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Persistence of Chlorpyrifos, Fenamiphos, Chlorothalonil, and Pendimethalin in Soil and Their Effects on Soil Microbial Characteristics

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Pesticides should be used in such a way that their residues do not build-up in the environment and that they have minimal effect on non-target organisms. There is a continuing need to evaluate the factors influencing pesticide dissipation rates from soil, and their potential to affect soil ecological characteristics in different situations. The effects of pesticides on soil microbial ecology have attracted particular attention over the years (Beare et al., 1992), since the structure and function of the soil microbial community have been correlated with general soil health. Soil microbial characteristics also influence rates of pesticide dissipation, and the ideal compound would persist long enough to give adequate pest, disease or weed control and then degrade to inert products. Most studies of the environmental fate and ecotoxicology of pesticides are done with a single application of the study compound, but in practice, particularly in tropical countries, pesticides may be applied repeatedly to the same crop. The objectives of the present experiments were to study the effects on degradation rates of repeated application of a number of soil-applied pesticides, and to investigate the effects of the selected pesticides on soil microbial community function. The compounds studied were a representative insecticide, nematicide, fungicide and herbicide (chlorpyrifos, fenamiphos, chlorothalonil and pendimethalin, respectively), all of which are used extensively world-wide.

MATERIALS AND METHODS

Commercial formulations of chlorpyrifos (Dursban 4, 48% a.i.), chlorothalonil (Cropguard, 44.1%, a.i.) and pendimethalin (Stomp, 32.8% a.i.) and analytical grade fenamiphos were used throughout these studies. Analytical grade samples of all four compounds plus their major degradation products were used to prepare standards for analytical quantification. Soil from a field site at Horticulture Research International, Wellesbourne, UK was used and it was sieved to pass a 3-mm mesh. Its major properties were: organic matter (loss on ignition), 4.61%; pH in 1:1 soil : distilled water, 6.8; biomass, 169 mg C/kg; gravimetric water holding capacity, 33.9%; clay, 18%; silt, 8%; sand, 74%.

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Three replicate top-soil samples were collected from the 0-10 cm layer of the site. Amounts of soil (1 kg) were treated separately with chlorpyrifos, chlorothalonil or pendimethalin suspensions in water (20 ml), or with a solution of fenamiphos in methanol (3ml) to give a concentration of 10 mg a.i./kg dry soil. Soil treated with the fenamiphos solution was left for 3 to 4 hours for the solvent to evaporate. Distilled water was added to adjust the moisture contents to 40% of water holding capacity. Samples of the three replicate soils were treated with water only and kept as controls. The soil/pesticide combinations were mixed by hand initially and then passed through a 3 mm mesh sieve after which they were transferred to loosely capped polypropylene containers and incubated at 20°C. Moisture contents were maintained by regular addition of distilled water. The treated soils were sampled periodically for 97 days and analyzed for pesticides and their metabolite concentrations. After 33 days, or when 75% of the initial concentration had disappeared if this occurred later, the soils were retreated with another dose of 10 mg/kg of the appropriate compound. A third treatment was done 33 days after the second treatment, irrespective of residue concentration in the soil samples.

Pesticides and their metabolites were extracted from samples of the treated soils (20 g) by shaking with acetonitrile:water 90:10 (25 ml) for one hour on a wrist action shaker. The samples were centrifuged for five minutes at 6000 rpm, after which sub-samples of clear supernatant were analyzed directly by HPLC using Kontron series 300 equipment. The column used was Lichrosorb-RP18 (250mm x 5mm; Merck) with an isocratic mobile phase flow rate of 1 ml min^{-1}. The mobile phases and wavelengths used for the analyses were as described by Singh et al (2001). The recovery of chlorpyrifos, fenamiphos, chlorothalonil and pendimethalin from soils treated with 10 mg/kg of the appropriate compound was 90%, 82%, 80% and 94%, respectively. The measured residues were not corrected for analytical recovery.

All of the soil-pesticide combinations described above, together with their respective controls, were investigated to determine possible effects of the pesticides on soil microbial communities and their activity. For this purpose, soil dehydrogenase, phosphatase, and total microbial biomass were determined 30, 60 and 90 days after the first pesticide applications. The functional potential of the microbial communities was also assessed by analysis of BIOLOG GN substrate utilisation patterns.

The methods suggested by Tabatabai (1982) were used with little modification for the measurement of soil dehydrogenase and phosphatase activities. Total microbial biomass was determined by measurement of ninhydrin-reactive N following fumigation with liquid chloroform and extraction with potassium chloride as described by Mele and Carter (1996).

Substrate utilisation patterns on BIOLOG GN microplates (BIOLOG Inc. Hayward, CA.) were assessed as described by Garland and Mills (1991). Sub-samples from each soil/pesticide combination (10 g) were shaken in sterile conical flasks for 25 min with 40ml Ringers solution. The samples were allowed to settle for 10 min when further Ringers solution was added to subsamples of the

supernatant to provide a final dilution of 1 part soil to 100. Samples of this solution (150µl) were used to inoculate each well of a BIOLOG GN microtitre plate. The solution was further diluted by factors of 10 and 100; and 200µl from these additional diluted samples were plated on R2A (Oxoid,Basingstke, UK) agar plates for bacterial colony counts. The BIOLOG plates and R2A agar plates were incubated at 25°C. BIOLOG readings were taken at 0 and 72 hours after inoculation at a wavelength of 600nm using an Anthos Labte HT-2 (version 1.22 E) plate reader. Bacterial colonies were counted after 72 hours of incubation. The utilization of the substrates by the BIOLOG-culturable microbial populations was analysed as described by Zak et al (1994) to give estimates of substrate richness (the number of substrates utilised) and substrate evenness (the distribution of utilisation among substrates). These were used to calculate Shannon's diversity index, which gives a measure of the metabolic diversity of the soil microbial population (Zak et al., 1994).

RESULTS AND DISCUSSION

The degradation patterns of chlorpyrifos, fenamiphos, and chlorothalonil in the soil are summarized in Figure 1. The formation and accumulation of metabolites are also shown. The individual decay curves for the parent compounds were fitted to the first-order rate equation by linear regression analysis of the logarithm of the residual concentration against time of incubation. The derived first-order rate constants and half-lives are shown in Table 1.

The half-life of chlorpyrifos was about 36 and 46 days for first and second treatments respectively. This agrees with results from previous studies that have suggested half-lives of chlorpyrifos in soil from 10 to 120 days (Racke et al., 1990). Concentrations of the degradation product trichloropyridinol (TCP) built up slowly but did not exceed 2 mg/kg during the 90-day experimental period.

Fenamiphos was rapidly converted into fenamiphos sulfoxide which was further oxidised to fenamiphos sulfone (Figure 1). The half-life of the first treatment of fenamiphos was 4.3 days (Table 1). Repeated application was associated with reduced rates of degradation, and the half-life of the third application of fenamiphos was 10.6 days. The dissipation of total toxic residues (fenamiphos plus the sulfoxide and sulfone oxidation products; TTR) was affected by repeated application. The overall half-life was about 30 days (Table 1) for the first treatment, but there was little change in TTR concentration during the 30-day period following the third treatment. Sequential treatment of fenamiphos therefore suppressed the overall rate of change in TTR in this soil which contrasts with previous findings where repeated treatment has resulted in enhanced degradation of fenamiphos (Smelt et al., 1996).

The disappearance of chlorothalonil from the soil was also rapid (Figure 1). The concentration of the metabolite hydroxy-chlorothalonil (OH-chlorothalonil) was always less than 2.5 mg/kg, even after repeated application of the parent compound. Repeated application considerably slowed down the degradation rate of chlorothalonil in the soil. The half-life of the first dose was 8.6 days and this

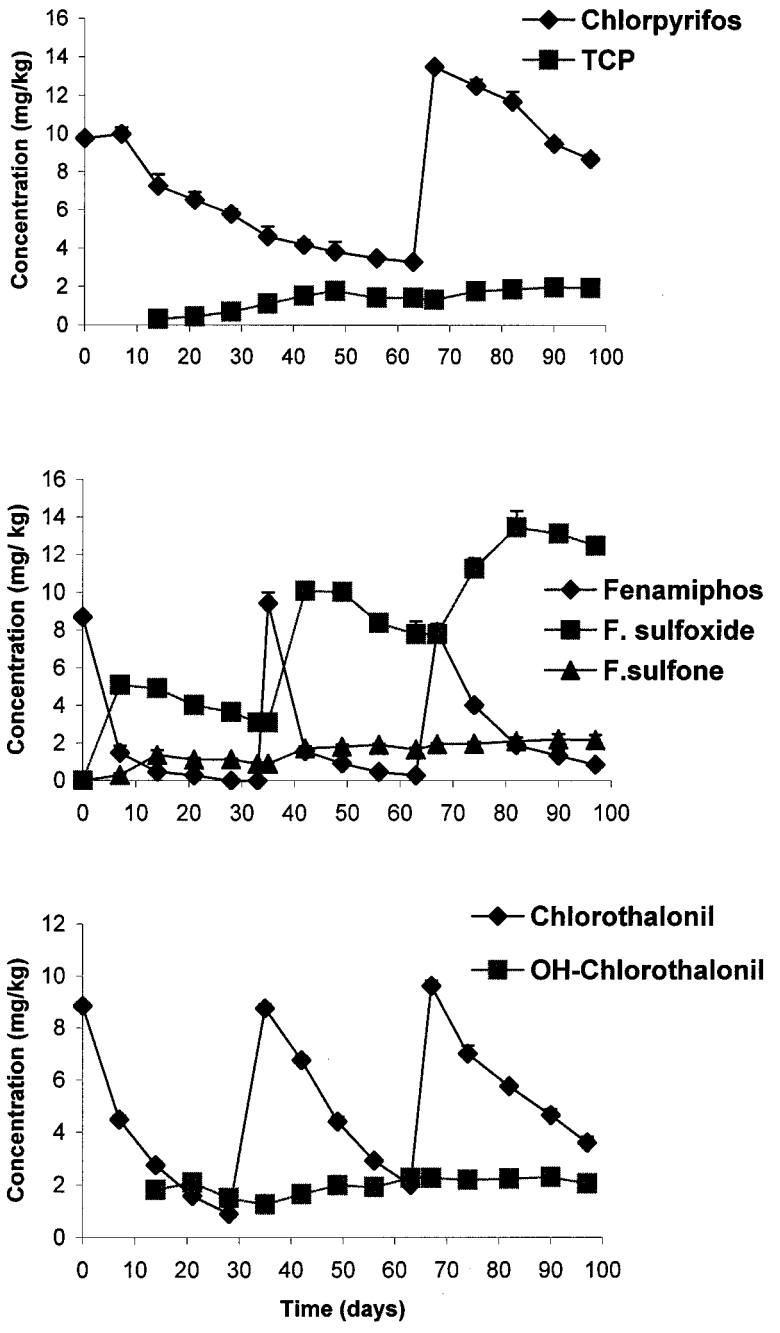

Figure 1. Degradation of chlorpyrifos, fenamiphos and chlorothalonil and formatio of their metabolites in soil

Table 1. First-order rate constants and half-lives for pesticide degradation.

Pesticide	Treatment number	R^2	Rate constant (day^{-1})	Half-life (days)
Chlorpyrifos	1	0.973	0.019	36.5 a*
	2	0.965	0.015	45.6 b
Fenamiphos (parent)	1	0.939	0.161	4.3 a
	2	0.920	0.117	5.9 b
	3	0.984	0.065	10.6 c
Fenamiphos (TTR)	1	0.972	0.022	31.5 a
	2	0.803	0.011	62.5 b
	3	0.789	0.009	165.0 c
Chlorothalonil	1	0.949	0.080	8.6 a
	2	0.984	0.051	13.6 b
	3	0.992	0.032	21.5 c
Pendimethalin	1	0.990	0.009	74.5

*For any one pesticide, half-lives followed by the same letter are not significantly different (P=0.05).

was extended to 21.5 days for the third treatment (Table 1). It has been reported previously (Motonaga et al., 1998) that repeated application of this pesticide suppresses its own degradation. However Motonaga et al cautioned that their pesticide application rate was rather high (40mg/kg), and that further studies are required with lower application rates with different soils. Our results suggest that inhibition can occur even with a lower application rate (10mg/kg).

The dissipation of pendimethalin was slow (data not shown) with a half-life of 74 days (Table 1). No repeat applications were made with this compound. Degradation of pendimethalin in soil has been shown in earlier studies to be slow (Walker and Bond, 1977), and this observation was confirmed in the present study.

Persistence of residues in the soil may have a significant impact on soil microbial communities and their functions like enzyme activity, which in turn are directly related to soil health and fertility. Dehydrogenase activity is a useful indicator of overall microbial activity of soils at any specific point in time and has been recommended as a measure of the side effect of agricultural chemicals (Gerber et al., 1991). In the present experiments, application of chlorpyrifos had a significant inhibitory effect on soil dehydrogenase activity after 30 days in the first treatment (Figure 2A). This inhibitory effect became highly significant after the second treatment. Fenamiphos had little effect on soil dehydrogenase activity after 30 and 60 days, but it had a small but signicant ($P<0.05$) negative effect after 90 days. A highly significant reduction in dehydrogenase activity was observed at all sampling times in the soil treated with chlorothalonil. Pendimethalin had little impact on dehydrogenase activity except at the 90 day sampling time. The dehydrogenase activity of the controls did not change during the 90-day period of the experiment.

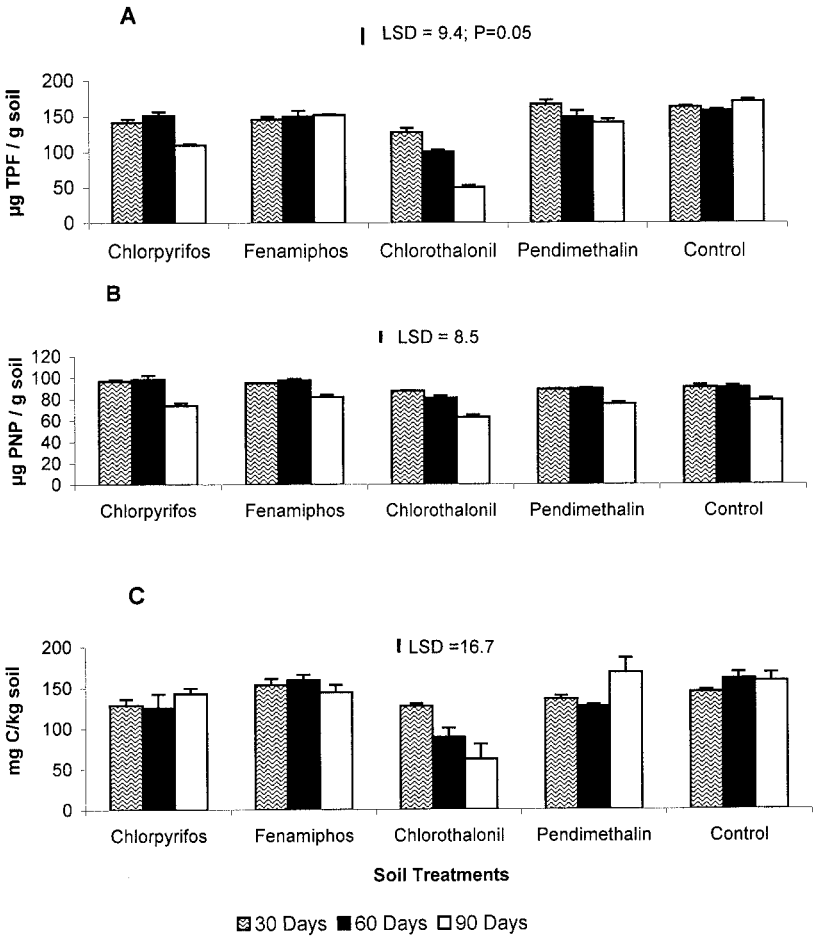

Figure 2. Effects of chlorpyrifos, fenamiphos, chlorothalonil and pendimethalin on A dehydrogenase activity, B phosphatase activity, and C total microbial biomass of soil. LSD (P=0.05) refers to comparisons with the appropriate control value.

Phosphatase activity in soil is also of importance due to its role in hydrolysing organic P compounds to inorganic P (Greaves and Webley, 1964). Phosphatase activity remained unchanged following application of chlorpyrifos or fenamiphos (Figure 2B). A highly significant reduction in phosphatase activity was observed at all sampling times in soils treated with chlorothalonil. Pendimethalin had no impact on phosphatase activity.

The microbial biomass represents only a small fraction of the total amount of soil N and C. However, its relatively rapid turnover, resulting in release of mineral N and other nutrients, makes its contribution to plant nutrition far greater than its size

might suggest. In the present experiments, treatment of soils with chlorpyrifos, fenamiphos or pendimethalin had little or no effect on microbial biomass. As with the other microbial characteristics, chlorothalonil again had a marked impact on measured microbial biomass, with a reduction of more than 50% at 90 days. Total microbial biomass in the control soils remained constant throughout the incubation period.

Degradation of organic matter is considered to be the soil process most sensitive to agrochemicals at recommended application rates (Domsch et al., 1983). As a measure of potential effects on turnover of organic substrates, we used BIOLOG plates. The results from the measurement of substrate utilisation patterns on BIOLOG GN plates and bacterial counts (Table 2) indicate significant adverse

Table 2. Microbial parameters derived from Biolog data and plate counts.

Pesticide	Time (days)	Biolog parameter Richness	Evenness	Diversity	Plate counts ($x10^7$ cfu/g)
Chlorpyrifos	30	85	0.94	4.09	1.05
	60	76	0.91	4.07	1.00
	90	68	0.83	3.87	0.88
Fenamiphos	30	93	0.94	4.22	1.16
	60	93	0.92	4.19	1.13
	90	92	0.91	4.23	1.08
Chlorothalonil	30	84	0.93	3.87	0.83
	60	54	0.82	2.88	0.78
	90	38	0.74	2.51	0.50
Pendimethalin	30	81	0.92	4.15	1.26
	60	83	0.95	4.04	1.06
	90	83	0.92	4.20	1.14
Control	0	90	0.95	4.33	1.13
	30	87	0.90	4.26	1.28
	60	78	0.93	4.09	1.06
	90	78	0.91	4.06	1.14
LSD (P=0.05)		8.0	0.060	0.303	0.063

effects on substrate utilization (richness), in overall metabolic diversity, and in the evenness of substrate utilization in soils treated with chlorothalonil after 60 and 90 days. A small but significant reduction in richness was also observed following the second treatment with chlorpyrifos. Treatment with pendimethalin or fenamiphos had little effect on any of the parameters measured in the BIOLOG study.

Overall, all of the measured microbial characteristics were adversely effected by chlorothalonil treatment, but the other pesticides had little or no effect. The significant observations of the present study are that repeated and sequential application of pesticides have inhibitory effects on their degradation rate and also

that some pesticides at realistic application rates may have interactive effects on the soil microbial community and its activity.

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