Toxicity, Uptake, and Transfer Efficiency of Fipronil in Western Subterranean Termite (Isoptera: Rhinotermitidae)

RAJ K. SARAN[1] AND MICHAEL K. RUST

Department of Entomology, University of California, Riverside, CA 92521

J. Econ. Entomol. 100(2): 495–508 (2007)

ABSTRACT The potential horizontal transfer of nonrepellent termiticides has become an important paradigm to control termites in recent years. In this study, ^{14}C-radiolabeled fipronil was used in a series of laboratory experiments to demonstrate the extent and ability of termites to transfer lethal amounts of fipronil to unexposed nestmates. Fipronil is an active and nonrepellent termiticide against western subterranean termites, *Reticulitermes hesperus* Banks, on sand at relevant doses. It exhibited delayed toxicity with the lowest $LD_{50} \approx 0.2$ ng/termite expressed between day 4 and 7. Both continuous and brief exposures to fipronil-treated sand seriously impaired the termite's ability to move and respond to a dodecatrienol trail, limiting potential horizontal transfer. In tunneling studies, fipronil prevented termite tunneling at concentrations as low as 0.5 ppm and was nonrepellent even at 500 ppm. Greater than 90% mortality was recorded by day 7 with concentrations ranging from 0.5 to 500 ppm. There was a linear relationship between the time of exposure and uptake of [^{14}C] fipronil when termites were continuously exposed to 0.5, 1.0, and 5.0 ppm for 24 h. However, uptake discontinued when the termites were immobilized. Maximum transfer of fipronil from donors to recipients occurred within the first 24 h. Fipronil was transferred by body contact and trophallaxis did not play a major role in horizontal transfer. In successive transfer studies, there was not enough fipronil on recipients for them to serve as secondary donors and kill other termites. In a linear arena study, there was an inverse relationship between the amount of fipronil on dead termites and their distance from the treated zone. Maximum mortality was observed within 1.5 m from the treated zone. Results in our laboratory studies suggest that horizontal transfer was not a major factor contributing to the efficacy of fipronil in the field.

KEY WORDS nonrepellency, delayed toxicity, termite movement, *Reticulitermes hesperus*

For more than six decades, soil treatments with termiticides have been the conventional technique for controlling subterranean termites (Su and Scheffrahn 1998). Even though baiting has become a popular termite control strategy in the past 10 yr, two thirds of the treatments by pest control companies are applications of liquid termiticides to soil (Curl 2004). Research and development has focused on the slow-acting insecticides in which mortality and the speed of kill are concentration dependent (Su et al. 1987). Two slow-acting insecticides, fipronil and imidacloprid, have become popular alternatives to the fast-acting and repellent pyrethroid barriers.

Fipronil belongs to the phenylpyrazole class of insecticides. It has low leaching potential with an octanol water coefficient of log 4.0 and low water solubility of 1.9–2.4 mg/liter at 20°C (Anonymous 1996). The mode of action involves blocking the γ-aminobutyric acid-gated chloride channel, with insects having a much greater sensitivity of this target site than mammals (Hainzl and Casida 1996). In the environment, fipronil degrades to its major metabolites via reduction to the corresponding sulfide, oxidation to the sulfone, hydrolysis to the amide, and photolysis to desulfinyl fipronil (Bobe et al. 1997). The sulfone, which is one of the metabolites in the soil, has proved to be as active as the parent molecule in toxicological and neurological studies with corn rootworms (*Diabrotica* spp.) (Scharf and Siegfried 1999). Even at the lowest label rate of 60 ppm in soil (aerobic conditions), fipronil exhibits a half-life of ≈200 d, which is affected by application rate (Saran 2001). All these physical and chemical properties make fipronil an efficacious and long-lasting termiticide.

Fipronil is inherently toxic to subterranean termites; the LD_{50} for *Coptotermes formosanus* Shiraki at 72 h is 1.33–1.39 ng per termite (Ibrahim et al. 2003). The LD_{50} for western subterranean termites, *Reticulitermes hesperus* Banks, at day 7 is ≈0.2 ng per termite (Rust and Saran 2006). A 24-h exposure of the eastern subteranean termite, *Reticultitermes flavipes* Kollar, on filter paper treated with 1 ppm fipronil provided 31% kill at 24 h and 84% kill at 7 d, after which the mortality did not increase significantly (Remmen and

[1] Corresponding author, e-mail: raj.saran@ucr.edu.

0022-0493/07/0495–0508$04.00/0

Su 2005b). These data provide support for the claim that fipronil provides delayed toxicity in termites.

Laboratory studies by Remmen and Su (2005a) and Hu (2005) concluded that fipronil was not repellent to *C. formosanus* and *R. flavipes* at concentrations as high as 100 ppm. Fipronil deposits of 250 ppm were reported to be repellent (Ibrahim et al. 2003). However, their data showed no statistical differences in mortality or the distribution of dead termites on sands treated with 20, 125, and 250 ppm. Thus, the concentration at which fipronil repels termites has yet to be determined.

Termites exposed to fipronil have the potential to transfer lethal doses to previously unexposed termites, the kill of recipients being dose dependent (Shelton and Grace 2003, Ibrahim et al. 2003, Tsunoda 2006). *C. formosanus* workers, exposed to 100 ppm fipronil-treated sand for 1 h and mixed with untreated workers, provided 39% kill of recipients at day 15 (Shelton and Grace 2003). Exposures to lower concentrations of fipronil did not produce a transfer effect. When the ratio of donors:recipients of *C. formosanus* was at least 1:4, there was significant transfer and kill of recipients (Ibrahim et al. 2003). Confining treated soldiers with untreated workers indicated that contact and grooming were probably responsible for the transfer and kill. As the ratio of donors:recipients decreased, the mortality of recipients also decreased (Tsunoda 2006).

Even though fipronil has been widely used as a soil treatment against subterranean termites, little is known about how they acquire and transfer it to nestmates. To understand its mechanism of toxicity better, we conducted a series of laboratory experiments with fipronil: to determine its effects of exposure on termite movement and potential transfer of fipronil to unexposed workers, repellency studies with a broad range of concentrations, and radiolabeled uptake from treated sand to determine its transfer and movement. In addition, we discuss the pick up and transfer of fipronil and its implications for controlling subterranean termites.

Materials and Methods

Termites. Termites were collected on the University of California, Riverside, campus in polyvinyl chloride traps provisioned with rolls of corrugated cardboard (Haagsma and Rust 2005). Termites were reared in the laboratory at 24°C and ≈100% RH. Pieces of brown paper towel (Fort James Corp., Deerfield, IL) were provided as food. Undifferentiated larval termites, the average worker weighing 1.6 ± 0.44 mg ($n = 50$), were used for bioassays and radiolabel studies. Termites were dyed by feeding on 0.02% Nile Blue-treated filter paper, and there was no mortality due to Nile Blue at the concentrations used in the study. A single colony was used for all bioassays.

Insecticide. Technical grade fipronil, 96.5% (AI), 5-amino-1-(2,6-dichloro-4-trifluoromethyl)-4-trifluoromethanesulfinyl-1*H*-pyrazole-3-carbonitrile), radiolabeled fipronil [Phenyl-U-^{14}C] (specific activity 56.4 μCi/mg), and formulated fipronil (Termidor, 2 SC) were obtained from BASF Corporation (Research Triangle Park, NC).

Topical Bioassay. To determine whether fipronil had delayed toxicity, workers were treated with acetone solutions of serially diluted technical grade fipronil. The droplets were deposited with a 27-guage needle in a glass tuberculin syringe (BD Biosciences, Franklin Lakes, NJ). Precise application was made with an Isco model M microapplicator (Instrumentation Specialties, Seward, NE). A 0.3-μl droplet was placed on the dorsum of the abdomen. The droplet was allowed to dry for ≈30 s, and the termites were placed in a plastic petri dish lined with a piece of moist paper towel. The petri dishes and termites were held in a chamber maintained at 100% RH. Ten termites were treated with each concentration, and a minimum of three replicates was conducted. The number of dead termites was counted daily for 1 wk. The data were analyzed by probit analysis using the Polo Plus program (LeOra Software, Menlo Park, CA).

Brief Exposure Studies. Termites were exposed to sand treated with different concentrations of fipronil to determine the effect of 1-h exposures on termite mortality over 7 d. These exposure studies were necessary to determine the time required to kill some but not all of the donor termites for later studies of locomotion, repellency, and horizontal transfer. To achieve the desired concentration of fipronil (wt:wt), 100 g of sterilized play sand (Oglebay Norton Industrial Sands, San Juan Capistrano, CA) was treated with aqueous suspensions of fipronil (2 SC) to provide two series of concentrations: 0.05, 0.1, 0.5, 1.0, and 5.0 ppm and 100, 200, 300, 400, and 500 ppm ([AI], wt:wt) deposits. These series were selected based on our initial observations and our objective to demonstrate the effect of dose (concentration × time) on delayed toxicity. The treated sand was allowed to dry in a laminar flow hood for 24–48 h. Approximately 2.0 g of sand was then placed in the bottom of a small plastic petri dish (3.5 cm in diameter, Becton Dickinson, Franklin Lakes, NJ). The sand was lightly moistened with 0.4 ml of water. Ten termites were confined on the treated sand for 1 h. The termites were removed and placed in plastic petri dishes (3.5 cm in diameter) provisioned with a disk of moist paper towel. The petri dishes were held inside a plastic container (43.2 by 29.9 by 17.7 cm, Rubbermaid Inc., Wooster, OH) on a plastic rack supported by three circular plastic rings (7.0 cm in diameter). Humidity indicator strips (Sud-Cheme Performance Packaging, Colton, CA) were taped to the walls of the container to ensure it was maintained at 100% RH. Dead termites were counted and removed daily for 7 d. Five replicates were tested for each concentration.

The mortality data were used to determine the Kaplan–Meier survivorship percentiles (St) for each concentration (Analytical Software 2005). This test accounts for right censored data or individuals not dead at the termination of the test. It also permits different treatments to be compared with one another. A pairwise comparison was made between the survival

probabilities of overlapping confidence intervals (95%) to determine nonsignificant differences.

Tunneling Study. The ability of termites to tunnel through sand treated with various concentrations of fipronil was studied by placing workers into sections of glass tubing (9 mm i.d. by 15 cm in length) filled with treated and untreated sand. A piece of cotton (1.8 cm in length) was loosely plugged into the bottom end of each tube. Each tube was filled with 7.5 cm of treated sand and a 3.9 cm layer of untreated sand. The top of the tube was plugged with a rubber stopper (# 000, Ward's Natural Science, Rochester, NY). A line was marked on the glass tubes with a permanent marker where the untreated and treated sand met. The controls were packed with untreated sand. The stopper was removed and 1.5 ml of deionized (DI) H_2O was added to the untreated sand in each tube and was drawn through the treated section with a gentle vacuum, producing a slight dampness to the entire sand column. The tubes were inverted and rested on the stopper.

Twenty worker termites were introduced in the bottom of each tube (3.9 cm of untreated sand) along with a piece of brown paper towel to serve as food. The distance tunneled was recorded after 2 d. The tubes were carefully broken and dead termites were counted. The live termites were transferred to a smaller petri dish (3.5 cm in diameter) provisioned with a moistened disk of brown paper towel and held at 100% RH. Dead termites were counted 1, 5, and 7 d after their removal from the tubes. Five replicates of each of 13 concentrations (0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 200, 300, 400, and 500 ppm [AI], wt:wt) of fipronil (2 SC) on sand were tested. The distances tunneled were analyzed with a one-way analysis of variance (ANOVA), and the means were separated by an all-pairwise comparison (Analytical Software 2005).

Movement Bioassay. To determine the effects of brief and continuous exposures of fipronil on termite trail-following behavior, workers were exposed to sand treated with fipronil (2 SC) (1 ppm [AI]; wt:wt) and then tested for their responses to (3Z,6Z,8E)-dodecatrien-1-ol (dodecatrienol henceforth) as described by Rust and Saran (2006). Two different exposure regimes were conducted. Eight hundred termites were confined to the treated sand (1 ppm, wt:wt; ≈15% moisture) in a petri dish (9.0 cm in diameter) and placed in the large plastic container maintained at 100% RH. For brief exposures, 400 termites were transferred after 1 h to individual clean petri dishes (4.25 cm in diameter) provided with a moist brown paper towel disk. Termites were then tested for trail-following behavior after 1, 4, and 8 h. For continuous exposures, groups of 125 termites were placed in each of three petri dishes after 4 and 8 h (as described above). The exposed termites were allowed to acclimate for 5 min before being tested. Controls (n = 400) were exposed to sand treated with acetone only.

To determine the effects of fipronil exposure on the trail-following response in termites, the time required for the termite to traverse the 10-cm trail treated with dodecatrienol was determined. Each trail was tested only once. Termites were tested for each exposure concentration and exposure period until 30 termites successfully traversed the trail. None of the termites followed the acetone trail. The travel times for termites completing the dodecatrienol trail were categorized as follows: 0–10, 11–15, 16–20, 26–30, and >30 s. The distribution of times required to traverse the 10-cm trail at each time period after exposure was analyzed with an r × c test of independence using the G-test (Sokal and Rohlf 1969).

Uptake of Fipronil. To study the uptake and horizontal transfer of fipronil, ≈5.84 mg of [^{14}C]fipronil (specific activity 56.4 μCi/mg) was diluted in 2 ml of acetone. When 10 μl of this stock solution was added to 10 ml of acetone, it resulted in a 1.65 μCi/10 ml (29.2 μg/10 ml) [^{14}C]fipronil solution and was used for our studies (stock A). For lower concentrations (0.5 and 1.0 ppm), the appropriate volume of stock A was added to 10 ml acetone so that when this solution was added to 25 g of sand, it resulted in a desired concentration (wt:wt) of fipronil. For higher concentrations (≥5 ppm), the appropriate volume of the stock A (29.2 μg/10 ml) was directly dissolved in 10 ml of acetone and was used to treat 25 g of sand. Four concentrations of fipronil (0.5, 1.0, 5.0, and 25.0 ppm wt: wt) were tested. Three aliquots of 5 g of treated sand were transferred to plastic petri dishes (5.0 cm in diameter). To ensure sufficient moisture on the sand, 1 ml of DI H_2O was added to each dish.

Seventy-five termites were placed in each of the three petri dishes (5.0 cm in diameter) containing one of the four concentrations of treated sand. The petri dishes were covered and transferred to the plastic container maintained at 100% RH. Sand treated with acetone was used as a control for the bioassays. Ten termites per each of three replicates were placed in 20-ml glass scintillation vials 1, 4, 8, 16, and 24 h after exposure. Termites were digested for 24 h in 200 μl of 15.8 N nitric acid. Ten milliliters of Cytoscint scintillation fluid (MP Biomedicals, Irvine, CA) was added to each vial and the vials were vigorously shaken. After 1 h, samples were placed in a liquid scintillation counter (LSC; LS 3801, Beckman Coutler, Fullerton, CA) and counted for 1 h or until the disintegrations per minute (dpm) had a σ value of 2 (95% confidence level on the channel readings based on repeated counts). A background count of 54 ± 2 dpm was subtracted from all readings. A Student's t-test was performed on samples (n = 20), and the minimum detection limit for a sample was 4.5 dpm above the background. Based on a number of preliminary trials, signals 5 times the standard deviation associated with the background counts were considered for our analysis. The instrument had a >95% efficiency for ^{14}C isotope. The amounts of ^{14}C label ≤0.01 ng per termite reported are the lowest limits with approximation.

Horizontal Transfer of Fipronil among Workers. To determine the amount of fipronil transferred from donors to recipients, a simple donor:recipient model (1:1) was used. Exactly 100 termites (donors) were exposed to sand treated with [^{14}C]fipronil at 0.5, 1.0, and 5.0 ppm for 1, 4, or 24 h. Each treatment concen-

tration and time combination was replicated three times. Controls consisted of donor termites exposed to acetone-treated sand for each exposure interval. After exposure, donor termites ($n = 15$) were placed in new plastic petri dishes (3.5 cm in diameter) each containing a moist brown paper towel disk. Fifteen recipient termites (blue dyed) were added to each petri dish. Both donor and recipient termites were held for 7 d inside the large plastic container maintained at 100% RH. Dead and live termites were counted for donor and recipient termites daily for 7 d. Dead termites were not removed from the petri dishes. At the end of 7 d, nondecomposing intact dead termites and live termites were randomly selected. Ten donor and 10 recipient termites from each replication of every treatment were placed into separate 20-ml glass scintillation vials and analyzed for ^{14}C label as described above. The amount of fipronil from donors and recipients was determined and the percentage of fipronil transferred was determined by the formula: % transfer = ng fipronil recipients × 100/ng fipronil (recipients + donors).

Survivorship percentiles for donor and recipient mortality 7 d postmixing were determined with a Kaplan–Meier Survival function test (Analytical Software 2005). The percentages of fipronil transferred from donors to recipients were analyzed with a two-way analysis of variance (ANOVA), and means were separated with Tukey's honestly significant difference (HSD) (Analytical Software 2005).

Maximum Transfer. The time in days required for the maximum transfer of fipronil from donor to recipient termites was studied. A group of 250 termites was transferred to each of three petri dishes (4.25 cm in diameter by 1.0 cm) containing 5 g of moistened sand treated with 0.5, 1, and 5 ppm [^{14}C]fipronil. Termites were exposed to the treated sand for 4 h. Groups of 15 exposed termites (donors) were placed in new petri dishes (3.5 cm in diameter by 1.0 cm) provisioned with a moistened brown paper towel disk. Other groups of 15 blue-dyed termites (recipients) also were placed in each of the petri dishes with 15 donors. Ten donors and 10 recipient termites were then analyzed separately for each concentration of [^{14}C]fipronil as described above. Four replicates were tested for each concentration and time period. Data were analyzed using a two-way ANOVA (Analytical Software 2005).

Transfer from Dead Workers. Donor termites exposed to 5 ppm fipronil treated sand for 24 h were unable to right themselves. However, when these termites were mixed with unexposed workers, there was increased mortality in unexposed workers, prompting us to test for fipronil transfer from moribund or dead termites to recipients. Groups of 75 termites were exposed to [^{14}C]fipronil-treated sand at either 5 or 50 ppm for 10 h. Four replications were tested. At the end of 10 h, a group of five termites from each replicate was analyzed for ^{14}C label as described above, to confirm [^{14}C]fipronil uptake. The remaining 70 dead termites from each exposure were frozen at −20°C for 2 h. Dead termites were thawed for 30 min. Four groups of 15 termites each were transferred to a new plastic petri dish (3.5 cm in diameter) provisioned with moist brown paper towel. Fifteen unexposed (dyed) termites also were transferred to each of the new dishes and were held together for 5 d inside one of the large plastic containers. Five termites each from both exposed dead and untreated termites were sampled and analyzed for ^{14}C label on days 1, 3, and 5 as described above for whole bodies. Volumes of HNO_3 and scintillation fluid were adjusted for the numbers of termites ($n = 5$). For the controls, 75 termites were exposed similarly to acetone-treated sand for 10 h and were then frozen at −20°C. Fifteen untreated dead termites were then mixed with 15 live dyed recipients in each of four replicates. The percentage of mortality of untreated termites was determined on days 1, 3, and 5 after mixing. The percentage of transfer at three different times was analyzed using two-way ANOVA (Analytical Software 2005). Means were separated using a Tukey's HSD at $P < 0.05$.

Transfer Due to Trophallaxis. To determine whether termite workers passed fipronil to other workers via trophallaxis, donor termites were confined with unexposed workers with sealed mouthparts. A group of 300 termites was starved for 3 d. One hundred and fifty termites were allowed to feed for 10 h on a filter paper disk (4.25 cm in diameter) treated with [^{14}C]fipronil at either 5 or 30 ppm (wt:wt). After 10 h, six groups of 15 termites each were randomly sampled. Three groups of 15 termites exposed to either 5 or 30 ppm fipronil were mixed with 15 blue-dyed workers (recipients) with sealed mouthparts. The other three groups of 15 termites exposed to either 5 or 30 ppm fipronil were mixed with 15 blue-dyed termites (recipients) with normal mouthparts.

To seal the mouthparts, workers were briefly anesthetized with CO_2, and the mouthparts were sealed with a small droplet of Bondini-2 cyanoacrylate glue (Pro-Tel Inc., Rancho Cucamonga, CA). The glue was fast cured by applying a microdroplet of cyanoacrylate accelerator (Pacer Technologies, Rancho Cucamonga, CA) with a single hair from a paint brush.

To confirm the presence of ^{14}C label in the hindgut of donors and recipients, donor termites were divided into three groups of 15 for each concentration and mixed with 15 blue-dyed recipients having normal mouthparts. After 3 d, the hindgut fluid of the 15 donor and 15 recipient termites was extracted by gently pressing the hindgut. The hindgut contents were blotted on a 5- by 5-mm piece of filter paper for both donors and recipients. Each piece of filter paper was placed in a 20-ml scintillation vial, and 10 ml of Hionic-fluor scintillation fluid (PerkinElmer Life and Analytical Sciences, Boston, MA) was added. Filter paper pieces were then counted by LSC as described above.

Controls were obtained by starving 60 termites for 3 d. Groups of 15 termites were placed into petri dishes (5.0 cm in diameter) with moist untreated filter paper disks (4.25 cm in diameter). A group of 15 dyed termites was added to each petri dish. Mortality was recorded daily for 7 d.

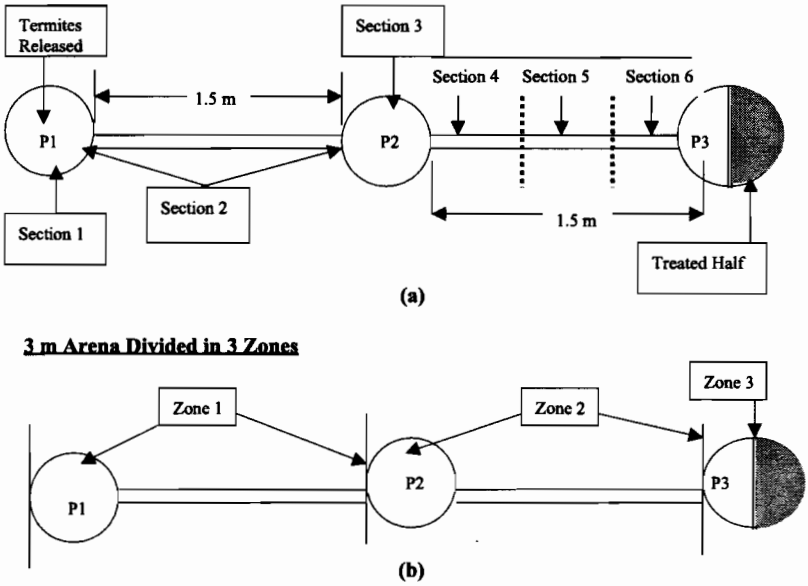

Fig. 1. Sketch of the 3-m foraging arena used to determine the effect of foraging distance on the horizontal transfer of [^{14}C]fipronil from the treated (shaded area) to untreated zones.

Successive Transfer Study. To determine whether fipronil could be transferred stepwise from donors to recipients several times after a single exposure (cascade event), a series of donor and recipient pairs of worker termites were tested (Rust and Saran 2006). Groups of (recipients) termites were mixed with the initial group of treated termites (donors) in a stepwise manner until the last recipients would have received fipronil after four transfers. Initially, 25 lots of 10 workers were exposed to 1 ppm fipronil on sand for 1 h based on our initial observations of donors in the single horizontal transfer study. Five lots of exposed termites were randomly selected and placed in plastic petri dishes provisioned with a piece of moist paper towel and held in the large plastic container at 100% RH chamber for 7 d. These served as a control for the exposed donors. Another five lots of 10 unexposed termites were selected and placed in separate petri dishes (3.5 cm in diameter) and maintained in a chamber at 100% RH for 14 d. These served as the untreated controls. Twenty lots of 10 workers (donor 1) were mixed with 20 lots of 10 blue-dyed termites (recipient 1) and placed in petri dishes provisioned with a disk of moist paper towel. These petri dishes were then placed in the large plastic container maintained at 100% RH. After 24 h, five lots of 10 termites were randomly selected and the donor 1 and recipient 1 (blue termites) were placed in separate petri dishes with a moist paper towel. The termites were maintained at 100% RH for 7 d. After the exposure, the recipient termites in the remaining 15 lots served as donor termites (designated 2) and were allowed in turn to mix with other recipients (designated 2) as described above. This alternate mixing of dyed and undyed termites continued until all the original 25 lots of termites were used (four transfers). Dead donor and recipient termites at each transfer were counted daily. The data were analyzed by a two-way ANOVA (Analytical Software 2005).

Movement of Fipronil in a Linear Foraging Arena. To study the effects of the potential horizontal transfer of fipronil in a simulated field situation, a linear foraging arena was set up in the laboratory. Three petri dishes (4.25 cm in diameter) were connected by two 1.52-m pieces of Tygon tubing (0.48 cm i.d.; St. Gobain Performance Plastics, Reading, PA) for a total length of 3 m (Fig. 1a). Petri dish 1 (P1) and petri dish 2 (P2) were covered with 5 g of sterilized sand at the bottom and provisioned with brown paper towel disks to serve as food for termites. Approximately 1 ml of water was added to the sand in each dish. Petri dish 3 (P3) was partitioned such that half was filled with 8 g of sterilized sand, and the other half was filled with 8 g of sand treated with [^{14}C]fipronil. Four concentrations of fipronil-treated sand (0.5, 5.0, 10.0, and 50 ppm) were tested. Each concentration was tested three times. To ensure sufficient moisture in both treated and untreated sands, 2 ml of DI H_2O was evenly added to the sands in the P3 petri dish. Exactly 100 termites were added to petri dish P1. Termite mortality in the arena was recorded over 7 d.

Our initial observations indicated that most of the dead termites were in the tubing. Thus, we divided the arena into unequal parts to determine the differences in the amount of fipronil on the termites in relation to their distance from the treated zone and provide sufficient numbers (n = 15) of termites per replicate (Fig. 1a). The position of each dead termite in the tubing between P2 and P3 was marked on the tubing with a permanent marker (Sharpie, Sanford, Bellwood, IL). The distance (centimeters) from the treatment dish (P3) was measured for each termite. Using

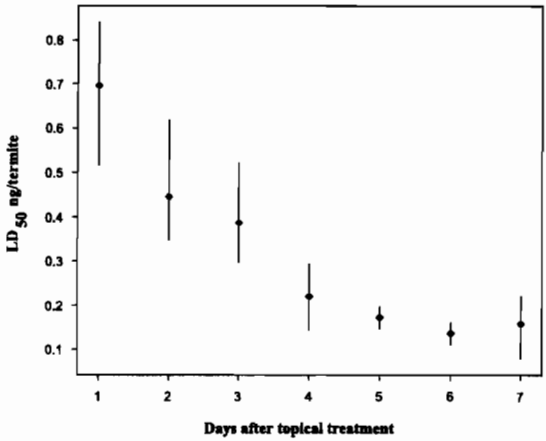

Fig. 2. Topical activity of fipronil against *R. hesperus*. Bars represent 95% CI.

a surgical blade, the tubing was then cut open from the top without disturbing the position of the termites. The termites were carefully removed from each 50-cm section of tubing and placed in each of three different 20-ml vials (sections 4, 5, and 6). A group of 15 dead or live termites collected from each section was then analyzed for ^{14}C label as described above.

The 3-m arena was divided into three unequal zones to determine the percentage of mortality of termites (Fig. 1b). The petri dish P1, where termites were released, and the tubing between petri dish P1 and P2 was designated zone 1 (Z1). Petri dish P2 and the tubing connecting dishes P2 and P3 were designated zone 2 (Z2). Petri dish P3 with the treated and untreated sands was considered zone 3 (Z3). Dead termites in each of these zones were counted daily.

A second test was conducted with a 6-m arena. This was similar to the 3-m arena except that the distances between P1, P2, and P3 were doubled (3 m). Two additional petri dishes (A and B, 4.25 cm in diameter) with brown paper towel disks as food and 5 g of moistened sterilized play sand were added at 1.5 m from P1 and P3 to prevent termites from desiccating in the longer arena. P3 was set up as described and 100 termites were transferred to dish P1. Termite movement and mortality were recorded over 7 d.

Data for the amount of ^{14}C label in termite bodies were analyzed using a two-way ANOVA. Percentage of mortality of termites was analyzed using a generalized linear model analysis (PROC GLM; SAS Institute 1999). Regression analysis between the amount of ^{14}C label on a termite body and the distance traveled was performed using Sigma Plot (SPSS Inc. 2002).

Table 2. Termite mortality after 1-h exposures of *R. hesperus* on sand treated with fipronil

Concn (ppm)	% termites dead after exposure			S(t)[a] (95% CI at day 7)
	Day 1	Day 5	Day 7	
5	47	100		
1	48	86	100	0.06 (0.03–0.12)a
0.5	14	72	78	0.24 (0.15–0.37)b
0.1	4	14	24	0.76 (0.63–0.86)c
0.05	2	6	4	0.84 (0.71–0.92)cd
Control	0	0	1	0.98 (0.90–1.00)d

[a] Five replicates of 10 termites were exposed to treated sand. Kaplan–Meier test determines the probability of survivors and test value is represented by S(t), where, t is days (Analytical Software 2005). Within columns, values of 95% CI on S(t) followed by different letters do not overlap.

Results

Topical Bioassay. A dose of 0.7 ng of fipronil killed 50% of the test population within 24 h. There was a gradual and significant decrease in the amount of fipronil required to kill 50% of the test population over the 7-d period. By day 3, the dose was reduced by ≈50% (0.22 ng per termite). After day 4, the dose of fipronil required to kill 50% of the termites stabilized between 0.1 and 0.2 ng per termite (Fig. 2).

Brief Exposure Studies. Brief exposures to higher concentrations (100–500 ppm) for 1 h resulted in significantly lower survivorship function [S(t)] values compared with the control within 4 h after exposure. Concentrations ≥300 ppm fipronil caused 100% kill within 24 h (Table 1).

One-hour exposures to 1 and 5 ppm fipronil provided 100% kill of workers by day 7 (Table 2). When exposed to 0.5 ppm, only 14% were killed at day 1, whereas 72% were killed by day 5. The S(t) at day 7 for 1 and 0.5 ppm was significantly lower than the two lowest deposits. The S(t) for 0.05 ppm fipronil was not significantly different from the control.

Tunneling Study. When the tunneling tubes were dismantled after 2 d, ≥69% of the termites were dead

Table 1. Termite mortality after 1-h exposures of *R. hesperus* to sand treated with high concentrations of fipronil on sand

Concn (ppm)	S(t)[a] with 95% CI			
	4 h	8 h	12 h	24 h
500	0.44 (0.33–0.54)b	0.10 (0.04–0.19)c	0	0
400	0.46 (0.36–0.55)b	0.10 (0.04–0.19)c	0.02 (0.00–0.07)c	0
300	0.66 (0.53–0.76)b	0.18 (0.10–0.29)bc	0.08 (0.03–0.17)bc	0
200	0.64 (0.51–0.75)b	0.38 (0.26–0.50)b	0.06 (0.03–0.11)c	0.04 (0.01–0.11)b
100	0.80 (0.68–0.88)b	0.42 (0.29–0.55)b	0.26 (0.16–0.38)b	0.08 (0.04–0.14)b
0	1.0a	1.0a	1.0a	1.0a

[a] Five replicates of 10 termites were exposed for to treated sand. Kaplan–Meier test determines the probability of survivors and the test value is represented by S(t), where t is hours (Analytical Software 2005). Within columns, values of 95% CI on S(t) followed by different letters do not overlap.

Table 3. Mortality and average distance tunneled by *R. hesperus* workers in sand treated with fipronil

Concn (ppm)[a]	% mortality of termites				Avg distance tunneled (cm)[b]
	Day 0	Day 1	Day 5	Day 7	
300	100				0a
200	97	100			0a
100	95	100			0a
50	93	93	97	100	0a
25	100	100	100	100	0a
10	86	89	100	100	0a
5	81	81	100	100	0a
1	69	86	91	91	0.3a
0.5	70	83	83	92	0.2a
Control	0	2	4	6	3.8b

[a] Twenty termites were used in each of five replicates.
[b] Means followed by the same letter are not significantly different (Tukey's HSD; $P < 0.05$).

for all concentrations tested (Table 3). Seven days after the termites were removed from the tubes >90% were killed even at the lowest concentration (0.5 ppm). Significantly more tunneling was observed in the control tubes compared with any of the concentrations tested ($F = 27.94$; df = 6, 28; $P < 0.001$). There was no significant difference in the distance tunneled in any of the fipronil concentrations. Even though the tunnels in the untreated sand stopped near the interface of the fipronil-treated sand, termites contacted a lethal dose of fipronil.

Movement Bioassays. Twenty-eight of 30 termites in the controls traversed the 10-cm pheromone trail within 10 s. Termites traversing the 10-cm trail after a 1-h exposure to 1 ppm fipronil showed significant changes in their responsiveness to trail pheromone at 1, 4, and 8 h ($G = 72.28$, df = 10, $P < 0.001$). After a 1-h exposure to 1 ppm, only 46% of the termites traversed the 10-cm trail within 10 s (Fig. 3). However, four of 30 termites took >30 s to traverse the trail. After 4 h, none of the termites previously exposed to 1 ppm fipronil for 1 h, completed the trail in <10 s and six of 30 termites took >30 s to complete the trail. At 8 h, 19 of 30 termites traversed the 10-cm trail in >30 s.

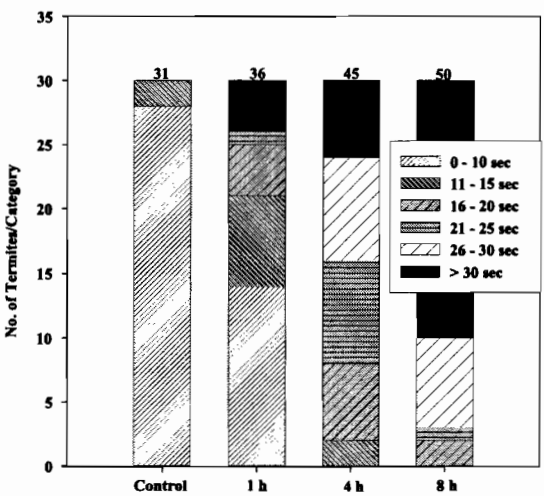

Fig. 3. Time (seconds) to traverse a 10-cm-long trail after a 1-h exposure to 1 ppm fipronil-treated sand. The actual numbers of termites tested to provide 30 complete runs are shown on the top of the stacked bars.

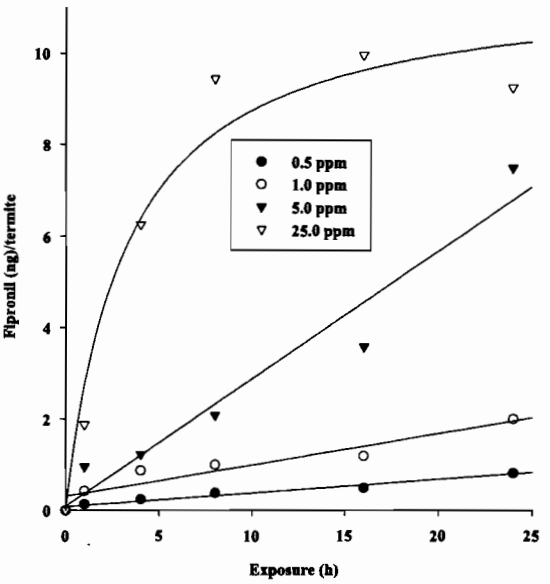

Fig. 4. Amount of fipronil measured by [^{14}C]fipronil taken up over time by termites on sand treated with fipronil at various concentrations. Uptake of fipronil by termites over 24 h to 0.5 ppm: $y = 0.083 + 0.03x$; $F = 90.67$; df = 1, 4; $P < 0.001$; $R^2 = 0.95$; 1.0 ppm: $y = 0.31 + 0.068x$; $F = 33.27$; df = 1, 4; $P = 0.004$, $R^2 = 0.89$; and 5.0 ppm: $y = 0.092 + 0.27x$; $F = 76.42$; df = 1, 4; $P < 0.001$; $R^2 = 0.95$. For 25 ppm fipronil, this relationship was represented by $y = -0.36 + 11.85x/(2.97 + x)$; $F = 43.43$; df = 2, 3; $P = 0.006$; $R^2 = 0.96$. Calculations of nanograms of fipronil per termite assume that all ^{14}C label is fipronil.

To complete each test with exposed termites, we had to use >30 termites. In almost all cases, termites that did not transverse the trail did not move after placing them on the trails. Those termites were allowed to remain at the starting point of trail for at least 15 min and were then discarded. As the time after exposure increased, the number of termites required to finish 30 replicates increased dramatically. At 4 and 8 h, 45 and 50 termites were tested, respectively (Fig. 3). In the controls, 30 of 31 termites tested traversed the trails. The effects of the continuous exposure on termite movement were nearly identical to brief exposures and were not included.

Uptake of Fipronil over Time. There was a direct linear relationship between the amount of fipronil picked up by termites and their exposure time for the three lowest concentrations (Fig. 4). When exposed to 25 ppm fipronil, termites rapidly acquired ^{14}C label over the first 10 h, and by 15 h there was no further significant uptake. The amount of ^{14}C label was ≈10 ng per termite. For 25 ppm, this relationship was represented by a hyperbolic equation (Fig. 4).

Horizontal Transfer. One-hour exposures to 0.5, 1.0, and 5.0 ppm provided 20, 89, and 100% kill of donors

Table 4. Percentage of mortality and survivorship function of donor and recipient termites 7 d postmixing

Concn (ppm)	Exposure (time)[a]	% dead		S(t)[b] (95% CI at day 7)		Mean ± SD (ng)[c]/termite		% transfer
		Donor	Recipient	Donor	Recipient	Donor	Recipient	
0.5	1	20	20	0.80 (0.66–0.88)c	0.66 (0.53–0.77)a	0.04 ± 0.01	0.01 ± 0.01c	20.8a
	4	42	31	0.57 (0.45–0.69)b	0.73 (0.56–0.83)a	0.09 ± 0.01	0.02 ± 0.01c	15.2b
	24	100	20	0	0.77 (0.65–0.86)a	0.27 ± 0.03	0.03 ± 0.01bc	10.5c
1.0	1	89	38	0.11 (0.06–0.19)a	0.57 (0.44–0.69)ab	0.28 ± 0.05	0.04 ± 0.01bc	13.3bc
	4	95	42	0.04 (0.01–0.12)a	0.40 (0.29–0.51)b	0.57 ± 0.06	0.06 ± 0.01bc	9.1bc
	24	100	27	0	0.57 (0.42–0.71)ab	0.92 ± 0.14	0.09 ± 0.03b	8.8bcd
5.0	1	100	47	0	0.48 (0.36–0.61)ab	0.44 ± 0.04	0.07 ± 0.01bc	13.1bc
	4	100	76	0	0.22 (0.14–0.33)b	0.78 ± 0.12	0.09 ± 0.01b	10.9bc
	24	100	100	0	0	3.92 ± 1.45	0.21 ± 0.06a	5.3d
Control	1	6	4	0.97 (0.88–0.99)c	0.97 (0.88–0.99)c			
	4	6	9	0.93 (0.82–0.97)c	0.91 (0.79–0.96)ac			
	24	2	4	0.97 (0.88–0.99)c	0.93 (0.82–0.97)ac			

Donors were exposed for different durations to the sand treated with various [^{14}C]fipronil concentrations (wt:wt).
[a] Fifteen donor termites were exposed to fipronil-treated sand for 1, 4, and 24 h before mixing with 15 recipient termites.
[b] Kaplan–Meier test determines the probability of survivors S(t), where t is days (Analytical Software 2005).
[c] Calculations of nanograms of fipronil per termite assume that all ^{14}C label is fipronil.

at day 7, respectively. When exposed for 4 h to 0.5, 1.0, and 5.0 ppm, 42, 95, and 100% of the donors were killed at day 7, respectively (Table 4). When donors were exposed for 24 h, there was 100% mortality at day 7 at all concentrations (Table 4). For all the treatments and exposures, donors showed significantly lower S(t) values compared with their corresponding controls.

Only 20–42% mortality was observed for recipients confined with termites exposed to sands treated with 0.5 and 1 ppm fipronil. Confinement with termites exposed to 5 ppm for 1, 4, and 24 h provided 47, 76, and 100% kill of recipients, respectively. Compared with corresponding controls, the S(t) value was significantly lower at day 7 for termites confined with donors in all cases except for a 4- and 24-h exposure to 0.5 ppm (Table 4).

There were significant differences in the amount of ^{14}C label recovered from recipients at day 7 when mixed with donors exposed to different concentrations of fipronil ($F = 44.19$; df = 2, 18; $P < 0.001$). The amount of time that donors were exposed to fipronil-treated sand had a significant effect on the amount of ^{14}C label transferred to recipients ($F = 22.3$; df = 2, 18; $P < 0.001$). There was an interaction between concentration and exposure period on the amounts of ^{14}C label that recipients picked up from donors ($F = 7.03$; df = 4, 18; $P < 0.001$).

Recipients picked up significantly higher amounts of ^{14}C label from donors exposed to 5 ppm deposits for 24 h compared with any other concentration and time period. Most of the exposures resulted in 0.01–0.07 ng of ^{14}C label being transferred to recipients. The least amount of ^{14}C label was transferred when donors were exposed for 1, 4, or 24 h on 0.5 ppm, 1, or 4 h on 1.0 ppm and 1 h on 5.0 ppm.

Both the concentration ($F = 13.51$; df = 2, 18; $P < 0.001$) and the duration of the donor's exposure ($F = 19.44$; df = 2, 18; $P < 0.001$) had significant effects on the percentage of ^{14}C label transferred to the recipients, but there was no interaction between concentration and exposure time ($F = 1.46$; df = 2, 18; $P < 0.26$). Donors exposed to 0.5 ppm for 1 h showed significantly higher percentage of transfer at day 7 compared with any other concentration and time combination (Table 4). When termites were exposed to 5 ppm for 24 h, the percentage transfer of ^{14}C label was the lowest compared with all other concentration–time combinations except for the donor's exposure to 1 ppm for 24 h (Table 4). There was a negative association between percentage of transfer and recipient survival probabilities (Kendall's tau [τ] = 0.428, $n = 9$).

Maximum Percentage of Transfer after Mixing Donors and Recipients. The greatest percentage of transfer of ^{14}C label occurred within 24 h for the three concentrations tested ($F = 201.11$; df = 1, 27; $P < 0.001$). On day 1, the percentage of transfer after exposure to 0.5, 1, and 5 ppm was 11.8, 8.9, and 9.2%, respectively. The concentration of fipronil had little effect on the percentage of ^{14}C label transferred at any given sampling day. There was a limited but significant interaction between concentration and days postmixing over 5 d ($F = 2.03$; df = 4, 27; $P = 0.037$). The percentage of transfer on days 3 and 5 was not significantly different from one another for donors exposed to 1 and 5 ppm.

Transfer from Dead Termites. The concentration of fipronil recovered from dead donors had a significant effect on the percentage of chemical transferred (^{14}C label) to recipients on day 1 after mixing ($F = 13.81$; df = 1, 6; $P = 0.009$). Recipient mortality was 100% within 24 h when mixed with dead donors exposed to 50 ppm. When mixed with dead donors exposed to 5 ppm, 100% mortality was achieved on day 5 (Table 5).

Transfer Due to Trophallaxis. The concentration to which the donors was exposed had a significant effect on the total amount of ^{14}C label recovered from donors, the donor's hindgut fluid, and recipients with and without sealed mouthparts ($F = 704.89$; df = 1, 16; $P < 0.001$). There were significant differences in the amount of ^{14}C label recovered from termite groups depending on whether they were donors or recipients, the condition of the recipient's mouthparts, and the amounts in the hindgut fluid ($F = 826.23$; df = 3, 16;

Table 5. Transfer of fipronil from dead termites (donors) to recipients over 5 d

ppm[a]	Days	% dead R	Amount of fipronil (mean ± SD [ng])[b]/termite		
			D	R	% transfer[c]
5	1	21	2.85 ± 0.29	0.21 ± 0.07	6.8a
	3	53	2.18 ± 0.45	0.23 ± 0.04	9.6b
	5	100	1.99 ± 0.51	0.28 ± 0.06	12.7b
50	1	100	9.14 ± 0.53	1.42 ± 0.30	13.5b

D, donors; R, recipients.

[a] Amount of fipronil in ppm on sand.

[b] Calculations of ng of fipronil / termite assume that all ^{14}C-label is fipronil.

[c] Percentage of transfer represents cumulative transfer; percentage of transfers followed by the same letters are not significantly different at $P < 0.05$.

$P < 0.001$). There was a significant interaction between concentrations and the condition of the mouthparts (sealed or normal) ($F = 222.32$; df = 3, 16; $P < 0.001$).

There were no significant differences in the ^{14}C label recovered from recipients with sealed or normal mouthparts. There were no detectable amounts of fipronil in the hindgut fluid of recipients with sealed or normal mouthparts. When exposed to 5 and 30 ppm deposits, the donors had 0.28 and 0.94 ng per termite, respectively, and significantly higher amounts of ^{14}C label than did the recipients with and without sealed mouthparts. The amount of ^{14}C label recovered from the donor's hindgut fluid was ≈ 15–25% of the total ^{14}C label recovered. There were no significant differences in the ^{14}C label recovered from recipients with sealed or normal mouthparts. There were no detectable amounts of ^{14}C label in the hindgut fluid of recipients with sealed or normal mouthparts. The mortality of termites with sealed and normal mouthparts was not significantly different over the 10 d period. All ^{14}C label was assumed to have been transferred by contact.

Successive Transfer Study. A 1-h exposure to 1 ppm fipronil-treated sand provided 100% kill of termites within 7 d. In the first transfer, 14% of the recipients were killed at day 1. By day 7, 68% of the recipients were killed. When a group of recipients of the first transfer (donor 2) were mixed with untreated termites (second transfer), 52 and 86% of the donor termites were dead by day 1 and day 7, respectively. Only 2% of the recipients were killed by day 7. When recipients of the second transfer were used as donors for the third transfer, only 2% of recipients died by day 7. The recipients of the third and fourth transfer were not killed.

Movement of Fipronil in a Linear Foraging Arena. There were few dead or live termites in petri dishes P1 or P2, most being in the Tygon tubing. Because maximum mortality was observed between petri dishes P2 and P3, the tubing (1.52 m) connecting them was divided into three equal parts, each measuring 50 cm (Fig. 1a). There was a significant effect of the concentration of fipronil on the amounts of ^{14}C labeled fipronil recovered from termites in the different sections ($F = 246.57$; df = 3, 48; $P < 0.001$) and significant differences in the amount of ^{14}C label recovered ($F = 191.45$; df = 5, 48; $P < 0.001$) from termites in each section. There was also a significant interaction between concentration and sections (the distance from the treated zone) ($F = 33.90$; df = 15, 48; $P < 0.001$), suggesting that the amounts of ^{14}C label recovered

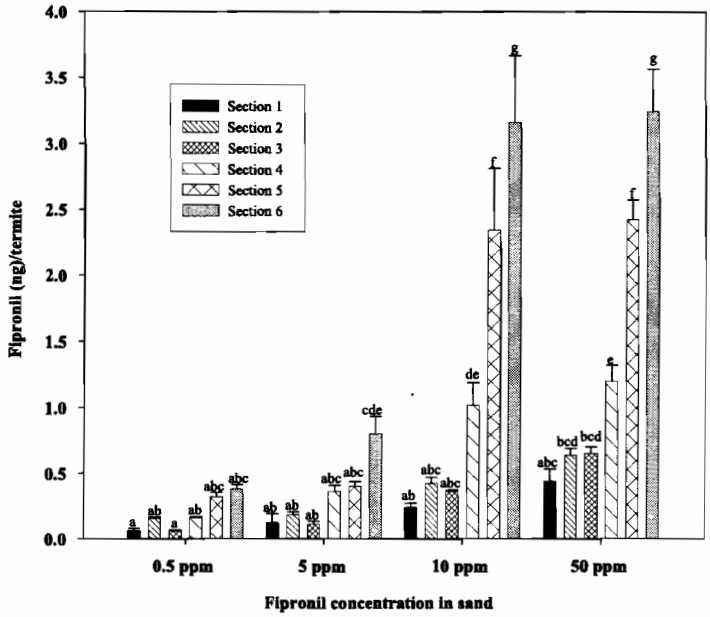

Fig. 5. Amount of fipronil recovered from termites in the six different sections. Bars with same letters are not significantly different at $P < 0.05$. Calculations of nanograms of fipronil per termite assume that all ^{14}C label is fipronil.

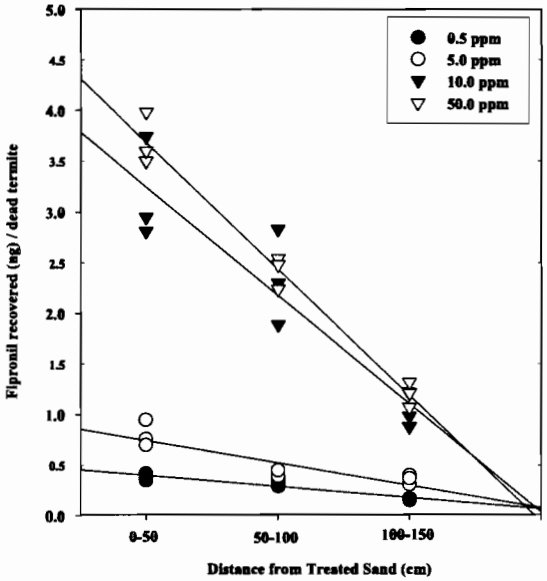

Fig. 6. Relationship between the distance traveled (location of dead termites after exposure) and the amount of fipronil recovered from termite bodies in each of the three sections. The regression equations were 0.5 ppm ($y = 0.508–0.002x$; $R^2 = 0.86$; $F = 45.19$; df = 1, 7; $P < 0.001$), 5.0 ppm ($y = 0.97–0.0044x$; $R^2 = 0.74$; $F = 20.15$; df = 1, 7; $P < 0.002$), 10.0 ppm ($y = 4.31–0.0214x$; $R^2 = 0.85$; $F = 42.68$; df = 1, 7; $P < 0.001$), and 50.0 ppm ($y = 4.93–0.024x$; $R^2 = 0.97$; $F = 308.64$; df = 1, 7; $P < 0.001$). Calculations of nanograms of fipronil per termite assume that all ^{14}C label is fipronil.

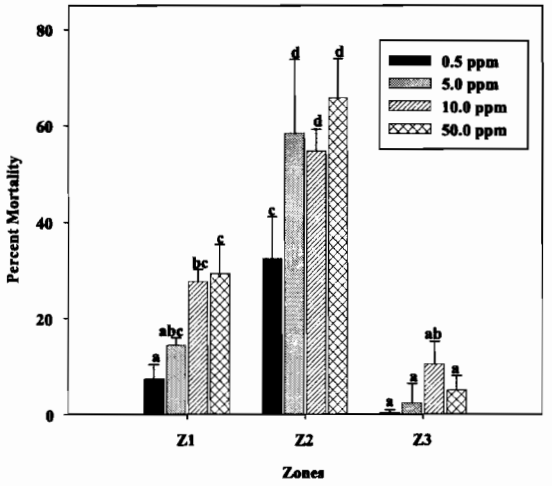

Fig. 7. Distribution of dead termites in the three zones of the 3-m arena. Bars with same letter are not significantly different at $P < 0.05$.

from the termites in sections and treatments show differing increasing trend (Fig. 5).

The amounts of ^{14}C label recovered from sections 1, 2, and 3 from all concentrations were not significantly different from one another. Significantly greater amounts of ^{14}C label were recovered from termites in sections 4, 5, and 6 than from sections 1–3 in 10 and 50 ppm treatments only (Fig. 5). Thus, only sections 4, 5, and 6 were subsequently analyzed.

There were significant linear negative regressions between the distances traveled (centimeters) by termites from the treatment zone and the amounts of ^{14}C label recovered from termite bodies ($F = 8.05$; df = 2, 33; $P < 0.001$). The regression slopes between the distance and amount of ^{14}C label recovered from a termite body were significantly different ($F = 18.7$; df = 3, 28; $P < 0.001$) (Fig. 6). Solving the equations for the x-intercept for each concentration provided the following distances: 0.5 ppm, 2.54 m; 5 ppm, 2.20 m; 10 ppm, 2.01 m; and 50 ppm, 2.05 m. No detectable amounts of ^{14}C label could be recovered from termites traveling distances >2.54 m from the treated zone.

The overall percentage of mortality in the 3-m linear arena was significantly different for the three concentrations ($F = 17.23$; df = 3, 24; $P < 0.001$). Percentage of mortality was significantly different in the three zones depending on their location in the arena ($F = 175.79$; df = 2, 24; $P < 0.001$). There was a limited but significant interaction between concentrations and zones ($F = 3.57$; df = 6, 24; $P < 0.01$). Intermediate levels of mortality were encountered in zone 1 (Fig. 7). Percentage of mortality was highest in zone 2 for each concentration. The percentage of mortality in zone 2 was not significantly different among 5, 10, and 50 ppm (Fig. 7). The lowest mortality was observed in zone 3 for all concentrations, nearest to the treated sand. The mean percentage of mortality and distances traveled by exposed termites in the 6-m arena were identical to those of the 3-m arena. Hence, the data are not shown.

Discussion

Currently, there is a perception in the pest control industry that delayed toxicity and nonrepellency of termiticides are responsible for horizontal transfer and areawide control of termites. One of the most commonly cited studies is a field study by Potter and Hillery (2002) in which fipronil and imidacloprid were applied as perimeter treatments. They concluded that the treatment effects extended beyond the treated zone, because of a reduction in live termites and the presence of dead termites in monitoring stations 0.3–4 m from the treatment. Areawide reductions in termites around fipronil treatments in conventional ground board studies in USDA–Forest Service plots also have been cited as evidence (Kard 2001). None of these studies demonstrated that horizontal transfer contributed to the control of termites away from the treatment. The new recommendations for termite treatments include perimeter alone treatments (exterior perimeter/localized interior treatment; Anonymous 2004) that were in part based on the claims of areawide effects. These recommendations assume that termites can be eliminated by treating with nonrepellent insecticides primarily around the exterior of buildings. Exterior treatments will not only reduce the cost of treatments but also lead to decreasing the

volume of insecticides applied, which is currently ≈60 million gallons annually (Curl 2004). However, our study suggests that the horizontal transfer of fipronil is limited to a short distance around the treatment.

Fipronil is toxic to termites, requiring as little as 0.16 ng per termite to produce 50% kill of *R. hesperus* workers. Topical bioassays provide an estimate of the amount of insecticide required to kill donors and recipients. Ibrahim et al. (2003) reported an LD_{50} of 1.33 ng of fipronil per termite for *C. formosanus* at day 3, which is comparable with our data considering that *C. formosanus* workers are ≈33% larger than *R. hesperus* and that their data were collected on day 3 versus day 7 as shown in Fig. 3. More fipronil would be required to kill *C. formosanus* on day 3 than day 7. Compared with other termiticides very small amounts of fipronil are required to kill either the donor or potential recipients, thereby increasing the likelihood of horizontal transfer (Rust and Saran 2006).

When exposed to high concentrations (>100 ppm), the termites were overwhelmed by the excessive amounts of fipronil; therefore, we did not observe any delayed toxicity. Unlike fast-acting insecticides such as pyrethroids or high concentrations of fipronil that kill termites quickly, ≤5 ppm deposits provided <50% mortality within 24. At day 5, 100% of the termites exposed to 5 ppm were killed. Brief exposures to ≥0.5 ppm provided 100% kill of termites by day 7. Our exposure studies with [^{14}C]fipronil showed that termites picked up lethal doses of fipronil within 1 h when doses were >0.5 ppm. However, it took ≈5 d for the full toxicity to manifest itself, corroborating the findings by Remmen and Su (2005b). Our topical bioassay and brief exposure data suggested that at lower concentrations, there was a delay of 72 h in mortality. Other slow-acting insecticides such as chlorfenapyr also provided a dose-dependent mortality with delayed toxicity (Rust and Saran 2006).

Even with 1-h exposures to higher doses from 100 to 500 ppm, fipronil toxicity symptoms were not obvious for at least 1 h. Symptoms occurred within 4 h after exposure to sands treated with as little as 1 ppm, especially those termites that failed to traverse the trails in the movement study. Their symptoms included reduced movement, and 10–20% of the termites lying on their backs and unable to right themselves. They exhibited typical symptoms due to a GABA antagonist intoxication, including hyperexcitation of the central nervous system, which led to convulsions and uncontrollable twitching of their legs and antennae (Hainzl and Casida 1996). Most of the fully intoxicated termites produced a small droplet of hindgut fluid. Intoxicated termites, typically on their backs with their tarsi curled together and antennae distorted, never recovered.

Fipronil did not repel termites at any of the concentrations tested, with >91% of the termites being killed in the tunneling studies. Remmen and Su (2005a) reported that *R. flavipes* and *C. formosanus* penetrated ≈1–2 cm into sand treated with 1 ppm fipronil. In our studies, there was no detectable penetration into sand treated with >1 ppm fipronil and very little tunneling (0.2–0.3 cm) in tubes with 0.5 and 1 ppm treated sand. In similar tunneling studies with chlorpyrifos (a nonrepellent but fast-acting insecticide), 96% of termites were killed within the first 48 h of a tunneling bioassay (Smith and Rust 1990). Contrary to Ibrahim et al. (2003), fipronil concentrations as high as 500 ppm did not repel *R. hesperus*, providing 100% kill of termites in our tunneling studies.

Termites remained in contact with fipronil-treated soils because of the lack of repellency and its delayed toxicity at low concentrations (≤1 ppm). Tunneling activities were suspended only after the onset of toxicity. Henderson (2003) reported that tunneling activity was not affected for up to 9 h after exposures to low doses of fipronil in soil. Chen and Allen (2006) found that the digging efforts of red imported fire ants, *Solenopsis invicta* Buren, in fipronil-treated sand were concentration dependent and that mortality was positively correlated with fipronil concentrations (1–10 ppm).

Signs of intoxication occurred between 4 and 8 h, the termite's movement being dramatically reduced. At 8 h, 40% of the termites tested never traversed the trail. Similar results were reported by Rust and Saran (2006) in *R. hesperus* after brief exposures to chlorfenapyr. Thorne and Breisch (2001) showed that sublethal exposures to another nonrepellent termiticide, imidacloprid, caused staggered walking in *R. virginicus*. Even though termites acquired much higher amounts of fipronil in the continuous exposure study, termites from both brief and continuous exposure studies performed similarly over the first 8 h because of delayed toxicity. The brief exposures to fipronil affected the termite's ability to perform normal walking activities long before morbidity. In the field, the lack of mobility of termites may dramatically limit the distance that exposed termites move after exposure to treated soils.

At lower concentrations (0.5, 1, and 5 ppm), termites were active for a longer period and were able to pick-up lethal doses. There was a direct positive linear relationship between the amount of fipronil recovered from the termite body and the time of exposure. At the highest concentration (25 ppm), most termites were immobilized within 8 h; thus, active uptake ceased long before the end of the 24-h exposure. The maximum amount of fipronil picked up by the termites on sand treated with 25 ppm was ≈10 ng. Even on the highest deposits termites only acquired ≈10 times more fipronil than the LD_{95} (0.75 ng per termite; Rust and Saran 2006). However, termites with doses as high as 10 ng would not be able to move very far from the treated zone. It is unlikely that such termites would return to the colony and serve as donors, because of the rapid toxicity of fipronil at this dose.

Several studies have concluded that the nonrepellent termiticides have the potential to be transferred to unexposed nestmates based on the mortality of the recipients. Donors were frequently treated (topically) with large and practically inappropriate amounts of insecticide, based on likely exposures in the field. Tomalski and Vargo (2004) topically treated

individual donor termites (100 ng per termite) and quantified the amounts transferred to recipients (in the digestive system and on the cuticle). Ibrahim et al. (2003) topically treated donors with technical grade fipronil (2.5 ng per termite) and mixed them with recipients in petri dishes to study the extent of horizontal transfer. Similarly, Hu et al. (2005) applied a range of doses (10, 20, 50, 100, and 200 ng per termite) of indoxacarb to donors to study horizontal transfer to recipients. Few studies have taken into account the amounts of insecticide actually acquired by donors from deposits and available for transfer. Haagsma (2003) reported that donors only acquired ≈34 ng of imidacloprid when exposed for 12 h to sand treated at 500 ppm [^{14}C]imidacloprid. In the current study, when termites exposed to fipronil acquired doses ≈5–10 times the lethal dose (≈10 ng), they were immobilized within 8 h. Such heavily intoxicated termites did not participate actively in horizontally transferring lethal amounts to recipients.

Donor and recipient mortality were dose dependent, being affected by concentration and exposure period of the donors. Greater than 75% of the recipient termites were killed when mixed with donors exposed to 5 ppm for 4 or 24 h. Donors died rapidly at the highest concentration (5 ppm), and they had higher amounts of fipronil on their bodies. Thus, more fipronil was available to the recipient termites touching and walking over them. Donors exposed to lower doses (0.5 and 1 ppm) produced <50% recipient mortality (Table 3). At lower doses, it was important for donor termites to remain alive longer to allow transfer of lethal amounts of fipronil.

The variability of recipient mortality was attributed to the mixing ratios of soldiers versus workers, colonies, and doses (Ibrahim et al. 2003, Shelton and Grace 2003). Our study also demonstrated that there were differences in donor mortality. In the transfer studies, donors had consistently lower mortality at day 7 than did termites exposed to the same doses (concentration × time) used in the brief exposure test. This difference may be directly attributed to the amount of fipronil transferred to recipients. Reduction in the amount of fipronil recovered from the donor's body by 10–35% resulted in lower mortality of donors, especially at lower concentrations. Similarly in successive transfer study, the mortality of the first and second set of donors was 94 and 86%, respectively, compared with 100% mortality of treated controls at day 7. At lower concentration (1–5 ppm) there was not enough fipronil on the body of the donors to kill more than one other termite (recipient).

The maximum transfer of fipronil took place within 24 h. On days 3 and 5, there was a negligible transfer of fipronil compared with the transfer that occurred on day 1. Haagsma (2003) and Suarez and Thorne (2000) proposed that multiple contacts between donors and recipients may be needed for horizontal transfer to occur. Our data suggest this occurs within 24 h.

Recipients were able to pick up lethal doses from dead termites in petri dishes, because fipronil was nonrepellent and unexposed termites touched and walked over the dead termites. The rapid mortality of recipients exposed to dead donors previously exposed to 50 ppm, was due to the higher availability of fipronil on the dead termites compared with donors exposed at 5 ppm. We did not observe other termites avoiding the dead termites during their movement in Tygon tubing over 7 d. This was contrary to findings of Su (2005) of necrophobic behavior of *C. formosanus*. However, in our arena the dead termites in the Tygon tubing were dry corpses (not decomposing).

Huang et al. (2006) showed that when termites consumed fipronil baits (40 ppm) their foraging population was suppressed. Thus, there is a possibility that fipronil transfer also may occur via trophallaxis. However, in our study the primary transfer of fipronil was due to body contact between donors and recipients. Haagsma (2003) and Rust and Saran (2006) reported similar findings in their studies with imidacloprid and chlorfenapyr, respectively. Termites fed on treated filter paper (visual feeding marks on filter paper) had detectable amounts (≈0.06–0.17 ng) of [^{14}C]fipronil in the hindgut fluid (anal droplets). However, there were no detectable quantities of ^{14}C label from the hindgut fluid of recipient workers with functional mouthparts. If a lethal amount of fipronil (0.16 ng/termite) had been transferred by trophallaxis, it would have been possible to detect it.

A lethal transfer of fipronil from donor to recipient occurred only to the first set of recipients in successive transfer studies. When donors exposed to fipronil-treated sand for 1 h were mixed with recipients at a 1:1 ratio, 68% of the recipients died by day 7. When the recipients of the first transfer served as donors, the amount of fipronil transferred to the next set of recipients did not result in significant mortality. This provides some additional corroboration of Ibrahim et al. (2003) findings that a minimum number of donors (40%) was required to observe maximum horizontal transfer. Approximately, one donor was needed to effectively transfer fipronil to each recipient termite.

The highest amounts of fipronil (2.5–4.0 ng per termite) from the termites within 0.5 m of the treated zone. There was a significant negative linear relationship between the distance from the treated zone and the amount of fipronil recovered from the termite bodies (nanograms per termite). Termites in section 6 (>2.5 m from the treated zone) had small amounts (≈0.5 ng) of fipronil on their bodies regardless of the exposure concentration. Once the toxicity symptoms started these termites were not able to transfer lethal amounts of toxicant to a recipient termite.

Few termites (≈10%) died in the treated zone. Even when exposed to the highest tested dose (50 ppm), most of them moved out of the treatment zone with the maximum mortality ≈65% occurring in the first 1.5 m of the arena. Regardless of the concentration, the onset of mortality began after 24 h and peaked at day 6. The length of the arena (3 or 6 m) and the concentration of the treated sand (10 or 50 ppm) did not affect the amounts of fipronil recovered from dead

termites at different distances away from the treated zone. Consequently, the time required for the onset of delayed toxicity and the time when termites left the treated zone may be the most important factors in determining whether horizontal transfer occurs.

Ibrahim et al. (2003) obtained the maximum horizontal transmission of fipronil when donors were topically treated with 2.5 ng per termite. In the linear foraging arena, the highest amounts of fipronil recovered from the termites in 10 and 50 ppm ranged from ≈2.8–4.0 ng per termite. Termites treated with such high doses were dead within 0.5 m of the treated sand and represented 25–35% of the total dead termites in the arena. To obtain doses in the range of 2.5–4.0 ng, termites must have contacted the treated sand directly. Live or dead termites that were ≈3 m away from the treated zone had <1 ng of fipronil on their bodies and may have received these doses through horizontal transfer from exposed donors. They represented 25–30% of the dead termites and were >1.5 m away from the treated sand (10 and 50 ppm). By the same token, in lower concentrations (0.5 and 5 ppm) horizontal transfer may be responsible for termites acquiring doses <0.5 ng. Such termites represented 6–10% of the total dead termites. In the linear arena with 0.5 and 5.0 ppm fipronil, none of the termites acquired 2.5 ng of fipronil. Mortality was probably due to both direct contact and transfer.

In another arena study, Su (2005) reported that maximum termite mortality (43%) either due to direct exposure to fipronil-treated sand or by social contact with exposed individuals was limited to a distance of ≤5m from the treated sand. Osbrink et al. (2005) in their study reported that none of the monitoring stations 1–3 m away from the treated structures had termites intoxicated by imidacloprid. Our laboratory studies corroborate these findings that the movement of these nonrepellent termiticides was limited. Even when a small percentage of termites (≈10–30%) were able to traverse 3 m, they contributed very little to the mortality of unexposed colony members via horizontal transfer. Each termite had <0.5 ng of fipronil on them.

The horizontal transfer of toxicants is a dynamic system. After a donor contacts soil treated with nonrepellent termiticides and acquires toxicant, there is a period before the toxicant adversely affects the donor. Transfer is most likely to occur within 24 h. Only a portion of the toxicant on the surface of the donor is available for transfer. If the toxicant is extremely active (lethal doses are very low) and nonrepellent, this would increase the likelihood that transfer from even dead termites may occur and result in recipient mortality.

Factors such as the inherent toxicity of the insecticide, the type of substrate, concentration of the insecticide, the exposure time of the donor, and time expired after exposure greatly affect the transfer process (Rust and Saran 2006). These factors determine whether donor termites will have sufficient toxicant to permit transfer of lethal doses to recipients. If sufficient toxicant is to be transferred to recipients over distances by exposed termites, the toxicant must exhibit delayed toxicity. There is a very narrow range of doses (concentration × exposure time) over which such transfers might occur in field situations. The transfer is most likely to occur at lower concentrations that permit the expression of the delayed toxicity. Thus, horizontal transfer is probably not the major contributing factor to the efficacy of nonrepellent termiticides with delayed toxicity in controlling termites in field situations.

Acknowledgments

We thank BASF for providing [^{14}C]fipronil and partial funding for this study. We also thank the Carl Strom/Western Exterminator Company Scholarship Fund for partial support of this research.

References Cited

Analytical Software. 2005. STATISTIX user's manual, version 8. Analytical Software, Tallahassee, FL.

Anonymous. 1996. Fipronil®, worldwide technical bulletin, pp. 1–27. Aventis CropScience, Lyon Cedex, France.

Anonymous. 2004. BASF receives EPA approval for Termidor EP/LIT label amendment. Pest Control Technol. 32: 11.

Bobe, A., C. M. Coste, and J. F. Cooper. 1997. Factors influencing the adsorption of fipronil on soils. J. Agri. Food Chem. 45: 4861–4865.

Chen, J., and M. L. Allen. 2006. Significance of digging behavior to mortality of red imported fire ant workers, *Solenopsis invicta*, in fipronil-treated sand. J. Econ. Entomol. 99: 476–482.

Curl, G. 2004. Pumped-up termite market. Pest Control. Technol. 32: 26, 28, 33.

Haagsma, K. 2003. Utilization and movement of toxicants and nutrients and their effects on the western subterranean termite, *Reticulitermes hesperus* Banks (Isoptera: Rhinotermitidae). Ph.D. dissertation, University of California, Riverside, CA.

Haagsma, K., and M. K. Rust. 2005. Effect of hexaflumuron on mortality of the western subterranean termite (Isoptera: Rhinotermitidae) during and following exposure and movement of hexaflumuron in termite groups. Pest Manag. Sci. 61: 517–531.

Hainzl, D., and J. E. Casida. 1996. Fipronil insecticide: novel photochemical desulfinylation with retention of neurotoxicity. Proc. Natl. Acad. Sci. U.S.A. 93: 12764–12767.

Henderson, G. 2003. Liquid learning. Pest Control Technol. 31: 48–59.

Hu, X. P. 2005. Evaluation of efficacy and nonrepellency of indoxacarb and fipronil-treated soil at various concentrations and thicknesses against subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 98: 509–517.

Hu, X. P., D. Song, and C. W. Scherer. 2005. Transfer of indoxacarb among workers of *Coptotermes formosanus* (Isoptera: Rhinotermitidae): effects of dose, donor:recipient ratio and post-exposure time. Pest Manag. Sci. 61: 1209–1214.

Huang, Q.-Y., C.-H. Lei, and D. Xue. 2006. Field evaluation of a fipronil bait against subterranean termite *Odontotermes formosanus* (Isoptera: Termitidae). J. Econ. Entomol. 99: 455–461.

Ibrahim, S. A., G. Henderson, and H. Fei. 2003. Toxicity, repellency and horizontal transmission of fipronil in the

Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 96: 461–467.

Kard, B. 2001. Gulfport studies stay the course. Pest Control 69: 30–33, 73.

Osbrink, W.L.A., M. L. Cornelius, and A. R. Lax. 2005. Effect of imidacloprid soil treatments on occurrence of Formosan subterranean termites (Isoptera: Rhinotermitidae) in independent monitors. J. Econ. Entomol. 98: 2160–2168.

Potter, M. F., and A. E. Hillery. 2002. Exterior-targeted liquid termiticides: an alternative approach to managing subterranean termites (Isoptera: Rhinotermitidae) in buildings. Sociobiology 39: 373–405.

Remmen, L. N., and N.-Y. Su. 2005a. Time trends in mortality for thiamethoxam and fipronil against Formosan subterranean termites and eastern subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 98: 911–915.

Remmen, L. N., and N.-Y. Su. 2005b. Tunneling and mortality of eastern and Formosan subterranean termites (Isoptera: Rhinotermitidae) in sand treated with thiamethoxam or fipronil. J. Econ. Entomol. 98: 906–910.

Rust, M. K., and R. K. Saran. 2006. The toxicity, repellency, and transfer of chlorfenapyr against western subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 99: 864–872.

Saran, R. K. 2001. Concentration dependent degradation of new generation termiticides in soil and their bioavailability to eastern subterranean termites. M.S. thesis, University of Nebraska, Lincoln, NE.

SAS Institute. 1999. SAS user's guide, version 8.2. SAS Institute, Cary, NC.

Scharf, M. E., and B. D. Siegfried. 1999. Toxicity and neurophysiological effects of fipronil and fipronil sulfone on the western corn rootworm (Coleoptera: Chrysomelidae). Arch. Insect Biochem. Physiol. 40: 150–156.

Shelton, T. G., and J. K. Grace. 2003. Effects of exposure duration on transfer of nonrepellent termiticides among workers of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). J. Econ. Entomol. 96: 456–460.

Smith, J. L., and M. K. Rust. 1990. Tunneling response and mortality of western subterranean termite (Isoptera: Rhinotermitidae) to soil treated with termiticides. J. Econ. Entomol. 83: 1395–1401.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco, CA.

SPSS Inc. 2002. SigmaPlot® user's guide, version 8.0. SPSS Inc., Chicago, IL.

Su, N.-Y. 2005. Response of the Formosan subterranean termites (Isoptera: Rhinotermitidae) to baits or nonrepellent termiticides in extended foraging arenas. J. Econ. Entomol. 98: 2143–2152.

Su, N.-Y., and R. H. Scheffrahn. 1998. A review of subterranean termite control practices and prospects for integrated pest management programs. Integr. Pest Manag. Rev. 3: 1–13.

Su, N.-Y., M. Tamashiro, and M. I. Haverty. 1987. Characterization of slow-acting insecticides for remedial control of the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 80: 1–4.

Suarez, M. E., and B. L. Thorne. 2000. Rate, amount, and distribution pattern of alimentary fluid transfer via trophallaxis in three species of termites (Isoptera: Termopsidae: Rhinotermitidae). Ann. Entomol. Soc. Am. 93: 145–155.

Thorne, B. L., and N. L. Breisch. 2001. Effects of sublethal exposure to imidacloprid on subsequent behavior of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 492–498.

Tomalski, M. D., and E. L. Vargo. 2004. Chain reaction. Pest Control 72: 51–53.

Tsunoda, K. 2006. Transfer of fipronil, a non repellent termiticide, from exposed workers of *Coptotermes formosanus* (Isoptera: Rhinotermitidae) to unexposed workers. Sociobiology 47: 563–575.

Received 22 Augsut 2006; accepted 26 December 2006.