

# Effect of vegetation on the longevity, mobility and activity of fipronil applied at the termiticidal rate in laboratory soil columns<sup>†</sup>

Chris J Peterson\*

## Abstract

**BACKGROUND:** Termiticides are applied at concentrations much higher than those used in agricultural settings. The longevity of fipronil has not been examined at the rates used for termite control, nor has the compound's movement in the soil been addressed.

**RESULTS:** Fipronil was detected in the eluates of treated soil cones, increasing initially and then decreasing to a steady concentration of about  $1 \mu\text{g mL}^{-1}$ . In larger PVC pipe plots, fipronil in the top treated soil depth (0–7.5 cm) dissipated more rapidly (half-life of 11–13 months) than in treated soil at the next treated depth (7.5–15 cm; half-life of 20–29 months). The presence of vegetation had no significant effect on the mobility, longevity or movement into untreated depths. Treated soil remained toxic to termites throughout the duration of the study. Fipronil moved into the 15–22.5 cm soil depth in sufficient concentration to cause 100% mortality to eastern subterranean termites in 3 day bioassays.

**CONCLUSION:** Fipronil remains in treated soil at levels toxic to termites for at least 30 months. Movement of the active ingredient was observed in sufficient amounts to kill termites in non-treated soil directly below the treated soil.

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**Keywords:** fipronil; soil mobility; longevity; termiticide

## 1 INTRODUCTION

The phenylpyrazole compound fipronil is a  $\gamma$ -aminobutyric-acid-gated chloride channel blocker with a high selectivity to insects owing to the presence of a trifluoromethylsulfinyl moiety.<sup>1</sup> Formulated as a  $91 \text{ g L}^{-1}$  SC, Termidor<sup>®</sup>, by BASF, fipronil is currently one of the best-selling termiticide active ingredients in the United States.

Soil-applied insecticides are used with the expectation of providing several years of protection from insect infestation. Fipronil is known to have a relatively long life in the soil, and, at the rate labeled for perimeter treatment for the prevention of termite infestation, Waite<sup>2</sup> found that there was no statistically significant dissipation of fipronil over the course of 18 months. The longevity of a treatment depends on several non-mutually exclusive factors, including irreversible binding to soil components, mobility of the active ingredient, microbial degradation and, if applicable, uptake by vegetation, acid/base hydrolysis and photodegradation.

Ying and Kookana<sup>3</sup> found that the sorption of fipronil to soil organic matter and to clay increased with increasing application concentration and with increasing polarity of the application solvent. The water solubility of an active ingredient is one of the factors that affect its movement in the soil. Compared with other termiticidal active ingredients, fipronil is much less water soluble than imidacloprid ( $2 \text{ mg L}^{-1}$  versus  $510 \text{ mg L}^{-1}$ ), but is as soluble as chlorpyrifos ( $2 \text{ mg L}^{-1}$ ) and is more soluble

than permethrin ( $0.2 \text{ mg L}^{-1}$ ) or chlordane ( $0.1 \text{ mg L}^{-1}$ ).<sup>4</sup> Because fipronil can be applied to the exterior of a structure, excessive degradation due to exposure to the elements is a legitimate concern, especially on the rainward side of the structure. Fipronil applied to the surface of the soil was detected to a depth of 60 cm within 22 months of application, but residues below 30 cm dissipated by 56 months.<sup>5</sup> Although only a few studies report uptake of fipronil into plants,<sup>6,7</sup> reduction of fipronil residues in soil through enhanced microbial degradation is a possibility. Soil microorganisms contain detoxification enzymes<sup>8</sup> and are associated with the root zone (rhizosphere) of such plants.<sup>9</sup> On the other hand, Gallaher and Mueller<sup>10</sup> found that the degradation of herbicides was slower in vegetated soil owing to reduced moisture content.

In the present study, the effects of the presence of vegetation, time and soil depth on longevity, mobility and termiticidal activity

\* Correspondence to: Chris J Peterson, USDA Forest Service, Insects, Diseases and Invasive Plants Research Unit, 201 Lincoln Green, Starkville, MS 39759, USA. E-mail: cjpeterson@fs.fed.us

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USDA Forest Service, Insects, Diseases and Invasive Plants Research Unit, Starkville, MS, USA

of the fipronil formulation Termidor were examined. A cone plot analysis was used to determine the effects of various factors on the concentration of fipronil in cone plot eluates. The concentrations in the eluates were determined by using gas chromatographic assays. The residues of fipronil in 15 cm of treated soil and in soil below the treated soil were examined in 45 cm packed soil columns at 3 month intervals for 30 months. The aged soil recovered from the columns was analyzed for toxicity to termites. This study addresses the longevity of fipronil treatments and the mobility of fipronil into untreated soil.

## 2 METHODS

### 2.1 Chemicals and soil

Fipronil 91 g L<sup>-1</sup> SC (Termidor) was purchased from a commercial retailer (Pest Control Depot, Palm Bay, FL). All organic solvents (acetonitrile, acetone and toluene) were Certified or Optima grade, purchased from Fisher Scientific (Hampton, NH). Deionized water was obtained from a Barnstead Nanopure ultrapure water system (Dubuque, IA).

The soil used was a sandy loam (14.75% silt, 75.25% sand, 10.00% clay, pH 7.4, 1.49% organic matter and a field capacity of 20.0% w/w moisture). This soil was that used in a previous examination of imidacloprid,<sup>11</sup> and bioassays and residue analysis of the soil showed it to be free of interfering pesticides.

### 2.2 Cone plot test

Cone plots consisted of 21.5 cm tall × 4 cm inside diameter (top) and 2.4 cm inside diameter (bottom) Ray Leach UV-stabilized Containers purchased from Hummert International (Earth City, MO). Each cone was fitted with glass wool at the bottom to prevent loss of soil.

Fipronil at the 0.06% labeled rate was prepared by diluting 6.4 mL of Termidor SC in 1 L of deionized water and mixing on a magnetic stir plate to constitute 0.58 mg mL<sup>-1</sup> fipronil. Soil (418.5 ± 0.25 g per each of three replications) was treated by adding 35 mL of the fipronil dilution (or 35 mL of water for the control) to the soil in plastic jars. The dilution was added in three 10 mL portions followed by the final 5 mL, with hand mixing between the addition of portions. Separate applications were made for each of the three replications. Following the addition of the last portion, the jars were placed on a jar roller for 30 min. The soil was left to sit for 2 h to allow the solution to diffuse through the soil. Cone plots were filled to a depth of 15 cm (about 140 g soil) with the appropriate soil treatment. Unused soil was reserved for extraction and analysis for fipronil by methods described in Section 2.4 and for bioassay as described in Section 2.5. The treated soil for each replication was divided into cones that would be vegetated and non-vegetated, so that the initial fipronil concentration of both treatments within a replication was the same. Vegetated cones received about 0.1 g of Bermuda grass [*Cynodon dactylon* (L.) Pers.] seed; non-vegetated cones received no seeds. Each cone received 25 mL of deionized water to wet the soil without resulting in saturation, followed 30 min later by the addition of 30 mL of water to provide a sufficient amount of eluate for the  $t = 0$  data, which were analyzed using the procedures detailed below.

The cones were kept in a greenhouse under natural light and 20–30 °C. Each cone was watered (30 mL) once weekly, simulating a 2.5 cm rainfall event. Once per month for 6 months, each plot received enough water so that >10 but <15 mL eluate collected

in a beaker. The eluates were cleaned up by using solid-phase extraction (SPE) cartridges as follows: one C-18 SPE (Thermo Scientific Hypersep, 500 mg) cartridge was conditioned by passing 5 mL of acetonitrile followed by 5 mL of water. The cartridge was allowed to dry by pulling a vacuum (67.7 kPa) through it for 15 min. Once dry, 10 mL of eluate was passed through the column, and pulling a vacuum for 15 min dried the column. The fipronil was washed off the column by using 5 mL of toluene. The toluene was reduced to less than 1 mL by using a stream of nitrogen, and then reconstituted to 1 mL with toluene.<sup>3</sup>

Fipronil concentration was quantified by gas chromatography with electron capture detection (GC-ECD) on an Agilent 6890 gas chromatograph by using the following method: injection volume 1 µL; injector temperature 250 °C; column Agilent 1909 1A-112 ultra 1 methyl siloxane 25 m × 320 µm inside diameter × 0.52 µm pore size; carrier gas helium at 20 mL min<sup>-1</sup> flow rate; oven temperature 50 °C for 1 min, ramped at 30 °C min<sup>-1</sup> to 200 °C and held for 10 min, ramped again by 30 °C min<sup>-1</sup> to 230 °C and held for 8 min; total run time 25 min; detector temperature 250 °C; equilibration time between runs 3 min; needle washes: two washes with hexane followed by two washes with acetone. The data were analyzed by using mixed analysis of variance for repeated measures on SAS.<sup>12</sup>

### 2.3 PVC pipe plot test

Polyvinyl chloride (PVC) pipe plots were used to examine the movement of fipronil in soil. A plastic plate with holes cut for drainage of water was affixed to the bottom of a 45 × 10.1 cm ID PVC pipe, with a piece of aluminum window screen fitted over the holes to prevent loss of soil. The pipes were filled to 30 cm with untreated soil, and the soil was then saturated with water to settle the soil and to prevent later compaction. The columns were allowed to drain for at least 24 h, and more soil was added to bring it up to the 30 cm mark.

Soil was treated at the recommended labeled rate with Termidor SC (24 mL Termidor SC concentrate in 3785 mL of water) or with water for the control. Between 40 and 45 kg of soil for each of the three replications was placed in a cement mixer, and the 3785 mL of solution was added. The soil was tumbled in the cement mixer for 15 min. The treated soil was placed in the pipes on top of the untreated soil to a depth of 15 cm. Unused soil was placed in large resealable plastic bags and reserved at ambient conditions in the dark for residue analysis and bioassay at 3 month intervals. This was done so that comparisons could be made between the reserved soil and soil that received weekly watering, was exposed to sunlight and included vegetation (if applicable). Vegetated plots were seeded with about 0.5 g of Bermuda grass seed, and the top 1 cm of soil was agitated to cover the seeds. Plots were watered (200 mL) immediately and once per week to simulate a 2.5 cm rain event. The plots were kept in a greenhouse under natural light conditions at 10–30 °C. At the start of the test and at 3 month intervals for 2.5 years, eight pipes (three vegetated and treated, three non-vegetated and treated, one vegetated and untreated and one non-vegetated and untreated) were cut into 7.5 cm sections, and the treated pipes were analyzed for fipronil content by using accelerated solvent extraction (as described in Section 2.4) and analysis by GC-ECD (described in Section 2.1). Collected soil was assayed for termiticidal activity as described in Section 2.5. This is a completely randomized design with a split-plot arrangement, with each pipe (combination of vegetated state, time and treatment) as the whole plot factor, and soil depth as the subplot factor. Mixed analysis of variance was used to determine

significance due to soil depth, vegetated state and time. This portion of the study had three replications.

### 2.4 Soil moisture determination and fipronil extraction

Fipronil was extracted by placing recovered soil into a foil weigh boat so that the total weight of the soil and the boat was between 35.0 and 35.5 g. The soils were dried at 90 °C overnight in an oven. After cooling, the soils and boats were reweighed and the percentage moisture (by weight) was found by subtraction.

For the extraction of fipronil, 25 ± 0.01 g of the dried soil was extracted by using a Dionex ASE 200 accelerated solvent extractor. This method used 60 mL of acetonitrile + acetone (70 + 30 by volume) passed through the 25 g sample at 100 °C and 10 342 kPa (1500 psi). The sample was concentrated to 10 mL under a nitrogen stream, and the resulting extract was analyzed by GC-ECD as described in Section 2.1. Percentage recovery of fipronil by using this method ranged from 98.2 to 113% at 20 µg g<sup>-1</sup> soil, depending on soil type.<sup>13</sup>

### 2.5 Termite bioassays

All bioassays used in this study used a 60 mm petri dish filled with 15–20 g of soil and moisturized to about 17–23% soil moisture by weight. Each dish had a 1 cm<sup>2</sup> cardboard square to provide food, and ten eastern subterranean worker termites (*Reticulitermes flavipes* Koll.) were placed in each dish. The dishes were held at room temperature in the dark, and the number of living termites was observed at 3 and 7 days.<sup>14</sup>

A preliminary toxicity test with three replications was conducted with fipronil at 100, 10, 1, 0.1 and 0.01% of the labeled rate (about 60, 6, 0.6, 0.06 and 0.006 µg g<sup>-1</sup> in soil, respectively). The dishes were destructively examined at 3 and 7 days, and the number of surviving termites was counted.

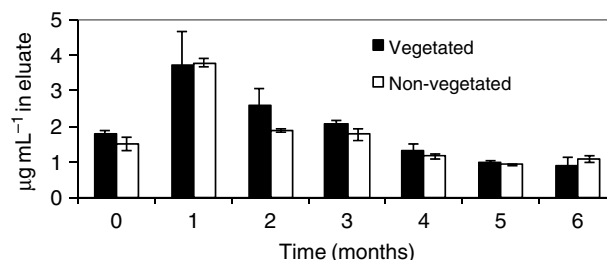
At 3 month intervals, i.e. when the pipe plots were cut, one 10–20 g portion of soil was removed from each pipe plot section (including the untreated pipe plots) and placed in a petri dish. Soil kept at ambient conditions since the time of treatment was bioassayed at these times as well. Ten worker termites from *R. flavipes* populations collected in the wild were placed in dishes set up as described above, and survival was recorded non-destructively at 3 and 7 days. The survival data were converted to mortality and square root arcsine transformed prior to mixed analysis of variance.<sup>12</sup>

## 3 RESULTS AND DISCUSSION

### 3.1 Cone plot test

The presence of vegetation had no effect on the fipronil concentration in eluates of the cone plots (Fig. 1). Time was the only factor to affect significantly eluate concentration ( $F = 40.49$ ;  $df = 6, 24$ ;  $P < 0.0001$ ).

Fipronil concentration in the eluate peaked at 3.8 µg mL<sup>-1</sup> at 1 month, and then decreased slowly from months 2 to 6 (Fig. 1). A similar increase in the first month was seen for imidacloprid.<sup>11</sup> The concentration of fipronil in soil declined from 15.7 µg g<sup>-1</sup> for vegetated and non-vegetated soil to 11.6 and 7.8 µg g<sup>-1</sup> (74 and 50% of the initial concentration, respectively) for vegetated and non-vegetated soil 6 months later. Mass balance of the fipronil was not attempted because not all of the eluates were collected. Soil treated at the initiation of the cone plot study and reserved for 6 months in the dark with no applications of water showed no dissipation of fipronil (data not shown). Soil recovered from



**Figure 1.** Fipronil concentration in the eluate of vegetated and non-vegetated cone plots. Error bars represent the SEM.

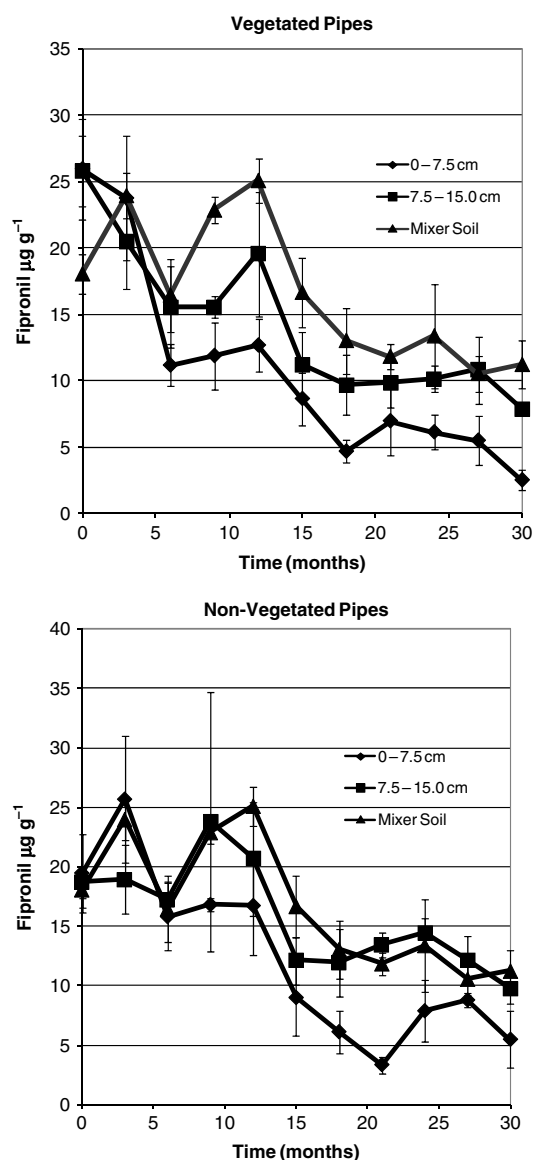
treated cone plots caused 100% mortality in 3 day bioassays after 6 months of weekly watering.

### 3.2 Soil extraction from PVC pipes

Figure 2 shows the concentration of fipronil in the 0–7.5 cm and 7.5–15 cm soil layers and in the soil reserved at the time of treatment. When all times and soil depths were analyzed together, there was a significant depth by time interaction; i.e. the rate of degradation of fipronil in the soil depended upon soil depth ( $F = 5.98$ ,  $df = 50, 219$ ;  $P < 0.0001$ ). The largest differences were seen in the top two (treated) depths and in the 15–22.5 cm depth. Settling of soil, as well as mobility of the active ingredient, might have caused the increase in fipronil concentrations at the 15–22.5 cm depth. Because little fipronil penetrated below 22.5 cm, differences in fipronil concentration at lower depths were not significant.

Fipronil dissipated more rapidly in the top depth (0–7.5 cm) than it did in the second depth (7.5–15 cm) (Fig. 2). The interaction of depth and time was significant ( $F = 2.36$ ;  $df = 10, 44$ ;  $P = 0.0245$ ), indicating that the effect of soil depth depended on time. The concentration was higher in the top depth for the first 6–9 months, but afterwards it was higher in the 7.5–15 cm depth (Fig. 2). Horwood<sup>15</sup> found increasing fipronil residues over time with increasing depth 15 months after soil treatment. The more rapid dissipation of fipronil in the top layer is likely due to a combination of exposure to sunlight, higher microbial activity and mobility of the active ingredient into lower layers. Because this effect was seen in both vegetated and non-vegetated soil in this study, the effect was not due to the presence of vegetation. Fipronil in the 7.5–15.0 cm depth dissipated at about the same rate as in the reserved soil, indicating that microbial degradation and soil binding were the primary mechanisms of dissipation in the PVC pipes. Indeed, microbial activity reduced the half-life of fipronil from about 32 to about 10 days.<sup>16</sup>

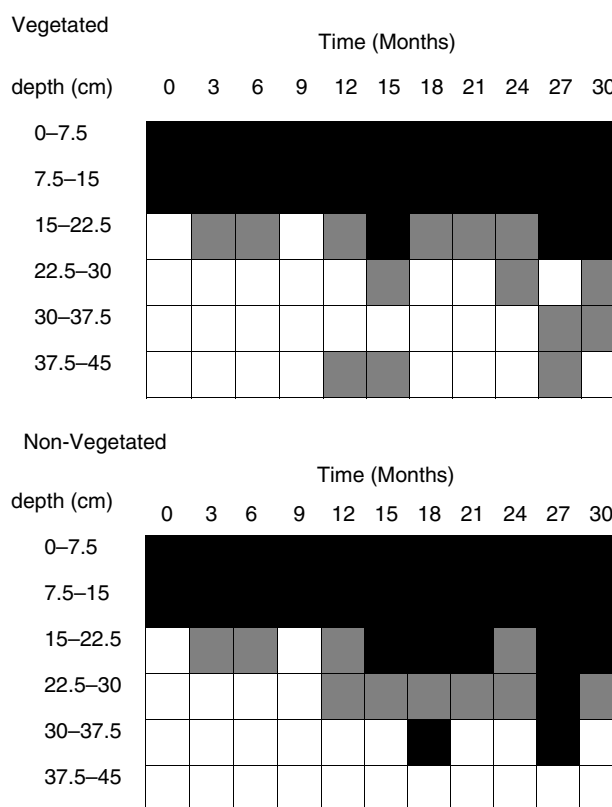
The half-life of fipronil was estimated by fitting an exponential trend line to the dissipation curves of the reserved soil and the soil recovered from the 0–7.5 cm and 7.5–15 cm depths. The half-life in reserved soil was estimated at about 28 months. In the 0–7.5 cm depth, the half-lives of fipronil in the vegetated and non-vegetated pipes were similar, or about 11 and 13.5 months, respectively. In the 7.5–15 cm depths, however, the half-life differed between the vegetated and non-vegetated pipes; about 20 months in vegetated pipes and about 29 months in non-vegetated pipes. In spite of this numerical difference, the effect of vegetation on dissipation at this depth was not statistically significant at the 0.05 level. In a field study, Horwood<sup>15</sup> found a 72 or 96% reduction in fipronil residues at a 7.5 cm depth 12 months after application, depending on study site location. The higher dissipation in Horwood's study<sup>15</sup> might be due to several factors: a slightly



**Figure 2.** Dissipation of fipronil in the top soil layer (0–7.5 cm), in the second soil layer (7.5–15 cm) and in soil reserved and kept in an incubator. Error bars represent the SEM.

lower initial fipronil concentration was used; the study allowed for lateral movement of the compound out of the treated soil; the overall air temperature was higher than in the present study; dissipation was presumed to be more rapid in the field. More precise half-lives for fipronil have been estimated in laboratory studies as 68–198 days in non-sterile soil, depending upon soil moisture,<sup>3</sup> or 122–128 days in sandy loam and 308–342 days in loamy sand.<sup>17</sup> The half-lives reported in the present study were generally longer (about 335 to about 884 days) than those reported by Ying and Kookana<sup>3</sup> because the initial concentrations here were much higher than those used in their study, and the effect of initial concentration on half-life has been documented for other compounds.<sup>18</sup> The initial soil concentrations in the studies reviewed by Tingle *et al.*<sup>17</sup> were not specified.

Fipronil was detected in all untreated soil depths by 3 months, and the concentration generally decreased with increasing depth and increasing time after 9 months (data not shown). The effect



**Figure 3.** Mortality of termites exposed to soil recovered from PVC pipes for 3 days: ■ soil portions resulting in 100% mortality; ▒ soil portions resulting in 70–99% mortality; □ soil portions resulting in less than 69% mortality.

of vegetation on fipronil concentration in untreated soil depths depended upon both soil depth and time, i.e. a significant three-way interaction ( $F = 1.79$ ;  $df = 30, 131$ ;  $P = 0.0140$ ). There were few differences between untreated depths within any time or between times for any given depth beyond 9 months. Fipronil is generally considered to be immobile in soil, with a general increase in mobility in progressively sandier soils.<sup>17</sup> This is attributed to the affinity of fipronil for soil particles, particularly organic matter and to a lesser degree clay.<sup>3</sup> A study of the initial penetration of fipronil into soils of varying moisture, however, showed no significant differences due to soil type, although the effects of soil type on long-term mobility were not investigated.<sup>13</sup>

### 3.3 Bioassay results

In the preliminary toxicity test, 100% mortality was seen in the 3 day bioassay at 1% of the labeled rate (about  $0.6 \mu\text{g g}^{-1}$ ), and in the 7 day bioassay at 0.1% of the labeled rate (about  $0.06 \mu\text{g g}^{-1}$ ). This is consistent with the results of Mulrooney and Gerard,<sup>19</sup> who found a 4 day  $LC_{50}$  value of  $0.49 \mu\text{g g}^{-1}$  in sandy loam soils.

The treated soil remained toxic to termites in 3 day bioassays for the duration of the study (Fig. 3). Soil from the 15–22.5 cm depth caused between 90 and 100% mortality to the termites at 3 months and after. Soil recovered from the 22.5–30 cm depth began to show toxicity from about 12 months onwards, with the greatest toxicity in all untreated depths from 15 to 21 months (Fig. 3). When comparing all depths, the effects of vegetation and time on mortality both depended upon depth; i.e. there was a significant depth by vegetation interaction ( $F = 3.32$ ;  $df = 5, 220$ ;  $P = 0.0065$ ) and a significant depth by time interaction ( $F = 4.87$ ;



df = 50, 220;  $P < 0.0001$ ), although the three-way interaction of depth, time and vegetation was not significant. Analyzing only the untreated depths did not change the significance of the factors. Soil from non-vegetated pipes was more toxic to termites at the 15–22.5, 22.5–30 and 30–37.5 cm depths, but soil from vegetated pipes was generally more toxic to termites at the 37.5–45 cm depth, although a statistical relationship was not seen.

Enough of the active ingredient moved into all but the lowest untreated soil depths to cause nearly 100% mortality in 7 day bioassays but not in 3 day bioassays (data not shown). Exposure to the air and the application of water lowered the concentration of the active ingredient in the top 7.5 cm, but dissipation in the 7.5–15 cm soil depth did not differ from that seen in reserved soil.

From the above bioassay data, if  $0.6 \mu\text{g g}^{-1}$  is taken as the lowest toxic exposure in 3 day bioassays, and by using the same dissipation curves as used to estimate the half-life in the different soil sections, then fipronil will dissipate to below effective levels in 57 and 71 months in the top depth of the vegetated and non-vegetated pipes, respectively, and in 106 and 161 months in the 7.5–15 cm depth of vegetated and non-vegetated pipes, respectively. Furthermore, had the top 7.5 cm of the pipes been sectioned into smaller increments, it is possible that by the end of the study the lowest fipronil concentrations would have been found at the surface. It is unknown if the top 1 cm or so of soil no longer contained enough fipronil to prevent termite infestation. Because it is likely that termites would only need a small depth of soil in which to tunnel, efficacy might be overestimated by examining the entire top 7.5 cm.

This study demonstrates that fipronil remains in treated soil in concentrations sufficient to kill termites for at least 30 months. By extrapolating the residue analysis data beyond 30 months and by comparing with fipronil toxicity data presented in Peterson,<sup>13</sup> much longer periods of efficacy can be expected. The cone plot study demonstrated that fipronil moves with soil water, and fipronil was detected in the PVC pipes at the greatest depth tested (37.5–45 cm). This mobility was not sufficient to cause high mortality in untreated soils in 3 day bioassays, but was sufficient in 7 day bioassays. The presence of vegetation in treated soil had no discernable effect on the longevity, mobility or termiticidal activity of fipronil, indicating that this product may be used in exterior perimeter applications with minimal concern for enhanced degradation or translocation due to turf grass or landscape plants.

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