# Toxicity, Repellency, and Transfer of Chlorfenapyr Against Western Subterranean Termites (Isoptera: Rhinotermitidae)

MICHAEL K. RUST AND RAJ K. SARAN

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

J. Econ. Entomol. 99(3): 864–872 (2006)

**ABSTRACT** Chlorfenapyr is a slow-acting insecticide against western subterranean termite, *Reticulitermes hesperus* Banks, when applied to sand. The $LD_{50}$ at day 7 for workers is 29.98 ng per termite and considerably higher than that of chlorpyrifos (14.01), cypermethrin (3.21), and fipronil (0.16). Brief exposures to sand treated with chlorfenapyr resulted in dose-dependent mortality over a broad range of concentrations. Brief 1-h exposures to ≥75 ppm provided >88% kill of termites at day 7. Chlorfenapyr deposits did not repel termites, even at 300 ppm. Termites tunneled from 0.1 to 1.8 cm into sand treated with 10- to 300-ppm chlorfenapyr deposits, resulting in ≥70% mortality. Within 1 h after being exposed to 50 ppm chlorfenapyr, ≈17% of the termites exhibited impaired responses to synthetic trail pheromone. By 4 h, nearly 60% of the workers were not able to follow a 10 fg/cm pheromone trail. There was a direct linear relationship of the uptake of [$^{14}$C]chlorfenapyr as concentration and duration of exposure increased. The percentage of chlorfenapyr transferred to recipients varied from 13.3 to 38.4%. Donors exposed for 1 h transferred a greater percentage of chlorfenapyr than did donors exposed for 4 h. A 1-h exposure on 100-ppm deposits provided sufficient uptake to kill 100% of the donors and sufficient transfer to kill 96% of the recipients. There was not enough transfer for recipients to serve as secondary donors and kill other termites. Horizontal transfer is limited to contact with the original donor and by the decreased mobility of workers within 4–8 h after exposure to treated sand. The effectiveness of chlorfenapyr barrier treatments is primarily due to its nonrepellency and delayed toxicity.

**KEY WORDS** chlorfenapyr, *Reticulitermes hesperus*, soil insecticide, insecticide transfer

Barrier insecticide applied to soils is one of the main strategies to protect structures from subterranean termites. Insecticides are categorized as repellent, toxic, and nonrepellent, or nonrepellent with delayed toxicity (Su et al. 1982). Repellent insecticides protect structures typically by repelling foraging termites, and sublethal exposures to pyrethroid insecticides inhibit termite tunneling (Smith and Rust 1990). The toxic and nonrepellent termiticides such as chlordane and chlorpyrifos kill termites by contact. With slow-acting insecticides, the level of mortality and the speed of kill are dependent on concentration (Su et al. 1987).

Chlorfenapyr is an aryl-substituted cyanopyrrole with broad-spectrum activity against insects and mites. It is a pro-insecticide being activated by the oxidative removal of the *N*-ethoxymethyl group (Treacy et al. 1994). The pyrrole metabolite uncouples oxidative phosphorylation by disrupting the mitochondrial membranes, thus affecting energy production and resulting in cell death and death of the insect or mite. Because chlorfenapyr binds to soil with a $K_{oc}$ = 11,500 ml/g and has low water solubility, the potential for leaching in soils is low (Hollingworth 2001), making it an ideal compound for termite barrier applications.

Although there are a number of publications dealing with chlorfenapyr and insects of agricultural importance, little has been published on the activity of chlorfenapyr against household insect pests since it was registered in 2001. Chlorfenapyr was found to be an effective nonrepellent barrier treatment against Pharaoh ant, *Monomorium pharaonis* (L.), with ants often relocating nests under treated materials increasing their exposure and kill (Buczkowski et al. 2005). Outdoor sprays of chlorfenapyr controlled Argentine ants, *Linepithema humile* (Mayr) (Suoja et al. 2000). Sprays of the chlorfenapyr provided good control against German cockroaches, *Blattella germanica* (L.) (Ameen et al. 2000). Soils treated with 0.5, 0.75, and 1.0% chlorfenapyr provided at least 5 yr of satisfactory protection against subterranean termites in the USDA Forest Service termiticide tests (Wagner 2003).

Because chlorfenapyr is considered to be a nonrepellent insecticide, it has the potential for transfer from exposed to unexposed termites. The insecticidal activity, repellency, and potential for horizontal transfer of chlorfenapyr were determined against workers of the western subterranean termite, *Reticulitermes hesperus* Banks. Concentrations and exposure times were determined to ensure that donor termites were

0022-0493/06/0864–0872$04.00/0 

able to interact with the recipients in transference studies. Studies were conducted with technical chlorfenapyr, technical [$^{14}$C]chlorfenapyr, and formulated chlorfenapyr (Phantom 2 SC). The relevance of the data in regard to controlling termites in treated soils is discussed, and the implications of horizontal transfer and control of termites beyond the treated zone are also discussed.

## Materials and Methods

**Insects.** Termites were collected from colonies on the campus of the University of California Riverside over a 9-mo period. Traps consisting of pieces of polyvinyl chloride pipe (9.7 cm i.d. by 15.0 cm) were placed in the ground at sites with confirmed termite activity. The traps were provisioned with rolls of corrugated cardboard (5.0 cm in length by 2.25 cm in radius). Termites feeding on the cardboard were extracted and transferred to plastic food containers provisioned with moist paper towels (Fort James Corp., Deerfield, IL). Termites were held for at least 2 wk at 25°C and 100% RH to ensure that the extraction process had not resulted in mortality. Undifferentiated larval termites, the average worker weighing 1.6 ± 0.44 mg ($n$ = 50), were used for the bioassays and radiolabel studies.

Termites were dyed for the repetitive stepwise transfer studies. Approximately 400 termites were placed in a petri dish (9 cm in diameter) provisioned with a piece of filter paper treated with 0.5 ml of an aqueous solution of 0.02% Nile blue (Sigma, St. Louis, MO). A small vial containing 0.02% Nile blue with a cotton plug served as a water source. After 7–10 d, most of the workers had turned a bright blue.

In the radiolabel transfer studies, termites were marked with a small dot of pink paint (Painty paint pens, EK Success Co., Clifton, NJ) diluted with acetone [1:1 (vol:vol)]. The mark was applied with a camel's-hair paint brush to dorsum of the abdomen. Termites were held for 7 d to ensure that the marking process did not kill the workers.

**Insecticides and Treated Sand.** Technical grade (99.3% [AI]) chlorfenapyr, [pyrrole-2-$^{14}$C]chlorfenapyr (132.17 $\mu$Ci mg$^{-1}$), formulated chlorfenapyr (Phantom 2 SC), and technical fipronil (BASF Corp., Research Triangle Park, NC) were tested. Topical applications of technical chlorpyrifos (Dow AgroSciences, Indianapolis, IN) and cypermethrin (91.5%, FMC Corp., Princeton, NJ) were tested in 1990 against western subterranean termites. Technical insecticides were dissolved in acetone, and suspensions with the chlorfenapyr 2 SC were mixed in water.

Sterilized play sand (Oglebay Norton Industrial Sands, San Juan Capistrano, CA) was selected because of its uniform particle size and light color. The light color aided in determining the distance that termites tunneled in the glass tubes.

**Topical Bioassay.** To determine the intrinsic insecticidal activity of insecticides, workers were treated with acetone solutions of serially diluted chlorfenapyr, chlorpyrifos, cypermethrin, and fipronil. The droplets were deposited with a 27-guage needle in a glass tuberculin syringe (BD Biosciences, Rutherford, NJ). Precise application was made with an Isco model M microapplicator (Instrumentation Specialties, Seward, NE).

Workers were carefully picked up with specimen forceps, and a 0.3-$\mu$l microdroplet of insecticide was placed on the dorsum of the abdomen. The droplet was allowed to dry for ≈30 s, and then 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, with a minimum of three replicates per concentration. The number of dead termites was counted daily for 7 d.

The data were analyzed by probit analysis (Robertson and Preisler 1992) by using the Polo program (LeOra Software, Menlo Park, CA).

**Brief Exposure Tests.** Worker termites were briefly exposed (1 and 4 h) to sand treated with chlorfenapyr to determine its contact activity. Aliquots of 100 g of sand were placed in petri dish bottoms (9 cm in diameter) and treated with serial dilutions of aqueous chlorfenapyr 2 SC to provide 1, 10, 50, 75, 100, and 200 ppm deposits (wt:wt). Excess water was applied to ensure the sand was uniformly treated and was frequently stirred with a glass stirring rod. The dishes with the treated sand were placed in a fume hood and allowed to dry for 24–48 h.

Approximately 1.2 ml of sand was placed in the bottom of a small plastic petri dish (3.5 cm in diameter, BD Biosciences, Franklin Lakes, NJ). The sand was lightly moistened with 1 ml of water. Ten termites were confined on the treated sand for 1 or 4 h. The termites were removed and placed in a plastic petri dish provisioned with a disk of moist paper towel. The petri dishes were held in a chamber maintained at 100% RH. The number of dead termites was counted daily for 7 d. Five replicates were tested for each concentration and exposure.

The data were used to determine the Kaplan–Meier survivorship percentiles (SPs) for each concentration with a Kaplan–Meier survival function test (Statistix 2005; Analytical Software, Tallahassee, FL). 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. The percentiles with confidence interval (95%) were compared with concentrations and exposure periods on survivorship.

**Repellency Tunneling Studies.** To determine the distances that termites tunneled into sand treated with chlorfenapyr, workers were placed 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 play sand. The top of the tube was plugged with a rubber stopper (#000). A line was marked on the glass tubes with 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 water added to the untreated sand in each tube was drawn through

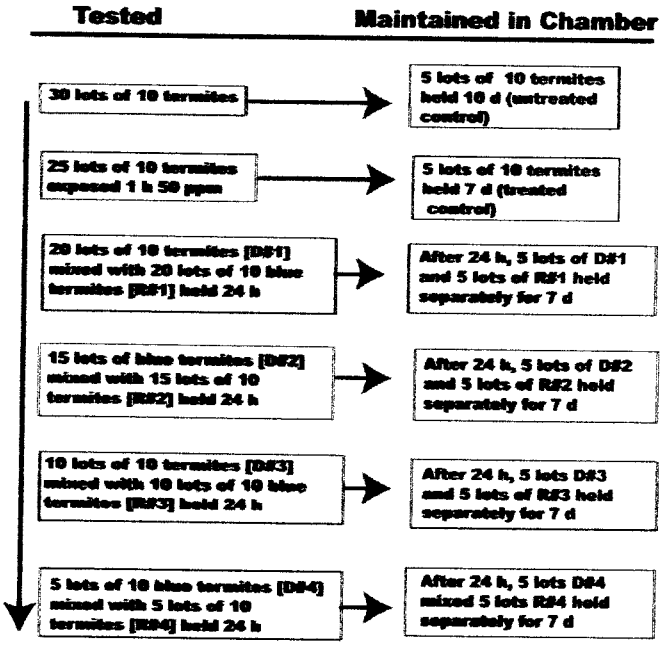


Fig. 1. Diagrammatic flowchart of the stepwise transfer studies with dyed termites. Initial donors exposed to 50-ppm chlorfenapyr deposits for 1 h.

the treated section with a 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 play sand) along with a piece of paper toweling to serve as food. The termites have an instinctive tendency to tunnel in the tubes. The distance tunneled was recorded after 2 d. The tubes were carefully broken, and the number of dead termites was 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. The number of dead termites was counted 1 and 7 d after breaking down the tubes. The distances tunneled by termites were analyzed with a Kruskal–Wallis test, and means were separated by an all-pairwise comparison (Statistix 2005).

**Movement Bioassays.** To determine the effects of chlorfenapyr on the movement of termites, workers were briefly exposed to treated deposits (50 and 100 ppm) for 1 h and then tested for their responses to the synthetic trial pheromone. The sand was moistened with deionized water. Two hundred termites were confined to the treated sands in petri dishes and placed in a plastic container (43 by 30 by 18 cm, Rubbermaid Inc., Wooster, OH) maintained at 100% RH. 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. After 1 h, the termites were gently transferred to a smaller petri dish (3.5 cm in diameter) provisioned with a moistened disk of brown paper towel. Termites were tested after 1, 4, and 8 h after being exposed.

A synthetic compound (3*Z*,6*Z*,8*E*-do-dectrien-1-ol) thought to serve as a trail pheromone was dissolved in acetone. Two 12-cm parallel lines exactly 2 cm apart were drawn with a no. 3 pencil on a piece of filter paper (12.5 cm in diameter, Whatman no. 1, Whatman International, Maidstone, England). One 10-cm line, exactly 1 cm from the end of the penciled line, was treated with the trail pheromone with a 10-μl syringe (Hamilton Co., Reno, NV) at a rate equivalent to 10 fg/cm. The other line was treated with acetone. A single termite was placed at the end of each line. The time required for the termite to transverse the 10-cm trail was determined. Each trail was only tested once. Thirty termites were tested per concentration and length of time after the exposure. The travel times were categorized as follows: 0–10 s, 11–15 s, 16–20 s, 21–25 s, 26–30 s, and >30 s.

The distribution of times required to transverse the 10-cm trace at each time period after exposure were analyzed with an r x c test of independence using the *G*-test (Sokal and Rohlf 1969).

**Insecticide Transfer Studies.** To determine whether lethal amounts of chlorfenapyr are transferred from one worker (donor) to another (recipient), donor termites were initially exposed to treated sands for 1 h. The sand was treated at 50, 100, 150, and 200 ppm. The 10 donor termites were removed from the treated sand and placed in a plastic petri dish (3.5 cm in diameter) provisioned with a piece of moist paper towel and 10 untreated termites dyed with Nile blue.

The donor and recipient termites were placed in a chamber at 100% RH, and the number of dead donor and recipients (blue) termites was counted at day 1,

2, and 7. Each exposure period and concentration was replicated three times.

**Repetitive Transfer Studies.** To determine whether chlorfenapyr could be stepwise transferred from donors to recipients several times after a single exposure (cascade event), a series of donor and recipient pairs of worker termites were tested (Fig. 1). Five lots of 10 termites that were selected and placed in a petri dish (3.5 cm in diameter) and maintained at 100% RH for 14 d served as untreated controls. Initially, 25 lots of 10 workers were exposed to 50 ppm chlorfenapyr on sand for 1 h. Five lots of exposed termites were randomly selected and placed in plastic petri dishes provisioned with a piece of moist paper towel and held at 100% RH chamber for 7 d to serve as a control for exposed donors. 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 and placed in a 100% RH chamber. 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 and were allowed in turn to mix with other "recipients" as described above. This was reiterated four times so that the last recipients would have received chlorfenapyr after three transfers from the original donor. This alternating of dyed and undyed termites continued until all the original 25 lots of termites were used (four transfers). The number of dead donor and recipient termites was counted daily. The data were analyzed by a two-way analysis of variance (ANOVA).

**Radiolabel Studies.** To study the uptake and horizontal transfer of chlorfenanpyr, serial dilutions of technical chlorfeanpyr mixed with [$^{14}$C]chlorfenapyr in acetone were prepared so that adding 10 μl of 0.171 mCi/ml [$^{14}$C]chlorfenapyr resulted in 0.02, 0.1, 0.15, 2.0, and 4.0 mg/ml. Exactly 10 ml of each solution was applied to 20 g of sand to produce 10, 50, 75, 100, and 200 ppm. Aliquots of 5 g of treated sand were placed into plastic petri dishes (5.0 cm in diameter). Approximately 1 ml of deionized water was added to each dish.

To study the uptake of radiolabeled chlorfenapyr, 75 termites were transferred to each of three petri dishes containing sand treated with 10 ppm chlorfenapyr. The petri dishes were covered and transferred to a plastic container maintained at 100% RH. Humidity indicator strips were taped to the sides of the chamber to ensure that the chamber remained at 100% RH. Five termites per replicate were removed from the petri dishes after 1, 2, 4, 8, 14, and 18 h. The termites were placed in 20-ml glass scintillation vials, and 100 μl of 15.8 N nitric acid was added to digest the termites. After 24 h, 10 ml of Cytoscint scintillation fluid (ICN Chemicals, Costa Mesa, CA) was added to each vial, and the vial was vigorously shaken. After 1 h, the samples were counted in a scintillation counter (Beckman LS 3801). Each sample was counted for 1 h or until the disintegrations per minute (dpm) had a $\sigma$ value = 2.

To determine the amount of chlorfenapyr transferred to the recipients, donor termites were exposed to sand treated with [$^{14}$C]chlorfenapyr for 1 and 4 h at five concentrations (10, 50, 75, 100, and 200 ppm). Donor termites ($n$ = 75) were placed in each petri dish (5.0 cm in diameter by 0.9 cm) containing treated sand, sufficient numbers for three replications for each concentration. Untreated termites (recipients) were marked by applying a small drop of pink paint (Painty paint pens, EK Success Co.) diluted with acetone [1:1 (vol:vol)] and applied with a camel's-hair brush to the dorsal side of termite abdomens. After 1 and 4 h, five exposed termites (donors) from each replication were transferred to a new plastic petri dish (3.5 cm in diameter by 1 cm) provisioned with a disk of moistened brown paper towel and five marked untreated termites (recipients). Petri dishes were transferred to a plastic container and maintained at 100% RH. To determine the amount of chlorfenapyr transferred to recipients after 7 d, five donor and recipient termites were placed in separate 20-ml glass scintillation vials and analyzed for [$^{14}$C]chlorfenapyr as described above. The amount of chlorfenapyr on donors and recipients was determined, and the percentage of chlorfenapyr transferred was determined.

The amounts of [$^{14}$C]chlorfenapyr picked up by the donors and recipients were analyzed with linear regression (Statistix 2005). The percentages of chlorfenapyr transferred from donors to recipients data were analyzed with a two-way ANOVA, and means were separated with a Tukey's honestly significant difference (HSD) test (Statistix 2005).

## Results

**Topical Bioassays.** Topical applications of 29.98 ng per termite provided 50% kill of termites at day 7 (Table 1). The fipronil was ≈187 times more active than the chlorfenapyr, with 0.16 ng producing 50% kill in 7 d. Chlorfenapyr was also less active than chlorpyrifos and cypermethrin, two termiticides that were widely used in the 1990s.

**Table 1. Intrinsic insecticidal activity of chlorfenapyr and several other insecticides (nanograms per termite) against *R. hesperus* workers**

| Insecticide[a] | $n$ | Slope ± SEM | $LD_{50}$ (95% CI) | $LD_{95}$ (95% CI) |
|---|---|---|---|---|
| Chlorfenapyr | 280 | 5.93 ± 1.313 | 29.98 (26.671–34.084) | 56.77 (45.234–101.595) |
| Chlorpyrifos | 900 | 6.70 ± 0.492 | 14.01 (11.491–15.776) | 24.67 (20.695–39.017) |
| Cypermethrin | 696 | 3.21 ± 0.354 | 2.81 (1.665–4.157) | 9.15 (5.416–139.397) |
| Fipronil | 190 | 2.42 ± 0.530 | 0.16 (0.090–0.221) | 0.75 (0.461–3.120) |

[a] Termites received applications of 0.3 μl of each dilution in acetone. The number of dead termites was counted at day 7.

**Table 2. Brief 1-h exposures of *R. hesperus* to sand treated with chlorfenapyr (2SC)**

| Concn (ppm) | % termites dead after exposure | | | Survivorship function $S(_t)^a$ (95% CI) at day 7 |
|---|---|---|---|---|
| | Day 1 | Day 5 | Day 7 | |
| 200 | 34 | 72 | 94 | 0.059 (0.0360–0.0948) |
| 100 | 54 | 90 | 88 | 0.120 (0.0665–0.2070) |
| 75 | 48 | 84 | 98 | 0.020 (0.0102–0.0390) |
| 50 | 6 | 42 | 68 | 0.308 (0.2248–0.4051) |
| 10 | 0 | 10 | 22 | 0.796 (0.6797–0.8781) |
| 1 | 4 | 8 | 18 | 0.824 (0.7052–0.9010) |
| | 0 | 0 | 0 | 0.961 (0.8704–0.9889) |

Five replicates of 10 termites exposed for 1 h to treated sand. Tests were conducted 5 January 2005. Kaplan–Meier survival function test determines the probability of survivors at day 7 (Statistix 2005).

**Table 4. Mortality and average distance tunneled by *R. hesperus* workers in sand treated with chlorfenapyr (2SC)**

| Concn (ppm)[a] | % mortality of termites | | | Avg distance tunneled (cm)[b] |
|---|---|---|---|---|
| | Day 0 | Day 1 | Day 3 | |
| 300 | 96 | 99 | 100 | 0.25ab |
| 250 | 84 | 88 | 91 | 0.2ab |
| 100 | 76 | 92 | 97 | 0.2b |
| 75 | 50 | 68 | 74 | 0.1b |
| 50 | 76 | 89 | 97 | 1.8ab |
| 10 | 50 | 56 | 70 | 0.3b |
| Untreated | 0 | 0 | 0 | 6.3a |

[a] Tests conducted 3 February 2005. Twnety termites in each of five replicates.

[b] Means followed by the same letter are not significantly different at $P < 0.05$ (Kruskal–Wallis all-pairwise comparison).

**Brief Exposure Tests.** One-hour exposures of termites on sand treated with 75–200 ppm chlorfenapyr provided 88–98% mortality at day 7 (Table 2). The survivorship function $SP_{(t)}$ was not significantly different for 75- and 200-ppm deposits. Exposure to 50 ppm chlorfenapyr produced ≈68% kill within 7 d and the $SP_{(t)} = 0.308$. Exposures to concentrations ≤10 ppm were not significantly different from the controls.

Four-hour exposures to deposits ≥50 ppm provided 100% kill of termites within 7 d (Table 3). Within 24 h, deposits as low as 10 ppm produced >50% kill. Even 4-h exposures to as little as 1 ppm chlorfenapyr provided 84% kill at day 7 and the $SP_{(t)} = 0.178$.

**Repellency Tunneling Studies.** Even sand treated with as little as 10 ppm chlorfenapyr provided 50% kill of workers on the day when the tubes were initially broken down. By day 3 after the tubes were broken down, significantly more termites were killed in all treated sands compared with the control tubes ($H = 20.48$, df = 6, $P < 0.0001$). Termites tunneled short distances (0.1–1.8 cm) into the treated sands (Table 4), whereas the termites tunneled significantly further in the untreated sand than they did in 10, 75, and 100 ppm chlorfenapyr ($H = 24.32$, df = 6, $P < 0.0001$). There was no consistent pattern of tunneling in any of the treated sands with most of the replicates not having any tunneling.

**Movement Bioassays.** Within 1 h after the brief exposure to the treated sand, there was no significant change in the termites locomotion on the pheromone trails (Fig. 2; $G = 5.808$, df = 5). After 4 h, there was a significant decrease in locomotion ($G = 57.806$, df = 5, $P < 0.05$), and none of the workers completed the trail in <10 s and 60% required >16 s. After 8 h, none of the termites completed the pheromone trail in <15 s ($G = 46.252$, df = 5, $P < 0.05$), and 80% of the termites required >30 s.

With the 1-h exposures on 100 ppm, none of the termites completed the trail within 10 s. Approximately 77% of the termites required 11–15 s to complete the trail, and 23% of the termites required 16–20 s. There was a similar distribution of responses between 50- and 100-ppm exposures at 4 and 8 h.

**Transfer Studies.** Sufficient amounts of chlorfenapyr were picked up by donors exposed for 1 h to 100-, 150-, and 200-ppm deposits to kill 100% of the workers by day 7 (Table 5). Sufficient amounts of chlorfenapyr transferred from donor termites exposed to 150- and 200-ppm deposits to produce 100% kill of recipients by day 7. Exposures to 50 ppm chlorfenapyr-treated sand provided ≈50% kill of both donors and recipients at day 7.

**Repetitive Transfer Studies.** The 1-h exposure of workers to 50 ppm chlorfenapyr-treated sand provided 78 and 96% kill at day 1 and 7, respectively (Table 6). After mixing with the dyed recipients for 24 h, only 4% of the recipients died. By day 7 after mixing, 68% of the recipients were killed. When the recipients of the first transfer were mixed with untreated termites, 22 and 70% of the donor termites were killed at day 1 and day 7, respectively. The recipients of the second and third transfer were not killed.

**Radiolabel Studies.** There was a direct linear relationship between concentration and the amount of chlorfenapyr picked up by termites exposed for 1 h ($y = 1.03 + 1.73x$; df = 1, 3; $P = 0.0023$; $R^2 = 0.96$) or 4 h ($y = 74.51 + 1.92x$; df = 1, 3; $P = 0.028$; $R^2 = 0.79$) on treated sand (Fig. 3).

When donors were exposed for 1 h, there was a linear relationship between the amount of chlorfenapyr picked and transferred and the concentration of the treated sand (Fig. 4). Donors picked up [$^{14}$C]chlorfenapyr ($y = -8.37 + 1.05x$; df = 1, 3;

**Table 3. Brief 4-h exposures of *R. hesperus* to sands treated with chlorfenapyr (2SC)**

| Concn (ppm) | % termites dead after exposure | | | Survivorship Function $S(_t)^a$ (95% CI) at day 7 |
|---|---|---|---|---|
| | Day 1 | Day 5 | Day 7 | |
| 200 | 98 | 100 | | |
| 100 | 60 | 100 | | |
| 75 | 84 | 100 | | |
| 50 | 98 | 98 | 100 | |
| 10 | 62 | 82 | 96 | 0.039 (0.0204–0.0719) |
| 1 | 4 | 80 | 84 | 0.178 (0.0995–0.2972) |
| | 8 | 10 | 12 | 0.887 (0.7757–0.9467) |

Five replicates of 10 termites exposed for 4 h to treated sand. Tests conducted 23 February 2005. Kaplan–Meier survival function test determines the probability that 10% survive at day 7 (Statistix 2005).

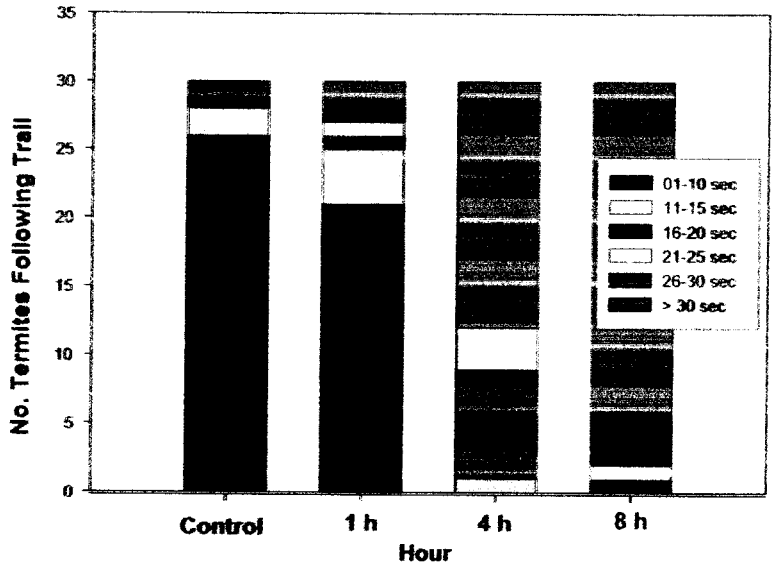


Fig. 2. Effect of a 1-h exposure to sand treated with 50 ppm chlorfenapyr on the responses of workers to trail pheromones.

$P = 0.0005$; $R^2 = 0.98$) from substrates about twice as fast as the recipients picked it up from the donors ($y = -4.08 + 0.49x$; df = 1, 3; $P = 0.0004$; $R^2 = 0.99$).

When donors were exposed for 4 h, the amount of chlorfenapyr picked up and transferred was directly related to the concentration of the treated sand (Fig. 5). Donors picked up and retained considerably more chlorfenapyr ($y = -42.98 + 12.04x$; df = 1, 3; $P = 0.0055$, $R^2 = 0.93$) than did the recipients ($y = -3.06 + 0.59x$; df = 1, 3; $P = 0.0002$; $R^2 = 0.99$).

The percentage of chlorfenapyr transferred from the donors to the recipients was significantly greater when the donors were exposed for 1 h ($F = 9.53$; df = 1, 18; $P = 0.0064$). There were significant differences between concentrations (Table 7, $F = 13.83$; df = 4, 18; $P < 0.0001$).

## Discussion

In our studies, we waited until 7 d to determine the topical activity of insecticides against termite workers. It is extremely important with nonrepellent and slow-acting insecticides to wait for latent effects to manifest themselves. Ibrahim et al. (2003) reported that the $LD_{50}$ values for fipronil against Formosan subterranean termite, *Coptotermes formosanus* Shiraki, were 2.93, 2.34, and 1.33 ng per termite at days 1, 2, and 3, respectively. It is likely that this would have been much lower at day 7. Considering that *C. formosanus* workers are ≈30% larger than *R. hesperus*, their calculations were very similar to ours if the amount of fipronil is divided by the average weight of the termite. Chlorpyrifos and cypermethrin were ≈2.1 and 10.7 times more active than chlorfenapyr (Table 1). The topical applications represent the minimal amount of insecticide that must be taken up by a termite or transferred to another termite to kill it. Consequently, ≈30 ng of chlorfenapyr must be taken up by a termite or transferred to provide 50% kill in 7 d.

Chlorfenapyr is a slow-acting insecticide. Su et al. (1987) defined slow-acting insecticides as those killing 90% of the treated individuals within 14 d, producing a broad effective lethal time 90%. Our brief exposures

Table 5. Percentage of mortality of donor and recipients termites after donors were exposed to sand treated with chlorfenapyr (2SC) for 1 h

| Concn (ppm) | % mortality of donors (D) and recipients (R) | | | | | |
|---|---|---|---|---|---|---|
| | Day 1 | | Day 2 | | Day 7 | |
| | D | R | D | R | D | R |
| 200 | 72 | 48 | 80 | 66 | 100 | 100 |
| 150 | 72 | 26 | 88 | 52 | 100 | 100 |
| 100 | 50 | 12 | 64 | 26 | 100 | 96 |
| 50 | 0 | 0 | 4 | 0 | 48 | 44 |
| Untreated | 0 | 4 | 0 | 8 | 8 | 16 |

The test was conducted 4 May 2005. Ten donor and 10 recipients were tested per each of five replicates per concentration.

Table 6. Repetitive transfer of chlorfenapyr between workers after donors were initially exposed to 50 ppm chlorfenapyr for 1 h

| Exposure | Transfer | % termites dead[a] | | | |
|---|---|---|---|---|---|
| | | Day 1 | | Day 7 | |
| | | D | R | D | R |
| 1 | 0 | 78 | 96 | | |
| | 1 | 74 | 4 | 92 | 68 |
| | 2 | 22 | 0 | 70 | 0 |
| | 3 | 0 | 0 | 2 | 8 |
| | 4 | 0 | 0 | 10 | 4 |
| Untreated | | 0 | | 2 | |

[a] D, donor; R, recipient. The recipients become the donors in the next transfer.

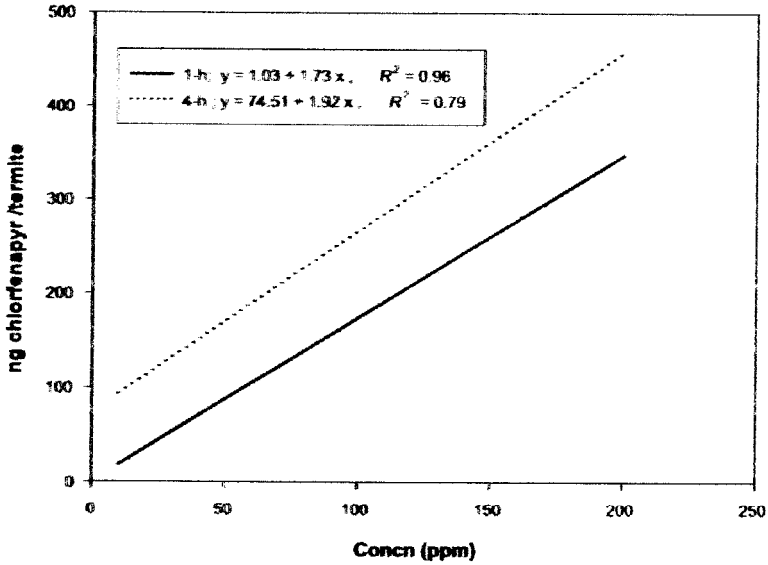


Fig. 3. Uptake of [$^{14}$C]chlorfenapyr by termites exposed to deposits for 1 and 4 h.

tests to chlorfenapyr resulted in dose-dependent mortality over a broad range of concentrations. Even with 4-h exposures, the mortality was dose dependent. The delay in toxicity permits movement of workers and social activities such as grooming and trophallaxis to occur. For example, exposure to fipronil barriers permitted *L. humile* to recruit nestmates for nearly 2 h, exposing greater numbers of ants (Soeprono and Rust 2004). In contrast, Su et al. (1982) reported that termites avoided dead and decaying termites that had been killed by fast-acting toxicants by sealing them off in tunnels. In addition, Smith and Rust (1990) reported that sublethal exposures of 5 min to pyrethroids such as bifenthrin and cypermethrin immobilized termites and inhibited tunneling behavior for 12 h.

Exposure studies were conducted with concentrations ranging from 1 to 200 ppm chlorfenapyr to determine the rates required to demonstrate horizontal transfer among termites. Brief exposures tests indicated that 1-h exposures to concentrations >50 ppm chlorfenapyr provided rapid kill of workers, limiting the possible transfer of toxicant. Consequently, 50 ppm was selected for the transfer studies. Exposures for 1 h provided ≈48–68% mortality at 7 d (Tables 2 and 5) when only 10 termites were exposed. When 250 termites were exposed to 50 ppm chlorfenapyr, 74–

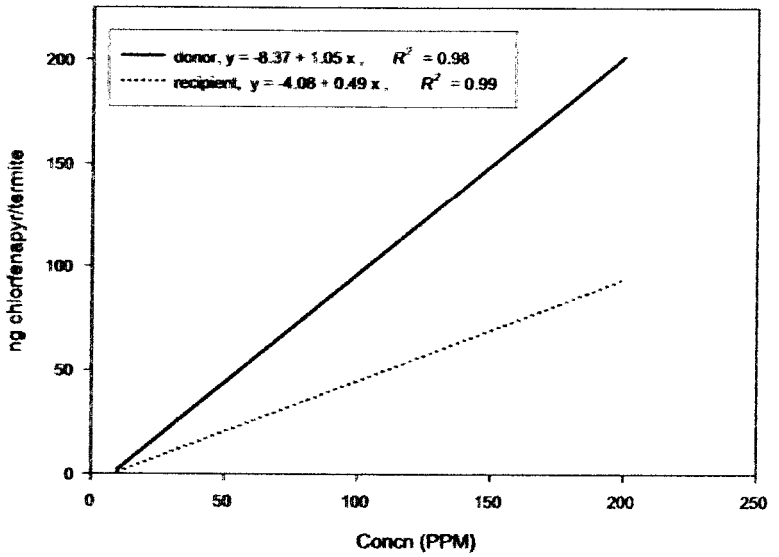


Fig. 4. Amount of [$^{14}$C]chlorfenapyr 7 d after donors and recipients were mixed together. Donors initially exposed for 1 h.

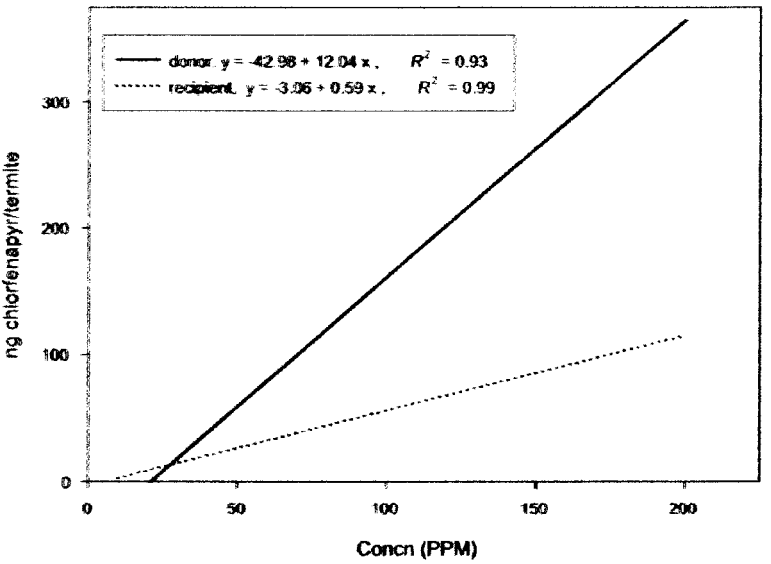


Fig. 5. Amount of [$^{14}$C]chlorfenapyr 7 d after donors and recipients were mixed together. Donors initially exposed for 4 h.

78% were killed at day 7 (Table 6). The higher mortality may be a result of increased contact and transfer between termites when 250 termites were exposed together.

The label-recommended rate of 15.1 liter of 0.25% chlorfenapyr applied to a trench (3.05 by 0.305 by 0.15 m) provides ≈197.4 ppm to the soil. Applications of 1.0 and 2.0% chlorfenapyr in both concrete slab and ground board studies have provided up to 7 yr of protection (Wagner et al. 2004). If the toxicants penetrated the soil only 5.1 cm, the deposits would be 2,826 and 5,652 ppm, respectively. Contact studies with sands treated with 50 ppm represent a biologically relevant exposure, especially aged treatments. This is important in determining whether horizontal transfer of insecticides might play a role in controlling termites under field conditions.

Table 7. Amount of chlorfenapyr (nanograms per termite) 7 d after donors and recipients mixed together and percentage transferred from donors to recipients

| Concn (ppm) | Donor Exp. (h) | Donor Avg (± SD) ng/termite | Recipient Avg (± SD) ng/termite | % transfer[a] |
|---|---|---|---|---|
| 200 | 1 | 208.2 ± 20.23 | 94.8 ± 5.48 | 31.3 |
| | 4 | 391.4 ± 30.88 | 115.5 ± 15.63 | 22.8bc |
| 100 | 1 | 91.8 ± 21.38 | 40.0 ± 9.12 | 30.3 |
| | 4 | 136.7 ± 32.3 | 55.5 ± 9.81 | 29.0a |
| 75 | 1 | 59.7 ± 8.14 | 34.8 ± 3.17 | 36.8 |
| | 4 | 73.8 ± 21.13 | 46.1 ± 9.34 | 35.4a |
| 50 | 1 | 45.5 ± 2.62 | 17.2 ± 2.32 | 27.4 |
| | 4 | 47.0 ± 10.73 | 22.5 ± 3.29 | 32.4ab |
| 10 | 1 | 11.1 ± 2.89 | 3.8 ± 0.90 | 25.5 |
| | 4 | 23.5 ± 5.73 | 3.6 ± 0.15 | 13.3c |

Donors were exposed either 1 or 4 h.

[a] Concentration followed by the same letter are not significantly different at $P < 0.05$ (Tukey's HSD).

Horizontal transfer of chlorfenapyr killed recipients when donors were exposed to concentrations ≥50 ppm. Only a single lethal transfer occurred, recipients not being able to transfer a lethal dose to another termite. As the concentration of chlorfenapyr increased, the amount picked up by donors increased in a linear manner. Approximately 30% of the chlorfenapyr was transferred to the recipients in 24 h.

Two factors that influence possibility of horizontal transfer are repellency and mobility of termites after exposure. *R. hesperus* workers did not avoid sands treated with chlorfenapyr and even tunneling in deposits as low as 10 ppm provided >70% kill of workers. Tunneling was limited, however, because extended exposures to chlorfenapyr killed termites in 2 to 3 d. In contrast *C. formosanus* avoided tunneling in soils treated with pyrethroids, resulting in reduced contact and kill (Su et al. 1993), whereas contact with soils treated with nonrepellent organophosphates such as chlorpyrifos and fenithrothin killed termites.

Within 4 h after exposure to 50 ppm chlorfenapyr, termite movement significantly decreased. The numbers of termites unable to follow the pheromone trail increased dramatically. Within 24 h, ≈50–75% of these termites were dead. The toxicity and its effects on locomotion reduced the likelihood that exposed termites might be able to return to nests, especially if they are more than a few meters from the treatment.

Potter and Hillary (2001) suggested that nonrepellent termiticides such as fipronil might kill termites away from the treated area. Kard (2001) also reported a decline in termites in untreated control plots near fipronil treatments. These two reports have been frequently cited as support for the concept of perimeter only treatments for termite control. Even though chlorfenapyr is transferred horizontal at biologically

relevant doses, our data would suggest that this is probably not the primary means of controlling subterranean termite infestations. Because chlorfenapyr is nonrepellent, lethal doses are readily obtained by workers. Locomotion is affected within 4 h, and it is unlikely that many workers successfully return to nests. The distance from the nest to the treatment becomes extremely important. For example, if a worker exposed to 50 ppm deposit for 1 h were to immediately return to the nest it might be able to move ≈15 m in the next hour before the toxic effects slow it down. However, this termite will only be carrying enough toxicant to kill one other termite. Of course, that is assuming that the termite can travel as fast in the tunnel as it can on a flat piece of paper treated with trail pheromone.

The success of the chlorfenapyr as a termite barrier is probably related to its nonrepellency and delayed toxicity. Foraging termites continue to contact these barriers and are killed. Horizontal transfer may play a role when the chlorfenapyr deposits have aged to around 50 ppm or less, allowing termites sufficient time to interact with nestmates before toxicity occurs. There is a dynamic relationship between the inherent toxicity of an insecticide, the exposure dose, the exposure time, and the onset of toxicity that dictates the likelihood of horizontal transfer. Other factors that might influence this relationship in natural conditions are the type of substrate treated and the species of termite. Additional research is warranted to determine how these factors might influence horizontal transfer and impart control under field conditions.

## Acknowledgments

We thank Jocelyn Millar (University of California, Riverside, CA) for synthesizing and providing the termite trail pheromone. We also thank BASF Corp. for providing the radiolabeled chlorfenapyr and partial support for the study.

## References Cited

**Ameen, A., W. Kaakeh, and G. Bennett. 2000.** Integration of chlorfenapyr into a management program for German cockroach (Dictyoptera: Blattellidae). J. Agric. Urban Entomol. 17: 135–142.

**Buczkowski, G., M. E. Scharf, C. R. Ratliff, and G. W. Bennett. 2005.** Efficacy of simulated barrier treatments against laboratory colonies of pharaoh ant. J. Econ. Entomol. 98: 485–492.

**Hollingworth, R. M. 2001.** Inhibitors and uncouplers of mitochondrial oxidative phosphorylation, pp. 1169–1261. *In* R. I. Krieger [ed.], Handbook of pesticide toxicology, 2nd ed. Academic Press, San Diego, CA.

**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.

**Potter, M. F., and A. E. Hillary. 2001.** Thinking "outside" the box. Pest Control Technol. 29: 68, 69, 72, 74, 76, 78, 80, 82, 84, 112.

**Robertson, J. L., and H. K. Preisler. 1992.** Pesticide bioassay with arthropods. CRC, Boca Raton, FL.

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

**Soeprono, A. M., and M. K. Rust. 2004.** Effect of delayed toxicity of chemical barriers to control Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97: 2021–2028.

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

**Statistix. 2005.** Statistix user manual, version 8. Analytical Software, Tallahassee, FL.

**Suoja, S., S. Garcia-Rubio, G. Chow, and V. Lewis. 2000.** Ant behavior impacts barrier efficacy. Pest Control 66: 65, 66, 68, 72.

**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.

**Su, N.-Y., R. H. Scheffrahn, and P. M. Ban. 1993.** Barrier efficacy of pyrethroid and organophosphate formulations against subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 86: 772–776.

**Su, N.-Y., M. Tamashiro, J. R. Yates, and M. I. Haverty. 1982.** Effect of behavior on the evaluation of insecticides for prevention of or remedial control of the Formosan subterranean termite. J. Econ. Entomol. 75: 188–193.

**Treacy, M., T. Miller, B. Black, I. Gard, D. Hunt, and R. M. Hollingworth. 1994.** Uncoupling activity and pesticidal properties of pyrroles. Biochem. Soc. Trans. 22: 244–247.

**Wagner, T. L. 2003.** U.S. Forest Service termiticide tests. Sociobiology 41: 131–141.

**Wagner, T., T. Shelton, C. Peterson, and J. Mulrooney. 2004.** Putting termiticides to the test. Pest Control 72: 23–29.

*Received 4 October 2005; accepted 1 December 2005.*