POSTER SESSION 4C

PESTICIDES IN THE ENVIRONMENT: MEASURING THEIR FATE AND EFFECTS

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Poster Papers: 4C-1 to 4C-7

Higher tier risk assessment of Agrochemicals, incorporating sediment into algal test systems

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ABSTRACT

Existing guidelines (OECD 201, OPPTS 850.5400 (draft)) for ecotoxicity testing of algae involve the exposure of the test organism to the test compound in sterile media. For compounds which rapidly dissipate, ecotoxicity tests performed under conditions of constant exposure may result in an overestimate of potential risk. Therefore, as an element of higher tier risk assessment, it may be appropriate to assess the impact of a chemical on organisms under more realistic environmental conditions. One approach to this is the inclusion of sediment in aquatic studies. This paper presents a method for testing the algal species *Selenastrum capricornutum* and *Navicula pelliculosa* in the presence of sediment. The methods used and results of two studies are presented.

INTRODUCTION

Assessment of algal growth inhibition is a standard testing requirement for product registration and is performed under a 'worst case' exposure scenario (EEC 1992, OECD 1984, US EPA 1996). This standard test is usually carried out in open flasks, containing sterile, nutrient rich, aqueous media, under continuous shaking and high light intensity, all of which are designed to promote high algal growth rates. At higher tiers of testing, the test can be modified to assess potential toxicity in the presence of sediment, which can result in a more realistic exposure simulation and hence a more 'real world' reflection of the potential for impact of a product in the environment (Campbell *et al*, 1998).

However, modification of the standard test causes a number of technical problems. This paper describes an algal growth inhibition test modified to include sediment in the test system. Wherever possible the test system has been designed to use existing equipment and to minimise additional costs.

METHOD DEVELOPMENT

The test design was based, as far as possible, on the standard EC and OECD methods, initially using the freshwater green alga *Selenastrum capricornutum*. Problems associated with the presence of sediment arose in both the level of algal growth and in the quantification of the algal cell density. Many of the sediments used in preliminary trials inhibited growth. Other problems included reduced growth due to 'shading' and the selection of a suitably sensitive, yet robust, growth assessment technique, capable of minimising the interference caused by the sediment particles.

Attempts to resolve problems and refine techniques showed that, for all but sediments of the coarsest sand, background interference meant that electronic particle counting was unusable as a means of determining algal growth. Spectroscopy of samples was also far too insensitive to assess algal growth, but fluorometry, using solvent extracted, filtered samples was shown to be effective. However, an increased initial inoculum density of algae was needed, both to aid early test fluorometric measurements and because algal growth rates are reduced in the presence of sediment.

Of a range of nine 'natural' sediments assessed, five were unusable and the sixth was eventually discarded, after showing variability between batches. Of the three remaining candidates one was eventually adopted, based on its preferred physico-chemical properties, including relative pH neutrality, organic content and low levels of suspended solids under test conditions.

Study design

The test, of minimum 72 hours duration, was close to the standard design, deploying six replicates of the control and triplicates of each concentration of the test substance. One blank (no algal inoculum) was incubated concurrently for each control and test concentration. The test vessels were conical flasks of 250 ml capacity, with foam bungs, containing 100 ml of test solution, together with 3 g of sediment. The cultures were incubated at 24°C, under continuous illumination of approximately 8000 lux, with orbital shaking, in an incubator.

Samples were removed daily from each vessel, including the blanks, for fluorometric measurement of algal growth. The appropriate blank measurements were subtracted from the algal culture values. Determinations were made on replicated samples, after acetone:DMSO solvent extraction (Mayer *et al*, 1997), on a fluorometer reading at the chlorophyll 'a' excitation and emission wavelengths. At the start of the test, the fluorescence of the inoculum culture and a range of dilutions of that culture were made to confirm the linear relationship between fluorescence and cell density (determined by electronic particle counting).

The study was run for a minimum of 72 hours and, if necessary, was continued beyond that period, to ensure that the entire algal logarithmic growth phase and potential recovery, was assessed.

Further development

The method was also examined to assess its suitability for testing with the freshwater diatom *Navicula pelliculosa*. The test material used was relatively insoluble and therefore, the study involved the use of a solvent carrier.

Initial growth trials concentrated on the three 'preferred' sediments previously identified and showed slower growth rates with *Navicula* than with *Selenastrum*. There was a reduction in fluorescence between the start of test and 24 hour observations, possibly due to the diatom sticking to the sediment. In the natural environment *Navicula* is known to be periphytic and associate with sediments (Round, 1965).

Overall, there was greater variation between replicates than in the *Selenastrum* studies. Despite this, growth factors exceeded the 16 fold increase (0 to 72 hours) required in regulatory studies. There were no deleterious growth effects associated with either of the two solvent carriers (dimethylformamide and acetone) tested.

The final test design for *Navicula* was exactly as previously detailed, except that six solvent control and three culture medium control replicates were used.

RESULTS

The results of a *Selenastrum capricornutum* growth study, in the presence of sediment, are presented in Figure 1. In an earlier standard study, without sediment, with the same test material (an established herbicide), the biomass and growth rate EC_{50}s were 11 $\mu g\ l^{-1}$ and 19 $\mu g\ l^{-1}$. In this study there was no significant reduction in growth (P=0.05), even at the highest test concentration of 320 $\mu g\ l^{-1}$.

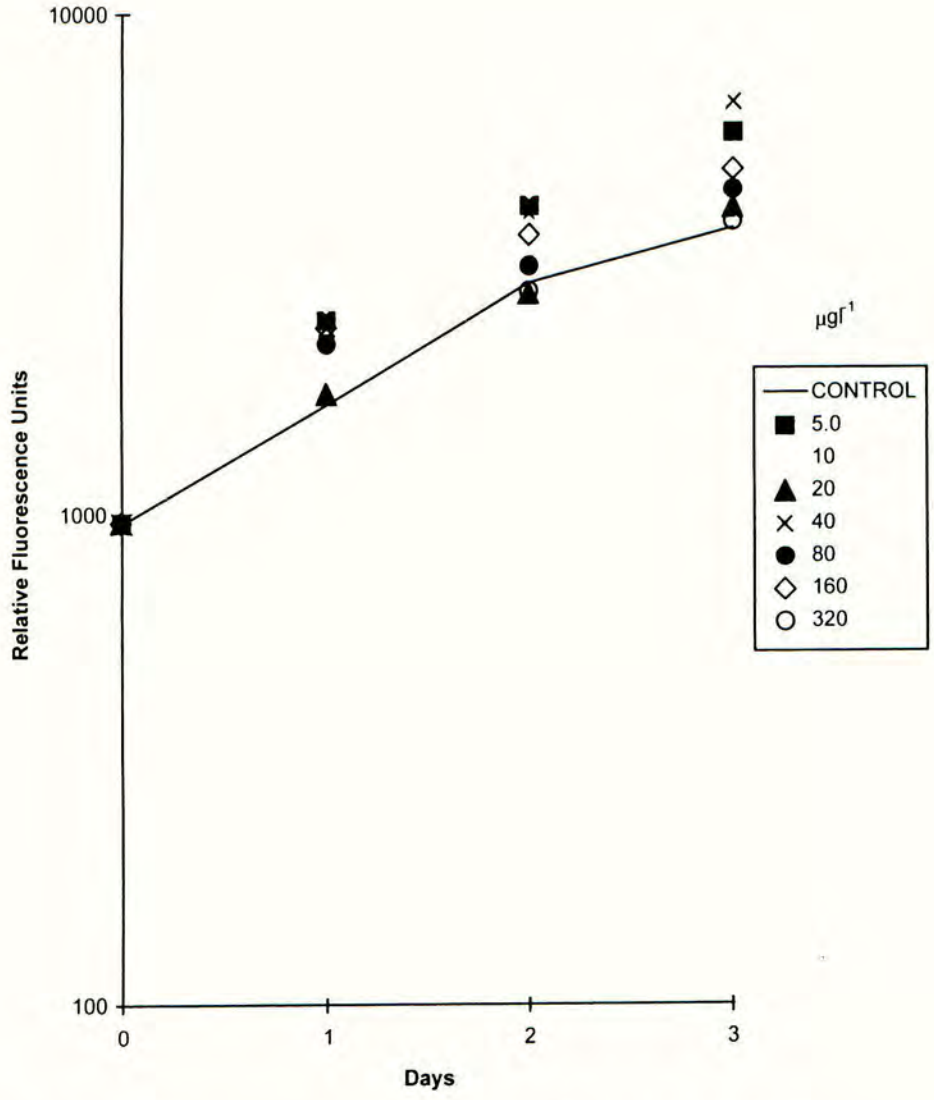

Figure 1. **Effects on growth of the green alga *Selenastrum capricornutum* over 3 days**

Figure 2 presents the results of a *Navicula pelliculosa* study on another herbicide, in the presence of sediment. The apparent dip in growth between day 0 and day 1, thought to be due to association of the algal cells with sediment, is obvious in all of the test concentrations. At 4 and 5 days there were no significant effects (P=0.05) at the highest test concentration 900 µg l^{-1}. In an earlier standard study, without sediment, biomass and growth rate EC_{50}s were 0.7 µg l^{-1} and 1.0 µg l^{-1} respectively.

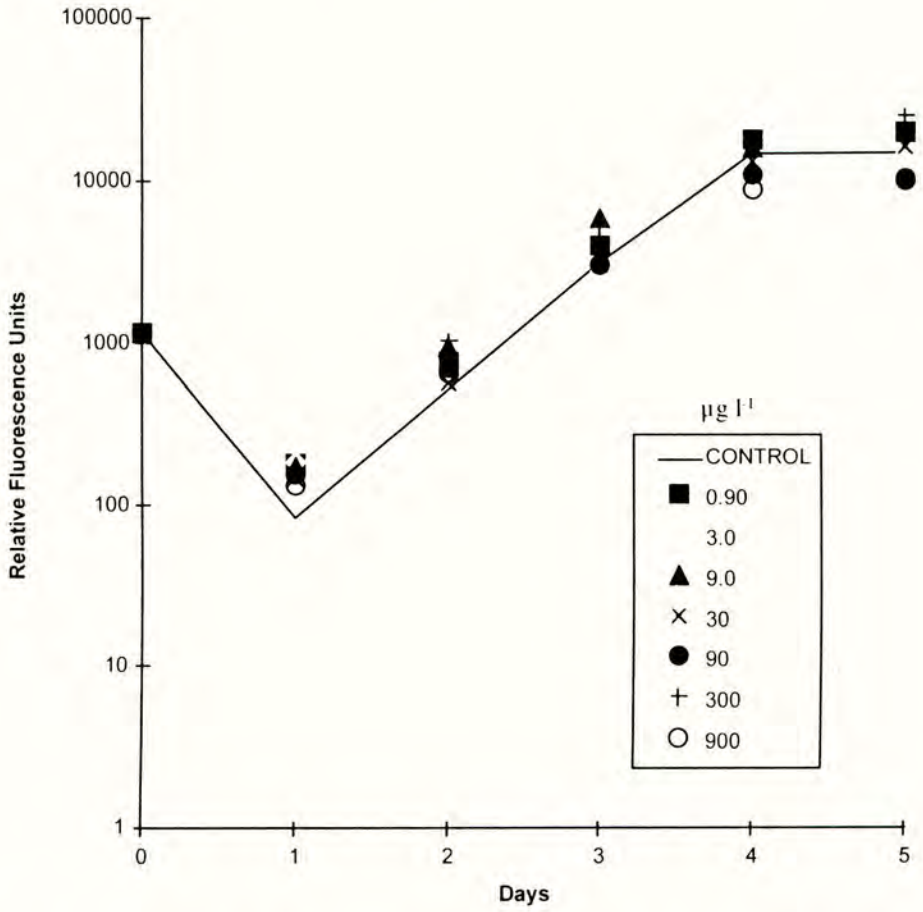

Figure 2. Effects on the growth of the freshwater diatom *Navicula pelliculosa* over 5 days

CONCLUSIONS

Methodologies suitable for the determination of the effects of agrochemicals on algae in the presence of sediment have been developed. Growth rates were lower and there was greater variation between replicates than in standard tests, but the design is suitable for modified exposure studies. The two studies described in this paper illustrate how fate processes may significantly mitigate risk under more realistic exposure conditions.

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The effects of the fungicide carbendazim in an innovative integrated terrestrial microcosm system

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ABSTRACT

In collaboration with scientists from Germany, Russia, UK and Portugal we have designed integrated terrestrial microcosms which can assess simultaneously the overall fate of a pesticide and its effects on representative soil organisms and ecosystem processes. This takes into account interactions between organisms in assessing the overall environmental impact of a pesticide on soil ecosystems. The ecosystem structural parameters measured include: microbial respiration rates, nematode populations, feeding activity using bait lamina sticks, earthworm, enchytraeid and microarthropod populations and effect on plant growth. The ecosystem processes assessed were soil ammonium-N and nitrate-N concentrations, soil dehydrogenase and phosphatase activity, and rate of organic matter decomposition.

Carbendazim was applied to soil in terrestrial microcosms at the Predicted Environmental Concentration (PEC) and at a range of doses 3, 9, 27, and 81 times the PEC value. The PEC was calculated based upon the recommended application rate, soil penetration to 5 cm, and a soil density of 0.95 g (dry weight)/cm^3. The endpoints most affected, usually at the higher doses of carbendazim, were earthworm weight and mortality, nematode populations, plant growth, rates of organic matter decomposition, phosphatase activity, and nitrate-N and ammonium-N concentrations.

INTRODUCTION

The maintenance of soil quality, fertility, and structure is essential to the protection and maintenance of the biodiversity and integrity of terrestrial ecosystems. The ability to protect these ecosystems depends upon the recognition and understanding of the potential effects that pesticides may have on the system (Edwards *et al.*, 1996). Laboratory methodologies have been widely used to assess effects of pesticides on key species of soil organisms or particular soil processes. However, these methodologies rarely adequately predict environmental consequences resulting from field use (Van Voris *et al.*, 1985). Often, field trials are hampered by high variability, poor reproducibility and relatively high costs (Edwards, 1992). The microcosm approach addresses the issues of variability and reproducibility whilst also providing an increased level of accuracy for predicting environmental effects on soil processes and structure (Edwards *et al.*, 1996).

Key ecosystem endpoint parameters measured include microbial and invertebrate populations, feeding activity, nutrient cycling, organic matter decomposition, and effects on plant growth.

The small integrated, homogenous microcosm used for this study can be classified as an open, homogenous terrestrial model ecosystem according to Edwards *et al.*, 1996. This type of microcosm uses sieved soil mixed with the contaminant, in this case carbendazim. Endogenous, as well as introduced organisms, can be included in the system. The small, manageable size of the soil microcosm means that many replicates may be used for each sampling timepoint.

METHODS AND MATERIALS

Microcosms, set up in a greenhouse, consisted of cylinders, 7.5 cm (inside diameter) x 15 cm high, made from high-density polyethylene pipe. A fine nylon mesh across the bottom of each microcosm allowed easy collection of leachate for analyzing nutrient and carbendazim concentrations. Earthworms, enchytraeids, and micro-arthropods were added to each microcosm. Field soil, (silty clay loam), collected from Florsheim, Germany, was sieved through a 5 mm screen and thoroughly mixed. Six batches of soil (30 kg each) were weighed out for a total of five dose levels and one control. Dose levels were based upon the predicted environmental concentration (PEC) of 0.76 mg a.i./kg soil dry weight. The PEC was calculated 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 dose (T1) was at the PEC, T2 was 3xPEC (2.28 mg a.i./kg soil dry weight), T3 was 9xPEC (6.84 mg a.i./kg soil dry weight), T4 was 27xPEC (20.52 mg a.i./kg soil dry weight), and the highest dose level (T5) was 81xPEC (61.56 mg a.i./kg soil dry weight). Each batch of soil was sprayed with 400 mL of the appropriate concentration of Derosal® solution (500 g/L carbendazim). The control (T0) was sprayed with 400 mL of deionized water. The treated soil was mixed thoroughly and packed gently into the cylinders. Five replicates for each dose level were sampled destructively 7, 14, 28, and 56 days after treatment (DAT).

Each microcosm was sown with approximately 10 wheat seeds, later thinned to 5 seedlings. Three earthworms (*Lumbricus rubellus*) and approximately 0.5 g of enchytraeids (*Enchytraeus albidus*) were added. Artificial rainwater was used to water seedlings as needed. Temperatures in the greenhouse were recorded approximately every two hours and ranged from a minimum of 20°C to a maximum of 38°C over 28 days. Microcosms were shaded with a 60% shade cover during the study. Lighting was supplemented as needed to maintain a 12 hour-12 hour light-dark cycle.

Microbial respiration rates were determined 7, 14, 28 and 56 DAT by measuring the basal and substrate-induced respiration (SIR) rates. Basal respiration was measured prior to adding glucose at 4000ppm. Carbon dioxide generated was trapped in a 0.075N sodium hydroxide solution. Traps were titrated with 0.05N hydrochloric acid using a phenolphthalein indicator. Nematode populations were assessed at 56 DAT using the

Baermann funnel technique (Baermann, 1917). Invertebrate feeding activity was measured by counting the numbers of holes eaten in the cellulose film of bait lamina sticks (Kratz, 1998) 7-9 days after insertion. Earthworms, sampled at 7, 14, and 28 DAT, were hand-sorted, weighed immediately after collection, and placed on wet paper towels overnight to clear their guts and then re-weighed. Plant growth was measured 7, 14, 28 and 56 DAT by recording the total shoot number and combined fresh weight immediately after sampling. Shoots were cut within 1 cm of the soil level and loose soil brushed off prior to weighing.

Soil ammonium and nitrate concentrations were measured by extracting 10 g of soil with 50 mL of 0.5M K_2SO_4. Extracts were analyzed using a microplate technique as described by Sims, *et al.*, 1995. Organic matter decomposition was measured using mesh litterbags containing wheat straw at 0 and 56 DAT.

RESULTS AND DISCUSSION

The average number of nematodes per gram of soil, after 56 days, for the control (T0) was 13.68. The treated soils averaged 11.45, 19.25, 12.18, 2.94 and 0.60 nematodes per gram of soil for dose levels T1 to T5 respectively. The average numbers for dose level T2 was significantly greater than the averages for T4 and T5, (see Figure 1). Higher concentrations of carbendazim had a more negative effect on nematode populations.

Earthworm weights were reduced significantly at the highest dose level (T5) by 14 DAT and at dose levels T4 and T5 by 28 DAT (see Figure 2). Survival rates were lower at levels T4 and T5 by 14 DAT reaching 0% survival for T5 at 28 DAT (see Figure 3). Earthworms were affected significantly by the highest concentrations of carbendazim.

Significant differences in invertebrate feeding activity between dose levels occurred as early as 7 DAT, with feeding activity in the two highest dose levels (T4 and T5) significantly lower than in the control and lower dose levels of T1 and T2. Feeding activity in T3 was significantly less than in levels T0, T1, and T2, and significantly greater than levels in T4 and T5, but no significant differences existed between most dose levels after 28 DAT (see Figure 4). High concentrations of carbendazim temporarily reduced feeding activity.

Shoot growth was measured at 7, 14, 28, and 56 DAT as the fresh weight, (g), per shoot. The highest carbendazim dose level (T5) resulted in significantly less shoot growth than in the control (see Figure 5).

High carbendazim concentrations (dose levels T4 and T5) increased soil ammonium-N concentrations significantly by 14 DAT compared to the lowest dose level (T1). The average ammonium-N concentrations at 14 DAT were 3.33, 10.91, and 16.43 mg/kg soil dry weight for dose levels T1, T4, and T5 respectively. Between 28 and 56 DAT, ammonium-N concentrations at those same dose levels decreased significantly below the

Figure 1. Effects of Carbendazim on Nematode Populations (56 DAT)

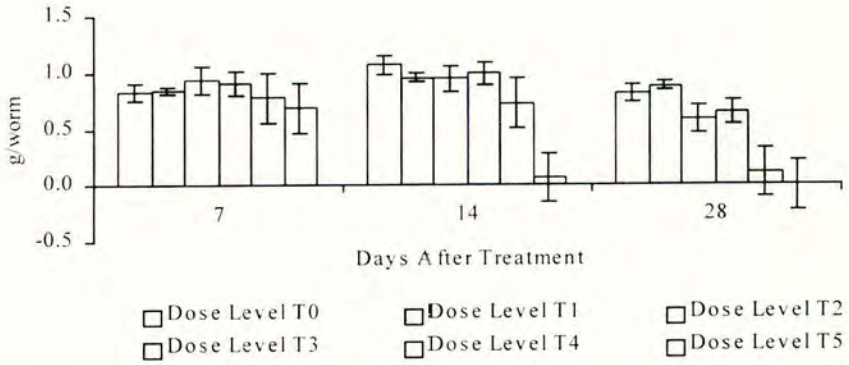

Figure 2. Effects of Carbendazim on Earthworm Weights

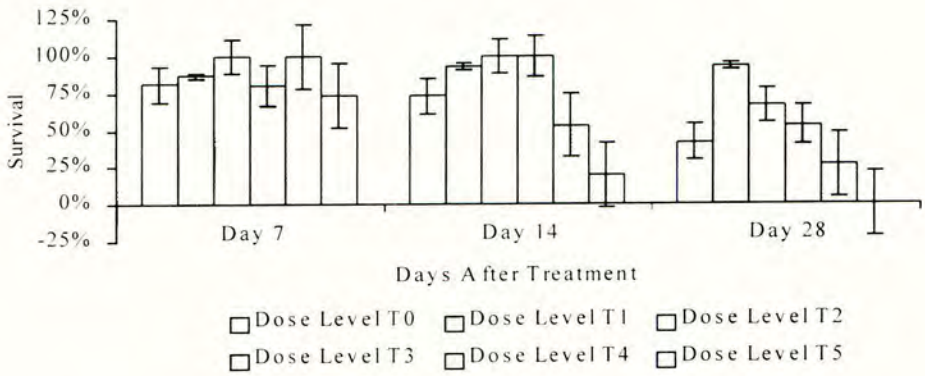

Figure 3. Effects of Carbendazim on Earthworm Survival

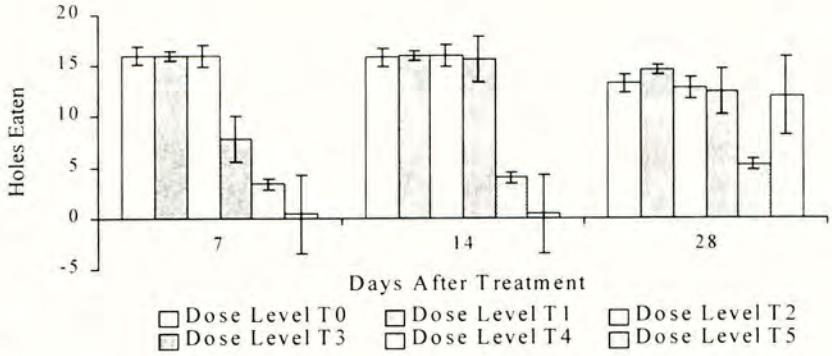

Figure 4. Effects of Carbendazim on Invertebrate Feeding Activity

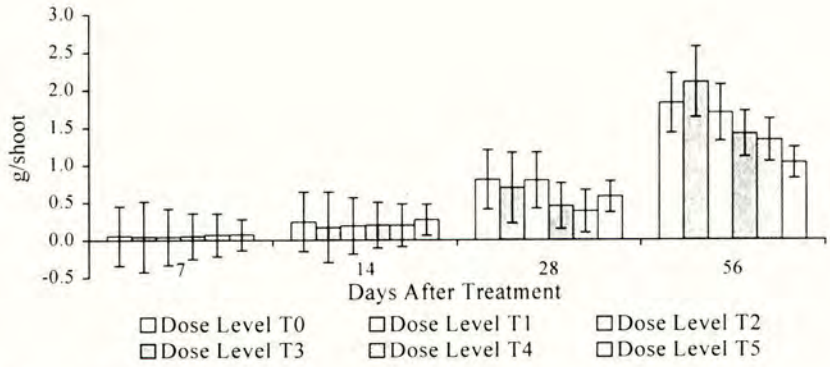

Figure 5. Effects of Carbendazim on Shoot Growth

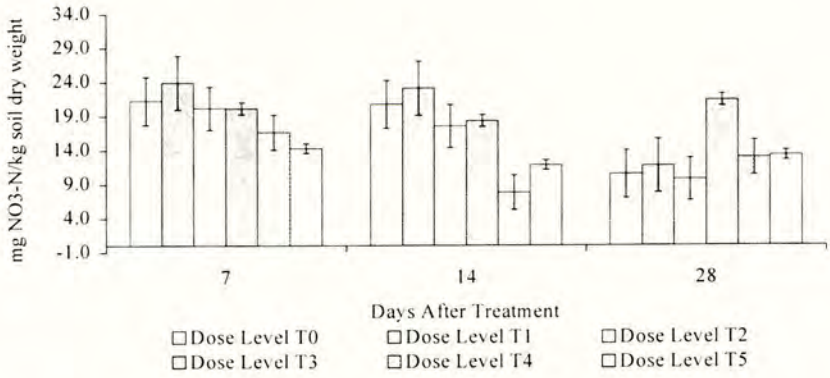

Figure 6. Effect of Carbendazim on Soil Nitrate-N Concentrations

control and lowest dose level (T0 and T1). The average ammonium-N concentrations at 56 DAT were 4.78, 3.47, 1.30, and 1.30 mg/kg soil dry weight for dose levels T0, T1, T4, and T5 respectively.

Nitrate-N concentrations at the highest dose levels (T4 and T5) were significantly lower than nitrate-N concentrations at the control and dose levels T1, T2, and T3 by 14 DAT. The concentration of nitrate-N in the soil recovered by 28 DAT with no significant differences between the control and treated soils. Carbendazim temporarily reduced the concentration of nitrate-N in the soil, (see Figure 6).

The homogenous microcosm system can be set up easily and maintained in a greenhouse or environmental incubator. Due to the small size, many replicates may be used to yield better reproducibility and an increased time range. Equipment may be easily obtained from general hardware or construction suppliers. Organisms and seed may also be easily obtained from seed companies or other biological supply companies. The results from this study were comparable to results from a field study conducted with identical soil, (Knacker *et al.*, unpublished data). Results from microcosm studies can be used to predict environmental risks more accurately by taking into account interactions between organisms, trophic levels, and ecosystem processes. These data presented demonstrate that the integrated soil microcosm may be used as a reliable and less expensive alternative or supplement to field research into the effects of pesticides and other pollutants on soil ecosystems.

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Field and laboratory studies on the effects of the fungicide Quinoxyfen on non-target and beneficial arthropods in cereals

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ABSTRACT

Quinoxyfen (DE-795) is a quinoline fungicide for the specific control of powdery mildews. The effect of quinoxyfen on non-target (beneficial) arthropods under both laboratory and field conditions has been extensively studied with the aim of addressing the data requirements of EU directive 91/414 and to provide important IPM information concerning the commercial use of quinoxyfen.

Under laboratory conditions quinoxyfen was harmless to *Aleochara bilineata* (rove beetle), *Orius insidiosis* (predatory bug), *Poecilus cupreus* (carabid beetle), *Episyrphus balteatus* (hover flies) and *Aphidius rhopalosiphi* (aphid parasitoid). Under large-scale field conditions, quinoxyfen had no detrimental effect on major groups of taxa. It was concluded that quinoxyfen is a highly selective fungicide compound which may be suitable for use in a wide range of arable IPM situations.

INTRODUCTION

The testing of effects of plant protection products (PPP) on non-target arthropods for regulatory and Integrated Pest Management (IPM) purposes has been the subject of many discussions in recent years. Growers, consumers, pressure groups and governments all require information on the side-effects of PPP's to the environment and to natural populations of predators in the field. Quinoxyfen (DE-795) is a quinoline fungicide for the specific control of powdery mildews (Longhurst *et al*, 1996). The effect of quinoxyfen on non-target (beneficial) arthropods under both laboratory and field conditions has been extensively studied. All studies were performed to IOBC principles and ESCORT guidelines (Barrett *et al*, 1994) with the aim of addressing the data requirements of EU directive 91/414 and to provide important IPM information concerning the commercial use of quinoxyfen. This poster outlines the findings from laboratory and field studies performed with quinoxyfen on a range of beneficial arthropods.

MATERIALS AND METHODS

Trials were performed under laboratory, extended laboratory and field conditions. Fortress[1] (EF-1186, a 500 g ai/L SC) a formulation of quinoxyfen for use on powdery mildew in cereals was used at an application rate of 250 g ai/ha. All studies included a water treated control and many contained a suitable toxic reference. The laboratory studies investigated lethal (mortality) and sub-lethal effects (feeding, fecundity or parasitism rate). Details of the laboratory test systems are given in Table 1.

Table 1. Test system details

Species	Common name	Study type
Aleochara bilineata	Parasitic rove beetle	Laboratory study, artificial soil
Aphidius rhopalosiphi	Aphid specific parasitoid	Laboratory study, glass plates
Aphidius rhopalosiphi	Aphid specific parasitoid	Extended laboratory study, barley seedlings
Orius insidiosus	Predatory bug	Laboratory study, glass plates
Poecilus cupreus	Carabid beetle	Laboratory study, artificial soil
Episyrphus balteatus	Hoverfly	Laboratory study, glass plates

A large scale study was conducted in three consecutive years in grassland in Devon, England to investigate the effects of spring and summer applications of quinoxyfen on epigeal arthropods (Brown, K.C. 1998). The same treatments were applied to the same experimental plots during each of the three years of the study. Field work was initiated in March 1994, and continued through three consecutive seasons, finishing in November 1996. Quinoxyfen was applied in the spring at 250 g ai/ha and again in the summer at 150 g ai/ha. Hostathion (420 g/l triazophos) was applied as a toxic reference at 820 g ai/ha in both the spring and summer of each year. Control plots were untreated. All treatments were applied in a volume of 200 l/ha. Surface active arthropods were sampled using pitfall traps, set within the central area of each plot. Samples were collected weekly for one week prior to each treatment date and for four weeks after treatment. Further samples were taken at monthly intervals.

[1] Trademark of Dow AgroSciences

RESULTS AND DISCUSSION

Laboratory studies

Under laboratory conditions quinoxyfen was shown to be harmless to *Aleochara bilineata* (rove beetle), *Orius insidiosis* (predatory bug), *Poecilus cupreus* (carabid beetle), *Episyrphus balteatus* (hover flies) and *Aphidius rhopalosiphi* (aphid parasitoid). A summary of the effects of quinoxyfen on non-target and beneficial arthropods is given in Table 2.

Table 2 Effects of quinoxyfen on non-target and beneficial arthropods – Laboratory studies

Species	Effect	Quinoxyfen	Control	Toxic ref.	Effect
Aleochara bilineata	R (beetles in F_1)	1610	1545	Not included	Harmless
	P (%)	31.2	28.5		
Aphidius rhopalosiphi	M (%)	63	3	Not included	Harmful
	P (mummies/wasp)	6	38.1	N/A	
Aphidius rhopalosiphi	M (%)	13.3	6.7	100	Harmless
	P (mummies/wasp)	16.7	17.2	N/A	
Orius insidiosus	M (%)	23.6	19.9	92%	Harmless
	R (eggs/female)	13	11.8	11.8	
Poecilus cupreus	M (%)	16.7	3.3	100	Harmless
	F (pupae/beetle)	8.8	8.6	N/A	
Episyrphus balteatus	M (%)	14	13	Not included	Harmless
	R (eggs/female)	60	70	N/A	

Note: M = mortality, R = reproductive effects, P = parasitism effects, F = feeding / predation effects

Field studies - epigeal arthropods

When examined on a year on year basis, from 1994 to 1996 in post-treatment plots quinoxyfen had no deleterious effects on arthropods compared with untreated or triazophos treated plots (Fig 1-4). There was a small reduction in lycosid spiders; these are likely to be indirect effects such as on reproduction, behaviour or feeding as they occurred 4 weeks after application. There was no carry over to the following field season pre-treatment trap catches indicating a recovery in the lycosid population. A comparison of pre-treatment numbers and post-treatment numbers, pooled over the three years (Fig 5) generally showed a similar or increased number of arthropods compared with triazophos treated or untreated plots. The bars on the graphs labelled 'others' covered a heterogeneous group of arthropods not belonging to the carabidae, staphylinidae, lycosidae, linyphiidae or colembola, identified separately. This artificial grouping covered all the other types of organisms caught in pitfall traps and is included for completeness. Examples of the types of organisms incorporated under this heading were acari, aphids, coleoptera, diplopoda, diptera, and lepidoptera.

Under conditions of the large-scale field trial conducted over three consecutive years in grassland, quinoxyfen had no detrimental effect on major groups of taxa.

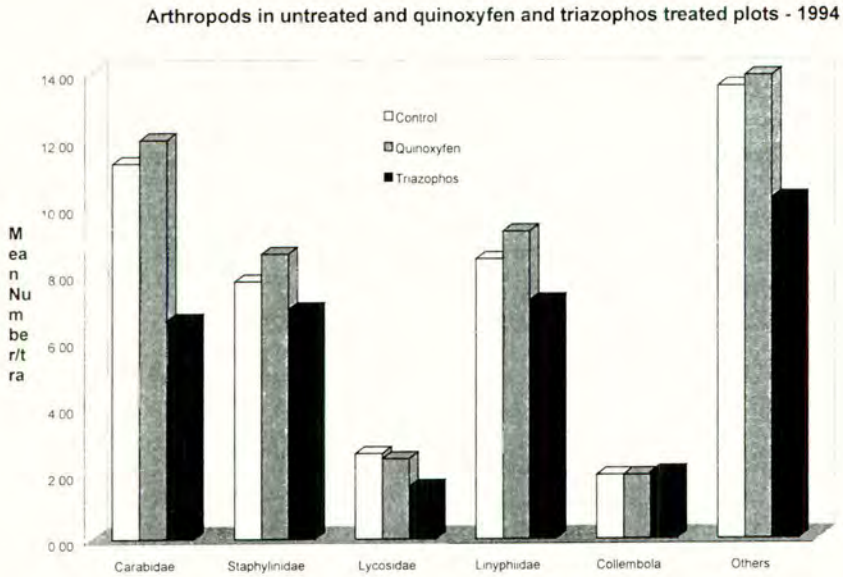

Figure 1 Effects of quinoxyfen on epigeal arthropods – Mean number per trap for each treatment in 1994.

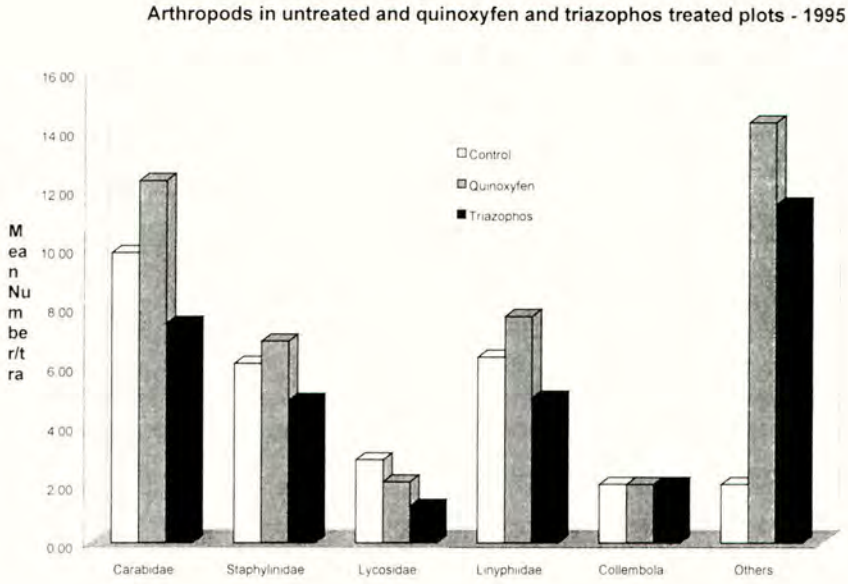

Figure 2 Effects of quinoxyfen on epigeal arthropods – Mean number per trap for each treatment in 1995.

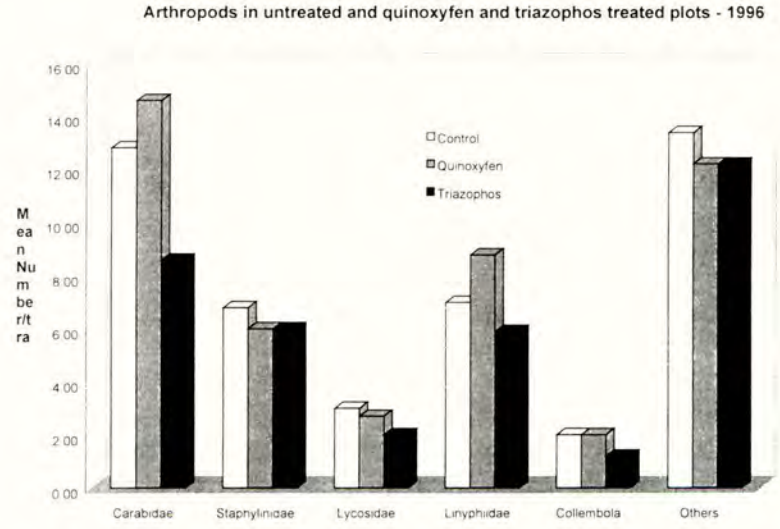

Figure 3 Effects of quinoxyfen on epigeal arthropods – Mean number per trap for each treatment in 1996.

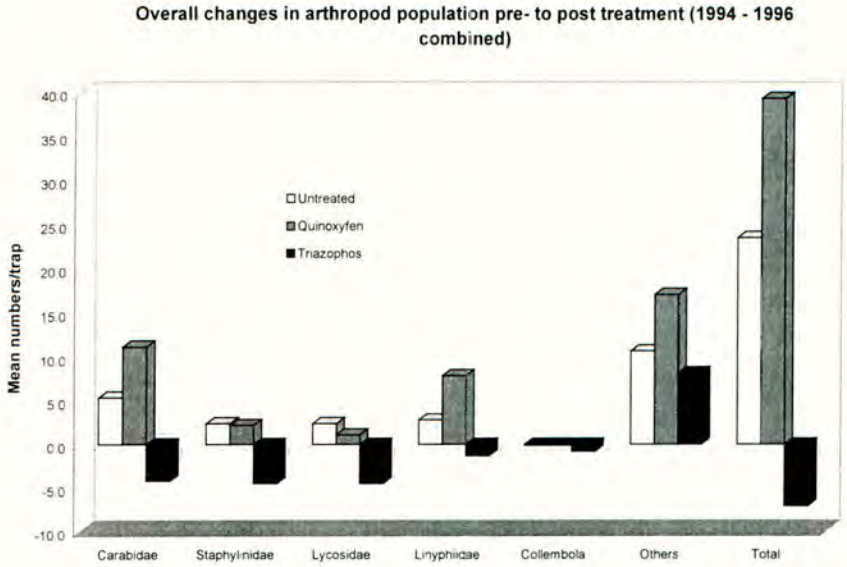

Figure 4 Effects of quinoxyfen on epigeal arthropods - A comparison of pre-treatment numbers and post-treatment numbers, pooled over the three years

CONCLUSIONS

It was concluded that quinoxyfen is a highly selective fungicide compound suitable for use in a wide range of IPM situations. The safety of quinoxyfen to non-target and beneficial arthropods was accurately predicted by laboratory studies and confirmed under field conditions.

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Enhanced biodegradation of metham sodium soil fumigant in Australia

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ABSTRACT

On a farm near Perth, Western Australia, where the soil fumigant metham sodium has been used annually for the past decade, a dramatic case of enhanced biodegradation has been observed. On an area of soil that had received several previous treatments, the maximum concentration of the active toxic compound methyl isothiocyanate (MITC) was less than half that reached in similar, previously untreated soil. Also, MITC was present in the soil for only a fraction of the time of the untreated soil. On sterilisation of the affected soil in an autoclave, followed by treatment with metham sodium, the MITC concentration-time relationship was similar to that of the previously untreated soil.

INTRODUCTION

Enhanced biodegradation of soil-applied pesticides occurs when a population of microorganisms, that by chance have the ability to utilise the pesticide as a food source, builds up (Racke, 1990). This results in more rapid removal of the pesticide from the soil environment than would occur under normal circumstances. The phenomenon is generally induced by multiple applications of the pesticide at relatively short intervals, although instances have been reported from a single application (Felsot AS, 1989, Smelt *et al.*, 1989).

Metham sodium, or sodium *N*-methyldithiocarbamate, is a widely used soil-applied fumigant. On contact with soil moisture it is hydrolysed to form methyl isothiocyanate (MITC) (Lloyd, 1962) which is the active pesticidal compound (Figure 1). It is broad-spectrum, acting on a wide range of soil-borne pests and diseases including insects, nematodes, fungi and weeds. The use of metham sodium in Australia is increasing rapidly as a substitute for methyl bromide because of that compound's phaseout under the Montreal protocol ban on ozone-depleting substances, as well as market demands for high quality, blemish-free produce.

In Europe, where metham sodium has been in use for several decades, instances of enhanced biodegradation have been reported, with the first case occurring in the Netherlands (Smelt *et al.*, 1989). This paper reports a severe example of enhanced biodegradation of metham sodium in a vegetable production enterprise in Australia.

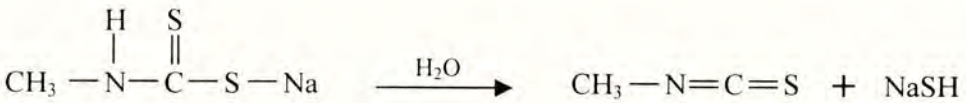

Figure 1. Equation showing the reaction of metham sodium with water to form methyl isothiocyanate (MITC).

MATERIALS AND METHODS

Soil samples were collected from two locations on a vegetable-producing property near Perth, Western Australia. Soil A had never been treated with metham sodium, while soil B had been treated with metham sodium annually for approximately the past ten years. A subsample of soil B (soil Bs) was sterilised in an autoclave at a temperature of 121°C and a pressure of 100 kPa for one hour.

For each experiment, three replicate 150 g samples of soil were placed in 250 ml Erlenmeyer flasks. A sufficient quantity (18µl) of metham sodium soil fumigant was added to each sample. This amount correlates with standard field usage, based on the manufacturer's recommended application rate of 500 litres/ha and assumes penetration to a depth of 30 cm in the soil.

Periodically, 10 g subsamples were removed from each flask and extracted with ethyl acetate (3×8 ml). The extracts were combined and 1 ml of a 11.25 ppm solution of benzyl isothiocyanate added as a normalisation standard. Samples were dried using magnesium sulfate and filtered prior to analysis using gas chromatography (GC) with a flame photometric detector in sulfur mode (394 nm).

The concentration of MITC in the soils was expressed as the percentage of the amount of MITC that could theoretically be produced from the metham sodium applied to the soil, assuming complete conversion of metham sodium to MITC.

RESULTS AND DISCUSSION

On treatment of soil A with metham sodium, the maximum measured concentration of MITC was 93% (Figure 2). The concentration of MITC in the soil subsequently decreased to zero over 17 days. When the same dose of metham sodium was applied to soil B, the maximum measured concentration of MITC was 42%, decreasing to zero in seven hours. For the sterilised sample of soil B (soil Bs), the maximum measured concentration of MITC was 88%, and the concentration reached zero in 18 days.

The maximum concentration of MITC measured in soil A after treatment with metham sodium (93%) correlated well with previous reports of the efficiency of conversion of metham sodium to MITC in the range 90-98% (Smelt and Leistra, 1974, Smelt *et al.*, 1989). The markedly lower value for soil B (42%) indicates that the MITC was being transformed very rapidly, almost as fast as it was being produced. The markedly lower residence time in the soil (seven hours versus 17 days) provides further evidence of rapid transformation of MITC.

In order to determine whether the rapid transformation was the result of a biological process, a sample of soil B was sterilised by autoclaving. This soil (Bs) was then treated with metham sodium and the concentration of MITC present measured. The dramatic increase in both the maximum MITC concentration (88% v 42%) and its persistence (18 days v seven hours) in soil Bs when compared with the unsterilised soil B confirms the microbiological basis of the rapid transformation in the unsterilised soil.

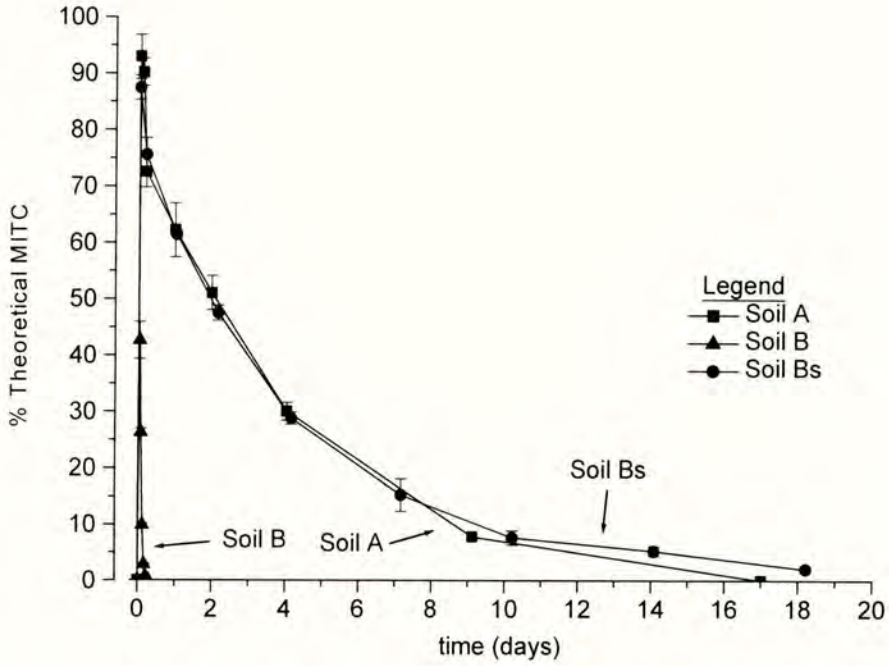

Figure 2. Change with time in the percentage of the theoretical amount of MITC produced in the three soils after treatment with metham sodium. Soil A was cultivated without previous exposure to metham sodium, soil B had a previous history of metham sodium use and soil Bs was a duplicate portion of soil B which was sterilised by autoclaving.

A measure of the toxic potential of MITC in the three soils was approximated by calculating the areas under each of the three curves shown in Figure 2. When normalised to soil Bs which gave an area of 100%, soil A gave an area of 94% and soil B gave 0.98%. The high value for soil A indicates that the effect of biological processes on the transformation of MITC in this soil was minimal. In soil B, biological processes are clearly very active in transforming the MITC, and thereby reducing its toxic potential.

ACKNOWLEDGEMENTS

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Comparison of sorption and degradation of imidacloprid in soils from a greenhouse and an open field in Spain

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ABSTRACT

Imidacloprid is extensively used in greenhouses and open fields. There is a lack of data regarding the fate of pesticides under greenhouse conditions. The increased use of pesticides may have implications for the contamination of ground water systems. The fate of imidacloprid in soils from a greenhouse in Almeria and an open field in Valencia were compared. Soils at these sites are very alkaline and have a low amount of organic carbon. Imidacloprid is very persistent in these soils, with estimated half lives of 165 to 247 days. Due to the low sorption (Koc=100.74) and low metabolic activity of these soils, imidacloprid could potentially leach. The loss of imidacloprid follows dual first order kinetics in Almeria and Valencia soils. Soil properties, such as clay and organic carbon content influence imidacloprid sorption and degradation. There was no significant difference between the degradation and sorption of imidacloprid in soils from the greenhouse and the open field.

INTRODUCTION

Imidacloprid is a popular insecticide currently used in Spain. Almeria has intensive horticulture based on a raised bed system under greenhouses irrigated from underground aquifers. There is concern that the aquifers are becoming depleted and contaminated (Martinez Vidal, 1993). The Valencia region has intensive citrus production in the field and pesticides have been detected in rivers.

Soils at these sites are very alkaline and have a low amount of organic carbon. Previous study in these sites showed that imidacloprid was very persistent and mobile in soil (Lopez-Capel & Wilkins, 2000; Flores-Cespedes *et al.*, 1999).

This study aims to evaluate the loss kinetics and soil-water partition coefficient of imidacloprid in these particularly alkaline soils. This study contributes to the understanding of imidacloprid movement and transformations in soil, and the influence of soil properties, water content and temperature on sorption and pesticide loss.

MATERIAL AND METHODS

Soils

The soils were provided by the university of Almeria (Spain). The topsoil layer from a citrus field in Sagunto (Valencia) was collected at the end of cropping season in July 1998. Four soil layers were collected from the greenhouse in La Mojonera (Almeria) between the two cropping seasons in February 1999. The four soil layers consisted of two layers on the raised bed, sand (0-10cm) and clay (10-18cm), and two layers of the original (native) soil, original 1 (18-50cm) and original 2 (50-100cm). Both greenhouse and open field are representative of the typical use of the compound monitored and of the agricultural practices. Some of the soil properties are described in Table 1. Soil pH was determined in a 1:2 soil/water suspension using a glass electrode, moisture holding capacity by the water outflow experiment using Haine's plates (-5Kpa), clay content by the pipette sedimentation method, organic carbon content by ANCA (Automatic Nitrogen and Carbon Analyser), microbial carbon by fumigation-extraction method and CEC (Cation Exchange Capacity) by Page's method. These soils have high pH, low organic carbon, and low microbial activity.

Table 1. Chemical and physical soil properties

Soil layer	% clay	%H_2O (70%MHC)	pH	% organic carbon	Microbial C µg C/g dry soil	CEC meq/100g soil
Top soil Valencia	30.2±0.6	16.11±0.11	8.22±0.03	0.56±0.02	193.7±15.3	6.35±0.36
Sand (Almeria)	2.5± 0.1	3.86±0.02	8.53±0.18	0.30±0.02	166.6±23.4	0.87±0.39
Clay (Almeria)	45±0.6	15.36±0.02	8.63±0.02	0.35±0.01	449.7±12.9	5.43±0.67
original 1 (Almeria)	46.5±1.0	11.05±0.13	8.41±0.06	0.67±0.03	397.8±29.9	7.90±0.24
original 2 (Almeria)	38.8±0.9	7.82±0.41	8.88±0.03	0.66±0.01	220.2±39.6	4.47±0.33

Degradation experiment

The soils were spiked with imidacloprid, 0.23 mg/ kg dry soil, and moisture was raised to 70%MHC (Moisture Holding Capacity) with de-ionised water.

Spiked soils (250g) were transferred into open glass containers. Triplicates of the five soils were incubated at 20 and 30°C in the dark, covered in black plastic bags and placed over a water bath to keep the soil moist. Soil samples of 12g were taken at 0, 1, 3, 5, 7, 15, 30, 60, and 90 days and stored in the freezer until analysed.

Soil samples (12g) were transferred to centrifuge tubes, shaken with acetone (25ml) for 4 h at 250 rev/min, and centrifuged at 4500 rev/min for 10 min. The solvent supernatants were then evaporated, redissolved in acetonitrile to a volume of 2ml, and filtered into HPLC glass vials for analysis.

Samples were analysed by Gilson HPLC-DAD at 269nm using a mobile phase of (35:65 v/v) acetonitrile:water and a flow rate of 1ml/min. Imidacloprid was detected at 3.89 minutes with a detection limit of 0.025 mg/kg.

Sorption experiment

Following the batch equilibration technique; two millilitres of imidacloprid solution prepared in calcium chloride (0.01M) were added to 1g of air-dried soil in 25 ml glass vials with caps. Triplicate samples of each soil were used with six imidacloprid concentrations ranging from 0.5 to 30mg/L. After shaking the samples to equilibrium, 24 h at 25°C, they were centrifuged at 10,000rev/min for 10 min.

Imidacloprid concentrations in the supernatant solutions (C_e) were determined by HPLC at 269nm using a mobile phase of acetonitrile:water (35:65). The amount of pesticide sorbed (C_s) was calculated from the difference between the initial and the equilibrium solution concentrations.

RESULTS

Degradation results

The average extraction efficiencies for imidacloprid (n=12, recovery as mean % recovery ± SE) in the Almeria and Valencia soils are 88.32±6.05; 86.62±3.73; 78.95±5.63; 81.28±5.62, and 82.32±7.41 in sand, clay, original 1, original 2 and Valencia respectively.

The percentage of remaining imidacloprid in soil slowly decreases with time. Figures 1 show the results of incubating imidacloprid (at 0.23mg/kg) with the four soil layers from the greenhouse and one from an open field at two temperatures (20 and 30°C).
The overall loss rate of imidacloprid is calculated by assuming that the pesticide loss follows a first order equation. DT50 values, shown in table 2, are estimated from 1st order rate constants, where k= Ln2/DT50.

The loss rate constant of imidacloprid increases with temperature. The change of the overall rate constant between 20 and 30°C is Q_{10} = 1.77± 0.19.
Although the loss of imidacloprid fits first order kinetics, it was observed that imidacloprid follows dual first order kinetics. There is fast degradation from 0 to 7 days, followed by slow degradation from 7 to 90 days.

Table 2. DT50 and Q_{10} values of imidacloprid in Almeria and Valencia soils.

soil layer	$DT50^{20°C}$	r^2	$DT50^{30°C}$	r^2	Q_{10}
sand	177.7	0.669	117.5	0.366	1.51
clay	247.5	0.917	121.6	0.953	2.03
original 1	165.0	0.929	99.0	0.719	1.66
original 2	173.3	0.762	96.3	0.531	1.80
valencia	187.3	0.693	101.9	0.654	1.83

r^2= correlation coefficient obtained from fitting first order kinetics. DT50 in days.

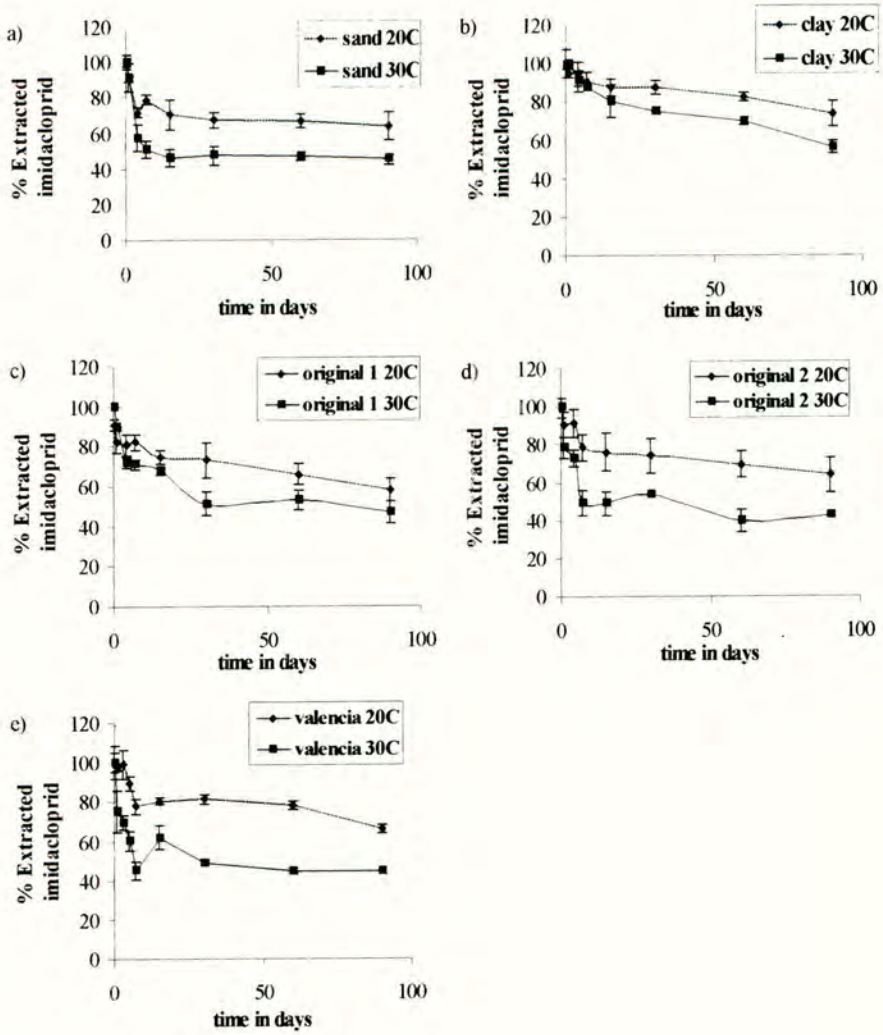

Figure 1. Percentage of extracted imidacloprid in the five soil layers: a) sand, b) clay, c) original 1, d) original 2 and e) Valencia.

Sorption results

The sorption isotherms for imidacloprid in Almeria and Valencia soils are shown in figure 2. The data fitted linear models of sorption. Imidacloprid sorption coefficients are shown in table 3.

K_d values were calculated using the linear isotherm equation $C_s = K_d C$,

where C_s= pesticide adsorbed per kg of soil, mg Kg^{-1}

C_e= equilibrium solution concentration, mg L^{-1}

K_d= adsorption coefficient, L Kg^{-1}

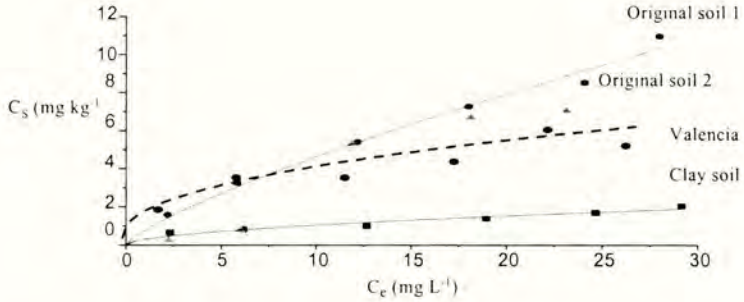

Figure 2. Sorption isotherms for imidacloprid on Almeria and Valencia soils.

Factors that affect sorption and degradation

In order to know the factors that influence the degradation and sorption of imidacloprid on the soil, soil properties of these soils were correlated to loss rate constants (-k) and K_d values by regression analysis. Table 4 shows the correlation coefficients between some soil properties and K_d and loss rate values.

Table 4 Correlation coefficients of K_d and (-k) values with soil properties

	r^2 (%OC)	r^2 (%clay)	r^2 (CEC)	r^2 (μC)	r^2 (%H_2O)
K_d	0.894	0.850[#]	0.871[#]	0.002	0.681[#]
(-k)	0.988[ϕ]	0.869[#]	0.092	0.758[#]	0.087

[ϕ] Excluding value from the sand layer. [#] Excluding value from the clay layer.
μC = Microbial carbon, %H_2O = % of soil moisture

The K_d and loss rate constant values correlated with the organic carbon and clay content of the soil. However, there were not significant correlations (95% CI) between the soil properties and K_d and (-k) values. In most cases correlation was only achieved by omitting the clay layer values. For comparative reasons a K_d value of 0.01 was arbitrarily selected for the sand layer, as little or no sorption was expected in this layer. The K_{OC} parameter is calculated as the slope of the regression line between K_d and the OC content. The function obtained was y=100.74x-0.2714 with r^2=0.8939, then K_{OC} =100.74. This K_{OC} value suggests that imidacloprid sorption in these soils is low.

DISCUSSION AND CONCLUSION

Imidacloprid is very stable in these soils, showing low degradation and low sorption. Half lives were not reached after 90 days incubation, and are estimated to be between 167 and 247 days. The loss of imidacloprid appears to follow dual first order kinetics. There is fast degradation from 0 to 7 days followed by slow degradation from 7 to 90 days. K_d and K_{OC} values suggest that imidacloprid sorption to the soil is low. The degradation and sorption results obtained in this experiment agree with previous studies (Lopez-Capel & Wilkins, 2000, Gonzales-Pradas *et al.*, 1999).

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As soils have low microbial activity and low carbon content, imidacloprid sorption and degradation were expected to be low. Although imidacloprid would be predicted to readily leach; previous studies concluded that it does not appear to leach below the surface soil (Rouchaund *et al.*, 1996; Hellpointer, 1998).

Environmental conditions such as temperature directly affect imidacloprid degradation rate by increasing pesticide loss with time. Soil properties, such as clay content and organic carbon correlated with sorption and degradation. However, other soil properties did not significantly correlate, and correlation could only be achieved by omitting the artificially raised clay layer, which was extracted from a local quarry.

There was no significant difference between the loss of imidacloprid in soils from a greenhouse and an open field in Spain (95% CI). Sorption values were also similar. This could be due to the fact that these soils had similar qualities.

ACKNOWLEDGEMENTS

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Behaviour of aldicarb in mediterranean irrigated soils

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ABSTRACT

Aldicarb is a very useful insecticide, acaricide and nematicide for citrus plagues, but under certain circumstances may pose an environmental risk in view of its potential to leach. Several indices such as PBT (percolation, bioaccumulation, toxicity), GUS (Groundwater Ubiquity Score) etc. have indicated aldicarb is in the high mobility category. This paper reports the degradation and leaching of aldicarb applied four times per year (February, April, June and July) at two rates (10 and 40 kg/ha per application) to Spanish soil. Aldicarb is almost immediately degraded to the sulfoxide and more slowly to the sulfone in soil. The study evaluates the degradation of aldicarb in both soil columns and in field microplots. The overall recommendation is that for safe use this product must be handled with care, by trained people and with a pre-harvest interval restriction in Spain of at least 100 days.

INTRODUCTION

Aldicarb is a well established methyl carbamate pesticide (Weiden *et al.*, 1965) used as an insecticide, nematicide and acaricide in several crops (citrus, cotton, peanuts, sugar beet, soybeans etc). In citrus, aldicarb controls aphids, whiteflies, mites and citrus nematodes.

The product, because of its toxicity, has restrictions on its sale and use. Operators must use appropiate protective clothing and there is a 100 day pre-harvest interval requirement for citrus in Spain. Aldicarb also has the potential to leach, with environmental indices such as **GUS** ($logDT_{50}$(4-log Koc) and **PBT** (5GUS +log Kow)/log DL_{50} values of 2.1 and 12.6 respectively placing it in the high mobility category.

Aldicarb (ALD) in soil oxidizes rapidly to aldicarb sulfoxide (ASO) and more slowly to aldicarb sulfone (ASN) (Smelt *et al.*, 1978; Bromilow *et al.*, 1980).

The purpose of this work is to show the degradation pattern in mediterranean irrigated soils, studied in intact soil columns and microplots in the field.

METHODS AND MATERIALS

Soil column experiment

Intact soil column samples (32 columns each from 0-25 cm depth) were taken with stainless steel open probes (10 cm i.d. and 25 cm height) from a sandy citrus orchard which had no previous history of aldicarb treatment. The soil characteristics of the citrus orchards are shown in Table 1.

Table 1. Soil characteristics

Organic matter (%)	0.25
Sand (%)	95.6
Silt (%)	1.6
Clay (%)	2.8
Calcium carbonate (%)	0.5
pH(1/2.5 soil/water)	7.9
CEC (meq/100g)	0.10
Density (g/ml)	1.68

To the surface of each column was applied the equivalent of 10 or 40 kg of aldicarb a.i./ha. The columns were irrigated with water, using a 50 mm pipette, until the water eluted from the base of the column (350 mm of total water). They were then maintained in the dark without further irrigation at 10°C (16 columns) or 25°C (16 columns). At timepoints 3, 7, 14 and 28 days after treatment (DAT), 2 columns of each rate and temperature were taken for analysis and the soil divided into sections equivalent to depths 0-7, 7-14 and 14-21 cm.

Microplot Experiment

Uncropped microplots of 2 x 4m were used in quadruplicate in a randomized experiment, designed and located at the IVIA farm (39°34´50´´N,00° 24´17´´W, at 55m altitude) at Moncada Valencia.-Spain). Main soil characteristics are shown in Table 2.

Table 2. Soil characteristics

Organic matter (%)	1.0
Sand (%)	63.4
Silt (%)	24.6
Clay (%)	12.0
Calcium carbonate (%)	21.1
pH (1/2.5 soil/water)	7.8
CEC (meq/100g)	13
Density (g/ml)	1.3

Two rates of application (10 and 40 kg aldicarb a.i./ha) were used. Applications were made three times in one year in February, April and June and once in July of the previous year. Immediately after treatment, the plots were irrigated with approximately 60 mm of water (measured in a water mill A.OTT-Kempten type C2"10.150" N°

84152). Further irrigation was carried out when 50 mm of accumulated evaporation was reached in a class A pan of the IVIA agrometereological field station located less than 100 m from the plots. Samples were taken with a soil probe to a depth of 35 cm (6 points in each subplot) without disturbing the soil layers, at timepoints of 1, 8, 14, and 28 days after treatment (DAT). The soil was divided into 0-5, 5-10, 10-15, 15-20, 20-35 cm layers. All the soil samples were air dried, sieved (2 mm diameter), wrapped with aluminium foil and stored in the dark at –25°C prior to analysis. The extraction was performed as described by Ou et al.(1988). Clean-up was as described by Page and French (1992). Analysis was carried out by HPLC (precolumn Guard-Pak Nova-Pak C18, column C18, pump 600E, 715 autoinjector, CHM oven, fluorescence detector 474, PCRS postcolumn pumps, Milenium data system, all from Waters).

RESULTS

Figure 1 shows the proportion of ALD, ASO and ASN in the different soil samples from the soil column experiment.

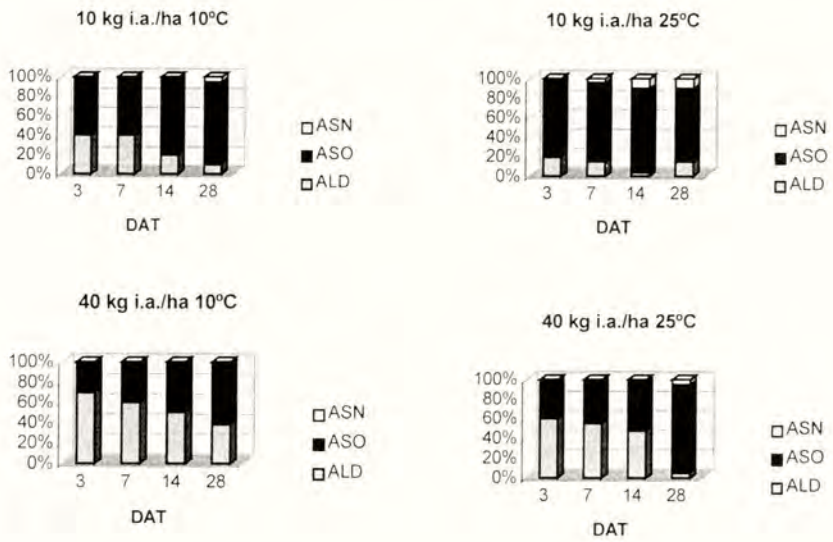

Figure 1. Proportions of ALD, ASO and ASN present in the total residue detected in the column experiment.

The effect of temperature on aldicarb degradation is clearly visible at both rates. The proportion of ASO and ASN in the soil column maintained at 25°C is greater than at 10°C for both rates. The predominant compound detected at 28 DAT is ASO. It is important, also, to note that application rate affects the rate of degradation of aldicarb. In the first soil samples (3 DAT) the proportion of ALD relative to the total compound

residues, is less than 40% in the lower rate (10 kg a.i/ha) but more than 50% in the higher rate (40 kg a.i./ha).

The results of the microplot experiments are shown in Figures 2 , 3 and 4.

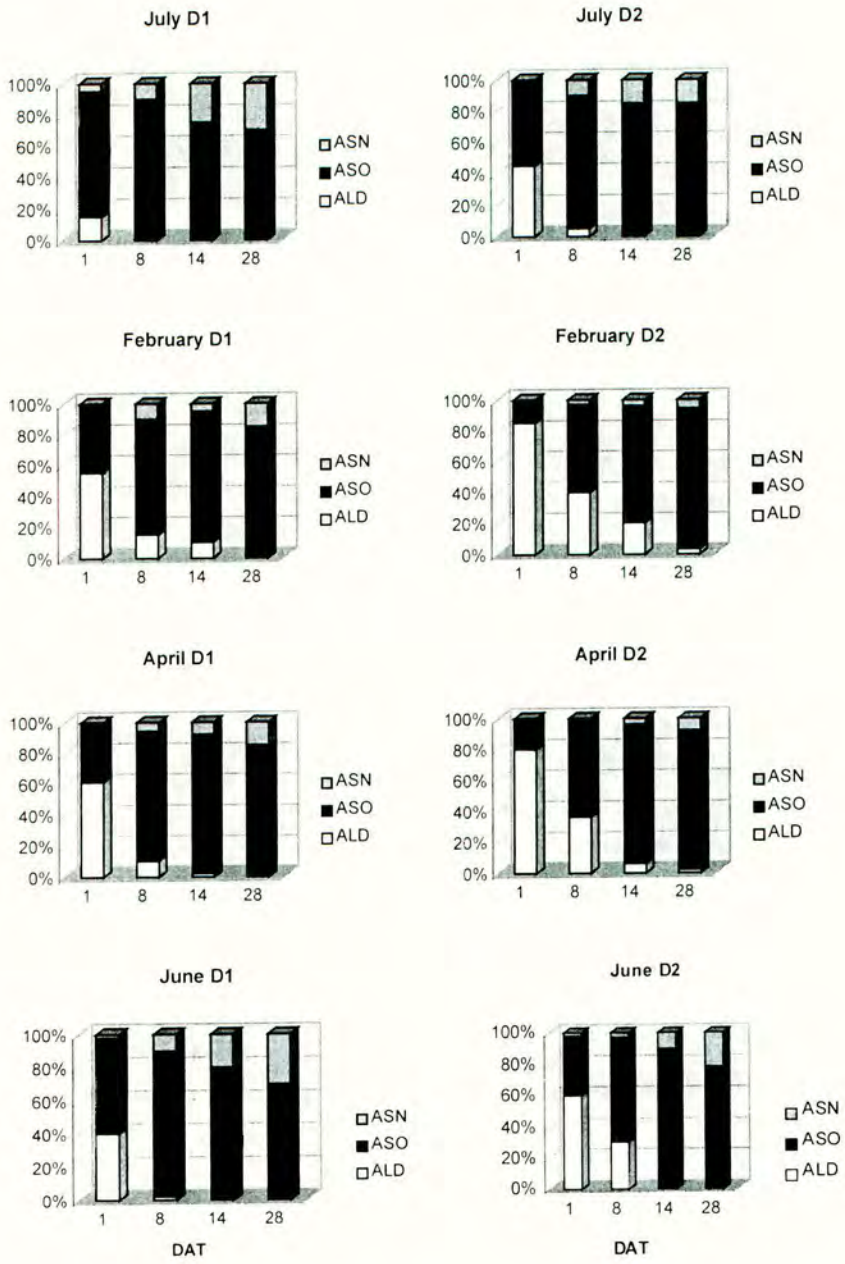

Figure 2. ALD, ASO and ASN from the total residue detected (TRD) in the microplot experiment.

These results show a clear influence of the season upon the degradation rate of aldicarb. Figure 2 shows that parent ALD at 1 DAT in July represents only 10% of the total residue whereas in February or April the corresponding figure is approximately 60%. Furthermore ALD is still detectable after the 28 DAT sampling in the colder seasons of application (February and April). The most soil persistent product is metabolite ASO. ASN also appears, but more slowly, and it represents a smaller proportion of the total than ASO.

Figures 3 and 4 show the concentrations of components in the soil samples, at the different depths and DAT, in the July and February seasons respectively. The profiles of the two figures are distinct from one another, with the July plot showing much lower residues of ALD, ASO and ASN at all depths and sampling times than February.

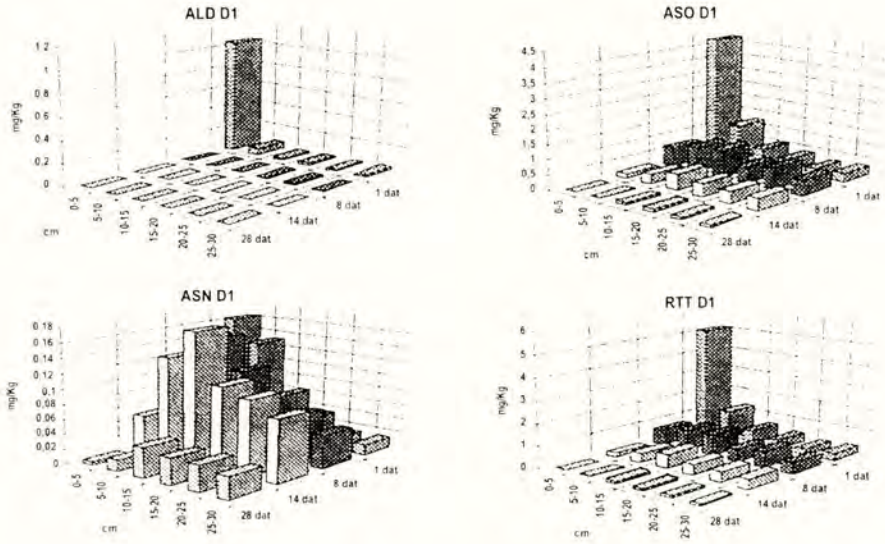

Figure 3. ALD, ASO, ASN and RTT (total residue detected) average concentration in the July microplot experiment.

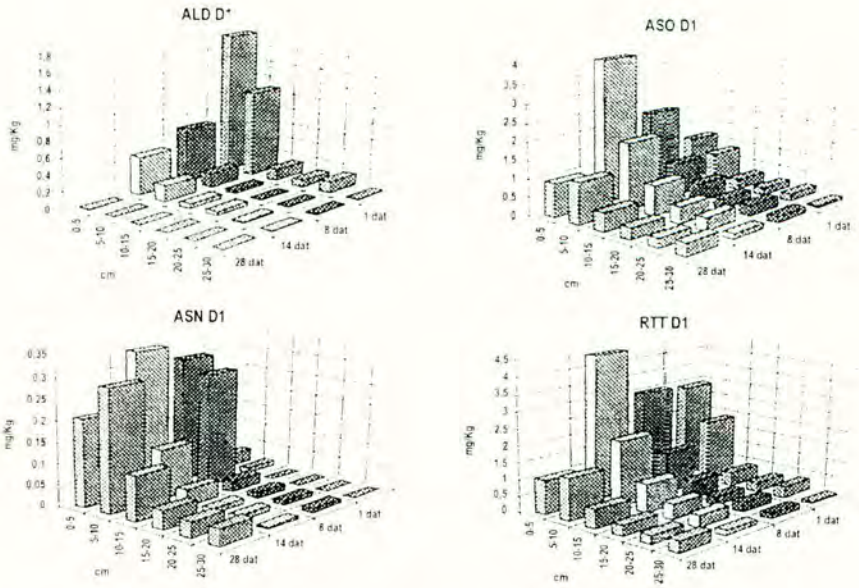

Figure 4. ALD, ASO, ASN and RTT (total residue detected) average concentration in the February microplot experiment.

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Degradation and adsorption of an anthranilate fungicide in soils from Northumberland, UK

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ABSTRACT

The degradation and adsorption of an anthranilate fungicide were examined in four soils from Northumberland, UK . These were selected to give a range of physical and chemical properties. Degradation studies were performed at two water contents. Although no significant correlation was found between degradation and soil properties, differences were observed between the two water contents. Adsorption was found to be correlated with soil cation exchange capacity.

INTRODUCTION

The fate of a pesticide in the soil environment is determined by the physiochemical properties of the compound and soil physical, chemical and biological properties, influenced by climatic and management factors. Within the soil environment a pesticide may be degraded or undergo transfer processes, including volatilisation, leaching and adsorption (Ashton & Monaco, 1991). The study of interactions between a pesticide and the environment are important in both predicting its fate and optimising its use.

Pesticide degradation in the soil system may be by biological, chemical or photochemical processes. The route and rate of degradation are influenced by a range of soil properties, including soil pH, organic matter content, texture and microbial population.

In addition to degradation, pesticide molecules may become associated with the solid phase of the soil matrix by the process of adsorption. Adsorption can reduce the availability of a pesticide in solution, and so influences both performance and degradation. Adsorption in soil is strongly influenced by soil organic matter and clay content, the soil components principally involved in adsorption processes (Arnold & Briggs, 1990).

As the properties which influence pesticide degradation and adsorption vary between soils, it follows that the extent of these processes in a particular soil will depend, to some degree, upon the chemical, physical and biological properties of that soil. The relationship between soil properties and pesticide adsorption and degradation can be examined through the study of these processes in soils which differ in their properties.

The present study was undertaken to investigate the influence of soil properties on the degradation and adsorption of the anthranilate fungicide AEC623892 (methyl - N - (5 chloro, 6 methoxynicotinoyl) - N - methyl anthranilate). This recently developed

compound has phenyl and pyridine moieties with ester and amide functional groups. It is anticipated to have moderately high dissociation constant (pKa) inferred by the pyridyl moiety. AEC623892 degrades to mainly two metabolites. Metabolite 1 results from microbial degradation and is the major metabolic product. Metabolite 2 is the product of chemical degradation (see Figure 1) (Aventis, 1998). Degradation and adsorption of AEC623892 were examined in four soils from Northumberland, UK at two water contents. The soils were selected to give a range of properties. Correlation and regression analysis were used to determine soil properties having the greatest influence on the degradation and adsorption of AEC623892.

MATERIALS AND METHODS

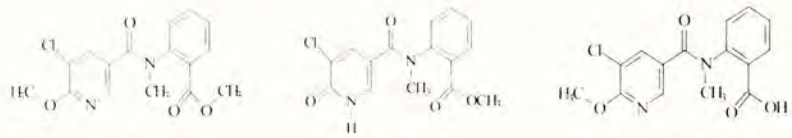
Soils and chemicals

Four soils were collected from the surface 10 cm of sites in Northumberland, UK. Soils were from the Rivington, Hallsworth, Brickfield and Wharfe series. The soils were air dried and sieved to <2 mm. Their 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 mixture of soil and water (1:3 soil water ratio) using a glass electrode. Total carbon was determined by automated nitrogen carbon analysis (ANCA) and cation exchange capacity (CEC), by the recommended MAFF method (MAFF, 1986). The gravimetric water content of air dry soil was determined by oven drying small samples of soil. The water content of soils at 0.1 bar suction was determined using Haines funnel suction plate apparatus.

Table 1: Soil physical and chemical properties, and % H_2O at air dryness and 0.1 bar suction

Soil	pH (in H_2O)	CEC (meq/100g)	% sand	% silt	% clay	% C	% H_2O (air dry)	% H_2O (0.1 bar)
Rivington	6.2	11.5	82.3	9.8	7.9	3.46	13.8	19.1
Hallsworth	7.1	19.6	44.5	22.8	33.1	2.84	22.4	41.1
Brickfield	6.9	21.8	59.1	19.6	21.3	2.73	15.03	33.2
Wharfe	7.5	9.2	65.2	22.7	12.1	3.52	14.1	33.0 .

AEC623892 (98% pure) was supplied by Aventis Crop Science UK Ltd. Acetonitrile was HPLC grade (Fisher). Other chemicals were technical grade.



AEC623892 Metabolite 1 (AEC631046) Metabolite 2 (AEC630627)

Figure 1: Chemical structures of AEC623892 and metabolites

Pesticide analysis

All analysis was by high performance liquid chromatography (HPLC). A Shimadzu SCL 6B HPLC was used, with a SPD - 6AV UV - vis spectrophotometric detector. A Sphereclone 5µm ODS (2) 250 x 46 mm column was used for analysis of samples from the degradation study. A Luna 5µm C18 (2) 150 x 46 mm column was used for analysis of samples from the adsorption study. The mobile phase was isocratic and comprised 55% acetonitrile, 45 % water. The flow rate was 1 ml min^{-1} . The HPLC detector was operating at 220 nm.

Degradation of AEC623892

Approximately 250g (dry weight) of sieved, air dry soil was treated with AEC623892 at an application rate of 30µg/100g dry soil. The compound was applied to the soil in acetonitrile and thoroughly incorporated. Treated soil was divided to give three replicates and each placed in glass vessels (250 ml capacity). The vessels were covered with Parafilm® and incubated at 20 °C in darkness. A further 250g dry weight of each soil was brought to 0.1 bar moisture through the addition of distilled water. These soils were treated and incubated as previously described. The incubation period was 49 days for air dry soil and 35 days for soil at 0.1 bar moisture. The mass of incubated soil was checked throughout the incubation period, and distilled water added where necessary to bring soil back to the initial water content.

Samples of approximately 10g dry weight were removed from the incubation vessels periodically throughout the incubation period. AEC623892 was extracted from the soil samples by shaking with acetonitrile. 12 ml acetonitrile was added to each sample and shaken at 250 rpm on an orbital shaker for 90 minutes. The samples were centrifuged at 2500 rpm for 15 minutes. The resulting supernatants were decanted and retained. A further 6 ml acetonitrile was added, and the samples shaken for 15 minutes followed by centrifugation. The supernatant was combined with that previously collected. This extraction method was found to give 76.5 % (± 6.04%) recovery of AEC623892 from the soils used in this study. The resulting extracts were concentrated to 2 ml under a stream of nitrogen and filtered <0.45µm prior to HPLC analysis.

Adsorption of AEC623892

Adsorption isotherms were obtained using the batch equilibrium method (OECD, 1997). Aliquots of 7g (dry weight) air dry soil were placed in centrifuge tubes (40 ml capacity) and shaken with 30 ml 0.01M calcium chloride for 12 hours at 250 rpm. Following this period, AEC623892 in aqueous solution was added to the soil - calcium chloride mixture to give five final concentrations of 0.7, 0.35, 0.07, 0.035 and 0.007 µg / ml. Triplicate samples were prepared for each concentration and each soil. The tubes were shaken for 24 hours. Previous studies showed that equilibrium was attained after 24 hours. The samples were then centrifuged at 4500 rpm for 15 minutes. Approximately 2 ml of the supernatant was removed and filtered <0.45µm prior to HPLC analysis.

RESULTS & DISCUSSION

Degradation of AEC623892

The % of AEC623892 and Metabolite 1 remaining at each sampling point, for each soil, are presented in Figures 2, a-d. Metabolite 2 was not detected in any case.

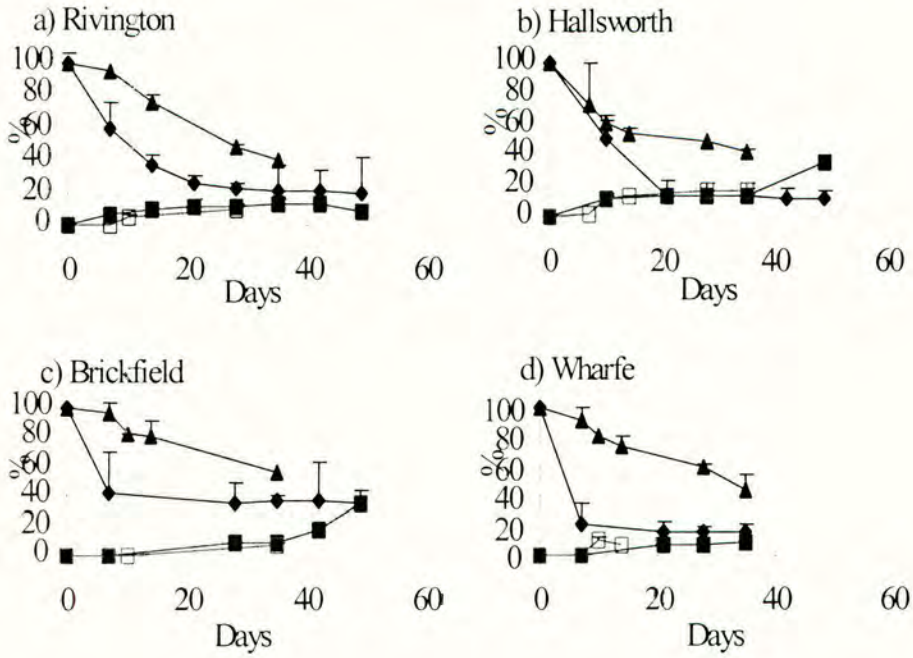


Figure 2. % AEC623892 remaining in air dry soil (◆) and soil at 0.1 bar moisture (▲), and % Metabolite 1 extracted from air dry soil (■) and soil at 0.1 bar moisture (□). Error bars represent the standard deviation of three replicates. Where these are not visible, they are obscured by the data point marker

The pattern of decline of AEC623892 differed between the two soil water contents. Loss of AEC623892 from air dry soil appeared to be more rapid than from soil at 0.1 bar moisture. However, the rate of formation of Metabolite 1 was similar in soil at air dryness and soil at 0.1 bar moisture indicating that the faster decline in AEC623892 extracted from air dry soil was not due to degradation alone. Rate constants were not derived for AEC623892 degradation in air dry soil.

Adsorption may contribute to these observed differences. Increased pesticide adsorption has been observed in dry soils. This has been attributed to the reduction in thickness of the water film coating soil minerals, so concentrating the compound close to adsorptive sites (Goetz *et al.* 1986). Furthermore, drying and rewetting of soil has been shown, in some cases, to reduce the extractability of pesticides from soils(García – Valcárcel & Tadeo,

1999). Through checking the total soil mass at each sampling point, some loss of water from soil between sampling dates was evident. This was rectified through the addition of distilled water. This slight drying and rewetting of the soil over the course of the incubation may have contributed to the decline in AEC623892 extracted from the air dry soil.

A rapid decline in % AEC623892 remaining was not observed in soil maintained at 0.1 bar moisture. Regression analysis of the log transformed AEC623892 concentrations against time gave a good fit over the complete incubation period. The correlation coefficient was statistically significant for all four soils ($P<0.05$). The first order rate constant (k, day $^{-1}$) was calculated. Values of k, presented in Table 2, did not differ significantly between soils ($P>0.01$). Regression analysis of k against soil properties did not indicate a strong relationship between k and any of the measured soil properties . Correlation coefficients resulting from the regression of k against soil properties are presented in Table 4.

Table 2: k (day $^{-1}$) and correlation coefficients (r) for soils at 0.1 bar moisture

Soil	k (day $^{-1}$)	r
Rivington	0.027	0.981
Hallsworth	0.021	0.931
Brickfield	0.018	0.973
Wharfe	0.023	0.980

Adsorption of AEC623892

Adsorption isotherms of AEC623892 in Rivington, Hallsworth, Brickfield and Wharfe soils were described using the Freundlich equation (OECD, 1997),

$$C_s = K_f \cdot C_{aq}^{1/n}$$

where C_s is the adsorbed amount 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: $\log C_s = \log K_f + 1/n \log C_{aq}$. The values of K_f, 1/n and correlation coefficients (r) derived for the adsorption of AEC623892 in the four soils are given in Table 3.

Table 3: Freundlich constants and correlation coefficients (r) for AEC623892 adsorption

Soil	K_f	1/n	r
Rivington	17.39	0.46	0.999
Hallsworth	46.84	0.47	0.976
Brickfield	57.70	0.36	0.986
Wharfe	8.24	0.83	0.960

The adsorption isotherm for AEC623892 was found to fit the Freundlich equation well, indicated by high values of r. The correlation coefficients (r) resulting from the correlation of K_f with soil properties are presented in Table 4.

Table 4: Correlation coefficients derived from the regression of K_f and k against measured soil properties. *** indicates significance at $P < 0.001$

	% sand	% silt	% clay	% C	CEC	pH
K_f	-0.640	0.277	0.754	-0.992***	0.999***	-0.029
k	0.198	-0.061	0.527	0.275	-0.108	0.680

The results of correlation of K_f with soil properties suggest a relationship between K_f and %C and soil CEC. A strong positive correlation between Kf and %C is usual. In this case, the relationship appears to be negative. The reliability of this result is questionable; it may be that the range of %C represented by the test soils (2.73% - 3.52%) was insufficient to provide an accurate representation of the effect of %C on adsorption of AEC623892. The effect of CEC upon adsorption is related to the ability of a compound to ionise and thus generate positively charged sites to interact with the negatively charged cation exchange complex. The apparently significant relationship between CEC and adsorption of AEC623892 may be partially attributed to the expected high pKa of this compound. The determination of soil properties influencing both k and K_f is complicated by the interrelation of soil properties. There appears to be some relationship between k and K_f; high values of K_f tend to correspond to low values of k in these soils, implying that adsorption does have some influence over degradation.

ACKNOWLEDGMENTS

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