling, as pathogens of plants, and pathogens of invertebrates.
Culture Techniques - Most soil microorganisms can be cultured on growth media,such as nutrient agar, so it is relatively easy to screen the effects of pesticides on cultures but results are difficult to interpret because this exposure to chemicals in is different from that in nature and cannot predict field results.
Populations - Pesticides can be applied to soil samples collected from the field, and the resulting effects can be assessed by counting populations of microorganisms in the samples at intervals after treatment and comparing them with populations in untreated control samples. However, such an assessment of whole populations of microorganisms is both difficult and time consuming. Microorganisms are more usually counted after they have been grown on synthetic organic media, either liquid (with gelose) or mineral (silicagel). Microorganisms are introduced from inoculum suspensions, diluted with water, into the medium. After a period of incubation, the numbers of microorganisms are estimated from the counts of the colonies on the plates or from an estimation of the highest dilution that permits growth of the microorganisms. Plating methods can provide estimates of the total populations of the major groups of microorganims in treated and control plots and samples. However, dilution counts often lead to errors, and are of doubtful value.

3. Dynamic Processes

The soil biota, consisting of the soil-inhabiting invertebrates and microorganisms, act together interactively in breaking down organic matter, releasing mineral nutrients, and incorporating these materials into the structure of the soil. This overall biological activity in soil is important in maintaining soil fertility and may be affected by chemicals such as pesticides. These key processes and other biological cycles may be vulnerable to pesticides that become introduced into soil.

Laboratory tests include:
Total Soil Respiration - Respiration measurements are probably the most common methods of assessing microbial activity in soil as an index of other soil processes, such as transformations of carbon, phosphorus,

and nitrogen. Measurements of oxygen uptake and carbon dioxide evolution can be made in a large Warburg respirometer, after small soil samples are treated with a pesticide at appropriate doses. One of the most serious criticisms of using respiration measurements to assess the effects of pesticides on soil respiration, is that a nil or small effects may conceal large, counterbalancing stimulation and inhibition of different major parts of the soil microflora.

Biomass - It is possible to assess the effects of pesticides on microbial biomass in the laboratory measurements of ATP and muramic acid. Alternatively, the biomass can be deduced from measurement of the flush of CO_2 evolved after fumigation of soil with $CHCL_3$. The CO_2 evolved comes from carbon mineralization of the bodies of microorganisms killed by the treatment; and enables biomass to be calculated.

Nutrient Transformations - Mineral nitrogen is a major plant nutrient, so there have been many studies on the effects of pesticides on mineralization of soil nitrogen. Ammonification and nitrification, as part of N-mineralization process, are the most useful systems in studying the side effects of pesticides on nitrogen cycles. Ammonification is the indicator for the release of nitrogen bound in organic matter and its availability for plant nutrition. Nitrification, i.e. the oxidation of ammonium to nitrate, is due to sensitive bacteria, and impairment of nitrification may be indicative of harmful influences of pesticides. It is also possible to assess the effect of pesticides on other mineral element transformations such as those of phosphorus and sulfur.

FIELD EXPERIMENTS

The exposure of whole soil ecosystems to a pesticide in the field is the most reliable method of assessing the overall effect of chemical. However, prediction of environmental hazard from field experiments depends on soil type and texture, degree of cultivation, amount of organic matter, and climatic conditions. The effect of pesticides on populations of soil organisms must be compared with those in a standard untreated control, and the treatments and controls replicated adequately. Size of plot must be related inversely to the size of the largest invertebrates being sampled. Plots up to 10m square are adequate for most purposes.

1. Invertebrates

Soil-inhabiting invertebrates differ greatly in form, size, number and habits; so different sizes of samples and methods of separation of invertebrates from soil are necessary for assessing populations. Soil samples can be from 2.5 to 20 cm in diameterand 10 to 15 cm deep.
Most species of arthopods can be extracted from soil in high-gradient Tullgren samples or by flotation, with efficiencies of up to 80%. Nematodes can be extracted from soil in Baermann funnels or by elutriation techniques. Earthworm populations can be sampled in the field by pouring dilute formaldehyde (50 ml in 9 l water) onto the soil surface in a defined quadrant to bring the worms to the soil surface. Populations of surface-living invertebrates can be assessed by wet or any pitfall traps. A drawback to using pitfall traps is that they measure both invertebrate population and activity. However, since they assess functional activity as well, they can provide useful index of

pesticide effects.

2.Microorganisms

Populations of microorganisms can be studied by plate and dilution techniques using soil samples taken from the field experiments.

3. Organic Matter Breakdown

The breakdown of organic matter is the most important process occurring in soils. There are many methods of assessing organic matter degradation including measurement of mineralization, and breakdown of cellulose, straw or leaf tissues burried in soil. All of these methods are suitable for assessment of how pesticides affect the process.

INTERPRETATION OF RESULTS

It is essential that results are interpreted in an integrative way (Figure 1) by considering initially the potential for environmental impact based on physio-chemical practices, recorded biological activity and proposed use

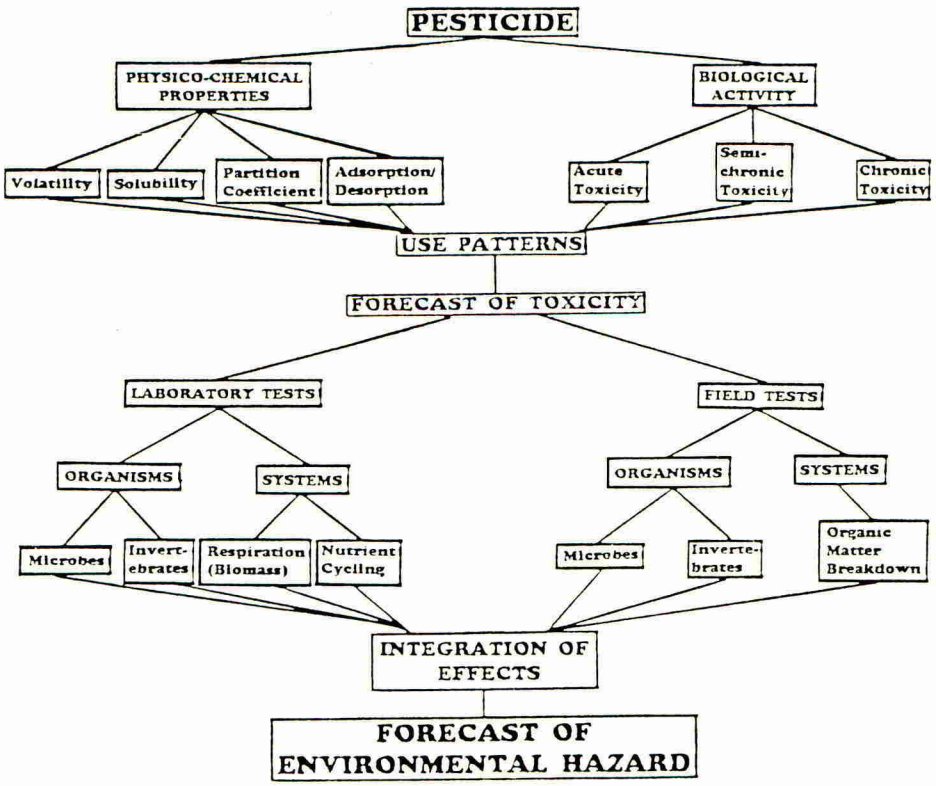

Figure 1. The integration of physicochemical properties, biological activity, laboratory and field tests in forcasting environmental hazards from pesticides.

patterns. Considerations involved must be (i) the potential toxicity to organisms with critical roles in important soil processes or as antagonists to pests and diseases, (ii) the potential effects on overall organic matter degradation and mineral cycling. Finally, the data generated from laboratory and field tests must be integrated to give a balanced forecast of potential environmental hazard. This can be demonstrated best by a practical example, comparing two well-established insecticides carbaryl and diazinon. (Table 1).

TABLE 1 Overall assesment of environmental hazard of two pesticides

	Toxicity to Microbes	Toxicity to Invertebrates	Respiration	Nutrient Cycling	Organic Matter Breakdown
Diazinon	*	**	*	--	--
Carbaryl	**	***	**	*	**

-- no effect * small effect ** intermediate effect *** strong effect

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LAMBDA-CYHALOTHRIN: LABORATORY AND FIELD METHODS TO ASSESS THE EFFECTS ON NATURAL ENEMIES

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ABSTRACT

The effect of lambda-cyhalothrin on a range of natural enemies was investigated in a series of experiments in the laboratory, small enclosures and in the field. Effects were compared to those of the broad-spectrum organophosphate dimethoate and the broad-spectrum carbamate methomyl. Using the laboratory test system of the aphid pest Rhopalosiphum padi and a range of natural enemy types, the broad-spectrum pyrethroid lambda-cyhalothrin was more selective than dimethoate or methomyl to representatives of three groups of natural enemies tested, the syrphid Episyrphus balteatus, the hymenopteran parasite Dacnusa sibirica and the carabid beetle Trechus quadristriatus, but not to the fourth, the linyphiid spider, Lepthyphantes tenuis. Detailed laboratory and field work on the parasite Aphidius uzbekistanicus and key predatory carabids demonstrated only transient effects on these natural enemies following lambda-cyhalothrin sprays (6.25 g AI ha^{-1}), but more prolonged or severe effects with dimethoate (400 g AI ha^{-1}). Linyphiid spiders were affected in the field by both treatments. There were no adverse effects on the aphid population in the summer following an autumn lambda-cyhalothrin spray. These results suggest that, with the exception of spiders, lambda-cyhalothrin is more selective than the broad-spectrum organophosphate and carbamate tested.

INTRODUCTION

Lambda-cyhalothrin (PP321, "KARATE") is a broad-spectrum pyrethroid insecticide which has been shown to be highly effective at very low rates against a wide range of pests, including aphids, beetles and caterpillars. Jutsum et al. (1984) have reported the excellent efficacy of lambda-cyhalothrin against aphids, both as vectors of Barley Yellow Dwarf Virus (BYDV) in the autumn and as leaf and ear pests in the spring and summer. Such treatments have proved highly cost effective and farmers have tended to make prophylactic applications which has led to concerns regarding the possible effects on the predation and parasitism of aphids. A series of laboratory, small plot and field studies were therefore designed to investigate the effects of lambda-cyhalothrin on a range of aphid natural enemies.

Recently there have been suggestions that pyrethroid insecticides are less selective (i.e. less toxic to the pest than the natural enemy) than organophosphates and carbamates. Contrary to this, Pickett (1988) proposed that compared to organophosphates and carbamates, pyrethroids are less toxic to natural enemies than to pests. As a general

hypothesis this cannot be completely true as Hassan et al. (1987) have shown pirimicarb, a selective carbamate aphicide, to be intrinsically more selective to a range of natural enemies than the pyrethroids permethrin, fenvalerate and deltamethrin. A laboratory study was therefore set up to compare the potential selectivity of lambda-cyhalothrin to that of a broad-spectrum carbamate, methomyl and the broad spectrum oganophosphate dimethoate.

This paper discusses the laboratory and field work performed on lambda-cyhalothrin to investigate its toxicity to aphid pests and a range of their natural enemies, compared to a broad-spectrum organophosphate and broad-spectrum carbamate.

LABORATORY STUDIES

Laboratory selectivity studies

The intrinsic toxicity of lambda-cyhalothrin, methomyl and dimethoate to the aphid pest Rhopalosiphum padi and a range of natural enemies was determined. Laboratory MLD values were estimated by applying measured drops of the pesticide solution to the arthropods using a microapplicator. Following the approach of Stevenson et al. (1984), a selectivity ratio was derived by dividing the MLD of the beneficial species by that of the pest, all MLD values were expressed in μg AI g^{-1} of arthropod by dividing by the mean weight of the arthropod. MLD values and selectivity ratios are given in Table 1.

TABLE 1: Relative toxicity of lambda-cyhalothrin, methomyl and dimethoate to an aphid and a range of its natural enemies

Species Treatment	MLD μg AI g^{-1}	Selectivity Ratio MLD Beneficial MLD Pest
Rhopalosiphum padi		
Lambda-cyhalothrin	0.003	-
Methomyl	0.44	-
Dimethoate	0.94	-
Episyrphus balteatus		
Lambda-cyhalothrin	>2200	>790000
Methomyl	4.1	9.5
Dimethoate	0.97	0.67
Trechus quadristriatus		
Lambda-cyhalothrin	22	8100
Methomyl	5.5	13
Dimethoate	15	16
Dacnusa sibirica		
Lambda-cyhalothrin	6.9	2500
Methomyl	2.3	5.2
Dimethoate	3.3	3.5
Lepthyphantes spp. (male)		
Lambda-cyhalothrin	0.10	35
Methomyl	39	89
Dimethoate	58	62

Laboratory and small plot parasite studies

The effects of lambda-cyhalothrin and dimethoate on cereal aphid parasites were investigated in the laboratory and field in France (Krepsi 1985, 1986). Detailed laboratory experiments were conducted on Aphidius uzbekistanicus where four replicates of ten aphid mummies were sprayed (lambda-cyhalothrin 6.25 g AI ha^{-1}, dimethoate 400 g AI ha^{-1}) and four replicates of ten adult parasites (five males and five females) were exposed to insecticide deposits on a glass plate for 10 minutes or one hour. The fecundity of the emerging and exposed adults was assessed (Table 2). Survival of the female parasites following exposure to a sprayed glass plate for one hour is shown in Figure 1. Similar survival curves were obtained for parasites emerging from sprayed mummies and following exposure to a sprayed glass plate for 10 minutes.

In small plot experiments where four replicate 100 m^2 plots were sprayed with lambda-cyhalothrin (6.25 g AI ha^{-1}) and dimethoate (400 g AI ha^{-1}) at field rate, aphids and mummies were collected in the field before and after spraying and cultured in the laboratory where parasite emergence and mortality were assessed. Parasites that had developed into mummies before spraying were unaffected by both treatments. There was no difference between lambda-cyhalothrin and control plots, in the mortality of younger parasites that had not developed into mummies at the time of spraying, but mortality of the parasites developing from aphids collected from the dimethoate plots was significantly greater than in the control or lambda-cyhalothrin plots ($p<0.001$).

TABLE 2: Mean fecundity (recorded as numbers of mummmies formed) of parasites following exposure to a sprayed plate for 10 minutes and one hour and of parasites emerging from sprayed mummies.

Treatment	Sprayed Mummies	Adults exposed to spray deposit (10 mins)	Adults exposed to spray deposit (1 hour)
Untreated Control	49.0	41.5	77.5
Lambda-cyhalothrin	58.5	52.3	62.8
Dimethoate	1.5*	0*	0*

* Significantly different to all other treatments $p<0.02$ (Mann-Whitney)

FIELD EXPERIMENTS

Autumn applications of lambda-cyhalothrin as "KARATE", to control the aphid vectors of BYDV were made in early November 1986 and 1987 to winter wheat at Beedon, near Newbury, Berkshire, U.K. Full details of the experiment are given in Brown et al. (1988). Applications were made at field rate (5 g AI ha^{-1}) to large plots at least 4 ha in size. There were four replicate treatments in the first year and three in the second. In the second year, dimethoate was applied at field rate (400 g AI ha^{-1}) as a positive control for comparison.

FIGURE 1: Survival of female Aphidius uzbekistanicus following exposure to spray deposits for one hour.

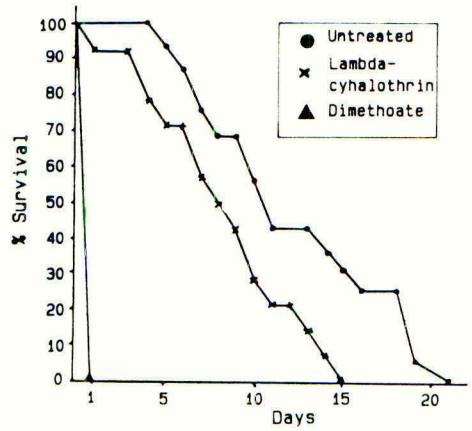


FIGURE 2: Abundance of Trechus quadristriatus adults.

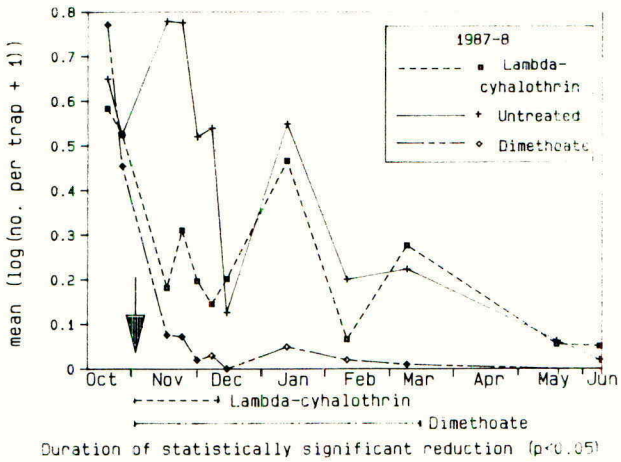
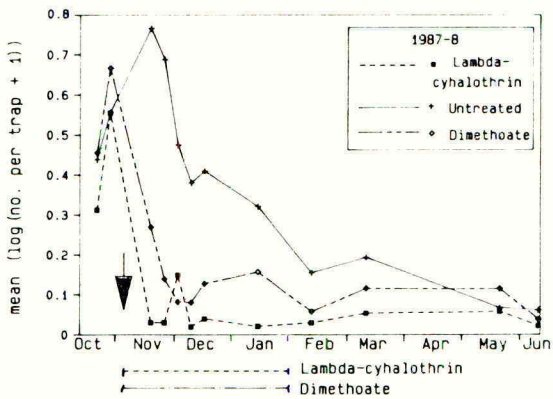


FIGURE 3: Abundance of Lepthyphantes tenuis adults.



Observations of the abundance of predatory arthropods were made using ten pitfall traps positioned centrally in each plot. Abundance of T. quadristriatus is shown in Figure 2. Abundance fell for about four weeks after spraying lambda-cyhalothrin, then returning to levels on the control plots. After spraying dimethoate, abundance fell and did not recover before the following summer. For the linyphiid spider L. tenuis (Figure 3) depressions in abundance were of a similar magnitude and duration after spraying both dimethoate and lambda-cyhalothrin.

Small enclosure studies with the carabids, T. quadristriatus, Nebria brevicollis and Bemdidion obtusum which are key aphid predators (Sunderland et al. 1987), showed only B. obtusum was susceptible to lambda-cyhalothrin while N. brevicollis and T. quadristriatus were not. Both N. brevicollis and B. obtusum were susceptible to dimethoate.

Aphid populations were monitored in the spring and summer following the autumn spray. Numbers of aphids along a transect of 100 tillers in each plot were counted. There was no difference in the development of aphids populations in the two treated plots compared to the control.

DISCUSSION

Lambda-cyhalothrin was selective to three of the four groups of natural enemies tested in the laboratory, the syrphid E. balteatus, the representative parasite D. sibirica and the carabid T. quadristriatus, but less selective to the linyphiid spiders Lepthyphantes spp.

Small plot and field studies conducted with lambda-cyhalothrin and dimethoate only, showed broad-spectrum pyrethroids were more selective than the broad-spectrum organophosphate dimethoate to key carabid species and the cereal aphid parasite A. uzbekistanicus. Following summer applications of lambda-cyhalothrin (6.25 g AI ha^{-1}) to 1 ha plots in France, no effects on egg laying behaviour of syrphids and only transient effects on carabid and staphylinid beetles were observed (ACTA 1985). Little selectivity to spiders was observed in the laboratory with either lambda-cyhalothrin or dimethoate. This was borne out in the field where L. tenuis abundance was similarly depressed by both treatments (Figure 3) laboratory data suggesting this is attributable to mortality. Other workers (Thomas et al. 1990) have shown that pyrethroids, such as deltamethrin, are not selective to spiders.

CONCLUSION

These data suggest the broad-spectrum pyrethroid, lambda-cyhalothrin is inherently more selective than the broad-spectrum organophosphate (dimethoate) and the broad-spectrum carbamate (methomyl) to three of the four groups of natural enemies tested in the laboratory and to two of the three groups of natural enemies tested in both the laboratory and field. In both the laboratory and the field, lambda-cyhalothrin was shown to exhibit some selectivity to hymenopteran parasites of cereal aphids and to most key carabid species, while dimethoate did not. Neither compound was selective towards linyphiid spiders in the field.

With the exception of spiders, the hypothesis that pyrethroids are more selective than organophosphates and carbamates is supported by these laboratory and field data on broad-spectrum compounds, but not with respect to selective ones such as pirimicarb.

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THE EFFECTS OF TEFLUTHRIN ON TERRESTRIAL NON-TARGET ORGANISMS.

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ABSTRACT

The effects of tefluthrin following its application as a granule or seed treatment have been assessed against the soil microflora, earthworms, beneficial arthropods, birds and mammals.

An exceptional feature of the environmental profile of tefluthrin as a granular insecticide is its low toxicity to birds. Birds may be exposed by feeding in treated fields and accidentally ingesting granules. Even small, vulnerable birds such as passerines would need to consume in excess of 4000 granules to experience an MLD, whereas larger birds such as ducks would have to ingest up to 3 million to reach such a level. Seed feeding birds would have to consume more than their own bodyweight in wheat seed to reach an MLD.

There were no effects on the soil microflora following an even incorporation of tefluthrin to give 1 mg AI kg soil^{-1} (equivalent to the excessive field rate of 1 kg AI ha^{-1}); populations of earthworms in the field after a band application of granules at the rate of 112.5 g AI ha^{-1} were unaffected. The effects on surface-dwelling beneficial arthropods in sugar beet are minimal and transitory.

At application rates suitable for the control of a wide range of soil pests, tefluthrin gives an exceptional margin of safety to a wide range of terrestrial non-target species.

INTRODUCTION

Tefluthrin is a highly effective soil insecticide (Jutsum et al., 1986) and has been shown not to suffer from loss of efficacy due to accelerated degradation when applied to soils which are known to exhibit accelerated degradation of carbofuran (Bewick et al.,1986). It is currently registered for use formulated as a granule (0.5% and 1.5% active ingredient) for application at rates up to 112.5 g AI ha^{-1} and as a seed dressing for sugar beet (12 g AI 100,000 seeds^{-1}) and wheat (20 g AI 100 kg^{-1} seed). Tefluthrin is readily degraded in soil (half-life c. 1 month) and neither it nor its degradation products are likely to leach (Bewick et al.,1986). The only anticipated significant exposure

route to organisms is from them feeding on or contacting the granules or treated seed. As both tefluthrin granules and treated seed are incorporated into the soil, the main organisms to be potentially exposed are soil-dwelling organisms such as earthworms, soil arthropods and the soil microflora. In addition, birds and mammals could be exposed to seed or granules spilled on the soil surface. Its safety to a variety of non-target terrestrial organisms, when applied as a granule or seed treatment, has been investigated. Laboratory studies have been carried out on the soil microflora, earthworms, bees, mammals and birds with additionally a field investigation on earthworms of a band application of granules to maize and on beneficial arthropods after in-furrow granule application and seed treatment in sugar beet.

STUDIES ON DIFFERENT ORGANISMS

Soil Microflora

In a laboratory study tefluthrin, as an aqueous emulsion was evenly incorporated into a sandy loam and a loam soil. Rates of application were 0.1 and 1.0 mg AI kg^{-1} soil (approximately equivalent to field applications of 0.1 and 1.0 kg AI ha^{-1}). An investigation of the effects on ammonification/nitrification, carbon turnover and soil microbial populations (bacteria, fungi and actinomycetes) was conducted. Procedures for investigating these microbially mediated processes followed the guidelines given by The 3rd International Workshop on Soil Microflora (Somerville et al., 1985). Microbial populations were determined by removing samples of soil at intervals post-treatment and assessing numbers of bacteria, fungi and actinomycetes by plate counts.

There were no effects on the turnover of nitrogen and carbon in the two test soils attributable to the tefluthrin treatments. No significant differences (P=5%) in microbial populations were detected between tefluthrin treated and control soils after incubation for 80 days.

Tefluthrin, even at the excessive rate of 1 kg AI ha^{-1}, had no effects on the abundance and activity of the soil microflora. These results indicate that effects from tefluthrin under field conditions would not occur.

Earthworms

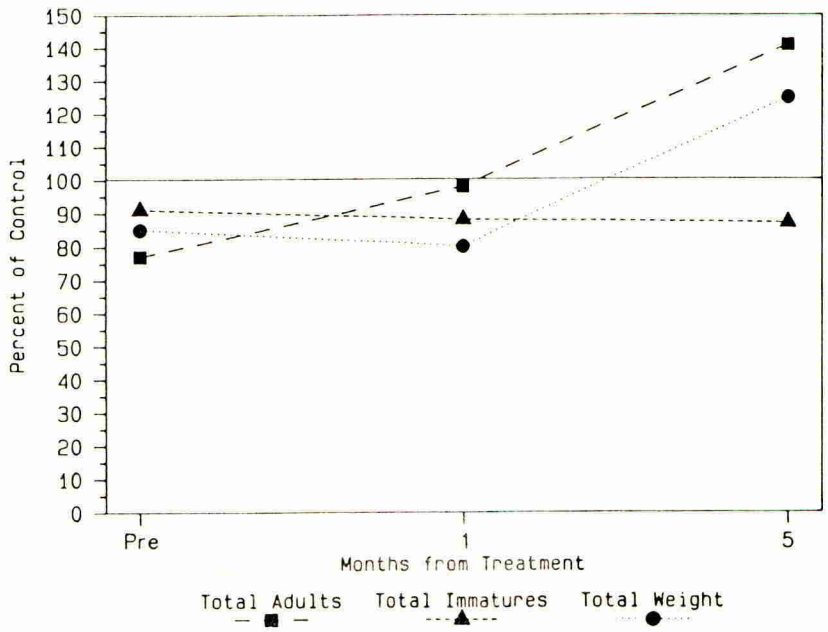
A laboratory and a field study have been conducted to investigate effects on earthworms (Lumbricidae). The laboratory study exposed the earthworm Eisenia foetida for 28 days to a range of concentrations of tefluthrin as technical material evenly incorporated into an artificial soil. This test was based on OECD guidelines (OECD, 1984). E. foetida were assessed for effects after 7, 14 and 28 days.

The laboratory study indicated that tefluthrin has a

relatively high toxicity to earthworms (2.0 mg AI kg^{-1} soil). Hence, in order to determine hazard in the field it was necessary to examine effects from an in-use field application where a typical exposure would occur. The field study exposed populations of earthworms to granules containing 1.5% tefluthrin, applied in 18 cm wide bands 75 cm apart, at a rate of 112.5 g AI ha^{-1}. Earthworms were sampled pre-treatment and 1 and 5 months post-treatment from tefluthrin treated and untreated plots using a formalin expellent method similar to that described by Raw (1959). As the granules were band-applied, sampling areas straddled these bands and included a representative portion of "untreated" soil between bands.

Results from the field test for sampling of the abundance of adult and immature earthworms and total bodyweight are presented in Figure 1 as ratios of numbers from the tefluthrin plots compared to the control numbers. Earthworms were identified to species and grouped into the two main genera Allolobophora and Lumbricus and numbers of adults and immatures of each genera and identified species were compared between untreated and tefluthrin treated plots. No significant reductions (P=5%) due to the application of tefluthrin occurred. This is probably due to the low application rate needed for effective soil pest control and the fact that band application is only affecting the area in the band around the seed leaving large numbers of earthworms unexposed in the "untreated" areas between bands.

Figure 1: Numbers and Weights of Earthworms from Tefluthrin Treated Plots Compared to Untreated Plots.



Beneficial Insects

In the laboratory, technical tefluthrin was tested on honey bees (Apis mellifera) to assess its acute oral and contact toxicity according to the methods described by Stevenson (1978). In the field, a detailed investigation of the effects of tefluthrin applied in-furrow in sugar beet as a 0.5% AI granule (50 g AI ha^{-1}) and as a seed treatment (10 g AI ha^{-1}) on beneficial arthropods was conducted and is reported elsewhere (Dewar et al., 1990).

The 24 hour contact and oral MLD values to bees were 0.28 and 1.88 ug ai bee^{-1}, respectively. In the laboratory, tefluthrin shows a high level of acute toxicity to bees, similar to that of other pyrethroids. However, granules and treated seed are soil applied and hence bees will not be exposed to tefluthrin and there will be no risk.

Tefluthrin applied in-furrow as a granule or seed treatment had only transient effects on beneficial arthropods. Where effects occurred, these tended to be immediately after drilling. Fewer effects were seen for the seed treatment; this is probably due to the low rate and the more discrete application of the seed treatment.

Mammals

Rats were dosed orally with tefluthrin as technical material and formulated as a 0.5% granule and as a 20% capsule suspension as used for seed treatment (all seed treatments are intended to be formulated in a similar manner). Mice were tested similarly with technical material only.

The acute oral MLD value for tefluthrin technical material to the rat and mouse was 22-35 and 46-57 mg AI kg^{-1}, respectively. MLD values to the rat for tefluthrin formulated as a 20% capsule suspension and as a 0.5% granule were greater than 400 and 10 mg AI kg^{-1} (the highest doses tested), respectively. Thus, it can be seen that formulating tefluthrin for seed treatment reduces its acute toxicity to the rat by at least one order of magnitude and it is realistic to assume to other mammals in a similar manner. Mammals are unlikely to ingest tefluthrin granules even if available to them and hence no risk is envisaged. However, they may feed on treated seed and it is known that woodmice (Apodemus sylvaticus) will unearth and eat sugar-beet seed. Woodmice open sugar-beet pellets and feed on the seed only (Green,1979). As the tefluthrin is present in the coat and not the seed, they will thus only ingest a small amount of the dose and not be affected. Using the MLD to the mouse of 46 mg AI kg^{-1} and increasing this ten-fold for the formulation effect it can be calculated that a 20 g woodmouse would have to eat over twice its bodyweight in treated wheat seed to achieve an MLD. This is unlikely to occur and hence tefluthrin-treated seed is unlikely to be a hazard to mammals even to small, vulnerable species such as the woodmouse.

Birds

Hazard to birds can be assessed using acute oral MLD values. These were determined for a typical passerine bird, the House Sparrow (Passer domesticus), a game bird, the Bobwhite Quail (Colinus virginianus) and a species of waterfowl, the Mallard Duck (Anas platyrhynchos). Birds were dosed orally with tefluthrin. In addition, LC50 values were derived for the Mallard Duck and Bobwhite Quail. Birds were offered food containing different concentrations of tefluthrin. Treatments were offered for 5 days and observations continued for 4 and 6 days post-treatment for quail and ducks, respectively.

The acute oral MLD values for the House Sparrow, Bobwhite Quail and Mallard Duck were 267, 734 and 4190 mg AI kg^{-1}, respectively. Sub-acute LC50 values to chicks of Mallard Ducks and Bobwhite Quail were 2317 and 15000 mg AI kg^{-1} diet, respectively.

The hazard to birds from the use of tefluthrin is best assessed by calculating the number of treated seeds or granules needed to be consumed to achieve an MLD. Table 1 shows this information for granules containing 1.5% AI (the most concentrated formulation and hence worst case, mean weight per granule of 0.10 mg) and wheat seed (20 g AI applied to 100 kg of seed, assuming each seed weighs 30 mg). Birds are not known to feed on pelleted sugar-beet seed and hence there will be no risk expected.

TABLE 1. Number of granules and seeds required to reach an MLD for three avian species.

Species	Bodyweight (g)	Number of items to reach an MLD	
		Granule	Wheat seed
Mallard Duck	1,000	2,800,000	700,000
Bobwhite Quail	200	98,000	24,500
House Sparrow	25	4,450	1,112

In several countries, the effects of soil-active insecticides on birds is an area of environmental concern; as a result, modification of application techniques and changes in formulation have been sought to reduce avian exposure and hence risk. Tefluthrin granules show a large safety margin and present an excellent way of reducing avian risk and will be especially useful where exposure cannot be lowered sufficiently by application or formulation technology. Sixteen granular insecticides were tested by Balcomb (1984) against wild-caught

songbirds. Fourteen of these insecticides caused mortalities when birds were dosed with forty or less granules. Tefluthrin granules compare very favourably with those tested in this study.

Tefluthrin-treated seed also shows a large safety margin to birds. Birds will not be able to ingest a lethal dose from seed feeding. They are not known to feed on sugar-beet seed where furthermore the tefluthrin is present in the unpalatable coating. Even a small bird would have to consume over a thousand wheat seeds to reach an MLD. Consumption of an MLD from tefluthrin granules or treated seed is unlikely and hence tefluthrin exhibits an exceptional margin of safety to birds.

CONCLUSION

Tefluthrin has been shown to have no adverse effects on a range of field-resident organisms and exhibits an exceptional margin of safety to birds.

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A COMPARISON OF A LABORATORY RESIDUAL TOXICITY TEST WITH A SEMI-FIELD 'TUNNEL' TRIAL TO ASSESS THE EFFECTS OF PESTICIDE RESIDUES ON HONEY BEES.

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ABSTRACT

A study is described which was designed to assess the effects of the residues of a pyrethroid insecticide, lambda-cyhalothrin, on honey bees (Apis mellifera). A laboratory residual toxicity test and a semi-field 'tunnel' trial were used in order that the results of the two tests could be compared. The laboratory residual toxicity test gave an extremely severe result, probably due in large part to the unnaturally prolonged and continuous exposure and stress that the bees experienced under the test conditions. The results were also highly variable, thus limiting the ability of the test to predict field hazard. The results of the laboratory test were not reflected in the 'tunnel' trial: there was no increase in mortality but some limited effects on foraging activity. The possible reasons for these results are considered, particularly in relation to the exposure of honey bees to pesticide residues. The implications for assessing the hazard of pesticides to honey bees are discussed.

INTRODUCTION

The 24-hour acute contact LD50 value of the pyrethroid insecticide, lambda-cyhalothrin ("KARATE") to honey bees (Apis mellifera) is 0.051 ug AI bee^{-1} (Gough & Wilkinson, 1984). It is thus classed as 'highly toxic' according to the toxicity classification of the International Commission for Plant-Bee Relationships. However, a series of field and semi-field or 'tunnel' trials (Gough & Wilkinson, 1984; Wilkinson et al, 1986) have shown that lambda-cyhalothrin is of low hazard to honey bees at rates of up to 15 g AI ha^{-1} when applied to flowering oilseed rape or to cereals sprayed with simulated honeydew (sucrose solution), even when the bees are actively foraging.

The effects on honey bees of lambda-cyhalothrin on lucerne (Medicago sativa) were assessed in a procedure analagous to that of Lagier et al (1974). The purpose of this test is to measure the toxicity of field weathered residues to honey bees in the laboratory. However, the results suggested a level of residual toxicity inconsistent with the results of the field and semi-field trials.

The study described here was designed to assess the effects of field-weathered lambda-cyhalothrin residues on honey bees using the laboratory residual toxicity test and a semi-field 'tunnel' trial. This was so that the results of the two tests could be directly compared and in particular to account for any differences observed, so aiding the hazard assessment process.

LABORATORY RESIDUAL TOXICITY TEST

Materials and methods

Two plant species, oilseed rape (Brassica napus) and lucerne were used in the laboratory residual toxicity test. Oilseed rape is a flowering crop highly attractive to honey bees (which is necessary for the 'tunnel' trial) and on which most field data regarding lambda-cyhalothrin is available. Lucerne was also included to allow comparison with the data from the previous laboratory residual toxicity test which used this crop.

Two tests were conducted based on the method of Lagier et al (1974). In each, three strips of oilseed rape and two of lucerne each measuring 10 m x 2.5 m were treated using a one-man hand-held plot sprayer. The plants were mature but not in full flower. With both crops, one strip was sprayed with lambda-cyhalothrin at a rate of 35 g AI ha^{-1} and another left untreated as a control. The third oilseed rape strip was treated at a rate of 15 g AI ha^{-1}. After residue ageing periods of 3, 8, 24, 48 and 96 hours, foliage samples were collected for bioassay: only the top halves of the plants were sampled so as to ensure no dilution effect from the lower, sheltered leaves. The oilseed rape samples were cut into pieces approximately 65 x 30 mm, while the lucerne samples were cut into approximately 50 mm lengths. Half litre portions of the chopped foliage were put into ventilated cages: these were constructed of a wire-mesh cylinder, 50 mm high, 140 mm diameter, with the top and bottom formed by 150 mm petri dishes. Thirty bees were added to each cage, there being three replicate cages for each treatment. The cages were supplied with sucrose solution in cotton wool, as food for the bees. This was located at the bottom of the cage so that the bees had to crawl through the foliage to reach it. Any effects on the bees were assessed after 24 hours of exposure, in darkness at 25 °C, to the residues.

Results

The change in percentage mortality of the bees, exposed to the various treatments, (corrected for control mortality) were plotted against time. From these curves, regression analysis was used to estimate the age of residue which was lethal to 50% (LT50) and 25% (LT25) of the bees in the test system (a linear regression model was found give the best fit). These estimates together with the 95% confidence limits are presented in Table 1.

SEMI-FIELD 'TUNNEL' TRIAL

Materials and Methods

The trial was based on a method developed in France by INRA (Delabie J, 1984). Oilseed rape only was used: a plot 50 x 40 m was grown, according to normal agricultural practices. One half was planted about six weeks later than the other in order to ensure an adequate supply of flowering plants for two successive replicate tests. Plots of the rape were enclosed by tunnel greenhouse frames, covered with a plastic mesh sufficiently fine to keep bees inside whilst still permitting weathering. The layout of each tunnel is shown in Fig 1.

TABLE 1. Estimates of the residue age (hours) lethal to x% (LTx) of bees in the residual toxicity test.

Treatment	Test	LT50 (95% confidence interval) / hours	LT25 (95% confidence interval) / hours
Rape/35 g AI ha^{-1}	1	11.5 (0.02-7640)	154 (0.02-1.42x10^{6})
	2	0.2 (0.04-1.03)	30.4 (0.1-9650)
Rape/15 g AI ha^{-1}	1	0.13 (0.04-0.41)	3.9 (0.12-123)
	2	0.0 (0.0-1.43x10^{46})	0.0 (0.0-1.18x10^{14})
Lucerne/35 g AI ha^{-1}	1	56.1 (0.002-1.72x10^{6})	542 (0.001-4.35x10^{8})
	2	44.7 (0.002-1.1x10^{6})	4756 (0.0002-1.05x10^{11})

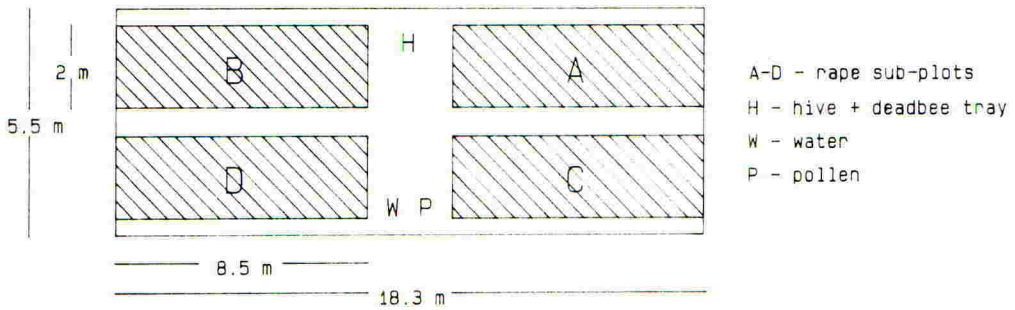
A single, queenright colony of honey bees was placed inside each tunnel, as shown, and left for up to one week to acclimatise to the new surroundings. Due to the limited foraging area available, supplementary food was supplied throughout the bees' confinement within the tunnel (sucrose solution in a contact feeder on the top board of the hive and dried pollen in a dish in front of the hive).

Treatment took place in the early morning, using a one-man hand-held plot sprayer, about one hour before the bees emerged, c. 07.30 h (this was ensured by closing up the hives the night before and then opening them up one hour after application). There were four tunnels each receiving a seperate treatment: control (water only), lambda-cyhalothrin at rates of 35 and 15 g AI ha^{-1} over all four sub-plots (A-D) in a tunnel and lambda-cyhalothrin at a rate of 35 g AI ha^{-1} over two sub-plots (B an D) only. The last treatment was designed to look at any differences in foraging between the treated and untreated ends. There were two successive replicate tests.

Assessments of the bees were carried out pre and post treatment. Mortality and behavioural assessments of the bees were carried out at the end of the first week (the acclimatisation period), for several days prior to treatment and were continued for up to a week after treatment. Hive inspections were carried out in the week prior to treatment and at about two and five weeks after.

Mortality in the hive and the tunnel was monitored by daily counts of dead bees from "dead bee trays" placed at the entrance to each hive and from polythene sheets placed along the longitudinal and transverse paths in each tunnel respectively. Foraging activity was assessed by counting the bees along a 1 m wide strip of each 8.5 m sub-plot: the observer walked alongside each sub-plot holding a 1 m cane horizontally to define the strip and to provide a reference point for counting, taking 90 seconds to cover the 8.5 m. All 4 sub-plots were counted twice, the first time counting bees in the air (flying), the second time counting bees settled on the flowers (and also noting any in contact with the rest of the plant). Activity was also monitored at the hive with observations, lasting 5 minutes, of bees at the

FIG. 1 TUNNEL LAYOUT



hive entrance and foragers departing and returning. The foraging and hive observations were made hourly on treatment day and every two hours on all other days. The state of the colony was monitored by a full inspection of the hive recording the approximate numbers of the different brood stages and the amount of stored food by visual counts.

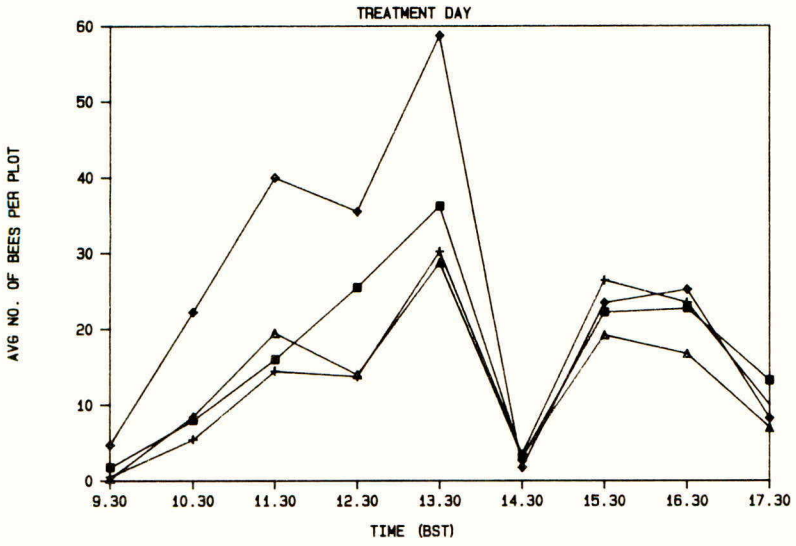
Results

There was no significant difference between mortality in the control and treated tunnels. The foraging results for treatment day are presented in Fig. 2 (total bees present - flying and on the plants) and Fig. 3 (percentage of total on the flowers). Results are shown for one of the tests only, the results for the two tests being similar. There is a reduction in the total number of bees recorded for about half a day after spraying as well as a reduction in the percentage of the foragers settling on the plants. Very few bees were seen on the foliage as distinct from the flowers. No harmful effects were seen in the hives from the lambda-cyhalothrin treatments when compared with the controls.

DISCUSSION

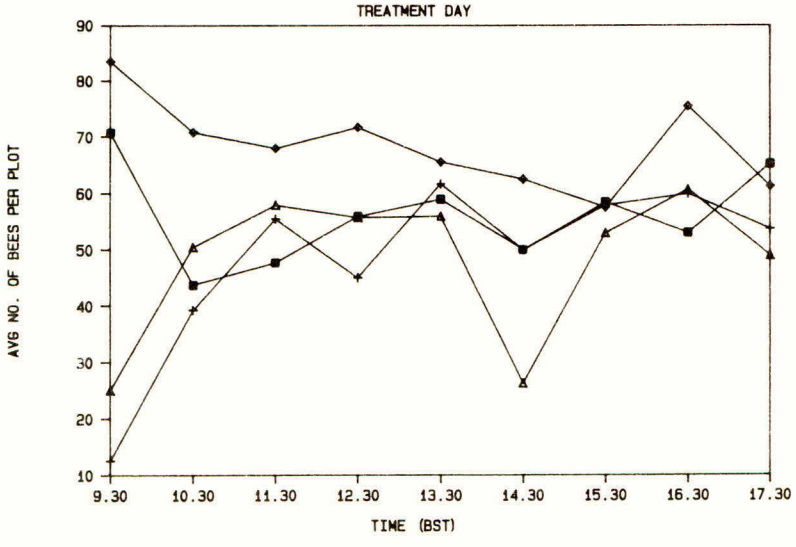
The laboratory residual-toxicity test is undoubtedly extremely severe and gives a highly variable result. The results indicate that there is a high level of toxicity on the day following treatment and marked toxicity for several days. The residue levels in this test are field derived and are therefore of the magnitude that foraging bees might normally encounter. However, in the laboratory cages the bees experience a very high degree of exposure throughout the test period, having to crawl through the chopped foliage for 24 hours. Also, they are unable to respond behaviourally as they might normally do in the field e.g. show repellancy, thereby reducing their exposure to the residues. In addition, the results themselves show a large amount of variability such that it is not possible to make any predictions from them with any degree of confidence. Thus the 95% confidence limits for the estimated LT50 and LT25 values vary in some cases from a few minutes to several years. This variability comes into the system at several stages, notably in the field (pesticide application, weathering and sampling of the residues) and in the laboratory (exposure, stress and response of the bees). Consequently, because of this high variability in the results, the ability of the test to predict field hazard is limited.

FIG. 2 TEST 2 FORAGING ASSESSMENTS - TOTAL
(BEES ON PLANTS + BEES FLYING)



◇ (CONTROL) + (15 g ai/ha) ■ (35 g ai/ha) △ (35 g ai/ha : 0)

FIG. 3 TEST 2 FORAGING ASSESSMENTS - PERCENTAGE
(BEES ON PLANTS / TOTAL BEES)



◇ (CONTROL) + (15 g ai/ha) ■ (35 g ai/ha) △ (35 g ai/ha : 0)

The results of the laboratory test were not manifested in the 'tunnel' trial, there being no increase in mortality. In this semi-field environment exposure is more realistic, with free flying bees operating from a hive and so subjected to minimal stress. It is still more severe than a full field experiment as they are confined to an area containing only treated plants. However, compared to the laboratory residual toxicity test, exposure of the bees to any pesticide residues will be much less. As the foraging results indicate, the proportion of a bee's time actually in contact with the plants is small as much of it's time will be spent in the air or in the hive. Further, most of this time on the plant is spent in contact with the flowers, not the foliage, and their turnover will further reduce pesticide exposure. Repellancy, shown both as a reduction in numbers of foragers and as a decrease in the proportion of foragers in contact with the plant, will also reduce exposure.

It is clear then that whereas the laboratory residual toxicity test indicates high toxicity over a significant period of time the hazard measured in the 'tunnel' trial is low. Similar contrasting results have been found in other studies (Atkins et al., 1977). This discrepancy is probably due to the excessively high levels of exposure experienced in the laboratory test compared to the field environment where the bees' contact with the residues is limited. The laboratory residual toxicity test is therefore potentially misleading if used in isolation to assess hazard and not as part of a sequential testing scheme, as has been realised with other laboratory derived toxicity data. However, the test may have improved value if the sprayed foliage is not chopped up but placed in a flight cage where exposure will be more realistic (Murray, 1985). This could give an indication of behavioural effects such as repellancy. Where laboratory studies indicate toxicity though, any realistic assessment of hazard must involve studies under field or semi-field conditions.

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THE EFFECTS OF TEFLUTHRIN ON BENEFICIAL INSECTS IN SUGAR BEET

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ABSTRACT

Tefluthrin applied as a seed treatment to sugar-beet pellets has very little impact on non-target beneficial organisms in sugar beet crops. Applied as a granule, it had some transient effects on carabid adults and larvae, spider adults and immatures, staphylinids, and symphypleone collembola, but much less than that of carbofuran and aldicarb, and very much less than gamma-HCH sprays which had pronounced effects on most arthropod groups studied. Tefluthrin seed-treatment or granules can therefore be used as alternatives to existing insecticides for soil-pest control in sugar beet with minimal effects on terrestrial beneficial organisms.

INTRODUCTION

Approximately two-thirds of the sugar-beet crop in the U.K. is treated at drilling with insecticide sprays or granules to control a wide range of pests (Dewar & Cooke, 1986). Much of this usage is as an insurance against possible attack. Recent developments in insecticidical seed treatments have offered the possibility of providing cheaper insurance protection against soil arthropod pests (Winder, 1990), which may have other environmental benefits. Tefluthrin, in particular, has shown good efficacy against the soil-pest complex in sugar beet in trials in the U.K. (Winder, 1990) and the continent (Dewar, 1989). As part of the evaluation of this insecticide prior to its introduction to the sugar-beet industry, the effects of tefluthrin on non-target organisms, when applied as a seed treatment or as a granule, were compared to three other insecticides, aldicarb, carbofuran and gamma-HCH, which are commonly used in sugar beet. This paper presents a summary of the more important findings from the vast amount of data collected.

MATERIALS AND METHODS

Trial design

The study, consisting of 6 treatments replicated 4 times in randomised blocks, was carried out at Broom's Barn Experimental Station in 1988. To minimise the difficulties associated with migration of insects between plots, they were surrounded by polythene barriers immediately after drilling. The barriered plots were 48 rows by 10m ($240m^2$). Herbicide and fertiliser applications were made possible by the provision of tramlines in discard areas between the blocks.

Insecticide Treatments

Sugar-beet seed, cv. Gala, treated with or without tefluthrin at 10g AI/unit (1 unit = 100,000 seeds), and also with standard fungicides, by Germaine's (UK) Ltd, was drilled on 12 April. Granular formulations of aldicarb (at 760g AI ha^{-1}), carbofuran (at 600g AI ha^{-1}) and tefluthrin (at 50g AI ha^{-1}), were applied with untreated seed at drilling using a Horstine Farmery "Microband" applicator mounted on the drill. A spray formulation of gamma-HCH (at 1.12kg ha^{-1}) was applied one day prior to drilling using a 3m two-man modular CO_2-pressurised boom delivering 200 l ha^{-1} through six Lurmark 02-F110 nozzles. This insecticide was then incorporated into the surface soil layers (top 5cm) using a dutch harrow in such a way as to minimise soil transfer between plots.

Assessments

Comparisons of treatment effects were made using pitfall traps, 'artificial' prey (*Drosophila* pupae) placed on cards, and direct counts on plants (e.g. of aphids). Only the pitfall trap results are presented in this paper.

RESULTS

The most severe deleterious effects on predatory groups were recorded in plots treated with gamma-HCH. The number of adult carabids caught in pitfall traps was significantly reduced by this treatment in the first six weeks after application (Fig 1a), but not thereafter (Fig.1b). The effect on carabid larvae was considerable, reducing their numbers to very low levels for 10 weeks after drilling (Figs. 1c & d). Staphylinid adults, spider adults (most of which were linyphiids) and immatures were also significantly reduced immediately after application (Figs. 1d, 2a & 2c) and transiently up to two months after treatment (Figs. 1e, 2b & 2d). Of the other non-target groups, Acari were severely reduced for up to 13 weeks after treatment (Figs. 2e & 2f), as were arthropleone Collembola (Figs. 3c & 3d). There were no effects on aphid-specific predators, dipteran larvae, earthworms (Figs. 3a & 3b) and symphypleone Collembola (Figs. 3e & 3f).

The carbamates, aldicarb and carbofuran, had less deleterious effects, but nevertheless significantly affected some groups. Carbofuran reduced the number of carabid adults and larvae for up to seven weeks after application (Figs. 1a - d) but had no effect on staphylinids (Figs. 1e & 1f) or on linyphiid spiders (Figs.2a-d). In contrast, aldicarb had little effect on carabid adults and only transient effects on their larvae, 2, 6 and 10 weeks after application (Figs 1a & b); staphylinids were only significantly reduced 2 weeks after drilling, and spider adults after 13 weeks. Both carbamates severely affected Acari (Figs 2e & f), arthropleone Collembola (Figs. 3c & d) and even symphypleone Collembola, the latter more than nine weeks after drilling (Figs. 3e & f). Carbofuran had longer lasting effects on all of these groups. In addition, earthworms were significantly reduced by both carbamates, on several trapping occasions up to 12 weeks after application (Figs. 3a & b). There were only minor transient effects of either carbamate on dipteran larvae.

Tefluthrin had the least effect of all insecticides on non-pest organisms, especially when applied to the seed pellet. The seed treatment had no effect on carabid adults or

larvae, only a minor transient effect on staphylinid adults, 2 weeks after drilling (Fig. 1e), and no effect on linyphiid spider adults or immatures, predatory Acari or arthropleone or symphypleone Collembola, dipteran larvae or earthworms (Figs. 1-3). Tefluthrin applied as a granule had some effects soon after drilling but these were mostly transient. Carabid adult numbers were reduced 2 weeks after application and again after 12 and 13 weeks (Figs. 1a & b). Carabid larvae were reduced significantly on only one occasion 4 weeks after drilling, although there were consistently fewer in tefluthrin-treated plots for 6 weeks (Fig.1c). Staphylinid beetles were also transiently affected 2 weeks after drilling but recovered quickly. Similarly, linyphiid adults, but not immatures, were decreased, though not significantly, immediately after drilling (Figs. 2a & 2c); numbers of Acari were consistently less throughout the sampling period, significantly so 7, 11, 12 and 13 weeks after treatment (Figs. 2e & f), but there were no consistent effects of tefluthrin granules on arthropleone or symphypleone Collembola, earthworms or dipteran larvae (Fig.3). As Broom's Barn farm is relatively free from sugar-beet soil pests there were no effects of treatments on plant stand, or, consequently, yield.

DISCUSSION

In this study, tefluthrin, applied as a seed treatment, had minimal deleterious effects on the main beneficial arthropod groups and other non-target organisms resident within sugar-beet crops. In addition to these environmental benefits it has proven efficacy against sugar-beet soil pests and is very safe to birds (Coulson, *et al,* 1990). As a granule applied at much higher rates, tefluthrin had greater effects on a wider range of species than the seed treatment, as might be expected from an insecticide which is applied along furrows, instead of discretely around the developing seedling; however, these effects were transient.

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FIGURE 1. The effects of insecticides applied to sugar beet at drilling on carabids and staphylinids: TGr - Tefluthrin granules; TST - Tefluthrin seed treatment; HCH - x-HCH; Car-Carbofuran: Ald - Aldicarb; Cntl - Control

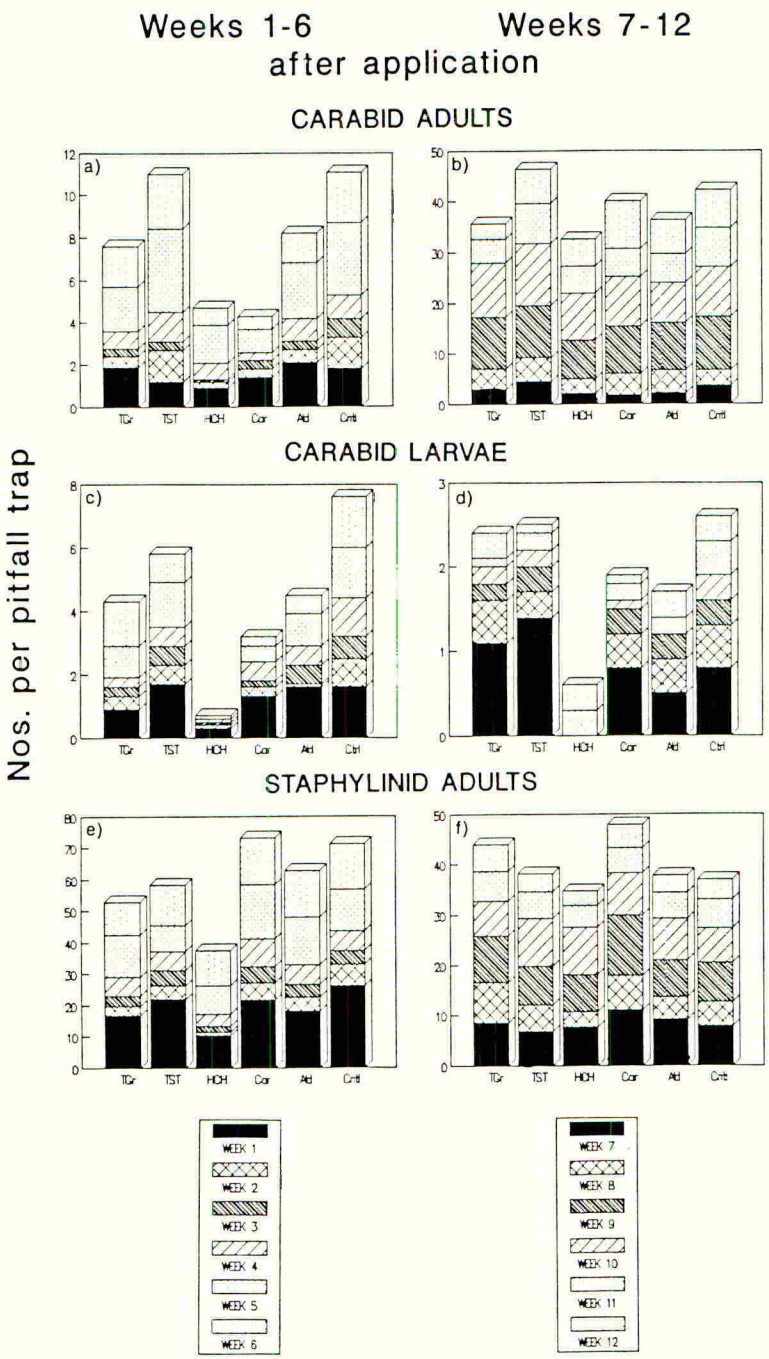


FIGURE 2. The effect of insecticides applied to sugar beet at drilling on spiders and acarid mites: TGr - Tefluthrin granules; TST - Tefluthrin seed treatment; HCH - x-HCH; Car-Carbofuran: Ald - Aldicarb; Cntl - Control

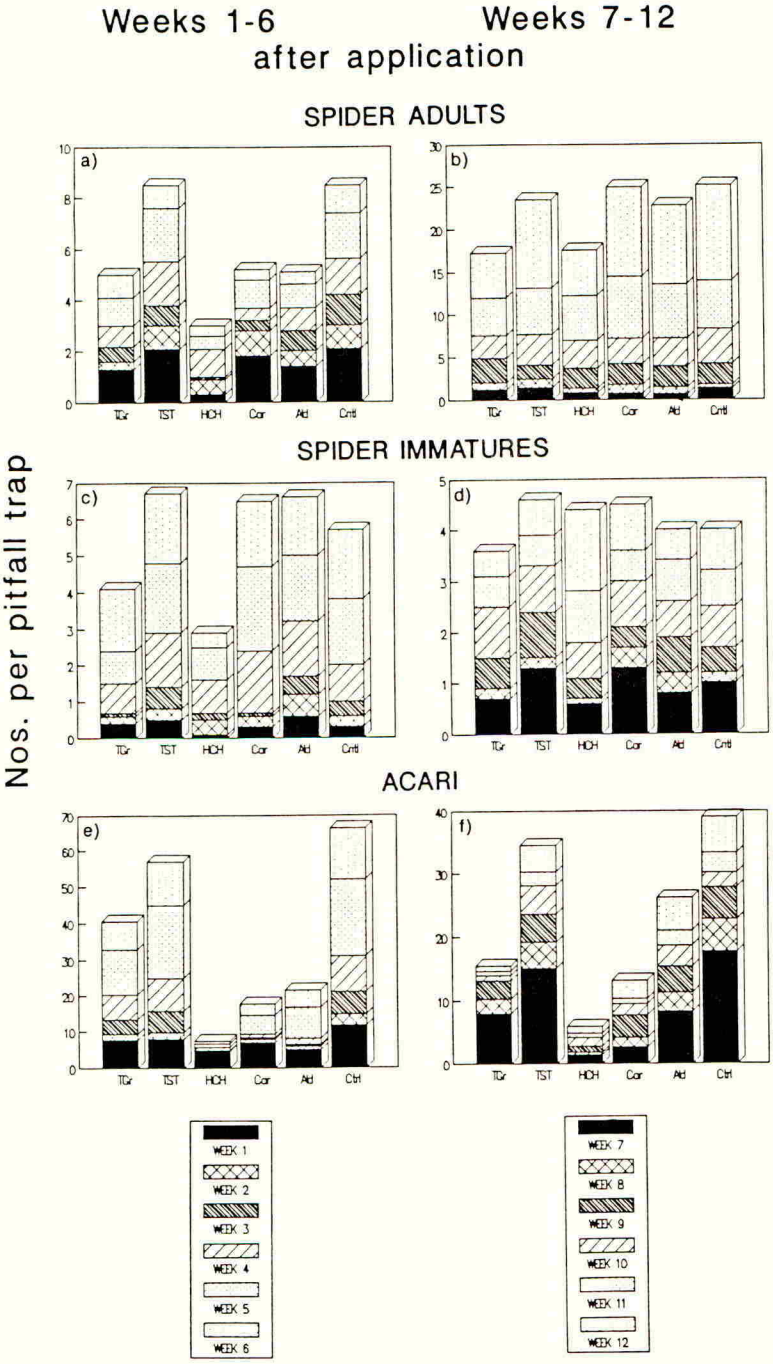
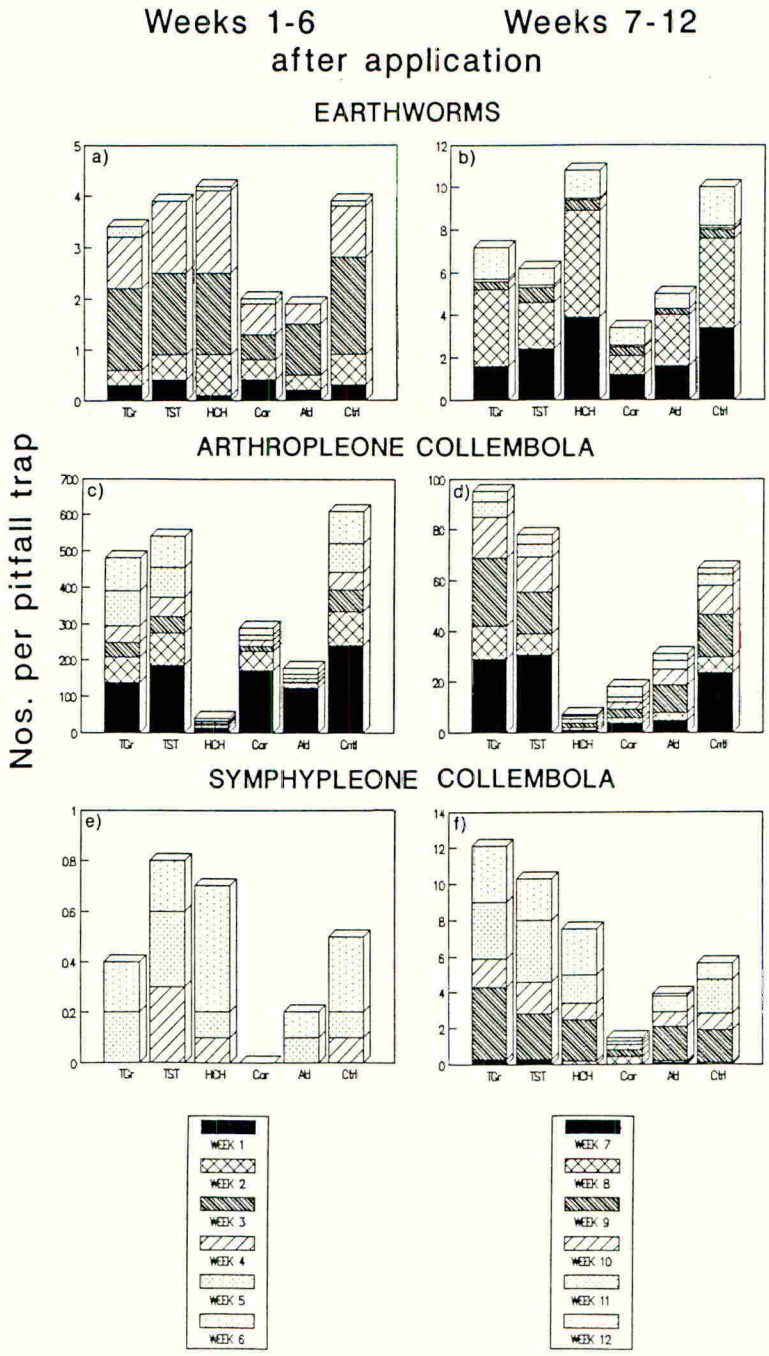


FIGURE 3. The effect of insecticides applied to sugar beet at drilling on earthworms and Collembola: TGr - Tefluthrin granules; TST - Tefluthrin seed treatment; HCH - x-HCH; Car-Carbofuran: Ald - Aldicarb; Cntl - Control



DEVELOPMENT OF A METHOD TO DETERMINE THE TOXICITY OF FOLIAR RESIDUES OF A BENDIOCARB FORMULATION TO THE WORKER HONEYBEE

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ABSTRACT

A novel test method by which the toxicity of pesticide foliar residues to honeybees may be assessed is described.

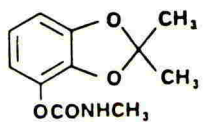
Glasshouse cultivated alfalfa plants are sprayed with the test compound and maintained outdoors under prevailing weather conditions, samples of the treated foliage are harvested at timed intervals and worker honeybees are exposed to this foliage in a laboratory study.

Using this method the foliar residue toxicity of a bendiocarb formulation was assessed. The results indicated residues reached a 'no-effect level' 7 days after spraying. The results of this study are reported and discussed.

INTRODUCTION

Bendiocarb is an insecticide effective against a wide range of insect pests. Under laboratory conditions bendiocarb has been shown to be toxic to honeybees with an oral LD_{50} of 0.1µg/bee.

Figure 1 Bendiocarb



2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate.

Turcam(R) is a wettable powder formulation containing 75% by weight bendiocarb. It has been developed for use in the USA on ornamental trees and plants to control pests such as aphids, whitefly, blackfly and florida wax scale. As honeybees could be exposed to bendiocarb residues, a study was designed to determine the toxicity of bendiocarb to bees from foliage residues derived from use of the above formulation.

METHODS

Treatment of the foliage

Alfalfa was selected as the source of foliage material, as the flowering plants are readily foraged by honeybees. Alfalfa has also been used in previous studies of the toxicity of pesticide residues to honeybees (Johansen et.al, 1977; Lagier et.al, 1974).

A 250g sample of alfalfa seed and 70g of Rhizobium innoculant were obtained from Elsoms Seeds Limited, Spalding, Lincolnshire, UK. Rhizobium was mixed with the alfalfa seed in order to induce rapid nodulation of the roots and therefore facilitate the fixation of nitrogen ensuring healthy plant growth. The Rhizobium treated seed was sown in sterilised John Innes potting compost in 9cm square pots. After approximately four weeks under glasshouse conditions the alfalfa had grown to a height of approximately 20cm and flower heads were developing. At this stage the plants were hardened off over a period of a few days and then kept permanently outdoors.

Bendiocarb treatment was carried out in the laboratory. Each pot of alfalfa was individually sprayed using a hand held air-driven spray gun, operated at a pressure of 15 psi, in a ventilated spray tower. Pots of plants were sprayed with either a solution of bendiocarb at a rate of 11oz/100 US gallons water (equivalent to 823.9mg/l) or water alone, as a control. All plants were sprayed to 'run off' as recommended in the application instructions. Approximately 12ml of solution were applied to each pot to achieve this. Bendiocarb was applied at the application rate recommended for the treatment of ornamentals to control thrips, azalea caterpillar, sycamore lacebug, oleander aphid and oleander caterpillar.

After treatment the plants were placed outdoors where they were maintained until used in the study. When necessary plants were watered to avoid wilting. Bottom watering was carried out to avoid 'artificial' wetting of the leaf surfaces.

Exposure apparatus

The exposure apparatus is shown in Figure 2. It comprised an outer aluminium mesh cage 46cm high, 29cm wide and 27cm deep, with a perspex roof and a front-opening perspex door. The cage was large enough to allow free flight of the bees without causing stress. The floor of the cage was lined with Whatman No. 1 grade filter paper to absorb any excreted material.

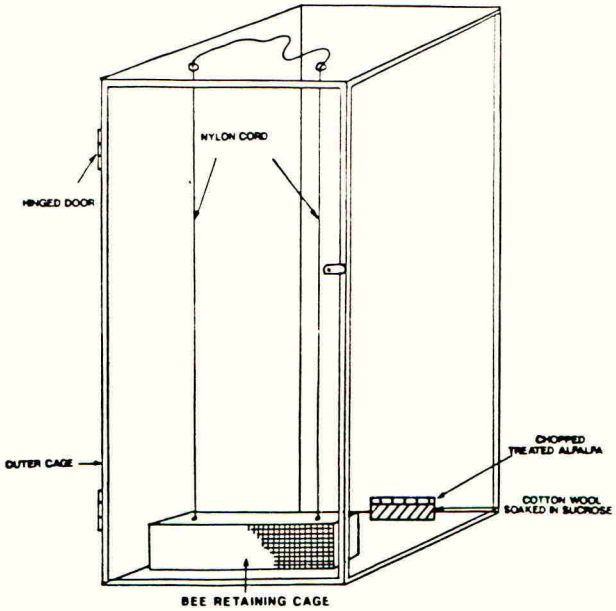
A smaller bee retaining cage, with an open base, was placed on the floor of the outer cage. This was constructed of stainless steel mesh and measured 24cm x 9cm x 5cm deep. Nylon cords were attached to the top of the bee retaining cage. These passed up through the top of the outer cage, and were used to raise the retaining cage thus releasing the bees for exposure. The test system was validated prior to use in the study.

Exposure of bees to the treated foliage

Adult worker honeybees (Apis mellifera L.) were collected each morning of use from free foraging hives. The bees were anaesthetised with carbon dioxide gas and batches of 100 were placed under each of the retaining cages. A cellulose pad saturated with 50% sucrose in water was placed on top of the retaining cage to provide an ad libitum source of food for the bees over the anaesthetic recovery period of approximately 2 hours.

During this period samples of the bendiocarb and water treated alfalfa foliage was harvested. The foliage from six control or bendiocarb treated pots was removed with scissors, chopped, combined and thoroughly mixed. From this prepared sample 7.5g aliquots ($50cm^3$ loose volume) was removed and spread over a layer of cotton wool saturated with 50% sucrose solution contained within a 9cm diameter glass petri dish.

Figure 2 Diagram of exposure apparatus



One petri dish containing either treated or control foliage was then placed on the floor of each outer cage. The bees were released by lifting the retaining cage using the nylon cords. Treatments were carried out in duplicate. Any bee mortalities or bees showing adverse effects were recorded at this time. 'Time 0' effects were therefore attributed to handling procedures prior to exposure.

Honeybee exposure was carried out in a constant environment room at 25°C ± 1°C in the dark, at a relative humidity of approximately 50%. Assessments of bee mortality and sub-lethal effects were made 18, 24 and 48 hours at the 3 hour and 1 day post-treatment time intervals, and after 24 and 48 hours at the 3, 7 and 14 day post-treatment time intervals.

RESULTS

The toxicity of bendiocarb residues on alfalfa declined rapidly with time from 100% mortality at the 3 hour post-treatment time interval to 0% at the seven day post-treatment time interval. It is assumed this was the result of rapid loss and/or metabolism of the applied insecticide. The number of mortalities recorded at each observation time at post-treatment exposure intervals is recorded in Table 1.

TABLE 1 Honeybee mortalities following exposure to treated and untreated foliage

a) Control

Foliage post-treatment harvest time	Cumulative mortalities/exposure time/treatment replicate							
	0 hours		18 hours		24 hours		48 hours	
	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)
3 hours	0	1	8	2	9	2	9	3
1 day	0	1	0	1	0	1	0	1
3 days	3	3	-	-	7	4	7	6
7 days	4	1	-	-	5	1	5	1
14 days	2	4	-	-	2	5	2	5

b) Bendiocarb

Foliage post-treatment harvest time	Cumulative mortalities/exposure time/treatment replicate							
	0 hours		18 hours		24 hours		48 hours	
	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)
3 hours	2	3	2	6	98	96	100	100
1 day	3	0	18	33	82	92	98	98
3 days	2	4	-	-	6	10	10	88
7 days	0	2	-	-	0	3	0	3
14 days	1	3	-	-	2	3	2	4

A few honeybees did not recover after anaesthesia. These mortalities were recorded as time 0 casualties. On no occasion did the number affected in each cage exceed 4% of the total population, and it is therefore considered these mortalities in no way affected the validity of the tests.

Table 2 shows the average percentage mortality per treatment at each observation and exposure time. These figures are based on the number of bees alive at the time of exposure initiation.

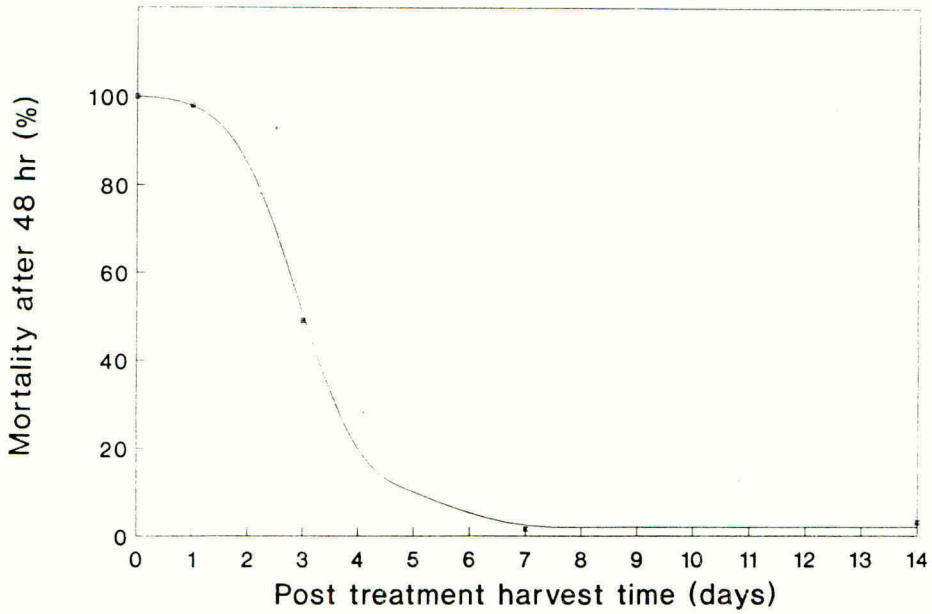
TABLE 2 Mean percentage mortality following exposure to treated and untreated foliage

Foliage post-treatment harvest time	Percentage mortality/treatment/observation time (hours)							
	0		18		24		48	
	Control	Bendio-carb	Control	Bendio-carb	Control	Bendio-carb	Control	Bendio-carb
3 hours	0.5	2.5	4.5	1.5	5.0	96.9	5.5	100
1 day	0.5	1.5	0	24.4	0	88.3	0	98.0
3 days	3.0	3.0	-	-	2.6	5.2	3.6	47.4
7 days	2.5	1.0	-	-	0.5	0.5	0.5	0.5
14 days	3.0	1.0	-	-	0.5	0.5	0.5	1.0

A plot of 48 hours percentage mortality against post-treatment time interval (Figure 3) indicates a steep decline in the toxic response observed, with residues reaching a no-effect level 7 days after spraying.

In addition to the acute toxic effect observed at the 3 hour and 1 day post-treatment time intervals, it was evident that the bees were repelled from the bendiocarb treated foliage. When the caged bees were observed after 18 hours exposure, the remaining live bees appeared to congregate away from the petri dish containing the treated foliage, a behaviour pattern which was quite unlike that observed in the controls. Repellency from the sole source of food may have indirectly led to a higher mortality rate in the bendiocarb treatments, brought on by honeybee starvation. This behaviourial observation should be taken into consideration when assessing the possible impact of this treatment on honeybees during normal use. It is possible that bees would actively avoid sprayed areas and therefore reduce exposure during the period of greatest foliar residue toxicity.

Figure 3 Plot of mean replicate 48 hours mortality figures against foliage post-treatment time interval



DISCUSSION

This method of determining the toxicity of foliar residues to bees proved to be relatively easy to carry out. Results from replicates were generally in good agreement. Minimal control and pre-treatment mortalities were observed indicating the bees were not unduly stressed by the test system. The study also indicated the potential influence of behaviourial responses which should be taken into account when predicting effects in the field from laboratory generated data.

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THE EFFECTS OF SIX INSECTICIDES USED IN UK CEREAL FIELDS ON SAWFLY LARVAE (HYMENOPTERA: TENTHREDINIDAE)

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ABSTRACT

A semi-field, manipulative experiment was carried out in June 1990 in southern England whereby batches of sawfly larvae (Hymenoptera: Tenthredinidae) were caged onto areas of spring wheat sprayed two hours earlier with either water or solutions of six insecticides at recommended field rates. These included two recently approved synthetic pyrethroids. This family of insects are important in the diet of wild gamebird chicks and are thought to be susceptible to broad-spectrum insecticides.

Pirimicarb proved to be the least toxic compound (32% mortality after 6 days) whereas the organophosphate compounds and the pyrethroids produced mortality rates of between 89% and 100%.

INTRODUCTION

The use of insecticides to control aphids in UK cereal fields in the summer has increased over recent years (Rands *et al.*, 1988). In a recent survey of UK cereal farmers conducted in 1988 by The Game Conservancy and the Department of Biology at Southampton University, over 55% of the 115,000 ha surveyed were treated with aphicides in the summer (Wratten & Mann, 1988). Over 60% of the treated area was sprayed with broad-spectrum organophosphate compounds often at levels of aphid infestation below ADAS damage thresholds. In 1988/89 the area of UK cereals treated with foliar insecticides (autumn and summer applications) increased by 87.2% over the previous year (Anon, 1990).

In 1982 the UK Advisory Committee on Pesticides recommended a moratorium on the summer use of pyrethroid insecticides because of a lack of data on the spectrum of activity of such compounds against a range of non-target invertebrates and because of fears about the toxicity of such compounds in the aquatic environment. Concern was also expressed about the potential wide scale use of such broad-spectrum products on a major UK field crop. Finally, such a restriction was thought necessary because of fears about cross-resistance potential with currently used organophosphate products. In 1990, this moratorium was partially lifted when two products, alphacypermethrin and deltamethrin, were given provisional approval for one year for summer use.

Much work has described the impact of pyrethroids upon the natural enemy complex of pests in UK cereals in the summer (Inglesfield, 1985; Shires, 1985; Cole *et al.*, 1986; Vickerman *et al.*, 1987). However, little work has been carried out to assess the impact of pyrethroids on the guild of insects of importance in the diet of farmland vertebrates. The chicks of the grey partridge (*Perdix perdix* L.) feed on a range of mostly phytophagous species in cereal fields in June-July (Southwood & Cross,

1969; Potts, 1986). As obligate insectivores for the early weeks of life they are susceptible to pressures (starvation, increased susceptibility to disease, predation, etc.) caused by reduced levels of insect feeding following the use of pesticides, leading to low levels of chick survival (Potts, 1986).

This paper describes a small, manipulative semi-field experiment in which six currently approved insecticides including the two provisionally approved pyrethroids were examined to discover the extent of their insecticidal activity against one of the most important chick food groups: the larvae of sawflies (Hymenoptera:Symphyta:Tenthredinidae).

MATERIALS AND METHODS

Insecticide application

The trial was conducted in a field of spring-sown wheat (cv Tonic) undersown with rye-grass on a farm on the Hampshire - Dorset border in southern England. Applications were made at GS 61 (Zadoks *et al.*, 1974) on 26 June 1990, between 11.10 am and 13.30 pm. The six insecticides were used at recommended field rates. Details are given in Table 1. The compounds were applied with a Oxford Precision Sprayer using a 2 m boom (medium nozzles) held about 70 cm above crop height. The sprayer was calibrated to deliver spray solutions at 2 bar, at a volume rate equivalent to 208 l/ha and at an average walking speed of 1.03 m/s. Applications of tap water were made to control plots prior to the six insecticides which were applied in the order given in Table 1. Conditions during spraying were dry and still with $^{5}/_{8}$ cloud cover, 20°C and 78% r.h.

TABLE 1. Details of the six insecticides and their rate of application (g AI/ha) used against sawfly larvae, in plots of spring wheat, Hampshire, June 1990.

AI	Product name	Rate (g AI/ha)
pirimicarb	Aphox	140.0
alphacypermethrin	Fastac	15.0
deltamethrin	Decis	6.25
phosalone	Zolone	490.0
demeton-S-methyl	Metasystox 55	121.8
dimethoate	BASF Dimethoate 40	340.0

Experimental design and analysis

Sawfly larvae (third-fourth instar *Dolerus* spp.) were collected by sweeping from a nearby field of rye-grass. An unbalanced randomised block design was used in which each treatment plot measured 2 x 10 m. Seven replicated treatments were incorporated into six blocks. Three of the blocks contained all seven treatments (six insecticides and water controls). A shortage of sawfly larvae meant that the remaining three

blocks only contained three treatments, namely the water control, the toxic standard (dimethoate) and the selective standard (pirimicarb). The success of the experiment required that the caging technique worked well to confine larvae within the treated areas and that pesticides were being delivered to the plots accurately, hence the use of the control or check plots only in the remaining three blocks.

Caging

Two hours after the plots were sprayed, cages were set up in the middle of each plot and 18 larvae introduced into them. Cages consisted of cylinders of transparent plastic (1 m high x 30 cm diameter) placed over an area of the crop. The tops were sealed with muslin and soil was banked up around the cylinder bases. After three days the cages were dismantled and larvae (alive and dead) were recovered. Live and moribund larvae were returned to the laboratory and fed uncontaminated wheat leaves for a further three days. Percentage survival was expressed as the number of actively feeding larvae present six days after spraying. In these experiments larvae were exposed to the insecticides via both surface residues and the ingestion of contaminated material. The larvae were never directly sprayed.

Analysis was conducted by two-way ANOVA in an unbalanced randomised block design using arc sine-transformed data, and pair-wise comparisons of the treatments carried out using the Tukey-Kramer procedure (Sokal & Rohlf, 1981).

RESULTS

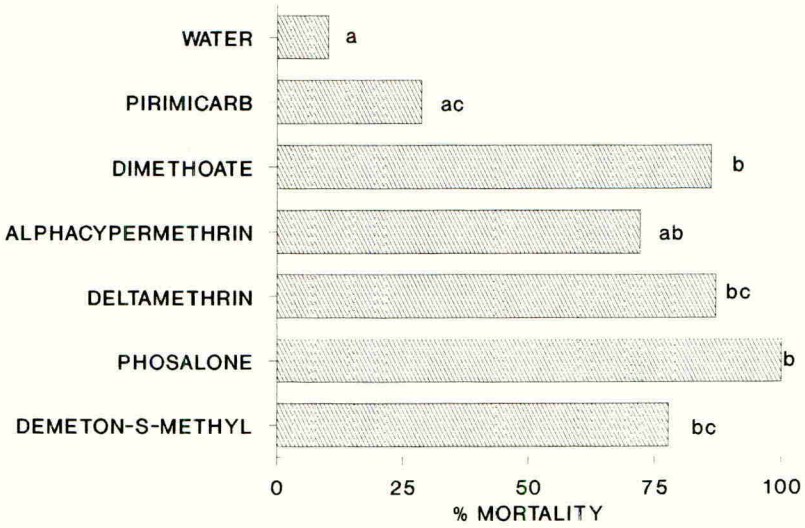
Mortality rates among batches of sawfly larvae treated with either water or solutions of six insecticides are presented at two dates post-treatment (Figures 1a & 1b).

Control larvae were well confined within the cages and very few live larvae (11 individuals of the original 108) could not be recovered. Subsequently missing larvae from treated plots were assumed to be dead even though not every dead body could be recovered after three days' field exposure. After three days, larvae exposed in field cages sprayed with the three organophosphate compounds (dimethoate, phosalone and demeton-S-methyl) and the two pyrethroids (alphacypermethrin and deltamethrin) suffered losses ranging from 72-100%. In contrast, larvae sprayed with pirimicarb suffered only a 29% loss (Figure 1a). It should be noted that, at three days although mortality under deltamethrin was slightly higher than under dimethoate, the difference between pirimicarb and deltamethrin was not significant whereas that between pirimicarb and dimethoate was. This was a direct consequence of the replication for dimethoate being twice as high as for deltamethrin (see experimental design).

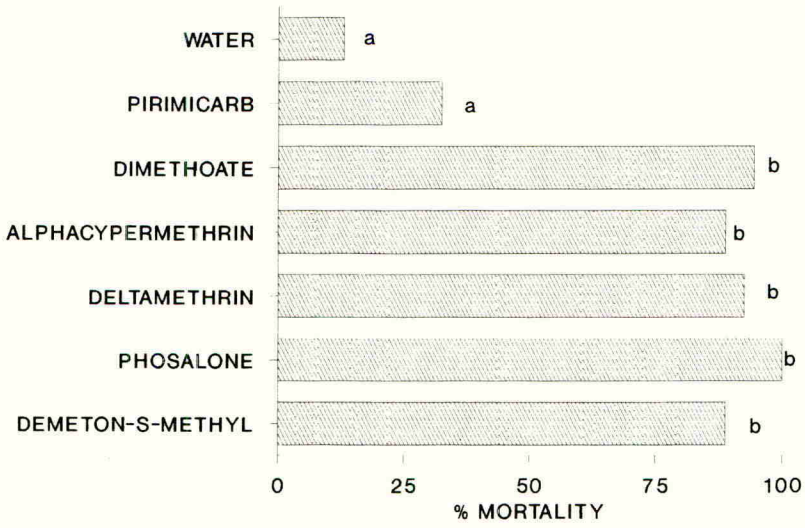
Many treated larvae recovered from the field were either moribund, exhibited abnormal movements, or stopped feeding. Losses estimated after a further three days showed that mortality among control and pirimicarb treated larvae were only slightly increased and that statistical differences between these two treatments could not be detected (Figure 1b). In contrast mortality among other treated larvae had risen to between 89% and 100%. All larvae treated with pyrethroid or

FIGURE 1. Mean mortality rates of batches of sawfly larvae exposed in caged areas of a spring wheat crop sprayed with either water or solutions of six insecticides at recommended field rates, Hampshire, June 1990. Columns with the same letter do not differ at the 5% level of significance.

a) After three days



b) After six days



organophosphate suffered significantly higher rates of mortality than those treated with water or pirimicarb (Figure 1b). Despite the differences in replication mentioned for the data collected after three days, after six days' assessment, all treatments gave significantly higher mortalities compared to pirimicarb.

DISCUSSION

This trial confirmed the broad-spectrum activity of organophosphate insecticides against chick-food insects (Vickerman & Sunderland, 1977; Sotherton, 1989; Aebischer, 1990). These data also confirmed the relative selectivity of pirimicarb against these beneficial species and now completes our knowledge of the impact of cereal aphicides on all the major groups of chick-food insects (Sotherton, 1989; in press). The need to continue assessment of larval mortality beyond the times of field exposure was clearly demonstrated. This was necessary to overcome any confusion arising from the characteristic knockdown and recovery phenomena of some species exposed to type II pyrethroids such as alphacypermethrin and deltamethrin. In the sawfly larvae, all individuals exhibiting lack of coordination or partial paralysis later died.

For all insecticides, including pirimicarb, the levels of sawfly mortality recorded in this trial were likely to have been underestimated. Larvae were not directly treated as would have been the case under more rigorous field conditions. Confirmation of these effects will require modification to the cage design and are urgently required.

ACKNOWLEDGEMENTS

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A COMPARISON OF LABORATORY, LYSIMETER AND FIELD STUDY TECHNIQUES TO DETERMINE ENVIRONMENTAL FATE OF PESTICIDES IN THE U.K.

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ABSTRACT

A wide range of soil physical characteristics, hydrological pathways and climatic conditions occur in the U.K. This means that laboratory and lysimeter studies on 'standard' soils, as well as more comprehensive field studies on the environmental fate of fungicides, molluscides or insecticides, are likely to be relevant only to specific and often limited situations. Knowledge of the range of soil and climatic conditions within the potential usage area of a product to be investigated can be used to select the most appropriate type of and conditions for environmental fate studies, so as to ensure their relevance in the U.K.

INTRODUCTION

Environmental fate studies in soil and water systems often comprise an important component of the final submission on safety of a pesticide to a registration authority. Two important aspects investigated are the degradation and leaching of a compound and studies can be carried out in the laboratory or the field. The aim of carrying out such studies is to simulate and predict the behaviour of a compound in the field situation, under a range of climatic and soil conditions.

Information on the spatial distribution of soil types in England and Wales, together with their physical and chemical properties is held by the Soil Survey and Land Research Centre (Carter 1989). Over 700 different soil types have been identified (Clayden and Hollis 1984) and since their agroclimatic (Jones and Thomasson 1985) and hydrological (Boorman and Hollis 1990) properties vary, a wide range of field conditions can occur. Some common U.K. conditions are, as yet, not considered by any regulatory body. In the light of this, the relevance of laboratory, lysimeter and field studies to the U.K. situation is discussed against the environmental background of potential usage areas.

SOIL AND AGROCLIMATIC CONDITIONS IN THE U.K. RELEVANT TO THE ENVIRONMENTAL FATE OF PESTICIDES

Most molluscides and some insecticides are applied directly to the soil. Other insecticides and most fungicides are applied to crops, but inevitably a proportion of the compound reaches the soil surface. Once within the soil their fate depends on the interaction between their inherent physico-chemical characteristics and a number of soil and climatic factors.

In the field, soil organic matter, as measured by organic carbon

content, varies according to clay content, moisture regime and land use. Average organic carbon contents for two topsoil textures under different moisture regimes and land uses are given in Table 1. The data is based on analyses from 6,000 topsoil samples taken at 5 km grid intersects throughout England and Wales.

TABLE 1. Variations in topsoil organic carbon content with texture, land use and soil moisture regime.

Texture	Moisture Regime	Arable	Temporary Grassland	Long term Grassland
Loamy sand	Free draining	1.1	1.8	2.3
Loamy sand	Seasonally wet	1.8	2.5	2.8
Clay loam	Free draining	2.5	3.2	4.2
Clay loam	Seasonally wet	2.5	3.1	4.1

Soil organic matter has a strong positive correlation with the physical adsorption of most pesticide compounds (Nicholls 1988). Because of this, soil/water partition coefficients (K_D, mL/g), as measured in batch equilibrium experiments, can be used to derive an accepted comparative measure of compound mobility (K_{OC}) by correcting them for the organic carbon factor (Gustafson 1989). However, in the field, a pesticide compound of given relative mobility as measured by K_{OC}, will be more mobile, and therefore more likely to move out of the soil zone, in free draining sandy soils than in seasonally wet loamy ones. On the same soil type it will be more mobile under continuous arable than under a rotational system that includes short or long term leys.

The relationship between the volume of water held and the soil suction from saturation to wilting point is known as the soil moisture characteristic. This varies according to particle size distribution, organic matter content and density and thus changes vertically and horizontally within soils. Seasonal changes in topsoil moisture content can have large effects on the degradation potential of a pesticide (Walker 1978). In addition, the moisture content of the soil when the pesticide is applied determines the concentration of the compound in the soil water fraction following partitioning. For a given soil type, topsoil moisture contents vary according not only to season and weather pattern but also density. Under arable cropping, density tends to have a cyclic variation from low/medium to medium/high. Such changes in soil density influence the amount of water retained at increasing soil water suctions (Fig. 1).

Temperature has a determining effect on microbial activity and hence the ability of a soil to degrade compounds. The half life of Propyzamide in topsoil with a moisture content of 9.8% ranges from 264 days at a temperature of 5°C, to 16 days at a temperature of 30°C (Walker 1978).

In the U.K. topsoil temperatures are very similar to air temperatures, the main differences occurring during the autumn and winter months when monthly averages of mean daily topsoil temperatures may be up to 1°C higher than their equivalent air temperatures. The year to year variation in mean monthly temperatures can be large, but in most U.K. arable situations, monthly average minimum daily temperatures rarely fall below 0°C, whereas monthly average maximum daily temperatures rarely

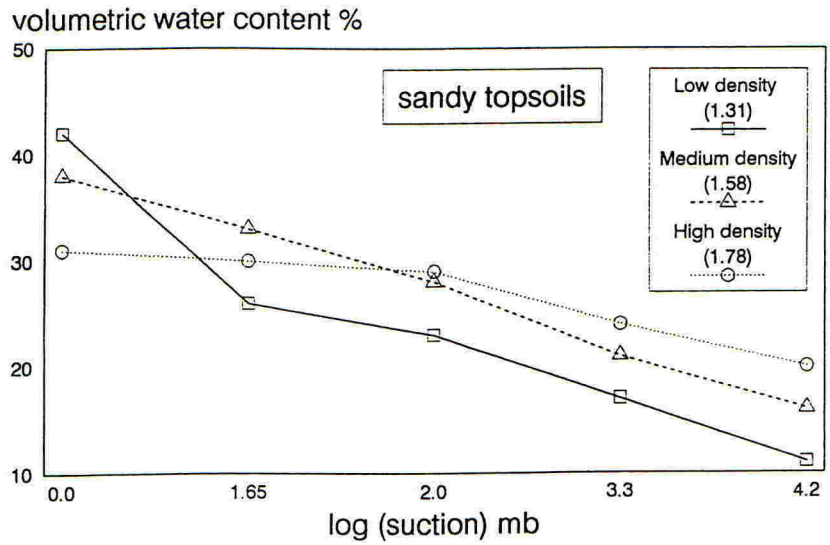


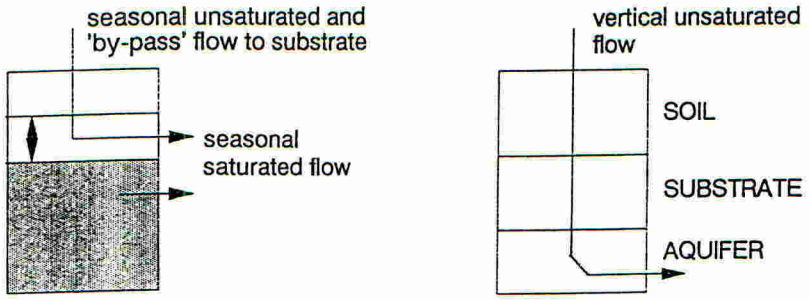
Figure 1 Moisture characteristics for a topsoil with 3.3% organic carbon

exceed 22°C. Average topsoil temperatures during February, March and April range from about 2 to 10°C and during September to November from about 16 to 5°C.

The principal routes and mechanisms by which water moves through soils and their geological substrates, determine the speed and frequency with which pesticide compounds dissolved in the soil water fraction, reach aquifers or surface waters. Seasonally or permanently waterlogged layers provide relatively rapid routes for water to move laterally out of the soil. They also produce anaerobic conditions under which degradation rates may be considerably different from those under aerobic conditions.

In the U.K., soil types have been grouped into 29 hydrological classes according to their main pathways and rates of water movement (Boorman and Hollis 1990). The system, known as HOST (Hydrology of Soil Types), is based on eleven simple models differentiating hydrological pathways, within which subdivisions are made according to the main mechanisms or rates of water movement. Examples of two of the models are given in Figure 2. The system has been calibrated against measurements of stream flow characteristics from over 600 catchments throughout the U.K. Each of the 29 HOST classes has a unique hydrological response to rainfall and this, in turn, is reflected in stream responses.

When rain enters the soil it displaces the more mobile parts of the soil water fraction. Rainfall in excess of evapotranspiration rates has the potential to move some of the soil water completely out of the soil zone so that the capacity for further degradation of any dissolved pesticide is severely reduced. In most years, any excess of rain that occurs during the summer months tends to be offset by the accumulated soil moisture deficit and little or no water moves out of the soil zone. During the autumn, however, the soil moisture deficit progressively



Soils with short seasonal saturation on slowly permeable or impermeable substrates.

Permeable well drained soils on permeable substrates. Aquifer below 2 m depth.

Figure 2 Two hydrological models within the HOST classification system.

reduces until a state approximating to field capacity is reached. From this point in time until the following spring, soil water will move downwards or sideways roughly in proportion to the amount of excess rainfall. The amount of Excess Winter Rain (EWR) during the period when the soil is at or near field capacity is thus an important agroclimatic property related to leaching potential and annual average values have been calculated at 5 km grid intersects within England and Wales (Jones et al. 1987). Within the main crop growing areas of England and Wales, the field capacity period ranges from less than 100 days to over 225 days. EWR ranges from less than 100 mm to over 1,000 mm and soil water fluxes from 0.9 mm per day to 4.4 mm per day.

LABORATORY, LYSIMETER AND FIELD STUDY TECHNIQUES IN THEIR U.K. CONTEXT

Laboratory studies

Laboratory studies on pesticide compounds are normally carried out to determine degradation and leaching potential both for the parent component and its metabolites. Studies are carried out on fresh but disturbed and sieved soil samples, usually repacked into laboratory containers. The advantage of such studies is that temperature and moisture conditions can be precisely controlled relatively cheaply, so that a range of soil climatic environments can be simulated. Soil type, its water content and potential, oxygen status and temperature are the operating conditions specified by many regulatory bodies but specific requirements vary. Some of the stipulated conditions, particularly those of temperature and the soil structural condition, have little relevance in the U.K. agricultural environment. Disturbed, sieved and repacked soil tends to have a granular type of structure in which water flow occurs mainly around individual particles. This type of structure occurs naturally only in relative loose sandy soils and, in most other cases, soil particles are aggregated into compact structures of varying shape, density and coherence. Within such soils water tends to flow preferentially along fine fissures or pores between aggregates. The more compact and dense the aggregate, the greater the likelihood that 'by-pass' flow will occur and that the soil moisture

characteristic will differ significantly from that of granular soils. For most laboratory environmental fate studies, therefore, U.K. field conditions will be best simulated using sandy soils at a range of organic carbon contents, moisture contents and temperatures likely to occur within the relevant crop growing areas. Even so, results will represent only about 7% of the agricultural environment, although they could be used as a 'worst case' situation to derive relative degradation and mobility base values beyond which further investigations may be deemed unnecessary.

Lysimeter studies

Increasingly, soil lysimeters are being used for leaching studies in order to work on undisturbed field soil whilst retaining the ability to control environmental conditions and degradation can also be investigated. Although lysimeters give a relatively accurate simulation of flow in both granular and structured soils, leaching study results are only applicable to situations where vertical flow to a groundwater-table or aquifer predominates. Only three of the HOST Models conform to this pattern and they represent between 30 and 40% of the area in which crops are grown. On the remaining land, soils have slowly permeable or impermeable layers within about 1.2 m depth which give rise to lateral flow, often under temporarily waterlogged conditions. Lysimeter studies cannot simulate leaching in these situations.

Sandy arable soils with low organic carbon contents are frequently selected for lysimeter studies, on the basis of a 'worst case' leaching scenario. Such soils cover about 5 to 6% of the cropped land in England and Wales. It is by no means certain that they represent a worst case for leaching. By-pass flow can occur in structured soils, particularly where individual aggregates are relatively large, dense and strongly developed. Where preferential 'by-pass' channels terminate in relatively free draining substrates, leaching is likely to be greater than in granular sandy soils. Soils with the potential for by-pass flow to a permeable substrate can be difficult to identify, but it is estimated that they may constitute about 2 to 3% of cropped land.

Field studies

Environmental fate studies carried out within individual fields or small catchments represent the ideal, but environmental conditions are difficult to characterize and control. They are also very difficult and expensive to monitor comprehensively.

The HOST classification of soils and their hydrological pathways enables field sites and catchments to be placed in their broad hydrological context so that sampling techniques and sites can be effectively targetted. For example, in a free draining sandy soil over soft weakly consolidated permeable sandstone, vertically moving leachate predominates as in the second model in Figure 2. Field conditions can be adequately characterized and sampled using inert soil water suction samplers at depth in conjunction with sequential soil sampling. Investigation is currently underway in this type of situation at Assarts Farm in Nottinghamshire, where work is being undertaken for the Water Research Centre as part of their research topic on pesticides in major aquifers, funded by the National Rivers Authority.

Conversely, in slowly permeable soils, lateral and by-pass flow are

likely to be dominant processes. In addition to sequential soil sampling, studies incorporating the sampling of saturated and unsaturated water phases, drain flow water and surface water may be necessary. Surface run-off may also be an important process that requires investigation and sampling. Multidisciplinary work at Rosemaund EHF in Herefordshire, funded by the Ministry of Agriculture Fisheries and Food is investigating the behaviour of pesticides in this type of situation. The site is dominated by soils corresponding to the first model in Figure 2, which represent about 10 to 15% of the cropped land in the U.K.

Initial results from the two studies suggest that the same pesticide is likely to be present at far greater concentrations in soil water moving out of the soil zone at the Rosemaund site than at the Assarts Farm site.

SUMMARY

The current trend to produce a standard data package which encompasses all possible environmental fate studies is neither cost effective nor necessary for the U.K. Environmental information is available to implement a stepwise decision process which will determine the optimum method or type of study for any specific pesticide and its target organism. Decisions should be based on a knowledge of the range of soil, hydrological and climatic conditions encountered within the potential usage area of the compound. In some cases simple laboratory studies, undertaken to comply with regulatory requirements in other countries, may indicate that no further work is necessary to cover U.K. situations. In other cases, however, additional lysimeter or even field studies may be required to take account of combinations of land use, soil type, hydrological pathways and climate characteristics specific to the U.K.

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WRc/SOIL SURVEY INERT SUCTION SAMPLER

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ABSTRACT

A difficulty in studying pesticide movement in the unsaturated zone of an aquifer is the need to obtain large volumes of water for analysis. WRc, in co-operation with the Soil Survey and Land Research Centre (SSLRC) has tackled this problem by using a modification of the SSLRC standard soil moisture suction sampler. The modified design is intended to render the installation as chemically inert as possible in order to avoid contamination of the water samples.

The samplers have been installed for field trials on the Sherwood Sandstone aquifer at Assarts Farm near Mansfield. The results of two trials are presented showing the successful application of the samplers.

INTRODUCTION

Concern has been growing in recent years over the contamination of groundwater supplies by pesticides. WRc are undertaking a survey of pesticides in the UK major aquifers on behalf of the National Rivers Authority. As part of this survey a site at Assarts Farm near Mansfield on the Sherwood Sandstone aquifer has been selected for the study of pesticide movement in the unsaturated zone of the sandstone aquifer.

The sampling methods used for tracing inorganic contaminants through the unsaturated zone rely mainly on recovering solid samples, then removing the contaminant by leaching or by centrifugation. These methods are suitable for inorganic contaminants because of their high (mg/l) concentrations and the small volume (ml) of samples needed for analysis. Detection limits for pesticides, to be useful in water supply studies need to be related to the EC MAC, that is, less than 0.1 µg/l. This requires large (over 1 litre) samples and makes centrifugation extremely time-consuming and open to contamination. Leaching of solid samples also is extremely difficult at low concentrations of pesticides.

The WRc/Soil Survey Inert Suction Sampler has been designed to overcome the problems of sampling porewater from the unsaturated zone. This paper gives the results of initial trials of the sampler and verifies its usefulness as a sampling tool.

THE SAMPLER

Construction

The Inert Suction Sampler is a modification of the SSLRC standard soil moisture suction sampler. The modified design (Figure 1) is intended to render the installation as chemically inert as possible, so the sampler shell is of stainless steel, tubes and caps of Teflon and the filter element ceramic. No adhesives or resins are used.

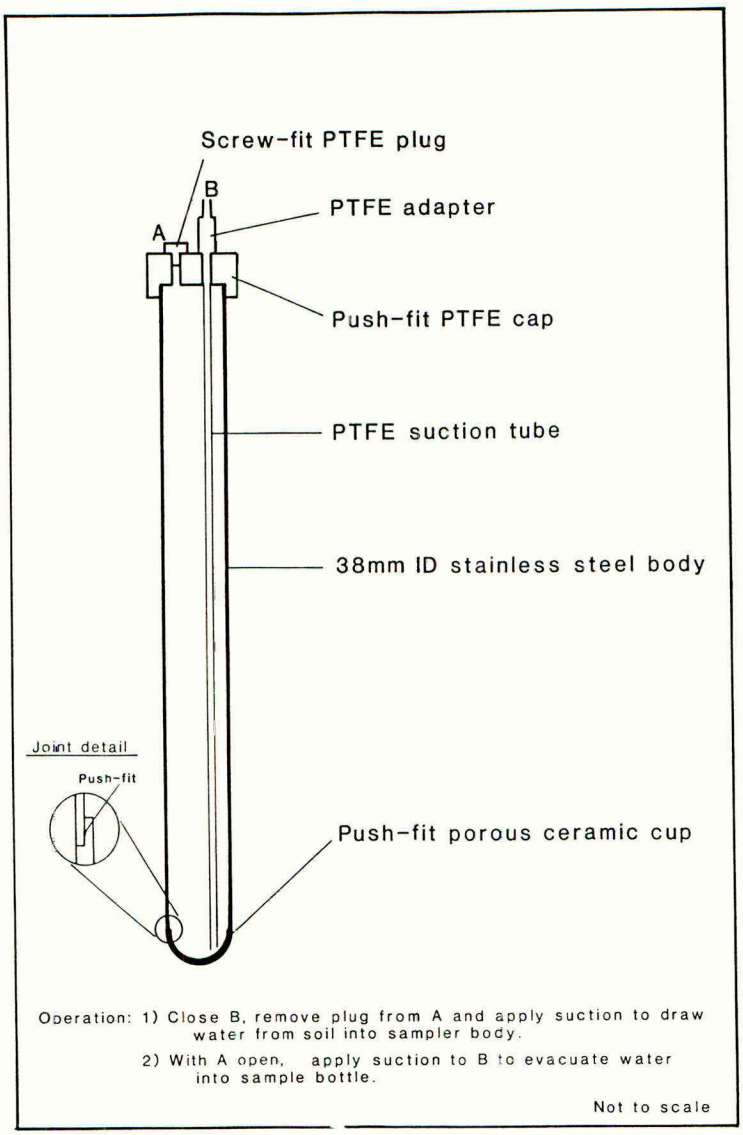

Figure 1. WRc / Soil Survey Inert Suction Sampler

Field Experiments

Each sampler, at the design stage, was expected to yield between about 100 ml and 500 ml depending on the soil moisture at the time of sampling. In order to obtain the necessary volume of water for analysis, a total of twelve samplers in four clusters of three (Table 1) were installed. If samples were small then the samples from clusters could be bulked to provide mixed samples.

TABLE 1 - Suction sampler performance

Sampler	Cluster	Volume recovered (ml) 18/19.12.89	9.1.90	30.1.90
1	P	950	750	700
2		1780	1500	1730
3		1070	400	830
4	Q	830	1000	1550
5		1450	1350	>1000*
6		20	1100	>1000*
7	R	200	550	1200
8		1090	1450	>1000*
9		710	1000	>1000*
10	T	650	350	trace
11		200	100	trace
12		10	0	trace

* less than 1200 ml

The samplers were set to a depth of 1.5 m below surface at the experimental site of Assarts Farm near Mansfield. This depth was judged to be below the active soil horizon so that the water sampled would be representative of that draining from the soil and entering the top of the unsaturated zone of the underlying aquifer. The concentration of pesticides measured in that water would be valuable as calibration data for pesticide transport models through the soil (as output) and the unsaturated zone (as input).

In 1989, the farmer at Assarts Farm sprayed the test plot with isoproturon in October but sampling could only begin following cancellation of the soil moisture deficit (SMD) in early December. The results of the sampling showed that the samplers were operating efficiently with three out of four clusters giving more than two litres of water (Table 1).

An application of mecoprop over the test site was planned for the spring of 1990 to act as a second pesticide trace for the samplers. This application was delayed by harsh frosts until 26 April when a SMD was already established in the area. Periodic sampling was undertaken until June when the high SMD prevented further sampling.

RESULTS

The recovery of samples (Table 1) shows some variability but three out of four clusters consistently gave more than 2 litres of water at each time. The analytical results of two field experiments are given in Table 2. In the first experiment isoproturon was detected at concentrations of about 1 µg/l in only two clusters, P and R&T on two occasions. This shows the leaching of the pesticide was for a short time and at low concentrations. The mecoprop used in the second experiment was not detected although the detection limit was lower, 0.05 µg/l. This could be due to the lack of infiltration during the dry weather that prevailed throughout that experiment

TABLE 2 - Results of pesticide analyses for porewater from Assarts Farm

		Concentration (µg/l) in sample clusters					
Sample date duplicate	Pesticides	P	P duplicate	Q	Q duplicate	(R&T)	(R&T)
19.12.89	Isoproturon	<1.0*	<1.0*	n.d.	-	1.20(±0.14)	-
	Linuron	n.d.	n.d.	n.d.	-	n.d.	-
	Mecoprop	n.d.	n.d.	n.d.	-	n.d.	-
	MCPA	n.d.	n.d.	n.d.	-	n.d.	-
	Atrazine	n.d.	-	n.d.	-	n.d.	-
	Simazine	n.d.	-	n.d.	-	n.d.	-
9.1.90	Isoproturon	n.d.	-	n.d.	n.d.	<1.0*	<1.0*
	Linuron	n.d.	-	n.d.	n.d.	n.d.	n.d.
	Mecoprop	n.d.	-	n.d.	n.d.	n.d.	n.d.
	MCPA	n.d.	-	n.d.	n.d.	n.d.	n.d.
	Atrazine	n.d.	-	.06+x	n.d.	n.d.	-
	Simazine	n.d.	-	.11+x	n.d.	n.d.	-
30.1.90	Isoproturon	<1.0*	-	n.d.	n.d.	n.d.	-
	Linuron	n.d.	-	n.d.	n.d.	n.d.	-
	Mecoprop	n.d.	-	n.d.	n.d.	n.d.	-
	MCPA	n.d.	-	n.d.	n.d.	n.d.	-
	Atrazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Simazone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22.2.90	Isoproturon	n.d.	-	n.d.	-	n.d.	-
	Atrazine	n.d.	-	0.41x	-	n.d.	-
	Simazine	n.d.	-	0.78x	-	n.d.	-
21.3.90	Isoproturon	n.d.	-	n.d.	-	n.d.	-
	Atrazine	n.d.	-	n.d.	-	n.d.	-
	Simazine	n.d.	-	n.d.	-	n.d.	-

n.d. - None detected x - detection limit 0.4 µg/l
* - Isoproturon detected but not quantifiable with confidence
(±) 95% confidence limit + - possible interference

Following the May application of mecoprop, samples were collected on 26 April, 8 and 23 May and 6 June but no mecoprop was detected in any samples.

DISCUSSION

The need to establish the pollutant pathways from the surface, through the unsaturated zone, to the water table and groundwater beneath is recognised as fundamental to understanding the environmental impact of land-use practices on groundwater quality. The study of such pathways has been the subject of intensive work over the past two decades with, in general, hydrogeologists studying the unsaturated zone of our aquifers and agriculturalists studying the more superficial soils. In terms of agrichemicals of importance to the water industry, this research has concentrated on the movement of nitrates from fertilisers.

The most common UK hydrogeological methodology for unsaturated aquifer material is to remove cores of rock by drilling, then to centrifuge the rock sample to recover the porewater from the rock. One centrifuge load can take about 1200 grams of sandstone and this may yield about 40 ml of water. This is perfectly adequate for a nitrate analysis (together with several other determinands). A pesticide analysis requires a minimum of 1000 ml per suite of pesticides so the inadequacy of centrifugation for sample preparation for such analyses is evident.

The work in agriculture tends to use leaching techniques to establish the chemical characteristics of the soil. This raises many problems with pesticides for the soil (or rock), leachates tend to be organic-rich liquids containing material that interferes with the analyses, particularly at low concentrations. A common detection limit for a single species of pesticide is 0.01 mg/kg. In a sandstone of 25% porosity, containing about 10% (by volume) porewater, and assuming all the pesticide is dissolved in the porewater, the pesticide concentration in the water at 0.01 mg/kg whole rock, would be about 0.2 mg/l. This 'detection limit' is about three orders of magnitude higher than what would be relevant to the concentrations of concern to the water industry - the EC MAC of 0.1 µg/l per pesticide.

The suction sampler has been designed to overcome some of the problems discussed above; it provides large water samples and as it is inert, avoids the interference found in leachate samples. The preliminary data presented here are very encouraging but more studies are needed into the integrity of the samples recovered. For example, what volume of rock is sampled? is the sample really percolating porewater? and what is the true relationship between concentrations of pesticides in solid aquifer samples and in porewaters.

CONCLUSIONS

The WRc/Soil Survey Inert Suction Sampler will provide samples of soil water of sufficient volume to be used for pesticide analyses.

The preliminary analyses of soil water suggest that, under normal cereal cultivation, herbicides may leach into the upper part of the unsaturated zone of our aquifers but for short periods and at low concentrations. This important conclusion needs validation by further field trials.

The field trials have been undertaken using herbicides of concern to the water industry; the samplers should be suitable for sampling other suites of pesticides such as the fungicides.

ACKNOWLEDGEMENTS

The work described in this paper has been funded by the National Rivers Authority.

CHARACTERISATION AND IDENTIFICATION OF FIBRE BOUND RESIDUES DERIVED FROM THE TREATMENT OF PEACHES WITH [^{14}C]-DICLORAN

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ABSTRACT

The metabolism of [^{14}C]-dicloran (2,6-dichloro-4-nitroaniline) was investigated in peaches. The treated fruit were extracted with a series of solvents which released 56.6% of the total residue. Fibre-bound residue (43.4%) could only be released by the strongly forcing conditions of 6M base hydrolysis, multiple Soxhlet extractions and 4M acid hydrolysis. Analysis of the fibre extracts (37.5% of total residue) revealed a multitude of products, including dicloran, six major identifiable components and several unknowns. Hydrolysis of [^{14}C]-dicloran in 6M base gave only 2,6-dichloro-4-nitrophenol. Addition of control fibre to the hydrolysis resulted in the same range of compounds recovered from the treated fibre. This result indicated that a significant component of the fibre-bound residue may have been dicloran which had decomposed under the extreme conditions required to release it. Thus over 67% of the residue in the fibre was either identified as known compounds or characterised as artefacts of the methodology.

INTRODUCTION

Previous studies on the metabolism of dicloran in peaches had established that a large proportion of the total residue (over 40%) comprised fibre-bound residue. This paper describes a new study in which methodology was developed to identify and/or characterise the fibre-bound residues

MATERIALS AND METHODS

Treatment, harvest and extraction of fruit

Peaches (Prunus persica; var. Windle's Weeping) were treated with [^{14}C]-dicloran (specific activity 10μCi/mg) formulated as a WP at the maximum field concentration of 130g a.i./hl. The fruit were given three treatments at 7 day intervals and harvested in samples of three, 18 days after the final application. The fruit surface was washed by ultrasonication in hexane/acetone (1 : 1) for 2 minutes. The stones were removed and the pulp was macerated with acetonitrile (3 times), acetonitrile/water (1 : 1; 3 times) and water (3 times). The radioactivity in the extracts was quantified by liquid scintillation counting (lsc). The residual fibre was washed with acetonitrile, air-dried and milled prior to being combusted to determine bound residue.

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Tlc analysis of extracts

Concentrated extracts were applied either in spots or bands using a Camag Linomat III Applicator to either 'normal phase' Machery Nagel GUV (0.25mm) silica plates (with or without a pre-absorption zone) and eluted with solvent systems 1-8 or 'reverse phase' Whatman LKC 18F (0.2mm) plates and eluted with solvent systems 9-11. The extracts were underspotted with standards (structures in Table 3) diluted in acetonitrile to a concentration of 10mg/ml. Solvent systems with preparations by volume were chosen as appropriate from the following :-

1 Toluene/acetone/triethylamine (10:10:1)
2 Toluene/diethylether/triethylamine (10:10:1)
3 Chloroform
4 Chloroform/acetic acid (95:5)
5 Chloroform/triethylamine (95:5)
6 Ethyl acetate/methanol (1:1)
7 Ethyl acetate/methanol/acetic acid (75:20:5)
8 Ethyl acetate/methanol/acetic acid (87.5:10:2.5)
9 Methanol/water (3:2)
10 Methanol/water/acetic acid (75:20:5)
11 Methanol/water/ammonia (59:39:2)

Tlc was conducted in closed tanks lined with filter paper. The marker compounds were visualised by U.V. light (254nm). Radioactive bands were quantified using an Isomess RITA Linear Analyser. Autoradiography (Osray M3 film) was used to confirm location of separated components.

Hplc analysis of extracts

Injector		:	Rheodyne
Pump		:	Merck Hitachi L-6200 or Perkin Elmer Series 3
UV detector		:	Merck Hitachi L-4000 or Pye Unicam LC 3 (254nm)
Radiodetector		:	Berthold LB 503
Fraction collector		:	Gilson 202
Chart recorder		:	Lab data RE 571.20
Column		:	30.5 x 0.7cm; PRP-1; 10 u
System 12	Flow	:	2ml/min
	Solvent	:	20% acetonitrile/ammonia (1%)(2 min)
		:	20% to 100% acetonitrile/ammonia (18min)
System 13	Flow	:	1ml/min
	Solvent	:	10% acetonitrile/ammonia (1%)(5 min)
			10% to 50% acetonitrile/ammonia (5 min)

For analytical purposes the eluant was collected in 25-28 fractions at 1 min intervals. The fractions were mixed with scintillation cocktail and measured by lsc. Lc/ms was conducted on the same column and similar solvent to System 12. The instrument was a VG7070E linked to a VG11250 data system and interfaced to the hplc by a moving belt.

Processing of treated peach fibre

Fibre from the extraction of treated peaches was heated at reflux with 6M NaOH for 20 hours (Figure 1). A trap containing ethyl acetate and water was included in the apparatus to collect volatile components. At the end of the hydrolysis this ethyl acetate was separated and the aqueous layer re-

extracted with ethyl acetate. The alkaline residue was filtered to remove the fibre. The filtrate was partitioned with ethyl acetate and then re-filtered to remove a fine precipitate. The precipitate was washed with water and the washings and alkaline filtrate were combined before sampling for lsc, to check that no losses had occurred. The aqueous filtrate was adjusted to pH <1 and re-extracted with ethyl acetate and water saturated butanol .

The residual fibre from the alkaline hydrolysis was processed further by Soxhlet extraction with acetonitrile (16 hours), ethyl acetate (24 hours) and water (24 hours). The acetonitrile extract was concentrated to aqueous and partitioned with ethyl acetate at pH 7, pH 13 and pH 2-3. A portion of the extracted fibre was heated at reflux with a 4M HCl for 6 hours, with a trap containing ethyl acetate and water being included in the apparatus. The acidic residue was filtered and the filtrate was partitioned into ethyl acetate. All the solvent extracts were sampled for lsc prior to concentration and chromatographic analysis.

2M Sodium hydroxide hydrolysis of fibre from treated peaches

Fibre from the extraction of treated glasshouse peaches was heated at reflux with 2M sodium hydroxide for 2.5 hours. The mixture was then filtered and the filtrate was partitioned with ethyl acetate. The aqueous fraction was adjusted to pH 1 and re-extracted with ethyl acetate and n-butanol. The extracts were sampled for lsc prior to concentration and chromatographic analysis. The concentrated ethyl acetate extract at pH 1 was purified by hplc in system 12. Repeated injections of approximately 200µl were made and the eluant was collected in 28 x 1 min fractions. Vial 4 and 6+7 from each analysis were combined. The two aqueous samples from combined vial 4 and 6+7 were concentrated by Rotavapor, adjusted to pH 1 with 4M HCl and partitioned into ethyl acetate for chromatographic analysis.

Control hydrolyses of [^{14}C]-dicloran with 6M sodium hydroxide

[^{14}C]-Dicloran (3.04mg) (specific activity 1.12µCi/mg) was diluted with 6M NaOH (20ml) and heated at reflux for 4 hours. A volatiles trap containing ethyl acetate and water was included in the apparatus. The alkaline residue was partitioned with ethyl acetate at pH <1 and pH 7. The solvent extracts were sampled for lsc before concentration and chromatographic analysis.

Fibre (1g) remaining after the extraction of control peaches was added to a second lot of [^{14}C]-dicloran (1.52mg), before the mixture was diluted with 6M NaOH and heated under reflux for 16 hours. A volatiles trap containing hexane and water was incorporated into the apparatus. On completion of the hydrolysis, the two layers in the volatiles trap were partitioned and the aqueous layer was extracted with ethyl acetate. The alkaline hydrolysate was filtered and the filtrate was partitioned with ethyl acetate. The aqueous fraction was re-filtered to remove the precipitate and then adjusted to pH 1. The acidic filtrate was extracted with ethyl acetate and n-butanol. All the extracts were sampled for lsc prior to concentration and chromatography.

RESULTS AND DISCUSSION

At final harvest 46.8% of the radioactivity applied to the peaches was recovered, of which 56.6% was extractable with solvent, leaving 43.4% in the fibre.Many techniques were evaluated to try and release the high proportion

of the residue (43.4%) which remained associated with the fibre. Mild extraction methods, including boiling with 0.5% EDTA, 1% NaOCl and 1% NaCl were unable to recover more than 15% of the fibre residue. Extremely forcing conditions were found necessary to release significant amounts of radioactivity involving boiling in 6M NaOH, acetonitrile, ethyl acetate and water soxhlets and finally heating with 4M HCl leaving only 5.9% of the residue in the fibre (Figure 1). The aqueous hydrolysates were partitioned into ethyl acetate and/or butanol at varying pH. All the organic solvent extracts containing 0.6% or greater of the fibre residue (0.3% of total residue) (extracts A-H) were analysed by tlc and hplc comparing with authentic non-labelled standards.

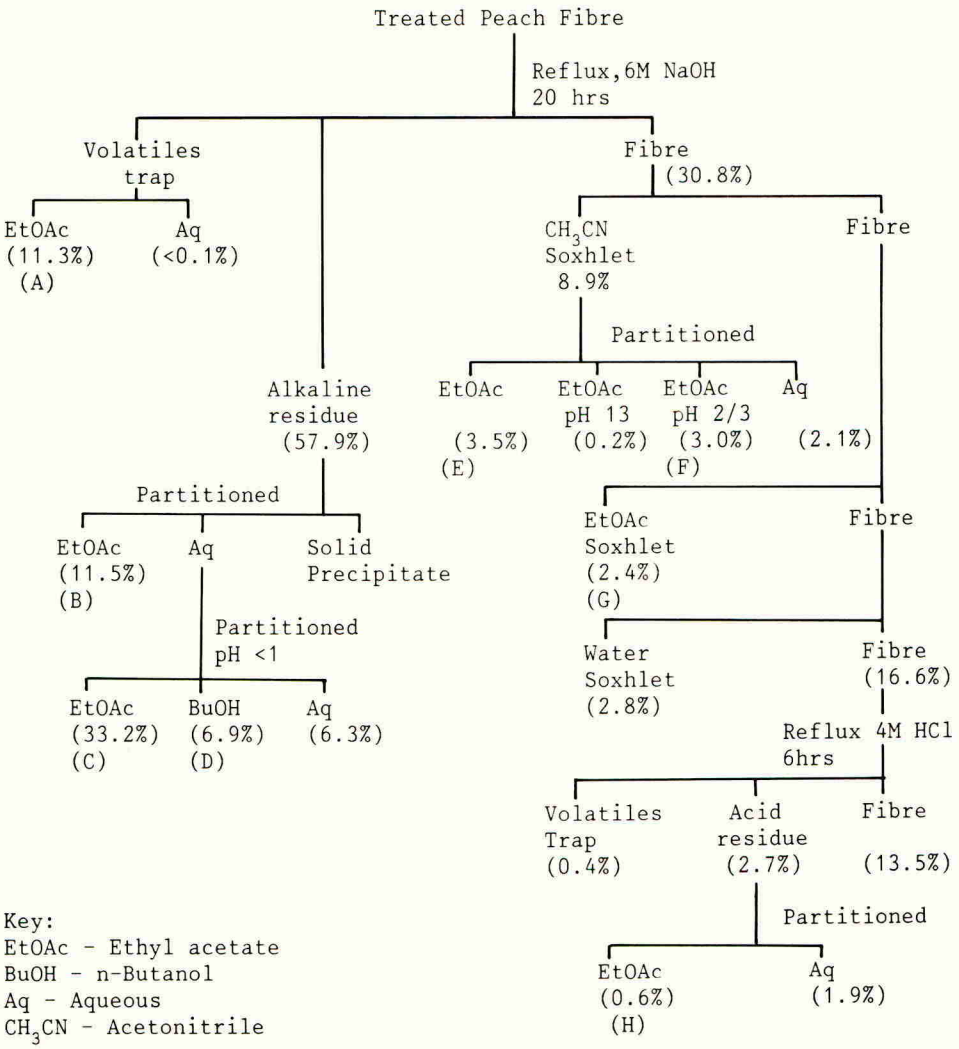
Large amounts of charred plant natural products in the extracts caused some or all of the radioactivity to adhere to the origin on TLC in the normal phase (Systems 1-8). Although this was partly overcome by using reverse phase tlc (Systems 9-11), the use of hplc (System 12) circumvented this problem, gave good recoveries and was able to separate all the standards, although standards VI and VII eluted very close to the solvent front. The results are summarised in Table 1. The hplc traces of all eight extracts were qualitatively very similar, each showing radioactive peaks which co-chromatographed with standards I to V. The identities of compounds I-V were confirmed by tlc in at least one solvent system and in addition dicloran (I) and 4-amino-2,6-dichloraniline (III) were fully characterised by lcms of extract A. There were also in each of the eight extracts two fast-eluting peaks (collected in vials 3/4 and 5/6). To facilitate the identification of these two components, cleaner (although less abundant) extracts were prepared by hydrolysing a second sample of fibre under slightly milder conditions, that is by heating with 2M NaOH for 2.5 hours. The alkaline hydrolysate was partitioned into ethyl acetate at pH 14 and 1 and n-butanol at pH 1. Analytical hplc analysis of the extracts (System 12) showed them to have a similar profile to those from the 6M NaOH hydrolysis. The two polar metabolites were isolated from the 2M NaOH hydrolysis by preparative hplc. The two unknowns were identified by hplc comparison with standards as 4-amino-2,6-dichlorophenol (VII) and 2,6-dichlorophenol (VI) (confirmed by lcms). Further confirmation by tlc could not be obtained due to the volatility of VI and the instability of VII on silica.

Several radioactive regions which did not co-chromatograph (hplc) with standards were observed in all the extracts. In an effort to reproduce these unknowns chemically, [^{14}C]-dicloran was hydrolysed with 6M NaOH. The volatiles trap containing 4% of the residue, was found to comprise unchanged dicloran. The majority of the radioactivity (90.2%) was recovered in the ethyl acetate extract of the alkaline residue and was found to be entirely 2,6-dichloro-4-nitrophenol (V). Hydrolysis of [^{14}C]-dicloran in the presence of 'control' (untreated) fibre however gave an entirely different result. Over 65% of the radioactivity was of a volatile nature and was either hexane (55.0%) or ethyl acetate (10.7%) soluble. The alkaline residue was partitioned into ethyl acetate at unchanged pH (16.0%) followed by ethyl acetate at pH 1 (6.8%) and butanol (8.7%). Analysis of the extracts by hplc showed 4-amino-2,6-dichloroaniline (III) to be the major product, with smaller amounts corresponding to the remaining standards. There were also small amounts of the unknowns identical to those obtained from hydrolysis of the treated fibre indicating that these unknowns and also portions of the other metabolites derived from treated fibre, may derive directly from dicloran by hydrolysis in the presence of fibre (comparison of treated and control experiments in Table 1).

CONCLUSION

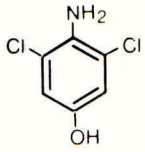
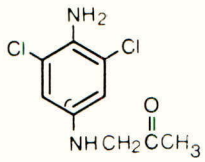
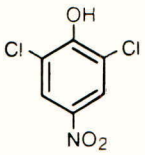
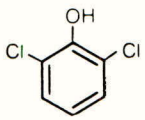
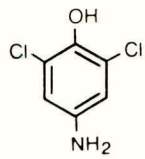
The residue levels in the [^{14}C]-dicloran treated, glasshouse peaches were high (14.07mg equiv./kg) due to the absence of weathering. Extraction with solvent recovered 56.6% of the residue, although a further 37.5% could be solubilised by extensive processing of the fibre, leaving only 5.9% fibre-bound. Dicloran and its metabolites were extensively sequestered within the plant fibre matrix, requiring highly forcing conditions to effect their release. Control experiments indicated that a significant proportion of the identified and unidentified radioactivity released from the fibre may have resulted from the breakdown of dicloran under these extreme conditions. Each unidentified component comprised no more than 3.6% of the total residue.

FIGURE 1 Extraction of residue in treated peach fibre (results expressed as a proportion of fibre radioactivity)



Key:
EtOAc - Ethyl acetate
BuOH - n-Butanol
Aq - Aqueous
CH_3CN - Acetonitrile

TABLE 1 Distribution of radioactivity in extracts from hydrolysis/extraction of treated fibre and [^{14}C]-dicloran with control fibre, determined by hplc

Metabolite	Structure	Distribution of residue (%) Treated Fibre	[^{14}C]-Dicloran/ Control Fibre
I		2.86	3.86
II		10.45	3.29
III		7.83	65.82
IV		7.02	2.01
V		2.71	1.66
VI		6.56	6.39
VII		12.58	1.12
Unknown 1		1.44	0.35
Unknown 2		5.11	1.10
Unknown 3		3.70	2.74
Remainder		5.82	7.30
Polar		13.1	2.90
Fibre-bound		13.5	-
Total		99.65	100.11

THE COMPARATIVE FATE OF (^{14}C)-AMITRAZ IN DIFFERENT SEDIMENT/WATER TYPES

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ABSTRACT

The fate of [^{14}C]-amitraz has been investigated in laboratory 'microcosms' using three different sediments and their associated water. The compound was applied to the water surface and the microcosms incubated at 25°C in a stream of moist air. Following application amitraz was rapidly dissipated from the water column via hydrolysis and adsorption to sediment. Times for 90% decline (DT 90 values) of amitraz in the microcosms ranged from 1.3 to 8 days. The initial products of hydrolysis (BTS 27271 and BTS 27919) were also degraded in the system, the rates of decline varying with sediment type. In an acidic sediment DT 50 values for the decline of BTS 27271 and BTS 27919 were 9 and 65 days respectively. However, corresponding values in alkaline loamy sand and clay sediments were much shorter (2 days and less than 30 days for BTS 27271 and BTS 27919 respectively). These hydrolysis products were further degraded leading to the formation of unextractable ('bound') residues and $^{14}CO_2$.

INTRODUCTION

As part of the assessment of the impact of amitraz (N-methylbis(2,4-xylyliminomethyl)amine) and its degradation products on the aquatic environment, the dissipation of radiolabelled amitraz has been investigated in the laboratory using three natural water and sediments.

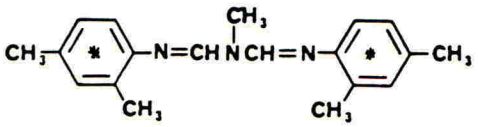


FIGURE 1 Amitraz

MATERIALS AND METHODS

Sediments

Three sediments and their overlying water were collected from the sites listed in Table 1. Large stones and plant debris were removed and appropriate physico-chemical properties determined.

TABLE 1. Sediment/water properties

Property		Sediment A	Sediment B	Sediment C
Location of site	:	River Granta, Cambridge.	Rampton ditch, Cambridge.	Wokefield pond Berkshire.
Water pH[a]	:	7.8	7.5	6.3
Sediment pH[b]	:	6.8	6.2	5.8
Organic matter (%)	:	1.2	2.4	3.8
Particle size distribution				
Sand (63 - 2000um)	:	87%	38%	89%
Silt (2 - 63um)	:	7%	28%	9%
Clay (< 2 um)	:	6%	34%	2%
Textural class (ADAS)		Loamy sand	Clay loam	Sand
CEC (meq/100g)	:	6.1	16.2	7.6

(a) Measured at time of collection.
(b) Determined with 1:2.5 sediment:solution ratio in 0.01M $CaCl_2$

Incubation system and treatment

The incubation system used was similar to that employed by Houx and Dekker (1987). Sediment was loosely packed into glass columns (30cm x 5cm i.d.) to a depth of 7cm and covered with 15cm of water. To maintain aerobic conditions in the surface water, a current of CO_2-free moist air was bubbled through the water via a dip tube extending 8cm below the water surface of sediments A and B. The use of CO_2-free air caused an increase in water pH and so CO_2 was not removed from the air supply to sediment C. Also, for this treatment the air was passed over the surface of the water rather than bubbling through it. The 'microcosms' were incubated in the dark at 25°C ± 2°C for between 6 and 18 days prior to treatment.

[^{14}C]-Amitraz was applied as a 'MITAC'[1] 20EC formulation to the water surface of the 'microcosms' at a rate of 1.68 kg AI/ha. Two batches of radiolabel were used: Sediments A and B were treated with material of specific activity of 136 µCi/mg and sediment C with material of specific activity 231 µCi/mg. Following application, effluent air from each microcosm was passed through two trapping solutions, one of ethanediol and one of ethanolamine.

Analysis

Radioactivity in the trapping solutions was quantified by liquid scintillation counting (lsc). At each sampling occasion the water was decanted off the sediment and radioactivity determined by lsc. The sediment

[1] Trademark of Schering Agrochemicals Limited

was extracted with dichloromethane followed by acetonitrile/water (4 : 1 by vol.) for 18 hours each using Soxhlet apparatus and radioactivity quantified by lsc. The solvent extracted sediment was dried, milled and oxidised using a Packard Tri-carb sample oxidiser.

Radioactivity in the surface water, dichloromethane and acetonitrile/water sediment extracts was characterised by hplc and tlc using the following systems:-

Hplc column	:	Dynamax C_{18} 250 x 10 mm i.d., 12 um.
Mobile phase	:	Solution A - 40% acetonitrile + 60% phosphate buffer (25 mM) pH 7.0. Solution B - acetonitrile. Both A and B contained tert-butyl ammonium bromide (2.5 mM).
Gradient	:	100% A @ 3.0 ml/min (for 15 min); 25% A @ 7.5 ml/min (for (15 min).
Tlc plates	:	Machery Nagel SILGUR 25 UV254 silica plates.
Solvent systems	:	System A - Toluene/triethylamine (9 : 1 by vol) System B - Cyclohexane/ethyl acetate/triethylamine (5 : 3 : 2 by vol)

RESULTS

Distribution of radioactivity

The distribution of radioactivity in the microcosms was similar for all three sediment types. Following application of the compound, radioactivity was rapidly dissipated from the water columns. Between 35 and 50% of applied radioactivity was associated with the sediments 1 day after application. The greatest adsorption was observed in the clay loam sediment B. Between one day and three to seven days post-application there was a desorption of radioactivity back to the surface water followed by a general decline in water and sediment extract associated radioactivity and a concomitant increase in volatile and sediment 'bound' radioactivity. The majority of volatile radioactivity was shown to be $^{14}CO_2$ (Figure 2).

Characterisation of radioactivity

Amitraz was rapidly eliminated from the microcosms as a result of hydrolysis. The products of hydrolytic cleavage were N-methyl-N'-(2,4-xylyl)formamidine (BTS 27271) and form-2',4'-xylidide (BTS 27919). Only negligible concentrations of BTS 27271 were observed in sediments A (<6%) and B (<7%), the principle degradation product being BTS 27919. Peak concentrations of this product were observed after 7 days (58%) in sediment A and 14 days (41%) in sediment B. However, much greater concentrations of BTS 27271 were observed in the acidic sediment C. The peak concentrations of BTS 27271 and BTS 27919 were observed after 3 days (28%) and 7 days (45%) respectively. These results are in agreement with those obtained from an abiotic amitraz hydrolyis study conducted over a pH range of 5 to 9. Under acidic and neutral conditions greater concentrations of BTS 27271 were

FIGURE 2 Distribution of radioactivity in the three sediment/water types following application of [^{14}C]-amitraz to the water surface

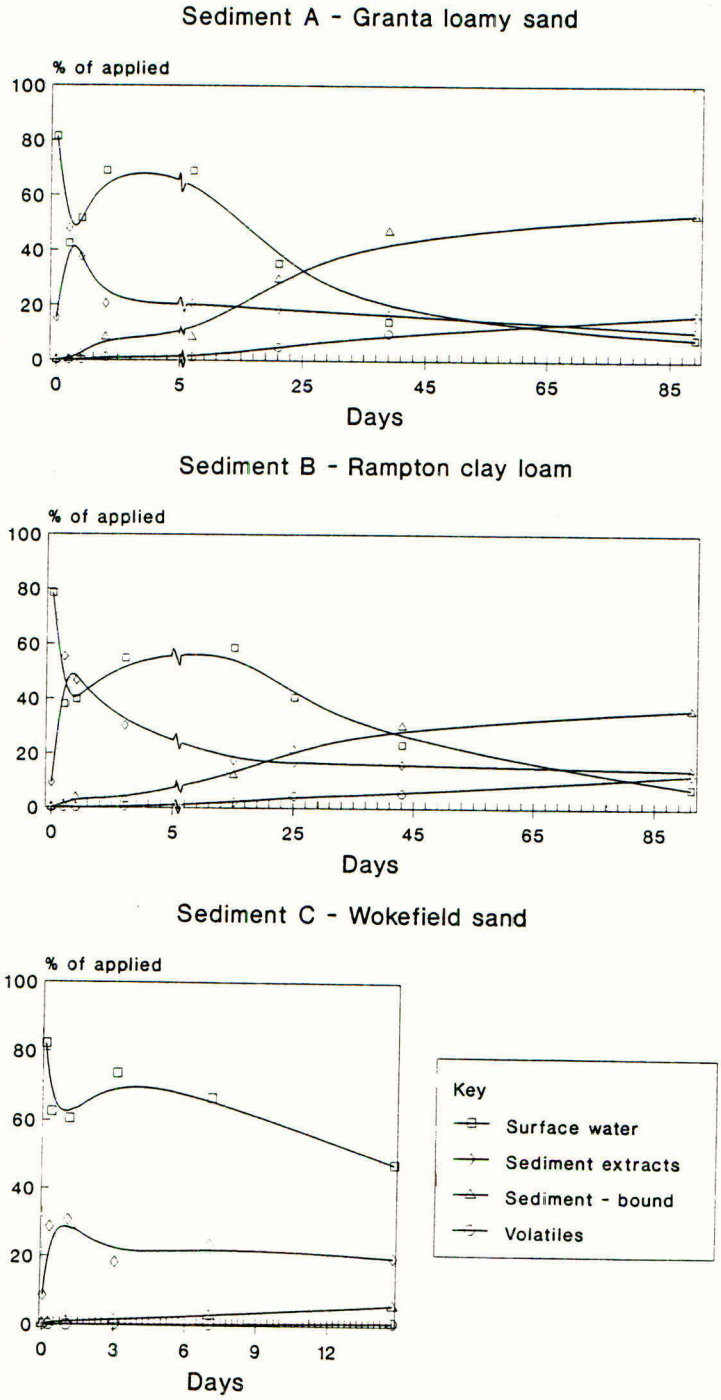
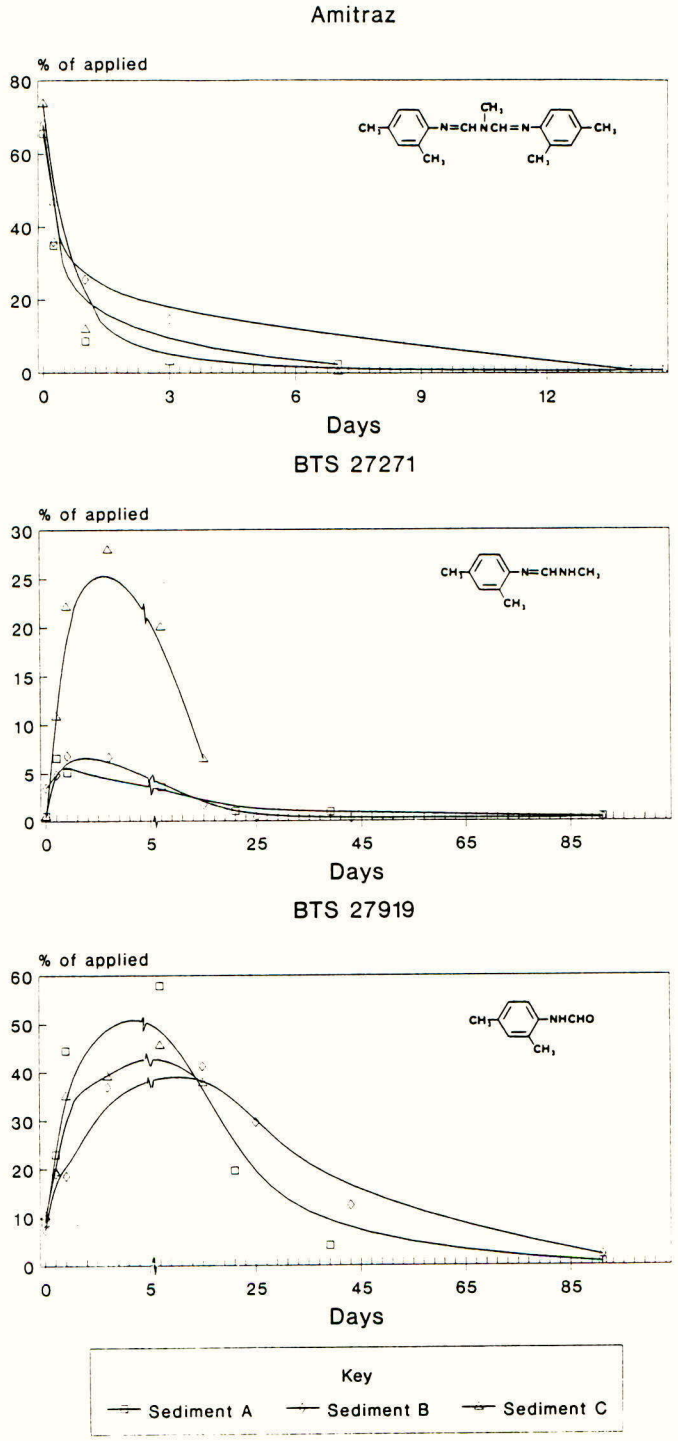


FIGURE 3 Degradation of amitraz and its principle hydrolysis products in sediment plus water.



detected compared with under alkaline conditions (J.K. Campbell, personal communication).

Subsequent degradation in all three sediments led to the formation of 2,4-dimethylaniline (BTS 24868) which was further degraded in all sediments to more polar products, sediment 'bound' residue and carbon dioxide.

Rates of degradation of amitraz, BTS 27271 and BTS 27919

Amitraz hydrolysis was rapid in all three microcosms. Times for 90% decline (DT90 values) ranged from 1.3 days in sediments A and C to ca. 8 days in the clay sediment B (see Figure 3).

The rate of degradation of BTS 27271 was also rapid. DT50 values ranged from 1.9 days in sediment A to ca. 9 days in sediment C.

BTS 27919 was relatively more persistent with DT 50 values for its decline being 16, 27 and 65 days for sediments A, B and C respectively.

DISCUSSION AND CONCLUSIONS

Any amitraz reaching surface waters as a result of spray drift, run-off or accidental overspraying will be extremely short-lived. Although degradation was slower in the clay sediment, this was due to greater adsorption of the compound to sediment therefore slowing the hydrolytic process but nevertheless still removing the compound from the water phase.

The quantities of the two products of hydrolytic cleavage in the sediment/water microcosms was dependent on the pH of the system. In alkaline water BTS 27271 was rapidly converted to BTS 27919. However, under more acidic conditions the rate of degradation was slower therefore BTS 27271 was detected at much higher concentrations. However, BTS 27271 did not persist in the microcosms.

The major product of amitraz breakdown in all three systems was BTS 27919. This compound has been shown to be broken down in soil by the action of microorganisms (D.J. Arnold, personal communication). The steady breakdown of this compound in the microcosm and the continued evolution of $^{14}CO_2$ during the incubation period indicated that biological activity was maintained for at least 13 weeks.

The route of breakdown of amitraz was identical in all three sediments. Variations in the quantities of degradation products formed were observed but overall there was only a four to five-fold range in the rates of decline of amitraz and its principal degradation products between the different sediment types.

REFERENCE

Houx, N.W.H.; Dekker, A. (1987) A test system for the determining of the fate of pesticides in surface water. International Journal Environmental Analytical Chemistry, 29, 37-59.

METABOLIC FATE OF THE INSECTICIDE 'KARATE' (LAMBDA-CYHALOTHRIN) ON COTTON AND SOYA LEAVES

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ABSTRACT

The metabolic fate of the synthetic pyrethroid insecticide Lambda-cyhalothrin has been studied in cotton and soya plants using ^{14}C-radiolabelled material. Degradation products have been identified in the foliage of cotton plants using tlc, and structural confirmation of selected metabolites has been carried out using a number of derivatisation techniques, followed by gcms analysis. Similar analytical techniques have been used to identify metabolites in soya leaves, and as a result it is possible to propose a single metabolic pathway for the degradation of Lambda-cyhalothrin which is common to both plants.

INTRODUCTION

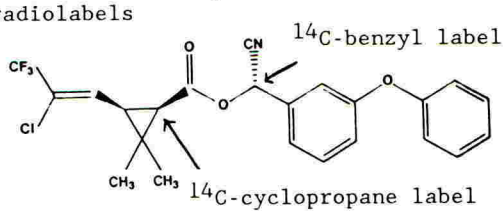
Lambda-cyhalothrin (I) (a 1:1 mixture of the enantiomers (S)-α-cyano-3-phenoxybenzyl (1R)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl (1S)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate) is a broad spectrum synthetic pyrethroid insecticide, developed by ICI Agrochemicals (Jutsum et al, 1984), which is active at low application rates against a wide range of foliar insect pests. Studies with ^{14}C-radiolabelled compound have been carried out to determine the metabolic fate of Lambda-cyhalothrin in cotton and soya plants, since the compound is used to control insect pests in both these crops.

MATERIALS AND METHODS

Test compound

^{14}C-Cyclopropane-labelled and ^{14}C-benzyl-labelled Lambda-cyhalothrin (Figure 1) were supplied by the Radiochemical Synthesis Group at Jealott's Hill Research Station. The specific activities, determined by glc, were 1.93 and 2.25 GBq mmol^{-1} respectively, and both materials had a radiochemical purity of ≥98%.

Figure 1. Structure of Lambda-cyhalothrin (I) and position of ^{14}C-radiolabels



and enantiomer (1:1 mixture)

Treatment of plants

Cotton plants were grown in pots in a glasshouse, and were treated with three applications of either ^{14}C-phenyl-labelled, or ^{14}C-cyclopropane-labelled Lambda-cyhalothrin. The compound was formulated as an emulsifiable concentrate, and was applied to each plant as a foliar spray. The rate of each application was 66 g AI ha^{-1}, equivalent to approximately one third of the commercial seasonal use rate. Leaves were harvested from mature plants approximately one month after the final application, and cotton seeds were harvested from the ripened bolls 51 days after the final application.

Soya plants were also grown in pots in a glasshouse, and were treated with two applications of either ^{14}C-phenyl- or ^{14}C-cyclopropane-labelled Lambda-cyhalothrin in the same manner. The rate of each application was 20 g AI ha^{-1}. Leaves were removed from mature plants 39 days after the second application of radiochemical, and beans were harvested 51 days after the second application.

Extraction of leaves

A representative subsample of leaves from each ^{14}C-radiolabelled experiment were extracted with either acetonitrile or acetonitrile/water (50/50, v/v) using a high speed tissue homogeniser. Solid and liquid fractions were separated by centrifugation, and the liquid extracts were diluted with water, acidified to pH 1 with hydrochloric acid, and partitioned with hexane. The amount of radioactivity in each liquid fraction was determined by liquid scintillation counting (lsc) and the amount of radioactivity in solid samples was determined by combustion and lsc.

Solid phase extraction

The various ^{14}C-radiolabelled fractions contained in the hexane extracts of cotton leaves were isolated using solid phase extraction. Concentrated extracts were loaded onto Silica solid phase extraction cartridges, and the cartridges were eluted with hexane, followed by hexane/ether mixtures of increasing solvent strength, and finally with methanol.

Acid hydrolysis of leaf extracts

All water-soluble fractions, after hexane partition, and all solid material remaining after acetonitrile or acetonitrile/water extraction were subjected to a series of acid hydrolyses of increasing strength (2M HCl, 2 hour reflux; then 4M or 6M HCl, 4 or 6 hour reflux). Following each acid hydrolysis, released radioactive material was extracted from the aqueous phase with dichloromethane.

Tlc analysis

Structural identification of all plant metabolites was carried out using normal phase tlc. Plant extracts were co-chromatographed with reference compounds of known structure using at least two dissimilar following solvent systems to develop each chromatograms.

Reference compounds were detected on UV-absorbent tlc plates (Machery-Nagel, SIL-G-UV_{254}, 0.25 mm) by visualisation under UV light. Radioactive areas on the chromatograms were located using a radiochromatogram scanner and by autoradiography.

Hplc analysis

Hplc was used to isolate a number of organosoluble metabolites from cotton leaf extracts prior to structural confirmation by derivatisation and/or gcms. Extracts were dissolved in the hplc mobile phase and analysed using the conditions shown below.

Column : Hichrom Spherisorb S5ODS (12.5 cm x 4.6 mm i.d)
Mobile phase : Acetonitrile/water (water adjusted to pH 2.5 with orthophosphoric acid) (40/60, v/v)
Flow rate : 2.0 ml min^{-1}
Detection wavelength : 235 nm.

Individual compounds were isolated by collecting column eluate fractions corresponding to each ^{14}C-peak as indicated by the radiodetector.

Some isomerisation of Lambda-cyhalothrin (probably photo-isomerisation) was expected on the leaf surfaces. The isomeric composition of the parent compound recovered from the leaves was measured by hplc using the conditions shown below:

Column : Hichrom Spherisorb S5W (25 cm x 4.6 mm i.d)
Mobile phase : Hexane/diethyl ether/tetrahydrofuran (98.9/0.84/0.25, v/v/v)
Flow rate : 2.0 ml min^{-1}
Detection wavelength : 230 nm.

Preparation of derivatives for gcms analysis

In order to facilitate structural identification by gcms, derivatives were prepared for several ^{14}C-radiolabelled metabolites isolated from cotton leaf extracts by hplc.

Methylation of ^{14}C-metabolites was carried out by addition of a freshly prepared solution of diazomethane in diethyl ether (DeBoer and Backer, 1956). The reaction solution was allowed to stand at room temperature for approximately 15 minutes, after which excess diazomethane was removed by evaporation under a stream of dry air. Acetylation was carried out by addition of acetyl chloride (BDH). The reaction mixture was allowed to stand overnight at room temperature, and excess reagent was removed by rotary evaporation. Silylation was carried out using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Pierce). The reaction was allowed to proceed at room temperature for 15 minutes.

Gcms analysis

Gcms analysis was carried out using a Finnigan Mat 8200 mass spectrometer and a Varian model 3700 gas chromatograph under the following conditions:

Column	: 25m OB101	Source temp	: 200°C
Initial temp	: 55°C	eV	: 70
Final temp	: 250°C	Accelerating voltage	: 3kV
Gradient	: 16°C min^{-1}.	Cathode	: 3.8
Ionisation mode	: Electron impact	Emission	: 0.5mA

RESULTS

Radioactive residues in cotton seed and soya beans were extremely low (0.01 - 0.03 mg kg^{-1} and $\leq$0.01 mg kg^{-1} respectively). Consequently, no further identification of these residues was carried out.

Radioactive residues in cotton leaves ranged from 2.9 to 4.1 mg kg^{-1}, and in soya leaves from 1.2 to 1.9 mg kg^{-1}. These residues were fractionated into organosoluble, water-soluble and unextractable material, as shown in Table 1. Tlc analysis of the hexane-soluble material showed that the majority of the radioactivity in this fraction was due to Lambda-cyhalothrin and its other isomers, although some unconjugated ^{14}C-metabolites and some polar material were present in the hexane fractions generated from cotton leaves. The radioactivity in these fractions which was due to Lambda-cyhalothrin and its isomers was isolated using solid phase extraction. Analysis of this material using normal phase hplc confirmed that changes in the isomeric composition had occurred, notably cis/trans isomerisation about the 1,3-bond of the cyclopropane ring, the trans-isomer accounting for up to 6% of the total residue in both cotton and in soya leaves, and R/Sinterconversion at the α-cyano position. This was more significant in cotton than in soya, the S-enantiomers accounting for up to 14% and 3% respectively in the two crops. Previous work with the structurally similar pyrethroid decamethrin (Ruzo et al, 1977) has shown that both these transformations can be photochemically induced.

Much of the remaining radioactive residue on the leaves was water-soluble material which was assumed to be conjugated metabolites of Lambda-cyhalothrin. A number of ^{14}C-metabolites were identified in the dichloromethane extracts generated by acid hydrolysis, and the metabolites were essentially the same in both cotton and soya, the major primary metabolic reaction being cleavage of the central ester linkage. Thus, the major metabolites identified from plants treated with ^{14}C-cyclopropane-labelled Lambda-cyhalothrin were conjugates of the cis- and trans-isomers of 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropanecarboxylic acid (compounds IIa and IIb), which accounted for up to 25% of the total radioactive residue in the leaf. Small amounts of both these compounds were also determined as unconjugated metabolites in the hexane-soluble fractions from cotton leaves. Small amounts (up to 4%) of conjugated 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2-hydroxy-methyl-2-methylcyclopropanecarboxylic acid (compound III), a product of hydroxylation at the one of geminal dimethyls on the cyclopropane ring, were also detected, although it is not clear whether hydroxylation occurred before or after ester cleavage.

For plants treated with ^{14}C-benzyl-labelled Lambda-cyhalothrin, ester cleavage generated α-cyano-3-phenoxybenzyl alcohol (IV), and conjugates of this compound accounted for up to 6% of the total radioactive residue in leaves. Further metabolism of compound IV occurs to give 3-phenoxybenzaldehyde (V), and 3-phenoxymandelamide (VIII). These compounds were detected at levels of less than 1% of the residue

TABLE 1. Fractionation of radioactivity on cotton and soya leaves

Fraction	Percentage of total leaf residue in:- Cotton		Soya	
	^{14}C-cyclo-propane	^{14}C-benzyl	^{14}C-cyclo-propane	^{14}C-benzyl
Hexane-soluble	53	57	60	56
Dichloromethane-soluble after 2M acid hydrolysis	27	27	35	38
Dichloromethane-soluble after 6M acid hydrolysis	3	2	<1	<1
Water-soluble after 4M and 6M acid hydrolysis	10	10	2	5
Unextracted	7	4	3	1

in cotton leaves, and were not detected at all in soya leaves. Both V and VIII were further metabolised. Compound V was either oxidised or reduced to form 3-phenoxybenzoic acid (VI), and 3-phenoxybenzyl alcohol (VII) respectively. Conjugates of VI and VII each accounted for approximately 8% of the residue in both cotton and soya leaves. For compound VII, this figure also includes a small amount of unconjugated material detected in the hexane-soluble fraction. Compound VIII was hydrolysed to 3-phenoxymandelic acid (IX), and conjugates of this compound accounted for approximately 7% of the residue in both cotton and soya leaves.

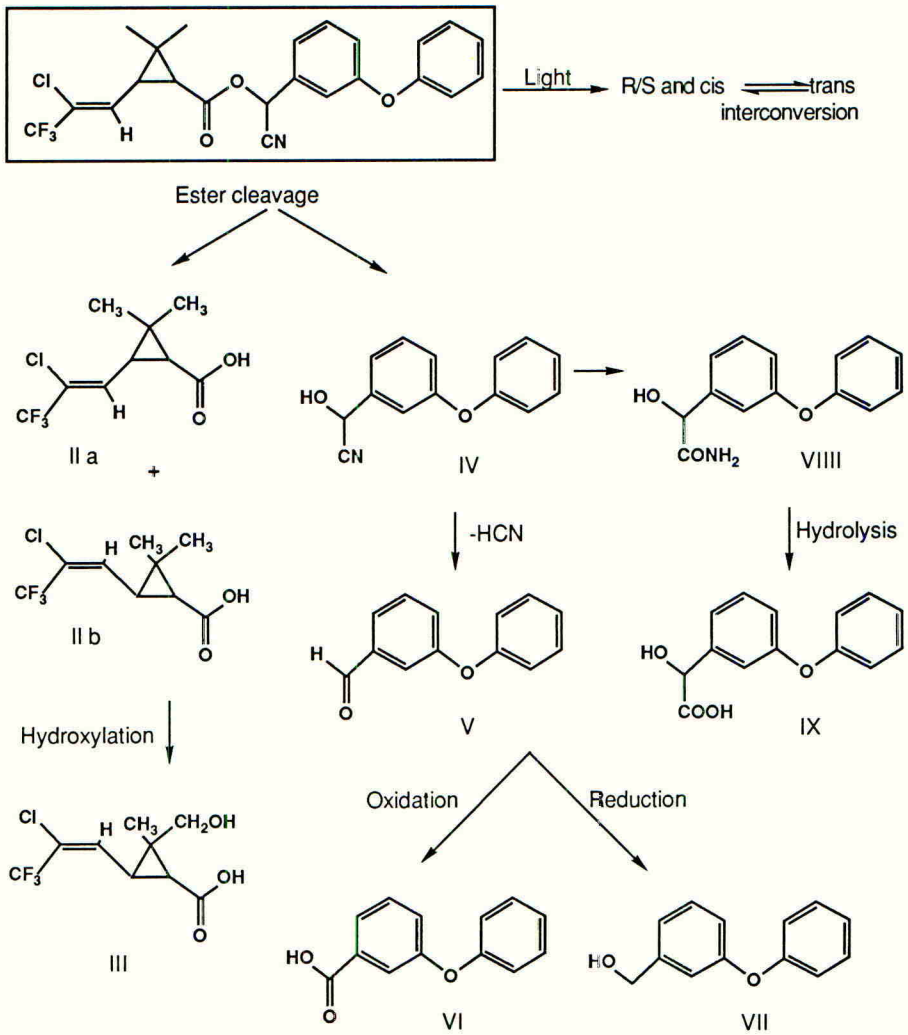
Structural confirmation of selected metabolites by gcms

Structural confirmation by mass spectroscopy was obtained for a number of ^{14}C-metabolites. Methylation of VI yielded the methyl ester and mass spectral analysis gave a parent ion at $m/_z$ 230 and characteristic fragment ions at $m/_z$ 199 $[M\text{-}OMe]^+$, $m/_z$ 169 $[M\text{-}OMe\text{-}^{14}CO]^+$ and $m/_z$ 141. Acetylation of VII yielded the acyl ester, which gave a parent ion at $m/_z$ 244, and fragment ions at $m/_z$ 202 $[M\text{-}CH_2CO]^+$ and $m/_z$ 185. Compound IX was methylated with diazo-methane, then silylated at the benzyl alcohol to yield a derivative which gave a small parent ion at $m/_z$ 332, as well as ions at $m/_z$ 317 $[M\text{-}CH_3]^+$ and $m/_z$ 273 $[M\text{-}CO_2CH_3]^+$. For all three metabolites, the mass spectra obtained for the ^{14}C-derivatives were shown to be in agreement with mass spectra obtained for authentic reference samples.

CONCLUSIONS

A pathway for the metabolism of Lambda-cyhalothrin in cotton and soya leaves is proposed in Figure 2. In total, this pathway accounts for between 60% to 80% of the total radioactive residue found in the leaves. Further identification of the remainder of the residue was not carried out, however, this remainder consists of a water-soluble fraction, possibly containing conjugated metabolites which are not cleaved by strong acid hydrolysis, and an unextractable or 'bound' residue which may be due to incorporation of ^{14}C-fragments into natural plant substances such as sugars and simple organic acids (Kaufman, 1976).

FIGURE 2. Metabolic pathway for the degradation of lambda-cyhalothrin in cotton or soya



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METABOLISM STUDIES OF HEXACONAZOLE IN TEMPERATE CEREALS AND THE SYNTHESIS OF THE METABOLITES

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ABSTRACT

Radioactive residues in wheat straw, following the application of ^{14}C-hexaconazole at intervals throughout the season have been characterised. The major biotransformation pathway is by oxidative metabolism of the alkyl side chain and subsequent conjugation with natural sugar molecules. The major components of the residue were hexaconazole, (±)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2,5-diol, (±)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2,4-diol, and (±)-5-(2,4-dichlorophenyl)-6-(1H-1,2,4-triazol-1-yl)hexan-1,5-diol, were characterised by thin layer chromatography, high performance liquid chromatography and gas chromatography multiple ion detection mass spectrometry of the trifluoroacetyl derivatives. Standard reference compounds of the putative metabolites were prepared by unambiguous synthesis.

INTRODUCTION

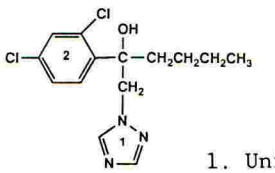
Hexaconazole [(RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexan-2-ol], 'Planète' is a broad spectrum systemic fungicide used to control a wide range of fungal diseases in cereal.

In order to investigate the biotransformation of hexaconazole on cereal straw ^{14}C-labelled hexaconazole was applied to wheat three times throughout the season. In this paper we describe the design, methodologies and results from the metabolism study and in particular how the support of skilled synthetic chemistry effort can greatly assist in the elucidation of biotransformation pathways by the preparation of putative metabolites using unambiguous synthesis.

MATERIALS AND METHODS

Hexaconazole radiolabelled with carbon-14 in either the triazolyl or phenyl ring, Figure 1 was used to treat two 0.3 m^2 plots of winter wheat (var. Galahad) grown under field conditions.

FIGURE 1. Structure of hexaconazole showing the location of the ^{14}C label.



(I)

1. Uniformly ^{14}C-triazolyl-labelled hexaconazole
2. Uniformly ^{14}C-phenyl-labelled hexaconazole

The two plots of wheat were sprayed with formulated ^{14}C-hexaconazole, one with ^{14}C-labelled triazolyl and the second with ^{14}C-labelled-phenyl material three times during the season. The first application, equivalent to 250 g/ha was applied at growth stage 30 (Zadoks decimal code) and the second and third sprays equivalent to 125 g/ha were applied at flag leaf and ear emergence (PHI 45 days). To protect the operator and the surrounding area from spray-drift during the application shelters were constructed using polythene sheet hung around a wooden frame. The polythene was removed when the spray was dry and the area netted to prevent bird and mammal damage. At harvest the wheat was separated into the raw agricultural commodities and stored at -15 ± 5°C prior to analysis. To quantify the total radioactive residue, straw was finely chopped and a known weight was extracted by homogenisation with two aliquots of acetonitrile followed by a further two volumes of acetonitrile/water (1:2). The liquid and solid phases were separated and quantified by liquid scintillation counting (lsc) and sample oxidation/lsc respectively. Total radioactive residues were calculated from a summation of the radioactivity found in the two phases.

Characterisation of the residues was carried out using the fractionation scheme shown in Figure 2. Acidic and basic hydrolyses were used to cleave conjugated exocons and to release bound residues from previously extracted plant debris respectively. Initial investigation showed that the metabolic profiles of the straw extracts contained one major and several less significant metabolites and the pattern was the same from each radiolabelled form.

The metabolism of 1,2,4-triazolyl fungicides in both plants and animals has been shown (personal experience and more recently reported by Bissig et al, 1988) to be primarily by oxidation of the alkyl side chain. In the case of hexaconazole hydroxylation can occur at any of the four carbons with the possible formation of several regio- and diastereo-isomers. Two of these compounds, 2,3-diol (V) (ICI, 1983) and 2,4-diol (IV) (ICI, 1982), each consisting of mixtures of diastereoisomers, were available as reference samples from previous work (Worthington, 1987).

In order to help identify the remaining metabolites of hexaconazole from this study it was necessary to prepare the other putative metabolites (II and III), and also the acid (VII). The 2,5-diol (III) was prepared (Worthington, 1990) as outlined in Figure 3 by the addition of the protected 3-butyn-2-ol to the substituted phenacyl bromide followed by reaction with 1,2,4-triazole, reduction and deprotection. The diol consisted of a mixture of diastereoisomers. Compound II was synthesised by a convenient route outlined in Figure 4 starting by reacting m-dichlorobenzene with glutaric anhydride to form the δ-ketoacid, which was converted by a series of standard chemical transformations (esterification, Wittig olefination, epoxidation, 1,2,4-triazole reaction and reduction) to the desired diol. The intermediate hydroxyester (VI) (Figure 4) could be hydrolysed to the hydroxyacid (VII).

Characterisation of the radioactive components was achieved by comparison of the extracts with standard reference compounds (II, III, IV, V and VII) using thin layer chromatography (tlc) and high performance liquid chromatography (hplc). Final confirmation of the presence of these components in the extracts was achieved by gas

chromatography multiple ion detection mass spectrometry after derivatisation of the extracts and reference compounds with trifluoro-acetyl imidazole (TFAI). Conditions for the chromatography are shown in Table 1

TABLE 1. Chromatographic conditions used to confirm the presence of the reference compounds

Chromatographic technique	Stationary phase	Conditions
Thin layer	Normal Phase Silica SIL G-25 UV_{254} (0.25mm)	Hexane:Acetone (1:1, v/v)
	Reverse Phase KC18F (0.20mm)	Acetonitrile: Ammonium Acetate (0.025M)(60:40, v/v)
High performance liquid	Spherisorb S50DS2	A. Methanol: Ammonium Acetate (0.025M) B. Water:Amm.Acetate (0.025M) Gradient 45-30%B in 25 min.
Gas (as TFA derivatives)	RSL 150 capillary (0.25mm i.d., 25m)	55°C/2 min, 55-265°C in 14 min, 265°C/10 min, Inj. 250°C

RESULTS

The radioactive residues found in the straw were 1.24 and 1.20 mg equivalents/kg for the phenyl- and triazolyl-labelled forms of hexaconazole respectively. Investigation into the nature of the residue showed that the components from each radiolabelled form were the same and were shown to comprise of hexaconazole (10%), (±)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2,5-diol (III, 40%), (±)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2,4-diol (IV, 8%) and (±)-5-(2,4-dichlorophenyl)-6-(1H-1,2,4-triazol-1-yl)hexan-1,5-diol (II, 6%). Compounds V and VII were not found. A biotransformation scheme for hexaconazole in straw is shown in Figure 5.

FIGURE 2. Scheme used to extract and fractionate the radioactive residues in straw and proportions of radioactivity in fractions from ^{14}C-phenyl (Ph) hexaconazole treated wheat.

Radioactivity residue in the sample taken was 1.24 μg equivalents/g calculated from a summation of the radioactivity in F2 and F1.

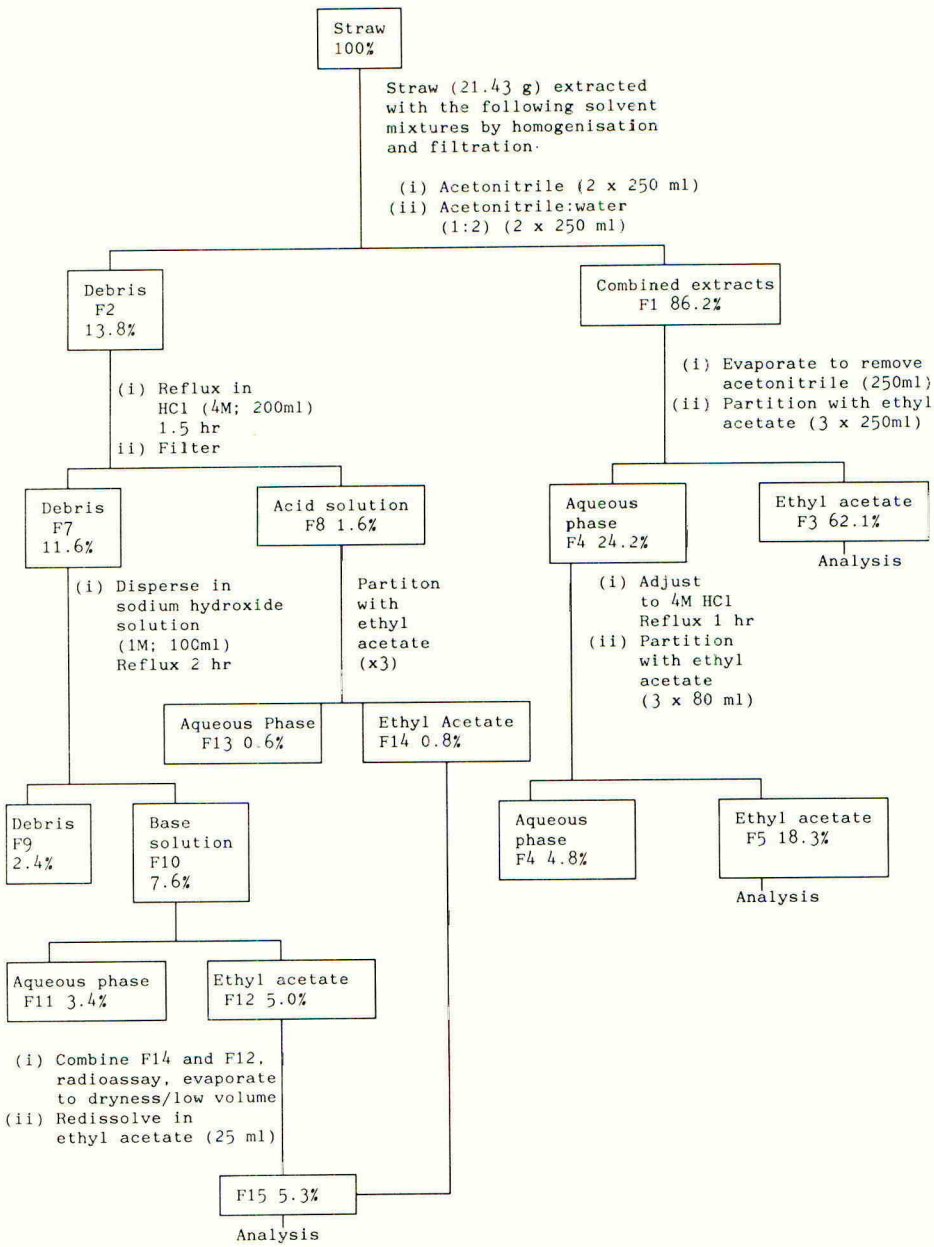


FIGURE 3. Preparation of (±)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2,5-diol (III)

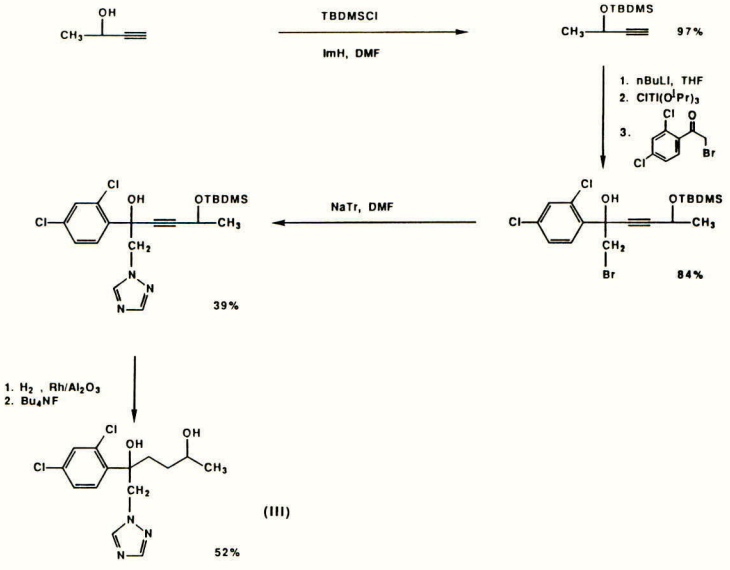


FIGURE 4. Preparation of (±)-5-(2,4-dichlorophenyl)-6-(1H-1,2,4-triazol-1-yl)hexan-1,5-diol (II) and 5-(2,4-dichlorophenyl)-5-hydroxy-6-(1H-1,2,4-triazol-1-yl)hexanoic acid (VII)

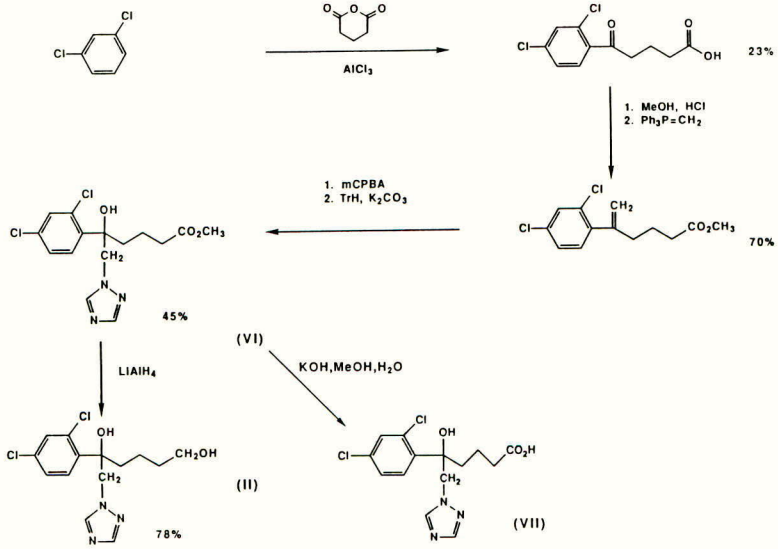
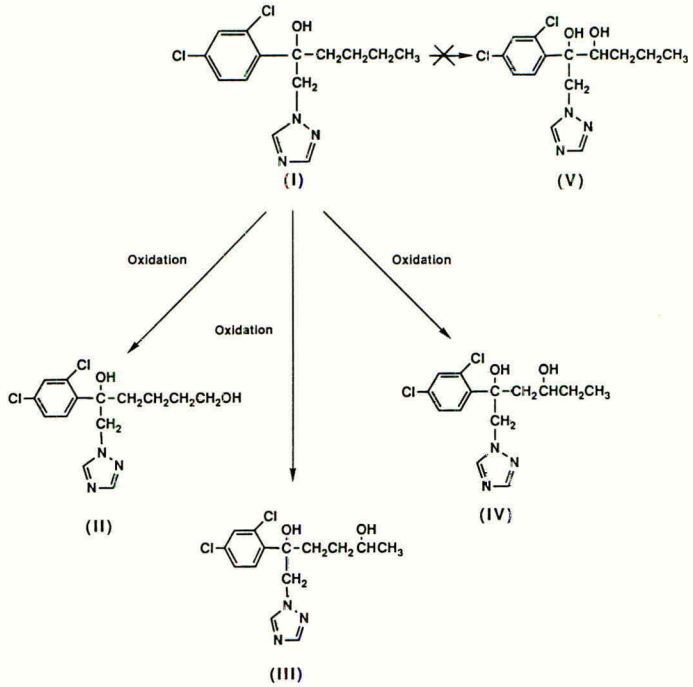


FIGURE 5. Biotransformation of hexaconazole in wheat straw.



CONCLUSIONS

The metabolism of hexaconazole in wheat straw was elucidated by chromatographic comparison of the predicted metabolites using tlc, hplc and gcms. The preparation of the reference compounds was carried out using unambiguous synthesis by skilled synthetic chemists and demonstrates the efficiency of a multi-disciplinary approach to solving metabolic problems.

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