Chapter 5

Screening insecticides for use as soil termiticides requires a series of bioassays: lessons from trials using *Reticulitermes flavipes* (Isoptera: Rhinotermitidae).

Incorporating termite behavior into termiticide bioassay design.

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Tests of soil termiticide efficacy should consider termite behavioral reaction to the toxicant. This chapter outlines a series of four separate bioassays that account for factors such as bioavailability, dose acquisition, route of entry, movement of intoxicated termites, and collective decision making that are important in modeling termite population response to field application. Results with seven different formulated termiticides indicate that the moniker repellent or non-repellent are a function of concentration not the purview of any particular class of chemistries. Bioavailability was evident in that mortality decreased with all active ingredients tested in bioassay with sandy loam soil compared to play sand with one exception, imidacloprid. Data included evidence that acquisition of a dermal dose is slow, requiring over one hour continuous exposure to treated sandy loam soil to produce >50% mortality at 10 ppm with all the formulations tested. Dermal and oral toxicities varied within all termiticides but one – fipronil - and all but one, the chlorfenapyr formulation, affected worker termite performance on an experimental trail compared to controls. Three behavioral conditions that must be considered when interpreting termiticide efficacy bioassay data are also discussed. First, the main route of entry for

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subterranean termites confronted with a soil-based insecticide is oral. Second, assignment of the task of gallery construction to specific workers and third, a collective decision making system of communication used by worker termites traveling through a network of galleries. It is therefore unlikely that consistent transfer of soil-borne toxicants will follow field application of any termiticide. A choice bioassay design is suggested as a more realistic approximation of termiticide field efficacy.

The process of protecting structures from subterranean termite infestation essentially involves breaking the chain of events and lines of communication that termites use to locate and establish feeding sites (*1*). Termite control tactics can be soil- or structure-based using methods such as termite resistant building materials, physical barriers, chemical barriers, or population management practices (*1, 2*). Application of a chemical barrier, however, has been the most common practice over the last 60 years (*2*). This is accomplished by placing an insecticide (termiticide) into the soil surrounding a structure for the purpose of excluding termites.

The screening of candidate insecticides for use with the exclusionary hypothesis employed in the last half of the 20th century was characterized by Ebeling and Pence (*3*) who determined efficacy in a constant exposure bioassay with the acceptable candidate producing rapid mortality. Twenty years later, Su et al. (*4*) demonstrated the need to incorporate termite behavioral reaction to the chemistry being tested in bioassay. This seminal work moved termiticide efficacy away from constant exposure mortality assays to experiments that allowed termites to construct galleries through treated soil in a "qualitative" tube bioassay system, which has become a commonly used evaluation tool (*5, 6, 7*). Su et al. (*4*) also defined the behavioral reaction of termites, using a petri dish (qualitative) assay, along a gradient as either 1 (I) – repellent, 2 (II) not repellent, or 3 (III) slow-acting stomach poison.

The termiticide active ingredients used between 1989-96 (several synthethic pyrethroids – cypermethrin, permethrin, bifenthrin, and two organo-phosphate isophenphos and chlorpyrifos) conformed to the exclusionary hypothesis. As defined by the tube bioassay, these termiticides, through rapid mortality or 'repellence', eliminated termite traffic into treated soil (*8, 9, 10, 11*). The so-called 'repellent termiticides' were envisioned to alter foraging, leaving the resident termite population unaffected and free to search for food resources. The lack of termite population impact implies that in the field, continued foraging will eventually, through application error, construction fault, or degradation of the active ingredient, allow termites to locate and exploit an untreated area around the structure/soil interface returning that structure to the list of active feeding sites – i.e. infestation.

In 1996, Bayer Environmental Science introduced a new class of chemistry, the neonicatinamides, that provoked a paradigm shift from the exclusion hypothesis to that of the 'treated zone'. That product, Premise®, with the active

ingredient imidacloprid, was described as neither repellent nor fast acting at label application rates and allowed subterranean termites to excavate galleries into the treated soil, bringing the term 'non-repellent termiticide' into the lexicon. Since 1996, two additional 'non-repellent' products have gained Environmental Protection Agency (EPA) registration, Termidor® (fipronil) and Phantom® (chlorfenapyr). Theoretically, use of a non-repellent termiticide allows termites to construct galleries through the treated soil and provides structural protection through attrition within the offending termite population. The attrition hypothesis has gained acceptance in the termite control industry due to successful field trials reporting termite population impacts following treatment (*12, 7*).

In order to fulfill the promise of the attrition hypothesis, a non-repellent termiticide should display delayed mortality and horizontal transfer between nest mates following contact with a lethal dose (*13*). The hypothesis of non-repellent termiticide efficacy, under the construct of attrition, predicts that structural protection is accomplished through population reduction achieved by transfer of a lethal dose through direct contact with treated soil or behavioral interactions (contact, grooming, or food exchange). Attaining structural protection with 'non-repellent' chemistries is predicated on termites tunneling through the treated soil and acquiring an effective dose of the active ingredient. Therefore, the question of termiticide efficacy under the attrition hypothesis can be tested in bioassay by examining five critical elements: concentration of active ingredient, time of exposure, lethal dose, route of entry, and behavior of an affected termite.

Field efficacy of termiticides, under the attrition scenario, could be predicted in bioassay with the most efficacious active ingredient being one which kills termites at the lowest concentration following the shortest exposure while not affecting the behavior of the intoxicated subject. The behavioral component is required to facilitate transfer and/or maintain communication, and therefore continued movement, to the 'lethal zone'.

I decided to examine the questions of concentration, time of exposure, lethal dose, route of entry, and termite mobility to test the hypothesis that termiticide efficacy could be predicted from bioassay under the attrition hypothesis. This manuscript reports data from five separate bioassays. Six non-repellent termiticide formulations were tested at four concentrations and four exposure time periods in two soil types to obtain Lethal Concentration values by time of exposure, termite excavation through treated soils were tested in a choice test bioassay system. Lethal Dose values were generated examining oral and dermal routes of entry, and movement of dermal-dosed termites was timed. Results are discussed in regard to predicting field efficacy assuming structural protection through termite population reduction**.**

Materials and Methods

Termites

Eastern subterranean termites, *Reticulitermes flavipes* (Kollar), were collected from infested logs at the University of Georgia Whitehall Forest in Athens, GA using extraction methods as described by La Fage et al. (*14*) and modified by Forschler and Townsend (*15*). Termite colonies were identified to species using soldier characteristics (*16*). Termites collected from logs were placed in clear plastic boxes (26 × 19 × 9 cm) containing moistened 9-cm No. 1 Whatman filter papers and several thin pieces of pine (11.25 × 3.75 cm and 1 mm thick). Plastic boxes with termites were maintained in an environmental chamber at 24 °C for no more than one month prior to beginning a bioassay. Only undifferentiated *R. flavipes* workers, fourth instar and older, were used in bioassay.

Termiticides

The five termiticide formulations tested in these trials were Chlorfenapyr (Phantom TM, BASF, Parsippany, NJ), Thiamethoxam (CGA, 25 WG, Syngenta, Greensboro, NC), Imidacloprid (Premise 75, Bayer, Kansas City, MO), Fipronil (Termidor, 80WG, BASF, Research Triangle, NC) and indoxacarb (Steward, 15 SC, DuPont, Wilmington, DE).

Soil Exposure-Time Bioassay

Treatment of Soil/Sand

Termiticide concentrations were determined by calculating the amount of active ingredient needed to reach a concentration of 10,000 parts per million (ppm, w of AI/w of soil) when 20 ml of solution were added to 100g of soil. Subsequent concentrations were reached by serial dilution of the aforementioned 10,000 ppm solution. Termiticides were tested in one of two substrates; Cecil series sandy loam soil (71% sand, 21% silt, 8% clay) or play sand purchased commercially (100% sand).

Termiticide solutions, prepared as previously described to obtain the desired concentration, were added to 100g of substrate to reach 20% soil moisture and the appropriate solution slowly added to the substrate in a plastic bag (16.5 × 14-cm). The solution/substrate was thoroughly mixed by hand, through the bag, until all of the substrate was evenly moistened. Untreated control substrates were brought to 20% moisture using distilled water only. The moistened substrate, for each solution/time/concentration combination tested, was then evenly divided (≈ 33 g) among three 9-cm petri dishes and spread to form a

continuous layer on the bottom using stainless steel spoons. Each petri dish was labeled with the termiticide solution used and concentration. Five concentrations (0.1, 1, 10, 100, and 1000 ppm) of each termiticide were tested and replicated at least 10 times per chemistry and substrate.

Exposure Time Period

Each termiticide and concentration was tested at four exposure time periods, 1, 10, 100, and 720 minutes. Petri dishes for the overnight exposure (720 minutes) treatments were placed in a plastic box (25 × 32.5 × 9-cm) and kept in an environmental chamber at 27 °C until time to remove the termites. Paper towels saturated with distilled water were placed in the bottom of the plastic containers to maintain high humidity conditions inside the overnight exposure arena. All other exposure times were maintained at room temperature.

One petri dish was used for all exposure times with a particular chemical and concentration combination for a single replicate. Ten termites were placed in the treatment petri dish at a particular termiticide concentration for the designated amount of time. There were 15 replicates performed for each solution/time/concentration combination. At the end of the time period termites were removed using featherweight forceps and placed into an observation petri dish (6 × 1.5-cm) that contained a 5.5-cm piece of No.1 Whatman filter paper moistened with 0.25-ml of distilled water. All observation petri dishes for each termiticide/concentration/time exposure combination were placed in separate plastic boxes (26 × 19 × 9-cm) in an environmental chamber at 27 °C. Paper towels saturated with distilled water were placed in the bottom of the plastic boxes to maintain high humidity. The number of living termites in each observation petri dish was counted every day for ten days to determine survivorship. All dead termites were removed to prevent transfer of toxicant due to cannibalism. Death was defined as lack of movement when touched by a probe.

Excavation Choice Bioassay

Nine round plastic containers (5-cm ID, 3.5-cm H) were connected using a 7-cm length of tygon tubing (2-mm ID). The central container was filled to a depth of 2.5-cm with a sand and vermiculite mixture (14:12 ratio) to provide a moisture-filled tunneling substrate and serve as the introduction chamber with the tubing entering the chamber at a height equivalent to the top of the sand/vermiculite mixture. The introduction chamber was connected to the base of four chambers, termed substrate chambers, containing play sand that was treated as described in the Time-Exposure section. Only one of the four substrate chambers, within any replicate, contained a treatment such that the termites introduced into each arena had a choice of three untreated and one treated substrate chamber. The four substrate chambers were connected by a 7-cm length of tygon tubing (placed on the opposite side from the tube leading

into that chamber) to the base of another chamber, termed the food chamber, containing a single block (2-cm^3) of pine wood.

The arrangement of chambers resembled a wheel with the introduction chamber at the center with four spokes (tube-defined paths) each leading to a separate substrate chamber with access to a final food chamber. Each bioassay arrangement of nine chambers was considered one replicate. Five hundred termites were placed into the introduction chamber at the start of the bioassay and confined in that chamber for 24-h using small (1.9-cm width) binder clips (Charles Leonard Inc., Glendale, NY). The binder clips were positioned on the tubes leading from the introduction chamber near the point of attachment to the introduction chamber to provide a period of acclimatization prior to release into the choice arena. Termites from a single laboratory culture were used for each replicate. At least three different termite colonies (laboratory cultures) were used in the 6-16 replicates that composed this series of tests. Termiticides were tested at 50-60 ppm's to simulate approximate labeled application concentrations.

Route of Exposure Bioassay

Individual termites were held under a binocular dissecting microscope using a vacuum venturi system. The system consisted of a Pasteur pipette attached to the end of a 3-mm ID piece of tygon tubing which was attached using a plastic t-connection to an open length of tubing at one end and a vacuum source at the other. Termites were picked up by placing the Pasteur pipette tip on the abdomen while placing a finger over the open tube to create a vacuum at the pipette tip. Termites were then treated using one of several concentrations of the appropriate termiticide solution using a micro-applicator. Termites were released following treatment by removing the finger from the open tube to break the vacuum suction at the pipette tip.

Termites were treated and maintained following treatment using one of four scenarios. Termites were treated by placing 0.15 microliters on the pronotum, which was allowed to dry before they were placed in either a Petri dish containing 9 other similarly treated termites or isolated in an individual tissue culture well in a standard 96-well plate. Both the Petri dish and tissue culture wells were lined with an appropriate disk of untreated #1 Whatman filter paper moistened with de-ionized water. These treatment regimes simulated a dermal exposure while allowing for grooming by nest mates (the 10 termites in a Petri dish) and no grooming (isolated tissue culture well termites). The oral route of exposure was simulated by using the vacuum venturi system and microapplicator to place 0.125 microliters of the appropriate concentration on the mouthparts of each termite and only including those where the droplet disappeared into the bucal cavity (not splayed across the head capsule or mouthparts) and assumed to have been consumed. Termites treated in the oral route assays were held in Petri dishes with 9 other likewise treated individuals or as isolated individuals as described for the topical assays. Mortality was recorded daily for 10-17 days all dead termites were removed daily from Petri dishes or tissue culture wells.

Termite Running Assays

Termites follow ink lines drawn by pens containing 2-phenoxyethanol, such as Papermate® pens (*17*). Photocopies were made of a sheet of paper that had four straight, 6 cm-long lines with a 1 cm diameter circle drawn at one end of each line. Seconds prior to performing the assay one line was traced with a disposable Papermate® pen. One termite from a group that had been treated with a topical dose close to the LD_{50} was gently placed inside the 1-m circle using the vacuum venture system previously described. As soon as the termite started running along the straight ink line it was timed over the distance of 6-cm with a hand-held stopwatch. Speed was recorded only when termites ran 6 cm without stopping or straying from the line. After running, the termite was placed in one well of a standard 90-well tissue culture plate which contained a piece of #1 Watman filter paper and labeled as to the chemistry, dose, and day when it was treated. Termites were timed one hour after treatment and for four consecutive days thereafter. The tissue culture plate was placed inside a plastic box containing wet paper towels and maintained in an environmental chamber at 24 °C. A new pen line was drawn for each termite tested.

Data Analysis

No replicate in any bioassay was included in analysis if the control survivorship was equal or less than 90%. Probit analysis was used to calculate LD_{50} values from topical and force-feeding assays for examination of route of entry. Probit analysis also was used to calculate LC_{50} values for each combination of termiticide concentration and exposure period (*18*). Mean corrected percent mortality was compared by time and concentration within substrate type by termiticide using Log10 transformed data with the General Linear Models Procedure (*18*). Mean separation was accomplished using Protected Least Significant Difference (*18*).

No statistical comparisons were made between termiticide formulations because the active ingredients represented different modes of action and various concentrations were often tested to obtain the appropriate approximation of a lethal dose. Chlorfenapyr is a metabolic inhibitor that affects electron transport in the mitochondria. The remaining three insecticides are nerve toxins. Acetamiprid, Thiamethoxam and Imidacloprid affect acetyl choline receptors, Fipronil impacts GABA-gated chloride channels, Bifenthrin is a sodium channel modulator, and Indoxacarb blocks voltage dependent sodium channels. Therefore, analysis was confined to comparisons within termiticide formulations.

Results

Timed exposure bioassay

The only treatment that provided a consistent statistically significant response within soil type was thiamethoxam with decreased LC_{50} values as exposure time increased in both sand and sandy loam soil (Table I).

Chlorfenapyr provided LC_{50} values that were not significantly different for the 1 and 10 minute exposures in either sand or sandy loam soil but the 100 minute and overnight exposures provided significantly lower LC_{50} values (Table I). The CI for the Chlorfenapyr overnight exposure treatment in sand was not calculated because more than 90% mortality was recorded at all concentrations, as indicated by the slope (22.1) (Table I). None of the Fipronil treatments in sand were significantly different (Table I). In sandy loam soil LC_{50} values for Fipronil at 1 and 10 minute exposure times were not significantly different but these were significantly lower than the longer exposure times (100 minutes and overnight) (Table I). A CI for the Fipronil overnight exposure treatment in sand and sandy loam soil was not calculated because 100% mortality was recorded at two of the four concentrations tested. imidacloprid treatments in either sand or soil were not significantly different with less than 36% mortality regardless of concentration or exposure time (Tables I & II). At the time termites were removed from exposure to Imadichloprid, they appeared intoxicated (sluggish and unresponsive to stimuli such as opening the petri dish lid and prodding with forceps), yet most recovered and appeared normal (compared to the control group) after 24 h in the pesticide-free observation petri dish. No LC values were calculated for Indoxacarb because the range of concentrations tested in these bioassays provided an all or nothing response (Table II).

Probit analysis also indicated that there was a significant increase in the LC_{50} values comparing time of exposure between substrates—sand and sandy loam soil—for all of the termiticides tested (Table I). In every case, where slope values allow a statistically valid comparison, the LC_{50} values were significantly higher in sandy loam soil compared to the same exposure time on sand.

The corrected percent mean mortality data are provided by concentration and time of exposure for each termiticide in Table II. The 10 and 100 ppm concentrations are highlighted because labeled application rates for these chemistries are $\approx$ 50 ppm and field application would likely provide concentrations within this range. Fipronil in sandy loam soil was the only termiticide, regardless of substrate, to provide statistically significantly higher (ANOVA, LSD) mortality when the 10-minute exposure is compared to the 1-minute exposure (Table II). Within each termiticide the two shortest exposure times provided significantly lower mortality in sandy loam soil compared to sand except for the Imadichloprid at 10 ppm/10 minute exposure combination (4.0 ± 1.63 sand and 2.67 ± 1.18 sandy loam) (Table II).

Trends within each termiticide were not evident. Thiamethoxam on sand provided 100% mortality only at the 100 ppm concentration for the overnight exposure, although at 10 ppm in sand that percentage was 95% (Table II). The mean corrected percent mortality data with Thiamethoxam did not provide 100% mortality at either 10 or 100 ppm at any exposure time in sandy loam soil (Table II). Chlorfenapyr provided 100% mortality after overnight exposure in sand at 10 and 100 ppm and in sandy loam soil at 100 ppm (Table II). The sandy loam/100 ppm treatment provided 100% mortality although it was not

Table I. Comparison of LC_{50} values, confidence intervals (CI) and slopes from the timed exposure bioassay by termiticide formulation, by exposure time and soil type

Time[1]	*LC_{50}*[2]	*CI*	*Slope ± SE*
IMIDACLOPRID			
		SAND	
1	11,290	2,844 to 119,867	0.45 ± 0.06
10	9,594	2,405 to 104,087	0.43 ± 0.06
100	10,950	2,032 to 278,547	0.33 ± 0.06
720	1,522	399 to 21,340	0.39 ± 0.08
		SANDY LOAM	
1	3×10^{12}	NA	0.11 ± 0.08
10	53×10^{6}	NA	0.25 ± 0.08
100	149×10^{12}	NA	0.03 ± 0.08
720	51,475	8,548 to 1,772,148	0.38 ± 0.07
FIPRONIL			
		SAND	
1	0.9	0.62 to 1.16	1.56 ± 0.20
10	0.9	0.71 to 1.13	2.35 ± 0.33
100	0.7	0.52 to 0.95	3.01 ± 0.57
720	0.9	NA	$20.84 \pm 1.46 \times 10^{5}$
		SANDY LOAM	
1	22	13 to 36	0.49 ± 0.06
10	17	11 to 24	0.69 ± 0.06
100	4	3 to 5	1.31 ± 0.12
720	2	NA	$9.07 \pm 1.75 \times 10^{4}$
THIAMETHOXAM			
		SAND	
1	35	25 to 49	0.84 ± 0.06
10	17	13 to 21	1.11 ± 0.07
100	5	4 to 7	1.35 ± 0.10
720	3	2 to 3	2.84 ± 0.36
		SANDY LOAM	
1	34×10^{6}	172,000 to 42×10^{6}	0.21 ± 0.07
10	1,870	990 to 4,516	0.67 ± 0.07
100	45	35 to 59	1.07 ± 0.07
720	12	9 to 15	1.19 ± 0.09

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CHLORFENAPYR			
		SAND	
1	31	25 to 41	1.14 ± 0.07
10	23	19 to 30	1.23 ± 0.08
100	4	3 to 5	1.49 ± 0.11
720	0.9	NA	$22.12 \pm 1.45 \times 10^5$
		SANDY LOAM	
1	32,801	6,966 to 566,109	0.43 ± 0.07
10	2,996	1,269 to 11,076	0.51 ± 0.06
100	94	70 to 128	0.96 ± 0.07
720	10	9 to 13	1.87 ± 0.15

[1] Numbers represent minutes of exposure time.

[2] Value in ppm.

statistically different than the 100-minute exposure/100 ppm treatment (at 92% mortality) (Table II). Chlorfenapyr provided, in sandy loam soil, more than 50% mortality at 10 ppm for the overnight exposure but 100% for the same exposure time at 100 ppm (Table II). Fipronil provided 100% mortality at the overnight exposure at 10 and 100 ppm in either soil type (Table II). Fipronil was also the only termiticide to provide no statistical difference between any of the exposure times for both concentrations in sand. Yet, as with the other chemistries, in the sandy loam soil Fipronil showed significantly less mortality at the shorter exposure times, 1 and 10-minutes respectively (Table II). Imidacloprid regardless of concentration and exposure time provided less than 36% mean mortality in sand and < 11% mean mortality in sandy loam soil (Table II).

Table II. Comparison of mean corrected percent mortality from timed exposure bioassay by termiticide formulation, soil type and time of exposure at two concentrations

	SAND		*SANDY LOAM*	
Time[1]	*Mean*[2] ± *S.D.*		*Mean*[2] ± *S.D.*	
Imidacloprid	*10 ppm*			
1	10.0 ± 6.55	B[a]	2.67 ± 1.18	A
10	4.0 ± 1.63	B	2.67 ± 1.18	A
100	16.67 ± 7.28	BA	10.67 ± 6.72	A
720	36.25 ±18.5	A	7.7 ± 3.78	A
Imidacloprid	*100 ppm*			
1	14.67 ± 6.46	A	5.41 ± 1.68	A
10	25.33 ± 8.39	A	5.41 ± 2.38	A
100	22.91 ± 5.78	A	5.33 ± 2.74	A
720	35.97 ±15.45	A	2.0 ± 1.07	A

Thiamethoxam	*10 ppm*			
1	29.31 ± 9.25	C	9.33 ± 3	C
10	43.24 ± 8.7	BC	2.74 ± 1.22	C
100	68.3 ± 8.57	BA	22.89 ± 5.05	B
720	94.81 ± 2.32	A	36.39 ± 6.22	A
Thiamethoxam	*100 ppm*			
1	54.67 ±10.95	B	9.33 ± 3.3	C
10	75.22 ± 8.28	BA	14.81 ± 6.1	C
100	94.04 ± 3.29	A	62.59 ± 8.68	B
720	100 ± 0	A	86.15 ± 3.87	A
Chlorfenapyr	*10 ppm*			
1	22.31 ± 8.28	B	5.4 ± 2.38	B
10	32.67 ± 8.97	B	10.22 ± 3.74	B
100	86.67 ± 7.22	A	11.33 ± 4.67	B
720	100 ± 0	A	37.22 ± 8.27	A
Chlorfenapyr	*100 ppm*			
1	68.8 ± 9.82	B	4.0 ± 1.63	C
10	75.33 ±10.55	BA	12.0 ± 4.28	C
100	92 ± 5.54	BA	50.59 ±10.0	B
720	100 ± 0	A	100 ± 0	A
Fipronil	*10 ppm*			
1	95.04 ± 3.45	A	40.67 ± 9.43	C
10	98 ± 1.45	A	27.78 ± 7.26	C
100	99.3 ± 0.67	A	71.33 ± 9.65	B
720	100 ± 0	A	100 ± 0	A
Fipronil	*100 ppm*			
1	100 ± 0	A	46.67 ± 9.5	C
10	100 ± 0	A	71.15 ± 8.08	B
100	100 ± 0	A	96.52 ± 1.71	A
720	100 ± 0	A	100 ± 0	A
Indoxacarb	*10 ppm*			
1	100 ± 0	A	0	C
10	100 ± 0	A	0	C
100	100 ± 0	A	74 ± 5.3	B
720	100 ± 0	A	98 ± 1	A

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Indoxacarb	*100 ppm*				
1	100 ± 0	A	100 ± 0	A
10	100 ± 0	A	98 ± 1.7	A
100	100 ± 0	A	100 ± 0	A
720	100 ± 0	A	100 ± 0	A

[1] Time of exposure in minutes

[2] Mean corrected percent mortality followed by the standard error for that mean.

[a] Means followed by the same letter within the same column for each concentration indicate no significant difference (P=0.05).

Excavation Choice Bioassay

None of the termiticides tested provided 100% mortality after 21 days in bioassay at approximate labeled application concentrations (50-60 ppm) (Figure 1). Statistical separation of the various formulations was not performed because the purpose of these data was simply to illustrate that no termiticide eliminated all termites in a small bioassay arena arrangement over a 21 day period. All termiticide treatments, with the exception of Acetamiprid (n = 6), provided evidence that termites tunneled into the treated sand – chamber B (Figure 2). The controls, over the 21 days in bioassay, provided evidence of tunnels in all four soil arenas while none of the treatments provided similar data, indicating that even 'non-repellent' concentrations can, in a choice test design, have replicates that indicate 'repellence'. All termiticides were successful at 'protecting' the wood opposite the treated sand at the concentrations tested (Figure 3). These data indicate that all of the formulations tested would be effective at providing a barrier to termite infestation if applied to play sand at concentrations equivalent to 50-60 ppm.

Route of entry biosassay

The oral/nestmate treatment data did not differ from the oral/isolated treatment, regardless of termiticide, therefore those data are not provided in Table III. Fipronil provided the lowest values for either route of entry and was equally toxic by either route (Table III). Imidacloprid and Thiamethoxam were less toxic by the dermal/isolated compared to the oral route while Acetamiprid, Indoxacarb, and Chlorfenapyr were the opposite (Table III).

Fipronil displayed no difference in toxicity between the oral or dermal/isolated treatments and provided an additive affect when the dermal/nestmate or isolated regimes are compared, indicating that grooming activity provided an additional dose (Table III). The remaining termiticides provided data indicating that the most toxic route of entry (determined by the isolation treatment regime) for each formulation dominates toxicity if the behavior of the termite allows for the ability to groom similarly treated dermal-dosed nestmates. Chlorfenapyr, acetamiprid, and indoxacarb were less toxic by the oral route and the value for the dermal/nestmate was more than the dermal/isolated treatments, indicating that grooming activity probably increased

the LD values in the dermal/nestmate treatment regime (Table III). Thiamethoxam and imidacloprid, despite a lower LD value for the oral route, provided no difference between the two dermal treatment regimes indicating that the intoxicated termites did not engage in grooming activity following application of the termiticide formulation (Table III).

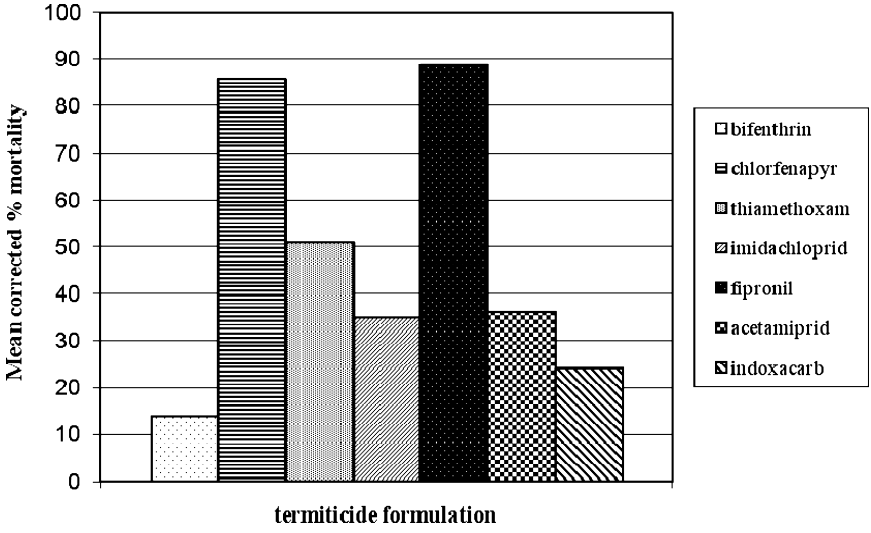

Figure 1. Mean corrected percent mortality from sand excavation choice bioassay at Day 21 by termiticide formulation.

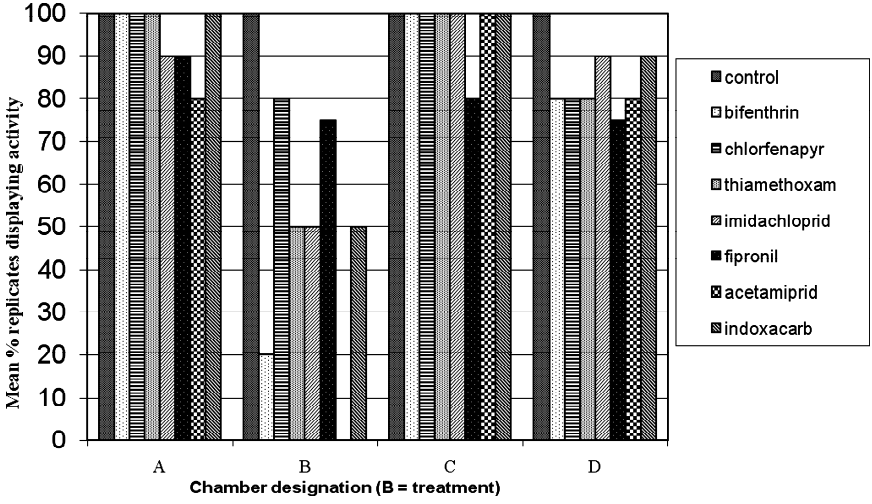

Figure 2. Mean percent of replicates from sand excavation bioassay that provided evidence of termite excavation by chamber and termiticide formulation at Day 21.

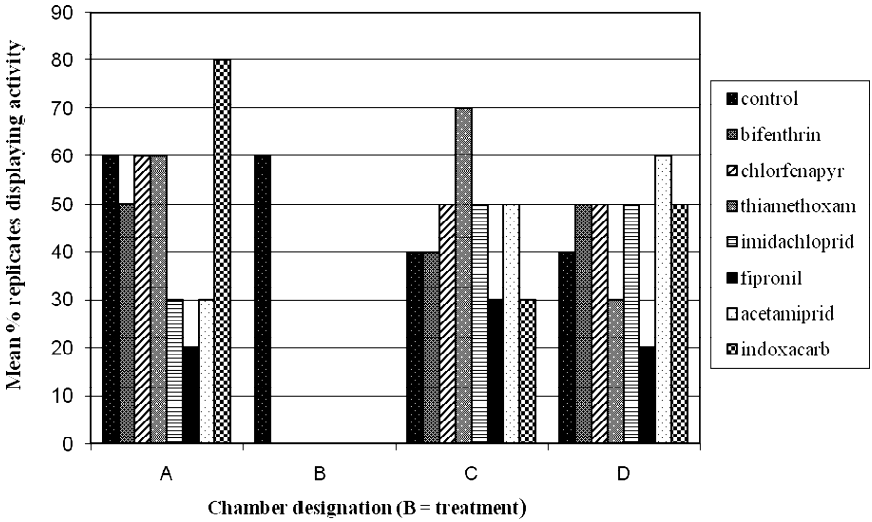

Figure 3. Mean percent of replicates from sand excavation bioassay that provided evidence of termite feeding on the wood by chamber and termiticide on Day 21.

Table III. Lethal Dose values at which 50% of the test animals died (LD50) for six selected termiticide formulations listed by active ingredient, route of entry (oral or dermal) treatment regime(dermal dose in isolation or with 9 similarly treated nestmates) in nanograms (ng) of active ingredient per gram of termite

Type of treatment regime	*LD50 VALUES (in ng AI per g of termite) Letters indicate significant difference based on CI overlap.*					
	Thiomethoxam	*imidacloprid*	*Acetamiprid*	*Fipronil*	*Indoxacarb*	*Chlorfenapyr*
Oral	238.8 A	931.7 A	277.34 A	22.57 A	303 A	11,152.9 A
Dermal nestmate	597 B	5,590 B	214.68 A	7.59 B	606 A	9,065.2 A
Dermal isolated	895.5 B	4,968.9 B	102.27 B	41.0 A	151.5 B	5,099.2 B

Timed running tests

All termiticides, within a respective chemistry, provided similar data trends comparing 1, 24 and 36-h after treatment and by 72-h a clear separation occurred. Therefore the 1- and 72-h data are provided in Figures 4 & 5. Termites dermal-dosed with imidacloprid were affected one hour after treatment as indicated by the high proportion of termites that did not run the full 6-cm 'test trail' and the longer time taken by those that did follow the trail (Figures 4 & 5).

The fipronil, indoxacarb, and chlorfenapyr treated termites were not affected one hour after treatment, as indicated by the high proportion of runners and fast running times (Figures 4 & 5). The Chlorfenapyr-treated termites were the exception in that they continued even up to 240-h (10 days after treatment) to provide results similar to the controls. Fipronil-treated termites, by 72-h, displayed 83% survivorship but the survivors were no longer responding to the ink; the few (17%) that did were sluggish with an average time of 22.3-s for the 6-cm. The survivorship of imidacloprid-treated termites was high (94%) at 72-h and a higher proportion (72%) of them ran compared to 1-h after treatment (33%). The lowest dose tested for acetamiprid and bifenthrin (0.02 ng/termite) provided immobilized subjects that did not survive 24 h so that data is not provided.

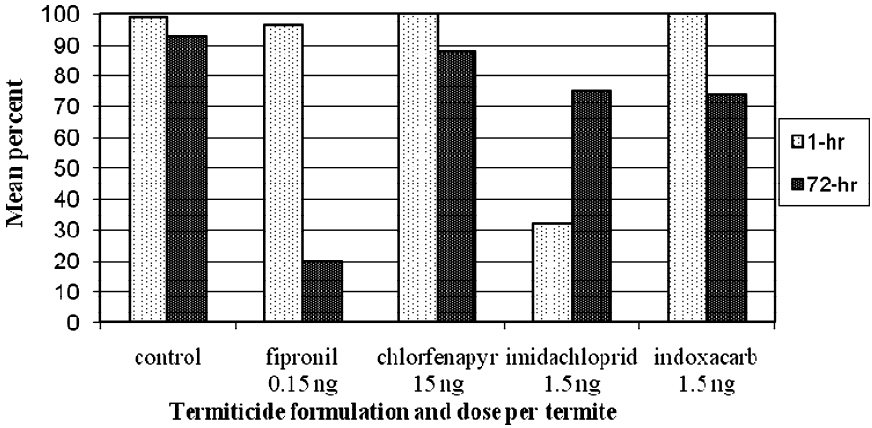

Figure 4. Mean percent topically-treated termites that ran along the ink pen line by termiticide formulation, dose in ng of active ingredient per termite and hours post-treatment.

Discussion

Congruence between laboratory screening bioassays and field efficacy is an important prerequisite for development of meaningful pesticide use patterns. This chapter attempts to provide data useful in modeling soil termiticide efficacy using a series of laboratory bioassays as a means of predicting field efficacy.

Attributes of termiticide chemistry that affect field efficacy of the end-use product would include persistence, bioavailability, solvent systems used in formulation, concentration, and active ingredient. Persistence is important for predicting the longevity of a soil barrier (*19*, *20*); The Chapter by Mulrooney, Wagner, and Gerard discusses appropriate methods for addressing this particular issue. Bioavailability is a complex issue and the comparisons between play sand and a sandy loam soil in the time exposure bioassay section of this chapter illustrate the importance of this phenomenon in understanding termiticide

efficacy. The use of sand in termiticide bioassay has the advantage of repeatability between laboratories yet it should be plainly stated that field efficacy will most likely require higher concentrations for field results to mimic laboratory data (*22*). Solvent systems used in formulation can impact efficacy in bioassay as demonstrated by Smith and Rust (*23*) and Rust and Smith (*24*), who mention that proprietary information complicates testing formulation affects. A recent study demonstrated that formulated soil termiticides required contact to produce adverse affects and prompted the use of commercial products in the tests described in this chapter (*24*).

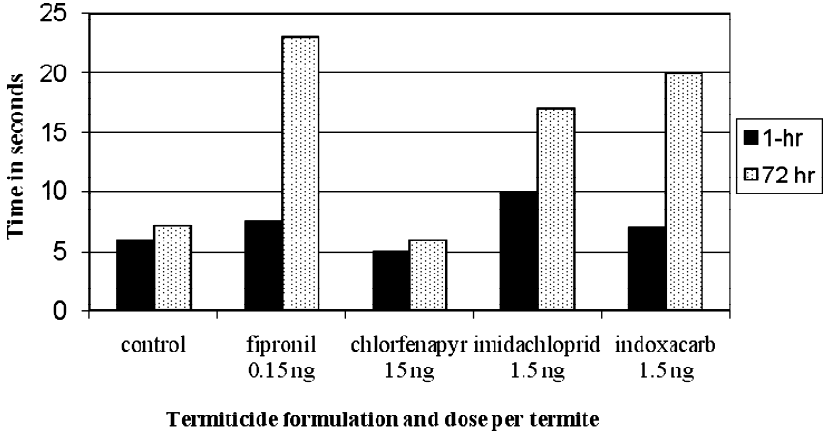

Figure 5. Time taken to run 6-cm by topically treated termites by termiticide formulation, dose in ng of active ingredient per termite and hours post-treatment.

Both concentration and the active ingredient are inexorably tied to their effect on the behavior of an individual termite. Intoxication of individuals, because of the collective decision making process employed by eusocial termites, must be considered within the context of what is known about termite behavior when interpreting bioassay data. It has long been recognized that insecticide concentration plays a significant role, as exemplified by studies attempting to find the 'repellent' limits in bioassay (*26, 133, 27, 28, 29*). However, concentration and route of entry are critical aspects of toxicity that have not been actively separated in previous studies. Dermal acquisition is generally assumed to be the major route of entry in the design of most termiticide bioassays (*13, 30, 28*). Saran and Rust (*29*) tested and supported the hypothesis that dermal contact is the major route of entry in bioassay involving termites exposed to treated substrates that cannot be excavated. The time exposure assays in this chapter, recent work by Green (*31*), and the fact that most of the published literature employ experimental designs that provide at least 1-hr exposure prior to examination of 'transfer' in bioassay (*13, 30, 28, 32*), illustrate that dermal acquisition is a slow process involving long-term (or multiple) exposure.

The mechanics, incidence and consistency of behaviors have an impact on designing bioassays with relevance to field efficacy. Two aspects of subterranean termite gallery construction are critical to interpreting bioassay data: the mechanics of the process and allocation of that task to individuals within a population. *R. flavipes* are known to manipulate soil particles with their mouthparts in the act of excavation (*33*). Therefore, termites involved in gallery construction following soil termiticide application or during foraging after application (curative or preventative scenario's respectively) would most likely be affected by an oral dose. Unfortunately there is a knowledge gap in how termites maintain galleries following excavation, but it can be assumed that this process also involves oral manipulation of gallery interior surface. Taken one step further these assumptions imply that termites not involved in gallery construction or maintenance (those using the gallery as an avenue of movement between feeding sites) would be the subjects that acquire a lethal dose through dermal contact.

It has been shown that not all worker termites are involved in gallery construction, indicating this task is performed by specific individuals (*33*); if they are killed or intoxicated, it can be assumed that communication of direction could eventually be lost to the main population. Applying the concept of swarm intelligence (*34, 35*) to termiticide bioassay would suggest that tests in perti dishes or tubes oversimplify the process used by intact termite field populations when confronted with a soil termiticide application. The system employed by subterranean termites to communicate traffic flow within the network of galleries connecting different feeding sites is unknown and without more knowledge a choice bioassay offers a better approximation of field events compared to a no-choice system.

This chapter attempts to illustrate relevant aspects of termiticides that require consideration when designing termiticide bioassay. The first is that the term "non-repellent" is not the purview of any particular class of chemistries but is a matter of concentration. The four-way choice bioassay data reported from these trials clearly demonstrated that all of the termiticides tested do not provide 100% mortality in small choice-test arenas after 21 days of exposure (Figure 1). Depending on concentration, all of the chemistries tested, including a 'repellent' formulation (bifenthrin), provided data where tunneling and mortality was limited (Figures 1-3). These data demonstrate the subjective nature of the non-repellent label and that this moniker should be used with a caveat to concentration. The second is bioavailability. It has been demonstrated in other studies that bioavailability is important for predicting the efficacy of a particular termiticide (*36, 37, 6, 38, 15, 22*). All of the termiticides tested in the studies outlined in this chapter, with the exception of imidacloprid, provided decreased termite mortality when comparing the same treatment between soil types (Table II). Bioavailability is a complex phenomenon that plays a role in termiticide field efficacy, especially at low concentrations. However, it is clear that one should use caution when extrapolating laboratory results obtained from bioassay in sand – especially in regard to field-use recommendations.

The third aspect of soil termiticide efficacy demonstrated by these tests involves route of entry. The dose-mortality assays, reported herein, clearly demonstrate the most toxic route of entry varies for each of the chemistries

tested (Table III). Termites can obtain a lethal dermal dose one of two ways: body-to-body contact with a dermal-contaminated termite or a contaminated substrate (gallery or soil). The timed exposure data (Tables I & II) indicate that in the field repeated exposures would be required to produce significant mortality by the dermal route of entry. Following a label application there would be, under ideal conditions, a 16-cm zone of treated soil that termites could traverse - once a gallery is constructed - in less than one minute. The one-minute exposure mortality data for sandy loam soils did not produce sufficient mortality by the dermal route of exposure to justify the attrition hypothesis (Table II). Shelton and Grace (*30*) demonstrated that, following exposures times ranging from 3-24 h on soil containing 1 ppm of fipronil or imidacloprid, transfer of a lethal dose was unpredictable and provided no more than 26% mortality in unexposed 'recipients'. Saran and Rust (*29*) exposed termites to treated sand for 1-hr and found limited dermal uptake of ^{14}C fipronil with most occurring by constant exposure for 24-h. The lessons from these and other studies is that the most likely route of entry following field application of a termiticide will be an oral dose through soil manipulation during gallery construction (*33*) and gallery maintenance - not the dermal route.

The potential that grooming could play to provide an oral dose for termiticide transfer has been demonstrated by Myles (*39*). Grooming has been documented as the most consistently performed behavior displayed by worker termites (*41*) and Whitman (*40*) found it occupied 16% of the active time of the average worker. These data indicate the potential grooming would have as a mechanism of oral dose transfer, but the dermal acquisition data would arguably relegate the grooming-oral route to a minor role unless the active ingredient provides a favorable oral/dermal toxicity profile (Table III). Another behavior that could contribute to transfer by the oral route would be food exchange, yet most studies of trophallaxis do not separate the potential modes - stomodeal or proctodeal (*41, 42, 43, 44, 45*). The trophallaxis oral route would be minimal because the amount of labeled food transferred is estimated from the literature to be below 15% on any given day (*44, 45*). Whitman (*40*) corroborates those data by describing stomodeal exchanges involved only food being chewed, eliminating it as a source of soil termiticide transfer, and proctodeal exchanges accounted for less than 1/3 of the food intake for the average worker over the course of several days. Cannibalism is the last potential route of oral dose acquisition but it has not been purposefully studied and seems an unlikely major contributor to any model of termiticide transfer given the reports of cadaver burying behavior in termites (*46*).

The fourth aspect of soil termiticide efficacy involves an understanding of termite behavior relative to collective decision making within termite populations. The importance of the decision making process is illustrated by data that incorporate distance in the experimental design whether laboratory bioassay (*47, 29*) or field studies (*48*) that do not provide evidence of transfer beyond a few meters. Any termite that is contaminated by contact with a termiticide represents a potential toxicant delivery system to other parts of the network of galleries and feeding sites maintained by a population of termites. The contaminated 'agent of transfer' must however be behaviorally unaffected long enough to exit the area of exposure. The timed running tests provided in the

chapter and the work of Saran and Rust (*29*) represent a starting point toward understanding the potential a particular termiticide has toward movement by contaminated termites as well as the range of doses that would allow transfer in the field. The data to date indicate that the dose response range is not only specific to a certain chemistry (and route of entry) but for most termiticides is represented by a small range of concentrations. The effective dose range required for any specific termiticide that would allow movement of 'lethal donors' from the point of insecticide application to a significant number of termites in a field population needs to be examined in more detail. It would appear, however, even with this level of detailed understanding that field application of a liquid soil termiticide could not, given the plethora of soil types and conditions at any single treatment site, provide consistent realization of the attrition hypothesis. Consistently attaining this range of concentrations in a field application would be impossible and combined with the impact of bioavailability, route of entry, population pressure, and persistence makes accurate predictions problematic.

In the field, aversion also may play a role in the efficacy of termiticides. Thorne and Breisch (*49*) determined that termites exposed to a sublethal dose of imidacloprid did not ‘learn’ to avoid treated soil. However, if termites get ‘sick’ in certain galleries those routes may receive less traffic and the chemical messages indicating a “path-to-follow” may deteriorate thereby mimicking aversion. Similarly, termites excavating into a soil termiticide treatment could die quickly, for example at the active end of gallery construction, and the chemical signal to travel down that path - at the fork in the system where termites decide which gallery to traverse - would be lost and activity redirected to another portion of the gallery system. The end result of collective decision making in termite gallery traffic patterns could result in structural protection at termiticide concentrations that kill termites (the traditional non-repellent concept) but do not impact termite populations (as in the traditional repellent paradigm). Repeated attempts to dig through treated soil could, however, reduce the number of termites, assuming all termites are involved in gallery construction, with structural protection achieved in a manner consistent with the attrition hypothesis although transfer played no role.

Summary

Advances in our understanding of termite biology and the new chemistries registered since 1982 call for a paradigm shift in bioassay design for testing termiticide efficacy. It is clear from the series of bioassays reported in this chapter, in addition to other studies (*47, 48, 32, 29*), that the attrition model of termiticide efficacy is unlikely to be consistently realized in the field using soil-based application of the formulations tested in this study. The mechanics of gallery construction and attendant oral dose, the realization that gallery construction is conducted by specific individuals combined with the assumption that traffic through the gallery system is dictated by collective decision making begs a reevaluation of soil termiticide efficacy. I propose that soil termiticides act as a preventative barrier following treatment because termites allocated to

exploring for new food resources (termites involved in gallery construction, foragers) are killed (slowly or otherwise) and the gallery leading to a soil treatment goes unexploited/unused by the remaining population whose traffic flow is directed elsewhere. Soil termiticides work in eliminating active structural infestations in much the same way by redirecting traffic in the soil in combination with killing termites 'trapped' in the structure through desiccation or contact with the treated soil.

Soil termiticides must be evaluated in conjunction with an understanding of subterranean termite behavior, as illustrated over 20 years ago by Su *et al.* (*4*), but advances in our understanding of termite behavior begs prudent interpretation of single-design bioassay data. Information from a bioassay series can be applied to what is known about termite foraging and colonization activities to formulate a hierarchy of outcomes based on the attrition or barrier hypotheses of soil termiticide efficacy and can be used to design active ingredients and application methodologies that would optimize transfer to realize widespread attrition. Determination of the oral and dermal toxicities combined with measures of the time-frame for toxicity-related behavioral changes could be used to predict useful insecticide candidates for realization of the attrition model However, actual field efficacy will always be subject to the vicarious conditions present at an individual treatment location, which may never be anticipated with certainty, highlighting the importance of attention to the details of application by the end user.

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