SESSION 7C

EFFECTS AND FATE OF PESTICIDES IN THE ENVIRONMENT

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A PROPOSED TEST METHOD FOR THE ASSESSMENT OF PESTICIDE IMPACT ON SEDIMENT DWELLING LARVAE OF THE MIDGE *CHIRONOMUS RIPARIUS*

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ABSTRACT

Representatives of government and industry regulatory and research groups have formed a working group to develop a test method to assess the potential impact of pesticides on sediment-dwelling organisms. Larvae of the midge *Chironomus riparius* were chosen as the test species.

It is proposed that compounds which have been shown to be persistent and readily adsorb to sediments may be further evaluated using such a test. The method in preparation will assess the development of larvae of the midge *Chironomus riparius* from first instar through to adult emergence using a natural-sediment, artificial-fresh water system. The pesticide formulation under investigation will be applied at a rate equivalent to the expected environmental concentration.

The following paper outlines the proposed test method and gives details of test method validation work currently in progress.

INTRODUCTION

During the normal use of an agrochemical product it is possible that some may reach surface waters as a result of drift and/or runoff. Side effects on non-target organisms, however, should be avoided. To test the potential impact of pesticides in surface waters, representatives of different biological groups - algae, daphnids, and fish, are tested routinely in aquatic studies. Under certain circumstances, when evaluating the effects of more persistent compounds (e.g. DT_{50} in water/sediment system >100 days) which in addition are likely to adsorb to a great extent to the sediment or organic material (POW >1000), sediment dwelling organisms become of particular interest.

At present there is no widely recognised test method available for evaluating the potential impact of pesticides on sediment dwelling organisms. There are test proposals to investigate contaminated sediments and particularly the bioavailability of toxic substances in sediments (Nebeker et al., 1984; Giesy et al., 1988; Ingersoll & Nelson, 1990); they are, however, less suitable for testing pesticides. Therefore a working group was formed to identify and develop a test method. The group comprises representatives of government and industry regulatory and research groups (P. Apel, UBA Berlin; L. Buhr, BBA Berlin; F. Heimbach, Bayer; R. Heusel, Hoechst; H. Köpp,

BBA Braunschweig; A. Müller, BBA Berlin and the authors). In this paper we outline the test method we propose for investigating the impact of pesticides on *Chironomus riparius* as representative of sediment-dwelling organisms and give details of method validation work currently in progress.

TEST ORGANISM

General

Chironomids are members of a widespread and species-rich family of Dipterans. Larvae of species of chironomids can be found in cold and warm, fresh and salt, oligotrophic and eutrophic waters. The life span of the imagines is usually only a few days during which copulation and egg laying occur. *Chironomus riparius* usually breed within 24 hours post-emergence and die soon after. The females extrude large gelatinous egg masses in helical strands. The larvae hatch after about 2-3 days at 20°C. They live in sediment constructing a tube of silk into which debris, sediment or algae may be woven. After four larval stages the larvae pupate. The pupae rise to the water surface and adults emerge about 18-26 days after egg hatch (at 20°C).

Culture conditions

Chironomus riparius larvae may be reared in glass crystallising dishes 14 cm diameter, 7 cm deep (ca. 1 l volume). Fine, acid washed quartz sand (e.g. Merck Art. No. 7536, washed and calcined, 0.2-0.8 mm) is spread in a thin layer about 2 cm deep on the bottom of the container. Kieselgur (e.g. Merck Art. No. 8117) has also been shown to be a suitable substrate.

Chironomids have been successfully cultured in a range of synthetic media, the same as used to culture *Daphnia* (e.g. medium M4, described in Elendt & Bias, 1990). Water is added to give a depth of about 3 cm above the substrate. Water levels should be topped up as necessary to replace evaporative loss. Gentle aeration (about 1-2 cm above the sediment to avoid disruption) should be provided via an airstone or pasteur pipette.

The culture can be started by allocating 50-100 first instar larvae to the prepared vessel.

Feeding

Food for the larvae can be prepared by adding 2.0 g of proprietary fish food (e.g. Tetra-Min) to 200 ml of dilution water and blending to give a homogeneous mix. Aliquots may then be added to the culture vessels (4-6 ml each day per 100 Chironomid larvae). Alternatively a small quantity of ground dry food may be finely distributed on the water. The amount of food given by both methods has to be adjusted according to water quality. Older larvae will require more, young ones less. If the larvae leave their tubes and start searching for food this indicates the feeding level is too low; on the other hand the water should not be overloaded with food and become cloudy or smell foul. Both will ultimately result in reduced growth rates (or even death of the larvae). The emerging adult midges do not require feeding.

Environmental conditions

The temperature within the breeding room should be maintained at 20°C (± 2°C). A photoperiod of 16 hours light and 8 hours dark should be established preferably under light of low intensity (< 100 lux). Under high

light intensities it may be necessary to include a 15-20 min dawn/dusk transition period to induce mating flights. The midges should be held in a gauze covered cage sufficiently large to allow swarming of the adults (a surface area of about 0.4-0.6 m^2 and a height of about 80-100 cm proved to be adequate).

Within a short period of emergence the adults will mate and egg laying may be observed within 1-3 days. The egg masses can be removed to start a new culture. This may be done using a widebore pipette which avoids damage to the eggs. To synchronise hatching of egg masses from different days the eggs may be transferred to a refrigerator (ca. 6°C) for a few days without reducing hatching rates severely.

First instar larvae should emerge after 2-3 days. They should be provided with a substrate for tube building at this time. If they are allowed to migrate out of the mucous sheath of the egg mass in the absence of a substrate they may attach to the glass and are then difficult to dislodge. The larvae go through four instar stages, pupate and emerge as adults in 18-26 days, the males usually being first to emerge. The time to emergence being dependent on the food available.

PROPOSED TEST METHOD

Principal of the test

First instar larvae obtained from established laboratory cultures will be introduced into acclimated natural-sediment, artificial-fresh water systems. The product under test will be applied as the formulation at a single rate equivalent to the expected environmental concentration based on the maximum recommended application rate. The development of the larvae to adult midge will be monitored.

Materials and methods

Test species

The test should be carried out using first instar larvae obtained from established laboratory cultures. Details of the culture methods are given above.

Sediment

The sediment should be collected from a suitable source, which will support growth and development of the test organism. It should not be contaminated e.g. with heavy metals or persistent organic materials.

The top 5-10 cm of the sediment should be sampled. Preferably, the sediment should be used without intermediate storage, however, if this is not possible it may be stored at about 4°C. The sediment should never be allowed to dry out.

The following information on the sediment and natural overlaying water at the point and time of sampling should be reported:

Water:
- pH
- oxygen content

- alkalinity
- hardness

(pH and oxygen should be determined both in the free water and close to the sediment)

Sediment:

- pH (measured with a 1:2.5 sediment: 0.01M $CaCl_2$ solution)
- particle size distribution
- organic carbon
- microbial biomass
- cation exchange capacity

Prior to the use and examination of the sediment it should be sieved through a 500 µm sieve to remove large particles and most of the fauna.

Preparation of the test

The water used in the sediment water system should be the same as that in which the test organism is cultured (see above). Its composition and physico-chemical properties (pH, alkalinity, conductivity, hardness) should be described. The test should be conducted in glass aquaria; a suitable size is ca. 25 x 34.5 x 21.5 cm (depth x length x width). These should contain a sediment layer ca 2.0 cm deep and a water column 20 cm deep. Gentle aeration should be provided through a glass pasteur pipette situated ca. 2.5 cm above the sediment layer. Once set up the aquaria should be covered (to avoid unnecessary evaporation) and allowed to acclimate for 1-2 weeks prior to treatment. During this acclimation period dissolved oxygen and pH of the water phase should be monitored. The exact volume of water added should be noted, and the level marked on the test vessel. Any loss of water should be replaced with deionised water prior to treatment.

About 24 hours before treatment, 50 first instar larvae should be allocated to each test vessel. Product treated and untreated control vessels should be replicated three times.

The tests should be conducted in a constant environment room at 20°C with a photoperiod of 16 hours light, 8 hours dark, and a 15 minute dawn/dusk transition period.

Pesticide application

The test compound should be applied as formulated product at a rate equivalent to the expected environmental concentration (to simulate a worst case scenario the calculation may be carried out according to BBA guidelines IV, 5-1, 1990). To improve homogeneous distribution the test substances may be diluted before application. Application should be carried out using a pipette. The substance should be evenly distributed into the upper water column and mixed gently without disturbing the sediment.

Assessment

All test vessels should be observed at least three times per week without disturbing the larvae and the sediment. Any differences in the construction of tubes or activity of the larvae should be noted. After day 20 of the experiment, observations should preferably be made daily (on working days) to record the time, number and sex of emerging adults. When 80% or more of the original larvae have emerged in the controls or when there has been no further emergence for five days, the test should be terminated. The sediment should then be examined for any remaining larvae. Their number and stage of

development should be recorded. Any egg masses deposited prior to the termination of the test should be recorded and removed to prevent re-introduction of larvae into the sediment.

Readings should be taken of the pH, temperature and dissolved oxygen levels in each test vessel at the start and the end of the study, and twice weekly during the experiment. The concentration of the test compound in the water columns should be analysed at the start and the end of the study.

METHOD VALIDATION

When developing a test method care has to be taken that the results obtained are relevant, reliable, replicable and interpretable. The method should be (relatively) easy to execute and, for regulatory studies, be designed to comply with the principles of GLP. In the following sections a stepwise procedure is given to develop and validate the previously described method for testing the potential impact of pesticides on sediment-dwelling organisms.

Range finding tests

A preliminary range-finding test will be conducted with first instar larvae in test solutions without sediment. Larvae will be exposed to a range of concentrations of a standard toxicant (a parathion formulation) for a time period of 48 hours. The test solutions should be prepared in the same standard dilution water in which the cultures are maintained. The test should be set up in triplicate for each concentration with 10 first instar larvae in each test vessel. Mortality should be recorded after 24 and 48 hours exposure and the EC_{50} calculated.

In a second range finding test, the midge larvae will be exposed to the test substance at a similar range of concentrations in the presence of sediment. Crystallising dishes will be set up containing 2 cm of the sieved natural sediment (for sediment collection and preparation see above). It will be covered by the same depth of water used in the culture (ca. 3 cm deep). The exact amount of water should be recorded and the level marked on the test vessels. The vessels will be covered with glass plates to reduce evaporative loss and prevent aerial contamination. They should be allowed to acclimate for 1-2 weeks before use. The water level should be topped up to the mark just before use. Approximately 24 hours prior to treatment, 10 first instar larvae will be added to each vessel. Triplicate dishes will be treated with each of the test concentrations. The water will be gently aerated via a glass pasteur pipette approximately 2 cm above the sediment (which should not be disturbed). At the end of the 10 day test period the larvae should be examined for mortality and larvae development.

Definitive test

Based on the results of the range finding tests, a suitable range of concentrations for the last step will be selected. This study will be conducted in glass aquaria as previously described (see preparation of test), only during the method validation phase will a range of concentrations of the test product be tested, to assess the sensitivity of the test method.

Conclusions

The method validation work will be carried out in six different laboratories. Based on the results of these studies the details of the test method will be finalised. Laboratories will be invited to participate in a second ring test, using a number of test substances with different physico-chemical properties. As the testing laboratories will be using natural sediments from different sources, the degree of variability which may be expected in the results will be reviewed.

Following this programme of work it is hoped a fully validated method can be recommended for testing the impact of agrochemical products, found to be persistent and having a high potential for accumulation, on sediment-dwelling organisms.

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INHIBITION OF THE DAPHNIA β-GALACTOSIDASE ENZYME COMPLEX AS A PREDICTIVE ASSAY FOR 21 DAY CHRONIC TOXICITY OF NEW CHEMICALS

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ABSTRACT

A new fluorimetric assay for assessing the *in vivo* inhibition of the β-galactosidase enzyme complex in *Daphnia magna* Straus was further developed and evaluated as a rapid assay for predicting the chronic toxicity of new chemicals to this species. After only 24 hours exposure of *Daphnia* neonates to five different crop protection products it was possible to detect significant reductions in enzyme cleavage of the substrate 4-methylumbelliferyl-β-D-galactoside. Furthermore, the concentration range over which reductions in enzyme cleavage occurred corresponded to the range over which reductions in growth and reproduction occurred over 21 days. Optimum methods for performing the assay and results thus far obtained are presented.

INTRODUCTION

Although the specific mechanisms by which toxicants affect individual organisms are in many cases unknown, it is generally assumed that the primary effects of toxicants are on cytological, biochemical and physiological processes. If exposure persists these may translate into longer-term life-history responses. Hence, the initial biochemical responses of organisms to toxicants can be indicative of potential longer term effects on individuals and populations if exposure persists. Of the biochemical processes investigated, enzyme stimulation or inhibition have in many cases proved to be sensitive indicators of toxic stress, for example acetyl cholinesterase (Weiss, 1961), aliesterase (Dortland, 1978), mixed function oxidases (Livingstone, 1985), electron transport system (Borgman, 1977), and lysosomal enzymes (Moore & Farrar, 1985). However, while changes in enzyme function may indicate an early sublethal response to toxic stress, they need not always imply a detrimental effect on survival, growth or reproduction of the individual if homeostatic compensation takes place (Livingstone, 1982).

Recently, inhibition of enzyme cleavage of the fluorigenic substrate, 4-methylumbelliferyl-β-D-galactoside (MUF galactoside) to 4-methylumbelliferone by toxicants in *Daphnia magna* has been shown to correlate qualitatively with conventional acute sensitivity (Janssen *et al.*, 1992). The aim of this work was to (a) develop a quantitative method for measuring enzyme cleavage of MUF galactoside in *D.magna*, and (b) evaluate whether inhibition of the *Daphnia* enzyme system can be used to predict the chronic toxicity of new chemicals to this species.

MATERIALS AND METHODS

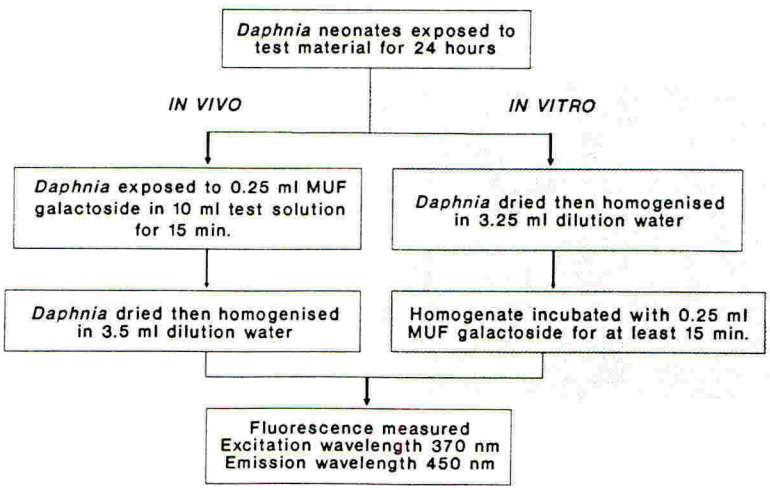
Chronic toxicity tests

Daphnia 21 day chronic toxicity studies were performed for five plant protection products; Galtak® 10 EC (AI : benazolin ethyl ester), Tramat® 50 SC (AI : ethofumesate), Sportak® 40 EC (AI : prochloraz) and two experimental fungicides, pyrimethanil and fluquinconazole. All studies were performed in accordance with international guidelines (OECD, 1984; USEPA, 1986) with a total of forty daphnids exposed to each test concentration, and at least four concentrations tested for each compound. Daphnids were fed approximately 1.35 mg carbon/l *Chlorella vulgaris* daily, and test solutions were renewed at least three times weekly throughout the studies. Actual test concentrations were determined periodically during the studies to ensure that nominal concentrations were achieved and maintained. At the end of each study a sample of the surviving daphnids in each treatment was removed and body length measured, from the tip of the head to the base of the spine, using a dissecting microscope and calibrated eyepiece micrometer.

β-Galactosidase enzyme assay

First instar daphnids were exposed for at least 24 hours, to the same concentrations of the test compounds, under the same environmental conditions, as were used in the chronic toxicity tests (see above). Following the exposure period to the test compounds, β-galactosidase activity was assessed by two alternative methods, represented schematically in Figure 1.

FIGURE 1. Schematic diagram of *in vivo* and *in vitro* measurement of β-galactosidase enzyme activity in *Daphnia magna*.



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For the first method enzyme activity was assessed *in vivo*, i.e. live daphnids were incubated with the substrate MUF galactoside, whereas for the second method enzyme activity was assessed *in vitro*, i.e. daphnid homogenate was incubated with MUF galactoside. For both assays, each replicate contained at least six animals, and a ca. 3 mg/ml stock solution of MUF galactoside was used. Control solutions, containing no daphnids were prepared for each method and used as blanks. For the benazolin ethyl and ethofumesate formulations, measurements of fluorescence were repeated, for each sample, for up to three days to establish the stability of the measurements obtained for the two methods.

RESULTS

Chronic toxicity tests

Concentrations of all test compounds were maintained within ± 20 % of nominal throughout the studies. ANOVA, followed by Tukey multiple range tests showed that all of the compounds caused significant reductions in growth ($p << 0.05$) and reproduction ($p << 0.05$) within the concentration ranges tested. The no observed effect and lowest observed effect concentrations (NOEC and LOEC) for each of the test compounds are given in Table 1.

TABLE 1. Effect concentrations (mg/l) for growth, reproduction and *in vivo* β-galactosidase enzyme activity in *Daphnia magna* exposed to different plant protection products.

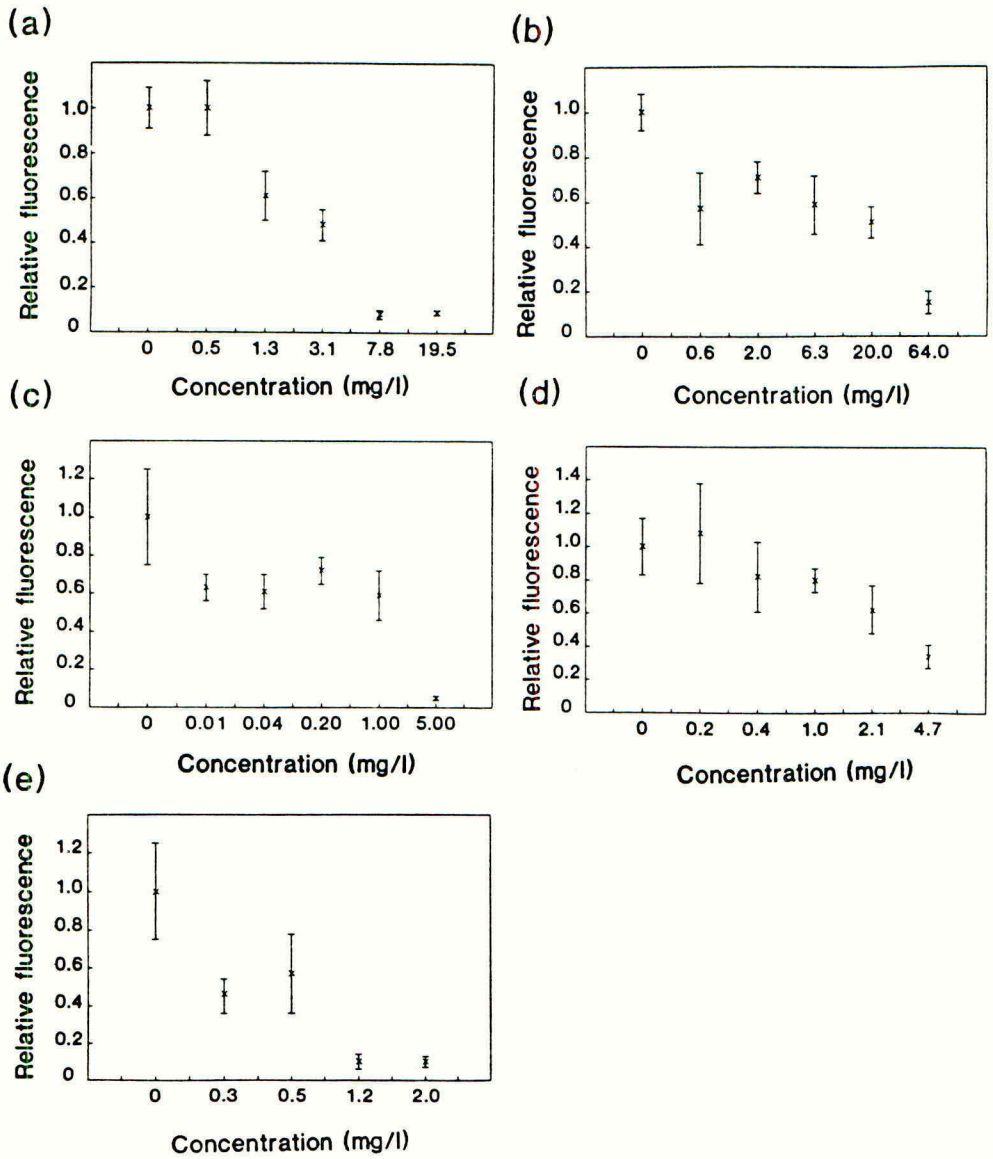
Active Ingredient/ Formulation	Reproduction NOEC	Reproduction LOEC	Growth NOEC	Growth LOEC	EC_{50} for Inhibition of Enzyme Activity
Benazolin ethyl[(a)]	7.8	19.5	7.8	19.5	2.2
Ethofumesate[(a)]	0.6	2.0	6.3	20.0	3.2
Fluquinconazole	1.0	5.0	1.0	5.0	0.2
Pyrimethanil	1.0	2.1	2.1	4.7	2.0
Prochloraz[(a)]	0.6	1.1	0.6	1.1	0.2

(a) - values given are for formulation concentrations

β-Galactosidase enzyme assay

The effect of the test compounds on *in vivo* enzyme activity, measured as fluorescence at 450 nm, is summarised in Figure 2 and Table 1. For the *in vivo* method, there was a concentration related reduction in relative fluorescence indicating inhibition of enzyme cleavage for all compounds tested. Indeed, at the higher concentrations tested, in all cases, there was a greater than 50 % reduction in fluorescence. Thus, in all cases substantial reductions in enzyme cleavage of the substrate (i.e. >50%) occurred within the same concentration range in which growth and reproduction were reduced in the chronic toxicity tests.

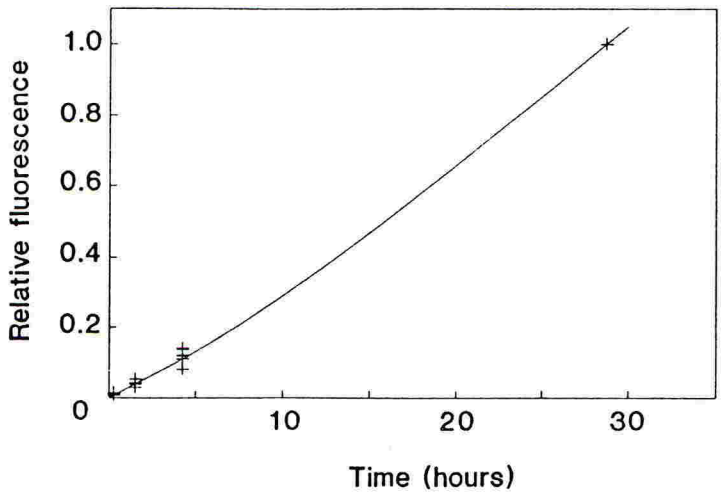
FIGURE 2. *In vivo* β-galactosidase enzyme activity (relative fluorescence) for *Daphnia* exposed to a) benazolin ethyl formulation, b) ethofumesate formulation, c) fluquinconazole, d) pyrimethanil and e) prochloraz formulation. Values given are mean ± standard error.



The pattern of fluorescence obtained for the *in vitro* method differed from that obtained for the *in vivo* method. While there was a concentration dependent increase in fluorescence for the benazolin ethyl formulation, there was no effect of the ethofumesate formulation or pyrimethanil on fluorescence over the concentration ranges tested. Thus the *in vitro* method was not performed for the prochloraz formulation or fluquinconazole.

There was also a difference between the two methods with respect to the stability of the fluorescence measurements. For the *in vivo* measurements, fluorescence of the daphnid homogenate was observed to be stable for at least 24 hours after preparation. However, for *in vitro* measurements fluorescence increased almost linearly with time irrespective of the treatment for up to 20 hours after addition of MUF galactoside (Figure 3). Indeed over 20 h the fluorescence increased by more than 100 fold and was independent of treatment.

FIGURE 3. Relative fluorescence of daphnid homogenate incubated with MUF galactoside at 20°C over 20 h.



DISCUSSION

Toxicant-induced reductions in β-galactosidase enzyme activity in *Daphnia magna* have recently been shown to correlate with acute sensitivity of this species to single chemicals (Janssen *et al.*, 1992). In this paper, the effect of plant protection products on *in vivo* and *in vitro* activity of the β-galactosidase enzyme complex were assessed. The effects of the compounds on enzyme activity were compared with the effects of the same plant protection products on *Daphnia magna* growth and reproduction. The results for *in vivo* activity demonstrated that at concentrations where growth and reproduction were reduced over a 21 day exposure, enzyme activity was reduced by greater than 50 % after only 24 h exposure. Hence, the *in vivo* assay provided a sensitive short-term measure of potential life history effects over long-term exposure.

In vitro enzyme activity was not inhibited by any of the compounds tested. Indeed fluorescence increased with benazolin ethyl formulation concentration. These results suggest that the site of action of compounds in the *in vivo* assay is not the β-galactosidase enzyme, but probably some function of substrate availability. The time dependent increase in fluorescence for the *in vitro* but not *in vivo* assay also indicates that *in vivo* fluorescence was dependent upon active uptake of the substrate by daphnids. Thus, the exact nature of the enzyme response to toxicants in the *in vivo* assay is unclear, and is worthy of further attention.

CONCLUSION

The results of *in vivo* inhibition of enzyme cleavage of MUF galactoside following short term exposure of *Daphnia magna* to a range of pesticides indicates the potential of this assay as an indication of possible longer term effects on growth and reproduction. The technique may therefore be helpful in screening compounds for relative toxicity to *Daphnia magna* as a replacement for the 21 day chronic toxicity test. It is, however, considered that the system should be further investigated using a wider range of compounds. In any investigations, fluorescence should be measured quantitatively in order to make valid comparisons between data derived from different experiments.

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TOXICITY OF SECOND GENERATION RODENTICIDES TO BARN OWLS

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ABSTRACT

The toxicity of three second generation rodenticides, brodifacoum, difenacoum and flocoumafen, to Barn Owls (Tyto alba) has been investigated. The owls were fed the rodenticides over a period of 15 days via rodenticide-fed mice to simulate the potential route of exposure in the wild. With each rodenticide the owls survived a relatively high total dose of 1.9 mg/kg owl bodyweight over 15 days (equivalent to two 25 g mice containing 1 mg/kg daily for 15 days). Residue analysis data were obtained from owl tissues and regurgitated pellets. The latter enabled the pellet analysis method developed for non-invasive monitoring of the exposure of owls to flocoumafen, to be extended to the other two rodenticides. Correlation of the toxicity data with results from field studies indicates a low risk to owls provided the rodenticides are used in a retrievable bait (eg. wax block) according to the minimal-use, pulse baiting method.

INTRODUCTION

Rodents are significant pests in and around farms and urban developments, and effective control relies largely on the use of rodenticide baits. Second generation rodenticides such as brodifacoum, difenacoum and flocoumafen are distinguished from the first generation chemicals by their effectiveness against resistant rodent strains and their ability to cause death after a single feed.

The risk to the environment following rodenticide use depends not only on the toxicity of the active ingredient to the non-target species, but also on the actual exposure of that species. 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 (Tyto alba) is a night hunting predator that feeds on live rodents and birds, and may be considered at risk from such baits.

This paper describes an investigation of the toxicity of three rodenticides to Barn Owls. It forms part of a large programme of work to ensure the safe use of rodenticides in the environment. The owls were fed on mice, which had previously been fed on either brodifacoum, difenacoum or flocoumafen wax block bait. This approach simulated their potential route of exposure in the wild. At post-mortem examination, tissues were removed for rodenticide residue analysis. In addition, regurgitated owl pellets were collected throughout the study and similarly analysed.

RESIDUE ANALYSIS METHODS

Whole treated mice, uneaten mice remains following owl feeding, owl tissues (liver, abdominal fat and breast muscle) and regurgitated owl pellets were analysed for residual rodenticide content. A reverse phase liquid chromatographic method was employed with fluorescence detection and post-column neutralisation to enhance sensitivity (Eadsforth et al, 1991).

For all matrices, the mean recoveries for brodifacoum ranged from 70%-111%, for difenacoum 80%-99% and for flocoumafen 86%-104%. Depending on the sample matrix the limit of determination ranged from 0.01 to 0.04 mg/kg.

MICE FEEDING PHASE

To provide dosed mice with a range of rodenticide concentrations for the owl toxicity study, batches of mice (6 batches per rodenticide) were allowed to feed on either brodifacoum, difenacoum or flocoumafen wax block bait of nominal concentration 0.005% wt/wt. Male mice were used, strain Harlan Olac Hsd/Ola:ICR, age about 6 weeks and mean weight 26 g. The bait was provided either as weighed amounts 1.0 g - 5.0 g over 23 hours or ad lib. for 24 or 48 hours. Representative numbers of mice from the individual batches were analysed, and the mean rodenticide content for the batches ranged from 0.95 - 5.2 mg/kg depending on the rodenticide and feeding regime.

The accumulation of residues in the mice varied considerably depending on the rodenticide. For the same amount of bait offered, the brodifacoum and difenacoum residues were respectively 2.3 and 1.3 times higher than those of flocoumafen. The lower retention of flocoumafen and difenacoum in the mice is presumably the result of faster metabolism and/or excretion of these two rodenticides than brodifacoum.

OWL FEEDING STUDY

A total of twelve captive bred Barn Owls of mixed sexes, weighing 320-455 g were employed. They were individually housed in outdoor flight pens (4.5 m x 2 m x 2 m) constructed of wire netting on a wooden frame with a base of concrete slabs and fitted with partial shelter, nest box, perching and a water bowl. Prior to treatment they were acclimatized for 15 days to a daily ration of up to four untreated mice (approximately 80 g of mice per day per bird).

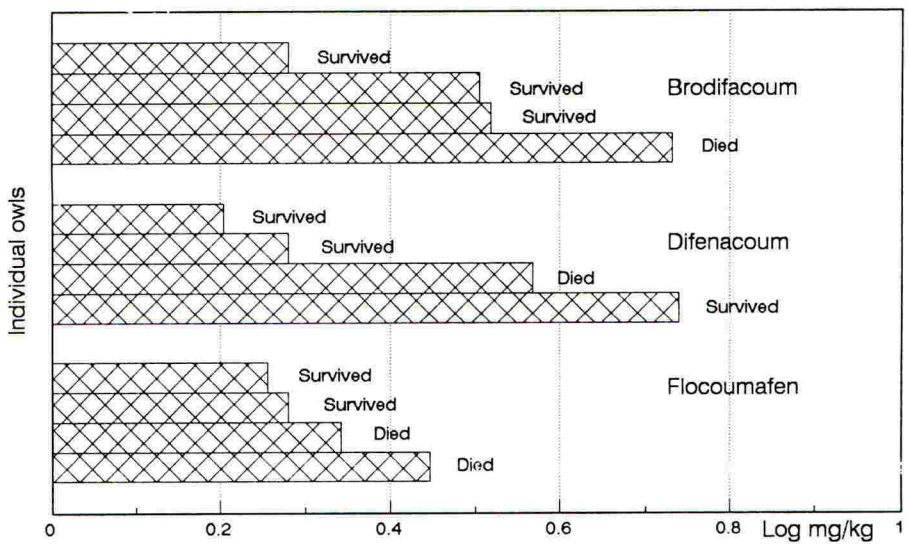
The owls were fed for 15 days in groups of 3, one per rodenticide. The amount of rodenticide offered to the owls was increased or decreased, depending on the result from the previously dosed group, by varying the numbers of treated mice offered and their residual rodenticide content. The balance of the owl feed was made up with untreated mice. Uneaten mice remains were collected and analysed to determine net rodenticide consumption. Owls surviving the 15 day treatment period were then fed on untreated mice for a further 15 days or until death. At the end of this period surviving owls were sacrificed, subjected to post-mortem examination and tissues (the liver, abdominal fat and breast muscle) were removed for

rodenticide residue analysis. Regurgitated owl pellets were collected throughout the study for residue analysis. Those obtained during acclimatization were used as control samples for the analysis.

For each rodenticide, the owls survived a cumulative dose of at least 1.9 mg/kg owl weight over the 15 days of treatment (Table 1, Fig. 1). Four owls died, one each after treatment with brodifacoum and difenacoum, and two with flocoumafen, on day 15 ± 1 of treatment. These owls had consumed a cumulative dose between 2.2 and 5.4 mg/kg depending on the rodenticide.

All three rodenticides are considered to have the same order of magnitude of toxicity to Barn Owls. This is because the difference in toxicity was observed over a narrow concentration range, only a small population of owls was tested and there was observed variability in individual susceptibility (eg. with difenacoum). In addition all owls with cumulative doses in excess of 1.9 mg/kg had multiple treatment related effects.

FIGURE 1. Owl feeding study cumulative dose (mg/kg) over 15 days



RODENTICIDE RESIDUES IN OWL TISSUE

The residues of all the rodenticides (Table 1) in breast muscle and abdominal fat were low. There was limited evidence of increased retention of brodifacoum and flocoumafen residues in the fat samples of owls treated at the highest levels.

The residues of flocoumafen in the liver samples, 0.5-0.7 mg/kg, are consistent with those found in chickens (Gallus domesticus), 0.6-0.9 mg/kg (Eadsforth, et al., unpublished data), and in Japanese Quail (Coturnix

coturnix japonica), 0.5-0.6 mg/kg (Huckle et al., 1989). The latter studies indicated the presence of a saturable high-affinity flocoumafen binding site in bird livers, and the liver residue data from the present study support this. The residues of brodifacoum are of a similar level to those of flocoumafen. In contrast the residues of difenacoum in liver are 3-4 times lower than those of the other two rodenticides, however, the residue level associated with owl death is correspondingly lower.

In assessing the relevance of rodenticide residues in owls found dead in the wild, it should be noted that owls survived with liver residues of 0.7 mg/kg brodifacoum, 0.15 mg/kg difenacoum and 0.5 mg/kg flocoumafen.

RODENTICIDE RESIDUES IN REGURGITATED OWL PELLETS

The residues of brodifacoum, difenacoum and flocoumafen in pellets collected during the treatment period were very consistent. They averaged respectively 29%, 26% and 21% of the amount consumed. These data are also comparable with flocoumafen residues present following a single dose study with radiolabelled flocoumafen, 15%, (Eadsforth et al., 1991) and 27% in a previous 6-day owl feeding study (Newton et al., in press). Post-treatment the residue levels of all three rodenticides in pellets declined rapidly, such that by day 3 post-treatment there was ≤10% of that during the dosing period and by the end of post-treatment (day 15) the levels were reduced to about 1%.

These data confirm that the pellet analysis method for non-invasive monitoring of potential exposure of owls to flocoumafen (Eadsforth et al., 1991), is also applicable to brodifacoum and difenacoum.

ASSESSMENT OF THE RISK OF THE RODENTICIDES TO OWLS

The results of this study indicate that brodifacoum, difenacoum and flocoumafen have approximately the same order of magnitude of toxicity to Barn Owls.

They also indicate that although the toxicity to Barn Owls of all three rodenticides is high, the risk of poisoning of the owls in the wild is low. With each rodenticide the owls survived a dose equivalent to the consumption of two 25 g mice containing a residue of 1 mg/kg per day for 15 days. This was the typical concentration of flocoumafen found in rats and mice collected in farm trials with flocoumafen wax block bait, employing the pulse baiting method (Harrison et al., 1988).

The total number of rodenticide treated mice, which the owls consumed (30 over 15 days) and survived, is far in excess of that which is likely to be consumed by owls in their natural habitat. The Barn Owl tends to hunt along hedgerows, river/ditch sides, roadside verges and at woodland margins, and its hunting range rarely overlaps with that of contaminated rodents around farm buildings. In addition, with the minimal-use, pulse baiting method using retrievable bait, the bait is only made available for limited, intermittent (weekly) periods. Thus contaminated rodents are potentially available for only a very limited period of time.

This conclusion is supported by results from a recent field study in Eire (Harrison et al., 1990) which monitored Barn Owl roosts and nests in an area where the three rodenticides were being used commercially. Regurgitated owl pellets were collected and no residues of the three rodenticides were found indicating that over the study period none of the owls was exposed to residues of the rodenticides in their prey. Also a survey was carried out by the Institute of Terrestrial Ecology (Newton et al., 1990) in which 145 owls found dead in Britain over a seven year period between 1983-1989 were examined. Although some 10% of the owls contained residues of brodifacoum or difenacoum in their livers, only four owls had residues in excess of 0.1 mg/kg and moreover, only one owl was adjudged to have been poisoned.

CONCLUSIONS

The use of all three rodenticides, brodifacoum, difenacoum and flocoumafen, in and around farms is likely to present a low risk to the owls, providing a retrievable (eg. wax block) bait is used in carefully covered and located bait stations using the minimal-use, pulse baiting method.

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TABLE 1 - Comparative toxicity data of rodenticides to Barn Owls and residues in owl tissues and regurgitated pellets

	Individual owls treated with:											
	Brodifacoum				Difenacoum				Flocoumafen			
Owl Survived	Yes	Yes	Yes	No	Yes	Yes#	No	Yes	Yes	Yes#	No	No
Mean owl weight during treatment (g)	415	415	448	343	345	385	358	350	320	345	377	455
Owl sex	F	F	F	M	M	F	M	M	M	M	F	F
Amount rodenticide offered/day (μg/day)	66	147	129	221*	115	56	221**	156	56	51	82	146
Net amount rodenticide consumed per day (μg/day)	52	88	99	133*	36	50	101**	128	39	43	56	85
Cumulative amount rodenticide consumed per owl weight over 15 days (mg/kg)	1.9	3.2	3.3	5.4*	1.6	1.9	3.7**	5.5	1.8	1.9	2.2	2.8
Residue (mg/kg) in: Liver	0.67	0.55	0.69	1.67	0.06	0.14	0.25	0.13	0.52	0.51	0.57	0.70
Fat	0.01	0.01	<0.01	0.13	<0.01	<0.01	0.01	<0.01	NS	0.01	0.07	0.08
Muscle	0.02	0.02	0.02	0.04	0.01	0.01	0.01	0.01	0.06	0.04	0.05	0.06
Mean amount of rodenticide regurgitated in pellet (μg/day)	19	NA	27	32	NA	10	31	35	5.5	10	NA	21
% rodenticide regurgitated	37	NA	27	24	NA	20	31	27	14	23	NA	25
Mean % rodenticide regurgitated in in pellets	Brodifacoum mean = 29%				Difenacoum mean = 26%				Flocoumafen mean = 21%			

Notes 1) The only owls which showed signs of ill-health during the study period were three of the four which died.

2) At post-mortem all owls except those marked # showed some treatment related effects (mainly minor).

3) NS - Insufficient sample for analysis.

4) * and ** Calculated, respectively, over 14 and 13 days feeding until death on the following day.

5) NA - Not analysed

6) All owls which died had survived the 15 days of treatment $\pm$ 1 days.

ACCEPTANCE STUDIES TO ASSESS THE HAZARD OF PESTICIDES FORMULATED AS DRESSINGS, BAITS AND GRANULES, TO BIRDS

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ABSTRACT

Hazard assessment procedures for pesticides formulated as dressings, baits and granules are reviewed. Several study designs assessing the palatability / repellency or hazard of granular formulations or treated seeds to birds in the USA, France, and a standardized guideline of the BBA in Germany are discussed and evaluated.

Although there are important differences in test design, all are valuable tools for the assessment of hazards to birds. The current data base from 10 years of studies according to the BBA guideline, allows new products to be comparatively ranked in terms of the potential hazard which they may pose to birds. Experience has shown that with this information a reliable and rather conservative prediction of the degree of safety to birds under actual farming conditions can be made.

INTRODUCTION

For the registration of pesticidal products, data on the toxicity to at least one bird species are required. The data to be submitted range from acute single dose toxicity values (LD_{50}, lethal threshold dose etc.) to no-observed-effect-concentrations (NOECs) in bird reproduction tests. Hazard assessment for birds is generally done by comparing the toxicity data with the maximum expected exposure concentrations. Seed dressings and granular formulations require special attention, because birds might either ingest treated seeds as food or the granular formulation as grit. In both cases, the total amount of active ingredient (AI) that can be taken up tends to be higher than the amount that is usually ingested as residues on sprayed feed items like insects or parts of plants. Concentrations on seed dressings and granular formulations are given by the application rate for seed dressing and the percentage of AI in the granules. Exposure can then be expressed as LD_{50} per m^2, corrected for the percentage of particles not exposed on the soil surface.

Some of the pesticides used for seed dressings or granules are toxic to birds. The AI are in many cases cholinesterase inhibitors (carbamates and organophosphates) like aldicarb, carbofuran, carbosulfan, isofenphos, terbufos, and methiocarb. Theoretically, a few particles containing one of these pesticides could be hazardous for an individual bird. However, taking into account their repellent properties, use and an appropriate formulation and/or mode of application, these particles can be handled without posing an unacceptable risk to birds.

For the pesticide manufacturer, it is essential to relistically evaluate the hazard of active ingredients that are toxic to birds. The tests for assessing the hazard potential have to be cost-effective, reproducible, and allow a reliable extrapolation to the field. An intermediate step between laboratory and full scale field studies may often be sufficient and may bypass the necessity for field studies which require much effort and time and whose results are often difficult to interprete (cf. Somerville & Walker, 1989). Various test methods have been used to assess the palatability/repellency, attractiveness or hazard of formulations/contaminated feed items to birds (Schafer *et al.*, 1983, Avery, 1991, INRA, 1990). Currently, the only standardized and ring-tested guideline addressing this issue is the BBA guideline 25 - 1 (BBA, 1981). This test guideline was developed in cooperation with experts from authorities, industry and research institutions. It was finalized in 1981 and is presently being updated. In a "round-robin" test, the method was calibrated (unpublished).

TEST PROTOCOLS

BBA (Biologische Bundesanstalt, Braunschweig) test guideline 25-1

In a two-step approach, the potential hazard of pesticidal end-use products is evaluated in a cage test (pen test). The standard test species is the Japanese Quail (*Coturnix coturnix japonica*) but the method can easily be applied to other species like home pigeons (*Columba livia domestica*), house sparrows (*Passer domesticus*), etc..

Variation A

This is an intensified version of the test. Its main purpose is to highlight any potential hazards of a test compound. This test does not account for actual farming conditions like application rate or the number of treated uncovered particles exposed on the soil surface. If no mortalities occur under these stringent test conditions, it can be assumed that the test formulation has a high margin of safety for birds.

At least 2 groups of 8 or more Japanese Quails are caged and accustomed to the untreated seed. Prior to the exposure phase of 8 hours they are starved for approximately 16 hours overnight. At the beginning of the exposure phase, untreated seed (25 % of the normal daily feed intake) and treated seed (75 % of the normal daily feed intake) are scattered on the floor of the cages which is covered with white quartz sand. The birds are observed periodically throughout the exposure period and thereafter for a 14 day observation period. Symptoms of intoxication and mortalities are recorded. Birds dying during the study are dissected and the ingested treated seeds are counted, if possible.

Variation B

In the case of mortalities in variation A, a second, less severe study is performed. Experience has shown that the results of this version reflect more realistically the hazard to birds under actual farming conditions. If no mortalities are observed, the margin of safety is sufficient to prevent bird death under good farming practice.

In an otherwise similar design, the birds are exposed to untreated and treated seed in a ratio of 90 : 10, respectively, of their daily feed

intake. Granular formulations are tested in the same way but without accustoming the birds to the blank formulation (granular formulation without AI).

Treated corn can be tested only with larger birds like pheasants (*Phasianus colchicus*) or pigeons because japanese quails do not ingest whole corn kernels. To represent small birds with a body weight around 20 g, house sparrows can be used.

INRA (Institut National de Recherche Agronomique, Versailles) test method

Individually caged birds (grey partridges (*Perdix perdix*) or red partridges (*Alectoris rufa*)) are accustomed for several days to the test seed which is dressed with a blank formulation. No alternative feed is provided. The seed consumption rate is recorded for each bird. During a 24 hour exposure period, those birds which consumed the blank formulation in sufficient quantity are offered exclusively seeds dressed with the AI containing formulation in feed cups. The individual consumption of treated seeds is used as a measure for the repellency of the formulation.

US Fish and Wildlife Service test method (Denver)

This method estimates repellency as a function of pesticide concentration in the feed expressed as the "R50" value (Schafer, 1983). The R50 is the concentration level in feed, at which the birds (redwinged blackbirds (*Agelaius phoenicus*)) would reduce consumption to 50 % of their approximate individual maximum food uptake capacity. By comparing the amount of AI ingested at that level (in mg/kg b.w.) with the LD50, a hazard factor can be calculated. No or little potential to cause bird poisonings is assumed if the ratio R50/LD50 is < 0.25. The results are currently used for supplementary information and no regulatory actions are triggered by these test results.

US Fish and Wildlife Service method (Gainesville)

As an intermediate step between laboratory and field studies, these tests are designed to assess the repellency and potential hazard of seed dressings to birds. Redwinged blackbirds are caged individually and are accustomed to the seed to be tested. For a 4 to 5 day period, the birds are offered treated seeds once daily for six hours, in addition to a permanent provision of untreated feed. The ratio of consumption of treated and untreated seed during the daily exposure periods is used as a measure for repellency (Avery, 1991).

COMPARISON OF RESULTS FROM THE DIFFERENT TEST METHODS AND EXTRAPOLATION TO ACTUAL FARMING CONDITIONS

Comparable results for several of the methods described above are available for the active ingredients imidacloprid, methiocarb and carbofuran.

Methiocarb

The hazard factor for methiocarb according to the method of the US Fish and Wildlife Service (Schafer *et al.*, 1983) was determined to be 1.47

which indicates a significant potential for avian toxicity. Methiocarb was also tested as a formulation containing 50% AI for dressing of corn in the intensified version of BBA 25-1 acceptance tests with home pigeons. The birds were strongly repelled by the test formulation and none of the pigeons showed signs of toxicity (Hermann, 1983).

Extensive experience with methiocarb seed dressings in the agricultural practice indicate that the "hazard factor" apparently overestimated the hazard to birds posed by using methiocarb as a seed dressing.

Imidacloprid

Wheat seeds dressed with imidacloprid (200 g/100 kg; red and blue coloured formulations) were offered in BBA 25-1 acceptance tests to japanese quails and house sparrows. No toxic symptoms were seen in house sparrows with both colors and in both variations of the acceptance test. Out of 8 japanese quails, 4 showed toxic symptoms in the intensified acceptance test with the red coloured dressing. All birds, however, recovered within one day. No effects were seen in variation B of the acceptance test with the red dressing and in both variations with the blue dressing (Pflüger & Schmuck, 1991).

In a test following the INRA-method, wheat seeds treated with 700 mg/kg diet of imidacloprid also proved sufficiently repulsive to grey partridges and crows, thus preventing those birds from ingesting a lethal amount of the AI.

These favourable findings were confirmed by the results of a field study in Great Britain. No bird casualties were recorded up to three weeks following the drilling of winter wheat seeds dressed with 700 mg/kg diet of imidacloprid on an 11 hectare field (CSL, 1992).

Carbofuran

The hazard factor for carbofuran as determined according to the method of the US Fish and Wildlife Service (Schafer *et al.*, 1983), was 5.1, indicating a high potential for avian toxicity.

Carbofuran was also tested as a 5% granular formulation in BBA 25-1 acceptance tests with japanese quails. Lethal effects in the intensified variation A tests were recorded for 19% of the tested birds. About 25% of the test birds exhibited toxic symptoms but showed partial recovery. These findings suggested that uncovered exposed carbofuran provides no large margin of safety to birds, thereby justifying the need for further tests.

In the variation B acceptance tests, sublethal toxic effects were noted in 10% of the birds and 2 out of the 32 test individuals died. Both test variations do not consider the actual application form, where the granules are incorporated into the soil thus mitigating the exposure conditions significantly. According to experience regarding extrapolation of test results to actual farming conditions, the findings do not indicate a significant risk to bird populations, whereas a hazard can not be excluded for all individual birds.

Observations of bird incidents in the field, arising from the use of granular carbofuran are listed in the field survey data report from the British Advisory Committee on Pesticides. According for example to the 1990 report on Pesticide Poisoning of Animals (Fletcher *et al.*, 1990), the number of bird carcasses reported under actual farming conditions from the use of granular carbofuran was less than 10 individuals in Great Britain. Similar conclusions can be drawn from other reports in Europe in general. These field observations confirm the conclusions from the BBA variation B tests and demonstrate that the limited hazard posed by application of granular carbofuran to birds could reliably be predicted. (The situation in the USA is not comparable due to the higher content of AI in the granules, different agricultural conditions and application techniques.).

CONCLUSIONS

Although there are important differences in the cage tests described above, they are all valuable tools for the assessment of the hazards to birds from the use of pesticides. The BBA guideline 25-1, in particular, has a solid foundation of more than 10 years of testing. This has shown that hazard predictions based on this guideline are reliable. Due to the more stringent exposure conditions compared with actual farming conditions, the effects observed in cage tests even tend to overestimate the actual hazard. This overestimation results from the fact that the density of seeds/granules offered to the test birds is much higher than in the field, the particles are not incorporated in the soil and the birds have no opportunity to change to another, untreated source of feed.

Besides hazard predictions, another more generalized advantage of standardized tests is the comparison and optimization of formulations with respect to safety for birds, e.g. by selection of different colorations, lower AI content per single item or by addition of a bird repellent.

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POST-REGISTRATION SURVEILLANCE TO DETECT WILDLIFE PROBLEMS ARISING FROM APPROVED PESTICIDES

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ABSTRACT

The Wildlife Incident Investigation Scheme (WIIS) operates in England and Wales as a post-registration procedure to monitor the effects of pesticides on wildlife by the investigation of cases of suspected pesticide poisoning. This is carried out by a combination of methods including field inquiries, disease assessment and laboratory studies. If the results of the Scheme demonstrate the existence of an unacceptable risk, the Advisory Committee on Pesticides may recommend withdrawal or modification of a product's approval. A similar scheme operates in Scotland.

This paper presents recent results from the Scheme, indicating that only a small proportion of confirmed cases of poisoning are caused by the approved use of agricultural chemicals. The majority of cases are due to misuse and deliberate abuse of pesticides. An outline is given of current research aimed at improving the efficiency, scope and reliability of the Scheme.

INTRODUCTION

Investigation of incidents started in the late fifties, when large scale deaths of grain-eating birds occurred following treatment of cereal seed with cyclodiene insecticides such as dieldrin. Since that time, a procedure has been developed for investigating wildlife incidents thought to involve pesticide poisoning. The Scheme now involves a field inquiry, to help identify the cause of an incident; a post-mortem examination, which may result in tests to ascertain whether disease contributed to the death; and chemical analyses of tissues from the dead animal to determine the presence of a range of pesticide residues (Hardy *et al.*, 1986).

The results are interpreted to assess the probable cause of the incident and whether any residues detected contributed to the death or illness of the animal. Mortality is generally attributed to a pesticide if residues or its derivatives are found above levels considered to represent lethal exposure. In some cases, the presence of residues in association with characteristic post-mortem findings may be used to assess mortality.

The results of investigations are reported to the Environmental Panel of the Advisory Committee on Pesticides (ACP). The information provided may result in a re-evaluation of the approvals previously granted to that product, or may affect the progress to full commercial use of products currently under provisional approval. A report which contains all the results for the year is published annually (Fletcher *et al.*, 1992).

In this paper only incidents involving vertebrate poisoning are examined, although the Scheme also examines the pesticide poisoning of beneficial insects such as honeybees.

FINDINGS

The cause of incidents in which pesticides are found can be assigned to one of four categories (Greig-Smith, 1988; Fletcher *et al.*, 1992). These are abuse of a product, in the form of deliberate, illegal attempts to poison animals; misuse of a product, by accidental, careless or wilful failure to adhere to the correct practice; approved use of the pesticide, according to the specified conditions for use; and unspecified use of a pesticide, where there is no evidence to assign the use to one of the above categories.

The number of pesticide poisoning incidents that have been reported by the Scheme from 1987 to 1991 is shown in Table 1.

TABLE 1. Pesticide poisoning incidents in England and Wales 1987-1991.

Year	Number of incidents reported	Abuse	Misuse	Approved use	Unspecified use
1987	193	57	11	7	9
1988	238	53	11	6	18
1989	258	71	10	7	9
1990	343	82	9	8	12
1991	361	79	11	10	18

The incidents arising from approved use were allocated to that category following examination of the evidence from the field inquiry and the analytical findings showed a particular pesticide was involved after being used in an approved manner. A breakdown of the use of products involved over the years 1987 to 1991 is shown in Table 2.

TABLE 2. The use of products involved in pesticide poisoning incidents attributed to approved uses (1987-1991).

Use of products	Number of incidents
Seed treatment	11
Insecticide crop spray	3
Granular treatment	5
Herbicide treatment	3
Molluscicide	3
Rodenticide	6
Lumbricide	2
Others	5

The post-registration surveillance provided by the Wildlife Incident Investigation Scheme reassuringly shows that few incidents of pesticide poisoning arise from their approved use (Bunyan & Stanley, 1983; Fletcher *et al.*, 1992).

Seed treatments

Over the years it is seed treatments which have most frequently been the cause of incidents attributed to the approved use of pesticides. In the past the Scheme has identified particular unpredicted problems with these compounds resulting in withdrawal or a change in practice; for example, poisoning of geese after ingestion of carbophenothion-treated seed (Hamilton & Stanley, 1975; Stanley & Bunyan, 1979).

Seed treatments present a particular hazard to birds, as full incorporation into the soil at the time of drilling is not possible, leaving seed available on the surface of the field.

Between 1987 and 1991, of the eleven cases identified, eight involved treatments to wheat (one gamma-HCH, the rest organophosphorus compounds); two, carbamates on peas; and one, a carbamate on maize. In six incidents with treated wheat, feral (or racing) pigeons, *Columba livia* var., were found poisoned, and in the other two, pheasants, *Phasianus colchicus*, died. In both dressed pea seed incidents, woodpigeon, *Columba palumbus*, were involved; and rooks, *Corvus frugilegus*, were found poisoned by dressed maize, which they had dug out of the ground.

Insecticide crop sprays

Only three incidents arose from insecticide crop sprays between 1987 and 1991. Considering the large area of crops that are sprayed every season, this is reassuringly small. Many birds and mammals are probably scared off fields while spray operations occur and as many of these compounds are non-persistent, wildlife may not be greatly at risk.

All three incidents involved organophosphorus compounds. One followed the spraying of a field against wheat bulb fly, *Delia coarctata*, at the recommended rate and resulted in the poisoning of woodpigeon and possibly other species. The second case involved several feral pigeons which were found dead with large amounts of rape seed in their crops. The field on which they were feeding had been sprayed just prior to their deaths. The third incident resulted in the poisoning of rabbits, *Oryctolagus cuniculus*, after a field of winter barley was sprayed for frit fly, *Oscinella frit*, control.

Granular treatments

As seen in Table 2, there were five incidents involving granular pesticides in the years 1987 to 1991. Four of these resulted from carbamate poisoning.

Pheasants and rooks were found dead following applications to sugar beet fields in two incidents; a little owl, *Athene noctua*, and thrushes, were poisoned after a field of cauliflowers was treated; and several passerines died as a result of a treatment for vine weevil control in a nursery.

Organophosphate poisoning occurred in mallard, *Anas platyrhynchos*, after a field of brussel sprouts was treated.

Herbicides

Hedgehogs, *Ericaceus europaeus*, were poisoned by paraquat after a roadside verge had been sprayed in one incident. In a second, a dog was poisoned after having access to a

field of potatoes which had been sprayed with diquat. A large number of passerines were found to contain residues of monochloroacetate after a field of onions was sprayed with this herbicide. The route of exposure was unknown, but the birds, all seed eaters, may have fed on the sprayed onion seeds or drank from puddles of spray formed on the dry ground.

Molluscicides

In all incidents of this category from 1987 to 1991, metaldehyde poisoning was diagnosed. Hedgehogs and hares, *Lepus capensis*, died after applications to fields in two incidents, and in a third over 50 pheasants were poisoned after pellets were broadcast on a field of winter wheat.

Rodenticides

With most of the rodenticide incidents, poisoning occurred after mammals or birds managed to obtain access to baits. These involved small passerines, such as dunnocks, *Prunella modularis*, which fed on the bait carrier and were small enough to get to the covered treated baits, or fed on bait moved out of the baiting sites by rats.

There was one incident involving a kestrel, *Falco tinnunculus*, which died after a treatment had been carried out. It is possible that this was a case of secondary poisoning.

Lumbricides

The two incidents from 1987 and 1991 involved poisoning of birds after chlordane had been used to control worms. Poisoning of birds by chlordane has been identified in the United States, where it was considered that bioaccumulation in the environment resulted in hazardous levels (Blus *et al.*, 1983).

The first incident involved members of the thrush family, which were found dead after applications to a cricket pitch. The other incident involved the deaths of tawny owls, *Strix aluco*, on a golf course which had been treated. The owls had probably been feeding on earthworms caught on the surface at night.

In both these incidents residues of oxidative metabolites of chlordane and metabolites of the major impurity found in technical chlordane, were detected in the tissues.

Other uses

A bat was found dead with large residues of dieldrin in its tissues in a roof space which had timbers treated with this persistent compound some years earlier. Another bat was poisoned after a wasp nest was treated, and a budgerigar also died after a similar control operation.

Approved veterinary products, particularly anti-parasitic treatments, have been shown by the Scheme to present some risk to wildlife. The veterinary pour-on treatment containing famphur, an organophosphorus insecticide, to eliminate warble flies, *Hypoderma bovis*, in cattle, has been found to poison birds feeding on dead insects found after treatment (Felton *et al.*, 1981).

DISCUSSION

It is almost certain that there are some additional incidents belonging to the 'approved use' category which, due to insufficient evidence, are placed in the 'unspecified use' group.

This will include poisoning cases involving rodenticides, where the origin of the residues detected are not found on field investigation as the animals may have taken some time to be affected; seed treatments, where poisoned birds may fly some distance from the source before dying; and incidents involving other types of compound with similar problems of identifying the usage.

As can be seen in Table 1, there are a large number of incidents where the cause is not attributed to pesticide poisoning. Some of these are found to be due to disease, trauma or starvation, or may have resulted from poisoning by non-agricultural chemicals (such as antifreeze). In others, the cause is not determined because there are inadequate or insufficient tissues available for analysis. Disease may not be identified in some cases, as it is usual for a gross pathological examination only to be carried out at post-mortem. This still leaves several incidents where the cause is unknown.

Not all incidents are analysed for the full range of pesticides in use. This may be because tissue residue analyses have not been developed for particular compounds or their metabolites or breakdown products. Also, as a result of examining the evidence, a decision may be taken to analyse only those compounds likely to be involved. Any common factors found from investigations of undetermined incidents which suggest involvement of a pesticide, may lead to the development of analytical techniques to determine the presence of that compound.

The Scheme is reactive and depends on land users and interested organisations to submit carcases from suspected wildlife poisoning incidents. The probabilities of reporting incidents will vary considerably between species, habitats and regions (Greig-Smith, 1988; Hart, 1990), resulting in biases which may overestimate some problems, while underestimating or even missing others. The probabilities of dead ringed birds being reported vary considerably depending on species (Baillie & Green, 1987).

These problems have led to current investigations into the reporting probabilities of birds killed by pesticides, being carried out by the British Trust for Ornithology. This will cover differences in species, such as body size and colour, and extent of geographical variations in reporting rates of selected species. It is hoped this will help in interpreting data gathered by the Scheme, and provide a more effective assessment of the safety of pesticides to birds of different species.

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VALIDATION OF ENVIRONMENTAL RISK ASSESSMENT PROCEDURES FOR PESTICIDES

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ABSTRACT

In the past, validation of procedures for predicting the environmental side-effects of pesticides has been informal. Quantitative comparison of predictions with data on actual effects is necessary to assess properly the degree of confidence that can be placed in the procedures used by regulatory authorities. We outline how this can be done using data from environmental monitoring schemes and from field experiments. The data must measure the type of effects which the assessment predicts, occurring under the conditions assumed in the assessment, and expressed in appropriate units. The sensitivity and representativeness of the data must be taken into account. Monitoring of environmental effects should be made more systematic, and extended to taxa not currently covered, if the full range of regulatory procedures is to be effectively validated.

INTRODUCTION

Environmental risk assessment of pesticides for regulatory purposes is a predictive process, based on a range of data which may include physico-chemical properties, standard laboratory toxicity data, and the results of field tests. These data are used to predict the side-effects which individual pesticides will have in normal use. Predictions rely strongly on expert judgement, though increasing use is being made of explicit, quantitative models.

As Berry (1990) stated when discussing pesticide hazards to human health, if we accept responsibility for providing data for risk assessment then 'we should be confident that our evidence is as good as we can get'. In fact we actually need a measure of how good the assessment procedures are, so that we can decide how much weight to place on them, and whether they need to be improved. This is also desirable to help regulators demonstrate to the public, and to pesticide manufacturers, that the procedures are appropriate, reliable and cost-effective.

In order to measure the reliability of assessment procedures it is necessary to compare their predictions with the effects which actually occur in normal use. We refer to this process as 'validation'. A distinction may be drawn between validating the assessment outcome for an individual pesticide use, and validating the assessment procedures themselves. The former provides confirmation of a particular prediction, whereas the latter requires the comparison of predicted with actual effects for a sufficiently wide range of pesticides and scenarios to provide confidence that the procedures will be reliable for pesticides in general. Both types of validation require data on the actual effects, which we refer to as 'validation data'. As they are to be used to test the reliability of

assessments, the validation data must themselves be reliable, providing a sensitive and unbiased measure of the actual occurrence of those effects which the assessments are intended to predict.

Reliability of predictions is not the only quality which is desirable in an assessment procedure, though it is the most crucial. Others include ease of use, consistency when used by different people, and the correctness of any mathematical components. These qualities also should be evaluated but in this paper we confine our attention, and the term 'validation', to evaluation of the reliability of predictions.

In the past, validation has been done informally on an *ad-hoc* basis, by experts reviewing assessments when reports of unexpected effects were received, and modifying their assessment procedures accordingly. In the UK, the low frequency of unexpected effects has suggested that the regulatory procedures for hazards to birds, mammals and honeybees are reliable (Bunyan & Stanley, 1983), though this has not been assessed quantitatively. This paper outlines how data from environmental monitoring and field studies can be used for quantitative validation.

VALIDATION USING ENVIRONMENTAL MONITORING DATA

In a number of countries the authorities responsible for the regulation of pesticides take steps to monitor effects caused by approved pesticides in the course of normal use. In principle, such monitoring can provide ideal validation data: a direct measure of the frequency of actual effects. In practice validation analysis entails requirements which few existing monitoring schemes can meet.

The fundamental requirement is that the validation compares 'like with like'. There are at least three issues to consider here. First, the validation data should provide a measure of the type of effects which the risk assessment procedure is intended to predict. For example, schemes which monitor the frequency of acute animal poisoning incidents, such as the UK Wildlife Incident Investigation Scheme (Greig-Smith, 1988a), produce data appropriate for validating predictions at the level of individual mortality, rather than other levels such as reproductive impairment or population decreases.

Second, the validation data should measure the occurrence of effects under the conditions which the risk assessment presumes. Individual risk assessments usually refer to specific formulations, used on specific crops and in accordance with instructions which specify the timing and rate of application and, in many cases, precautionary measures such as the removal of excessive spillage. The assessment may take account of reasonable variation in the conditions of use, but usually not the deliberate misuse or abuse of the pesticide. The assessment may include separate predictions for different groups of species. To compare like with like, the validation data should be restricted and stratified in the same way as the assessment. In practice, it is frequently not possible to obtain full information on poisoning incidents, so many of the incidents recorded by monitoring schemes may need to be excluded when conducting a validation, unless the analysis can be carried out at a more general level.

Third, measures of actual effects should be expressed in units which are appropriate for comparison with the predicted effects. These may be

difficult to decide, as the final predictions of assessment procedures are usually not expressed quantitatively: instead pesticides have been classified as 'low', 'medium' or 'high' risk, or as 'acceptable' or 'unacceptable'. However, classification is sometimes largely determined by a quantitative index of risk, which can be used to indicate appropriate units for the measure of actual effects. For example, a frequently-used index of risk for birds feeding on treated seeds is the number of lethal doses an individual animal is expected to consume in a day. This is effectively an index of the risk per exposed individual. Therefore, to make a fair comparison the total numbers of poisoning incidents should be divided by the total number of exposed individuals, to estimate the frequency of actual effects per exposed individual. This is impracticable, because the total number of individuals actually exposed is unknown. However, if it can be assumed that the number of individuals exposed is a linear function of the area of crops treated, then the number of poisoning incidents divided by the area treated will provide an index of effects per exposed individual. The usefulness of weighting by area treated has been demonstrated in comparative studies of data on poisoning incidents (eg. Greig-Smith, 1988b). This produces a relative measure will be adequate for validation of the assessment procedure, but not for the quantitative validation of an individual assessment which requires an absolute measure of effects in the same units as the prediction.

An important additional requirement is to take account of the sensitivity and representativeness of the validation data. It is recognised that only a proportion of animal poisoning incidents are likely to be reported and that various biases may occur: for example, the probability of reporting may be disproportionately high for pesticides which cause a rapid death, for species of birds which are large and conspicuous, for crops in which corpses are easily visible, and in places where the frequency of human visitation is high (Greig-Smith, 1988a). It will rarely be possible to make a quantitative correction for such biases, but it may be possible to stratify validation analysis so as reduce their influence. In any case, careful account should be taken of the potential for bias when interpreting the results of validation.

These requirements have been illustrated by reference to data on the frequency of animal poisoning incidents, but the same principles apply to other types of environmental monitoring data used for validation. For example, risk assessment for the aquatic environment might include predictions of the concentrations of pesticides in surface water, which could be validated by comparison with data from a national scheme for monitoring water quality. These data would have to meet the same general requirements, though the issues raised might be different.

Systematic schemes exist in the UK for monitoring pesticide effects on birds, mammals and honeybees (Greig-Smith, 1988a), but not for effects on other animals or non-target plants. It will be necessary to devise methods of monitoring effects on other taxa if the full range of regulatory procedures are to be subjected to effective validation. There is also a general need to assess and improve the sensitivity of monitoring, and obtain more information on the cause of individual incidents. These improvements would also enhance the performance of monitoring schemes in their other role, as a safety net for identifying effects not predicted by risk assessment.

VALIDATION USING DATA FROM FIELD STUDIES

Another potential source of validation data is from field studies. These are designed to measure the effects of experimental applications of pesticides on an agricultural scale in a representative environment.

The requirement to compare like with like in validation applies in the same ways to field studies as to environmental monitoring, but may be more easily met. The types of effects predicted by risk assessment tend to be the same as those which are usually measured in field studies, such as individual mortality of birds. The experimenter has a degree of control over conditions in a field study, and can attempt to ensure that they are representative of those assumed in the risk assessment. Any deviation from the intended conditions can be measured and allowed for when interpreting the validation analysis. It is still necessary to consider carefully the appropriate units for the measure of actual effects. Relative measures for validating an assessment procedure may need to be weighted by the number or area of sites tested, if they are based on data for a number of pesticides subjected to different programmes of field testing. Absolute measures of effects in appropriate units for validating an individual assessment should be easier to obtain from specially-designed field studies, than from monitoring schemes.

The requirement to allow for the sensitivity and representativeness of validation data also applies equally to field studies. It may be possible to assess sensitivity quantitatively, and thus to correct for it: for example, by estimating the efficiency of searching for corpses and applying an adjustment to the numbers found, though this is not straightforward. It is more difficult to assess and allow for the extent to which field studies are representative of the full range of conditions and effects which occur in the normal use of a pesticide (Hart, 1990). In this respect field studies are less useful than environmental monitoring, which in principle measures actual effects directly.

Data from field studies which are used as part of a risk assessment cannot legitimately be used for validating that assessment, because they are not independent. This means that additional field studies would be required for validation purposes. Owing to the cost involved, few pesticides are subjected to detailed field studies after they have passed through the regulatory procedures and are approved for use. Therefore it is unlikely to be practicable to validate assessment procedures using field studies, although it should be possible to attempt validation of individual assessments of the few pesticides for which adequate independent field studies have been conducted.

Where a regulatory procedure involves an initial assessment based on toxicity tests and estimates of exposure, followed by a second stage of assessment based on field tests, the latter can be used to provide a test of criteria used in the former. If successful, this would effectively validate the first part of the assessment procedure, but not the second part. The impracticality of validating the whole assessment procedure is a further limitation of using validation data from field studies, rather than from environmental monitoring.

COMPARISON OF PREDICTED AND ACTUAL EFFECTS

Ideally, the validation of an assessment procedure should provide a quantitative measure of the agreement between predicted and actual effects. If the measures of predicted and actual effects are qualitative (eg. 'acceptable' or 'unacceptable' risk) then the degree of agreement can be expressed as the percentage of risk assessments which are correct. If the measures are quantitative (eg. frequency of mortality) then agreement can be measured by statistics such as the correlation coefficient, or the percentage of variance explained by regression of the validation data on the predictions. Poor agreement between predictions and validation data may be due to bias or lack of sensitivity in the validation data, rather than failure of the assessment predictions. Therefore comparisons must be interpreted very carefully, in order to identify the cause of disagreements. Even when deficiencies are found in the validation data, this will be useful in focussing attention on ways of improving the methods used in field studies and monitoring schemes, so as to provide more reliable data for validation and other regulatory purposes in the future.

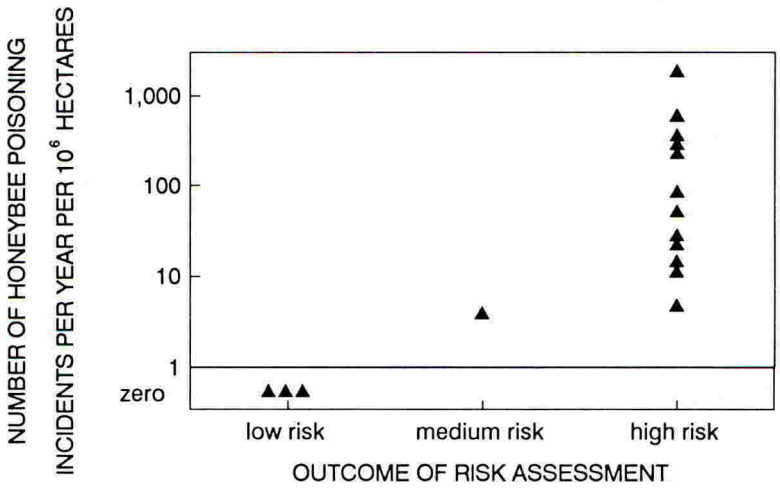

Figure 1. Validation of a risk assessment procedure for the toxic effects of pesticides on honeybees, by comparing predicted risk (low, medium or high) with a measure of actual effects based on the numbers of incidents of poisoning detected in a national monitoring scheme in the UK. Each point represents one pesticide/crop combination (Hart *et al.*, in prep.).

AN EXAMPLE: PESTICIDE HAZARDS TO HONEYBEES

Incidents of honeybees poisoned by pesticides are monitored by a national scheme in the UK. We have used this to provide a measure of actual effects for validating new risk assessment guidelines, which are being developed under the auspices of the European and Mediterranean Plant Protection Organisation (EPPO) and the Council of Europe (CoE) (Greig-Smith, 1991). The guidelines were used to assess a range of insecticide sprays, including all those for which poisoning incidents have been detected. Because the guidelines in effect predict the risk per

exposed individual the incident data were weighted by the areas of crops treated, to allow for differences between pesticides in the numbers of honeybees exposed. Both incident data and areas treated were expressed as annual averages, in order to allow for changes in relative use of the pesticides from year to year. Comparison of predicted and actual effects shows that the risk assessment procedure accurately identified those products which caused the highest frequencies of poisoning incidents (Figure 1). More pesticides are being assessed for this analysis, to confirm whether the guidelines reliably identify 'low risk' (Hart *et al.*, in prep.). We are also using data from the UK Wildlife Incident Investigation Scheme to validate assessment procedures for risks to birds and mammals from pesticide seed treatments.

IMPROVEMENT OF RISK ASSESSMENT PROCEDURES

The types of analysis proposed here for validation may also be used to suggest improvements to risk assessment procedures. Detailed analysis of the validation data used in Figure 1 has shown how some of the criteria for classifying risk to honeybees, proposed in early versions of the EPPO/CoE guidelines, could be adjusted to enable more pesticides to be accurately classified without the need for field studies. The validation data were also used to test the predictive value of potential determinants of risk which are not currently incorporated in the assessment procedures. The attractiveness of crops to honeybees, and the presence of flowering weeds, were found to contribute to the occurrence of poisoning incidents. These methods offer an empirical way of deriving reliable risk assessment criteria, based on simple measures of potential risk, without requiring that the underlying mechanisms are fully understood.

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THE LONG-TERM ENVIRONMENTAL FATE AND EFFECTS OF FLUFENOXURON IN ORCHARDS

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ABSTRACT

Two long-term studies were established in Kent (U.K.) apple orchards to investigate the environmental fate and effects of annual applications of the acaricide/insecticide flufenoxuron (CASCADE 100 g/l DC). Each orchard was divided into three blocks, with two treatments per block, a water control and flufenoxuron. Flufenoxuron was applied once per year at the immediate post-blossom stage, at a commercial rate (nominal concentration 15 g AI/hl) by tractor-driven mist-blower.

The fate of flufenoxuron was investigated, principally by quantifying residues in soil and earthworms. After three years, concentrations in soil (0-2 cm depth) were around 0.1-0.2 mg/kg. Concentrations in earthworms initially increased rapidly, but stabilised around 0.1-0.2 mg/kg. After three years, sampling detected no deleterious effects of flufenoxuron on earthworm populations. Decomposition of leaf litter in soil was assessed and indicated that organisms responsible for the comminution of organic matter were also unaffected by the treatments.

INTRODUCTION

As part of continuing investigations into the environmental fate and biological effects of flufenoxuron, two large-scale field studies were established in apple orchards in the U.K. The objectives were to study the fate of the compound in orchard soils and in earthworms, and to investigate possible effects on earthworms and other soil-dwelling fauna. This paper describes the first three years of these long-term experiments.

METHODS

The study sites, experiment design and application of treatments

The studies were conducted in commercial orchards, on the Shell Research Ltd. Farm, Sittingbourne, Kent (SRC site) and at Conyer Farm, Teynham, Kent. The SRC site was flat and the whole orchard floor was bare earth, the Conyer site was on a gentle slope and the surface beneath the trees was bare earth with a mown grass strip between each row. The trees at both sites were around 2-3 m tall. The orchards received normal inputs of fertilisers and crop protection products, but products known to be harmful to earthworms or non-target arthropods were avoided. The orchard soils were Clay Loams (c. 20% clay), though the sand content varied; 21% at SRC and 40% at Conyer. Organic carbon contents were 2.2% at SRC and 1.7% at Conyer.

Each orchard was divided into three blocks, with one control plot and one flufenoxuron treated plot in each block. The plots were 40 m x 45 m at

the SRC site and 35 m x 70 m at Conyer. In each year, prior to treatment, an enclosure designed to restrict the surface movement of earthworms was erected in the central area of each plot. The enclosures were located in different positions within the plots each year. They consisted of polythene sheeting buried to a depth of 100 mm with a further 100 mm protruding above ground. Each enclosure was 9 m x 9 m at SRC and 10 m x 15 m at Conyer.

Each year, a single application was made, when the trees were at the immediate post-blossom stage. The three control plots were sprayed with water using a tractor-driven mist-blower calibrated to deliver 650 l/ha. Flufenoxuron was then applied to the three treated plots at a nominal concentration of 15 g AI/hl, using the same equipment. The theoretical deposition rate of flufenoxuron was thus 97.5 g AI/ha.

Sampling and analytical procedures

Samples of the flufenoxuron spray-tank mixture were taken to confirm the nominal dose. To measure deposition onto the soil surface, 16 aluminium foil targets (25 cm square) were placed on the ground before applications were made. After spraying, the targets were washed with acetone to produce a single sample per plot. Sample analyses were by reversed-phase hplc.

Apple leaves were collected at random from the trees within the enclosure in each plot and analysed for flufenoxuron residues by normal-phase hplc. The results are expressed as mass of flufenoxuron per mass of leaves (wet weight). Soil cores (15 cm deep) were collected using augers from within the enclosure in each plot and divided into sections. Only the 0-2 and 2-4 cm layers were analysed in years 1 and 2; in year 3 the 4-6 cm layer was also analysed. The samples were analysed by normal-phase hplc and the results expressed as mass of flufenoxuron per mass of dry soil.

Earthworms were sampled within the enclosure in each plot using the formaldehyde extraction method (Raw, 1959). Earthworms were taken to the laboratory and identified, counted and weighed. Numbers and biomass of mature and immature Lumbricus terrestris collected per plot were compared using Student's two sample t-tests. Mean weights per individual were analysed in a similar fashion.

The mature L. terrestris were retained for residue analysis following a 24 h holding period to allow voiding of their gut contents. Samples were macerated, extracted and analysed by normal-phase hplc and the results expressed as mass of flufenoxuron per mass of earthworms (wet weight). In year 3 of the experiments the frass produced by the mature L. terrestris during the 24 h holding period was also retained and analysed as for soil.

Effects on soil organisms responsible for the comminution of organic matter were investigated using a method designed to assess leaf litter breakdown in soil (Heath et al., 1964). Weighed batches of discs, cut from Sweet Chestnut (Castanea sativa) leaves were placed inside mono-filament polyester mesh bags (100 x 100 mm) of three different mesh sizes; 0.025 mm (to admit soil micro-organisms only), 0.5 mm (to admit soil mesofauna but exclude earthworms) and 3.36 mm (to admit all soil organisms including earthworms). Throughout the studies, five bags of each mesh size were buried in each plot (100 mm deep) for periods of 6 months after which they were exhumed and replaced by a new set. The exhumed leaf disc remains were weighed and the percentage weight loss within each bag was calculated. Student's two sample t-tests were then used to analyse the data.

RESULTS AND DISCUSSION

Application data

The concentration of flufenoxuron in the spray tank-mixtures was in the range 72-119% of the nominal concentration (15 g AI/hl). Mean deposition on the ground in the flufenoxuron treated plots was in the range 29-61% of the nominal application rate (97.5 g AI/ha) and therefore 39-71% of the applied dose was deposited on the trees or lost to the atmosphere.

Residues in leaves, soil, L. terrestris and frass

Figure 1 illustrates the concentrations of flufenoxuron found in leaves, soil, mature L. terrestris and frass at each site during year 3 of the studies. Concentrations in leaves were highest (c. 10 mg/kg) immediately after application, falling steadily to around 1 mg/kg by the time of leaf-fall in the autumn. Concentrations in soil decreased with depth at both sites, being around 0.1-0.3 mg/kg in the top 2 cm layer, 0.01-0.06 mg/kg in the 2-4 cm layer and at or just above the limit of determination (0.01 mg/kg) in the 4-6 cm layer. Averaging the soil concentrations over year 3 at all depths analysed, and assuming a soil density of 1.4 g/cm^3, some 25% of the nominal applied amount of flufenoxuron could be accounted for in the soil at the SRC site and 18% at the Conyer site. Concentrations in mature L. terrestris were around 0.1-0.2 mg/kg during year 3 at both sites. Since concentrations in frass were higher than in soil and earthworms, with peak concentrations around 0.5-0.6 mg/kg, it is unlikely that residues in frass were principally derived from soil. It is more likely that L. terrestris were feeding on material with a higher flufenoxuron concentration than the soil. Concentrations in apple leaves were c. 1 mg/kg by the time of leaf-fall, so that leaves and other vegetation were probably a major source of the compound for L. terrestris.

FIGURE 1. Mean concentrations of flufenoxuron in samples from the flufenoxuron treated plots (Year 3).
(Soil, frass: mg/kg dry weight. L. terrestris, leaves: mg/kg wet weight.)

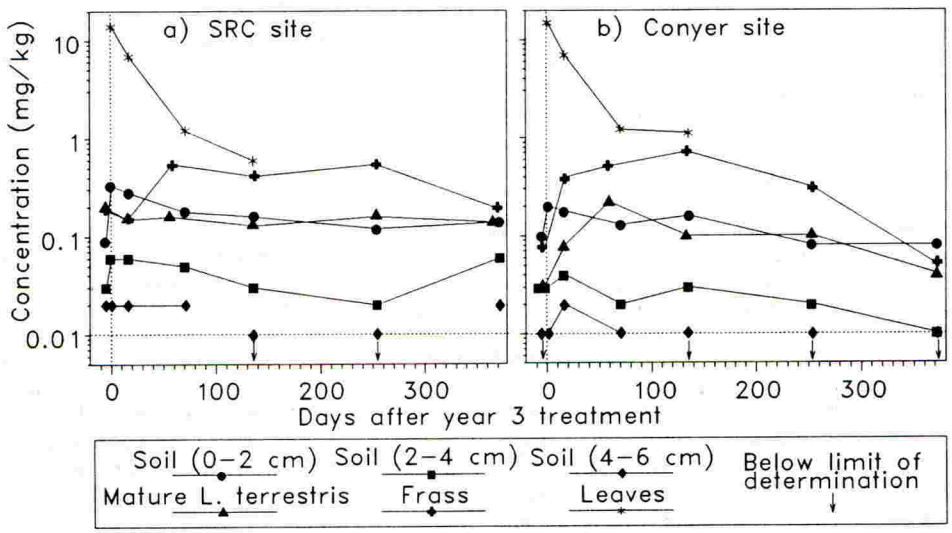
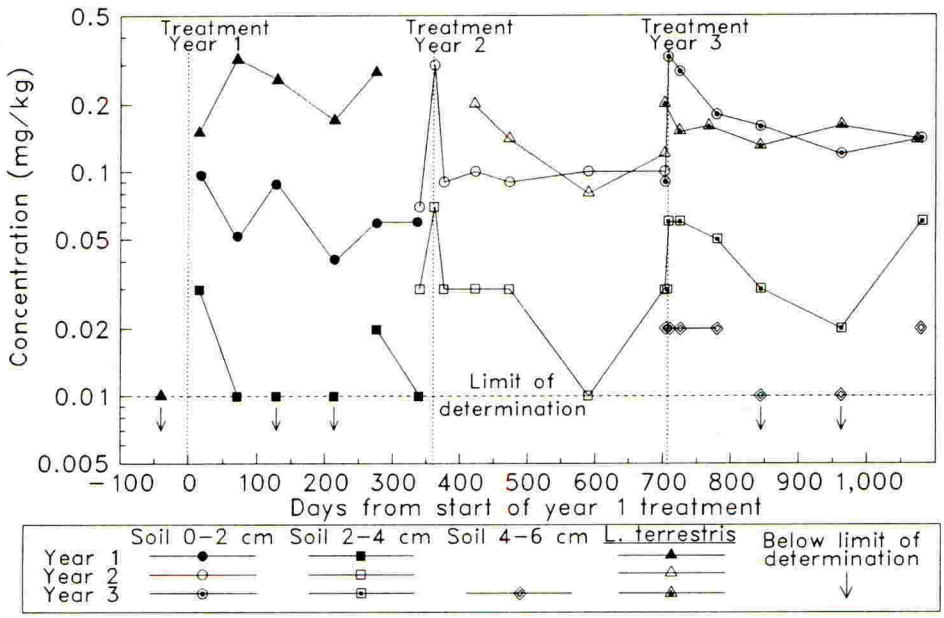


Figure 2 illustrates the concentrations of flufenoxuron found in mature L. terrestris and soil, over the three years of study at the SRC site. The Conyer site exhibited similar trends. Flufenoxuron concentrations in the upper (0-2 cm) soil layer increased, by approximately 0.05 mg/kg, following each application. Concentrations in the 2-4 cm layer were barely above the limit of determination in year 1, rising gradually to c. 0.02-0.06 mg/kg by year 3. In year 3 the 4-6 cm layer was also analysed, showing concentrations at or just above the limit of determination. Since the leaching potential of flufenoxuron is very low (K_{OC} c. 3200, Webb et al., unpublished results), the most likely explanation for this downward movement of residues through the soil profile is as a result of natural processes, such as the activities of invertebrates. At both sites, concentrations in mature L. terrestris increased rapidly after the first application to c. 0.2 mg/kg and remained relatively constant (0.1-0.2 mg/kg) thereafter.

FIGURE 2. Flufenoxuron concentrations in soil and L. terrestris - SRC site.



Earthworm populations

The formaldehyde sampling technique is dependent on soil moisture conditions. During periods of dry weather fewer earthworms were collected than following periods of prolonged rain. L. terrestris was the most abundant species found at both sites and throughout the three years of the study the data for the control and flufenoxuron treatments were generally similar. Biomass of earthworms collected on each sampling occasion in year 3 (expressed in kg/ha) is shown in Figure 3; data for mature and immature L. terrestris indicated no significant differences ($p>0.05$) in their biomass between control and flufenoxuron treated plots. Mean numbers of mature and immature L. terrestris collected per plot in year 3 were similar in the control and flufenoxuron treated plots (Table 1); there were only two significant differences and these did not follow a treatment

FIGURE 3. Estimated earthworm biomass in the orchards (Year 3).

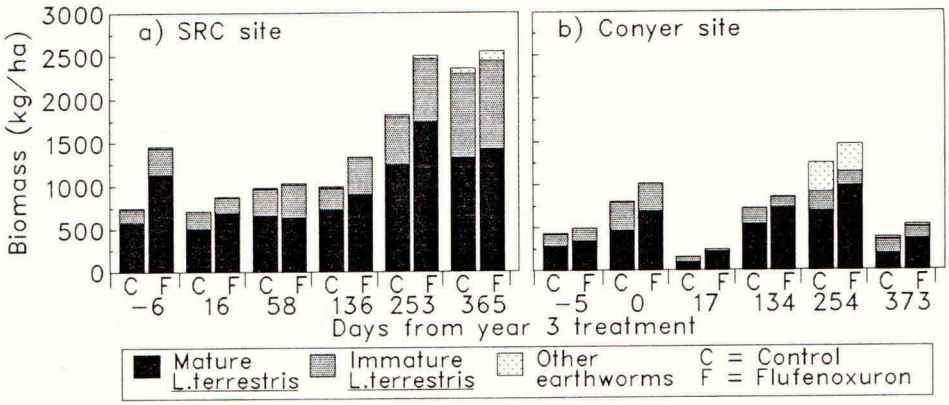


FIGURE 4. Mean individual wet weights of L. terrestris (Year 3).

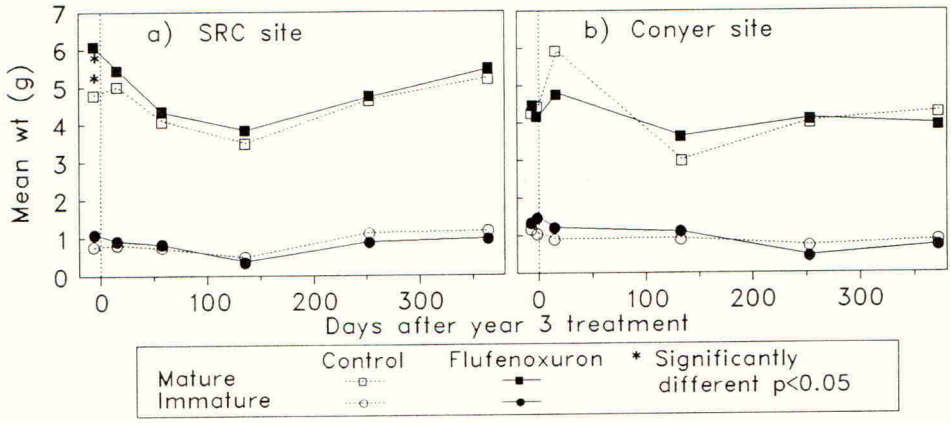


TABLE 1. Mean number of L. terrestris collected per plot (Year 3).

a) SRC site

DAT	Mature		Immature	
Year 3	Cont.	Fluf.	Cont.	Fluf.
-6	5.2	9.0	9.7	13
+16	4.4	6.2	13	9.6
+58	7.4	7.2	20	22
+136	10	11	25*	54*
+253	13	17	23	41
+365	13	13	41	50

b) Conyer site

DAT	Mature		Immature	
Year 3	Cont.	Fluf.	Cont.	Fluf.
-5	3.2	2.6	5.8	5.3
0[1]	5.3	8.3	15	10
+17	1.3	4.2	6.3*	1.5*
+134	8.5	9.2	9.1	6.0
+254	8.0	12	14	16
+373	1.7	4.1	9.0	8.5

* Significantly different ($p<0.05$) Student's t-test [1] pre-treatment

2 quadrats per plot except at Conyer, +17 DAT, when there were 4 quadrats per plot (Quadrat size - 0.25 m²)

related trend. In comparison with the control, the flufenoxuron treatment had no deleterious effects ($p>0.05$) on the mean weights per individual of L. terrestris for both stages of maturity (Figure 4).

Comparison between earthworm populations in the control and flufenoxuron treated plots on each sampling occasion thus indicated that the populations of L. terrestris were not affected by the flufenoxuron treatments. Moreover, since numbers of immature specimens were similar in both the control and flufenoxuron treated plots the data indicated that population recruitment was not affected.

Leaf litter breakdown

Over the three years of the studies, the data for each size of mesh bag, for each burial period, indicated no significant differences ($p>0.05$) in the rates of leaf disc breakdown in samples from the control and flufenoxuron treated plots. The data for year 3 are shown in Table 2. The coarse mesh bags, which allowed the entry of all soil organisms including earthworms, had the highest rate of leaf disc breakdown and the fine mesh bags, which only admitted soil micro-organisms, had the lowest rate of breakdown. The data indicated that flufenoxuron had not affected the soil organisms responsible for the comminution of organic matter.

TABLE 2. Mean percentage breakdown of leaf discs (Year 3).

Site	Treatment	Burial period May 1990 - Nov. 1990 Bag mesh (mm)			Burial period Nov. 1990 - May 1991 Bag mesh (mm)		
		0.025	0.5	3.36	0.025	0.5	3.36
SRC	Control	52	57	90	38	40	95
	Flufenoxuron	50	59	90	36	49	94
Conyer	Control	45	49	73	52	70	97
	Flufenoxuron	47	51	82	54	67	99

No significant differences ($p>0.05$) between control and flufenoxuron treatments

CONCLUSION

The use of flufenoxuron in orchards over three years at a dose rate of 97.5 g AI/ha gave rise to low residues in soil and earthworms which did not significantly affect soil organisms, including earthworms.

ACKNOWLEDGEMENT

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LAMBDA-CYHALOTHRIN: EFFECTS ON NATURAL PEST CONTROL IN BRAZILIAN SOYBEANS

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ABSTRACT

The effects of lambda-cyhalothrin on soybean pests and their natural enemies was investigated in the laboratory and field. Laboratory tests showed lambda-cyhalothrin to be less toxic than monocrotophos to the carabid beetles tested and similarly toxic to the predatory bug tested.

In the field, lambda-cyhalothrin (3.75 g AI/ha) and monocrotophos (120 g AI/ha) were applied to control the velvetbean caterpillar Anticarsia gemmatalis. A. gemmatalis populations were effectively controlled by both treatments. Following applications of both insecticides, there was no evidence for effects on chrysopids, ants, staphylinid beetles, parasitic wasps or spiders but effects were observed on some carabid beetles and predatory bugs. Typically, effects lasted 24 days and there was recovery to control plot levels at the time of subsequent A. gemmatalis infestations, which did not reach economic threshold levels. There was no evidence for a reduction in the potential for natural control of subsequent infestations of A. gemmatalis or other late season pests.

INTRODUCTION

Lambda-cyhalothrin (PP321, KARATE) is a broad spectrum synthetic pyrethroid insecticide used to control a wide range of pests, including aphids, beetles and caterpillars in a variety of crops worldwide. In Brazil, insecticides are generally applied twice to soybean during the season, once in the late vegetative stages, against defoliators such as the velvetbean caterpillar Anticarsia gemmatalis and again at the end of the season to control pod damage by the stink-bug complex. Lambda-cyhalothrin has been shown to give good control of A. gemmatalis and the green stink bug Nezara viridula (Belarmino et al., 1991). Natural enemies are considered important in the control of these pests (Gazzoni et al., 1988). Early season applications against A. gemmatalis have caused concerns regarding the possible disruption of natural control of subsequent generations of A. gemmatalis, secondary defoliator pests and late season pests. There is evidence for up to 80% reduction in the abundance of natural enemies five days after application of some broad-spectrum insecticides (Oliveira et al., 1988). However, few detailed assessments regarding the significance of this in terms of pest control have been made. A laboratory and field programme was therefore set up in southern Brazil to investigate the effects of lambda-cyhalothrin on the key soybean pests and their natural enemies over a complete season.

LABORATORY STUDIES

The intrinsic toxicity of lambda-cyhalothrin and monocrotophos to the soybean pests, Anticarsia gemmatalis and Nezara viridula and a range of natural enemies, the carabid beetles Lebia concina and Calosoma spp. and the predatory bug Tropiconabis capsiformis, was determined. Measured drops of pesticide were applied to the dorsal surface of the insects and the LD50 determined. Following the approach of Stevenson et al. (1984), the potential selectivity of a compound to a species was estimated by using the ratio of field rate to LD50 value. LD50 values and ratios are given in Table 1. The smaller the ratio, the greater the potential selectivity to the arthropod in the field. The order of decreasing selectivity of both insecticides to the natural enemies was; Calosoma spp.> L. concina > T. capsiformis, although lambda-cyhalothrin appears to be more selective than monocrotophos to carabid beetles in particular to L. concina.

TABLE 1: Relative toxicity of lambda-cyhalothrin and monocrotophos to a range of pests and beneficials in soybean. (Field Rates: Monocrotophos = 120 g AI/ha; Lambda-cyhalothrin = 3.75 g AI/ha)

Species Treatment	LD50 (µg AI/insect)	Ratio ($x10^{-6}$) Field Rate / LD50
Anticarsia gemmatalis		
(3rd instar larvae)		
Lambda-cyhalothrin	0.002	1875
Monocrotophos	0.308	390
Nezara viridula		
(3rd instar nymphs)		
Lambda-cyhalothrin	0.001	3750
Monocrotophos	0.014	8571
Calosoma spp.(a)		
(adults)		
Lambda-cyhalothrin	>1.00 (b)	<3.75
Monocrotophos	30.0	4.00
Lebia concina		
(adults)		
Lambda-cyhalothrin	0.011	341
Monocrotophos	0.019	6316
Tropiconabis capsiformis		
(4th instar nymphs)		
Lambda-cyhalothrin	0.003	1250
Monocrotophos	0.042	2857

(a) Calosoma spp. comprises Calosoma alternans and Calosoma retusum
(b) LD50 not reached

FIELD STUDIES

In the 1990-91 soybean season a trial was conducted in the

municipality of Passo Fundo in the state of Rio Grande do Sul in Southern Brazil, in direct drilled soya (variety BR4). The site was divided into four replicate blocks of three plots, lambda-cyhalothrin, untreated control and monocrotophos. Each plot was greater than 1 hectare in area. In early February, applications of lambda-cyhalothrin as KARATE 5EC, 3.75 g AI/ha and monocrotophos as AZODRIN 400, 120 g AI/ha, were made using a tractor mounted boom sprayer, to control Anticarsia gemmatalis. It was intended to make a second insecticide application in early March to control the stink bug complex, but stink bugs remained below economic threshold levels throughout the season, so a second application was not made. Average spray deposition on lambda-cyhalothrin plots, at both crop canopy and soil level, measured by residue analysis of filter paper squares mounted on supports in the crop (8 deposition cards per level per plot), was 98% at crop canopy level and 56% at soil level. Full details of the methods are given in White et al. (1992).

Crop

Due to the drought in the state of Rio Grande do Sul, the leaf area index of the crop was approximately half that of a normal year. At the end of the study, yields on lambda-cyhalothrin plots were statistically significantly greater (27% greater $p<0.05$) than on the controls while yields on monocrotophos plots were not.

Pests

Crop dwelling arthropods were sampled by crop beating. Ten 1 metre crop-beating samples were combined to give each plot sample. Anticarsia gemmatalis larvae were separated into instars based on head capsule width and larval length (Heineck, 1989) and then separated into cohorts, each new infestation was considered the beginning of a new cohort. A. gemmatalis populations were divided into three cohorts, cohort I (all pre-treatment), cohort II, the sprayed cohort and cohort III the re-invading cohort which appeared on the treated plots only. To investigate mortality from sprays and natural enemies, a life table was then constructed for each cohort and survivorship curves constructed using the logarithms of the lx values from the life tables (lx = number of individuals surviving out of a starting number of 1000) (Southwood, 1978). The mortality rates of each cohort, given by the slope of the linear component of the survivorship curves, were compared between treatments.

The first infestation of Anticarsia gemmatalis was sprayed in early February. Both lambda-cyhalothrin and monocrotophos gave over 90% control for 14 days after treatment (DAT) (Figure 1). There was a drought induced crash of the control populations 10 DAT, numbers of all instars falling simultaneously. A second A. gemmatalis infestation was observed 21 DAT on the treated plots. However, numbers remained below the economic damage threshold and appeared to be controlled naturally, falling again 28 DAT. Other fields on the farm also experienced a second A. gemmatalis infestation in late February. At this time the control plots were heavily defoliated (>60%). Division of the A. gemmatalis populations into three cohorts revealed A. gemmatalis mortality rates on lambda-cyhalothrin and monocrotophos treated plots in cohorts II and III to be greater than on the control ($p<0.01$).

Natural enemies

Arthropods were sampled pre and post-treatment, weekly samples being taken between mid January and mid April. In each plot, crop dwelling arthropods were sampled in the same way as pests and ground dwelling arthropods were sampled using 10 pitfall traps containing 4% formalin. In addition, Anticarsia gemmatalis larvae were collected before and after treatment and reared in the laboratory to determine the percentage parasitism. All time series data were analysed using a 2-way ANOVA, to detect differences between the treatments over time.

In general, chrysopids, ants, staphylinid beetles and spiders were unaffected by either treatment. Effects were observed on 3 of the 5 carabid species sufficiently abundant to analyse and 3 of the 4 predatory bug species. Effects were transient typically lasting 24 DAT (Figures 2 and 3). No effects were observed on parasitic wasps.

DISCUSSION

In the laboratory, the ratio of field rate to intrinsic toxicity (Table 1) was 2 to 3 orders of magnitude lower for Calosoma spp. than for the pests, Anticarsia gemmatalis and Nezara viridula suggesting there is a potential for selectivity to this species with both lambda-cyhalothrin and monocrotophos. With both compounds, high ratios were observed for the pests tested, to the predatory nabid bug Tropiconabis capsiformis and with monocrotophos to Lebia concina, indicative of a lack of selectivity to these species.

Field data showed a different pattern of abundance for Calosoma alternans and T. capsiformis following spraying. Numbers of T. capsiformis fell on the treated plots following spraying, increasing to control plot levels 4-5 weeks after treatment. C. alternans abundance, however, appeared to follow pest abundance, high catches being observed on control plots during the Anticarsia gemmatalis outbreak and then later on treated plots during the A. gemmatalis reinvasion. Laboratory data suggest mortality is unlikely with this species following application of either insecticide and it is possible that the effects observed on C. alternans are due, at least in part, to migration from areas of low to high prey density, although hatching of larvae into adults may have been a contributory factor.

Laboratory data suggest there is a potential for selectivity to some beneficial species. In the field, any effects observed on natural enemies were transient and natural enemy abundance was increasing at the time of the second Anticarsia gemmatalis infestation. A. gemmatalis mortality rates on treated plots during the second infestation were greater than mortality rates on the control plots and this second infestation did not require chemical control and appeared to be controlled naturally. In conclusion, these data show A. gemmatalis was satisfactorily controlled by lambda-cyhalothrin and any effects observed on natural enemies were sufficiently transient not to reduce the potential for natural enemies to control subsequent infestations of A. gemmatalis or other late season pests.

FIGURE 1: Percentage Anticarsia larvae remaining following spraying

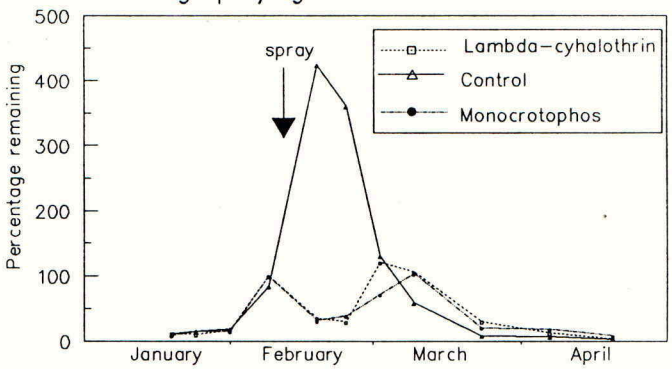


FIGURE 2: Tropiconabis capsiformis adults

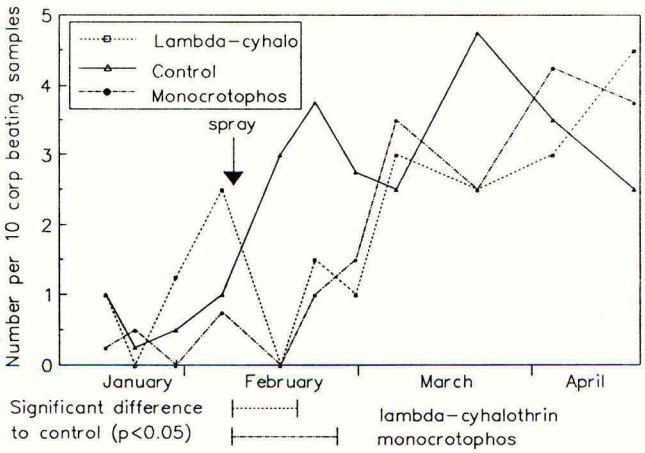
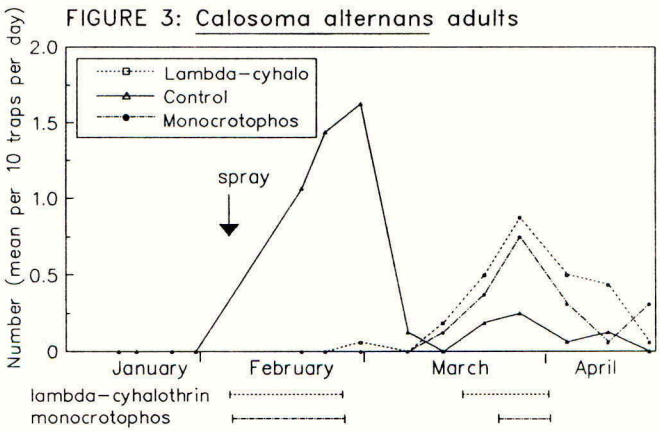


FIGURE 3: Calosoma alternans adults



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PESTICIDE EXPOSURE OF BIRDS BREEDING IN VEGETABLE CROPS IN ONTARIO, CANADA

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ABSTRACT

Two species of birds were examined for possible pesticide exposure in an Ontario agricultural wetland. Both the red-winged blackbird (Agelaius phoeniceus) and the barn swallow (Hirundo rustica) nest in the Holland Marsh Polder, an area of intensive vegetable production, and in the adjacent wetlands. Residues of DDE were higher in pooled egg samples from Polder-nesting red-winged blackbirds (22.3 and 27.1 ppm pp'-DDE lipid weight, in 1990 and 1991, respectively) than in eggs from an upstream control wetland (3.0 and 13.9 ppm). Polder swallow eggs contained 59.1 ppm DDE, versus 4.6 ppm in controls. Cholinesterase enzyme (ChE) activity, an indicator of organophosphate or carbamate exposure, was measured in nestling plasma and brain. The increase of ChE activity with age was curvilinear in red-winged blackbirds and linear in swallows. The fitted curves of Polder nestlings did not differ significantly from controls, although 32 % of the individuals showed depressed ChE activity. Reproduction was not affected.

INTRODUCTION

In southern Ontario, wetlands often share water with agricultural areas, and wildlife inhabiting wetlands is potentially exposed to an array of the old and new agrochemicals. The persistent organochlorines (OCs), mostly no longer in use, are still in the ecosystem, and are found in eggs of local birds (Hébert et al., 1991). Modern organophosphate (OP) and carbamate pesticides, although generally short-lived, are the most acutely toxic to wildlife, and birds appear to be especially sensitive (Walker, 1983). Concern for the effects that these agrochemicals could have on birds motivated an examination of breeding populations of red-winged blackbirds (Agelaius phoeniceus) and barn swallows (Hirundo rustica) in a market garden environment.

The selected experimental area was the Holland Marsh, Ontario's largest vegetable producing region, created in the wetlands of the Holland River. Its marshy soil is highly organic, and it has a long history of heavy pesticide use (Miles et al., 1978). Crops often receive weekly applications of OP or carbamate insecticides (personal observation), and the intensity of insecticide applications on vegetables in Ontario is surpassed only by that in fruit orchards. The vegetable fields of the Holland Marsh are maintained

[1]currently at: Springborn Laboratories (Europe) AG, Seestrasse 21, CH-9326, Horn, Switzerland

as polders; a polder being an area of reclaimed land with an artificially lowered water level. The Holland Marsh Polder, situated 40 km north of Toronto, covers about 2900 ha of predominantly onion and carrot crops. It is surrounded by dykes and two canals that divert excess water around the Polder. The original watercourse is now a drainage and irrigation ditch running down the centre of the Polder, to a large pumping station at the north end, where the canals meet. From there, the drainage water again joins the Holland River, flowing north through marshes and into Lake Simcoe.

The red-winged blackbird (RWBB), an insectivorous early breeder, was chosen as the study species for 1990 and 1991, for its abundance, reliance on aquatic invertebrate prey, and ease with which to work. Since pesticide applications are more frequent later in the season, the barn swallow (BASW) was included in 1991, as an insectivorous late breeder. Unlike the RWBB, it is double-brooded in southern Ontario.

The objective of the study was to assess exposure of RWBB and BASW nestlings to some groups of agricultural chemicals, and to compare survival and reproductive success of both species breeding within the Polder, to populations in control wetlands within the same watershed.

Previous studies on pesticide residues in the Holland Marsh drainage system found the Polder to be heavily contaminanted with organochlorine compounds (Miles & Harris, 1978). Levels of DDE and Dieldrin were still expected to be high, and could accumulate to levels affecting reproduction in birds. To measure the exposure of breeding birds to OCs, eggs were collected from nests in the Polder, and from various other sites in the Holland River. The egg contents were analysed for residues of OC compounds. The relative contaminant levels might also serve as a measure of the reliance of the different bird populations on the Polder for foraging, prior to egg-laying.

To measure current exposure to the cholinesterase inhibiting pesticides (OPs and carbamates), nestlings of all ages were sampled for total cholinesterase (ChE) activity in blood plasma and in whole brain. In birds, a measured depression in brain ChE activity of 20 % less than control birds, is considered a sign of OP or carbamate poisoning, and 50 % enzyme inhibition in a dead individual is diagnostic for cause of death (Ludke et al., 1975). Plasma ChE activity has the advantage of being a non-lethal indicator, but is more difficult to interpret than brain ChE activity. It is more variable, and recovers faster after exposure.

METHODS

Sample populations

RWBB nests were found by canoe in cattails (Typha sp.) along the drainage ditch in the middle of the Holland Marsh Polder, and in the vegetation along control streams within the same watershed. After a nest was located, it was mapped, and the vegetation was tagged 3 meters downstream. Nests were revisited every 4 days in 1990, and once a week in 1991, to establish nesting success and productivity.

In 1990, RWBB populations in 3 areas of the Holland River were monitored: the Polder, with 98 active nests (a.ns.), South Control (just upstream from the Polder; 42 a.ns.), and the North Cell (edge of a cattail marsh, 4 km downstream; 44 a.ns.). In 1991, RWBB nests were monitored in the

Polder (50 a.ns.), and in a new control area (50 a.ns.) that was more similar in nesting habitat and water nutrient loading. This area, the Marina Control, was situated 2 km up the East Branch, a lower tributary of the Holland River. Its nutrient source was non-agronomic, mostly urban.

Three BASW populations were monitored in 1991; 65 active nests were followed in the Polder, namely in farm sheds adjacent the drainage ditch. The control population of 22 active nests was located under a bridge in the previously mentioned East Branch. To control for differences between bridge- and barn-nesting birds, two well-populated bridges over the Polder canals were also monitored (Canal Bridges; 50 a.ns). Previous observations showed that these birds spent time foraging over the Polder fields, as well as over the canals.

Egg collections

In 1990, a 5-egg sample was taken from marsh-nesting RWBBs in all three study sites. In 1991, a similar sample was taken once again from the Polder and from the South Control, as well as from nests directly downstream from the Polder, at the main Pumping Station. For each sample, one egg was removed at random from 5 fresh, complete (3-4 eggs) clutches. BASW eggs were collected from the Control bridge in the East Branch, from either of the Canal bridges, and from the Polder barns. These samples were comprised of 7 randomly chosen, fresh eggs.

Contaminant analysis

Eggs were refrigerated until analysis by the CWS Laboratory Services at the National Wildlife Research Centre (NWRC) in Hull, Quebec. The eggs were pooled, per species and per area, and their whole contents were homogenized and analyzed for residues of 65 OC compounds, using a Hewlett-Packard 7673A gas chromotograph with auto-injector (Won & Turle, 1987). The levels of the three most important contaminants, DDE, dieldrin and total PCBs, are reported here.

Cholinesterase sampling

In 1991, RWBB nestlings of all ages were taken from the South Control site to determine the base-line nestling ChE activity. Twenty-four control samples of both plasma and brain were obtained, as well as 12 potentially exposed samples from the Polder. For a plasma sample, about 0.2 ml of blood was drawn from the jugular vein using a disposable 0.5 ml syringe with a 28.5 gauge needle. The sample was divided among 4 heparinized capillaries, centrifuged for 5 minutes, and frozen on dry ice immediately. For the brain sample, the nestling was euthanized using CO_2, after which the head was severed and frozen. Eleven BASW chicks from the Polder barns, and nine from the Canal Bridges were collected and similarly sampled for brain and plasma samples.

ChE activities were determined spectrophotometrically with a microplate reader by the CWS Laboratory Services, NWRC, using a modified version of the Ellman method (Ellman et al., 1967; CWS, 1992). Brain weight was used to classify nestling age, as the best measure of the developmental stage.

RESULTS

OC contaminant levels in RWBB and BASW eggs

Concentrations of DDT and its metabolites, were significantly higher in eggs from birds breeding inside the Polder, than in eggs from birds immediately outside the Polder. The concentration of pp'-DDE, the main DDT metabolite, is presented on a lipid weight (l.w.) basis in Table 1, in geographical order, from upstream to downstream.

TABLE 1. Levels of pp'-DDE (ppm l.w.) in pooled egg samples of Red-winged Blackbirds (RWBB) and Barn Swallows (BASW) from the Holland Marsh, Ontario.

Location	RWBB '90 % lipid,(N),ppm	RWBB '91 % lipid,(N),ppm	BASW '91 % lipid,(N),ppm
South Control	8.0 (5) 2.95	5.6 (5) 13.89	*no data*
Polder	6.7 (5) 22.28	4.8 (5) 27.08	8.4 (7) 59.10
Canal Bridges	*no data*	*no data*	6.8 (7) 34.22
Pumping Station	*no data*	5.2 (5) 12.48	*no data*
Control Bridge	*no data*	*no data*	7.4 (7) 4.64
North Cell	6.6 (5) 3.66	*no data*	*no data*

The pooled BASW sample from the Polder showed the highest levels of DDE, while the Canal Bridge levels are intermediate between this, and the Control Bridge. This difference among sites is also reflected in the RWBB levels. The dramatic differences in such a small geographical area strongly suggest that the contaminants are indeed picked up very locally, supporting the hypothesis that RWBB and BASW eggs reflect local contaminant conditions rather than those of their wintering grounds.

TABLE 2. Levels of dieldrin (ppm l.w.) in pooled egg samples of Red-winged Blackbirds (RWBB) and Barn Swallows (BASW) from the Holland Marsh, Ontario.

Location	RWBB '90 % lipid,(N),ppm	RWBB '91 % lipid,(N),ppm	BASW '91 % lipid,(N),ppm
South Control	8.0 (5) 0.129	5.6 (5) 0.105	*no data*
Polder	6.7 (5) 1.759	4.8 (5) 1.763	8.4 (7) 5.576
Canal Bridges	*no data*	*no data*	6.8 (7) 5.557
Pumping Station	*no data*	5.2 (5) 0.596	*no data*
Control Bridge	*no data*	*no data*	7.4 (7) 0.216
North Cell	6.6 (5) 0.061	*no data*	*no data*

The swallow egg levels showed that birds nesting under the Canal Bridges were more exposed to contaminants than Control Bridge birds, probably through their diets. This is consistent with earlier observations of these birds foraging over Polder fields.

Dieldrin levels in eggs from the Polder sites were significantly higher than in eggs from any other site (Table 2). Again, the BASW eggs accumulated more than the RWBB eggs, which is probably a reflection of their different feeding strategies. There were more BASWs observed feeding on aerial insects directly over Polder fields, than there were RWBBs foraging on the fields. Dieldrin showed up in both BASW Polder and BASW Canal samples as 5.5 ppm l.w. The sum of the PCBs was also higher in BASW eggs (from 2.127 ppm l.w. at Canal Bridges to 3.176 at Control Bridge) than in RWBB eggs (from 0.895 at Control, to 1.098 at Polder sites).

RWBB reproduction

In 1990, RWBB nesting success in the Polder was 35 %, almost half that of the North Cell (downstream) and the South Control (up-stream), (60 % and 58 %, respectively). Productivity was 0.1 fledgling per breeding pair in the Polder, and 1.7 in the South Control. Due to the habitat differences that this could well be attributed to, the Marina Control was selected as the 1991 control. Productivity in the Polder was similar in 1991 (0.1), and nesting succes was lower (14.3 %), but these parameters were not significantly different in the Marina Control (T-test), where productivity was 0.5, and nesting succes was 20.8 %.

BASW reproduction

Nesting success in BASWs was higher than in any of the tested RWBB populations; 77.3 % in the Polder, 75.0 % at the Control Bridge, 76.6 % at one Canal Bridge. The exception was the other Canal Bridge, Simcoe Bridge (28.6 %). Because Simcoe Bridge suffered a total failure of the first nests due to abandonment, it was omitted from the reproductive study. The brood failure was most likely due to the disturbance from a single recreational activity at the bridge in June. With the exclusion of Simcoe Bridge, all the tested reproductive parameters did not show significant differences among BASW populations. More details will follow in a later publication.

ChE activity-RWBB nestlings

The relationship between brain weight and total cholinesterase activity in young RWBB brains is a curvilinear quadratic one. Base-line brain ChE activity increases with nestling development (age) and appears to peak at a brain weight of 0.6 g. The fitted curve of Polder nestling brain ChE did not differ significantly from the fitted curve of the control population. However, when individual Polder brain ChE samples were compared with the 95 % confidence interval of the fitted control curve, 9 Polder nestlings were below that area (<twice S.E.), showing significantly depressed ChE levels. Thus, although the Polder population as a whole did not differ from the controls, 32 % of the sampled nestlings were exposed to ChE-inhibitors to a certain degree.

Plasma ChE activity levels increased in the same way with nestling brain weight, and this increase showed no difference in nestlings from the South Control area and from the Polder. The margin of error was greater than that of the brain samples, and the number of individual Polder nestlings showing reduced plasma ChE activity(< twice S.E.) was just 3 of 16, or 19 %

ChE activity-BASW nestlings

Barn Swallow brain ChE activity increased linearly with brain weight, as

did plasma ChE. However, the small number of birds taken from the control area was insufficient to show a change in the slope at the higher brain weights. This was also the case for establishing a good base-line for this species.

CONCLUSION

On the basis of DDE and dieldrin residue patterns in their eggs, and evidence of individual exposure to ChE inhibitors, it appears that RWBBs and BASWs breeding or feeding in the Holland Marsh Polder are exposed to agrochemicals of past and present. With recommended use of registered pesticides, the current levels of exposure appear not to be affecting passerine reproduction as much as ecological factors (habitat loss, prey availability, recreational disturbances).

Both species are practical monitors for locally occurring persistent contaminants. BASWs are interesting monitors of exposure to ChE inhibitors at agricultural locations, although more information is needed on the way this species reacts.

ACKNOWLEDGEMENTS

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EVAPORATION OF 1,3-DICHLOROPROPENE FROM SOIL IN LABORATORY AND FIELD STUDIES

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ABSTRACT

Experiments to determine the rates of evaporation of 1,3-dichloropropene (1,3-D), the major component of the soil fumigant D-D 92, from the surface of soil in the laboratory and an outdoor enclosure are outlined.

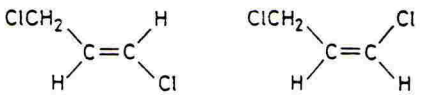
Two methods based on the use of pumped charcoal tubes and passive sampling organic vapour monitors (badges) were developed for the determination of 1,3-D in air.

Air exchange rates needed to calculate the amount of 1,3-D emitted from the surface of the soil in enclosures were determined by the same air monitoring methods using emitters containing 1,2,3-trichloropropane as reference. Up to 50% of the 1,3-D applied was found to be evaporated from the soil in these experiments over a period of about a month.

Outdoor air monitoring for 1,3-D evaporated from a treated plot and a commercially treated field has also been undertaken. Air concentrations down wind of the field fell rapidly following treatment and at no time was the 8 hour TLV of 5 mg/m^3 for 1,3-D exceeded even at the edge of the field.

INTRODUCTION

The soil fumigant D-D 92* contains 1,3-dichloropropene E- and Z-isomers (1,3-D) as the active ingredient (minimum 92% m/m). It is a clear amber coloured liquid with vapour pressure 3.7 kPa at 20°C and density 1.17-1.22 g/ml at the same temperature.



E Z

The product is used widely for the control of plant nematodes. It is applied prior to planting by soil injection (15-20 cm depth) at a dose rate in the range 120-600 litres/ha. A period, normally not less than 2 weeks, is allowed for the fumigant to disperse from the soil before sowing or transplanting crops.

Because of the volatility of 1,3-D a significant route for this dispersal is by evaporative loss to the atmosphere. Studies undertaken to

* Shell trade mark

develop air monitoring methods and to examine rates of loss by this mechanism under laboratory and outdoor conditions are described.

METHODS FOR THE DETERMINATION OF 1,3-D IN AIR

Pumped charcoal tubes for active sampling (150 mg, SKC 226-01) and passive carbon based Organic Vapour Monitors (badges, 3M 3500) were evaluated for their suitability for trapping 1,3-D from ambient air. Up to 120 litres of air at ~200 ml/min is typically drawn through the trapping tube and exposed badges sample passively at a rate equivalent to 31.5 ml/min when the air flow across the face of the badge is >10 cm/sec. Trapped 1,3-D is desorbed with carbon disulphide (CS_2) and is determined by capillary GC with flame ionisation detection using a 25 m x 0.22 mm BP10 fused silica column. Nonane is the internal standard for the method and 1,2,3-trichloropropane (TCP) is used as a reference material for calculation of air exchange rates in test room and enclosure studies. All four components including the Z- and E-isomers of 1,3-D are separated under the conditions used.

The suitability of the two types of samplers for both 1,3-D and TCP was examined in a combination of laboratory and test room experiments where desorption efficiency, storage stability, break through from tubes, loss from badges and trapping efficiency was studied.

Desorption efficiency for 1 to 1000 μg of 1,3-D or TCP added to either device was essentially quantitative. Storage stability at + 4°C and ambient temperature showed no appreciable loss of sorbed chemical over 3 weeks. Breakthrough from tubes only occurred under conditions where large amounts of compound (>10 mg) and high air flows (e.g. 2 litres/min), in excess of those expected in normal air monitoring, were used. Badges showed no loss of added compounds and no reduction in trapping efficiency after 6 days exposure even under conditions of high temperature (32°C) and humidity (up to 95% relative humidity).

From these initial development studies the 3M Organic Vapour Monitor and the SKC 150 mg charcoal tube were considered suitable for further evaluation as devices for quantitative monitoring of 1,3-D in air.

Direct comparison of the two procedures was therefore made over an extended period in a test room. This room was of ca. 36 m^3 overall volume (dimensions 4.5 m x 3 m x 2.7 m high) maintained at a temperature of 20-25°C, 50-60% relative humidity, with an air circulation provided by two electric fans and ventilation provided by a window and door. Tube and badge samplers were set up side by side at two sampling points, one 0.95 m high at the front of the room and the other at 1.6 m height at the rear. A mixed atmosphere of 1,3-D and TCP was generated by one emitter consisting of a glass tube with a 7 mm orifice containing D-D 92 and two similar emitters containing TCP with 12 mm orifices placed in the middle of the room at a height of 0.4 m. Typical comparative results for badges and tubes sampled simultaneously under these conditions (Table 1) show there is generally good agreement between the two techniques for both 1,3-D and TCP.

TABLE 1. Comparison of badges and tubes sampled simultaneously in the test room.

Sampling period (hours from start)	Air concentrations (mg/m^3)					
	1,3-D (Z-)		1,3-D (E-)		TCP	
	Badge	Tube	Badge	Tube	Badge	Tube
0-7	0.35	0.37	0.17	0.20	0.48	0.49
71-79	0.78	0.75	0.46	0.43	1.52	1.45
119-127	0.64	0.82	0.38	0.51	0.90	1.15
127-143	0.84	1.15	0.50	0.70	1.15	1.56

It was therefore possible to develop these techniques for monitoring evaporation of 1,3-D from D-D 92 in enclosed ventilated spaces by using TCP as a simultaneously trapped reference material for calculation of ventilation rates.

To confirm the use of this approach in a controlled test room experiment, badge monitors which were changed at intervals were exposed throughout a period of 10 days to provide continuous measurement of air concentrations. 1,3-D and TCP emitters placed in the room were weighed at the start of the experiment and at the start and end of each badge exposure period.

Using the measured air concentration of TCP and the mass loss from the TCP emitter, the following equation may be applied to determine the number of air changes per hour in the test room.

$$\text{Air changes/hr} = \frac{\text{Rate of emission (mg/hr)}}{\text{Room volume } (m^3) \times \text{air concentration } (mg/m^3)}$$

In a similar way, using the same equation, the results of the TCP measurements were applied to obtain expected air concentration of 1,3-D based on mass loss from the 1,3-D emitter. Comparison of measured and calculated results for 1,3-D air concentrations with badge monitors showed good agreement with differences no greater than ±15% over a 10 day period as would be expected if the sampling procedure is working effectively. Conversely, the air concentration data for 1,3-D could have been used to calculate the mass loss from the emitter.

These initial experiments with 1,3-D emitters confirmed that the air monitoring approach could be used to determine mass loss of 1,3-D by evaporation and the procedures developed were next applied to study evaporation of 1,3-D from soil surfaces.

VOLATILIZATION OF 1,3-D FROM THE SOIL SURFACE

Two experiments were carried out to investigate this process, in one, D-D 92 was applied to soil in a tank in the test room, in the other, a small scale field treatment was made in an outdoor enclosure.

Tank study

A stainless steel tank (1 m square and 0.4 m deep) was placed in the middle of the test room described in the method development studies. The tank was filled to within 5 cm of the top with clay loam soil (28.4% sand, 42.9% silt, 28.7% clay, 2.9% organic matter; pH 6.6) with a moisture content of 20.8% (m/m) prepared to give a tilth as expected prior to field treatment. This was allowed to equilibrate for a period of several weeks.

A total of 19.8 g of D-D 92, equivalent to 198 kg AI/ha was injected uniformly into the soil at the intersections of a square grid with 10 cm spacings to a depth of 15 cm using a syringe to simulate a soil treatment on laboratory scale. Immediately afterwards the soil was pressed down firmly to seal the surface as in the field. TCP emitters, identical to those used in the test room method development studies, were placed on the soil surface at two points. During the experiment a total of 15 litres of water were sprayed onto the soil as simulated rainfall.

Air concentrations of 1,3-D and TCP obtained by continuous monitoring with a series of badges throughout the experimental period and by spot monitoring at intervals with charcoal tubes ranged from between 3 to 4 mg/m^3 at the start of the experiment to 0.02 mg/m^3 after 35 days for combined Z- and E-1,3-D isomers and from 0.67 to 0.12 mg/m^3 for TCP. Badges and tubes compared favourably. There was an initial delay of a few hours in build up of 1,3-D in air as it travelled to the soil surface and a predominance of the more volatile Z-isomer was detected during the first few days when the Z- to E-isomer ratio exceeded 1.5.

Using the procedures described the cumulative amount of 1,3-D lost from the surface of the soil in the tank was calculated from the continuous badge monitoring data. The results showed that about 30% of the applied 1,3-D was lost by evaporation in the first week, after which the rate of loss decreased considerably such that at the end of 5 weeks some 50% loss could be accounted for by this mechanism.

At the end of the experiment the soil was analysed and a further 2% of the applied D-D 92 could be accounted for as 1,3-D or its breakdown product 3-chloroallyl alcohol (3-CAA). This relatively small amount of material remaining in the soil is not unexpected as studies on the behaviour of D-D in soil using radiolabelled 1,3-D (Roberts and Stoydin, 1976) have shown that 3-CAA degrades to 3-chloroacrylic acid which further degrades to chloride ion.

The behaviour of D-D in soils has been modelled mathematically (Leistra & Frissel, 1975) using physico-chemical data. The model allowed for loss by volatilization and degradation in the soil. In this case the predicted loss by volatilization from a sandy loam soil over a period of four weeks was about 40% (m/m).

Enclosure study

The same monitoring procedures were used in an outdoor enclosure (semi-circular polythene tunnel) where D-D 92 was applied to an area of bare soil (3.5 m x 6 m) by hand applicator to a depth of 15 cm using a rectangular 30 cm square grid as a guide. A total of 470 g of fumigant, equivalent to a dose of 224 kg AI/ha was applied and the hole closed after each injection. TCP was released into the atmosphere from 5 emitters placed at random on the surface of the treated area.

Air inside the tunnel was monitored continuously for 1,3-D and TCP using badge monitors at two sampling points, 1.5 m high at each end of the tunnel, over a period of 4 weeks from treatment of the soil. Electric fans were used to ensure sufficient air flow across the faces of the badges. Soil moisture content was maintained close to 20% (m/m) using a sprinkling system.

Air concentrations of 1,3-D E- and Z-isomers ranged from close to 1 mg/m^3 at the start of the experiment to about 0.01 mg/m^3 by the end of the 4 weeks. The ventilation rate was typically in the range 7 to 14 air changes per hour.

Results calculated for loss of 1,3-D by evaporation from the soil surface showed a similar pattern to those found for the test room experiment (40-50% evaporation). A further 4.5% (m/m) of the D-D 92 was found in the soil.

In these studies, badge monitors proved their value in providing a continuous sampling record throughout the experimental period. Also, being passive samplers, they did not require any power supply or other services and consequently they could be used in relatively large numbers for sampling simultaneously at different locations.

FIELD STUDIES

Following the successful work in the test room and outdoor enclosure, badge monitors have been used to determine 1,3-D air concentrations from a small scale treatment and a commercial application of D-D 92 in the field.

Small plot field study

In the small plot experiment D-D 92 was injected over an 8 m x 8 m plot at a dose rate equivalent to 328 kg AI/ha using the same procedures described for the enclosure study.

Continuous measurement of air concentrations of 1,3-D over a period of 2 weeks from treatment was made with badge monitors located at pre-determined sampling points in the vicinity of the treated area, at the edge of the plot and 2, 5, 10 and 20 m from the edge. Badges were positioned 1.5 m off the ground on canes under the protection of a plastic funnel placed above, but not over the sampler. Badges were changed at intervals from 1 to 3 days during the first week depending on location but were allowed to sample for a period of 7 days during the second week. Meteorological data, in particular wind speed and direction, were recorded and badge monitors were mostly placed in down wind locations. The maximum concentration of 1,3-D of 0.03 mg/m^3 was found at the edge of the plot

during the middle of the first week and, as in the test room and enclosure studies, there appeared to be a delay period before fumigant reached the soil surface. The average concentration during the second week had fallen to <0.002 mg/m^3. A limited number of charcoal tube samples, taken over 6 to 8 hour periods, gave very similar results.

Commercial field treatment

A similar approach to that described for the small plot study was used to monitor 1,3-D air concentrations resulting from a commercial scale field treatment in Southern France, as an indication of possible bystander exposure. The field, an elongated rectangle of 1.1 ha area, was treated with D-D 92 using a tractor drawn applicator at a high dose rate of 550 litres/ha prior to planting vines.

Continuous measurement of the air concentration of 1,3-D from 1 day prior to treatment to 10 days following treatment was made using Organic Vapour Monitors which were changed on a daily basis. These were set out in parallel arrays of samplers positioned down wind of the treated area at distances of 0, 20, 40 and 80 m (up to 3 times the width of the field) from the edge of the field. A limited number of up-wind sampling points were located on the opposite side of the field. Charcoal tube samples were also taken for up to 8 hours per day at selected sampling points.

The mean result from each array of badge monitors positioned at a height of 1.5 m (approximate height of the breathing zone) at down wind locations showed that the maximum average concentration of combined Z- and E-isomers of 1,3-D of 1.2 mg/m^3 (maximum for an individual sampler was 2.2 mg/m^3) occurred on day 1 of the study close to the edge of the plot with only about one third of this value at a distance of 80 m. After day 1, air concentrations fell rapidly at all distances and were generally below 0.1 mg/m^3 by day 5. At no time was the 8 hour TLV of 5 mg/m^3 for 1,3-D, as listed by the ACGIH (American Conference of Governmental Industrial Hygienists) exceeded even at the perimeter of the field.

For most of the duration of the trial the wind was blowing across the width of the plot, such that the results of each sampler in an array would have been expected to be similar and this was found to be the case. For the same reason 1,3-D concentrations for up-wind samples were mostly all less than the limit of determination (0.02 mg/m^3).

Results for tube and badge samples taken side-by-side generally agreed well. As found in the small scale studies, the more volatile Z-isomer was evaporated fastest during the first few days after treatment.

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VOLATILIZATION BEHAVIOUR OF PESTICIDES IN FIELD TRIALS

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ABSTRACT

A field trial was designed to study the volatilization of pesticides from agricultural ecosystems. For this purpose, apparatus for practice-relevant application of radioactively labelled, formulated active ingredients and for reproducible sampling of plants and soil was built.

Volatilization trials were carried out with pesticides using appropriate target crops and at commercial application times to ensure typical weather conditions for the experiment. The formulated active ingredients methabenzthiazuron, aminotriazole, betacyfluthrin, oxydemeton-methyl, triadimenol and tebuconazole showed either no or little volatilization (< 20%). Diuron showed no volatilization from a soil without vegetation but approximately 24% evaporated after application to a closed stand of weeds. About 27% of the reference compound parathion-ethyl had been volatilized after 24 hours from an ecosystem simulating a field of winter barley in the early stages of development.

INTRODUCTION

According to the guideline 6-1 of the Federal Biological Institute for Agriculture and Forestry of the Federal Republic of Germany (BBA, July, 1990), the volatilization behaviour of pesticidal active ingredients must be investigated either in a laboratory experiment or in a field trial, if the hydrolytic or photolytic half-lives exceed 4 days.

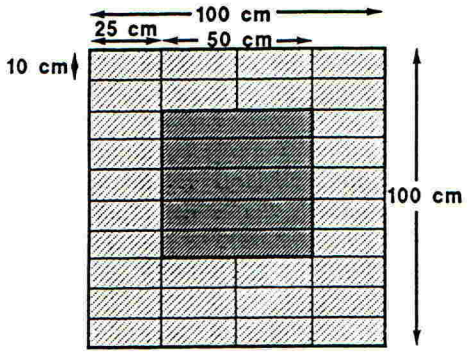
The test discribed in this paper was designed so that radioactively labelled pesticides could be used under the practical conditions of a field trial. After application, the loss of radioactivity and the active ingredient from the target plants or soil was measured. The radioactive labelling allowed the quantitative determination of non-volatile degradation products, and metabolites, or material derived from parent compound bound to the plant or soil matrix. These would not be detectable without using labelled chemicals and would otherwise enter the material balance as volatilized active ingredient. Since the use of radioactive isotopes in the field is not usually permitted, the trials were carried out in plant containers maintained under field conditions.

MATERIAL AND METHODS

Plant containers

The 1m x 1m plant container was composed of 35 individual vessels. The sampling space consisted of 5 individual vessels being 50cm x 10cm x 30cm (depth) in size. These vessels were surrounded by 30 vessels (25cm x 10cm x 30cm). These smaller containers simulate a closed plant stand and were included in the tests to minimize "edge effects". All plant vessels were fixed on a palette. Prior to the start of the trial, the plant container was surrounded with a steel collar which enabled it to be driven into the application apparatus by means of a fork-lift truck.

FIGURE 1. Plant container with individual boxes for volatilization trials in the field



30 plant vessels:
25cm x 10cm x 30cm

5 plant vessels:
50cm x 10cm x 30cm

vessel material: ®Hostalit Z

Application apparatus

The application apparatus consisted of a mobile steel frame to which a vertically adjustable platform with the arrangement of spray nozzles was attached. The space between plant container and nozzle platform was enclosed in order to avoid contamination of the surrounding area during application. Practice-relevant application was simulated by spraying with 3 individual nozzles 50cm apart. The spray boom was driven by an electronically controlled pulse motor. The entire distance to be covered by the spray boom was 120cm. At a speed of 6km per hour the acceleration distance and the stopping distance were 10cm each, i.e. the spray boom was moved at a constant speed over a distance of 100cm. Nozzles, hoses, valves and the supply vessel containing the application solution were attached to the spray boom. For the spraying process the supply vessel was put under pressure by means of compressed air and the solution was kept in motion by means of a magnetic stirrer. This avoided separation of the components of the spray solution in the supply vessel. After the production of a uniform spray pattern, the spray boom was moved over the plant stand at the predetermined speed.

Application

Applications were made under the following conditions:

- Number of spray trips: 1
- Distance between nozzle and plants: 50cm
- Set of nozzles: 3 nozzles
- Distance between nozzles: 50cm
- Type of nozzles: Lechler LU 120-04
- Pressure in the supply vessel: 4.9 bar
- Pressure at the nozzle: 3.4 bar
- Speed: 6km per hour
- Amount of spray applied: 260-315 l/ha

About 1-2 minutes after application, the plant container was driven out of the application apparatus,without vibration, by means of a fork-lift truck.

Commercial formulations prepared with ^{14}C-labelled active ingredients were used for volatilization trials. Three parallel trials were carried out with the same active ingredient on different days. Rate and time of application followed the directions for use and were in accordance with "good agricultural practice". As far as possible, the target crops were grown in the field until the date of treatment. The soil used was a loamy sand and met the specifications of the German lysimeter guideline (BBA, February, 1990).

Active ingredients

The volatilization behaviour of the herbicides, insecticides and fungicides listed in Table 1 was investigated under varying weather conditions.

TABLE 1. Rates, formulations, target crops and times of application of pesticides in volatilization trials.

Active ingredient	Rate of application	Formulation	Soil/Crop	Time of application
Methabenzthia-zuron	3.5 kg AI/ha	wettable powder	soil/pre-emergence	November
Diuron	5.6 kg AI/ha	wettable powder	soil, dolomite	April
Diuron	5.6 kg AI/ha	wettable powder	weeds	June
Aminotriazole	4 kg AI/ha	soluble powder	weeds	June
Betacyfluthrin	15 g AI/ha	emusifiable concentrate	winter wheat	May
Triadimenol	250 g AI/ha	emulsifiable concentrate	winter wheat	May
Tebuconazole	250 g AI/ha	emulsion, oil in water	winter wheat	April
Oxydemeton-methyl	125 g AI/ha	emulsifiable concentrate	winter wheat	May
Parathion-ethyl	105 g AI/ha	emusifiable concentrate	winter barley	September

Sampling/processing/analytical procedures

The 5 individual vessels in the centre of the 1m x 1m plant container were used for sampling. Within 2 minutes after application, one of these containers was removed (vibration-free sampling) from the plant stand, in order to determine the initial spray deposit. The plants were pulled out of the soil together with the complete root system, transferred into suitable solvents and extracted.

The top 2-3cm of the soil was scraped off with a spoon and extracted. Subsequently, the radioactivity and the amount of active ingredient were determined. The unextractable portions of radioactivity were determined by combustion. These measured values were defined as applied radioactivity and/or applied amount of active ingredient. The steps described here were carried out immediately to minimize volatilization during sampling. Further samples were taken after 1, 3, 6 and 24 hours. One plant container was removed and processed at each sampling interval. The sequence of removing the plant vessels was randomized to avoid systematic errors.

The plant and soil extracts were analyzed by hplc and/or tlc to determine the proportion of AI in the samples.

Recording of climatic data

Duration and intensity of sunshine and rainfall were recorded during the 24 hour test period. Air temperature, humidity and wind velocity were recorded at the level of the plant stand and the prevailing wind direction and wind velocity were recorded at 2m above groundlevel.

RESULTS AND DISCUSSION

The evaporation of radiolabelled herbicides, insecticides and fungicides from plant and soil surfaces was investigated. Physico-chemical properties and results from the volatilization trials are summarized in Table 2.

TABLE 2. Vapour pressure, water solubility of pesticidal active ingredients used in volatilization trials and their recoveries, with standard deviations, 24 hours after application.

Active ingredient	Vapour pressure [Pa] 20°C	Water solubility [g/l] 20°C	Recovery after 24 hours (in % of applied radioactivity)
Methabenzthiazuron	5.9×10^{-6}	5.9×10^{-2}	107.0 (9.2)
Diuron	2.3×10^{-7}	3.5×10^{-2}	108.2 (8.6) (soil)
Diuron	2.3×10^{-7}	3.5×10^{-2}	75.9 (1.8) (grass)
Aminotriazole	2.8×10^{-5}	339	90.6 (1.5)
Betacyfluthrin	$0.1\text{-}0.9 \times 10^{-7}$	2×10^{-6}	106.5 (11.0)
Oxydemeton-methyl	3.8×10^{-3}	miscible	94.2 (10.9)
Parathion-ethyl	8.9×10^{-4}	1.0×10^{-2}	73.4 (6.4)
Triadimenol	$4.1 \times 10^{-8}/2.4 \times 10^{-7}$	$6.2\text{-}3.2 \times 10^{-2}$	89.0 (11.7)
Tebuconazole	1.3×10^{-6}	3.2×10^{-2}	98.0 (14.4)

Since these were field trials, the quantitative analysis of the volatilized portions of active ingredients was not possible. However, the volatilization rate could be calculated indirectly from the difference between applied and recovered radioactivity. The proportion of radioactivity measured on the target area immediately after application was equated with 100% and the losses due to volatilization from the plants and/or the soil surfaces occuring in the course of the trail were calculated in percent of the initial deposit.

Figures 2 and 3 show the rates of volatilization of some ^{14}C-labelled pesticides from the ecosystem sections; 1, 3, 6 and 24 hours after application. The data were mean values from 3 parallel trials. The variation seen in the results was due to the prevailing weather conditions on the day of application and the type and size of the target crop.

FIGURE 2. Volatilization kinetics of methabenzthiazuron, diuron and aminotriazole from agricultural ecosystems.

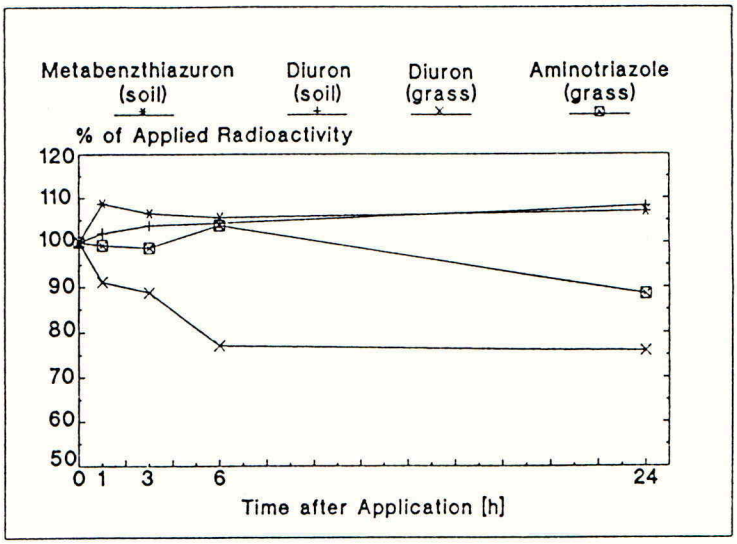
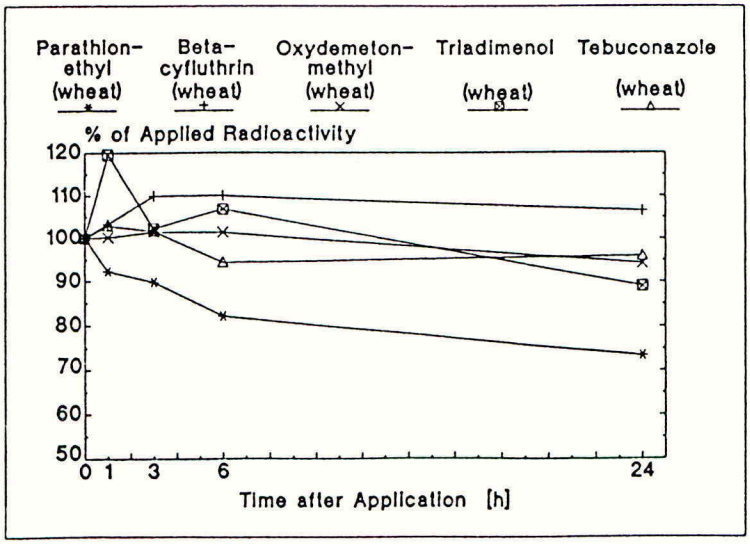


FIGURE 3. Volatilization kinetics of betacyfluthrin, oxydemeton-methyl, parathion-ethyl, triadimenol and tebuconazole from agricultural ecosystems



The larger the plants the greater the variation in the structure of the plant canopy.The plant cover was almost 100% at the time of the application of diuron and aminotriazole on weeds, whereas the coverage was only about 50% in the case of parathion-ethyl on winter barley at the stage of tillering. A further factor contributing to differences between trials was the sampling immediately after the application to determine the initial spray deposit on plants. At this sampling time the leaves were still wet and therefore cross-contamination from another plant vessel was possible.

Methabenzthiazuron, applied pre-emergence in the autumn, did not show any volatilization within 24 hours. Similarly diuron did not volatilize when it was applied on bare ground but in contrast to this, only about 76% of the applied active ingredient were recovered after 24 hours, when the chemical was applied to a closed stand of weeds, i.e. about 24% was volatilized as parent compound or metabolites.

The volatilization of the active ingredients betacyfluthrin, triadimenol, tebuconazole and oxydemeton-methyl from the ecosystem section simulating a winter wheat field in the period April to June was <20%. The volatilization of the reference compound parathion-ethyl from the ecosystem section simulating a winter barley field in the early stages of development was about 27%. It was not possible to attempt to correlate the measured volatilization rates to physico-chemical properties such as vapour pressure and water solubility due to the limited data available.

When volatilization rates >20% within 24 hours are determined in these trials an assessment of the photochemical-oxidative degradability of the chemical in the air is required (BBA, July, 1990). This may be determined experimentally or by calculation. For example, based on calculations according to Atkinson, the half-life of diuron in the troposphere is in the range of a few hours (Hellpointner, 1991).

CONCLUSION

The volatilization of ^{14}C-labelled herbicides, insecticides and fungicides was investigated under field conditions. The test design allowed a practice-relevant treatment of field crops, taking into account the target crop, the degree of ground coverage and the weather conditions typical for the time of application. These factors are of great importance for the distribution of the spray deposit on soil and plants and allows an environmentally relevant assessment of volatilization behaviour.

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Bayer-Bericht Nr.: PF-3658

A SIMPLE PROCEDURE TO MEASURE THE VOLATILITY OF AGROCHEMICALS FROM SOIL AND LEAF SURFACES

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ABSTRACT

A simple procedure to measure quantitatively the volatility of agrochemicals from soil and leaf surfaces is described. ^{14}C-Radiolabelled agrochemicals were prepared as their commercial formulations. An aliquot of a formulation was sprayed quantitatively at maximum field rate onto each leaf of a single French bean plant and onto moist soil contained in glass pots. Air was passed over the leaves (2m/s) and the soil surface (1m/s) for 24 hours. The temperature and relative humidity of the air was measured during this time. Duplicate leaves and duplicate soil pots were analysed at regular intervals to determine the amount of radioactivity remaining on the sample. Recoveries were expressed as a percentage of the radioactivity recovered from samples analysed immediately after treatment.

INTRODUCTION

As our understanding of the behaviour of agrochemicals in the environment increases, additional areas emerge as worthy of study. One such area is the measurement of the volatility of agrochemicals from soil and leaf surfaces. To this end the Federal Republic of Germany has issued a new guideline : BBA Guideline Part IV, 6-1 "Tests for the volatilisation behaviour of plant protection products and their fate in the atmosphere".

This guideline requires that an assessment be made of the losses, by evaporation, from leaf and soil surfaces of all agrochemicals which are relatively stable in water and sunlight (half-life >4 days). Where losses exceed 20% in 24 hours, further information will then be required on the rate of degradation of such compounds in the atmosphere. It is therefore necessary to develop an experimental procedure to assess evaporative losses of agrochemicals from leaf and soil surfaces. This paper describes a very simple experimental system to make these measurements. The procedure allows the temperature, relative humidity and linear velocity of the air passing over the treated surfaces to be monitored throughout the experiment. The experiment is also carried out in a non-enclosed system so that the air passing over the treated surfaces cannot become saturated with the test compound.

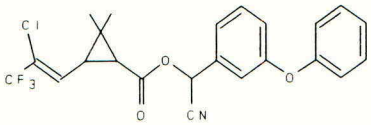
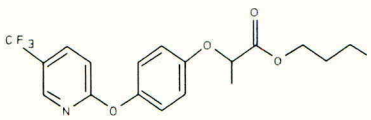
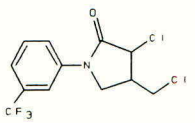
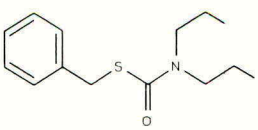
MATERIALS

The soil used was Speyer 2.1, a sandy soil obtained from the Agricultural Investigation and Research Institute, Obere, Langgasse, GDR. It was passed through a 2mm sieve and adjusted to 60% of its moisture holding capacity (60% MHC) by the addition of distilled water.

The plants used were dwarf French beans (*Phaseolus vulgaris*) at the flowering/1st fruiting growth stage.

The details of the agrochemicals used are given in Table 1.

TABLE 1. Structures, formulations and vapour pressures of the test agrochemicals

Compound	Structure	Formulation	Vapour Pressure
Lambda-cyhalothrin		5.0% EC	0.0002mPa at 20°C
Fluazifop-P-butyl		12.5% EC	0.003mPa at 20°C
Flurochloridone		25.0% EC	0.44mPa at 25°C
Pirimicarb		50.0% WP	2.1mPa at 20°C
Prosulfocarb		80.0% EC	6.91mPa at 25°C

To facilitate the measurement of agrochemical remaining on soil or leaf surfaces at various time intervals after application, all the chemicals tested were labelled with ^{14}C-carbon. The radiochemical purities of all the compounds used were >97%.

METHODS

Measurement of radioactivity

Radioactivity in solutions was quantified by liquid scintillation counting (lsc) using LKB Optiphase 'Safe' scintillation cocktail and LKB 1219 scintillation spectrometers (Pharmacia Limited, Uppsala, Sweden). Unextracted radioactivity was measured by combustion of samples in a Harvey OX300 biological sample oxidiser prior to measurement by lsc.

Application technique

A Linomat III tlc applicator (Camag, Muttenz, Switzerland) was modified so as to allow an exactly known amount of the formulated test compound to be sprayed onto a leaf or soil surface. The Linomat III is designed to spray a radioactive solution, from a microlitre syringe, onto a tlc plate. This is achieved by using a stepper motor to slowly depress the plunger of the syringe. Then a jet of gas directed over the end of the syringe needle is used to nebulise the radioactive solution as it exits from the needle and direct the spray onto the tlc plate. By removing the unit which creates this spray from the tlc applicator it is possible to use this unit to direct the spray onto a leaf or a soil surface rather than a tlc plate.

Plant experiment

A circle of diameter 5cm was marked on each of 12 leaves of a single dwarf French bean plant. Formulated agrochemical (~50μl) was applied, at its maximum field rate, to each circle using the tlc applicator. Two leaves were removed from the plant and were analysed immediately after treatment to determine how much radioactivity had reached the leaf surface.

The treated plant was placed in a glasshouse and air was blown over the leaves at a speed of >2m/s using an electric fan. The air speed was monitored by an Airflow Edra Five anemometer (Airflow Developments Limited, High Wycombe, England). The air temperature and relative humidity were measured with an ACR "Stick-on" XT-101 data logger and a Model EH-010 probe (ACR Systems Incorporated, Surrey, Canada). Extractor fans were used to prevent the air in the glasshouse from being saturated by pesticide vapour. Duplicate leaves were analysed at regular time intervals (eg. 0, 1, 3, 5, 7 and 24 hours after treatment) throughout the 24 hour period. At the end of the experiment, the remainder of the plant (ie. stem, roots and untreated leaves) were also analysed to determine if loss of radioactivity from the treated leaves was due to translocation. In this study no significant levels of radioactivity were detected in these untreated plant parts.

The radioactivity remaining on a leaf was quantified by extracting it with acetonitrile (50cm^3) using an Ultra-Turrax tissue macerator (Janke and Kundel GmbH, Ika Werk, Staufen i Breisgau, Germany). The extract was

centrifuged and the supernatant was transferred to a volumetric flask, made up to volume and analysed by lsc. The solid debris was dried, weighed and combusted prior to quantitation of the unextracted radioactivity by lsc.

Soil experiment

Aliquots (~50g) of moist (60%MHC) Speyer 2.1 soil were weighed into glass pots (35mm x 48mm i.d.). Formulated agrochemical (~50μl) was applied, at its maximum field rate, to each soil pot using the tlc applicator. Two soil samples were analysed immediately after treatment to determine how much radioactivity had reached the soil surface.

Treated soil pots were placed at the front edge of a fume hood and the sash adjusted until an air speed of >1m/s over the soil surface was obtained. The air speed, temperature and relative humidity of the air were measured throughout the experiment, as described for plants. In order to prevent the soils drying out during the experiment, distilled water was dripped slowly onto each soil surface using a Type 202S multichannel peristaltic pump (Watson-Marlow Limited, Falmouth, Cornwall, England). Duplicate soil pots were analysed at regular time intervals (eg. 0, 1, 3, 5, 7 and 24 hours after treatment) throughout the 24 hour period. As each pot was sampled it was weighed and the moisture content calculated. The moisture contents achieved throughout the experiments were in the range 48.2% - 67.3% MHC.

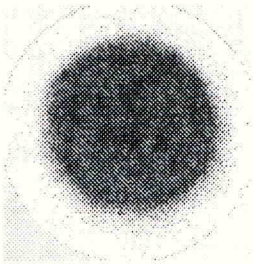
The radioactivity remaining on the soil was quantified by extracting the soil with acetonitrile; the soil was transferred to a round bottom flask to which acetonitrile (150cm^3) was added, the flask was shaken by hand for ~15 seconds and the soil was filtered through a Whatman No.4 paper. The filtrate was transferred to a volumetric flask, made up to volume and analysed by liquid scintillation counting (LSC). The solid debris was dried, weighed and combusted prior to quantitation of the unextracted radioactivity by lsc.

RESULTS

Validation of the application technique

In order to demonstrate that reproducible amounts of radioactivity could be sprayed onto surfaces by the Linomat III, an aqueous solution of a non-volatile radiolabelled compound was prepared. Eight aliquots (50mm^3) from this solution were then sprayed onto eight polystyrene discs. The discs were transferred to scintillation counting vials, dissolved in scintillation cocktail and analysed by lsc. The amount of radioactivity in the aliquot taken for spraying was 3773Bq and the mean radioactivity recovered on the discs was 3626Bq with a coefficient of variation of 0.9%. This corresponds to a recovery of 96%. These results clearly demonstrated that this technique allows small volumes of radioactive solutions to be sprayed quantitatively onto small surface areas. In addition, the uniformity of the spray application was investigated by using the Linomat III to spray ink onto a filter paper. The result, pictured in Figure 1, shows that a very good spray distribution can be achieved.

FIGURE 1. Application of ink onto a filter paper using a Linomat III tlc applicator



Evaporation from plants and soil

The radioactive recoveries from the treated leaves and soil at intervals up to 24 hours are listed in Tables 2 and 3 respectively.

TABLE 2. Radioactive recoveries from plants within 24 hours of treatment

	Percent of Applied Radioactivity Recovered After :					
Compound	0h	1h	3h	5h	7h	24h
Lambda-cyhalothrin	100	97.9	100.8	90.0	91.3	87.6
Fluazifop-P-butyl	100	102.9	97.5	92.0	N/A	75.0
Flurochloridone	100	101.6	104.0	97.8	98.8	93.7
Pirimicarb	100	83.1	76.3	65.0	60.9	47.4
Prosulfocarb	100	97.1	91.7	88.9	78.5	53.3

TABLE 3. Radioactive recoveries from soil within 24 hours of treatment

	Percent of Applied Radioactivity Recovered After :					
Compound	0h	1h	3h	5h	7h	24h
Lambda-cyhalothrin	100	103.9	100.8	101.1	N/A	101.9
Fluazifop-P-butyl	100	98.7	95.4	98.1	87.5	84.9
Flurochloridone	100	99.1	98.4	97.8	95.6	93.5
Pirimicarb	100	98.2	97.7	96.8	95.2	89.5
Prosulfocarb	100	103.3	99.2	97.6	101.9	82.0

N/A = not analysed at this time interval

The air temperature and relative humidity were measured during each study. The minima and maxima for each study are summarised in Table 4.

TABLE 4. Temperature and relative humidity of the air during each study

	Temperature		Relative Humidity	
Compound	Plant	Soil	Plant	Soil
Lambda-cyhalothrin	12 - 23°C	16 - 19°C	40 - 67%	41 - 81%
Fluazifop-P-butyl	14 - 22°C	17 - 20°C	31 - 37%	33 - 47%
Flurochloridone	13 - 29°C	19 - 26°C	34 - 61%	44 - 57%
Pirimicarb	13 - 21°C	17 - 20°C	66 - 82%	55 - 64%
Prosulfocarb	14 - 22°C	16 - 20°C	21 - 35%	42 - 57%

CONCLUSIONS

An understanding of the possible significance of the evaporation of agrochemicals into the atmosphere is an area of current investigation. It is probably not yet clear if this could represent a significant source of agrochemical redistribution, but experimental data is certainly needed before a scientific assessment can be made. The experimental system described in this paper provides a very quick and simple method to gain experimental data on evaporative losses.

The major contribution to the simplicity of this method is the development of a system to spray reproducibly a measured amount of formulated, radiolabelled agrochemical onto a small area of leaf or soil. The treated leaves and soil are then exposed to a constant wind speed for a 24 hour period. This exposure is carried out under conditions of total equilibrium with the atmosphere and with a temperature and humidity variation which can occur over a 24 hour period under field conditions.

The results obtained show that evaporative losses can occur, especially from leaf surfaces. These losses do not correlate simply with the vapour pressures of the compounds tested. This is not unexpected since volatilisation from surfaces is also controlled by the adsorption properties and, for moist soils, by the water solubility (Burkhard & Guth, 1981) of the compound.

Pirimicarb was shown to be the most readily volatilised compound from a leaf surface. This result demonstrates the validity of the proposed method as pirimicarb's aphid control has been shown to be due to it's movement in the vapour phase.

REFERENCE

Burkhard, N.; Guth, J.A. (1981) Rate of Volatilisation of Pesticides from Soil Surfaces; Comparison of Calculated Results with Those Determined in a Laboratory Model System. *Pesticide Science*, **12**, 37-44.

VOLATILITY TESTING OF PESTICIDES IN A WIND TUNNEL

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ABSTRACT

In the Federal Republic of Germany data on the volatilisation potential of pesticides out of soil and from plant surfaces are required for registration (BBA, guideline part IV/6-1). To fulfill the requirements of the guideline, a wind tunnel was constructed. The system allows a broad range of test conditions. Experiments described here were performed using the following parameters: air velocity 1.0 - 1.2 m/s, relative air humidity 30 - 50 %, 20 - 25 °C. Volatilisation was determined by diverting a proportion (*ca.* 2.5 %) of the air stream through polyurethane foam and measuring the quantity of adsorbed chemical. Results from experiments with model chemicals (lindane, simazine, di-(2-ethylhexyl)phthalatic acid ester, deltamethrin) are presented. While volatilisation within 24 h for simazine, di-(2-ethylhexyl)phthalatic acid ester, and deltamethrin was below 1 % (from soil and plants), approximately 50 % of the lindane deposit was volatilised from plant surfaces.

INTRODUCTION

Investigations into the dissipation of pesticides in soil, water, and air are necessary for the assessment of the fate of active ingredients (AI). Besides photolysis, soil degradation, leaching, and run-off, direct volatilisation into the atmosphere may be a major dissipation pathway (Taylor & Spencer, 1990). Volatilisation of applied pesticides is a significant source for long-range transport of these chemicals (Spencer & Cliath, 1990).

In compliance with the BBA-guideline part IV/6-1 "Testing the volatilisation potential and the fate of pesticides in air" (BBA, 1990) the tests were performed in a wind tunnel. The amount of volatilised compound was determined over 4 - 5 time intervals by diverting a proportion of the air stream (*ca.* 2.5 % of the total air stream) through an adsorbent.

MATERIALS AND METHODS

Test substances

Experiments were performed with ^{14}C-simazine, ^{14}C-DEHP (di-(2-ethylhexyl)phthalatic acid ester), ^{14}C-deltamethrin and with non-labelled lindane (gamma-1,2,3,4,5,6-hexachlorocyclohexane; in formulation NEXIT flüssig SC with 80 % AI, wt/V).

Soil and plants

A sieved silty sand soil was used for all soil experiments (fraction < 2 mm). The soil fulfills the requirements of the BBA-guideline part IV/6-1 (sand content > 70 %; organic carbon content ≤ 1.5 %). The maximum waterholding capacity (MWC) was 26 g water/100 g dry weight. Experiments were performed at 60 % of the MWC (16 g/100 g dry weight).

The beans (garden beans, *Phaseolus vulgaris*, "Saxa, green") were planted in unsieved soil. The treatment was performed when the plants were in flower and/or first fruit bearing.

Application solutions

^{14}C-Labelled compounds were added to blank formulations (simazine in GESATOP 500, DEHP in a commercial EC-formulation, deltamethrin in DECIS). After 5 min treatment in an ultrasonic bath, water was added. For lindane the formulation was used according to the manufacturer's instructions.

Application

The application solution was sprayed on a target area of 1 m^2 where bowls with soil or plants were placed. A full cone nozzle made of stainless steel was used (Lechler FC4-448). The nozzle was mounted at a height of 66 cm and the application was performed at 2 bar pressure.

Stainless steel bowls (44.5 x 31.5 cm surface area) were used for all experiments. The bowls were filled with a 4 cm-layer of burnt clay granulate and 500 ml of water. A nylon gauze was placed on this layer. Then a 3 cm-layer of sieved soil was added, up to the rim of the bowl. This arrangement ensured that the soil remained wet during the experiment.

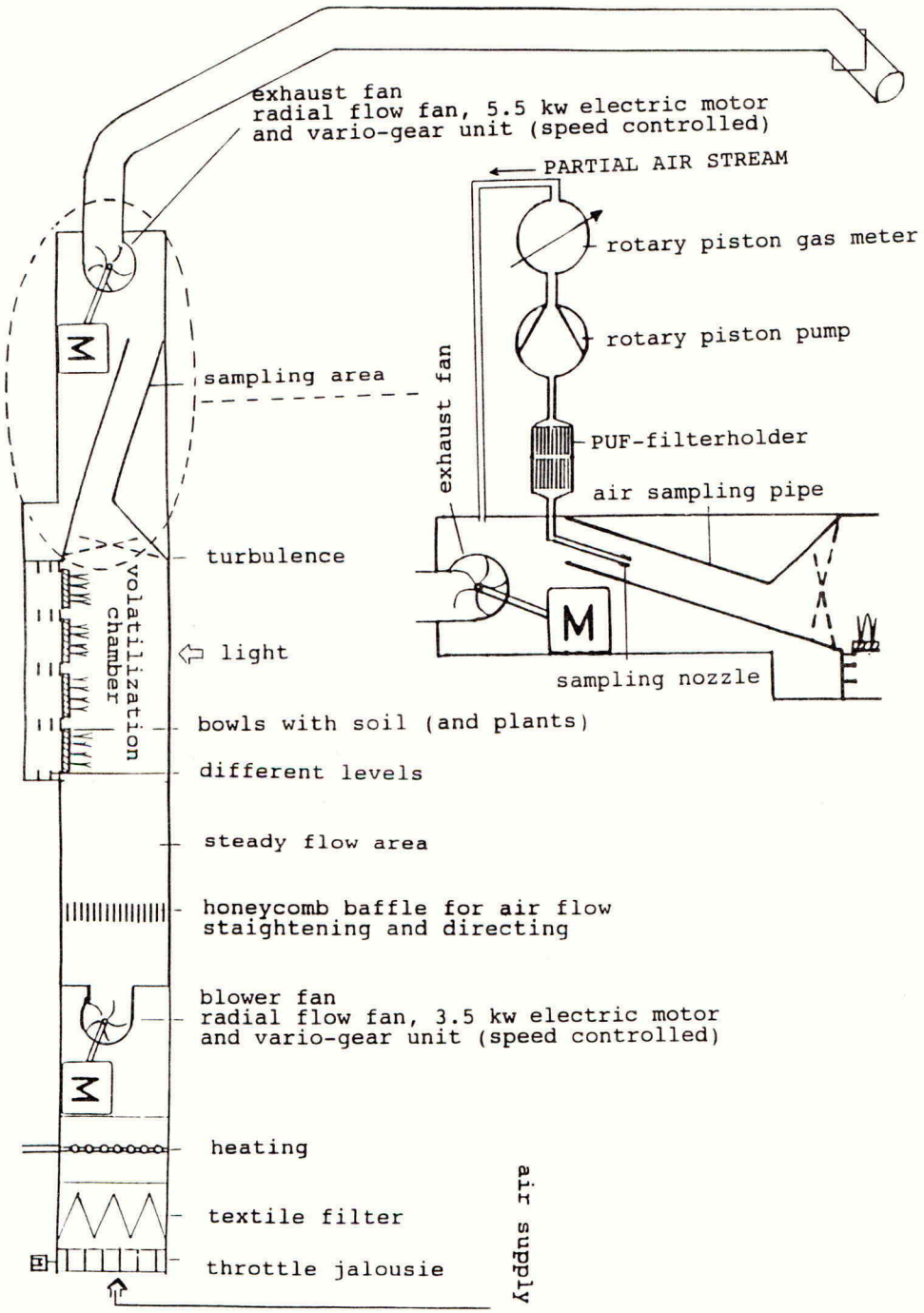
The bottom of the application chamber was covered with crepe paper before the bowls were put in. The paper was extracted with methanol after application in order to determine the amount of radioactivity or AI which missed the soil or plants in the bowls. The cartridge and the nozzle were rinsed with methanol and the AI content of the rinsing solution was determined.

Wind tunnel

A scheme of the wind tunnel is shown in Fig. 1. It was located in a hall (1500 m^3 volume). The ambient air was sucked in through a textile filter. The air was heated and blown by a fan into a steady flow area. Then it passed through a honeycomb grid for air flow laminarisation. Behind it was the volatilisation chamber (stainless steel; cross section 0.85 m x 0.85 m, length 2 m). After the volatilisation chamber the air stream passed through a specially formed sheet steel blade to cause turbulence. The turbulent air was passed through a steel tube of 1.6 m length. The air sampling was performed at the end of a straight section (1 m) of this tube. The waste air was blown with a second blower fan through a pipe (35 cm diameter) to the outlet.

The air was moisturized with a steam humidifier. The regulation was performed manually in relation to the humidity of the ambient air. The following parameters were monitored approximately 25 cm in front of the

FIGURE 1: Wind tunnel; inset: air sampling equipment.



sample bowls: air velocity with a vane anemometer; temperature and relative humidity with a thermohygrometer. The bowls with the treated soil or plants were placed 10 cm above the bottom of the volatilisation chamber.

Air sampling

The air sampling device is shown in the inset of Fig. 1. Part of the air (*ca.* 2.5 %) was sucked out of the main stream using a stainless steel sampling probe. Suction was provided by a rotary piston pump. The filter holder contained two pieces of polyurethane foam (PUF; with 10 cm diameter and 10 cm layer thickness, density 30 kg/m^3) for each sampling period. The first piece of PUF was the sampling plug, while the second piece of PUF was used for the detection of breakthrough.

The PUF pieces were extracted with toluene after sampling. The first piece of PUF from each sampling was extracted three times and the second piece of PUF once with 100 - 200 ml toluene, respectively (Niehaus *et al.*, 1990).

Determination of the main air stream volume

The main air stream volume was determined according to the German VDI guideline 2066 (VDI, 1975). A velocity profile was recorded at 16 positions, which represent plane segments of identical area. From this profile the mean velocity of the waste air was calculated. The velocity of the waste air at one point (representing the nominal value of the mean velocity) was recorded continuously during each volatilisation experiment (using a thermoanemometer; tolerance 2.5 %). Velocity values measured were automatically corrected to a standard temperature of 21 °C (294 K).

The volume of the main air stream was calculated using the following equation: $A \times V_M \times 3600 = H$ with A, area (circle area, diameter 35 cm) in m^2; V_M, mean air velocity of the waste air; H: main air stream in m^3/h; 3600, converting factor from seconds to hours. The air velocity of the waste air was averaged over the total time of each experiment.

Determination of the partial air stream volume

The volume of the partial stream was determined using a calibrated rotary piston gas meter (tolerance 1 %). The values obtained from the gas meter had to be corrected for temperature using the following equation:

$$V_{corrected} / V_{measured} = 294\ K / T_{partial\ stream}.$$

Analyses

^{14}C-Compounds: Aliquots of application solutions, rinsing solutions and PUF extracts were analysed by liquid scintillation counting. Lindane: All analyses were performed by GC-ECD (Brücher, 1991).

RESULTS AND DISCUSSION

The volatilisation experiments (both soil and plants) with the ^{14}C-labelled compounds simazine, DEHP, and deltamethrin clearly showed that the volatilisation potential of these substances with vapor pressures of 8.1×10^{-7} (simazine; at 20 °C; Perkow, 1990), 6×10^{-6} Pa (DEHP; at

20 °C; Streit, 1991), and 2 x 10^{-6} Pa (deltamethrin; at 25 °C; Perkow, 1990) is very low. The volatilisation (maximum 0.6 % in 24 h) was lower than the radiochemical purity of the chemicals.

In contrast, higher values were found for the volatilisation of lindane. In four experiments lindane formulation (150 µl) dissolved in H_2O (40 ml) was applied to a 1 m^2 area (equivalent to 1.5 kg formulation in 400 l/ha). Between 79 - 96 mg lindane was applied to the test surfaces, depending on the position of the bowls in the spray cone and the amount of bean leaves hanging over the bowls.

The amounts of lindane volatilised were determined by analysing the toluene extracts of the PUF pieces. The second piece of PUF of each sampling contained only low amounts of lindane. In no case was more than 4 % of the total amount of lindane from one air sampling found in the extract of the second piece of PUF (average from 19 samples: 1.3 ± 0.8 % standard deviation).

Table 1 presents an overview of the environmental conditions prevalent at the time of the four experiments. The results are given in Table 2.

TABLE 1: Wind tunnel conditions for experiments with lindane.

experiment:	beans I	beans II	beans III	soil
temperature [°C]	20.0 ± 0.3	24.6 ± 0.3	24.7 ± 0.3	25.0 ± 0.7
air velocity [m/s]	1.02 ± 0.02	1.11 ± 0.01	1.13 ± 0.01	1.12 ± 0.02
air humidity [%]	49.7 ± 4.2	41.6 ± 0.6	35.0 ± 1.0	40.2 ± 3.5

TABLE 2: Volatilisation of lindane.

experiment:	beans I[a]	beans II	beans III	soil
volatilised 0 h - 1 h [%]	2.4	4.0	4.5	1.6
volatilised 1 h - 3 h [%]	5.1	8.2	8.0	2.9
volatilised 4 h - 6 h [%]	6.9	11.9[b]	9.2	3.4
volatilised 7 h - 24 h [%]	21.0	26.7[c]	31.3	16.9
volatilised 25 h - 29 h [%]	2.4[d]	6.7[e]	4.9	3.6
sum 0 h - 29 h [%]	37.8[d]	57.5	57.9	28.4

a, small beans with low covering, lower temperature; b, sampling 4 h - 7 h; c, sampling 8 h - 23 h; d, sampling 25 h - 30 h; e, sampling 24 h - 29 h.

Lindane (vapor pressure 1.2 x 10^{-3} Pa; Perkow, 1990) had a remarkable volatilisation potential. Volatilisation from plant surfaces was significantly higher than from soil, probably due to the greater surface in contact with the air.

Lindane residues (amount applied minus amount volatilised) were used to calculate the volatilisation rates as described by Timme *et al.* (1986). The half-lives for the volatilisation of lindane from beans (experiments II and III) were 22 h and 21 h, respectively (second order kinetics).

CONCLUSIONS

The wind tunnel which was developed in our institute is a valuable test system for determining the volatility of pesticides under controlled conditions. Using this system the volatility of non-labelled as well as ^{14}C-labelled compounds can be determined.

Additionally, the wind tunnel is suitable to investigate the influence of several parameters on the volatilisation, e.g., relative humidity, temperature and air velocity.

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