POSTER SESSION 4C

MEASURING THE FATE AND EFFECTS OF PESTICIDES IN THE ENVIRONMENT

Session Organiser: Dr A S Terry
                       Cambridge Environmental Assessments,
                       Boxworth, UK

Poster Papers: 4C-1 to 4C-8

Does triticonazole affect microbial activity?

E Börjesson
Swedish University of Agricultural Science, Department of Microbiology, P A Box 7025, SE-750 07 Uppsala, Sweden
Email: Elisabet.Borjesson@mikrob.slu.se

L Johnsson
Swedish University of Agricultural Science, Plant Pathology and Biocontrol Unit, P¹ Box 7035, SE-750 07 Uppsala, Sweden

ABSTRACT

The long-term fate and the influence on biomass of the fungicide triticonazole (TTZ), 5-[(4-chlorophenyl)methylene]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol, applied via seed or soil treatment, were studied. TTZ was sprayed on bare soil or applied on wheat grains as a disinfectant before sowing. The seeds were germinated and grown in pots in a greenhouse at $22^{\circ}C$. The dissipation speed was studied by chemical analysis of the residues in soil every fourth week until no TTZ could be detected. The microbial activity was measured as substrate-induced respiration (SIR), at the start, at the half-way point and at the end of the study.

The microbial biomass was initially decreased in the soil treatment but recovered after 56 days. The active part of the biomass did not change during the experimental time.

INTRODUCTION

Triticonazole (TTZ), 5-[(4-chlorophenyl)methylene]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol, is a relatively new fungicide developed by Rhône-Poulenc (now Bayer CropScience) and patented in 1988. It is a broad-spectrum systemic triazole acting by inhibition of demethylation in the sterol biosynthesis pathway found in most fungi except *Oomycetes*. It is used as a seed disinfectant against seed-borne diseases and as a preventative treatment against a number of foliar pathogens such as rusts *(Puccinia spp)*, powdery mildew, leaf-spots, eye-spot, leaf and net blotch of cereals, seedling diseases, head smut of corn *(Ustilago spp)* and bunt *(Tilletia caries)*. Seed treatment combines disinfection of the seeds with longer-term protection of the plant. The seed are coated with a film of formulation containing the fungicide. Such targeted deposition of the fungicide allows for reduced rate doses and minimized environmental risks.

The objective was to conduct a pot study in the greenhouse to find out the degradation rate of the fungicide triticonazole (TTZ) in soil, when TTZ is applied via seed treatment compared to soil treatment. In addition, the influence on the biomass was also to be investigated. The size of the biomass was measured as substrate induced respiration (SIR) (Stenström et al, 2001). The initial respiration rate obtained when a substrate at a saturating concentration is mixed into a soil sample is assumed to be proportional to the concentration of the substrate-responsive biomass. The intention was to extrapolate the findings to current field studies.

MATERIALS AND METHODS

Experimental design

A total of 96 Mitscherlich pots (6.3 dm^3 per pot) were prepared, 48 for the soil treatment (A) and 48 for the seed treatment (B). The pots were put in a greenhouse at about 22°C, watered continuously and sampled every fourth week for chemical and biological analysis.

Soil preparation

The soil was taken from the province Dalarna in the middle of Sweden on 26th June 2001. Below are listed some characteristics of the soil.

Table 1. Soil Properties

Property	
Texture (mm):	
% <0.002	29
% 0.002-0.02	56
% 0.02-0.2	10
% 0.2-2	5
pH	6.5
Organic matter (%)	2.8
Maximum Water Holding Capacity (g/100 g)	44
Cation Exchange Capacity (meq/g)	16.8
P-AL (mg /100g dry weight)	4.7
K-AL (mg /100g dry weight)	12
Mg-AL (mg /100g dry weight)	35.1
Ca-AL (mg /100g dry weight)	177
Base saturation (%)	69.6

TTZ application on seed

A winter wheat seed was used, cultivar Stava from Svalöf Weibull AB, with a germination capacity of 95% and a thousand kernel weight of 41 g. One kg of the seed was treated with 4.0 ml of a mixture of the formulation ´Premis 25 FS` (10 ml) and distilled water (10 ml). The original formulation contained 25 g/litre of triticonazole. The kernels were extracted and analysed by hplc (Console, 1993) to measure the TTZ applied.

Pot preparation

A total of 96 Mitscherlich pots with individual pot numbers were used for the experiment, each containing 4800 g of soil. In 48 pots, 112 ml of a diluted ´Premis 25 FS` suspension were sprayed on each pot. The treatment suspension was made by mixing 1.0 ml formulated product and 99 ml distilled water and from this suspension 6.0 ml was taken and mixed with 5994 ml of distilled water. After the TTZ application the pots were covered with 800 g of soil.
Fourteen treated wheat kernels were placed in each of the other 48 pots and covered with 800 g soil. The application rate was equivalent to 8.9 g/ha, both in the soil and the seed treatments.

Plant care and soil sampling

At the start of the experiment, soil samples were taken from both soil and seed treatments for chemical analysis. The pots were placed in a greenhouse and the light was switched on for 12 hours a day. During the experiment the soil moisture was adjusted to about 40% of the MWHC value. Three pots with plants and three pots without plants were sampled every fourth week.

Chemical and biological analysis

Before the study started, TTZ was applied to the seeds and they were analysed by hplc. Each soil sample was thoroughly mixed before analysis and the water content was measured. Then 50 g of each sample was extracted, cleaned up and analysed by gc-ms (Guillet & Simonin, 1992). By use of the mass spectrometer as detector instead of the electron capture detector it was possible to lower the detection limit compared to that in the method by Guillet & Simonin. The method was validated with soil samples spiked at the quantification level 0.002 µg/g, as well as 0.01 µg/g and 0.02 µg/g.

The soil samples were analysed on the sampling day. When the concentration of TTZ in all pots from one treatment at a given sampling point was below the quantification level, no further sampling was conducted.

The microbial activity was measured as in samples from Days 0, 56 and 140. The activities were measured in thawed samples at the end of the study.

RESULTS AND DISCUSSION

Chemical analysis

The mean TTZ content of the treated kernels was 21.6 µg TTZ/14 kernels when the study started. The theoretical concentration of TTZ applied in the seed treatment was calculated to be 21.6/1750 = 0.01234 µg/g soil for the upper part (1750 g) of the pot.

Degradation of TTZ in the upper part of the pot is shown in Figure 1.
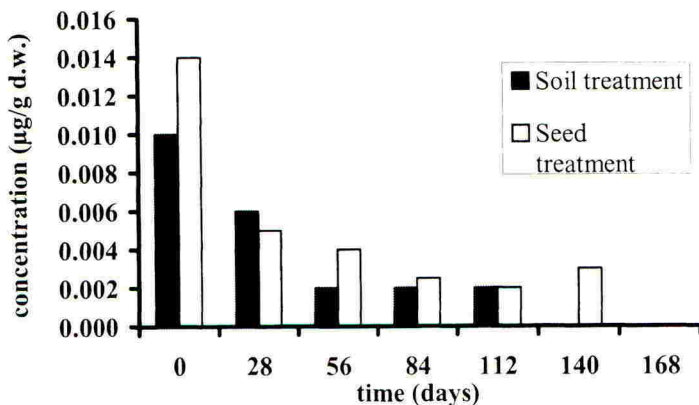

Figure 1. Residues of TTZ in the upper part of the pot.

The dissipation rate was slightly faster in the seed treatment than in the soil treatment from 0-28 days. The relatively low TTZ application rate in this study led to a rapid dissipation between 0-28 days and only very low residue concentrations, just above the quantification level, could be found in both treatments after 28 days. After 168 days, no detectable amounts were found.

The degradation rate, $DT_{50,}$ in the soil treatment was calculated as $t_{1/2} = \ln 2/k \sim 0.69/0.0257 = 27$ days and in the seed treatment $0.69/0.0236 = 29$ days. The value for k was calculated by the mean square error method.

Values from the three first sampling dates were used to calculate the degradation rate, as beyond 56 days some of the analysed concentrations were <0.002 µg/g, and concentrations based on them were not statistically valid.

Biological activities

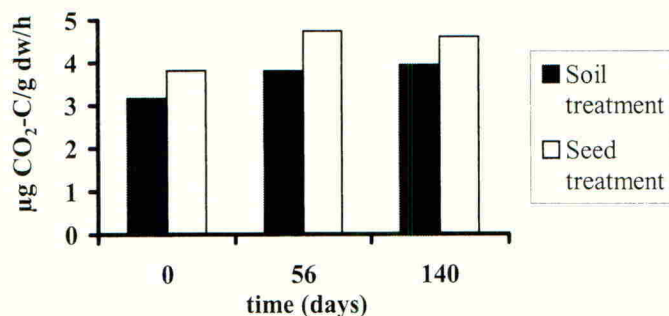The respiration rate measured by the SIR method is presented in Figure 2.

Figure 2. The SIR (the height of each bar) obtained in both treatments

The rate of respiration did not decrease in either of the treated soils during the time of incubation. This indicates that the degradation capacity of the microflora remained unchanged throughout the whole incubation.

The biological activity, measured as SIR, remained almost unchanged during the experiment and did not affect the microbial degradation in neither of the treatments.

REFERENCES

Consol E (1993). HPLC analysis of triticonazole in simple formulations and on treated seeds. *Rhône-Poulenc Secteur Agro.*

Guillet; Simonin B (1992). Analytical method for the determination of residues in soil. *Rhône-Poulenc Secteur Agro.*

Stenström J; Svensson K; Johansson M (2001). Reversible transition between active and dormant microbial states in soil. *Microbial Ecology* **36**, 93-104.

The fate and uptake of the fungicide carbendazim into organisms in soil microcosms

L A Burrows, C A Edwards
The Ohio State University, 1735 Neil Avenue, Columbus, Ohio, 43210, USA
Email: soilecol@osu.edu

T Knacker, B Förster
ECT Oekotoxokologie GmbH, Böttgerstr. 2-14, D-65439 Flörsheim am Main, Germany

ABSTRACT

Integrated soil microcosms (ISM) were constructed from high-density polyethylene (HDPE) cylinders, 7.5 cm id x 15 cm deep, with a fine nylon mesh across the bottom to collect leachates. They were packed with sieved soil, three earthworms (*Lumbricus rubellus*) added, and 10 wheat seeds sown. Carbendazim application rates, based on a predicted environmental concentration (PEC) of 0.76 mg a.i./kg soil dry weight, were 1, 3, 9, 27, and 81xPEC, (T1 to T5, respectively). Carbendazim residues in soils, plants and leachates were analyzed after 7, 14, 28, and 56 days. Carbendazim residues in earthworms were analyzed after 7, 14, and 28 days.

Soil carbendazim residues remained steady for 28 days then decreased between 28 and 56 days. Mean soil carbendazim residue concentrations were 0.20, 0.45, 1.1, 2.4, and 6.4 mg/kg soil for T1, T2, T3, T4, and T5, respectively after 56 days. Carbendazim leachate concentrations did not exceed 1.1% of the amount applied for any treatments at any time-point. Mean carbendazim concentrations in earthworms after 7 days were 1.0, 1.1, 2.8, 6.7, and 39.4 μg/g earthworm for T1, T2, T3, T4, and T5, respectively. Soil to earthworm bioaccumulation ratios ranged from 0.5 to 7.3. Plant uptake of carbendazim was low except at 81xPEC when concentrations reached 150 mg/kg. Residues in soils and plant tissues were correlated in soil microcosms, terrestrial model ecosystems (TME) and field experiments.

INTRODUCTION

The movement, persistence and degradation of a pesticide in the terrestrial environment may be influenced by: the adsorption and desorption of the pesticide from soil particles, its aqueous solubility, biological or chemical degradation pathways, and the uptake of the pesticide into plants or animals. Pesticides that sorb strongly to soil may become unavailable biologically for uptake into organisms or for use as a substrate by microbial communities (Pierzynski *et al.*, 1994). Pesticides that are very soluble in water are usually more available biologically for uptake into organisms and can leach more easily through the soil (Edwards *et al.*, 1998). Plants, by taking up pesticides, may remove significant quantities from the soil, thereby reducing the exposure of soil organisms to the pesticide residues. The potential for assessing the overall impacts of pesticides on soil systems using integrated soil microcosms (ISM) and terrestrial model ecosystems (TME) was described by Edwards *et al.* (1998) and the effects of carbendazim on structural and process parameters in soil microcosms, in the

same experiments described here, were reported by Burrows and Edwards (2000). The objective of this experiment was to investigate the fate and uptake of carbendazim in soil, leachates, plants and earthworms in an integrated soil microcosm, and to compare results with those from terrestrial model ecosystems (TME) and field sites with the same soil type to assess their value as predictive methodologies in risk assessment.

METHODS

Integrated soil microcosm

General. Field soil (silty clay loam) was sieved through a 5 mm screen, and mixed thoroughly. Six 30 kg batches of soil were weighed for five application rates and one control. Application rates were based upon the predicted environmental concentration (PEC) of 0.76 mg carbendazim/kg soil dry weight (based upon the recommended application rate, soil penetration to 5 cm, and a soil density of 0.95 g (dry weight)/cm^3). The lowest treatment (T1) was equal to the PEC, T2 was 3xPEC, T3 was 9xPEC, T4 was 27xPEC, and T5 was 81xPEC. Each batch of soil was sprayed with 400 ml of the appropriate concentration of 'Derosal$^®$' solution in deionized water. The soil was sprayed and mixed thoroughly and packed gently (700 g soil/microcosm) into high-density polyethylene (HDPE) cylinders, 7.5 cm id. Fine nylon mesh was placed across the bottom of each microcosm. Greenhouse temperatures were recorded every two hours with a 12 h-12 h light-dark cycle. Artificial rainwater was used to water microcosms daily, (25 ml/microcosm). Each microcosm contained 10 wheat seeds, later thinned to 5 seedlings, and three adult earthworms (*Lumbricus rubellus*) were added. Five replicate microcosms of each application rate were sampled destructively 7, 14, 28, and 56 days after treatment. Soil, plant, earthworm, and leachate samples were stored frozen (≤-15°C) until extracted and analyzed.

Soil residues. Soil samples, 10 g wet weight, were extracted with methanol (1:1 weight:volume) by shaking for 15 hours 0, 7, 14, 28, and 56 days after treatment. Samples were centrifuged to pellet the soil, the supernatant extracts filtered (0.45 µm), and injected into an hplc. Isocratic conditions (0.8 ml/min flow rate) were used with a mobile phase mixture of 65:35 methanol: 0.1% KH_2PO_4(aq). The injection volume was 50.0 µl on a Spherisorb, ODS-2, 4.6 x 250 mm, 0.5 µm column with u.v. detection at $\lambda = 284$ nm. Retention time for carbendazim ranged from 6-9 minutes. Extraction recoveries of fortified samples ranged from 71% to 84%, averaging 77%. The limit of quantitation for soil residue extraction was 0.50 mg/kg.

Carbendazim leaching was assessed 7, 14, 28, and 56 days after treatment. Leachate samples were filtered (0.45 µm) to remove particulate matter and injected directly into an hplc system using the same conditions as for the soil residue analyses.

Earthworm residues of carbendazim were analyzed 7, 14, and 28 days after treatment (Bernal *et al.*, 1997). Samples were acidified with 3 ml of 0.05 N HCl and homogenized thoroughly with a Polytron homogenizer, then extracted three times with 10 ml of ethyl acetate by shaking for 15 minutes, centrifuging, and pipeting off the organic supernatant. Samples were neutralized with 3 ml of 0.1 N NaOH and mixed thoroughly and extracted once again with 10 ml of ethyl acetate. All four organic layers were combined, concentrated to dryness using a TurboVap (50°C water bath), and reconstituted with 2 ml of an 80:20 ethyl

acetate:hexane mixture. The extracts were cleaned up by solid phase extraction with an aminopropyl cartridge (500 mg) and eluted with 3 ml of a 95:5 ethyl acetate:methanol mixture. The eluates were concentrated to dryness and reconstituted in 1 ml of mobile phase solvent. Samples were injected into a hplc system using the same conditions as for the soil residue analyses except the mobile phase was a 60:40 mixture of methanol: 0.1% $KH_2PO_{4(aq)}$. Extraction recoveries of fortified samples ranged from 52% to 153%, averaging 102%. The limit of quantitation for earthworm residue extraction was 0.35 mg/kg (wet weight).

Plant tissue residues of carbendazim were analyzed 7, 14, 28 and 56 days after treatment (Fernández-Alba *et al.*, 2000). Replicate samples in individual microcosms were combined due to the small size of plants. Plant samples were prepared by homogenizing with dry ice in a blender. Carbendazim was extracted into 60 ml of ethyl acetate by homogenizing with 10-15 g of anhydrous sodium sulfate using a homogenizer for 30-45 seconds. The organic layer was vacuum-filtered through a glass fibre filter covered by anhydrous sodium sulfate (8-10 g) and the sample extracted a second time with 25 ml of ethyl acetate. The final combined extract was concentrated to <3 ml using a Savant evaporator, then transferred quantitatively to a centrifuge tube with methanol and concentrated to almost dryness in a TurboVap (60°C water bath). Samples were air-dried and reconstituted with 2 ml of a 50:50 mixture of acetonitrile and water. Extracts were sonicated, vortexed, filtered (0.45 μm) and injected into an LC/MS-MS system. These analyses used 10 μl injection volumes on a Zorbax Rx-C8 column (150 x 4.6 mm, 5 μm) heated to 30°C. Mobile phases were 50 mM ammonium formate in a 95:5 mixture of deionized water and acetonitrile at pH 4 (Mobile Phase A), and acetonitrile (Mobile Phase B). A gradient elution used a flow rate of 1 ml/min over 15 minutes starting with 20% B for 4 minutes, moving to 80% B over 8 minutes and holding for 3 minutes before returning to initial conditions. A 1:1 split was used to divert excess flow into a waste container before entering the mass spectrometer. The retention time for carbendazim was 4.5 minutes. Detection and quantitation were done using a mass spectrometer in electrospray (+) mode with a drying gas flow rate of 10 litres/min at 325°C, capillary voltage of 3 kV, and source temperature of 110°C. Carbendazim was quantified at m/z 192.2 fragmenting to a daughter ion at m/z 160.6. Extraction recoveries of fortified samples ranged from 32% to 39%, averaging 36%. The limit of quantitation for plant residue extraction was 0.15 μg/g.

TME and field validation of soil microcosm results

The TME and field validation experiments, conducted in Flörsheim a.M. Germany, used the same soil type and application rates as those in the microcosm experiment. Intact soil cores were removed from the field and placed in specially-designed carts equipped for leachate collection and located in a climatic chamber maintained at 23°C ± 5°C, 50-80% humidity, with a 16h-8h light-dark cycle. Each TME consisted of a 40 cm deep HDPE cylinder, 17.5 cm id, with a HDPE bottom plate with holes and a thin piece of inert gauze placed between it and the soil core. Leachates were collected using polyvinylchloride tubing connected to a widemouth polyethylene bottle. Appropriate amounts of carbendazim were diluted in demineralized water to produce a total volume of 50 ml for each TME and 3 litres of spray solution for each field plot (~1200 litres/ha). The solutions were applied uniformly drop-wise to each TME using a pipette. The field plots were sprayed using a PL1 plot sprayer equipped with ten flat ray spray nozzles. Immediately after application, each TME was irrigated with 150 ml of artificial rainwater and each field plot sprayed with 20-30 litres of water.

RESULTS AND DISCUSSION

Integrated soil microcosm (ISM)

Carbendazim leachate concentrations ranged from 0.0 ± 0.0 µg/litre to 16 ± 5.3 µg/litre with increasing application rate (Figure 1). Significant differences in leachate carbendazim concentrations ($P < 0.05$) occurred only between samples from treatment levels 1xPEC and 3xPEC, 28 days after treatment. The highest carbendazim concentrations in the leachates corresponded with the highest application rates, but did not exceed 1.1% of the amount of carbendazim applied, indicating that carbendazim has a very low mobility in soil.

Soil carbendazim residues did not change significantly over all application rates for the first 28 days after treatment with two exceptions. Soil treated at application rates of 3xPEC and 9xPEC had significantly lower carbendazim residues ($P < 0.05$) 14 days after treatment. Between 28 and 56 days, the carbendazim residues in soil decreased significantly for all application rates, (Figure 1), possibly because microbial and fungal communities adapted to carbendazim as a new energy source.

Carbendazim plant residues increased with the amount applied but decreased over time (Figure 1). Concentrations were highest after 14 days and lowest 56 days after treatment, with the exception of samples treated with 81xPEC, which had higher carbendazim residues 7 days after treatment. The reductions in carbendazim concentrations were probably due to plant growth and represent a dilution of carbendazim rather than a loss from plant tissues.
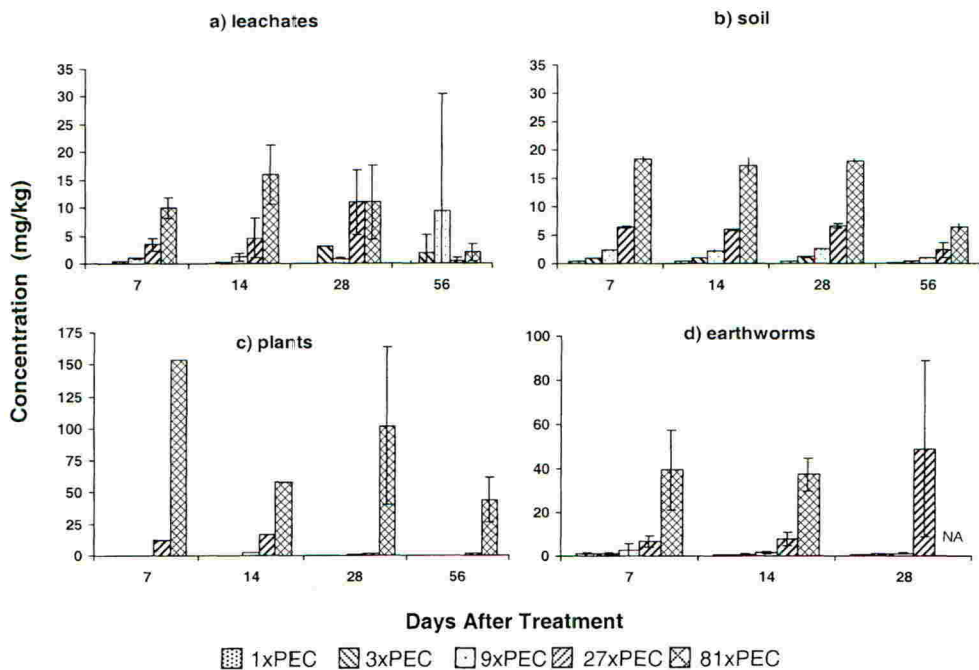

Figure 1: Carbendazim residue concentrations (mean $\pm$ SD) in: (a) leachates (µg/litre), (b) soil (mg/kg), (c) plants (mg/kg), and (d) earthworms (mg/kg) from a soil microcosm. PEC = predicted environmental concentration = 0.76 mg/kg. NA = 81xPEC data not available.

Earthworm residues increased with higher application rates but did not change significantly over 28 days (P < 0.05, Figure 1), indicating that carbendazim was absorbed rapidly by the earthworms and reached a steady state within seven days. The increase in residues at the 27xPEC application rate after 14 days was not statistically significant and probably due to small sample size. There were no significant correlations between carbendazim residue concentrations in the earthworms and soil moistures. Soil to earthworm bioaccumulation ratios ranged from 0.5 to 7.3 and averaged 1.6, 0.9, 0.8, 3.2, and 2.2 for the application rates 1xPEC to 81xPEC, respectively. The consistent differences in ratios at the higher carbendazim application rates indicate that carbendazim is accumulated by earthworms only when soil concentrations reach high levels, probably due either to the inability of the soil to retain and adsorb large amounts of carbendazim or for the earthworms to excrete carbendazim (Figure 1).

Comparison of soil microcosm, TME and field validation results

Pesticide residues in soil and plants were analyzed in microcosms, TME and field experiments. Plant tissues from the microcosms were analyzed for carbendazim using a different analytical method than for the TME and field plant tissue samples (Jones *et al.*, 2002). Soil samples were analyzed using similar methods for the three experiments. Soil carbendazim concentrations were consistently higher in the microcosms than in the TME and field experiments possibly due to erroneous assumptions in calculation of application rates for the microcosms. Soil carbendazim residues decreased between 4 and 8 weeks after treatment in the microcosms and between 8 and 16 weeks after treatment in the TME and field experiments (Figure 2). Plant tissues from the TME and field experiments contained much higher concentrations of carbendazim than those from the microcosms (Table 1).
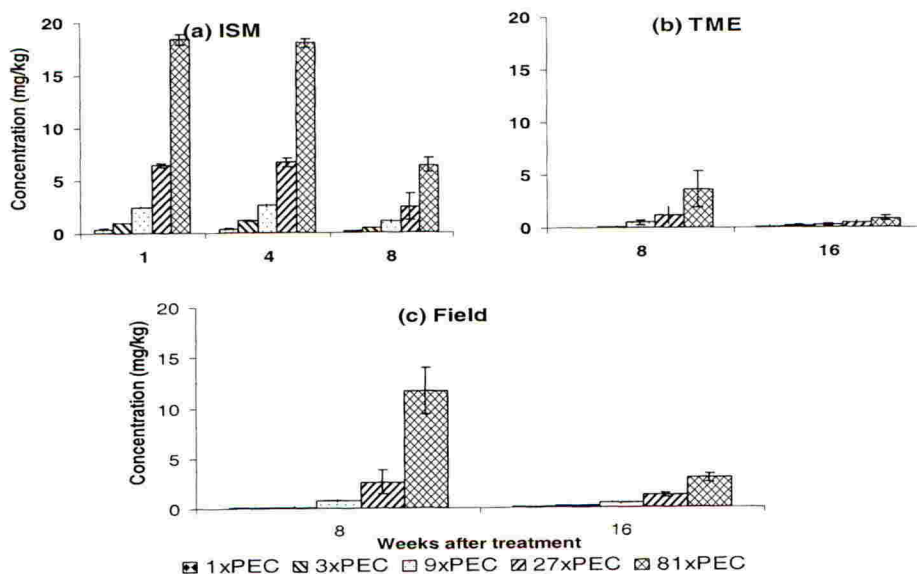

Figure 2: Carbendazim residues in soil (mean ± SD) for ISM, TME and Field systems (mg/kg) at 1, 4, 8, and 16 weeks after application. PEC = predicted environmental concentration = 0.76 mg/kg for ISM and 0.36 mg/kg for TME and Field.

Table 1: Carbendazim residues in plant tissue for ISM, TME and field systems (mg/kg) at 1, 2, 4, 8, and 16 weeks after application. (NA = Not Applicable). PEC = predicted environmental concentration = 0.76 mg/kg for ISM and 0.36 mg/kg for TME and Field. BLOQ = Below Limit of Quantitation.

System	Weeks after Treatment	1xPEC	3xPEC	9xPEC	27xPEC	81xPEC
ISM	1	BLOQ	BLOQ	BLOQ	12.3	154
	2	BLOQ	BLOQ	2.54	16.6	58.0
	4	BLOQ	BLOQ	1.04	1.93	102
	8	BLOQ	BLOQ	BLOQ	1.60	44.2
TME	8	BLOQ	0.31	1.3	9.9	14
	16	BLOQ	BLOQ	BLOQ	2.7	1.9
Field	8	0.26	1.3	7.8	13	74
	16	BLOQ	BLOQ	BLOQ	BLOQ	3.0

Both integrated soil microcosms and terrestrial model ecosystems can provide a cost-effective alternative to field experiments in providing data for risk assessment. A tiered approach to testing using single species or process tests as Tier I, integrated soil microcosms as Tier II and terrestrial model ecosystems or field experiments as Tier III would be a practical approach to environmental impact assessments (Edwards *et al.*, 1998).

ACKNOWLEDGEMENTS

REFERENCES

Bernal J; Nozal M; Toribio L; Jimenez J; Atienza J (1997). High-performance liquid chromatographic determination of benomyl and carbendazim residues in apiarian samples. *Journal of Chromatography* **787**, 129-136.

Burrows L A; Edwards C A (2000). The effects of the fungicide carbendazim in an innovative integrated terrestrial microcosm system. *Proceedings of the BCPC Conference – Pests & Diseases* **1**, 365-370.

Edwards C A; Knacker T; Pokarzhevskii A (1998). The prediction of the fate and effects of pesticides in the environment using tiered laboratory terrestrial model ecosystems. *Proceedings of the BCPC Conference – Pests & Diseases* **1**, 267-271.

Fernández-Alba A R; Tejedor A; Agüera A (2000). Determination of imidacloprid and benzimidazole residues in fruits and vegetables by liquid chromatography-mass spectroscopy after ethyl acetate multiresidue extraction. *Journal of AOAC International* **83**, 748-755.

Jones S E; Williams D J; Holliman P J; Taylor N; Baumann J; Forster B; Van Gestel C A M; Rodrigues J M L (2002). Ring-Testing and Field Validation of a Terrestrial Model Ecosystem (TME) – An Instrument for Testing Potentially Harmful Substances: Fate of the model chemical carbendazim. *Ecotoxicology*, (in press).

Pierzynski G M; Sims J T; Vance G F (1994). *Soils and Environmental Quality*. CRC Press Inc.: Boca Raton, FL, USA.

Influence of organic amendments on soil sorption of the fungicides metalaxyl and tricyclazole

L Cox, M C Fernandes, M C Hermosín, J Cornejo
Instituto de Recursos Naturales y Agrobiología, CSIC, PO Box 1052, 41080-Seville, Spain
Email: lcox@irnase.csic.es

ABSTRACT

The influence of two commercial humic amendments of agricultural origin (LF and SF) on metalaxyl and tricyclazole soil sorption was assessed. Two soils of different physicochemical properties were treated with 10% w/w organic amendment and sorption studies performed by batch equilibration procedure. Sorption of both fungicides greatly increased with the solid amendment SF, whereas sorption of both fungicides remained unaffected or slightly increased with the liquid amendment LF, which has been attributed to interactions between dissolved organic matter molecules of LF and soil surfaces, giving rise to competition with fungicide molecules for sorption sites.

INTRODUCTION

Pesticide contamination of surface and groundwaters is a present concern encouraging research to understand the fate and redistribution of these chemicals in soil and water. Because sorption processes directly or indirectly determine the amount of pesticide in solution, adsorption and desorption of the pesticide control degradation and movement in soil to a great extent.

Soils of low organic carbon content have a low capacity for retarding pesticide mobility (Guo et al., 1993), since soil organic matter is the primary adsorbent for pesticides and sorption is one of the main processes reducing the mobility of these chemicals in the soil (Chiou, 1989). Thus, organic amendments can modify surfaces of soils promoting adsorption and reducing pesticide contamination of ground water (Barriuso et al., 1995; Cox et al., 1997).

The aim of this study was to assess the influence of two commercial humic amendments, liquid and solid Fertiormont (Fertilizantes Montaño, Antequera, Spain) on soil sorption of the fungicides metalaxyl and tricyclazole.

MATERIALS AND METHODS

Fungicides

Metalaxyl (methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate) (97.7% purity) was supplied by Novartis International AG (Basel, Switzerland). Tricyclazole (5-methyl-1,2,4-triazolo[3,4-*b*][1,3]benzothiazole) (95.5 % purity) was supplied by Eli Lilly (Indianapolis, USA). Structural formulae and physicochemical properties are given below.

Metalaxyl
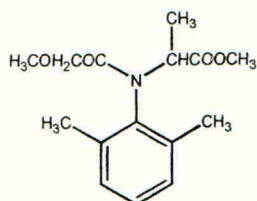
Tricyclazole

M_r= 279.3 g/mol
v.p.= 0.293 mPa (30 °C)
water solubility = 7.1 g/l (20 °C)
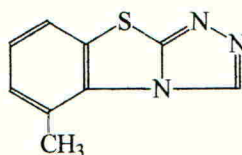
M_r= 189.2 g/mol
v.p.= 0.027 mPa (25 °C)
water solubility= 1.6 g/l (25 °C)

Soil and Amendments

Two soil samples (P2 and A) were collected from the 0-10 cm upper layer of the horizon, air-dried, sieved to pass a 2 mm mesh and stored at room temperature. Soil samples were amended (10% w/w) with two organic amendments, one liquid (LF) and one solid (SF), thoroughly mixed and air-dried. Some physicochemical properties of the organic amendments are given in Table 1, and physicochemical properties of the soils (original and amended) in Table 2. Dissolved organic carbon (DOC) of the organic amendments and soils was determined after extraction (soils and SF) or dilution (LF) with a 1 N solution of $CaCl_2$ (1:2 w/v) and clay mineralogy (I= illite, M= montmorillonite, K= Kaolinite) calculated by X-ray diffraction on oriented specimen.

Table 1. Chemical properties of the organic amendments

Parameter	LF	SF	Units
pH (1/5)	5.09	9.40	
Dry matter	312.0	--	%
Organic C	14.9	18.3	%
Dissolved organic C	68000	2000	mg/litre

Table 2. Physicochemical properties of original soils (P2 and A) and amended soils

Soil	pH	OM %	DOC mg/litre	Fe$_2$O$_3$ %	Silt %	Sand %	Clay (I, M, K)[*] %	CaCO$_3$ %
P2	7.1	0.99	20.1	4.3	8.9	70.7	16 (59, 20, 20)	0.6
P2+SF	7.7	1.82	200				--	-
P2+LF	6.5	3.34	2600		-		--	-
A	7.9	1.31	28.2	2.6	23.0	54.0	23 (40, 10, 50)	7.6
A+SF	7.8	2.41	100	-	-		--	-
A+LF	7.2	1.72	300	-	-		--	-

[*]I=illite, M= montmorillonite, K= kaolinite

Sorption studies

Sorption isotherms were measured using a batch equilibration method. Duplicates of 5 g (metalaxyl) or 2 g (tricyclazole) of each soil were treated with 10 ml of fungicide solutions with concentration (C_i) ranging from 5 to 100 µM made up in 0.01 M $CaCl_2$. The suspensions were shaken at 20 ± 2°C for 24 h and centrifuged at 12 000 rpm at the same temperature. Supernatants were filtered and equilibrium concentrations (C_e) determined by hplc with a photodiode array detector. The following conditions were used: Nova-Pack C18 column, 150x3.9 mm; flow rate, 1 ml/min.; eluent system, 50:50 methanol/ water (metalaxyl) and 20:80 acetonitrile/water (tricyclazole); detection wavelength, 230 nm; injection volume, 25 µl.

Sorption isotherms were obtained by plotting the amount of fungicide sorbed (C_s= µmol/kg) vs the equilibrium concentration (C_e = µM) and fitted to the Freundlich equation (1).

$$C_s = K_f.C_e^{n_f} \qquad (1)$$

Sorption constants K_f and n_f, which indicate adsorption capacity evaluated at C_e=1 µM and intensity, respectively, were calculated. The amounts of metalaxyl or tricyclazole sorbed at 20 µM equilibrium concentration, which falls between the C_e range used, were calculated as a distribution coefficient K_d (2).

$$K_{d20} = C_s/C_e \qquad (2)$$

RESULTS AND DISCUSSION

Metalaxyl and tricyclazole sorption isotherms are given in Figure 1, and sorption coefficients after fitting isotherms to Freundlich equation in Tables 3 and 4, respectively. There are no significant differences in metalaxyl sorption coefficients (K_f and K_{d20}) between original soils P2 and A (Table 3), despite the higher organic matter (OM) content of soil A when compared with P2. This can be attributed to the contribution of mineral surfaces in metalaxyl sorption (Andrades et al., 2001) or to different nature of soil OM rendering different sorption capacity.

When OM of P2 and A is increased with the amendments, sorption significantly increases in P2+SF and A+SF, whereas no significant increase was observed with the liquid amendment LF in P2 or A soils. Previous studies have indicated that the dissolved OM of LF contains great amounts of relatively non-humified material with high affinity for montmorillonite (Cox et al., 2000), which represents 20% and 10%, respectively, of the clay fraction of P2 and A soils (Table 1). This fact suggests competition between dissolved organic carbon (DOC) from LF and metalaxyl molecules for sorption sites, since previous studies have indicated that metalaxyl sorbs on montmorillite (Andrades et al., 2001). Although DOC sorbs to a higher extent on A soil, as indicated by the lower DOC of A+LF soil extract when compared with P2+LF (Table 1), it should be noticed (Table 3) that sorption of metalaxyl is slightly higher in A+LF than in P2+LF. This suggests that the addition of LF to soil also generates available sorption surfaces which compensate the competition of DOC from LF for fungicide sorption sites. In contrast, the SF amendment contains high amounts of highly humified material, which has been shown to sorb other polar pesticides (Cox et al., 2000, 2001) and greatly increases sorption of metalaxyl.
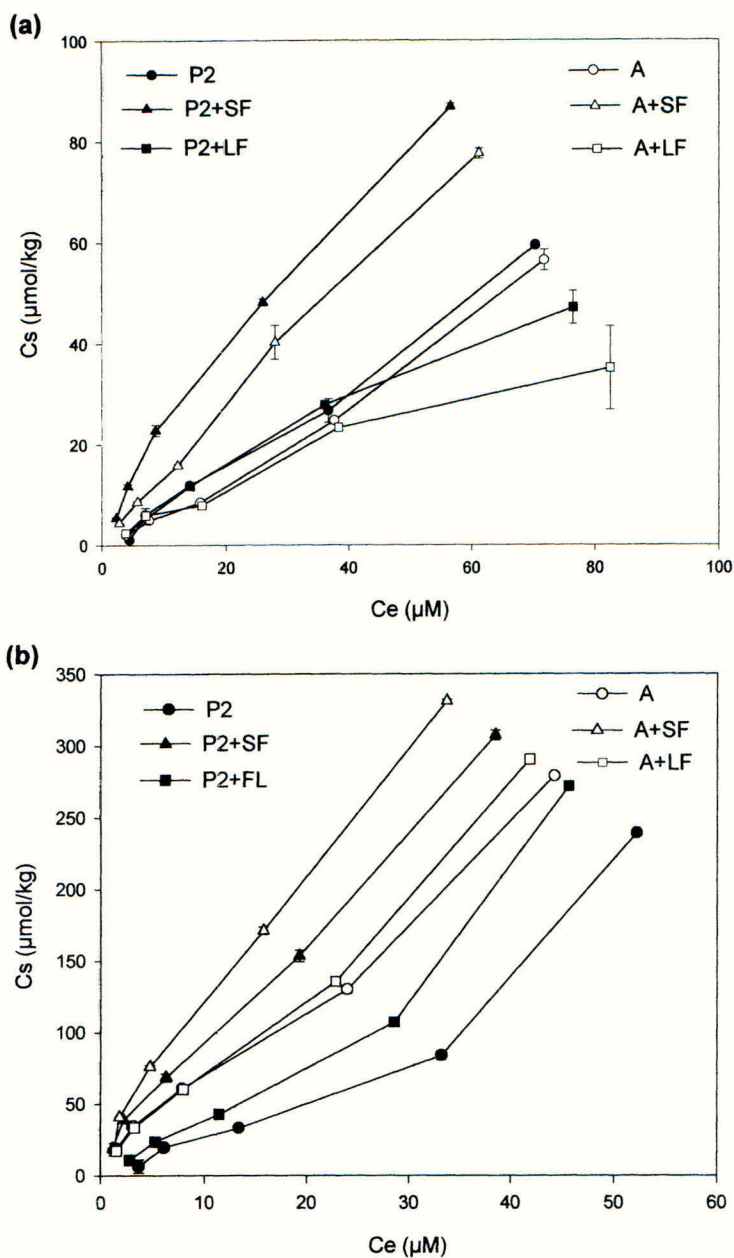
Figure 1. Metalaxyl (a) and tricyclazole (b) sorption isotherms in original
P2 and A soils and soils amended with FS or LF.

Table 3. Sorption coefficients for metalaxyl on original soils (P2 and A)
and amended soils

Sample	Kf_a	Nf_a	r^2	Kd_{20}
P2	0.405 (0.65-0.25)	1.152 ± 0.16	0.944	0.64
P2+SF	1.605 (1.86-1.38)	0.968 ± 0.05	0.990	1.46
P2+LF	0.302 (0.42-0.18)	1.161 ± 0.07	0.988	0.49
A	0.429 (0.51-0.36)	1.132 ± 0.06	0.993	0.64
A+SF	1.652 (2.51-0.80)	0.933 ± 0.02	0.998	1.35
A+LF	0.648 (0.87-0.49)	0.997 ± 0.09	0.974	0.64

Tricyclazole sorbs on soils to a much higher extent than metalaxyl, due to its lower water solubility (Kanazawa, 1989). Sorption on soil A is much higher than on soil P2 (Table 4), due to the higher OM content of A, which is the most important soil component affecting sorption of nonpolar pesticides (Chiou, 1989). The much lower sorption on P2 can be also due to its high content in iron oxides (Table 1), which can be tightly bound to soil organic matter reducing sorption capacity of this soil component (Celis *et al.*, 1996).

When soils were amended, sorption of tricyclazole increased in both soils with SF amendment, specially in the case of P2+SF (Table 4). With LF amendment, sorption slightly increased in P2+LF when compared with original soil P2, whereas no significant differences were observed between A and A+LF. This can be attributed to the higher OM content of P2+LF soil, but also to the high amount of the highly polar DOC molecules in solution, which would favour sorption of the low water soluble tricyclazole molecules on soil surfaces. Again competitive sorption between DOC molecules from LF and fungicide molecules can explain the differences between SF and LF amendments.

Table 4. Sorption coefficients for tricyclazole on soils P2 and A unamended
and amended with FS and FL

Soil	K_f	N_f	r^2	Kd_{20}
P2	1.584 (2.313 – 1.085)	1.213 ± 0.134	0.964	3.00
P2+SF	18.281 (20.233 – 16.517)	0.752 ± 0.044	0.990	8.70
P2+LF	3.511 (4.430 – 2.782)	1.082 ± 0.087	0.981	4.49
Al	13.274 (14.888 – 11.834)	0.767 ± 0.047	0.989	6.60
AL+SF	19.498 (23.228 – 16.368)	0.81 ± 0.082	0.970	11.04
AL+LF	12.050 (13.474 – 10.777)	0.816 ± 0.0459	0.991	6.94

CONCLUSIONS

The addition of organic wastes to soil can modify its sorption capacity for pesticides such as the fungicides metalaxyl and tricyclazole, and this is highly dependent on the nature of the organic amendment and on soil composition. In some cases sorption is increased but in other cases, specially when the amount of dissolved organic carbon introduced is high, sorption can be decreased due to competition with pesticide molecules for sorption sites. The effect of this on leaching is that organic amendments do not always reduce leaching potential of fungicides.

ACKNOWLEDGEMENTS

REFERENCES

Andrades M S; Sánchez Martín M J; Sánchez-Camazano M. (2001). Significance of soil properties in the adsorption and mobility of the fungicide metalaxyl in vineyards soils. *Journal of Agriculture and Food Chemistry.* **49**: 2363-2369.

Barriuso E; Calvet R; Houot S (1995). Study of the effect of sewage sludge application on atrazine behaviour in soil. *International Journal of Environmental Analytical Chemistry* **59**, 107-121.

Celis R; Cox L; Hermosín M C; Cornejo J (1996). Retention of metamitron by model and natural particulate matter. *International Journal of Environmental Analytical Chemistry* **65**, 245-260.

Chiou C T (1989). Theoretical considerations of the partition uptake of nonionic organic compounds by soil organic matter. In: *Reactions and Movement of Organic Chemicals in Soils.* Soil Science Society of America Special Publication N 22, pp. 1-29.

Cox L; Celis R; Hermosín M C; Becker A; Cornejo J (1997). Porosity and herbicide leaching in soils amended with olive-mill waste water. *Agriculture, Ecosystems & Environment* **65**: 151-161.

Cox L; Celis R; Hermosín M C; Cornejo J; Zsolnay A; Keller K.(2000). Effect of organic amendments on herbicide sorption as related to the nature of the dissolved organic matter. *Environmental Science and Technology* **34**, 4600-4605.

Cox L; Cecchi A; Celis R; Hermosín M C; Koskinen W C; Cornejo J (2001). Effect of exogenous carbon on movement of simazine and 2,4-D in soils. *Soil Science Society of America Journal* **65**: 1688-1695.

Guo L; Bicki J J; Felsot A S; Hinesly T D (1993). Sorption and movement of alachlor in soil modified by carbon-rich wastes. *Journal of Environmental Quality* **22**:186-194.

Kanazawa J (1989). Relationship between the soil sorption constants for pesticide adsorption and their physicochemical properties. *Environmental Toxicology and Chemistry* **8**: 477-484.

Effects of azoxystrobin on soil microorganisms under laboratory conditions

V A Kalinin, K V Bykov
Moscow Timiryazev Agricultural Academy, Moscow, 127550, Russia

A G Osman
Environment and Natural Resources Research Institute (ENRRI), The National Centre for Research (NCR), Khartoum, Sudan

ABSTRACT

Laboratory experiments were carried out to study the effect of azoxystrobin on growth of some beneficial soil microorganisms and on three strains of *Phytophthora infestans* in pure cultures. The experimental results revealed no risk to microorganisms in comparison with different strains of *Phytophthora infestans*. These were: *Pseudomonas putida, Klebsiella planticola, Azotobacter chrococcum and Clostridium acetobutilicum.* EC_{50} values for these microorganisms were in 3-5 orders of magnitude higher in comparison with EC_{50} values for different strains of *Phytophthora infestans*. Laboratory studies with soil cultures showed that azoxystrobin strongly inhibited soil fungi and stimulated growth of soil microorganisms, which are able to grow in poor media. Inhibition and stimulation were correlated with the content of azoxystrobin in soil negatively and positively, respectively. The results also indicated that there were no regular and clear effects of azoxystrobin on soil microorganisms, which use organic or mineral forms of nitrogen. Azoxystrobin degraded slowly in soil under laboratory conditions with an initial lag-period in the range of four to eight weeks depending on its concentration and incubation temperatures. This indicates that the azoxystrobin degradation in soil occurs by microbial processes.

INTRODUCTION

During the last two decades a new class of fungicides, "the strobilurins", have been developed. Strobilurins (including azoxystrobin) have a mode of action on plant disease infestants which is entirely different from that of the most well-known fungicides; they block mitochondrial respiration in fungal infestants by blocking electron exchange between cytochrome B and cytochrome C (Clough, Godfrey, 1998). This property of the fungicide azoxystrobin gives rise to an extremely wide spectrum of its action upon disease agents. Azoxystrobin reveals both contact and less expressed systemic effects combined with long-lasting protective action. It might seem that azoxystrobin having a wide spectrum of fungicidal activity and high biological activity should cause essential changes in soil microbial association. This idea of a great theoretical and practical interest has, however, not been studied yet.

In the relevant literature, there is no clear information about concentrations of azoxystrobin exhibiting toxic action upon soil microorganisms and their individual physiological groups. There is no information about microrganisms taking part in decomposition of azoxystrobin in soil. These present investigations sought to address these points.

MATERIALS AND METHODS

Azotobacter chroococcum (strain 265), *Pseudomonas putida*, (strain 91-96), *Klebsiella planticola* (strain TCXA-91 Rif^{200}), *Clostridium acetobutylicum* (strain 18), and three strains of *Phytophthora infestans* (ОДП-12.3; 3ВК-2.6 and 3ВПТ-15.2) received from the microbiological collection of the Department of Microbiology and the Department of Botany of Moscow Timiryazev Agricultural Academy were used in the present pure culture experiments. Aerobic and facultative aerobic bacteria were cultivated in corresponding standard culture medias; anaerobic *Clostridium acetobutylicum* was cultivated in a liquid medium according to the method of limiting dilutions. The three strains of *Phytophthora infestans* were cultivated according to the method of Shattock (Shattock, 1988). Azoxystrobin in varied concentrations prepared by the method of sequential dilution of 'Amistar' formulation (Syngenta Agro AG) was applied to the culture media for growing the microorganisms studied.

Dose-effect relationship was evaluated by the method of "probit-analysis". The rate of increase of the effect was determined by the slope of the curves. EC_{50} (effective concentration required to inhibit 50% of growth of test organisms) index was used to evaluate comparative toxicity of azoxystrobin for different microorganisms and to determine selectivity index of the fungicide with several species. The degree of danger of the fungicide to beneficial microorganisms was estimated by Krouglov's safety coefficient (SC)(Krouglov, 1991) representing a ratio of EC_{50} to the concentration of a pesticide in soil after its application at a recommended rate. Such a concentration of the fungicide studied in our experiments amounted to 1 mg/kg of the soil, or 0.0001% (*m/m*).

The influence of azoxystrobin at rates of 1, 50, 200 and 500 mg/kg of the soil on *Klebsiella planticola* and other microorganisms with incubation at temperatures 18 and 40°C during 150 days was studied in soil culture experiments. *Klebsiella* microbial density was determined in the agarised culture medium of Luria Bertoni (LB) with addition of rifampicinum on the 8, 15, 30, 60, 100 and 150 days of incubation. The density of micromyces was determined in Czapek's medium. The number of autochthon microorganisms was counted in nitrite-agar medium under a microscope at a magnification of 80 times (100 fields of view in the dish). Microorganisms utilising organic forms of nitrogen were studied in meat infusion agar (MIA). Microorganisms utilising mineral forms of nitrogen, including actinomyces, were determined in starch-and-ammonia agar (SAA). Soil samples for determination of azoxystrobin residues by hplc were taken at the same intervals.

RESULTS

Effects of azoxystrobin on pure cultures of soil bacteria and the phytopathogenic fungus *Phytophthora infestans*

The results of studying the influence of azoxystrobin upon growth and development of pure cultures of soil bacteria and *Phytophthora infestans* are represented in Figures 1 and 2.

Figure 1 shows that for *Pseudomonas putida* (strain 91-96), *Azotobacter chroococcum* (strain 265) and *Clostridium acetobutylicum* (strain 14), the graphs of dependence of the biological effect of the fungicide on its dose are very similar as for the angle of inclination and, correspondingly, the rate of rise of the effect. At the same time, the slope of the curve for

Klebsiella planticola (strain TCXA-91Rif^{r200}) is more steep than that of the other curves, suggesting that even a slight increase of the dose of the fungicide can cause a very strong negative effect.
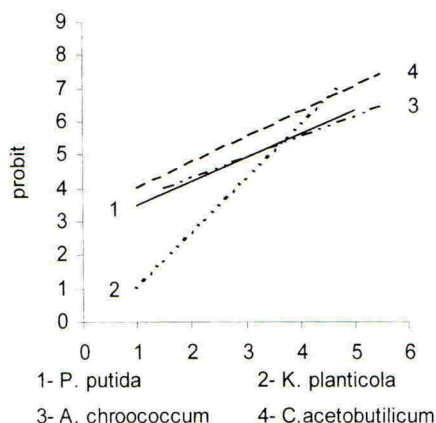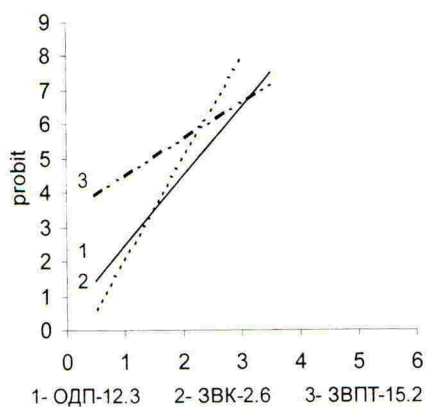

1- ОДП-12.3   2- ЗВК-2.6   3- ЗВПТ-15.2   1- P. putida   2- K. planticola
3- A. chroococcum   4- C.acetobutilicum

Figure 1. Dose-reaction curve for *P. infenstans*. Figure 2. Dose-reaction curve for soil microorganisms.

The concentrations of the fungicide causing 50% destruction of the cells of pure culture microorganisms, EC_{50} values, were calculated on the basis of these graphs (Table 1). According to the values calculated, *Pseudomonas putida* (strain 91-96), *Azotobacter chrococcum* (strain 265) and *Klebsiella planticola* (strain TCXA-91Rif^{r200}) with selectivity indexes (SI) in the range of 13.5 – 20 are a little more resistant to azoxystrobin in comparison with *Clostridium acetobutylicum* (strain 14). At the same time pure cultures of *Pseudomonas putida* (strain 91-96), *Klebsiella planticola* (strain TCXA-91Rif^{r200}), *Azotobacter chroococcum* (strain 265) and *Clostridium acetobutylicum* (strain 14) proved to be more resistant to the fungicide in comparison with various strains of *Phytophthora infestans*. The EC_{50} values for these microorganisms were 3 – 5 orders of magnitude higher in comparison with that for the strain ОДП-12б3. According to the selectivity indexes calculated in base of data of Table 1, it can be concluded that azoxystrobin is characterized by a very strong selective ability.

Table 1. EC_{50} of azoxystrobin for different microorganisms.

Species	EC_{50}
Pseudomonas putida (strain 91-96)	0.03177
Klebsiella planticola штамм (strain TCXA-91Rif^{r200})	0.02965
Azotobacter chroococcum (strain 265)	0.02148
Clostridium acetobutylicum (strain 18)	0.001589
Phytophthora infestans (strain ОДП-12,3)	0.000001782
Phytophthora infestans (strain ЗВК-2,6)	0.0000006761
Phytophthora infestans (strain ЗВПТ-15,2)	0.000000023

Azoxystrobin at concentrations exceeding more than 200 times those estimated following use on-the-farm, only slightly inhibited the growth of the *Pseudomonas putida* (strain91-96*), Klebsiella planticola* (strain TCXA-91Rif^{r200}) and *Azotobacter chroococcum* (strain 265) cultures (SC > 200); growth of *Clostridium acetobutylicum* (strain 14) was depressed by the fungicide in concentrations exceeding 15 times those estimated following on-the-farm use (SC>15). Thus, the present investigations with pure cultures have shown that the fungicide azoxystrobin represents little danger to *Pseudomonas putida*, *Klebsiella planticola*, *Azotobacter chroococcum* and *Clostridium acetobutylicum.*

Evaluation of the toxicity of azoxystrobin to the associative nitrogen-fixing bacteria of the genus *Klebsiella planticola* applied to the soil

On incubation of an unsterilized soil with application of a bacteria *Klebsiella planticola* culture and azoxystrobin, there was an essential inhibition of the density of the bacteria studied in 8 days of incubation at concentrations of azoxystrobin significantly exceeding its estimated concentrations used in on-the-farm-conditions. From the 15th day of incubation, however, the density of the bacteria cells in the treatments with azoxystrobin exceeded their density in the control (nil treatment); the differences between these treatments evened out by the 100th day. On the 150th day of incubation there was a significant decrease of the density of *Klebsiella planticola* cells in all the treatments with various azoxystrobin concentrations, particularly in the treatments with its higher rates. A remarkable decrease of the number of bacteria cells could be observed in the control.

At a temperature of 18°C, a remarkable inhibition of bacterial growth occurred in sterilized soil on the 8th day of incubation, the inhibition being, however, much slighter than that in the treatment with unsterilized soil. Azoxystrobin, at the rate 500 mg/kg, for example, inhibited bacterial cell growth only by 14.4% in comparison with 63.2% in the experiment with unsterilized soil. On the 15th day, the bacterial density practically evened out in the treatments with azoxystrobin and in the control. Then, in the course of the experiment (with the exception of the treatment with 1 mg azoxystrobin per kg of the soil on the 30th day, and the treatments with 1 and 50 mg/kg on the 60th day of the experiment, where a slight inhibiting effect occurred), the density of *Klebsiella planticola* cells in the treatments of the experiment with the fungicide significantly exceeded that in the control. Azoxystrobin in a concentration used in on-the-farm conditions practically showed no inhibiting effect upon bacterial growth of *Klebsiella planticola* at a temperature of 18°C, both in sterilized and unsterilized soils. With increasing concentration in soil, the toxic effect of azoxystrobin on *Klebsiella planticola* became much stronger. With time, however, the toxic effect of the fungicide became weaker and the bacterial density increased substantially, seemingly due to its partial degradation.

The effect of the fungicide azoxystrobin on soil microflora

The results of our experiment have shown that in 8 days of incubation of the soil at a temperature of 18°C, the growth of fungi was inhibited up to 44.0; 63.8; 73.9 and 78.3%, respectively, by azoxystrobin applied at the rates of 1, 50, 200 and 500 mg/kg of the soil (Table 2). Then, the percentage of inhibition increased and reached its highest point on the 30th day of incubation. The toxic effect of the fungicide was seen even at the 150th day of the experiment and averaged 21.2 and 38.6% at the 1 and 50 mg/kg rates, respectively.

Table 2. Percentage of inhibition of the growth of soil fungi by different concentrations of azoxystrobin (18°C)

Concentration of azoxystrobin, mg/kg	Days of incubation					
	8	15	30	60	100	150
	Number of fungi					
0.0 (control)	80.5	188.8	165.5	567.4	264.9	457.0
	Percent of inhibition					
1.0	44.0	61.2	67.1	60.8	44.5	21.2
50	63.8	86.1	82.9	71.0	47.1	38.6
200	73.9	89.1	87.9	81.6	48.9	63.7
500	78.3	91.5	90.0	88.0	70.5	87.1

At concentrations of 200 and 500 mg/kg the percentage of inhibition remained high and amounted to 63.7 and 87.1%, respectively. At a temperature of 40°C, even the lowest rate of the fungicide azoxystrobin (1 mg/kg of the soil) inhibited more than 86% of fungi. This phenomenon can apparently be explained not only by the action of the fungicide but also by a high incubation temperature. The results obtained show that azoxystrobin possesses a high fungicidal activity and, depending on the temperature of the environment, inhibits the growth of microscopic fungi for 100 – 150 days after its application in concentrations close to those used in on-the-farm conditions.

Studies of the effect of increasing rates of azoxystrobin on soil microflora in a nitrate-agar (NA) culture showed, that at a temperature of 18°C on the 8th day of incubation, the fungicide stimulated the growth of microorganisms of this group. The total amount of microorganisms of this group in the treatments with application of the fungicide exceeded that in the control treatment and reached the highest point when the concentration of the fungicide was 500 mg/kg. This phenomenon caused mainly by the bacteria of the genus *Mycobacterium* could be observed during 60 days. At all the dates studied with incubation at a temperature of 40°C, there was a significant increase of the absolute number of the bacteria of the genus *Mycobacterium* and *Nocardia*, particularly when the concentration of the fungicide was 500 mg/kg.

When applied to soil samples, the fungicide stimulates the growth of microorganisms growing in poor culture medium – including the bacteria of the autochthonous group – and mineralizing humus substances. Moreover, the higher the rate of the fungicide, the stronger the increase of the amount of microorganisms that could be observed in treated variants in comparison with that in the control. This is mainly due to the increase of the amount of the bacteria of the genus *Mycobacterium, Nocardia* and *Arthrobacter.* There could not be observed any definite inhibiting or stimulating effect of azoxystrobin on soil microorganisms using organic or mineral forms of nitrogen for nutrition.

Degradation of azoxystrobin in the soil in laboratory conditions

Under laboratory conditions, the rate of degradation of the fungicide depended on its concentration and on the soil temperature. With application to soil in low concentrations, the decomposition percentage of the fungicide during the whole period of the experiment remained significantly higher than that in the treatments with higher concentrations. When

applied at a rate of 1.0 and 500 mg/kg of the soil, 71.3 and 45.6% of azoxystrobin, respectively, decomposed during 150 days at a temperature of 18°C (Table 3). The intensity of decomposition at 40°C was much stronger than that at 18°C. This can be explained by the fact that some groups of soil microorganisms did not grow at high temperatures while the others, mainly thermophilic ones with a powerful enzymatic system, grew and decomposed the fungicide intensively. In addition, high temperature could also accelerate decomposition of azoxystrobin. An intensive microbial decomposition of the fungicide could be observed from the 60th and 30th days of incubation of soil samples at temperatures 18°C and 40°C, respectively. The data obtained in this experiment clearly show the predominance of microbial decomposition of azoxystrobin in soil.

Table 3. Rate of the degradation of azoxystrobin in soil ($t° - 18°C$).

| | Days of incubation | | | | | |
	8	15	30	60	100	150
Concentration of azoxystrobin, mg/kg	Percentage of degradation					
1	4.3	6.3	9.6	67.2	68.1	71.3
50	0.4	2.3	2.4	40.2	45.6	62.8
200	0.2	0.6	1.6	33.2	40.4	58.5
500	0.1	0.4	0.8	27.1	40.0	45.6

Decomposition of azoxystrobin in soil under laboratory conditions occurs at a rather low rate, which appears to be related to the time necessary for adaptation of a number of soil microorganisms to new environmental conditions and for synthesis of enzymes providing them with the tools to utilise this synthetic fungicide. Azoxystrobin decomposed in soil very slowly and remained intact during 150 days of the experiment where the rate of its decomposition depended on its concentration in soil. In addition, it should be stressed that a complete decomposition of the fungicide did not occur even after 5 months. Furthermore, the concentration of azoxystrobin in soil remained relatively high (0.25 mg/kg) even at the treatment rate (1 mg/kg) estimated to be very close to the rate resulting from on-the-farm use.

ACKNOWLEDGEMENTS

REFERENCES

Clough J M; Godfrey C R A (1998). The strobilurin fungicides. In: *Fungucidal activity: chemical and biological approaches,* eds D H Hutson & J Miyamoto, pp109-148. (Wiley series in agrochemicals and plant protection). John Wiley & Sons; Chichester.

Krouglov Y V (1991). Microflora of soil and pesticides, p. 128. *Agropromizdat*, Moscow. (in Russian).

Shattock R C (1988). Studies on the inheritance of resistance to metalaxyl in *Phytophtora infestans. Plant Pathology* **37**, 4-11.

Comparison of soil sorption measurement techniques for a ^{14}C anthranilate fungicide

A Kennedy, R M W Wilkins, E Lopez – Capel

Department of Agricultural & Environmental Science, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK
Email: Angela.Kennedy@ncl.ac.uk

ABSTRACT

Sorption is one of the most important processes influencing pesticide fate in soil. Sorption of a ^{14}C labeled anthranilate fungicide was examined in 5 soils differing in their physical and chemical properties. Measurements were made using both the batch equilibrium method (low solid: solution ratio), and a centrifugation method, allowing direct sampling of soil solution from unsaturated soil (high solid: solution ratio). The batch method suggested more sorption than that observed using the centrifugation method. The extent of discrepancy between the results of the two methods appeared to be related to soil organic carbon content and pH.

INTRODUCTION

Sorption of pesticide molecules by soil is of great importance regarding the distribution of such chemicals in the environment. The retention of pesticide molecules by the solid phase of the soil matrix reduces availability and therefore influences effectiveness; it controls and is controlled by degradation processes and influences the movement of organic substances through the soil profile, potentially to ground water (Koskinen & Harper, 1990). It is important to determine pesticide concentration in the soil solution as it is in this phase that pesticides are mobile within the soil profile (Garcia – Valcerel & Tadeo, 1999). Sorption of pesticides in soil is particularly influenced by soil organic matter and clay content.

Sorption is commonly assessed using the batch equilibrium method (OECD, 1997). Soil is shaken with an aqueous solution of pesticide at a low solid: solution ratio. Following a period of equilibration, usually 24 hours, the concentration in the aqueous phase is measured and sorption calculated. Although frequently used, the batch equilibrium technique has several shortcomings. Conditions do not reflect those found typically in the field, where the period of contact between pesticide and soil is prolonged, degradation may occur and under field conditions higher soil: solution ratios, than used in these types of studies, are usual (Garcia – Valcerel & Tadeo, 1999). Higher concentrations of pesticides than encountered in the field are normally used. Sieved, air dried soils are commonly used in batch equilibrium studies, but in the field aggregates are of varying size. The thorough mixing of soil and aqueous phase by shaking in the batch method may also result in unrepresentative measurement of sorption (Walker, 2000).

Previous studies have indicated discrepancies between results where sorption is measured at low solid: solution ratios using the batch method, and high solid: solution ratios using centrifugation to extract soil solution (Walker & Jurado – Exposito, 1998; Walker, 2000).

This study was undertaken to compare sorption of an anthranilate fungicide in 5 soils using two measurement techniques. The standard batch equilibrium technique was compared to a centrifugation method in which sorption was measured in unsaturated soil at three solid: solution ratios. Sorption was measured after 1 hour and 24 hours contact time between fungicide and soil. Desorption of the fungicide from soil was measured using the batch equilibrium method only. Sorption measurements from each method were also correlated with soil physical and chemical properties.

MATERIALS AND METHODS

Pesticide

An anthranilate fungicide was used in this study, supplied by Bayer CropScience UK Ltd. The fungicide has phenyl and pyridine moieties with ester and amide functional groups. The compound was labeled with ^{14}C on the pyridyl ring with a specific activity of 177 μCi/mg. The pKa of the molecule, measured by titration, was estimated to be 8.

Selection and characterization of soils

Soils from the Rivington, Hallsworth, Brickfield, Wharfe and Dunkeswick series were used in this study. Samples, collected from sites in Northumberland, UK, were taken from the upper 20 cm of the soil profile and sieved to <2 mm. Soil physical and chemical properties are summarised in Table 1. Particle size distribution was determined using the pipette – sedimentation method. Soil pH was measured in a 1:3 soil: water mixture using a glass electrode. Total organic carbon was determined by automated nitrogen carbon analysis, and cation exchange capacity (CEC) using the recommended MAFF method (MAFF, 1986). The maximum water content (MWC) of soils was determined using Haines funnel suction plate apparatus. The solid: solution ratio for the batch method, and the solid: solution ratio equivalent to 50, 60 and 80% MWC used in the centrifugation method are presented in Table 1.

Table 1: Soil physical and chemical properties, and solid: solution ratios for batch method and centrifugation method.

Soil	% OC	CEC meq/100g	% clay	pH	batch	centrifugation 50% MWC	centrifugation 60% MWC	centrifugation 80% MWC
Rivington	3.2	17.0	16.4	6.6	0.2	2.8	2.5	2.0
Hallsworth	2.7	26.4	26.2	6.7	0.2	1.9	1.7	1.2
Brickfield	4.1	33.3	43.0	6.7	0.2	1.0	0.9	0.6
Wharfe	2.7	12.1	3.9	7.3	0.2	2.2	1.9	1.5
Dunkeswick	2.7	25.1	14.1	7.0	0.2	1.9	1.7	1.3

Columns under "Solid: solution ratio under experimental condition:" are batch, centrifugation 50% MWC, centrifugation 60% MWC, centrifugation 80% MWC.

Sorption - batch equilibrium method

The standard batch equilibrium method (OECD, 1997) was used. Soil (0.3 g dry mass) was placed in a centrifuge tube (2.5 ml capacity) with 0.01 M $CaCl_2$ (1.5 ml) thus giving a solid: solution ratio of 0.2. The soil and $CaCl_2$ solution was placed on an orbital shaker at 100 rpm for 12 hours. An aqueous solution of unlabelled (9 – 30 μl) and radiolabelled (30 μl)

fungicide was then added so that 6000 dpm was added to each tube and concentrations of 1.63, 0.81, 0.3, 0.24 and 0.16 µg/g dry soil were obtained. Triplicate samples for each concentration and each soil were prepared. Soil – pesticide mixtures were then returned to the shaker. After 1 hour, samples were removed and centrifuged at 10000 rpm for 3 minutes. A portion (0.5 ml) of the supernatant was transferred to a scintillation vial with 3 ml scintillation cocktail. These were subject to liquid scintillation counting (LSC) for 10 minutes using a Packard Tricarb 2100 TR liquid scintillation analyzer.

This sampling procedure was repeated after 24 hours, after which, in order to assess desorption, the remaining aqueous phase was removed and replaced with 1.5 ml 0.01 M $CaCl_2$. The samples were returned to the shaker for 24 hours then sampled as described. Blank samples without soil were also prepared to check for sorption of the compound onto the tubes. The experiment was undertaken at room temperature (20°C). Previous studies indicated degradation to be negligible over the 48 hour time period used in this experiment (Kennedy & Wilkins, 2000).

Sorption – centrifugation method

Sorption was measured using a slight variation on the method outlined by Walker (2000). Soil (0.3 g dry mass) was placed in an eppendorf tube (0.5 ml capacity), the tip of which had been pierced and packed with a small amount of glass wool. Several hours prior to the addition of pesticide, distilled water was added to the soil to give the solid: solution ratios presented in Table 1. Unlabelled and radiolabelled fungicide were then added as described above and thoroughly incorporated using a metal spatula. Samples were prepared in triplicate for 1 hour and 24 hour sampling times.

After 1 hour, the eppendorf tubes were placed inside centrifuge tubes (2.5 ml capacity), into which a pre-weighed glass microfibre disc had been placed. The resulting unit was centrifuged at 10000 rpm for 10 minutes. Following centrifugation, the glass microfibre disc was retrieved from the centrifuge tube, and re-weighed, thus allowing determination of volume of soil water extracted. Wet discs were placed in 3 ml scintillation cocktail and counted as described previously. This procedure was repeated after 24 hours. Recovery of fungicide from the discs was checked through dipping a pre-weighed disc into an aqueous solution of ^{14}C fungicide of known activity. The disc was then re-weighed, placed in a scintillation vial with 3 ml scintillation cocktail and counted using LSC. The experiment was conducted at room temperature (20°C).

RESULTS & DISCUSSION

Description of isotherms

Sorption isotherms of the fungicide in the 5 soils, measured by the two methods previously outlined, were described using the Freundlich equation (OECD, 1997),
$$C_s = K_f.C_{aq}^{1/n}$$

where C_s is the amount sorbed at equilibrium (µg/g), C_{aq} is the equilibrium concentration in the aqueous phase (µg/ml) and K_f and $1/n$ are constants representing the slope and intercept of the isotherm in the linear form: $\log C_s = \log K_f + {}^{1/n} \log C_{aq}$. Values of K_f, $1/n$ and r^2 for each

of the methods are presented in Table 2. Generally, data fit the Freundlich isotherm well, although r^2 for the data obtained using the centrifugation method was in some cases lower than for that generated using the batch method.

Batch method

Measurements of sorption made at 1 hour and 24 hours using the batch method showed that 39 – 60 % of the sorption measured after 24 hours occurred within the first hour of the experiment. No statistically significant relationship was found between K_f and any of the measured soil properties presented in Table 1. K_f at 1 hour and 24 hours were found to be significantly different (p=0.035). Brickfield and Wharfe soils showed significantly more sorption than the others. In the case of Brickfield this may be attributed to higher % OC. On comparing sorption and desorption isotherms, K_f was found to be significantly greater (p=0.001) for the latter than the former, indicating some degree of hysterisis between the two processes. This discrepancy was significantly greater in Brickfield than the other soils used in the study.

Centrifugation method

Comparing sorption measured at 1 hour and 24 hours showed 80 – 100% of sorption measured after 24 hours to have occurred within 1 hour. K_f did not differ significantly between the two sampling intervals. The more rapid sorption in the centrifugation method compared to the batch method may be due to the concentration of the compound closer to sorptive surfaces in the unsaturated soils. A significant relationship was found between K_f and soil clay content, % OC and CEC. (p=0.006, p=0.001 and p=0.017, respectively). These soil properties are commonly related to sorption. Sorption was not found to vary significantly between the three soil: solution ratios used (p = 0.522), neither was any significant difference observed between the 5 soils (p = 0.929).

Table 2: Freundlich constants and correlation coefficients (r^2) for sorption in 5 soils measured using a batch and centrifugation method

Soil	Batch			Cent. (50% MWC)			Cent. (60% MWC)			Cent. (80% MWC)		
	K_f	1/n	r^2	K_f	1/n	r^2	K_f	1/n	r^2	K_f	1/n	r^2
Rivington	7.9	0.89	0.998	4.4	0.89	0.993	6.6	0.95	0.996	4.5	0.83	0.988
Hallsworth	5.6	0.88	0.980	5.7	0.85	0.945	3.3	0.73	0.982	3.3	0.65	0.962
Brickfield	20.2	1.05	0.988	2.2	1.14	0.802	1.2	0.3	0.987	3.6	1.4	0.910
Wharfe	20.1	0.98	0.997	4.7	0.98	0.984	3.6	0.90	0.844	4.7	0.96	0.940
Dunkeswick	10.4	1.03	0.992	1.5	0.66	0.713	3.5	0.92	0.806	2.1	0.72	0.973

Comparison of the measurement techniques

As presented in Table 2, values of K_f were found to be higher for the batch method than for the centrifugation method, indicating more sorption in the former. Although not consistently, values of 1/n were generally lower for the centrifugation method, indicating greater curvature of the sorption isotherm. Shaking in the batch method may increase availability of sorption sites. The increased curvature of isotherms in the centrifugation method may result from the diffusion of molecules to less available sites at high solid: solution ratios (Walker & Jurado – Exposito, 1998). Figure 1 (a) compares the sorption isotherm derived using the batch method

to those obtained using the centrifugation method at the solid: solution ratios described in Table 1 for Wharfe soil.
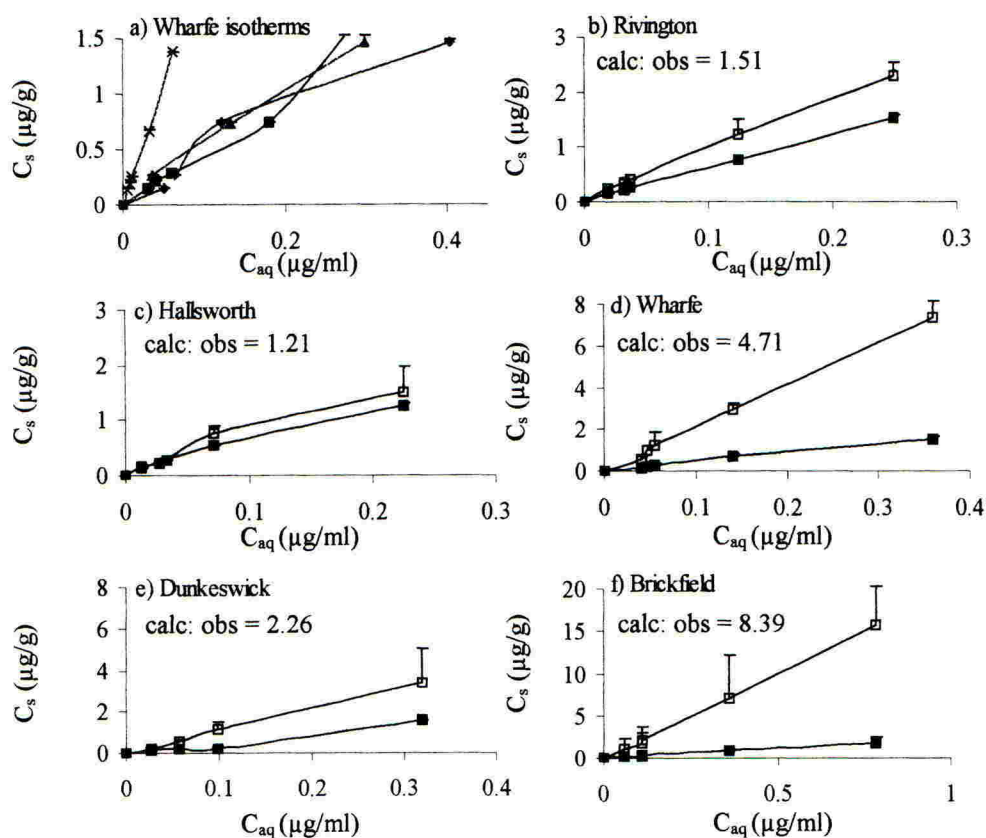

Figure 1, a) sorption isotherms for Wharfe soil at 0.2 (x), 2.2 (■),1.9 (▲) and 1.5(♦) solid: solution ratio. b) – f): $C_{s\ obs}$ (■) and $C_{s\ calc}$ (□) for sorption of a fungicide in Rivington, Hallsworth, Wharfe, Dunkeswick and Brickfield soils. Bars represent the standard deviation of triplicate samples. Where these are not visible they are obscured by the data point marker. Calc: obs represents the ratio of the slope of the calculated isotherm to that of the observed isotherm

Values of K_f and $1/n$ derived from the description of the batch method isotherm using the Freundlich equation were used to calculate the expected values of C_s ($C_{s\ calc}$) from C_{aq} for soils at the three solid: solution ratios used in the centrifugation method. This calculated value was then compared to the observed value ($C_{s\ obs}$). Figure 1, b – f compares the observed and calculated isotherm for each soil at the solid: solution ratio equivalent to 50% MWC. The difference between observed and calculated values of C_s was expressed as a ratio of the slope of the calculated isotherm to the slope of the observed isotherm. This ratio did not differ significantly between the three solid: solution ratios used in the centrifugation method, but was found to be significantly lower ($p = 0.001$) for Hallsworth compared to the other soils, indicating a closer agreement between the observed and calculated isotherms for this soil. When correlated with soil properties, a significant relationship was observed with % OC and

pH (p=0.032, p = 0.001, respectively). Soils with lower organic carbon and lower pH gave a better agreement between the two values, suggesting the batch method to overestimate sorption to a greater extent where sorption is already expected to be comparatively high due to high %OC.

CONCLUSIONS

The results of this study strongly suggesting the batch equilibrium method overestimates sorption when compared to a method which measures sorption under conditions considered to be more representative of the field situation. Further work is necessary to determine the aspects of the methodologies which contribute to these differences. The extent of discrepancy between the two methods appears to be related to soil properties.

ACKNOWLEDGMENTS

REFERENCES

García – Valcárcel A I; Tadeo J L (1999). Influence of soil moisture on sorption and degradation of hexazinone and simazine in soil *Journal of Agricultural and Food Chemistry* **47**, 3895-3900.

Kennedy A; Wilkins R M W (2000). Degradation and adsorption of an anthranilate fungicide in soils from Northumberland, UK *BCPC Conference Proceedings* volume 1, pp. 393-398 British Crop Protection Council

Koskinen W C; Harper S S (1990). The retention process: mechanisms. In: *Pesticides in the Soil Environment: Processes, Impacts and Modeling* ed, H H Cheng, pp. 51-73 SSSA book series 2: Soil Science Society of America, Inc., Madison, Wisconsin, USA.

MAFF (1986). *The Analysis of Agricultural Materials* MAFF Reference Book 427, 3rd ed. London, HMSO.

OECD (1997). OECD guidelines for testing of chemicals: Proposal for updating guideline 106: Adsorption / Desorption using a Batch Equilibrium Method. Revised draft document, October 1997.

Walker A (2000). A simple centrifugation technique for the extraction of soil solution to permit direct measurement of aqueous phase concentrations of pesticide. In: *Pesticide Soil Interactions – some current research methods*, eds J Cornejo & P Jamet, pp. 173-178: INRA.

Walker A; Jurado – Exposito M (1998). Adsorption of isoproturon, diuron and metsulfuron methyl in two soils at high soil: solution ratios *Weed Research* **38**, 229-238.

Fate of the dicarboximide fungicide procymidone in alkaline greenhouse soils from Almeria (Spain) and Albenga (Italy)

E Lopez-Capel, A Kennedy, R M Wilkins
Department of Agricultural and Environmental Science, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK
Email:elisa.lopez-capel@ncl.ac.uk

ABSTRACT

The fungicide procymidone is currently used in greenhouses and open fields in Southern Europe for the control of plant diseases in vegetables, ornamental crops and grapevine. Procymidone has been reported to be persistent in soil and resistant to microbial degradation after repeated application. However, little is known about its fate in alkaline south European soils where it is extensively used and where it may potentially leach and contaminate the highly used ground water system.

The typical soils chosen from a greenhouse in Almeria (Southern Spain) and a greenhouse in Albenga (Italy) are very alkaline and of low organic carbon content. Procymidone is unstable under alkaline aqueous conditions, and hydrolyses at high pHs. The fate of procymidone in soils from the greenhouse in Almeria was studied at various temperatures, under sterilized and non-sterilized conditions and compared to its dissipation in soils from a greenhouse in Albenga. The dissipation of procymidone was also determined at several pHs under aqueous conditions. Procymidone was not persistent in Almeria and Albenga soils, with estimated half-lives of 6 and 5 days, respectively. Due to its high reported sorption and short half-life, procymidone is not expected to leach and contaminate groundwater.

INTRODUCTION

Procymidone, N- (3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide) is a systematic fungicide used for the control of *Botritis, Cinerea, Molinia* and *Sclerotinia* species in fruit growing, strawberry, vine, horticulture and flower and ornamental cultivations.

The importance of procymidone as a fungicidal treatment in preference to other dicarboximide fungicides has increased due to absence of microbial degradation after repeated application (Slade *et al.*, 1992). However, the soils of Almeria (Spain) and Albenga (Italy) greenhouses where it is now extensively used are very alkaline and rapid hydrolysis may affect the degradation and sorption of procymidone (Villadieu et al, 1994).

The fate of procymidone in soils from a greenhouse in Almeria was studied at various temperatures, under sterilized and non-sterilized conditions to determine if soil microbes degraded procymidone. As the hydrolysis of procymidone would be expected in alkaline soils this was studied in aqueous solutions at various pH values. The dissipation of procymidone in Almeria and Albenga greenhouse soils was compared, and the potential leaching of procymidone to groundwater assessed.

MATERIALS AND METHODS

Materials

Stock solutions of analytical grade procymidone (99.9% purity, Qmx Environmental Standards UK) at 100 mg/L were prepared in acetonitrile. The calibration standards were prepared in acetonitrile by serial dilution of the stock solution. Analar grade acetone was supplied by Fisher; and HPLC grade acetonitrile, sodium dihydrogen phosphate dihydrate 99%, di-sodium hydrogen phosphate dihydrate 99%, sodium hydroxide and glycine 97% by Sigma-Aldrich.

The soils used in these experiments were provided by the Universidad de Almeria (Spain) and by the Universita Cattolica del Sacro Cuore, Piazenza (Italy). The Almeria soils used consisted of four soil layers (*sand 0-10 cm, clay 10-20 cm, native 1 20-60 cm and native 2 60-100 cm depth*) from the artificial raised bed of a greenhouse in La Mojonera (Almeria-Spain). The Albenga greenhouse soils consisted of three soil layers (0-30 topsoil, 30-60 middle and 60-90 cm bottom). All soils were air-dried at room temperature and sieved to 2mm. Determined properties of Almeria and Albenga soils are presented in Table 1.

Table 1. Soil Properties of Almeria and Albenga soils

Soil layer	% clay	% H_2O	pH	% OC	Micr. C	CEC	Soil type
Almeria Sand	2.5	3.8	8.5	0.30	166.6	0.87	sand
Almeria Clay	45.6	15.3	8.6	0.35	449.7	5.43	clay
Almeria Native 1	46.5	11.1	8.4	0.67	397.8	7.90	clay
Almeria Native 2	38.8	7.8	8.8	0.66	220.2	4.47	clay loam
Albenga Topsoil	8.0	21.7	7.9	1.30	93.8	n.a.	silt loam
Albenga Middle	14.7	25.1	8.3	0.50	n.a.	n.a.	silt loam
Albenga Bottom	15.0	24.9	8.5	0.10	n.a.	n.a.	silt loam

% H_2O (wt/wt) at 70% Moisture Holding Capacity, n.a. = not available
Microbial Carbon in µg C/g dry soil, CEC in meq/100 g soil, soil type (USDA).

Determination of the dissipation rate of procymidone in Almeria and Albenga soils.

Almeria soils were sterilized by autoclaving twice at 1000 psi and 200°C for 30 minutes. Soil sterility was checked prior to treatment. Sterilized and non-sterilized Almeria soils were fortified with procymidone at a spiking rate of 0.23 mg a.i./kg dry soil, and the Albenga soils at 10 mg a.i./kg dry soil. Soil moisture was raised to 70% MHC (Moisture Holding Capacity) with de-ionized water. The soils were spiked, thoroughly mixed and covered for 1h to equilibrate.

Triplicates of fortified sterilized and non-sterilized Almeria soils (250 g) were transferred into open glass containers and incubated at 20, and 30°C and 70% MHC. Triplicates of fortified Albenga soils (250 g) were transferred into open glass containers and incubated at 20°C and 70% MHC. They were kept at constant temperature in the dark, covered with black plastic

bags and placed in a water bath to keep the soil moist. Soil samples of 12 g were taken at 0, 7, 15, 30, 60, 90 and 120 days and stored in the freezer until analyzed.

Residues of procymidone were extracted from wet soil by shaking samples (12 g) with acetone (25 ml) for 4 h at 250 rpm. The samples were centrifuged at 4500 rpm for 10 min. The solvent supernatants were evaporated, redissolved in acetonitrile to a volume of 2.2 ml, and filtered into a hplc glass vial for analysis. Samples were analyzed by Gilson HPLC-DAD at 212 nm using a reverse phase Aqua column 15 x 4.6 mm, a mobile phase of 65:35 v/v acetonitrile:water (0.4M phosphoric acid) and a flow rate of 1ml/min. Procymidone was detected at 4.3 minutes with a limit of detection of 0.01 ppm.

Determination of the dissipation rates of procymidone in aqueous solution at various pHs

Aqueous buffer solutions (100 ml) of pH 6, 7, and 8 were prepared using di-sodium hydrogen phosphate dihydrate (0.2M) and sodium dihydrogen phosphate dihydrate (0.2M) solutions. Aqueous buffer solutions of pH 9 and 10 were prepared using glycine (0.2M) and sodium hydroxide (0.2M) solutions (Dawson *et al.*, 1986). Procymidone was added to the buffer solutions to give a concentration of 3 mg Procymidone/ litre solution.

The aqueous buffer solutions containing procymidone were incubated in the dark at 20°C, and aliquots of 5 ml were taken at 0, 1, 2, 3, 4, 7, 9, 11, 23, and 50 days after treatment. The aliquots were passed through solid phase extraction (SFE) cartridges. SFE cartridges were air-dried and procymidone residues from the cartridges extracted with methanol (2 ml). Samples were analysed by hplc as previously described.

RESULTS

Dissipation of procymidone in Almeria and Albenga soils

Figure 1 shows the percentage of procymidone remaining in four soil layers from the Almeria greenhouse at 20°C and 70% MHC under non-sterilized (NS) conditions. Figure 2 show the percentage of procymidone remaining in three soil layers from the Albenga greenhouse at 20°C and 70% MHC under non-sterilized (NS) conditions. The percentage of procymidone remaining in Almeria and Albenga soils decreased with time, increased with temperature and varied between soil layers.
The overall loss rate of procymidone is calculated by assuming that the pesticide loss follows pseudo-first order kinetics with a residue of procymidone remaining after the initial loss. The equation being: $y = A*exp(-k*x) + residue$, where y = % procymidone remaining, $-k$ = loss rate constant, x = time in days, and A, residue = constants. DT_{50} (half-life) values, shown in Table 2, are estimated using the equation $k = Ln2/DT_{50}$.

No significant differences (N.S., 95% CI,) were found between the loss of procymidone in non-autoclaved and autoclaved Almeria soils and between the loss of procymidone in Almeria and Albenga soils, using general linear model analysis.

Table 2. Loss rate constants and estimated half-lives of procymidone in Almeria and Albenga soils.

soil layer	conditions	(-k) days^{-1}	DT_{50}	Residue	r^2
Almeria sand	20C NS	0.0622	11.15	44.97	0.9557
Almeria sand	20C S	0.0394	17.60	42.05	0.9357
Almeria sand	30C NS	0.1385	5.00	39.25	0.9805
Almeria sand	30C S	0.1640	4.23	53.10	0.9359
Almeria clay	20C NS	0.1226	5.65	27.74	0.9704
Almeria clay	20C S	0.1081	6.41	32.56	0.9804
Almeria clay	30C NS	0.1975	3.51	33.78	0.9802
Almeria clay	30C S	0.2024	3.42	32.38	0.9452
Almeria native 1	20C NS	0.1574	4.40	42.21	0.9831
Almeria native 1	20C S	0.0579	11.97	35.57	0.9640
Almeria native 1	30C NS	0.2796	2.48	31.10	0.9602
Almeria native 1	30C S	0.1316	5.27	24.97	0.9858
Almeria native 2	20C NS	0.1763	3.93	32.72	0.9888
Almeria native 2	20C S	0.0766	9.05	24.51	0.9922
Almeria native 2	30C NS	0.2969	2.33	27.78	0.9632
Almeria native 2	30C S	0.2006	3.46	32.90	0.9548
Albenga top	20C NS	0.1586	4.37	30.35	0.9676
Albenga middle	20C NS	0.1279	5.42	30.20	0.9553
Albenga bottom	20C NS	0.1258	5.51	40.57	0.9669

-k= loss rate in days^{-1}, DT_{50}= estimated procymidone half-life in soil in days by Pseudo First Order Kinetics. Residue = % procymidone remaining in soil over time, r^2= correlation coefficient.
NS= Non-sterilised, S= Sterilised

Hydrolysis of procymidone in aqueous solution at various pHs

Procymidone loss increased with pH following pseudo first order kinetics. The rate of procymidone hydrolysis and half-life in aqueous buffers at various pH values are shown in Table 3. The rate of hydrolysis increased with an increase in pH (pH = 0.7206(-k) +7.915) with a correlation coefficient of 0.9933. The % of procymidone residue decreased with pH (pH = -0.9672(residue)+10.113) with a correlation coefficient of 0.9553.

Table 3. Rate of procymidone hydrolysis and half-life in aqueous buffers at various pH values.

pH	(-k) (days^{-1})	DT_{50} (days)	DT_{50} (hours)	Residue	r^2
6	0.0789	8.78	210.84	58.08	0.9307
7	0.2811	2.46	59.18	34.08	0.9782
8	0.8452	0.82	19.68	7.96	0.9815
9	5.3976	0.13	3.08	2.09	0.9987
10	17.7252	0.04	0.94	1.68	0.9997

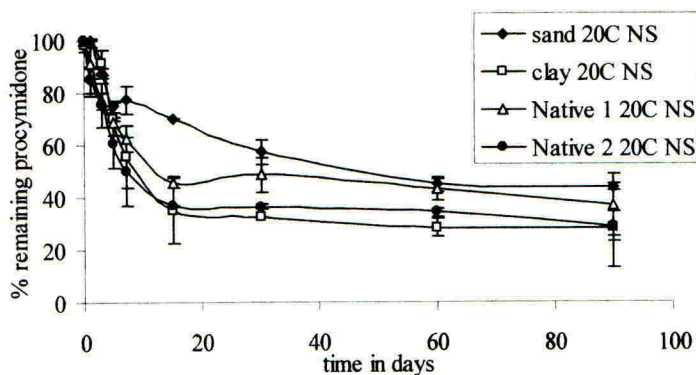DT50= half-life, residue =% pesticide remaining, r^2=correlation coefficient.

Figure 1. Percentage of remaining procymidone in four soil layers from the Almeria greenhouse at 20°C and 70% MHC under non-sterilized (NS) conditions.
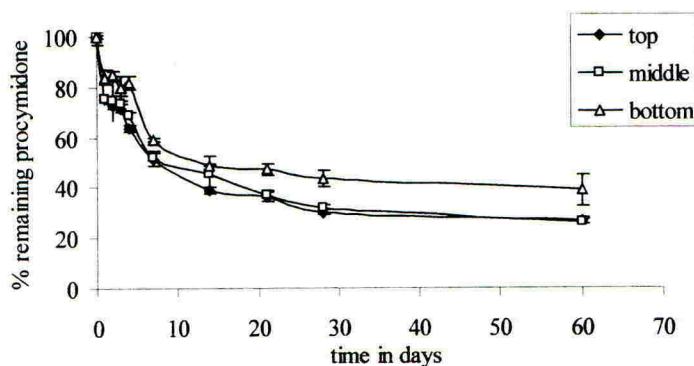

Figure 2. Percentage of remaining procymidone in three soil layers from the Albenga greenhouse at 20°C and 70% MHC under non-sterilized (NS) conditions.

Leaching potential of procymidone in Almeria and Albenga soils

The leaching index model Ground Water Ubiquity Score (GUS) was applied to the procymidone data. This was achieved by combining the effect of degradation and sorption processes, where GUS = $\log_{10}$ (t $_{1/2}$soil) x (4-$\log_{10}$ K_{oc}). Procymidone had an average half-life of 5.7 days in Almeria and Albenga soils and a K_{oc} value of 533 (Gonzales-Pradas *et al.*, 1999), giving a score of 0.96 in the GUS model. This classifies procymidone as a non-leacher. Using procymidone literature review values (DT_{50}=7 days and K_{oc}=1500 from ARS-USDA), gave a score of 0.69 in the GUS model.

DISCUSSION AND CONCLUSION

Procymidone was not found to be persistent in Almeria and Albenga soils and Pseudo First Order (PSDFO) Kinetics described its dissipation. There was an initial rapid dissipation rate followed by a much slower process. The first step could be related to hydrolysis, while the second step may be associated with low or negligible microbial degradation and/or desorption.

The rapid loss of procymidone at pH 6, 7, 8, 9, and 10 in aqueous buffer solutions could be attributed to hydrolysis and is estimated to follow pseudo-first order kinetics. It is possible that the high pH of the Almeria and Albenga soils (pH 8.6) enhances the degradation of procymidone, and that chemical hydrolysis is the main degradation pathway in the first step. Hydrolysis in soil is four times slower than in aqueous solution, therefore sorption to the soil slows down the process. The second step, which is normally related to microbial degradation, would by minimal in Albenga soils and almost negligible in Almeria soils. No significant difference was observed in the dissipation of procymidone in Almeria soils under sterilized and non-sterilized conditions and between the dissipation of procymidone in Almeria and Albenga soils. Therefore, microbial degradation would be unlikely. A residue of procymidone was observed to persist over time.

Procymidone has a shorter half-life and lower sorption value in the alkaline Almeria and Albenga soils than in literature reviews (ARS-USDA). Despite this, procymidone is not expected to leach to ground water. However, Gonzalez-Pradas *et al.* (2002) observed procymidone movement through the Almeria soil profile after repeated applications. Therefore, instantaneous or short sorption equilibrium values may not always be applicable to field conditions, especially in soils with low organic carbon content (Beulke *et al.* 2001).

The results suggest that due to the alkaline conditions of the Almeria and Albenga soils, procymidone dissipates by chemical degradation and sorption to the soil will be lower than in non-alkaline soils. Although procymidone won't be expected to leach further studies should look into the effect of long-term sorption kinetics on leaching.

ACKNOWLEDGEMENTS

REFERENCES

ARS-USDA database (1995) http:\\ncsr.arsuda.gov\rsml\ppdb\html
Beulke S; Brown C D; Dubus IG; Walker A (2001). Characterisation of sorption for the modelling of pesticide fate. *BCPC Symposium proceedings* No. **78**, pp. 51-56.
Dawson R N C; Elliot D C; Elliot WH; Jones KM (1986). Data for biochemical research. Oxford Science publications, 3rd Edition, pp. 417.
Slade E A; Fullerton R A; Steward A; Young H (1991). Degradation of the dicarboximide fungicides iprodione, vinclozolin and procymidone in Patumahoe clay loam soil, New Zealand. *NZ J. Crop Hortic. Science*, **19**, 129-34.
Gonzales-Pradas E; Flores-cespedes F; Fernandez-Perez M; Garratt J A; Wilkins R M (2002). Pesticide leaching in a greenhouse in Almeria. *J. Soil Sci. Society of America (accepted for publication)*
Gonzales-Pradas E; Flores-cespedes F; Urena-Amate M; Fernandez-Perez M; Grazia-Camisa M; Capri E; Glass R C (1999). Adsorption of diuron, imidacloprid, procymidone and pyrimethanil on mediterranean soil. *XI Symposium Pesticide Chemistry*, pp. 313-319.
Villadieu J C; Calmon M; Calmon J P (1994). Mechanisms of dicarboximide ring opening in aqueous media-procymidone, vinclozolin and chlozolinate. *Pesticide Science,* **41** (2): 105-115.

Field studies to determine the effects of the fungicides Mancozeb and Dinocap on predatory mites in orchards and vineyards in Europe

M Miles, E Green

Dow AgroSciences, European Development Centre, 3 Milton Park, Abingdon, OX14 4RN, UK

ABSTRACT

Mancozeb and dinocap are key strategic fungicide molecules in resistance management programmes. Both are registered for use in a wide range of crops globally. An important use for both products is in orchards and vineyards where the conservation of predatory mites is often a high priority with growers. The effects of mancozeb and dinocap on predatory mites has been extensively researched. Data has been compiled from field trials conducted in Europe on the effects of these two products to predatory mites. Using data obtained from studies conducted in Germany, France, Italy, UK and Belgium a range of responses has been defined. Using the database of effects and higher tier risk assessments methods, application scenarios compatible with Integrated Pest Management programmes and safety to non-target arthropods have been identified.

INTRODUCTION

Mancozeb and dinocap are fungicides with multisite modes of action against economically important fungal diseases. Mancozeb is a broad spectrum contact fungicide with high protectant activity and dinocap has preventative, curative, and eradicant action against powdery mildews. To date there are no recorded incidences of resistance to either product despite many years of use on high resistance risk diseases. Both fungicides may be used to control diseases in orchards and vineyards where the conservation of predatory mites is an important component of Integrated Pest Management (IPM). Additionally, there are governmental and environmental pressures to develop and use products safely with minimum impact on non-target arthropods. Predatory mite species such *Typhlodromus pyri* are recognised as both important antagonists of pest species and sensitive indicators of ecologically significant effects.

MATERIALS AND METHODS

Mancozeb

A large number of field studies on the effects of mancozeb to predatory mites have been conducted. Data was collected from 18 reports from which 41 records of effect were obtained. All studies were conducted to internationally recognised guidelines in vines (32 records) or orchards (9 records). Each test consisted of four to five replicates of 10 to 12 vines or trees. For vines, between two and six sprays were applied per season with total annual application rates between 0.8 to 14 kg mancozeb/ha, and for apples, between one and six sprays per season covering 6.4 to 28.8 kg mancozeb/ha. All tests contained an appropriate control (water or 'soft' standard) and many contained products known to be harmful to populations of predatory mites. Leaves were sampled before and after applications and at the

end of the season. The number of motile mites were counted typically using a leaf washing method. Data collected came from tests performed between 1986 and 1994. All trials were performed in Europe within fruit growing regions of Germany (26 records), France (6 records), UK (5 records) and Belgium (3 records). An additional record from a published source was also included (Blumel *et al.*, 2000). From the 41 observations eight came from GLP compliant studies. Each test was classified according to the effect observed on the mite population at the end of the season compared to the control.

Dinocap

Three GLP compliant field studies were initiated in 2001 in vines in two regions of France and one in Italy. Dinocap (as the purified xylene free formulation) was applied either two, five or eight times per season at an application rate of 210 g dinocap/ha. Water was included as a control treatment and propineb was applied on seven occasions at the maximum recommended rate as a toxic standard. Leaves were sampled before and after application, at the end of the season (2001) and the following year (2002). Mites were counted using a leaf washing method and the number of motile forms recorded. Each application scenario was classified according to the effect on the mite population compared with the control.

Classification of effects

Three thresholds of effect were applied to the data, up to 25% (harmless), 25% to 50% (acceptable level of effect compatible with IPM) and 50% to 75% (harmful but next season recovery may occur).

RESULTS AND DISCUSSION

Mancozeb

In all studies the test population was identified as *T. pyri* with the exception of one location in France where the mites were *Cydnodromus californicus*. The percent effect at the termination of the study corrected for control, (Abbott, 1925) against the total annual amount of mancozeb applied per ha in the trial is presented in Figure 1.

The data indicated a rate-related response between the amount of mancozeb applied per season and the effect on the mites. Below 5.0 kg/ha/year only two observations exceeded 25% effect out of a total of 14, indicating that in 86% of the observations, mancozeb was classified as harmless. Applications between 0.8 to 8 kg as/ha/year were virtually always safe to *T. pyri*. Only one record out of 21 provided an effect slightly exceeding the 50% trigger (54% effect at 6.0 kg as/ha/year). This indicated that where up to 8.0 kg as/ha/year was applied, mancozeb was classified as compatible with IPM 96% of the time and in no cases was the 75% threshold ever reached. Even at application rates between 8.0 and 28.8 kg as/ha/year (18 records), there were five records (28%) indicating that the 50% threshold was not exceeded at all and the product was safe. The majority of the records, 13 out of 18 (72%) were above the 50% but below the 75% level indicating that harmful effects could occur but were unlikely to be persistent. In only four cases out of 18 (22%) for field trials conducted between 8.0 and 28.8 kg as/ha/year was the 75% level exceeded.

This analysis allowed safe uses to be predicted according to the level and importance of effect to be tolerated by the grower and examples are given in Table 1. All scenarios cited are

predicted to be safe to off-crop communities of mites with a maximum drift due to applications in vines over a whole season unlikely to exceed 0.5 kg mancozeb/ha/year.
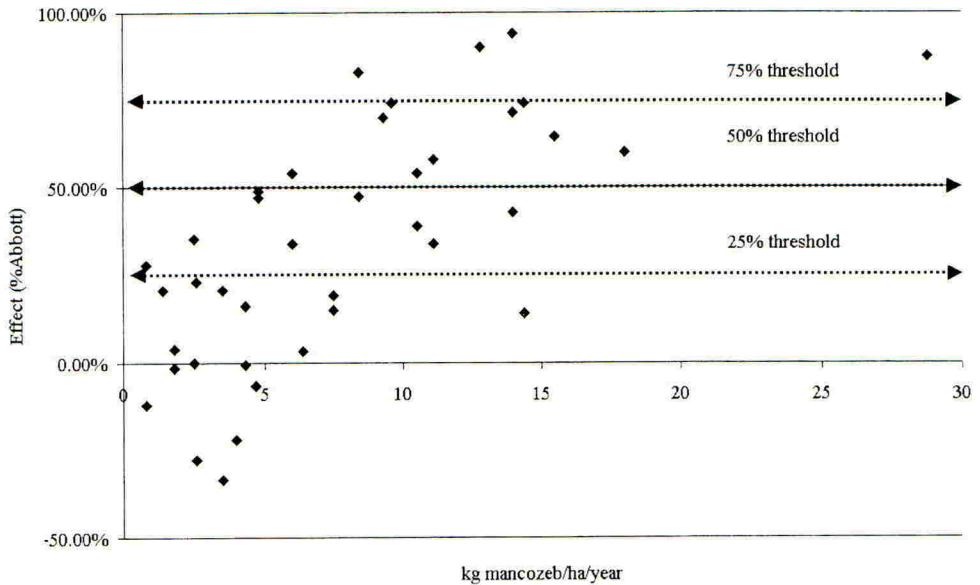

Figure 1.   Frequency analysis for effects of Mancozeb on predatory mites under field conditions. Results from trials in vines and apples - Europe 1986 -1994.

After re-coding of negative values (population increases) as 0.0%, a non-linear regression analysis was undertaken. LR_{50} and LR_{70} values of 9.13 kg /ha/year (95% C.I. 7.06 - 13.0) and 18.3 kg /ha/year (95% C.I. 9.26 - 518) were estimated respectively for mancozeb. The suggested safe range of 4.0 - 8.0 kg mancozeb/ha was supported by the calculation of the LR_{50} and confidence limits.

Table 1.   The risk of applications of mancozeb to vines and orchards in Europe to cause harmful effects to populations of predatory mites

Total annual application rate kg mancozeb/yr	Predicted effect	Frequency of predicted effect	Example treatment scenario (vines)
0.8 - 4.0	Less than 25%	86%	1 - 4 applications
4.0 - 8.0	Less than 50%	96%	4 - 6 applications
8.0 - 16	Less than 75%	88%	6 - 8 applications

Dinocap
Results from field trials conducted in France (Horcher and Renck) and Italy (Aquiterme) are given in Figures 2 - 4. In France the species were *T. pyri* at Horcher and *T. pyri* and *Paraseiulus talbii* at Renck. At the Aquiterme site, the mite species were *T. pyri* and *Euseius finlandicus*. At all sites the propineb treatments caused clear harmful effects which lasted through to the next season. At Horcher, none of the dinocap treatments had an adverse effect on mite populations. The highest recorded effect level was only 28.9% after three sprays in

the five application treatment regime. At all other sampling timings less than 25% effect was observed. At Renck mite population reached nearly 50% effect after the first application but at all subsequent samplings less than 25% effect was recorded for all dinocap treatments. Overall the dinocap treatments were safe to predatory mites in France. In Italy, a higher level of effect was noted for dinocap and was rate related. The majority of the observations made over the year were in excess of the 25% effect threshold. In the two application regime, the 50% threshold was exceeded briefly after each spray but quickly recovered to levels below the threshold. In the five application regime, effects built slowly after each spray and exceeded 50% effect after four applications, remaining close to that level until late summer. A greater level of effect was seen in the eight application treatment which increased over the season and approached but did not exceed the 75% effect level. At the end of the year, mite populations in the two and five spray regimes had recovered to control levels, however in the eight spray treatment, mite levels were at 39.3%. At the start of the following year, all populations were equal to, or in excess of, the control.

A difference in the response of the mite populations to applications of dinocap was observed. French mite populations were less susceptible than those from Italy. This regional variation has been observed by other researchers. Studies conducted in Italy (Anon, 1998) and Austria (Redl et al, 1996) have shown that between two and three applications per season in vines had limited effects on predatory mites, and recovery was apparent at the end of the season. In contrast, trials conducted in the UK in apples (Cross and Berrie 1994) and in France in vines (Kreiter et al, 1996) have indicated that four to five applications per season of dinocap are selective to the predatory mite T. pyri. In the UK situation, the mites present in the orchards were organophosphate-resistant T. pyri and had a history of exposure to crop protection products. In France tolerance to dinocap of certain field populations of T. pyri was confirmed in laboratory studies (Kreiter et al, 1998). It is unlikely that acquired tolerance to dinocap by populations of predatory mites is the only reason for the levels of variation seen, and other factors such as the species of mite present, pesticide history, agronomic practice and environmental considerations may also be important.

Due to this variation in response it is difficult to make an overall recommendation for the safe use of dinocap with respect to predatory mites. In some regions (e.g. Italy and Austria) two to three sprays per season (420 to 630 g dinocap/ha) would appear to be safe and compatible with IPM. In other areas (UK and France) five or more applications would be appropriately protective (1050 to 1680 g dinocap/ha) to predatory mites. The limited effects seen in-crop indicated that dinocap poses no unacceptable risk to off-crop mite communities. Dinocap is currently authorised for use in IPM programmes in France, Portugal, Italy and Austria at rates similar to those proposed in this paper (Reboulet, 1998, Anon, 1998, Redl et al., 1996, Dow AgroSciences unpublished data 2000). This highlights the safety of the purified xlyene free formuation.

CONCLUSIONS

Application scenarios compatible with Integrated Pest Management programmes and safety to non-target arthropods have been identified for the use of the fungicides mancozeb and dinocap in orchards and vineyards. These scenarios may be modified to adapt to local conditions.
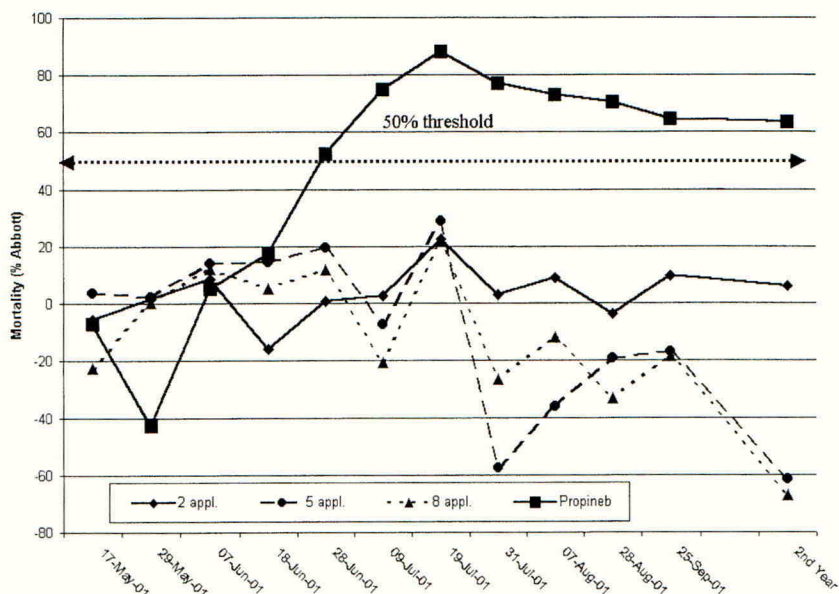
Figure 2.   The effect of different number of applications of dinocap at 210 g/ha to predatory mites in vines. Field trial in France 2001 - 2002 (Horcher).
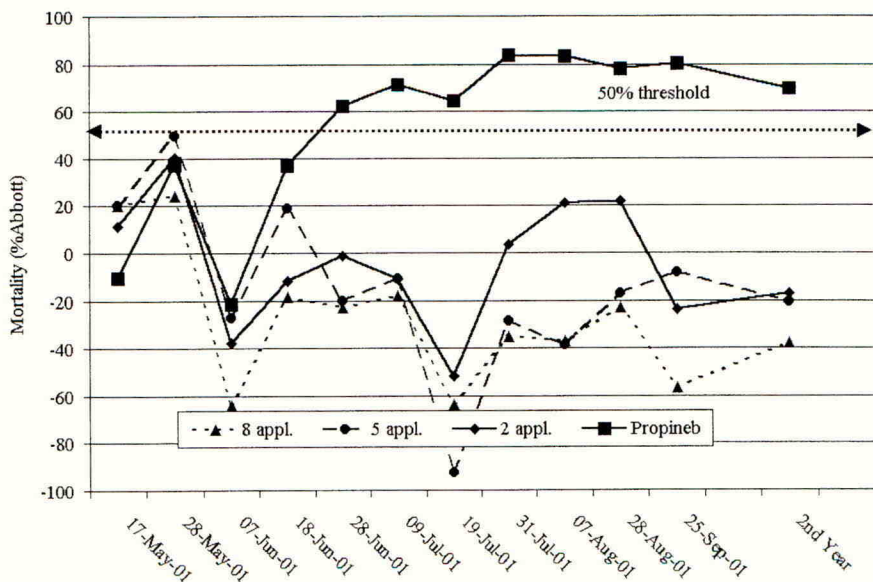

Figure 3.   The effect of different number of applications of dinocap at 210 g/ha to predatory mites in vines. Field trial in France 2001 - 2002 (Renck).
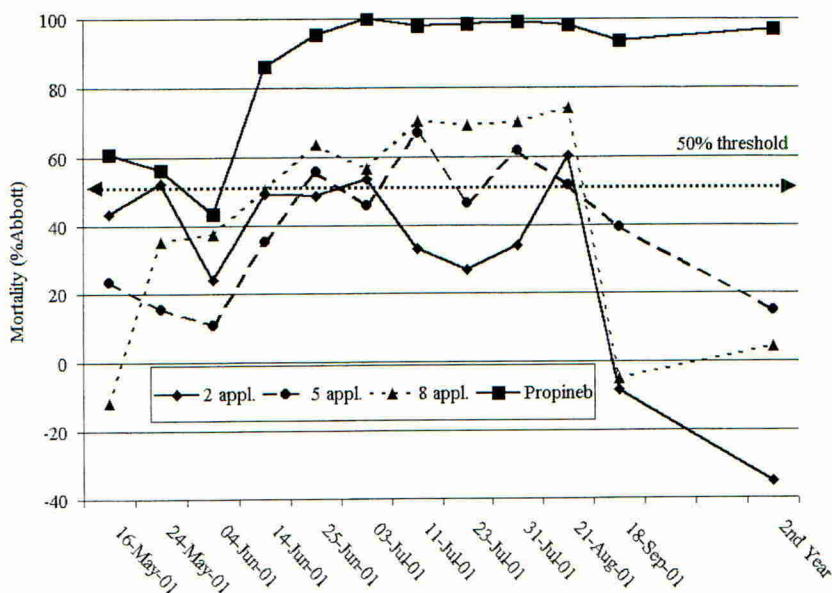
Figure 4 The effect of different number of applications of dinocap at 210 g/ha to predatory mites in vines. Field trial in Italy 2001 - 2002 (Aquiterme).

REFERENCES

Abbott W S (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**, 265-265.

Anon (1998). Il dinocap nei programmi difesa antioidica e compatibilita con I fitoseidi. *L'infomatore Agario* **20**, 1-14.

Blumel S; Pertl C; Bakker F M (2000). Comparative trials on the effects of two fungicides on a predatory mite in the laboratory and in the field. *Entomologia Experimentalis et Applicata* **97**, 321-330.

Cross J V; Berrie A M (1994). Effects of repeated foliar sprays of insecticides or fungicides on organophosphate-resistant strains of the orchard predatory mite *Typhlodromus pyri* on apple. *Crop Protection* **13** 39-44.

Kreiter S; Sentenac G; Barthes D; Auger P (1996). Premier cas de resistance de *Typhlodromus pyri* a un fongicide a base de dinocap. *Phytoma* **483**, 53-56

Kreiter S; Sentenac G; Barthes D; Auger P (1998). Toxicity of four fungicides to the predaceous mite *Typhlodromus pyri* (Acari:Phytoseiidae). *Journal of Economic Entomology.* **91** 802-811.

Reboulet J (1998). Choix des prodiut phytosanitares en vignoble. *Phytoma* **503**, 42-45.

Redl H; Koschier E; Steinkellner (1996). Untersuchungen über die raubmilbentoxische Nebenwirkung des Oidium-Bekampfungsmittels Dinocap in osterreichischen Rebanlagen. *Miteilungen Klosterneuburg* **46**, 1-7.

Persistence and mobility of aldicarb in a simulated red clay soil profile

T Vrahimi-Hadjilouca, R M Wilkins
Ecotoxicology Research Group, Department of Agricultural and Environmental Science, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

ABSTRACT

The persistence and mobility of aldicarb (Temik® 10G) under the alkaline red clay soils of Cyprus is not well known and this was investigated in a simulated red clay soil profile in segmented columns over a 140 day period at a moisture level of $85 \pm 10\%$ of soil field capacity. Aldicarb transformation to aldicarb sulfoxide was slow and partial while aldicarb sulfoxide transformation to aldicarb sulfone was slow and surprisingly low. Disappearance half-lives calculated from pseudo-first-order rate constants were 4.01 ± 0.446 days for aldicarb and 16.1 days for 72.9% of total toxic residues and 78.1 ± 3.23 days for the remaining 27.1% of aldicarb equivalent applied. Aldicarb was the least and aldicarb sulfoxide was the most mobile and had broken through all 60-cm depth segments including leachate (0.1% of applied) by the 71st day. Generally, the highest toxic residues were in the top soil layer. The risks to leaching were estimated to be low from the use of aldicarb in the potato growing area.

INTRODUCTION

Aldicarb has environmental problems but its use continues as it fills an important role in many crops. This includes its use in Mediterranean soils such as the alkaline red clay soils of Cyprus (and elsewhere) where much potato production is located. The concern of aldicarb residues in soils and groundwaters arose in the USA (Zaki et al., 1982) and the environmental and hydrological conditions were associated with potato cultivation (Jones and Estes, 1995). Many studies on soils from around the world have expanded the data on fate of aldicarb but those in alkaline conditions, such as the important red clay soils of the Mediterranean area where potatoes may be grown are limited. The objectives of this study were to determine the persistence of aldicarb in the red clay soil of Cyprus through understanding the disappearance rates of aldicarb and its toxic residues and to evaluate the mobility and leaching of aldicarb, aldicarb sulfoxide and sulfone.

MATERIALS AND METHODS

Soil collection

Soil was collected from a field near Xylophagou in the red clay soil Kokkinochoria area of Cyprus where no cultivation or pesticide application had occurred for at least 4 years. No aldicarb or metabolite residues were detected in the field. Surface materials were discarded and horizontal sections to 1-12, 12-24, 24-36, 36-48 and 48-60 cm depths were dug out and collected separately. The soils were separately air dried, sieved to 4 mm and mixed. Selected properties of the soils are in Table 1.

Preparation of stacked columns

Polyvinyl chloride pipe (15 cm inside diameter) was cut into 4 sections of 12 cm length and 1 of 17 cm which were then re-assembled with silicone sealant such that a ridge (2-5 mm) extended inside the column to disrupt water movement. A wire mesh was heat sealed to the base of the column and the upper 17 cm section supported a water delivery device. Thirty such columns were prepared; six of these were used for field capacity measurements and water content monitoring which determined the amounts of water added to all columns during the experiment to maintain the required water content.

The lowest section of the column was packed with soil from the lowest layer of the soil profile (48-60 cm) to simulate the bulk density of the natural soil. The remaining sections were each packed with their corresponding part of the soil profile in a similar manner. The columns were kept under cover and saturated with water and allowed to drain; over 10 days 12 litres of water leached through each column. The base of each column was placed on a pan containing filter paper to promote water movement.

Each column was treated by abstracting soil (700g) from the top of the column, granular 'Temik 10G' (1.75 g, measured as 172 mg aldicarb) was incorporated, and the abstracted soil was replaced. Water was delivered to the top of the column once a week (at 360 ml per hour) to maintain a moisture content of 85 ± 10% of field capacity. This was applied before sampling, allowing 2-3 hours for equilibration.

Table 1. Soil properties by depth

Depth (cm)	Particle size distribution (%) diameter (mm)			Organic matter (%)	pH (1:5)	Calcium carbonate (%)	CEC (meq /100 g)
	Sand (2.0 - 0.02)	Silt (0.02-0.002)	Clay (<0.002)				
0-12	27.2	22.0	50.8	0.85	7.8	4.16	30.0
12-24	27.0	16.0	57.0	0.81	7.9	5.20	29.0
24-36	26.4	15.0	58.6	0.77	8.2	6.40	29.0
36-48	20.5	15.1	64.4	0.74	8.0	1.05	32.5
48-60	19.5	15.9	64.6	0.70	7.7	0.0	32.7

Column sampling

Soil sampling was done at 1, 3, 7, 14, 28, 49, 71, 102 and 140 days after application. At each time 3 columns were retrieved, cut with a nylon thread into their sections which were capped at both exposed ends, weighed and frozen (-20°C) until analysed. The soil cake was thawed and cut perpendicularly into 4 quarters and opposite segments were combined and mixed prior to sampling. Any leachate was determined through corresponding extraction and analysis of the filter papers at the base of each column.

Extraction from soil and analysis

Moist soil (35 g) was weighed into screw capped centrifuge bottles with anhydrous sodium sulfate, hplc grade methanol (75 ml) added and shaken for 2 hours. After centrifugation the supernatant was analysed by hplc using a Waters Nova-Pak® C8 column with u.v. absorption at 200 nm. The mobile phase for aldicarb was acetonitrile/water (40:60 v/v, 1ml/ min) and for aldicarb sulfoxide and sulfone was acetonitrile/water (13:87 v/v, 0.9 ml/min). Retention times were 5.7 min (aldicarb) and 4.7 and 7.6 min (for sulfoxide and sulfone). With no methanol concentration the limits of detection were 40 ng/g (aldicarb and aldicarb sulfoxide) and 50 ng/g (aldicarb sulfone) soil. Overall recoveries from spiked soils were $94 \pm 1.6\%$ (aldicarb), $85 \pm 0.7\%$ (aldicarb sulfoxide) and $93 \pm 1.9\%$ (aldicarb sulfone). Filter papers were analysed in a similar manner.

RESULTS AND DISCUSSION

Aldicarb disappearance

Residue analyses of aldicarb were obtained as a function of depth (and column leachate) and time after treatment and were converted to absolute amounts for each column section. Thus, the total amount present in each column, for each sampling time, was available and expressed as a percent of that applied (172 mg/column). The disappearance curve for aldicarb is shown in Figure 1 and it had a 50% disappearance time of about 7 days. There was an initial fast rate from day 0 to 28 followed by a very slow rate up to day 140. During the fast rate period 99.0% of the aldicarb had been transformed. The disappearance rate constants calculated by pseudo-linear regression of total mean aldicarb concentrations over time were 1.73×10^{-1} day^{-1} for the fast stage and 2.59×10^{-1} day^{-1} for the slow stage, the corresponding half-lives were 4.0 ± 0.45 and 268 ± 54.5 days. These parameters relate to surface soil temperatures where air temperatures varied between 31.6 and 10.1°C with a mean daily temperature of 21.5°C. These values are of a similar order to reported studies, for example, half lives of 3-5 days found in field lysimeters (Bowman, 1988).

Transformation to aldicarb sulfoxide and sulfone

Transformation to aldicarb sulfoxide was slow and only partial, reaching a maximum of 29.8% of aldicarb equivalent applied at 14 days after treatment (Figure 1). At the end of the study (140 days) aldicarb sulfoxide was still present (at 8.5%). Further transformation to aldicarb sulfone was very slow and incomplete, reaching a maximum of 3.5% from the 49th to 71st day, and declining to 2.4% at the end of the study. As aldicarb and the two oxidation metabolites are toxic to animals, the three compounds together in soil form the total toxic residue (TTR) and the disappearance of this is environmentally important. The 50% disappearance time for the TTR was about 13 days corresponding to a first order rate constant of 5.33×10^{-2} day^{-1}. However, it is better described (as for aldicarb alone) as two processes, the faster up to 28 days and a following slower step up to 140 days. The resulting pseudo first order degradation rates were 4.3×10^{-2} and 8.9×10^{-3} day^{-1}, giving half-lives of 15 ± 2.9 and 78 ± 3.2 days, respectively. Based on the first order model 73% of the TTR (as aldicarb equivalent) had degraded with the initial fast rate and 27% with the subsequent slow rate.
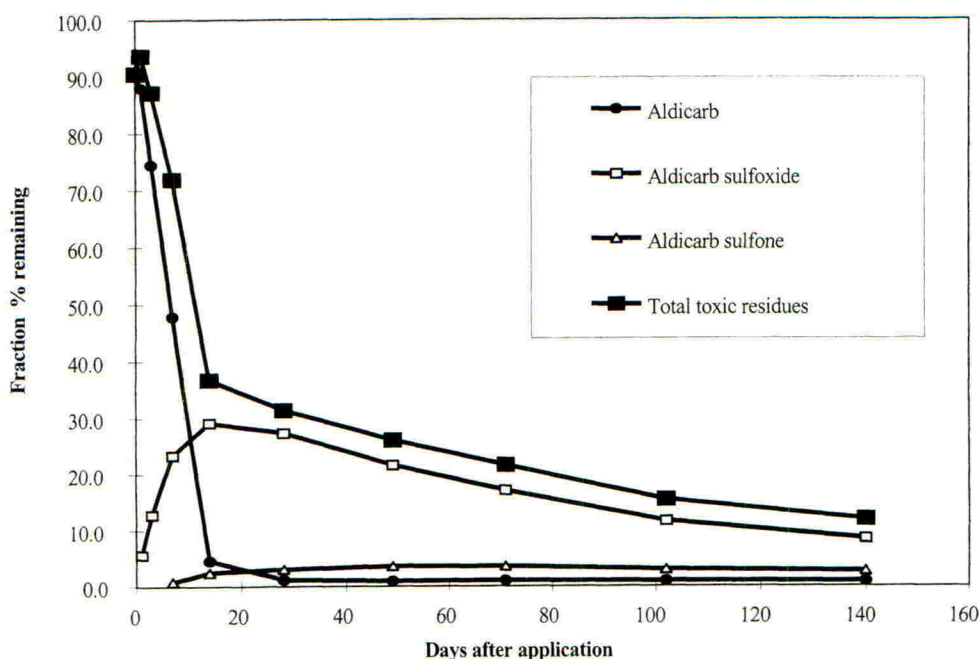
Figure 1. Percent (mean total per column) of aldicarb and oxidation products

The disappearance rate constant for aldicarb (and for TTR) was high with only partial oxidation to the sulfoxide and lower oxidation to the sulfone. The losses of the parent molecule and metabolites were likely to be due to hydrolysis, enhanced by the high pH of the soil and by the high moisture content. The application rate used (75 μg/g soil, based on the top 12 cm depth) was high and corresponds to about 100 kg a.i./ha. Evidence for retardation of microbial activity by high soil concentrations of aldicarb is mixed (Read, 1987) but is unlikely in the present study as retardation started 28 days after application, when TTR concentrations were lower. The deeper levels were lower in organic matter and pH (Table 1) and there was some indication that degradation in these soils was reduced as suggested by Daoji et al, 1993. These kinetics were largely in agreement with Hornsby et al. (1990) for aldicarb losses in the unsaturated zone of an acid soil (initial fast rate half-life 11 days 0-86 days, slower rate 23 days 86-120 days) and with alkaline soils of pH 8.5-9.2 (Daoji et al., 1993).

Mobility of aldicarb and oxidative metabolites in the soil profile

Aldicarb

Aldicarb was the least mobile of the residues recovered and was always higher in the top 1-12 cm of the simulated soil profile (shown with the percent of applied aldicarb equivalent of aldicarb sulfoxide and aldicarb sulfone in Table 2). The greatest depth of leaching (0.1% of applied dose) was the 24-36 cm zone reached at 7 days. The most leaching (16%) from the top layer, into the second layer, occurred on the first day and this persisted until day 7. Very little aldicarb was ever detected in the 24-36 cm, and none in the 36-48 cm, or lower, zones.

Table 2. Percent of aldicarb applied (172 mg) to each column as aldicarb, aldicarb sulfoxide, aldicarb sulfone (means ± SEM, n=3) according to time and soil depth (ND = not detectable)

Residue	Soil depth (cm)	Days after application									
		Day 0	1	3	7	14	28	49	71	102	140
Aldicarb	0-12	90.5 ±0.93	72.0 ±5.84	62.3 ±6.31	33.4 ±4.40	4.3 ±0.24	1.0 ±0.06	0.9 ±0.14	0.8 ±0.04	0.8 ±0.10	0.8 ±0.01
	12-24	ND	16.0 ±6.21	12.1 ±5.29	14.3 ±4.88	0.2 ±0.01	0.1 ±0.01	traces	0.2 ±0.01	0.1 ±0.02	ND
	24-36	"	ND	ND	0.1 ±0.04	traces	ND	ND	traces	ND	"
	Total	90.5 ±0.93	88.0 ±0.69	74.4 ±4.45	47.8 ±2.28	4.5 ±0.28	1.1 ±0.06	0.9 ±0.14	1.0 ±0.05	0.9 ±0.12	0.8 ±0.01
Aldicarb sulfoxide	0-12	ND	3.6 ±0.25	6.9 ±1.13	12.8 ±0.26	13.5 ±1.26	10.5 ±0.85	10.6 ±1.53	8.1 ±1.44	5.2 ±0.74?	4.6 ±1.14
	12-24	"	2.1 ±0.53	5.9 ±1.68	10.4 ±1.53	14.7 ±1.52	12.3 ±0.58	7.6 ±0.27	2.8 ±0.24	1.0 ±0.22	0.2 ±0.05
	24-36	"	ND	ND	0.1 ±0.00	1.6 ±0.42	4.5 ±0.49	3.4 ±0.66	5.0 ±0.31	2.8 ±0.14	1.2 ±0.20
	36-48	"	"	"	ND	ND	ND	ND	0.9 ±0.34	2.4 ±0.25	2.3 ±0.06
	48-60	"	"	"	"	"	"	"	0.2 ±0.04	0.2 ±0.05	0.2 ±0.10
	leachate	"	"	"	"	"	"	"	0.1 ±0.01	traces	traces
	Total	ND	5.7 ±0.28	12.8 ±1.22	23.3 ±1.71	29.8 ±2.09	27.3 ±0.65	21.6 ±1.13.	17.1 ±1.03	11.6 ±0.47	8.5 ±1.07
Aldicarb sulfone	0-12	ND	ND	ND	0.3 ±0.02	0.7 ±0.02	0.8 ±0.09	0.9 ±0.03	0.9 ±0.03	0.6 ±0.03	0.6 ±0.02
	12-24	"	"	"	0.5 ±0.02	1.6 ±0.11	1.7 ±0.22	1.6 ±0.02	0.9 ±0.06	0.3 ±0.06	traces
	24-36	"	"	"	ND	0.1 ±0.01	0.5 ±0.04	1.0 ±0.06	1.3 ±0.18	1.2 ±0.07	0.8 ±0.10
	36-48	"	"	"	"	ND	ND	ND	0.3 ±0.10	0.8 ±0.11	1.1 ±0.09
	48-60	"	"	"	"	"	"	"	0.1 ±0.02	traces	0.1 ±0.05
	leachate	"	"	"	"	"	"	"	ND	ND	ND
	Total	ND	ND	ND	0.8 ±0.00	2.4 ±0.13	3.0 ±0.31	3.5 ±0.11	3.5 ±0.32	2.9 ±0.08	2.6 ±0.28
Total/column		90.5 ±0.93	93.7 ±0.82	87.2 ±3.63	71.9 ±3.70	36.7 ±2.05	31.4 ±0.53	26.0 ±1.17	21.6 ±0.78	15.4 ±0.43	11.9 ±0.97

Aldicarb sulfoxide

Aldicarb sulfoxide was the most mobile of the three residues (Table 2). It was found in the 12-24 cm depth one day after application (2.1%). It broke through to the bottom of the column and in the leachate by day 71 after application. This leaching was entirely due to its mobility rather than oxidation of aldicarb which did not realistically pass the 12-24 cm layer. However, the highest concentrations remained in the upper 2 layers with maxima on day 14. For deeper soil the maxima were on day 71. This compound formed the major residue (8.5%) at the end of the experiment at 140 days after application.

Aldicarb sulfone

This was first detected in the upper 2 layers of the simulated profile on day 7 (Table 2). Thereafter, it was recovered wherever the sulfoxide was found, but at lower concentrations. Unlike aldicarb, and for most of the time aldicarb sulfoxide the soil zone with the highest sulfone concentration was not the top 1-12 cm but the 12-24 cm for the period 7-49 days, the 24-36 cm for 71-102 days and the 48-60 cm depth by day 140. No aldicarb sulfone was leached from the columns.

The comparative mobility of aldicarb, aldicarb sulfoxide and aldicarb sulfone was as expected, in line with previous studies (e.g. Bowman, 1988) and with published K_{OC} values and water solubilities (Fava et al, 2001). The maximum depth was reached by aldicarb sulfoxide to 60 cm and was much less than most other studies have suggested (Wyman et al., 1987), especially allowing for the high application rate. This indicates that the leaching potential of aldicarb, and TTR, in the red clay soils is low.

REFERENCES

Bowman B T (1988). Mobility and persistence of metolochlor and aldicarb in field lysimeters. *Journal of Environmental Quality* **17**, 689-694.
Daoji C; Feng X; Xinming J; Zhonglin Z; Xiaomei H; Zhenke D (1993). Fate of aldicarb in the vadose zone beneath a cotton field. *Journal of Contaminant Hydrology* **14**, 129-142
Fava L; Bottoni P; Crobe A; Caracciola A B; Funai E (2001). Assessment of leaching potential of aldicarb and its metabolites using laboratory studies. *Pest Management Science* **57**, 1135-1141.
Hornsby A G; Rao P S; Jones R L (1990). Fate of aldicarb in the unsaturated zone beneath a citrus grove. *Water Resources Research* **26**, 2287-2302.
Jones R L; Estes T L (1995). Summary of aldicarb monitoring and research programs in the USA. *Journal of Contaminant Hydrology* **18**, 107-140.
Read D C (1987). Greatly accelerated microbial degradation of aldicarb in re-treated field soil, in flooded soil and in water. *Journal of Economic Entomology* **80**, 156-163.
Wyman J A; Jones R L; Medina J; Curwen D; Hansen J L (1987). Environmental fate of aldicarb and aldoxycarb applications to Wisconsin potatoes. *Journal of Contaminant Hydrology* **2**, 61-72.
Zaki M H; Moran D; Harris D (1982). Pesticides in groundwater. The aldicarb story in Suffolk County, NY. *American Journal of Public Health* **72**, 1391-1395.