

Chapter 9

Fipronil: Toxicity to Subterranean Termites and Dissipation in Soils

J.E. Mulrooney¹, T. L. Wagner¹, and P. D. Gerard²

¹USDA Forest Service, Starkville, MS 39759

²Clemson University, Clemson, SC

Fipronil (Termidor 80 WG) was applied to covered and exposed plots at one secondary and four primary USDA Forest Service termiticide test sites in the U.S. Residue analyses and bioassays of soil samples were conducted over 5 y. Fipronil had an exponential decay at all sites. The DT_{50} of fipronil in a silt loam soil in Oktibbeha Co., MS in covered and exposed plots was 202 and 177 d, respectively. Fipronil dissipation appeared to be faster at the secondary site (Oktibbeha Co, MS) compared to the primary tests sites. Dissipation was faster in covered plots in AZ and MS compared to FL and SC; while that in exposed plots was similar among sites. In 7 d bioassays, termite penetration of soil cores from primary test sites was significantly greater in exposed plots (33.9 ± 1.2 mm) than that from covered plots (25.7 ± 1.2 mm). Differences in termite penetration and termite mortality between covered and exposed plots at the secondary test site were not significant. Average distance penetrated by termites, averaged over treatments and primary sites, significantly increased during the last three years (37.2 mm) of sampling compared to the first three sampling times (20.7 mm). Termite mortality averaged over sites and years for covered and exposed plots was not significantly different. Freundlich adsorption coefficients (K_f), determined from adsorption isotherms, ranged from 0.14 on a gravelly sand (Pima Co., AZ) to 5.47 on a silt loam (Oktibbeha Co. MS).

Fipronil, a halogen-substituted thioether containing phenylpyrazole insecticide, was developed by Rhone Poulenc in 1987. It acts as an agonist at the gamma-aminobutyric acid (GABA)-gated chloride channel/ionopore complex, and possesses a high level of toxicity to insects because of its specificity to this target site (1). Laboratory and field studies conducted by Rhone Poulenc on different soil types under different temperature conditions identified five principal metabolites (desulfinyl, sulfone, sulfide, amide, and a photodegradation product), which occur through degradation pathways of hydrolysis, photolysis, oxidation, and reduction (2). A comprehensive review of the environmental fate and toxicology of fipronil can be found in Gunasekara et al. (3).

The behavior of a pesticide in the soil and its dissipation in the environment are dependent on its adsorption, which in turn depends on the physical-chemical properties of the pesticide, the climate, and the nature of the soil. Adsorption processes control the availability of pesticides for adsorption by plant roots or soil organisms, and their leaching through soil (4). Therefore, adsorption is a major influence on the balance between pesticide efficacy and leaching to groundwater. It has also been shown that adsorption limits the degradation of pesticides by reducing their partitioning into the soil liquid phase (5). Bobe et al. (6) studied the adsorption of fipronil to soils varying in organic matter. Their results showed that adsorption to soil decreased with decreases in organic matter content of the soil. The effect of organic matter content of soil on adsorption of fipronil was also demonstrated by Mulrooney and Gerard (7). In contact bioassays of fipronil treated soils, termite mortality (LC_{50}) decreased as organic matter in the soil increased. For example, LC_{50} 's ranged from 0.49 ppm on sandy loam soil with low organic matter (1.8 %) to 6.99 ppm on a silt loam with higher organic matter (2.6 %). Increased adsorption of fipronil to soil with higher organic matter content decreased the amount of fipronil available for transfer to termites.

The movement and degradation of fipronil were investigated in Australian soils following standard termiticide treatment methods (surface application under slab and trenching treatments along walls). Surface application studies in three field sites showed slow dissipation and little movement for fipronil in all three soils under the simulated slab during a three-year period. The greatest mass of the chemical residues remained in the quartzite sand layer (thickness, 5 cm), and only small amounts of these were found to have migrated into the soil layers (depth, 0 -15 cm) underneath the quartzite sand layer. Of the three metabolites (desulfinyl, sulfide, and sulfone) found in soils, the sulfone derivative had the highest concentration. One year trenching studies at two sites in Adelaide, Australia, showed that vertical movement and dissipation of fipronil occurred in the soils. The average concentration of fipronil in the trenches (depth, 0-30 cm) decreased from 33.7 to 14.9 mg/kg in the loam soil at one site and from 39.4 to 14.6 mg/kg in the clay soil from the other site over the year (8). Ying and Kookana (9) determined the sorption of fipronil and its two main metabolites, desulfinyl and sulfide, on a range of soils from South Australia. The Freundlich sorption coefficient (K_f) values, a measure of the relative adsorption capacity of soil, for fipronil on these soils ranged from 1.94 to 4.84. The metabolites had a higher sorption to soils, with K_f -values ranging from 11.09 to 23.49 for the sulfide derivative and from 4.70 to 11.77 for the

desulfynyl derivative. The sorption coefficients of fipronil and its metabolites were found to be better related to soil organic carbon than clay content.

In a study on open field behavior of fipronil under Sahelian conditions (Niger, Africa), the amide and trifluoromethylpyrazole derivative were the principal degradation products recovered from the soil (10). This study also included a measure of the mobility of fipronil in soils and showed that fipronil did not migrate below the first 10 cm.

The toxicity of fipronil and its metabolites to several insect species has been investigated. Fipronil sulfone is the major metabolite of fipronil in Southern armyworm larvae and presumably in other insects (11). Desulfynyl fipronil, a significant contributor to the effectiveness of fipronil, is the principal photoproduct on plants and soils and is as potent as or more potent than fipronil in toxicity to houseflies (12). Mulrooney and Goli (13) in topical applications of fipronil and its metabolites determined the order of toxicity (LD_{50}) to boll weevils (*Anthonomus grandis*) to be: sulfide > fipronil > sulfone > desulfynyl.

Fipronil (Temidor 80 WG) was registered for use as a termiticide in September 1999 and became available for use in pre- and post-construction applications in 2000. Unlike the pyrethroid termiticides which are repellent to termites, termites can not detect fipronil and thus enter treated areas where they are poisoned. The toxicity of fipronil to subterranean termites has been documented by several researchers. Ibrahim et al. (14) determined the 72 h LD_{50} of fipronil to be 1.36 ng/insect in topical bioassays using *Coptotermes formosanus* Shiraki. Osbrink et al. (15) determined the LT_{50} of fipronil to *Reticulitermes virginicus* to be an average of 271 min when termite workers were placed on filter paper treated with 630.65 $\mu\text{g}/\text{cm}^2$ of fipronil. Remmen and Su (16) obtained an LC_{50} of 0.04 ppm after *R. flavipes* workers were exposed to fipronil treated sand for 1 wk. Shelton and Grace (17) in a simple donor-recipient test exposed *C. formosanus* workers to sand treated with fipronil at 1, 10, and 100 ppm for 1 h. Mean mortalities of termite donors after 14 d were 36, 36, and 98%, respectively.

Ibrahim et al. (14) found fipronil to be repellent to *C. formosanus* at 0.125%. However, Remmen and Su (16) observed that fipronil concentrations as high as 64 ppm did not repel *R. flavipes* (Kollar) and *C. formosanus* termites. They observed 89% mortality at 1 ppm and failure of termites to completely penetrate treated sand. They concluded that 1 ppm fipronil may provide an adequate barrier for both *R. flavipes* and *C. formosanus*.

Hu (18) found 100% mortality of eastern subterranean and Formosan termites within 3 d at treatment concentrations of 50 and 100 ppm and after 28 d at 1 ppm. Her results also showed that penetration into 50 mm thicknesses of treated sand decreased with increasing concentration. Penetration failure was due to rapid mortality, rather than repellency of fipronil.

Although the toxicity of fipronil to termites is well documented, long term studies of the degradation and toxicity of fipronil applied at termiticidal rates have not been reported. The purpose of this study was to determine the dissipation of fipronil in different soils found at U.S. Forest Service test sites and its efficacy against subterranean termites in laboratory bioassays.

Materials and Methods

Test Sites

The four primary Forest Service termiticide test sites are located in Pima Co., Arizona; Calhoun Co., Florida; Harrison Co., Mississippi; and Union Co., South Carolina. The test site in Arizona is on the Santa Rita Experimental Range managed by the University of Arizona near Greenvalley, AZ. The other sites are in the Chipola (near Panama City, FL), the Harrison (near Saucier, MS), and the Calhoun (near Union, SC) Experimental Forests. These sites represent semiarid (Arizona), temperate (South Carolina), and subtropical climates (Florida and Mississippi). Soil pH ranges from approximately neutral (6.9, Arizona) to moderately acidic (4.8, Florida) (Table 1). In 2001, a secondary test site was established in Mississippi State University's John W. Starr Memorial Forest in Oktibbeha Co. just outside Starkville, MS.

Experiment 1

An approximate area of 10 by 15 m was cleared of small trees and shrubs in the Forest Service's secondary test site in Oktibbeha Co., Mississippi. A 6 by 8 grid consisting of 1.5 M square plots was then measured and marked. Treatments consisting of fipronil (Termidor 80 WG) at 0.06% A.I and water controls in covered and exposed plots were randomly assigned to plots. There were five replicates of each treatment. An approximate 60 by 60-cm area in each plot was cleared of vegetation and duff to expose the mineral soil. A 43 by 43-cm metal treating frame was placed on the soil and rocks and roots in the upper 7 cm of soil were removed. The 0.06% fipronil solution (764 ml volume) was applied within the treating frame using a watering can. This volume corresponds to a standard 3.785 L per 9.29 m² (1 gal. per 10 sq. ft.) pretreatment application of termiticide. After treatment, black plastic sheets and 40.6-cm square concrete stepping stones were placed over fipronil and water-only control plots, while five plots of each treatment were left exposed. The design was completely randomized with five replicates of fipronil and water controls in both covered and exposed plots, for a total of 20 plots. Because of the close proximity of this site to the laboratory, soil samples were collected for residue analysis immediately after application and at monthly intervals for the first year, thereafter at yearly intervals for 5 y. Samples were collected at 0 and 6 mo, and at yearly intervals for bioassay. Samples were collected using a sampling probe in which the soil core was collected in 2.54-o.d. by 10.16-cm butyrate (Tenite[®], U.S. Plastics, Lima, OH) tubes.

Table 1. Test site characteristics

| <i>Site</i> | <i>Location</i> | <i>Soil Type</i> | <i>Bulk Density (g/cm³)</i> | <i>pH</i> | <i>% OM</i> | <i>% Clay</i> | <i>% Silt</i> | <i>% Sand</i> | <i>Ave. Yearly Rainfall (cm)</i> |
|-------------------------------|--------------------|------------------|--|-----------|-------------|---------------|---------------|---------------|----------------------------------|
| Santa Rita Experimental Range | Pima Co., AZ | Gravelly sand | 1.47 | 6.9 | 0.6 | 7.5 | 15.1 | 77.4 | 35.5 |
| Chipola Experimental Forest | Calhoun Co., FL | Sand | 1.16 | 4.8 | 0.8 | 2.8 | 2.7 | 94.5 | 163 |
| Harrison Experimental Forest | Harrison Co. MS | Sandy loam | 1.11 | 5.1 | 1.8 | 4.9 | 25.2 | 69.9 | 170 |
| John Starr Memorial Forest | Oktibbeh a Co., MS | Silt loam | 0.97 | 4.8 | 2.6 | 15.0 | 72.2 | 12.8 | 130 |
| Calhoun Experimental Forest | Union Co., SC | Sandy loam | 1.09 | 4.7 | 1.3 | 3.8 | 29.0 | 67.2 | 127 |

Experiment 2

Application of a 0.06% A.I. rate of fipronil (Termador 80 WG) in a volume of 3.785 L per 9.29 m² was made to soil at the four primary termiticide test sites maintained by the Forest Service.

At each site, plots were laid out in completely randomized design on 1.5 M centers in a 2 by 10 arrangement. In this test, the soil was prepared and fipronil (0.06%) was applied to covered and exposed plots in the same manner as described above.

Installation of each test and site visits each year were made in February, April, June, and September to Florida, Arizona, Mississippi, and South Carolina, respectively. Immediately after application and each following year for 5 y, three soil samples were collected from each plot. Two of the samples were used for bioassays, the third for residue analysis. Soil samples collected from untreated soil were used as controls. Soil samples were held at -20°C until bioassays and residue analyses were conducted.

Residue Analysis

Analysis of fipronil residue was done using an Agilent[®] (Santa Clara, CA) 5990 gas chromatograph equipped with electron capture detector. The parameters of the analysis method were as follows: injection volume, 1 µl; carrier gas, helium; make-up gas, argon/methane; injector temperature, 250°C; detector temperature, 250°C; oven program, 50°C initial temperature with a 30°C/min ramp to 230°C for 8 min. An Agilent[®] 25-m Ultra-1 methyl siloxane phase column (I.D. 0.32 mm) with 0.52-µm film thickness was used. Retention time was 17.924 min.

Sampling tubes were emptied of soil (~45.9 cm³); the soil core was mixed and oven dried at 100°C. Then 25 g samples were randomly collected for extraction. All solvents used in extractions were HPLC grade. Extraction of fipronil from soil was made with an Accelerated Solvent Extractor, ASE-200 (Dionex[®], Sunnyvale, CA) using a 70:30 mixture of acetone:acetonitrile at a total volume of 50 ml. Oven temperature and pressure were 100°C and 105.4 kg/cm², respectively, with a 5 min static time. Extraction volume was reduced to 10 ml under nitrogen using a Rapid Vac (Labconco[®], Kansas City, MO). Percent recoveries of fipronil (Termidor 80 WG) spiked in soils from the different sites were: Pima Co. AZ, 108.2 ± 2.4; Calhoun Co., FL 94.5 ± 11.2; Harrison Co., MS; 113.3 ± 1.9; Oktibbeha Co., MS, 98.2 ± 8.7; and Union, Co., SC, 98.3 ± 7.5.

Adsorption

Adsorption isotherms were obtained using a batch equilibrium method (6, 9). Two grams of soil from each test site was treated with 5 ml of 5% acetonitrile/water solutions (0 – 10 ppm) of technical fipronil (98%) (Chem Service, Inc, West Chester, PA) in 20-ml scintillation vials. Vials were shaken in a shaker/water bath for 4 h at 200 rpm and 22°C. After centrifugation at 2800

rpm for 30 min, the supernatant (3 ml) was separated and passed through solid phase extraction (C₁₈) cartridges (AccuBond^{II}, Agilent Technologies Inc., United Kingdom). The cartridge was first conditioned with 5 ml of acetonitrile followed by 5 ml of distilled water before 3 ml of the supernatant was loaded. Elution of fipronil was obtained with 5 ml of acetonitrile. The eluate was brought to dryness under a constant stream of nitrogen and then re-dissolved in 1 ml toluene. Fipronil concentration was then determined by GC-ECD as described above. Percent recovery of fipronil from eluate was $89.4 \pm 2.8\%$.

The amount of fipronil adsorbed was evaluated as the difference between that initially present in the solution and that remaining after equilibration with soil. The adsorption isotherms were obtained by plotting the equilibrium content of fipronil adsorbed to soil against the equilibrium concentration of fipronil in the liquid phase. These isotherm data were described by the Freundlich equation:

$$S = K_f C^n \quad (\text{equation 1})$$

Where S is the concentration of fipronil adsorbed by the soil ($\mu\text{g/g}$), C is the equilibrium concentration ($\mu\text{g/ml}$). Values of the parameters of sorption, K_f (Freundlich coefficient) and n (Freundlich exponent), were estimated by linear regression after log-log transformation.

Bioassays

Two 10.0-cm deep soil samples from each plot were bioassayed with termites from two *Reticulitermes* spp colonies. Termites were collected from fallen pine logs separated from each other by at least 1000 m on the Noxubee National Wildlife Refuge near Starkville, MS and held at ambient temperature in galvanized trashcans in the laboratory. Different colonies were used each year.

The bioassay method used was similar to that described by Su et al. (19). In our bioassay, the 10.0 cm of soil in the sample tube was reduced to 5.0 cm by pushing out the bottom 5.0 cm of soil. Two 3.0-cm agar segments were placed on either side of the soil core to provide moisture during the bioassay. Then the tube containing the 5.0 cm of soil was connected by a Tygon[®] tubing collar to another tube containing 80 workers and one soldier. Wooden sticks of southern yellow pine and paper strips provided food and harborage for termites in both the tube containing termites and the tube with soil, so that termites had a source of food both above and below the treated soil.

The bioassay was terminated after 7 d when mortality as well as distance tunneled through treated soil (penetration) was determined.

Data Analysis

The experimental design of both experiments was completely randomized. Distance penetrated by termites into soil cores and termite mortality, adjusted

for control mortality (20), were analyzed using the mixed procedure (PROC MIXED) of SAS (21). Mean separation was made using the PDIFF option.

Results and Discussion

Residue Analysis

Experiment 1

Fipronil applied to sandy loam soil in the John W. Starr Memorial Forest in Oktibbeha Co., MS showed an exponential decay over the five years of the study (Figure 1). Time to 50% dissipation (DT_{50}) in covered and exposed plots was 202 and 177 d, respectively. These values are within the half-life range, 91 – 222 d, in soil determined by Rhone Poulenc (2). Fipronil residues in exposed plots leveled off at 2.11 ppm after 12 mo, while those in covered plots did not level off until 24 mo when levels in the soil were 0.79 ppm.

A study conducted between 1990 and 2002 at the Harrison Co. test site determined the half-lives of termiticides applied at label rates to soil in trenches around miniature foundations. These data are presented to give some perspective to the dissipation of fipronil: chlorpyrifos 1.0%, Dursban[®] TC (1,254 d); fenvalerate 0.5%, Tribute[®] (831 d); permethrin 0.5%, Dragnet[®] FT (768 d); cypermethrin 0.3%, Prevail[®] FT (488 d); cypermethrin 0.25%, Demon[®] TC (399 d); isofenfos 0.75%, Pryfon 6 (301 d); and permethrin 0.5%, Torpedo[®] (138 d) (22). Fipronil at about one tenth the application rate (0.06%) dissipated slightly slower than the Torpedo formulation of permethrin (0.5%).

Experiment 2

It was not possible to determine DT_{50} 's in this experiment because samples were collected at yearly intervals and 50% of the residue had dissipated by the time the 1 y samples were collected. As in Experiment 1, the dissipation of fipronil at primary test sites in Pima Co., Arizona; Calhoun Co., Florida; Harrison Co., Mississippi; and Union Co., South Carolina appears exponential (Figure 2). Parameters of the regressions are given in Table 2. All regressions were significant ($P < 0.0001$). The initial residues collected from all primary test sites were higher than that at the secondary test site in Oktibbeha Co., MS (Experiment 1). The dissipation of fipronil in soils at the primary test sites in Arizona, Florida, Mississippi, and South Carolina does not appear to be as rapid as that in the soil in Experiment 1 from Oktibbeha Co., MS (Figures 1 and 2). The silt loam soil from Oktibbeha Co., MS had higher organic matter (OM) than the other soils in this study. Soils with higher OM could be expected to have higher populations of microbes to hasten the degradation of fipronil.

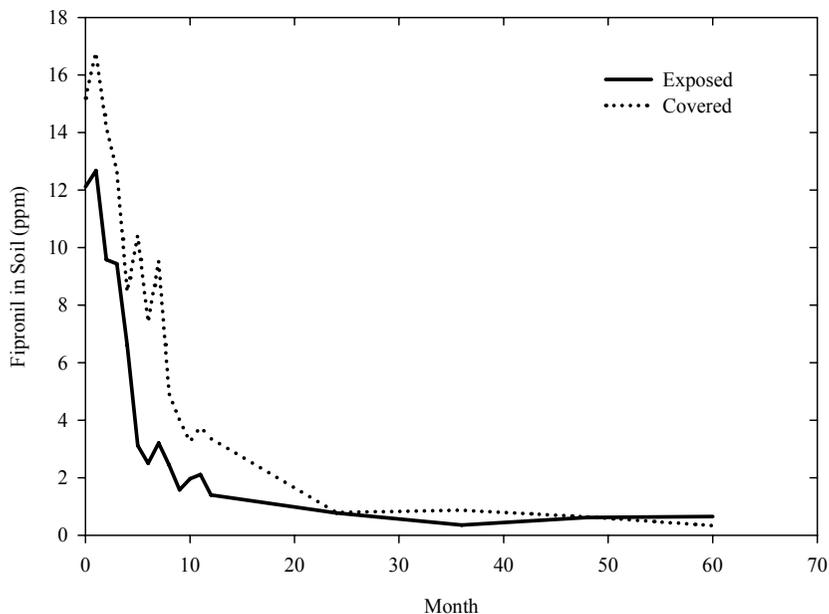


Figure 1. Fipronil residues extracted from covered and exposed plots at U.S. Forest Service secondary test site (Oktibbeha Co., MS).

Degradation of fipronil by microbes in a clay loam soil was demonstrated by Zhu et al. (23). They showed that the half-lives of fipronil in a non-sterile clay loam soil were 9.72 and 8.78 d at 25 and 35°C, respectively compared to 33.51 and 32.07 at 25 and 35°C, respectively in the sterile soil. This study demonstrated that microbial degradation was an important factor for the metabolism of fipronil in the non-sterile clay loam soil.

Residues in covered plots did not level off until around 4 y after application, when residues in Florida and South Carolina were about twice those in Arizona and Mississippi (Figure 2). Increased dissipation in Arizona could be the result of high temperatures; while Mississippi's high rainfall and warm temperatures produce a favorable environment for microbes. Residues in exposed plots in Arizona, Florida, and Mississippi leveled off 1 y after application. Those in exposed plots in South Carolina were about ten times those from the other sites after 1 y and did not level off until 3 y after application.

Initial (year 0) amounts of fipronil recovered from soil samples varied among sites. In Experiment 1, 12 to 16 ppm of fipronil were found in the silt loam cores from Oktibbeha Co. collected at time 0 (Figure 1). The amount of fipronil recovered could be a result of the penetration of the fipronil solutions into the soil at application. As will be discussed below, fipronil readily adsorbed to the silt loam in Oktibbeha Co. in Experiment 1; therefore, the penetration of fipronil into the soil would likely be very shallow. Fipronil residue would be limited to the upper portion of soil samples (10.0 cm) collected after application.

Because fipronil was not uniformly distributed within the soil sample, a dilution effect occurred as only a portion of the residue was collected when the 25 g of soil was randomly taken from the sample for residue analysis. The amount of fipronil relative to the amount of soil collected is small; therefore low concentrations in the soil were observed. A similar situation existed at the Pima Co. site in Arizona, in that penetration was limited by application to a dry soil in April. Higher initial concentrations of fipronil in soil were found in sand in Calhoun Co., FL and sandy loam in Union Co., SC (Figure 2). The application of fipronil at the Florida site was made in February, a time of year when the soil would be expected to have a high moisture content which would aid in the penetration of fipronil through the soil (24). When samples were collected after application at this site, the amount of fipronil relative to the amount of soil collected was high, therefore higher concentrations of fipronil were observed. Also, Carter and Stringer (25), in laboratory studies of penetration of chlorinated hydrocarbon termiticides through soils from seven states, observed greater penetration of sand and sandy loam soils from the Florida and South Carolina test sites.

Table 2. Parameters of linear regressions of year (log) on fipronil residue (log) in soil in covered and exposed plots for each Forest Service test site

| <i>Site</i> | <i>Intercept</i> | <i>Slope</i> | <i>r</i> ² |
|------------------|------------------|--------------|-----------------------|
| | <i>Covered</i> | | |
| Pima Co., AZ | 2.86 | -2.26 | 0.85 |
| Calhoun Co., FL | 3.76 | -1.21 | 0.51 |
| Harrison Co., MS | 3.64 | -2.41 | 0.73 |
| Union Co., SC | 3.66 | -1.22 | 0.39 |
| | <i>Exposed</i> | | |
| Pima Co., AZ | 2.78 | -2.90 | 0.83 |
| Calhoun Co., FL | 2.91 | -2.54 | 0.84 |
| Harrison Co., MS | 2.53 | -2.12 | 0.52 |
| Union Co., SC | 3.52 | -2.19 | 0.67 |

Adsorption

An adsorption isotherm, which describes the relation between the activity or equilibrium concentration of the adsorptive (fipronil) and the quantity of adsorbate (fipronil solution) on the soil surface at constant temperature, is generally used to describe adsorption (26).

The parameters of the Freundlich equation (equation 1) are given in Table 3. S is the amount of adsorbed fipronil, C is the equilibrium concentration of dissolved fipronil and K_f and n are two constants characteristic of the fipronil adsorption capacity (26). K_f is the amount of fipronil adsorbed at an equilibrium concentration, which is a measure of the relative adsorption capacity of soil and n is the intensity factor of the adsorption (6).

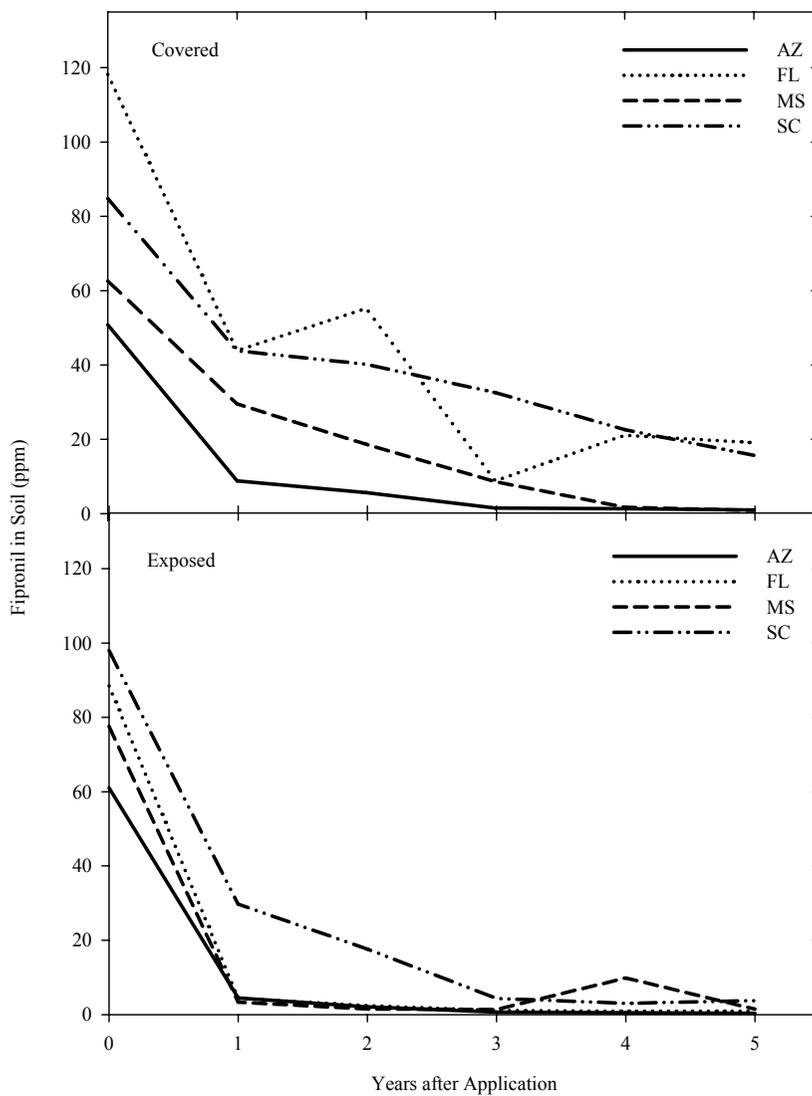


Figure 2. Fipronil residues extracted from covered and exposed plots at U. S. Forest Service primary test sites

Table 3. Parameters of the Freundlich equation ($S = K_f C^n$) describing the adsorption isotherms of fipronil in soils at Forest Service termiticide test sites

| <i>Site</i> | K_f | n | r^2 |
|-------------------|-------------|-------------|-------|
| Pima Co., AZ | 0.14 ± 0.02 | 1.73 ± 0.21 | 0.87 |
| Oktibbeha Co., MS | 4.82 ± 0.34 | 1.08 ± 0.19 | 0.76 |
| Calhoun Co., FL | 0.15 ± 0.02 | 1.47 ± 0.25 | 0.78 |
| Harrison Co., MS | 1.01 ± 0.04 | 0.93 ± 0.13 | 0.83 |
| Union Co., SC | 0.88 ± 0.08 | 1.02 ± 0.13 | 0.85 |

The silt loam soil from Oktibbeha Co., MS had the highest adsorption coefficient (5.47). Sandy loam soils from Harrison Co., MS and Union Co., SC had coefficients that were similar; 1.21 and 1.20 respectively, but were five times lower than that of the silt loam from Oktibbeha Co. The lighter soils from Pima Co., AZ (gravely sand) and Calhoun Co., FL, (sand) had coefficients much lower than the other soils, 0.14 and 0.15 respectively. As can be seen from Table 2, the soil with the greatest K_f , the silt loam from Oktibbeha Co., had the highest percent OM. This soil also has the highest clay content of the soils included in the test. Soil OM has been shown to be highly correlated with pesticide adsorption. For example, Ying and Kookana (9) reported that Freundlich sorption coefficients (K_f) of fipronil on a range of soils from South Australia ranged from 1.94 to 4.84 and were better related to soil OM than clay content. Bobe et al. (6) in another study of fipronil adsorption on two Sahelian soils (Sagua and Banizoumbou) from Niger, Africa and a Mediterranean soil (Montpellier) also determined that adsorption was dependent on OM: the adsorption coefficients were 4.3 (Sagua 0.1% OM), 7.3 (Banizoumbou 0.3% OM) and 45.5 (Montpellier 6.5% OM). For unknown reasons, sorption coefficients observed for soils from Pima Co. and Calhoun Co. (0.14 ± 0.02 and 0.15 ± 0.02 , respectively) in our study, which had lower percent OM than that of the Sagua soil, were much lower than that of the Sagua soil in Bobe et al. (6). Ahmad et al (27) reported that the nature of OM is a determining factor of the adsorption capacity of a soil. They found that variation in adsorption of pesticides could be explained only when variations in the aromatic components of OM were taken into consideration. More than likely, the components of OM in soils from Arizona and South Carolina are different from those found in Niger, Africa; to what extent is beyond the scope of the present study.

Bioassays

Experiment 1

Average distance penetrated by termites through 50-mm untreated soil cores was 45.1 ± 2.2 and 48.9 ± 1.5 mm for covered and exposed plots, respectively. Differences in distance penetrated by termites and termite mortality between

covered and exposed plots treated with fipronil were not significant; therefore, penetration and mortality were averaged over test type (Table 4). Distance penetrated through soil samples and termite mortality had similar trends in that both increased over time since application. Increases in termite penetration through termiticide treated soil over time should be expected as residues dissipate; however, mortality generally decreases over time due to dissipation. One possible explanation for these results is that for the first 2 y fipronil remained in the upper portion of the soil core and termites only penetrated the core to the edge of the fipronil residue (ca. 24 mm). Over time, fipronil migrated down into the soil, became less concentrated, and thus termites were able to penetrate greater distances into the core. Also, it is possible that roots growing through treated plots and/or excavations by other soil invertebrates create guides and/or passage ways through the soil over time that prompt termites to penetrate through treated soil.

Table 4. Average mortality and distance penetrated through silt loam soil cores (covered and exposed plots combined) treated with fipronil (Termidor 80 WG) in Oktibbeha Co. MS

| <i>Month</i> | <i>Mortality (%)</i> | <i>Distance (mm)</i> |
|--------------|----------------------|----------------------|
| 0 | 68.1 ± 7.8 b | 22.2 ± 5.7 b |
| 6 | 63.6 ± 8.9 b | 25.8 ± 5.5 b |
| 12 | 72.4 ± 6.9 b | 46.6 ± 3.6 a |
| 24 | 89.3 ± 4.7 a | 26.8 ± 6.0 b |
| 36 | 95.1 ± 2.9 a | 52.0 ± 0.0 a |
| 48 | 96.6 ± 2.8 a | 42.1 ± 4.6 a |
| 60 | 86.9 ± 5.9 a | 51.8 ± 0.2 a |

Means within a column followed by the same letter are not significantly ($P < 0.05$) different as determined by PDIFF (21)

As fipronil became more dispersed in the soil and penetration increased, termite mortality significantly ($F = 5.19$; $df = 6, 133$; $P < 0.0001$) increased (Table 2). A slight, but non-significant, decrease in mortality occurred at 60 mo after application. Mortality at 24 mo ($89.3 \pm 4.7\%$) and thereafter was significantly greater than that at 0, 6, and 12 mo; 68.1 ± 7.8 , 63.6 ± 8.9 , and $72.4 \pm 6.9\%$, respectively. Control mortality in covered ($30.1 \pm 3.6\%$) and exposed ($37.6 \pm 3.8\%$) plots was unexpectedly high in this soil. The abrasiveness of the clay in this soil could have contributed to the mortality of controls. Smith and Rust (28) observed that a 2 h exposure of termites dry sand containing 10% kaolin clay resulted in 34% mortality.

Experiment 2

Averaged over years and sites, termites penetrated an average of 49.6 ± 0.6 mm out of a possible 50.0 mm through control soil samples with an overall average mortality of $17.2 \pm 1.6\%$. Distance penetrated through fipronil treated

soil samples from covered plots (25.7 ± 1.2 mm) was significantly less ($F = 46.06$; $df = 1, 752$; $P < 0.0001$) than those from exposed plots (33.9 ± 1.2 mm).

Average distance penetrated by termites through fipronil treated soil samples each year, averaged over treatments and sites, significantly ($F = 54.86$; $df = 5, 904$; $P < 0.0001$) increased during the last three years (37.2 mm) of sampling compared to the first three sampling times (20.7 mm). This is similar to the results from Experiment 1 and provides additional evidence that downward movement of fipronil may have occurred over time.

Termite penetration through soil samples from the four sites, averaged over treatments and years, was significantly different ($F = 37.76$; $df = 3, 4$; $P = 0.0032$). Average termite penetration of fipronil treated soil followed the order: Mississippi (36.6 ± 1.4 mm) > South Carolina (35.0 ± 1.3 mm) > Florida (28.3 ± 1.6 mm) > Arizona (15.8 ± 1.4 mm).

The interaction of years*sites*treatment was significant ($F = 3.74$; $df = 12, 752$; $P < 0.0001$) for distance penetrated by termites through soil samples. Unlike the results of Experiment 1, there were no consistent trends of penetration distances with time since application (Table 5). Average soil penetrations for each year*site combination included the entire gamut of possibilities, 0 to 50 mm, during the 5 y of the study.

Termite mortality in samples from covered and exposed plots in penetration bioassays averaged over years and sites was not significantly different. Mortality for years and sites was significant, $F = 19.03$; $df = 5, 896$; $P < 0.0001$ and $F = 3.71$; $df = 3, 298$; $P = 0.0121$, respectively. Termite mortality on soil samples collected during the second year ($73.8 \pm 3.0\%$) was significantly lower than that seen during the other years of the study which ranged from 87.6 ± 1.8 to $93.3 \pm 1.3\%$ and mortality on samples collected at year 0 ($87.6 \pm 1.8\%$) was significantly different from year one ($93.3 \pm 1.3\%$). Termite mortality, averaged over years and treatments, on samples from Arizona ($76.8 \pm 2.1\%$) and Mississippi ($89.1 \pm 1.4\%$) was significantly lower than that from Florida ($90.8 \pm 1.6\%$) and South Carolina ($94.7 \pm 0.9\%$). These mortalities are somewhat reflective of the lower fipronil residues recovered from soil samples from Arizona and Mississippi compared to those from Florida and South Carolina (Figure 2).

The three-way interaction of years*sites*treatments was significant ($F = 2.28$; $df = 15, 896$; $P = 0.0035$) for mortality (Table 6). As was the case with penetration, there were no consistent trends of termite mortality over time since application. Except for an uncharacteristic low mortality on soil collected from exposed plots 2 y after application in Arizona ($18.2 \pm 7.1\%$), mortalities were between 76 and 100%.

Fipronil is a slow-acting nonrepellent termiticide. The lethal time to kill 50% of the population (LT_{50}) of *Reticulitermes virginicus* was determined for fipronil by Osbrink et al. (15). Termite workers placed on filter paper treated with $630.65 \mu\text{g}/\text{cm}^2$ of fipronil had an average LT_{50} of 271 min compared to an average LT_{50} of 13 min for workers exposed to $526.13 \mu\text{g}/\text{cm}^2$ of chlorpyrifos, a fast-acting organophosphate termiticide. Fipronil's lack of repellence was demonstrated by Remmen and Su (16). They observed that fipronil concentrations as high as 64 ppm did not repel *R. flavipes* or *C. formosanus*.

Table 5. Average distance (mm) penetrated by termites through soil treated with fipronil (Termidor 80 WG) at U.S. Forest Service termiticide test sites

| Year | Covered | | | |
|------|----------------|----------------|----------------|----------------|
| | Arizona | Florida | Mississippi | |
| | | | South Carolina | |
| 0 | 10.1 ± 2.9 Bc | 13.0 ± 3.5 Cc | 33.2 ± 4.0 Bb | 45.0 ± 3.3 Aa |
| 1 | 1.9 ± 1.9 Bb | 7.2 ± 3.9 Cb | 5.2 ± 2.8 Cb | 30.8 ± 3.9 Ba |
| 2 | 0.8 ± 0.8 Bc | 7.8 ± 4.3 Cc | 46.8 ± 3.6 Aa | 18.8 ± 5.0 Cb |
| 3 | 10.4 ± 4.2 Bc | 30.2 ± 5.0 Bb | 33.4 ± 4.4 Bb | 44.9 ± 3.6 Aa |
| 4 | 9.0 ± 2.9 Bc | 20.6 ± 5.0 Bb | 52.0 ± 0.0 Aa | 20.6 ± 3.1 Cb |
| 5 | 34.9 ± 5.0 Ab | 47.9 ± 2.0 Aa | 50.5 ± 1.5 Aa | 41.0 ± 4.3 Aab |
| | <i>Exposed</i> | | | |
| 0 | 0.0 ± 0.0 Bb | 9.8 ± 3.0 Db | 39.2 ± 3.4 BCa | 42.9 ± 3.6 Aa |
| 1 | 4.0 ± 1.5 Bb | 33.0 ± 5.6 BCa | 6.2 ± 3.0 Cb | 44.8 ± 1.9 Aa |
| 2 | 2.9 ± 1.5 Bb | 27.5 ± 5.8 Ca | 37.2 ± 5.1 Ba | 28.4 ± 5.7 Ba |
| 3 | 41.6 ± 4.2 Aa | 39.0 ± 5.2 Ba | 48.2 ± 2.7 Aa | 50.2 ± 0.9 Aa |
| 4 | 38.1 ± 4.8 Ab | 52.0 ± 0.0 Aa | 51.6 ± 0.4 Aa | 24.3 ± 4.4 Bb |
| 5 | 35.6 ± 5.4 Ab | 52.0 ± 0.0 Aa | 36.2 ± 4.9 Bb | 28.2 ± 5.4 Bb |

Means within a treatment (covered or exposed) and within a column (sites) followed by the same upper case letter are not significantly ($P < 0.05$) different as determined by PDIFF (21). Means within a treatment (covered or exposed) and within a row (years) followed by the same lower case letter are not significantly ($P < 0.05$) different as determined by PDIFF (21)

termites and that mortality at 1 ppm was high (89%) indicating that lack of penetration into the treated sand was due to mortality.

Because fipronil is a relatively slow acting termiticide, termites entering treated areas would not die immediately but have time to leave treated areas before death and therefore may transfer fipronil to nestmates through grooming and trophalaxis. These characteristics of fipronil, slow-activity and ability to be transferred, could possibly explain some of the variability between the degree of termite penetration and the resultant mortality seen in experiment 2. Mortality would not only be due to the amount of toxicant taken up by termites as they penetrate treated soil, but could also be due to the amount of fipronil transferred during social interaction between nestmates exposed to fipronil residues and those that never entered treated soil. In addition, the year-to-year variability in mortality and soil penetration could also be due to differences in colonies used in bioassays.

Protection of structures is dependent upon the presence of an effective termiticide barrier to termite attack. Fipronil has been the most effective new termiticide in Forest Service field tests in recent years. It has been 100% effective for 13 y against termite attack in small plot studies at the lowest label rate of 0.06% at all primary Forest Service test sites (29). Even though fipronil, applied at the lowest label rate (0.06%), had a faster rate of dissipation than that reported for older termiticide chemistries applied at rates ranging from 0.25 to 1.0%, fipronil's toxicity showed little decrease over the 5 y of this study. This is most likely due to fipronil's toxicity to termites at very low dosages.

Acknowledgements

We thank Craig Bell and Don Fye for assisting in the installation of this study and for collecting soil samples. We also thank Blossie Boyd for conducting residue analyses and laboratory bioassays.

References

1. Gant, D. B., A. E. Chalmers, M. A. Wolf, H. B. Hoffman, and D. F. Bushey. *Rev. Toxicol.* **1998**, *2*, 147-156.
2. Anonymous. 1996. *Fipronil: Worldwide Technical Bulletin*. Rhone Poulenc. Research Triangle Park, NC.
3. Gunasekara, A. S., T. Trung, K. S. Goh, F. Spurlock, R. S. Tjeerdema. *J. Pest. Sci.* **2007**, *32*, 189-199.
4. Coquet, Y. *Pest. Manag. Sci.* **2002**, *58*, 69-87.
5. Guo, L., W. A. Jury, R. J. Wagnet, and M. Flury. *J. Contam. Hydrol.* **2000**, *43*, 45-62.
6. Bobe, A., C. M. Coste, and J. F. Cooper. *J. Agric. Food Chem.* **1997**, *45*, 4861-4865.
7. Mulrooney, J. E., and P. D. Gerard. *Sociobiol.* **2007**, *50*, 63-70.
8. Ying, G. and R. S. Kookana. *Environ. Toxicol. Chem.* **2006**, *25*, 2045-2050.

9. Ying, G. and R. S. Kookana. *J. Environ. Sci. Health.* **2001**, B36, 545-558.
10. Bobe, A., J. F. Cooper, C. M. Coste, and M. A. Miller. *Pestic. Sci.* **1998**, 52, 275-281.
11. Brookhart, G. and D. F. Bushey. 1994. *Eight International Union of Pure and Applied Chemistry International Congress of Pesticide Chemistry*, July 4-9 1994, Washington, DC, abstract 189.
12. Hainzl, D., L. M. Cole, and J. E. Casida. *Chem. Res. Toxicol.* **1998**, 11, 1529-1535.
13. Mulrooney, J. E. and D. Goli. *J. Econ. Entomol.* **1999**, 92, 1364-1368.
14. Ibrahim, S. A., G. Henderson, and H. Fei. *J. Econ. Entomol.* **2003**, 92, 461-467.
15. Osbrink, W. L. A., A. R. Lax, and R. J. Brenner. *J. Econ. Entomol.* **2001**, 94, 1271-1228.
16. Remmen, L. N. and N. Y. Su. *J. Econ. Entomol.* **2005**, 89, 906-910.
17. Shelton, T. G. and J. K. Grace. *J. Econ. Entomol.* **2003**, 96, 456-460.
18. Hu, X.P. *J. Econ. Entomol.* **2005**, 98, 509-517.
19. Su, N. Y., R. H. Scheffrahn, and P. M. Ban. *J. Econ. Entomol.* **1993**, 86, 772-776.
20. Abbott, W. S. *J. Econ. Entomol.* **1925**, 18: 265-267.
21. SAS Institute. 2001. *User's Guide, ver. 8e*. SAS Institute, Cary, NC.
22. Mulrooney, J. E., T. L. Wagner, B. M. Kard, and P. D. Gerard. *Sociobiol.* **2006**, 48, 117-133.
23. Zhu, G., H. Wu, J. Guo, and F. M. E. Kimaro. *Water, Air, and Soil Pollution* **2004**, 153: 35-44.
24. Beal, F. H. and F. L. Carter. *J. Econ. Entomol.* **1968**, 61, 380-383.
25. Carter, F. L. and C. A. Stringer. *Bull. Environ. Contam. & Tox.* **1970**, 5, 421-426.
26. Sparks, D. L. *Environmental Soil Chemistry*. Academic Press, Inc. San Diego, CA. 1995.
27. Ahmad, R., R. S. Kookana, A. M. Alston, and J. O. Skjemstad. *Environ. Sci. Tech.* **2001**, 35, 878-884.
28. Smith, J. L. and M. K. Rust. *J. Econ. Entomol.* **1993**, 86, 53-60.
29. Wagner, T., T. Shelton, C. Peterson, and J. Mulrooney. *Pest Control.* **2007**, 75, 58-60, 62, 64, 66-69.