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Concentration-Dependent Degradation of Three Termiticides in Soil Under Laboratory Conditions and Their Bioavailability to Eastern Subterranean Termites (Isoptera: Rhinotermitidae)

RAJ K. SARAN¹ AND SHRIPAT T. KAMBLE²

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ABSTRACT Degradation and bioavailability of imidacloprid, fipronil, and bifenthrin applied at label rates ([AI], wt:wt in soil) in the loamy soil of Nebraska were determined over a 6-mo duration. Based on the calculated half-lives of the three termiticides, it was concluded that the degradation rate was lowest when a termiticide was applied at the highest label rate. Bioassays of *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) conducted at 8, 31, 65, 90, 135, 160, and 180 d posttreatment showed an inverse relationship between the LT_{90} values and the variable concentrations. At day 180, exposures to all the termiticide-treated soil samples (concentration \times termiticide) resulted in 100% mortality of *R. flavipes* workers. However, lower LT_{90} values were observed for termites exposed to soils treated with highest label rates even when the treated soils were aged in the lab for 6 mo. This suggested a higher bioavailability of these three termiticides when applied at higher application rates. Termite mortality was fastest for bifenthrin followed by fipronil and imidacloprid.

KEY WORDS toxicity, bifenthrin, fipronil, imidacloprid, half-life

For more than six decades, treatment of soil with termiticide has been the conventional technique for the control of subterranean termites (Su and Scheffrahn 1998). In majority of cases (two-thirds), pest control companies use termiticides instead of baits and wood treatments in preventing and controlling termite damage (Curl 2004). In recent years, insecticides such as chloronicotinyl (imidacloprid), neonicotinoid (thiamethoxam), phenyl pyrazole (fipronil), and pyrole (chlorfenapyr) have become popular alternatives to organophosphates and pyrethroids. These compounds are less hazardous than chlorinated hydrocarbons and organophosphates and have a limited life in soil. These compounds were reported to be nonrepellent and slow acting (Shelton and Grace 2003, Ibrahim et al. 2003, Remmen and Su 2005a, 2005b; Saran and Rust 2007). It has been suggested that due to this nonrepellency and delayed toxicity, exposed foraging termites transfer lethal amounts to nestmates. These termiticides are considered to be more effective in controlling termites by killing them some distances away from the treated structures (Potter and Hillery 2002). However, recent studies have shown that the extent of horizontal transfer of termiticide among foragers was limited to 5–6 m from the treated barrier (Osbrink et al. 2005, Su 2005, Rust and Saran 2006, Saran and Rust 2007). Thus, the residual amounts and

bioavailability of termiticides over time may be the primary factors contributing to the termiticide efficacy in soil. Studies with other soil insects have indicated that soil type, soil pH, insecticide type, moisture, temperature, microbial communities, and target insect affect insecticide degradation, bioavailability, and its efficacy in the soil (Harris 1972, Tashiro and Khur 1978, Chapman et al. 1982, Macalady and Wolfe 1983, Felsot and Lew 1989).

Su et al. (1993) and Gold et al. (1994) reported that termiticides lose effectiveness over time. Gold et al. (1996) further showed differences in termiticide concentrations through time, indicating that within 180 d, all termiticides (chlorpyrifos, imidacloprid, and deltamethrin) included in the tests had significantly decreased in concentration (wt:wt basis in soil). Austin (1999) has demonstrated that exposure to chlorpyrifos, deltamethrin, and imidacloprid and subsequent mortality of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) were affected by soil type. Also, the initial concentration at which the termiticides were applied affected degradation rate. Chlorpyrifos exhibited lower degradation rate when applied at $\approx 1,000 \mu\text{g/g}$ soil than when applied at typical agricultural levels of 0.3–32 $\mu\text{g/g}$ soil (Racke et al. 1994).

Currently registered termiticides have soil organic partition coefficients values (K_{oc}), which place them in the immobile classification, implying they do not readily leach through the soil profile (Helling and Turner 1970, McCall et al. 1979). This indicates the potential for interactions between these compounds

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and components of the soil matrix that may affect the biological activity of these insecticides (Forschler and Townsend 1996).

Bioavailability of the chemical has two components. The first is availability to the insects in the soil. The second component is due to the physical distribution of a compound in the soil after initial application. Therefore, the efficacy of an insecticide in soil is governed by the intrinsic toxicity of an insecticide, its ability to penetrate into the insect, and its bioavailability (Simmons et al. 1992). A compound may be present in the soil well above detection limits but not be available to insects in a sufficient quantity to control them.

Variable application rates for termiticides are meant to accommodate a wide range of environmental conditions (soil type, pH, and soil moisture content), and they are presumably based on the physicochemical properties of the termiticides. However, applicators in real-life situations are often confused about application rates, and some opt for lower doses to reduce costs. But when applied at lowest recommended rates, failures of termite treatments are common and the durability of treatments is often reduced. However, little is known about the degradation and bioavailability of fipronil, bifenthrin, and imidacloprid over time when applied to the soil as a termiticide at different application rates.

We proposed a hypothesis that the half-life of termiticides (calculated from degradation rates) in soil is dependent on the applied concentrations. Also, the toxicity of termiticides is correlated to their bioavailability over time in soil. This laboratory study was conducted to predict the effect of application rates on termiticides efficacy in soil over time. Experiments were designed to address the following objectives: 1) determine half-lives of termiticides, applied at various concentrations in similar soil type and environmental conditions; and 2) evaluate the toxicity and bioavailability of termiticides to eastern subterranean termite over time.

Materials and Methods

Termites. Eastern subterranean termites were collected from infested logs (*Pinus* sp.) in Henderson, NE (50 km west of Lincoln, NE). Termites were identified using morphological keys for soldiers and workers (Snyder 1954). Termites were maintained in the dark at 25°C in 60- by 15- by 30-cm glass aquaria containing 7- by 3- by 1-cm wood blocks of white pine (*Pinus alba* L.). Undifferentiated termite workers were used for bioassays.

Soil and Soil Analysis. In spring 2000, soil representing a residential area was collected near Lincoln, NE. For analysis, soil (500 g) was pulverized and passed through a 2-mm sieve and autoclaved (1 h at 120°C and 1 atmosphere) on two successive days. Soil was analyzed for particle size, pH, organic matter content, cation exchange capacity (CEC), phosphorus (Bray-1), and potassium by the Soil and Plant

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Termiticides. Termiticides representing three chemical classes were selected as follows: 1) chloronicotinyl (imidacloprid, Premise), 2) phenyl pyrazole (fipronil, Termidor, and 3) pyrethroid (bifenthrin, Talstar). Formulated products Premise 75 WSP (Bayer Environmental Science, Research Triangle Park, NC), Termidor 9.1% SC (BASF Corporation, Research Triangle Park, NC), and Talstar 7.91% SC (FMC Corporation, Philadelphia, PA) were purchased from a local distributor. Technical grade imidacloprid, fipronil, and bifenthrin were purchased from Chem Service Inc. (West Chester, PA).

Treatments. Termiticides were applied to 400 g of soil (passed through a 2-mm sieve) and stored in 1.89-liter Ziploc bags (S. C. Johnson, Inc., Racine, WI). Soil aliquots were previously weighed on an oven-dried basis and adjusted to 10% moisture content. Stock solutions in deionized distilled water (1,000 ml) were prepared for each termiticide (formulated product). A required amount of stock solution was added to 500 ml of deionized water such that when applied to 400 g of soil in bags it provided the desired concentration (w:w) of active ingredient (AI). Three insecticide concentrations (low, medium, and high) were used for each termiticide. Imidacloprid was applied at 50, 75, and 100 μg (AI)/g soil; fipronil at 60, 95, and 125 μg (AI)/g soil; and bifenthrin at 60, 90, and 120 μg (AI)/g soil. Four replications were used for each concentration. For controls (untreated checks), 400-g soil samples were treated with 500 ml of deionized water alone.

Treated soil samples in Ziploc bags were allowed to air-dry in a fume hood overnight (≈ 12 h) and moisture was adjusted to $25\% \pm 1.2$. Soils were maintained at $25 \pm 2^\circ\text{C}$ and $98.0 \pm 3.2\%$ RH in growth chambers (Percival Scientific, Boone, IA). Temperature and humidity data were collected using a Traceable hygrometer/thermometer (model 11-66-21, Hart Scientific, Friendwood, TX). Deionized water (5–10 ml) was sprinkled on the samples once a week to maintain $\approx 25\%$ soil moisture content. At 0, 8, 31, 65, 90, 135, 150, and 180 d, the soil was thoroughly mixed, and a 10-g soil was randomly sampled from treated lots and controls for chemical analysis.

Sample Preparation, Extraction and Analysis of Termiticides. Using the extraction procedure of Steinwandter (1992), 10 g of soil was placed in 125-ml Erlenmeyer flasks. Forty milliliters of high-performance liquid chromatography (HPLC) grade acetonitrile was added to each soil sample, and the flasks were stoppered and placed in an incubator/shaker (G25-KLC, New Brunswick, Edison, NJ) maintained at 20°C and agitated overnight (≈ 12 h) at 250 rpm. The samples were allowed to stand for 1 h so as to allow soil particles to settle. A 1.5 ml of the clear supernatant was transferred to a 2.0-ml microcentrifuge tube. Aliquots were centrifuged at $12,000 \times g$ for 20 min, and supernatants were transferred into a new 2.0-ml microcentrifuge tube after passing through a 3-cc glass syringe equipped with a 0.2- μm Acrodisc CR PTFE syringe

filter (Pall Corporation, East Hills, NY). Finally, 1 ml of the solution from each microcentrifuge tube was pipetted into a 2.0-ml auto-injector vial, sealed with a PTFE lined screw cap (Varian Inc., Walnut Creek, CA). Samples were analyzed soon after extraction, and unused vials were stored at -20°C until analysis. Standard curves for each of the termiticide were prepared using serially diluted stock solutions 1,000, 1,250, and 1,200 ppm (wt:vol) of technical grade imidacloprid, fipronil, and bifenthrin in acetone, respectively.

Three extracted termiticides were analyzed separately using HPLC (Varian 9012 pump, 9050 variable length detector UV/VIS, and 9100 autosampler). Data collection and peak analyses were performed using Varian Star version 4.5 chromatography workstation connected to a computer. For imidacloprid, a mobile phase 70:30 (water:acetonitrile) under isocratic conditions at a flow rate of 1.0 ml/min was used. A reverse phase C18 column (250- \times 4.6 mm i.d.; 5- μm particle size; Luna Phenomenex, Torrance, CA) was used. The UV/VIS detector was set at (λ) 270 nm, as described by Placke and Weber (1993) and Baskaran et al. (1999). For fipronil, a methanol:water gradient (78:22-72:28 over 12 min) with 1.0 ml/min flow rate was used to separate fipronil from the sulfone metabolite (Hainzl and Casida 1996) and the detector UV/VIS was set at 280 nm. A reverse phase C18 column (250 by 4.6 mm i.d., 5- μm particle size; Luna Phenomenex) was used. Bifenthrin samples were analyzed using a reverse phase Luna C18 column (150 by 4.6 mm i.d., 5- μm particle size; Luna Phenomenex). Mobile phase was acetonitrile:water (95:5) at a flow rate of 1.0 ml/min under isocratic conditions. UV/VIS detector was set at 204 nm.

Using these extraction procedures and analysis conditions, the method sensitivity for all the three termiticides was ≈ 0.5 mg/kg or ≈ 100 -fold lower than lowest application rate.

Extraction Efficiency and Recovery Rate. To determine the extraction efficiency and analytical quality control, recovery tests were performed for each of the insecticides, at each sampling interval. A 100-ml solution (in acetone) of the technical grade termiticide was added to an 80-g sterilized soil sample. The final concentration of each termiticide in 80-g soil samples was 100 ppm ([AI], wt:wt). The acetone was allowed to evaporate overnight in a fume hood. The treated soil was then stored in 50-ml centrifuge tubes (30 by 11.5 cm Falcon, BD Biosciences, Franklin Lakes, NJ). Soil moisture content was adjusted to 25% by adding deionized water. These tubes were then stored at 0°C to prevent further degradation. Five replications per treatment were made. On each sampling day of the degradation study, 10 g of soil samples were used for residual analysis and to determine percentage of recovery. Analytical results of insecticides were finally corrected for recovery efficiency determined at each sampling interval. An external standard was used for confirmation and calculation of the total residue because of its accuracy and reproducibility (Poole and Poole 1997).

Degradation Rates and Half-Life of Termiticides. A half-life model, previously described by Su et al. (1999), $C_t = C_0 \times (0.5)^{(t/k)}$ was used to estimate the degradation rate (k) of each termiticide in treated soil at different concentrations, where C_t is termiticide concentration at time t , C_0 is initial concentration, and k is half-life index or time required for 50% termiticide degradation. The model was linearized by taking natural logarithm, $\ln C_t = \ln C_0 + (t/k) \times \ln (0.5)$ or $Y = A + B \times t$, where $Y = \ln C_t$, $B = [\ln (0.5)]/k$, and $A = \ln C_0$. A linear regression (SAS Institute 2000) was used to estimate the slope parameter B and associated variance, $Var (B)$. A half-life index, k , was calculated for each termiticide concentration by using estimated B , $k = [\ln (0.5)]/B$, and variance associated with k , $Var (k) = \{[\ln (0.5)]^2/B^4\} \times Var (B)$, from the variance propagation theorem. Because k and B were mathematically related, two half-life indices, k_1 and k_2 , were compared if the corresponding slopes, B_1 and B_2 , were different. All pairwise comparisons of slopes (B) estimated from samples collected from each soil-termiticide concentration combination was based on the standard normal distribution: $Z = (B_1 - B_2) / \sqrt{Var (B_1) + (B_2)}$. The two slopes (and hence the two half-life indices) were different at $\alpha = 0.05$ when $Z > 1.96$.

Lethal Times (LT₅₀ and LT₉₀) and Bioavailability of Termiticides. At each time interval, 10 g of treated soil sample was transferred to petri dishes (5.0 cm in diameter). Twenty worker termites were placed into these petri dishes containing five-mm-diameter moistened filter paper disks (Whatman No. 1) as a food source. Dead termites were removed from the petri dishes at intervals of 2, 4, 6, 8, 12, 16, 20, and 24 h. The experimental units were held in constant darkness at $28 \pm 1^{\circ}\text{C}$ and 100% RH. Exposure of termite workers to untreated soil served as control.

Data Analysis. Difference between degradation slopes and concentration data were analyzed using analysis of variance (ANOVA) and PROC GLM (SAS Institute 2000). PROC MIXED Procedure was used to determine the interaction among residues using LT₉₀ values. Fisher's least significant difference (LSD) was used to test significant differences among the means whenever F values were significant. Linear regression for determination of termiticide half-life was performed using PROC REG (SAS Institute 2000). PROC GLM models procedure was used to compare the slopes of the three different termiticides when applied at three different concentrations and also the interaction between the concentrations and insecticides. Time \times dose-response data were analyzed for each concentration by PROC LIFEREG procedure (SAS Institute 2000). This model provided a better fit for the LT₅₀ and LT₉₀ values on the observed value, compared with the PROBIT₁₀ procedure.

Results

Soil Analysis. Based on the elemental and particle size analysis, the soil used in this study was classified as loam (Kamble and Saran 2005). The soil sample was a mix of top layer (18 cm) of soil with 0.91% organic matter, 25.9% sand, 26.7% clay, coarse silt 23.1, fine silt 19.8, and 4.4%

Table 1. Degradation slopes and calculated half-lives (based on regression equations) for three termiticides used at three different concentrations in the study

Termiticide	Concn (µg/g soil)	Half-life (± SE) ^a (d)
Imidacloprid	50	166.64 ± 3.44a
	75	210.09 ± 2.28b
	100	280.98 ± 4.35c
Fipronil	60	223.12 ± 4.45b
	95	364.74 ± 3.18cd
	125	544.18 ± 4.71d
Bifenthrin	60	213.67 ± 3.30b
	90	298.17 ± 2.50c
	120	433.97 ± 4.71d

Within a column, numbers followed by same letters are not significantly different at $\alpha = 0.05$.

^a Half-lives are estimates based on the degradation patterns during 180 d in a laboratory study.

very fine silt. The cation exchange capacity (CEC) was 22.8 Cmol/kg and pH was 7.0. The average percentage water content (% ± SD) for the soil samples used in these experiments was 8.1 ± 0.5 and contained 17.2 ppm phosphorus (P) and 178.0 ppm potassium (K).

Termiticide Behavior in Soil. Time (days after treatment) had a significant effect on the amount of imidacloprid recovered at each sampling interval ($F = 370.33$; $df = 7, 72$; $P < 0.001$). Initial concentrations of imidacloprid applied to soil had a significant effect on the amount of residue recovered ($F = 335.79$; $df = 2, 72$; $P < 0.001$). There was a significant interaction between the time (in days) after the treatment and concentration applied ($F = 4.97$; $df = 14, 72$; $P < 0.001$).

There were significant differences for both the days after treatment ($F = 13337.03$; $df = 7, 72$; $P < 0.001$)

and concentrations of fipronil applied to soil ($F = 11430.1$; $df = 2, 72$; $P < 0.001$). There was a significant interaction between the days after treatment and initial concentration at which fipronil was applied to the soil ($F = 70.94$; $df = 14, 72$; $P < 0.001$).

For bifenthrin, days after treatment had a significant effect on the amount of residue recovered at different sampling intervals ($F = 2167$; $df = 7, 72$; $P < 0.001$). Residues recovered also were dependent upon initial concentrations of bifenthrin applied to soil ($F = 17742$; $df = 2, 72$; $P < 0.001$). There was a significant interaction between the days after treatment and concentration of bifenthrin initially applied to the soil ($F = 78.02$; $df = 14, 72$; $P < 0.001$).

Extraction Efficiency and Recovery Rate. Mean recovery rate (% ± SD) of imidacloprid, fipronil, and bifenthrin was 87.18 ± 0.25 , 94.01 ± 0.1 , and 98.0 ± 0.3 , respectively. The recovery rate of the imidacloprid, fipronil, and bifenthrin, calculated at different time intervals (0, 65, 135, and 180 d after application), remained unaffected.

Degradation and Half-Life of Termiticides. Half-lives for each termiticide (Table 1) were calculated based on the slopes of regression equations in Figs. 1–3. The calculated half-life for each concentration applied was longer than the incubation period (180 d); therefore, these half-lives were only the estimates.

Significant differences were observed among the regression slopes of all the three termiticides at the applied concentrations ($F = 123.96$; $df = 2, 8$; $P < 0.001$), suggesting that the degradation rates of the different insecticides in the treated soil were different at different applied concentrations. For imidacloprid, fipronil, and bifenthrin, applied at different rates, significant differences were observed among the regres-

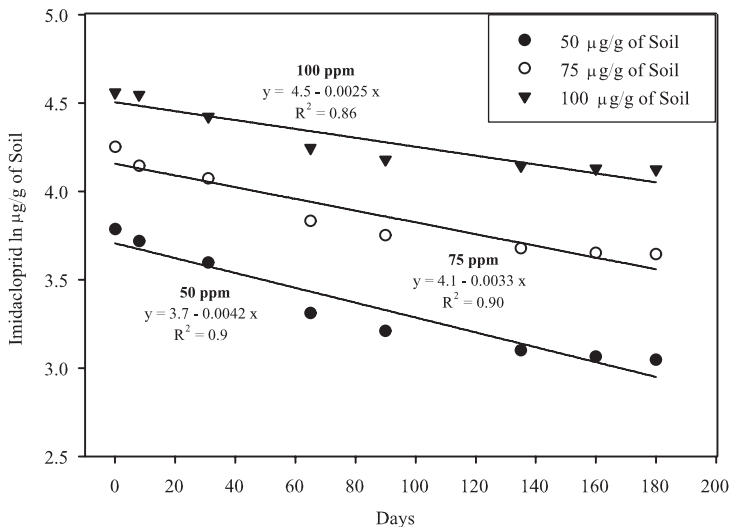


Fig. 1. Imidacloprid degradation curves for different concentrations based on the amount of imidacloprid recovered from the soil samples on different sampling intervals from 0 to 180 d. The regression between time (days after treatment) and amount recovered (micrograms per gram of soil) for different concentrations are provided next to each regression line: 50 ppm: $R^2 = 0.92$, $F = 71.5$, $df = 1, 6$, $P < 0.001$; 75 ppm: $R^2 = 0.90$, $F = 54.0$, $df = 1, 6$, $P < 0.001$; and 100 ppm: $R^2 = 0.86$, $F = 39.9$, $df = 1, 6$, $P < 0.001$.

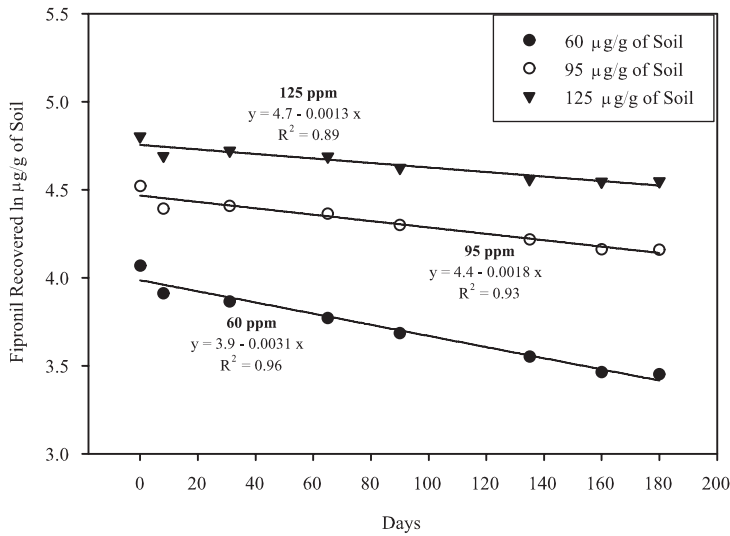


Fig. 2. Fipronil degradation curves for different concentrations based on the amount of imidacloprid recovered from the soil samples on different sampling intervals from 0 to 180 d. The regression between time (days after treatment) and amount recovered (micrograms per gram of soil) for different concentrations are provided next to each regression line: 60 ppm: $R^2 = 0.96$, $F = 1161.7.5$, $df = 1, 6$, $P < 0.001$; 95 ppm: $R^2 = 0.93$, $F = 1257.1$, $df = 1, 6$, $P < 0.001$; and 125 ppm: $R^2 = 0.89$, $F = 255.2$, $df = 1, 6$, $P < 0.001$.

sion slopes of residues recovered for each insecticide concentration, imidacloprid ($F = 65.87$; $df = 2, 9$; $P < 0.001$), fipronil ($F = 123.77$; $df = 2, 9$; $P < 0.001$), and bifenthrin ($F = 209.55$; $df = 2, 9$; $P < 0.001$).

Overall comparisons of the mean degradation rates of the three insecticides at three different concentrations, imidacloprid applied at 50 ppm (micrograms per gram of soil) revealed significantly higher rate of degradation than any other termiticide-concentration combinations. Fipronil at 95 and 125 ppm and bifenthrin at 120 ppm indicated the lowest degrada-

tion rates of any termiticide-concentration combinations.

Lethal Time (LT₅₀ and LT₉₀) and Bioavailability. The lethal time values (LT₅₀ and LT₉₀) for workers of *R. flavipes* exposed to soils treated with imidacloprid, fipronil, and bifenthrin at various concentrations are shown in Tables 2–4 respectively.

For imidacloprid, significant differences were observed among the LT₉₀ values for the sampling interval (8, 31, 65, 90, 135, 160, and 180 d after initial application) on which the bioassays were conducted ($F = 3446.69$;

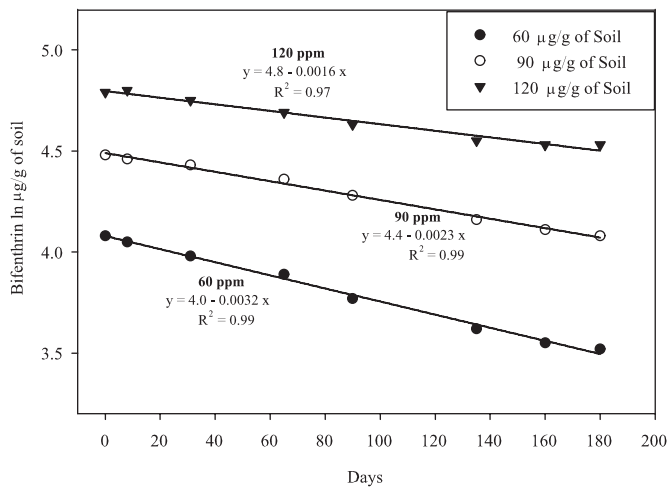


Fig. 3. Bifenthrin degradation curves for different concentrations based on the amount of imidacloprid recovered from the soil samples on different sampling intervals from 0 to 180 d. The regression between time (days after treatment) and amount recovered (micrograms per gram of soil) for different concentrations are provided next to each regression line: 60 ppm: $R^2 = 0.99$, $F = 1091.1$, $df = 1, 6$, $P < 0.001$; 90 ppm: $R^2 = 0.99$, $F = 895.8$, $df = 1, 6$, $P < 0.001$; and 120 ppm: $R^2 = 0.97$, $F = 250.1$, $df = 1, 6$, $P < 0.001$.

Table 2. Toxicity of imidacloprid treated soil to termites at different time intervals over a period of 180 d after the initial application

Termiticide	Concn (µg/g soil)	DAT	n	Slope ± SE	LT ₅₀ (95% FL) ² (h)	LT ₉₀ (95% FL) (h)	χ ²
Imidacloprid	50 ppm	8	100	5.25 ± 0.95	15.39 (12.6–18.1)	21.70 (19.2–24.1)	195.5
		31	100	5.98 ± 1.02	16.55 (13.5–19.6)	23.73 (21.0–26.4)	184.6
		65	100	19.56 ± 3.11	36.34 (26.2–46.4)	59.82 (51.0–68.6)	89.3
		90	100	26.26 ± 4.19	48.24 (34.6–61.8)	79.77 (68.0–91.6)	87.7
		135	100	24.78 ± 4.11	58.85 (46.0–72.3)	88.60 (77.3–99.8)	134.6
		160	100	28.38 ± 4.99	78.60 (63.9–93.3)	112.6 (99.6–125.7)	176.8
	75 ppm	180	100	29.30 ± 4.75	90.63 (76.6–104.6)	125.3 (113.3–138.2)	268.5
		8	100	6.02 ± 1.04	13.93 (10.8–17.1)	21.16 (18.4–23.9)	128.7
		31	100	6.56 ± 1.12	15.20 (11.8–18.6)	23.80 (20.0–26.1)	128.7
		65	100	17.83 ± 2.80	32.62 (23.4–41.8)	54.04 (46.0–62.0)	85.4
		90	100	23.78 ± 3.73	43.50 (31.2–55.8)	72.05 (61.4–82.8)	85.4
		135	100	23.01 ± 3.93	55.49 (43.5–67.4)	83.11 (72.6–93.6)	139.3
	100 ppm	160	100	25.48 ± 4.40	64.50 (51.3–77.7)	95.15 (83.4–108.0)	150.4
		180	100	31.55 ± 5.64	84.56 (68.2–100.9)	122.45 (107.9–137.0)	166.8
		8	100	5.56 ± 0.96	13.22 (10.3–16.1)	19.90 (17.3–22.4)	136.0
		31	100	5.90 ± 0.99	13.43 (10.3–16.5)	20.52 (17.8–23.2)	125.1
		65	100	13.02 ± 2.16	28.05 (21.3–34.8)	43.68 (37.8–49.6)	113.8
		90	100	17.35 ± 2.88	38.59 (28.8–48.4)	61.30 (52.7–69.8)	113.8
		135	100	21.94 ± 3.84	52.86 (41.5–64.2)	79.02 (68.8–89.2)	139.1
		160	100	25.11 ± 4.46	58.67 (45.6–71.7)	88.82 (77.2–109.6)	131.4
		180	100	31.30 ± 5.43	72.10 (55.9–88.3)	109.69 (95.3–124.0)	127.5

^a DAT, days after treatment.
^b 95% FL, 95% fiducial limits.

df = 6, 54; $P < 0.001$). There were significant differences in LT₉₀ values for different concentrations ($F = 74.10$; df = 2, 9; $P < 0.001$). There was a significant interaction between days after treatment and initially applied concentrations ($F = 18.31$; df = 12, 54; $P < 0.001$).

Time (days after treatment) had a significant effect on the LT₉₀ values ($F = 2705.19$; df = 6, 54; $P < 0.001$) for fipronil. There were significant differences among the LT₉₀ values for different applied concentrations of fipronil ($F = 133.33$; df = 2, 9; $P < 0.001$). There was a significant interaction between days after applica-

tion and concentrations applied ($F = 33.11$; df = 12, 54; $P < 0.001$).

There were significant differences among the LT₉₀ values of bifenthrin estimated at different time intervals after initial application ($F = 1583.54$; df = 6, 54; $P < 0.001$). Significant differences were observed among LT₉₀ values of different concentrations at which the bifenthrin was applied to the soil ($F = 95.54$; df = 2, 9; $P < 0.001$). It was observed that the sampling interval and the initially applied concentration exhibited significant interactions ($F = 12.71$; df = 12, 54; $P < 0.001$).

Table 3. Toxicity of fipronil-treated soil to termites at different time intervals over a period of 180 d after the initial application

Termiticide	Concn (µg/g soil)	DAT ^a	n	Slope ± SE	LT ₅₀ (95% FL) ^b (h)	LT ₉₀ (95% FL) (h)	χ ²
Fipronil	60 ppm	8	100	4.71 ± 0.79	10.67 (8.2–13.1)	16.33 (14.2–18.5)	124.5
		31	100	7.40 ± 1.23	14.61 (10.7–18.4)	23.51 (20.2–26.7)	99.2
		65	100	6.77 ± 1.18	16.97 (13.4–20.4)	25.0 (22.0–28.2)	151.1
		90	100	13.37 ± 2.34	33.90 (27.0–40.8)	49.95 (43.8–56.0)	155.3
		135	100	14.17 ± 2.53	40.77 (33.4–48.1)	57.8 (51.2–64.3)	190.2
		160	100	17.13 ± 3.05	47.45 (38.6–56.3)	68.0 (60.1–75.9)	177.1
	95 ppm	180	100	17.90 ± 3.12	49.65 (40.4–58.9)	71.14 (62.9–79.3)	177.9
		8	100	4.59 ± 0.78	10.13 (7.7–12.5)	15.64 (13.5–17.7)	119.2
		31	100	6.13 ± 1.01	13.41 (10.2–16.5)	20.70 (17.9–23.5)	118.9
		65	100	5.68 ± 0.99	16.11 (13.1–19.0)	22.94 (20.3–25.5)	187.2
		90	100	11.37 ± 1.98	32.23 (26.3–38.1)	45.88 (40.7–51.0)	187.2
		135	100	13.35 ± 2.33	36.15 (29.2–43.0)	52.17 (46.0–58.3)	171.1
	125 ppm	160	100	13.98 ± 2.42	40.13 (32.9–47.3)	56.92 (50.5–63.3)	188.8
		180	100	15.37 ± 2.71	44.26 (36.30–52.2)	62.7 (55.6–69.8)	189.6
		8	100	3.77 ± 0.64	9.81 (7.8–11.8)	14.35 (12.6–16.0)	160.7
		31	100	5.58 ± 0.95	12.45 (9.5–15.3)	19.15 (16.6–21.7)	120.3
		65	100	5.81 ± 1.00	13.21 (10.2–16.2)	20.19 (17.5–22.8)	124.3
		90	100	11.62 ± 2.00	26.42 (20.4–32.4)	40.38 (35.0–45.7)	124.3
		135	100	12.17 ± 2.08	29.89 (23.6–36.1)	44.51 (38.9–50.0)	143.0
		160	100	13.42 ± 2.34	35.23 (28.2–42.2)	51.34 (45.2–57.5)	162.5
		180	100	13.69 ± 2.39	37.31 (30.2–44.4)	53.75 (47.5–60.0)	173.3

^a DAT, days after treatment.
^b 95% FL, 95% fiducial limits.

Table 4. Toxicity of bifenthrin treated soil to termites at different time intervals over a period of 180 d after the initial application

Termiticide	Concn (µg/g soil)	DAT ^a	n	Slope ± SE	LT ₅₀ (95% FL) ^b (h)	LT ₉₀ (95% FL) (h)	χ ²
Bifenthrin	60 ppm	8	100	2.40 ± 0.40	5.76 (4.5–7.0)	8.65 (7.5–9.7)	140.3
		32	100	4.03 ± 0.70	9.33 (7.2–11.4)	14.17 (12.3–16.0)	128.8
		65	100	5.55 ± 0.94	12.65 (9.8–15.5)	19.33 (16.8–21.8)	125.1
		90	100	6.47 ± 1.15	16.41 (13.0–19.7)	24.18 (21.19–27.1)	151.4
		135	100	7.40 ± 1.31	19.03 (15.2–22.8)	27.92 (24.5–31.3)	155.1
		160	100	8.50 ± 1.51	21.36 (16.9–25.7)	31.58 (27.6–35.5)	149.9
	90 ppm	180	100	9.34 ± 1.61	22.72 (17.9–27.5)	33.94 (29.7–35.7)	142.0
		8	100	2.58 ± 0.43	5.57 (4.2–6.9)	8.67 (7.5–9.8)	113.2
		32	100	3.89 ± 0.68	8.33 (6.30–10.35)	13.01 (11.2–14.8)	112.1
		66	100	5.34 ± 0.92	11.64 (8.8–14.4)	18.06 (15.6–20.5)	115.7
		90	100	6.64 ± 1.16	14.69 (11.2–18.1)	22.67 (19.6–25.7)	120.4
		135	100	7.55 ± 1.32	17.44 (13.5–21.3)	26.50 (23.0–29.9)	130.3
	120 ppm	160	100	7.65 ± 1.36	18.65 (14.7–22.6)	27.84 (24.3–31.3)	142.6
		180	100	8.17 ± 1.44	20.60 (16.3–24.8)	30.42 (26.7–34.1)	150.6
		8	100	2.60 ± 0.43	5.15 (3.8–6.5)	8.28 (7.1–9.4)	100.8
		31	100	4.08 ± 0.71	7.97 (5.8–10.0)	12.87 (11.00–14.7)	96.5
		65	100	4.45 ± 0.77	9.14 (6.8–11.4)	14.50 (12.4–16.5)	104.2
		90	100	5.58 ± 0.98	12.16 (9.2–15.0)	18.87 (16.3–21.43)	116.7
		135	100	6.96 ± 1.21	15.30 (11.7–18.9)	23.65 (20.4–26.8)	119.9
		160	100	7.71 ± 1.35	17.05 (13.0–21.0)	26.31 (22.7–29.8)	120.5
		180	100	7.99 ± 1.39	17.92 (13.8–22.0)	27.52 (23.9–31.1)	122.5

^a DAT, days after treatment.
^b 95% FL, 95% fiducial limits.

Bioavailability of Termiticides. The regression analysis between the log₁₀-transformed values of recovered termiticides (micrograms per gram of soil) and log₁₀LT₉₀ values (hours), resulted in regression values (R²) ranging between 0.87 and 0.98 for all the tested termiticides and concentrations (Figs. 4–6). For imidacloprid (100 ppm), fipronil (125 ppm), and bifenthrin (120 ppm), the slopes of the regression equations were -4.0, -6.8, and -4.1, respectively. However, at the lowest applied concentrations of imidacloprid (50 ppm), fipronil (60 ppm), and bifenthrin (60 ppm), the slopes of the regression equations were

-2.6, -3.1, and -2.3, respectively. This indicated that the termite mortality was higher at higher concentrations due to enhanced bioavailability of the applied termiticides.

Discussion

Our recovery rates and extraction efficiencies were consistent with those reported by Bobe et al. 1998 (85 ± 5% for fipronil), and Baskaran et al. 1999 (81.6 ± 2.4% for bifenthrin and 85.2 ± 2.4% for imidacloprid). Thus, the decrease in amount of termiticides recov-

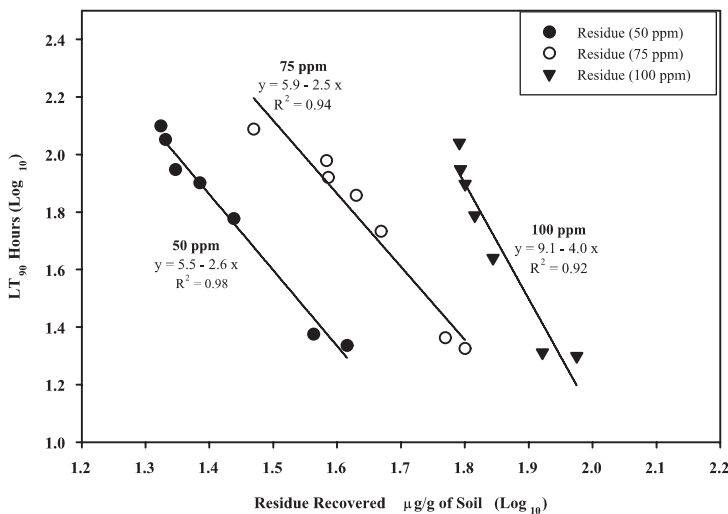


Fig. 4. Relationship between the amounts of imidacloprid recovered at different intervals and LT₉₀ values for termites exposed to these samples. The regression equations between different concentrations and LT₉₀ values are provided next to each regression line: 50 ppm: R² = 0.98, F = 290.3, df = 1, 5, P < 0.001; 75 ppm: R² = 0.94, F = 85.0, df = 1, 5, P < 0.001; and 100 ppm: R² = 0.92, F = 60.0, df = 1, 5, P < 0.001.

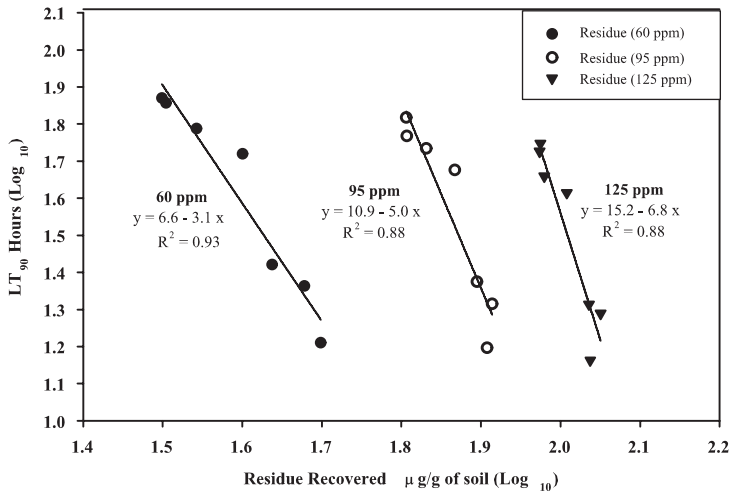


Fig. 5. Relationship between the amounts of fipronil recovered at different intervals and LT_{90} values for termites exposed to these samples. The regression equations between different concentrations and LT_{90} values are provided next to each regression line: 60 ppm: $R^2 = 0.93$, $F = 72.7$, $df = 1, 5$, $P < 0.001$; 95 ppm: $R^2 = 0.88$, $F = 38.3$, $df = 1, 5$, $P < 0.001$; and 125 ppm: $R^2 = 0.88$, $F = 38.7$, $df = 1, 5$, $P < 0.001$.

ered over time from soil samples was due to degradation only and was not due to a time-dependent adsorption phenomenon. Baskaran et al. (1999) reached similar conclusions in their study with bifenthrin, imidacloprid, and chlorpyrifos.

The initial concentrations of the termiticides applied to the soil affected the degradation rates and consequently the half-lives based on these degradation rates were significantly different. It has been suggested that the prolonged persistence of the insecticides when applied at higher concentrations was associated with a temporary decrease in bacterial and fungus numbers resulting in a prolonged inhibition of

soil dehydrogenase and esterase activities (Felsot and Dzantor 1995).

Few data are available on the degradation of fipronil (Bobe et al. 1997) and even less so for the rates applied as termiticides. When applied at field application rates (8 g [AI]/ha), 75% of the fipronil degraded within 3 d, and the four metabolites were detected (Bobe et al. 1998). Fipronil when applied at termiticidal rates did not show much degradation, and no metabolites were detected in our residue analysis even after 180 d. We considered fipronil residue recoveries as "toxic total" (fipronil + metabolites A, B, C) as reported by Bobe et al. (1998) at field application rates.

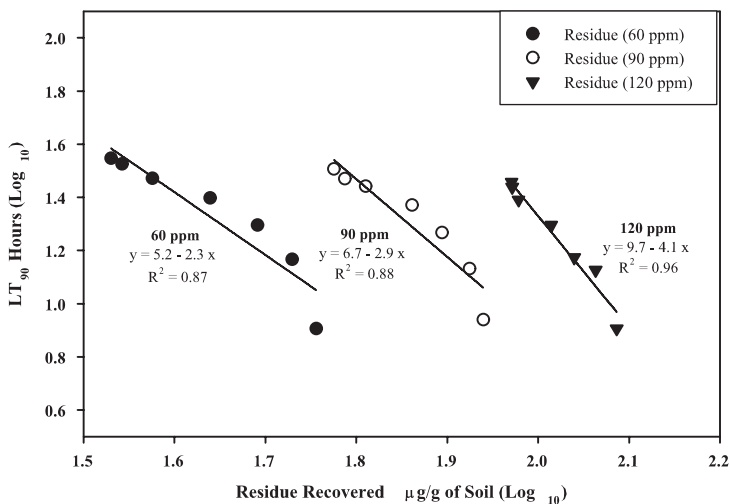


Fig. 6. Relationship between the amounts of bifenthrin recovered at different intervals and LT_{90} values for termites exposed to these samples. The regression equations between different concentrations and LT_{90} values are provided next to each regression line: 60 ppm: $R^2 = 0.87$, $F = 34.9$, $df = 1, 5$, $P = 0.002$; 75 ppm: $R^2 = 0.88$, $F = 37.7$, $df = 1, 5$, $P < 0.001$; and 120 ppm: $R^2 = 0.96$, $F = 121.0$, $df = 1, 5$, $P < 0.001$.

Differences in the initial application rates of termiticides and chemical and physical properties of the soil affect degradation rates. In the current study, because the insecticides were applied to the soil under similar laboratory incubation conditions, differences in degradation rates were more likely due to the chemical and physical properties of the insecticides. The degradation of these insecticides followed a linear pattern initially, but it became more flattened toward the end (Figs. 1–3). This suggested that the initial degradation rates were much higher, they slowed over time. The slowing degradation rates may have affected the estimated half-lives of the termiticides and their availability to the termites in the soil. Bobe et al. (1998), Baskaran et al. (1999), Racke et al. (1994), and Su et al. (1999) observed similar degradation trends in their studies.

There was an inverse relationship between the initial applied concentrations of all three termiticides in our study and their LT_{50} and LT_{90} values against *R. flavipes*. Bioavailability of the termiticides over time was higher at higher concentrations. The greater bioavailability of the termiticides may explain similar trends observed in previous studies (Edwards et al. 1957, Peterson et al. 1971, Smith and Rust 1992, Forschler and Townsend 1996, Ramakrishnan et al. 2000). However, bioassays with treated soil demonstrated that the differences in LT_{50} and LT_{90} were partly explained by the differential availability of termiticides in soil because bioactivity varies among soil types. Felsot and Lew (1989) suggested that partitioning of the insecticides between soil organic matter and soil solution (i.e., the sorption process, which is inversely related to the water solubility), affects the availability of the insecticide to target organisms. Because termiticide application rates are very high in comparison to rates used in agriculture, the bioavailability and the efficacy of relatively water-soluble termiticides such as imidacloprid will not be significantly lower than other classes of less soluble compounds (e.g., pyrethroids and phenyl pyrazoles). Oi (1999) demonstrated increased adsorption of imidacloprid in soil with time, which resulted in increased K_{oc} values. However, over time and depending on the degradation rates, the concentration of the applied termiticides might reduce to levels at which the bioavailability decreases considerably providing only sublethal or no effects on termites.

Fipronil exhibits low water solubility (1.9 mg/liter) at 20°C in distilled water. At low rates in soil, it has low soil affinity, due to strong competition from the aqueous phase. Adsorption increases as concentration increases (Bobe et al. 1997). However, at termiticide application rates of 0.06–0.125%, the adsorption process exhibits a reverse phenomenon, whereby there is a decrease in adsorption coefficient with an increase in concentration (Kamble and Saran 2005). The result is that over a certain range of concentration, more fipronil molecules will be present in the aqueous phase. In the case of fipronil it was also demonstrated that there was a significant decrease in adsorption coefficient as the soil organic matter and clay content

decreased (Bobe et al. 1997). This explains more bioavailability of the fipronil in soils with lower organic matter because of the small amount of organic phase available for fipronil molecules. Lower water solubility of fipronil offers a lower leaching potential as demonstrated by Bobe et al. (1997) and a higher persistence, the latter being more important for a durable termiticide treatment.

Bifenthrin has low water solubility (0.1 mg/liter), is strongly adsorbed (K_{oc} 1,000,000, Xia and Brandenburg 2000) in soil, and shows no leaching potential. However, there was some increased efficacy against mole cricket when golf fields were watered immediately before or after application (Xia and Brandenburg 2000). Bifenthrin molecules are tightly bound to soil particles and may not be competitively absorbed by termite body. But even in a tightly bound condition, it has been demonstrated previously (Smith and Rust 1992) that termites were quickly killed at 20% soil moisture. Increased clay content increases the toxicity of certain pyrethroids, such as cypermethrin (Smith and Rust 1993). Cypermethrin and clay apparently interacted creating a formulation similar to a wettable powder (Smith and Rust 1993). Such wettable powder may have increased affinity to the nonpolar termite integument and could easily penetrate the termite integument. Because bifenthrin and cypermethrin belong to the same class of insecticides, the higher clay content in the soil used in current study, (>25%) might explain higher toxicity of bifenthrin compared with fipronil and imidacloprid. However, some background information about the behavior of bifenthrin in soil and its toxic action on insects will help us in understanding the bioavailability of bifenthrin to termites. Smith and Rust (1990) reported the high mortality rate when termites were directly exposed to soil treated with bifenthrin. In their direct exposure studies, bifenthrin had the greatest activity compared with other pyrethroids, because as little as 1 ppm killed all the insects within 3 h. It also was observed that pyrethroids bifenthrin, cypermethrin, and permethrin acted much faster than chlorpyrifos and chlordane. In their tunneling study, termites did not tunnel into soil treated with formulated bifenthrin even at the lowest concentration of 1 ppm (wt:wt) due to repellency against bifenthrin. Certain termiticides, especially pyrethroids, were reported to be repellent to termite workers (Su et al. 1982, Jones 1989, Smith and Rust 1990, Rust and Smith 1993). Su and Scheffrahn (1990) found that nine different pyrethroids were repellent at sublethal doses.

The differential bioavailability in different soil types may affect the field performance of a termiticide over longer periods than tested in our study. Therefore, termiticide soil bioassay must be reported in relation to soil type (Forschler and Townsend 1996). The particular mode of action of the various termiticides used in this research reaffirms what we presently know about the speed in which they knock down insects in general. The response time to intoxication was observed in a descending order of pyrethroids < phenyl pyrazoles < chloronicotinyls. Further knowledge

about efficacy and bioavailability of the newer compounds like imidacloprid and fipronil will be more relevant in terms of understanding their ability to protect treated structures over time. We did not test for any microbial activity in our soil samples but microorganisms play a major role in the degradation of many pesticides (Harris et al. 1988), yet little is known about the type of microbes involved specifically. The knowledge about the abundance and survival of such microorganisms will definitely provide us more information about the degradation of new generation termiticides in different soil types and locations.

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