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Effect of hexaflumuron on mortality of the Western subterranean termite (Isoptera: Rhinotermitidae) during and following exposure and movement of hexaflumuron in termite groups

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Abstract: The effects of hexaflumuron consumption on the mortality of workers of the Western subterranean termite, *Reticulitermes hesperus* Banks, were observed following various exposures to a substrate treated with ^{14}C-labeled hexaflumuron at 5 g kg^{-1}. Uptake of hexaflumuron by workers was rapid, peaking at approximately 280 ng hexaflumuron per termite 12 days after exposure. The onset of mortality began at day 8, with cumulative mortality reaching approximately 96% by day 45. Excretion of hexaflumuron from worker termites was rapid following various exposure periods, half-lives ranging from 2.1 to 4.7 days. Trophallaxis among worker termites was efficient, hexaflumuron levels in recipient termites approaching two-thirds of levels present in donor termites feeding continuously on hexaflumuron-treated filter paper. The effect of donor-to-recipient group ratios was negligible on the amounts of hexaflumuron transferred from donors to recipients. In laboratory tests, hexaflumuron diffused from a feeding source within 7 days. The presence of additional food sources appeared to inhibit movement of hexaflumuron. Movement of hexaflumuron by cannibalism and coprophagy occurred, but was significant only when termites were starved. Hexaflumuron also appeared to adversely affect egg development by preventing hatch. Hexaflumuron was readily transferred through termite groups, effectively suppressing laboratory populations of *R hesperus*. Variable efficacy in field situations employing baiting with hexaflumuron in southern California may be a consequence of sporadic feeding at bait stations, the rapid clearance of hexaflumuron from individual termites, and the difficulties in bait presentation (low foraging activity, poor bait station foraging fidelity) inherent in the foraging characteristics of the Western subterranean termite.

Keywords: *Reticulitermes*; Isoptera; hexaflumuron; baiting; trophallaxis

1 INTRODUCTION

Baiting for the suppression or elimination of subterranean termites is not a new idea. Esenther and Beal[1,2] used attractants in conjunction with mirex to suppress populations of subterranean termites. Jones[3] attempted to use fenoxycarb as a bait material for subterranean termites. Baiting technology became commonly used for termite control with the registration of hexaflumuron and adoption of the Sentricon® termite baiting system for the suppression or elimination of subterranean termites by DowAgrosciences in 1994.[4] Introduction of the Sentricon termite bait system has been mirrored by the introduction of a myriad of termite control systems based on the same principle of monitoring termite activity and applying treatment following detection of termite activity, using a variety of active ingredients.[5]

The ultimate goal of termite baits is to eliminate termites from structures. In practice, hexaflumuron is an attractive option because, when applied in baiting systems, it is not likely to be translocated to areas unintended for pesticide application[6] and requires small amounts of active ingredient per treatment. Part of this goal involves suppression or elimination of termite colonies near the structure so that the risk of termite attack is low, and a further aspect is, if colonies are successfully suppressed or eliminated, that the resurgent attack in structures might be thwarted.[7] Primary considerations in determining efficacy of treatment include absence of alate flights within the

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area or structure treated, absence of termite activity at locations where baits were introduced and within the structure to be protected, and sustained absence following removal of treatment.[7]

Successful control or suppression of *Reculitermes hesperus* Banks using hexaflumuron has been documented relatively rarely compared with other North American species of termite. Termite colony suppression or elimination was documented at several commercial and residential structures infested with *R hesperus*.[8] Colony elimination of *Reticulitermes* spp (presumed to be *hesperus*) following extensive baiting in several locations in northern California has also been reported.[9] Subsequent invasions of treated areas were assessed to be invasions by colonies not subject to treatment. In southern California, a population of *R hesperus* defined by mark-release recapture studies was eliminated.[10] Termite activity in the defined limits of the original colony eliminated was nil up to 4 years post-treatment (Haagsma, unpublished data), although subsequent termite activity has been noted. In each of these cases, care was taken to maximize termite exposure to bait material by providing many bait stations for termites to forage on, and minimizing adverse conditions such as excessive moisture and predation by non-target species.

Baiting with the Sentricon system with hexaflumuron as the active ingredient has had mixed results in commercial applications. Of 48 individual field test sites of commercial bait installations over a 2-year period in southern California from 1996 to 1998, only three of these sites met the unofficial criteria set out by Thorne and Forschler[7] for bait efficacy and 29 sites met the unofficial criteria set out by Su.[11]

Several factors may account for actual or perceived lack of efficacy. In one study in southern California, a cumulative average of only 17% of offered feeding sources were attacked by *R hesperus* over a 2-year period.[12] Another study in southern California showed that termites attacked approximately 6% of wooden stakes offered and activity in bait stations dropped by approximately 50% after replacing monitoring stakes.[13] With such low attack rates, the likelihood of introducing bait into a colony would be reduced. Other factors, such as a seasonal periodicity of feeding,[12,13] poor fidelity to monitoring/bait stations (Haagsma, unpublished data) or termite exclusion from baiting stations by opportunistic predators[14] might result in poor field efficacy of hexaflumuron-based termite baiting systems for *R hesperus*.

Herein, we considered aspects of hexaflumuron uptake in individuals and in laboratory groups of *R hesperus*. Termite mortality resulting from hexaflumuron dosages over time was investigated. Factors affecting hexaflumuron presence and action in termites, such as hexaflumuron clearance rates and mortality following removal of treatment, were considered. In addition, movement of active ingredient within termite groups as a result of trophallaxis, cannibalism and recycling of fecal material was quantified. Finally, we considered the effect of hexaflumuron treatment on reproduction by quantifying egg development when reproductives were confined with larvae and nymphs feeding on hexaflumuron. These data were considered as possible factors and discussed in explaining the variable field baiting results of *R hesperus* in southern California.

2 EXPERIMENTAL METHODS

2.1 Insects

Termites were collected from a single colony on the Riverside campus of the University of California. Termite traps, consisting of lengths of PVC pipe (9.72 cm ID × 15.0 cm) were placed in the ground at sites with confirmed termite activity, and baited with rolls of corrugated cardboard (5.0 cm length × 2.25 cm radius). Termites feeding on cardboard were extracted and transferred to plastic food storage boxes provisioned with moistened paper towel. Termites were held for at least two weeks in laboratory culture at 25 °C and approximately 100% RH before use in experiments to ensure that the extraction process resulted in no mortality.

2.2 Chemical

Acetone (5 ml) was mixed with [*2,6-difluorophenyl*-U-^{14}C]hexaflumuron (specific activity 33.0 mCi mmol^{-1}, 0.103 mCi total activity; Ref: FAPC994006 Dow AgroSciences, Indianapolis IN) to make a stock solution. Exactly 30 µl of stock solution was added to 1 ml of acetone and technical hexaflumuron (Dow AgroSciences, Indianapolis IN) was added until the final amount of labeled and unlabeled hexaflumuron resulted in a 5 g kg^{-1} concentration when applied to a disk of filter paper (Whatman #1, 4.5 cm diameter). The ratio of labeled to unlabeled hexaflumuron was approximately 1:71.

2.3 Hexaflumuron uptake and clearance

To determine the amount of hexaflumuron taken up by workers, termites were fed on ^{14}C-hexaflumuron-treated deposits as described in Section 2.2. The bottom of a plastic Petri dish (9.0 cm diameter) was roughened with 400-grit sandpaper to provide solid traction for termites and permit ease of movement in the arena. Treated filter paper was moistened and placed in the bottom of the Petri dish. Two hundred and fifty workers were added to each of three replications. Five termites were randomly selected from each replicate approximately every day for up to 45 days after initial introduction. Groups of five termites were placed into separate scintillation vials (20-ml) (Kimble Glass, Vineland, NJ) and digested with 80 µl of nitric acid.[15] Scintillation fluid (Cytoscint®; ICN chemicals Costa Mesa, CA; 10 ml) was added to each sample and samples were then counted by liquid scintillation counting (LSC; Beckman Instruments LS 3801). Dead termites were removed from treatments on a daily basis and counted. Cumulative mortality of the remaining termites in each

replicate was also recorded. Data on initial uptake of hexaflumuron by live termites and the amount present in dead termites were analyzed by linear regression on $\log(Y + 1)$ transformed data.[16]

Two hundred and fifty termites were confined to treated filter papers (4.5 cm diameter) in each of four replicates to determine clearance rates of hexaflumuron in individual termites. Groups of 40 termites from each replicate were removed at 1, 3, 5, 9, 15 and 30 days and held on untreated, moistened filter paper. Sub-samples of five live termites for each replication and exposure time were taken at 1, 3, 5, 9, 15 and 30 days, digested, and counted by LSC. Cumulative mortality in each subset was recorded, and clearance rates of hexaflumuron were analyzed by linear regression on $\log(Y + 1)$ transformed data.[16] One-way ANOVA was performed on arcsin-square-root-transformed mortality data.[16]

2.4 Quantification of transfer

To determine the amount of hexaflumuron transferred between termites, workers were confined to treated surfaces and then mixed with unexposed workers. Termites were placed on filter paper (Whatman #1, 4.5 cm diameter) treated with 1 ml of an acetone solution containing 30 µl of stock solution of ^{14}C-hexaflumuron + technical hexaflumuron to provide a 5 g kg^{-1} concentration on the filter paper. Termites (donors) were held for 1, 3, 7 and 12 days to feed on the treatment. Treated termites were then mixed with untreated termites marked with paint (Painty Paint Pens, EK Success Co, Clifton, NJ). Donor-to-recipient ratios were 1:1, 1:2 and 1:4. Donors and recipients were placed on an untreated filter paper food substrate in feeding arenas and allowed to mix. The holding times for each ratio were 1, 3 and 7 days. For each combination, four replications of five donor and recipient termites were digested and counted with LSC. Percentage transfer was calculated as [ng recipients/(ng donors + ng recipients)] * 100. Data for hexaflumuron uptake by the donors and the recipients were analyzed with a factorial ANOVA.[16]

To determine the maximum amounts of hexaflumuron obtained by recipients via trophallaxis, recipient termites were provided new donor termites every 24 h. Donor termites had fed continuously on treated filter paper for 7 days before mixing with recipients. For each of these groups, termites were confined on and off a filter paper feeding substrate to determine if available food affected rates of trophallaxis. Donors and recipients were mixed in a 1:1 ratio for 1, 5, 9, 12, 15 and 20 days. Four replications of five termites were used for each treatment. Data from donors were analyzed by one-way ANOVA and data from recipients held on or off a feeding substrate were compared with a *t*-test within days post-mixing using log-transformed data.[16]

2.5 Multiple transfers

To determine if trophallaxis is a cascade event, whereby donors transfer material to recipients and these recipients in return act as donors to other termites, marked (painted) donor termites were held on treated filter paper to feed for 7 days. These were then allowed to mix with recipients (1:1 ratio) for 1, 3 and 7 days. Following exposure, the recipient termites then served as donor termites, and were in turn allowed to mix with other 'recipients' as above. This was reiterated four times so that the last recipients would have received hexaflumuron after three trophallactic transfers from the original donor. All termites commingled on untreated filter paper that served as a feeding substrate. Four replications of ten termites were done for each treatment. Data from donors and recipients for each iteration were analyzed by a one-way ANOVA.[16]

2.6 Distance and rate of transfer of hexaflumuron

To determine the movement of hexaflumuron over a given distance by trophallaxis or the movement of treated termites, either 3, 5 or 7 Petri dishes (4.5 cm diameter) were connected by 243 cm lengths (0.45 cm ID) of sterilized Tygon tubing (St Gobain Performance Plastics, Reading, PA) to create small, medium and large foraging arenas, respectively (Fig 1). Tubes were connected to Petri dishes by creating a small hole on the edges of the Petri dish, inserting and hot-gluing a polyethylene pipette tip (Molecular Bio-products, San Diego, CA) with the distal end cut off such that termites would be able to pass. Sterilized play sand was added to the interior of the Tygon tubes so that it occupied approximately one-half of the interior volume. Disks of filter paper (Whatman #1, 4.5 cm diameter) were placed in the bottom of the Petri dishes and approximately 4 g of sterilized play sand were added to each dish. Sand in the Petri dishes and Tygon tubes were lightly

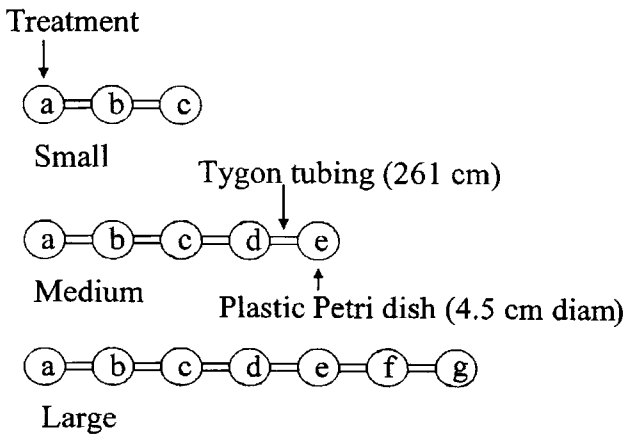

Figure 1. Diagram of a simulated field study considering movement of hexaflumuron within various group sizes of *Reticulitermes hesperus*. Petri dishes containing sand were connected by 261-cm lengths of Tygon tubing and 200 termites were introduced into the apparatus. A Petri dish with ^{14}C-hexaflumuron-treated filter paper replaced a Petri dish with no treatment (in section 'a') following an acclimatization period, and termites were subsequently sampled at 3, 7 and 14 days during feeding and 7, 14 and 30 days after removal of treatment.

moistened by applying water with a small hypodermic syringe.

Two hundred termites were introduced into the apparatus (section 'a', Fig 1), and were held for one week to allow for acclimatization and distribution. After one week, the clean filter paper disks in the Petri dishes at the end of the apparatus (section 'a') were replaced with filter paper treated with 5 g kg^{-1} ^{14}C-hexaflumuron. Each of three replicates was maintained for 3, 7 and 14 days. After each experiment, Petri dishes were separated from Tygon tubing. The tubing was further subdivided into two equal subsections and if termites from the sections were unavailable for sampling, termites were taken from the sub-sections of tubing most proximal to the section in question. Five termites were removed from each section or sub-section where applicable and destructively sampled as described in Section 2.3, and counted with LSC. Data were analyzed with a two-way ANOVA on $\log(Y + 1)$ transformed data.[16]

To test for secondary movement of hexaflumuron, where termites are not actively feeding on a treated material and all movement is by either trophallaxis or the movement of termites containing hexaflumuron, apparatus similar to that described above was used. Exactly 200 termites were introduced into the apparatus and allowed to acclimatize for one week. Additional termites were internally dyed by feeding them filter paper dyed with 0.1 g kg^{-1} Nile Blue A. This dye produced a visibly distinct termite that lasted for greater than 30 days with little mortality.[13] These termites were subsequently allowed to feed on filter paper impregnated with ^{14}C-hexaflumuron for 7 days. Two hundred marked and labeled termites were then introduced into a Petri dish on one end of the apparatus (section 'a', Fig 1) and termites were allowed to mingle for 7 and 14 days. Sub-sets of non-marked termites were destructively sampled at the end of each period and counted by LSC. The number of dyed termites in each arena was counted 7 days following their introduction. Hexaflumuron levels for dyed and undyed termites at 7 days of treatment exposure and 7 days of mixing exposure were analyzed by a one-way analysis of variance within treatments and distribution of termites within individual size apparatus were analyzed with a Kruskal–Wallis test for goodness of fit.[16]

2.7 Movement of hexaflumuron by cannibalism

Termites marked with a small drop of paint (as described in Section 2.4) were confined for 3, 7 and 12 days to feed on filter paper treated with 5 g kg^{-1} ^{14}C- hexaflumuron. After exposure, four replicates of ten termites were killed by flash freezing them in an ultra-cold refrigeration unit. Frozen termites were thawed and introduced into Petri dishes (50 × 9 mm) lined with a filter paper disk. (Whatman #1, 4.25 cm diameter) or Petri dishes without a filter paper disk (no food source). An additional five termites for each exposure period were digested with nitric acid and counted to confirm ^{14}C-hexaflumuron uptake. Five untreated termites which had been fed *ad libitum* or had been starved for 7 days were introduced into the Petri dishes. All arenas were held at 100% RH and approximately 25 °C. Five non-treated termites were sampled at 1, 3 and 7 days, digested and counted by LSC. Controls for arena tests utilized similar numbers of workers incapable of feeding. Termites were anesthetized with carbon dioxide and their mouthparts sealed with a small drop of cyanoacrylate glue. LSC counts from those termites provided a baseline with which to compare termites capable of cannibalism. Data from donors in all treatments and data from recipients within exposure treatment were analyzed with one-way ANOVA.[16]

2.8 Movement of hexaflumuron by fecal ingestion

To determine if larval termites feed on fecal material (coprophagy), termites were exposed to fecal material produced by termites actively feeding on ^{14}C-hexaflumuron-treated filter paper, and the amount of radiolabeled hexaflumuron consumed by naïve termites was determined. One hundred termites were allowed to feed on small filter paper disks (4.5 cm) treated with 5 g kg^{-1} ^{14}C-hexaflumuron. Disks were placed in the bottom of plastic Petri dishes (7.5 cm diameter). Treated paper and termites were removed from the arenas after 3, 7 and 14 days. The treated paper was lightly scraped to remove fecal material deposited on the paper. This fecal material was added to that already deposited in the dish. Groups of ten untreated termites, which had been starved for 7 days or fed *ad libitum*, were introduced into the arena and held for 1, 3 and 7 days. Termites collected at each time period were destructively sampled and counted by LSC. Controls consisted of termites with non-functioning mouthparts to prevent feeding and trophallaxis as described in Section 2.7. Data were analyzed with a one-way ANOVA within each treatment.[16] The amount of hexaflumuron present in fecal material was determined by taking a small sub-sample, then weighing and counting it. Counts were corrected for the entire frass sample weight.

2.9 Transfer to reproductives and eggs

To determine whether hexaflumuron was transferred by trophallaxis to queens or eggs, five female secondary reproductives were held with 200 worker termites on filter paper disks (7.5 cm diameter) treated with 5 g kg^{-1} ^{14}C-hexaflumuron. Eggs produced by secondary reproductives were collected as they appeared, and any dead larvae were removed and replaced weekly. When 2–7 eggs accumulated in each replicate, groups of eggs were digested in nitric acid and counted using LSC.

Additional groups of eggs produced at least 15 days after initial treatment were held with 100 untreated workers in petri dishes provisioned with untreated filter paper to determine egg viability. Controls consisted

of eggs produced by secondary reproductives reared on untreated filter paper. Secondary reproductive mortality was recorded at one-week intervals for up to two months. Secondary reproductives were not digested or counted.

3 RESULTS

3.1 Hexaflumuron uptake and clearance

Figure 2 shows hexaflumuron levels in termites feeding continuously on labeled hexaflumuron-treated filter paper. By day 1, individual termites (2.97 ±0.053 mg) had taken up approximately 60 ng of hexaflumuron. These levels increased and peaked at day 12 at approximately 289 ng after which levels dropped to approximately 225 ng by day 40. Uptake during the first 12 days was approximately linear and regression analysis for this period was significant ($R^2 = 0.55$; $F = 29.97$; $P < 0.0001$). Mortality significantly different from controls was not observed until 12 days after the initial exposure at which time approximately 17% succumbed to the treatment. Mortality increased thereafter in a logistic fashion, with 93% mortality occurring by day 45. Hexaflumuron levels in dead termites removed from populations ranged from approximately 58 to 141 ng (mean = 113.2 ± 43.2), with no apparent association to feeding time. Regression analysis of data from day 12 to day 45 was not significant ($R^2 = 0.003$; $F = 0.11$; $P < 0.74$).

Hexaflumuron clearance by workers following various feeding times is shown in Table 1. Initial post-exposure levels of hexaflumuron increased with increasing feeding time, ranging from approximately 177 ng at 1 day to 307 ng after 30 days. Regression analyses for post-feeding sampling periods were significant in all cases (Table 1). Rate of hexaflumuron clearance increased as the initial feeding time increased. The half-life of hexaflumuron in termites ranged from 2.1 days to 4.65 days following 15-day and 3-day feeding exposure, respectively. In general, half-life decreased as feeding period increased. Cumulative mortality of termites remained low for groups feeding up to 5 days on treated filter paper. Within these treatments, there was no significant difference in mortality at 1 day initial feeding, whereas mortality was significantly different at 30-days post feeding versus 7 and 14 days post-feeding for 3- and 5-day exposures. Cumulative mortality increased to 11.6% at 7-day with 9-day exposures, but there was no significant latent mortality at 14 and 30 days. Cumulative mortality at 15 days ranged from approximately 14 to 27% and from 55 to 63% at 7 and 30 days, respectively. When mortality data were considered for each exposure and post-exposure situation, exposure and post-exposure factors were significant ($F = 108.87$; $P < 0.0001$; $df = 7, 46$).

3.2 Quantification of transfer

Hexaflumuron uptake by donor termites confined for various times on hexaflumuron-treated filter paper and the amount transferred to recipient termites held for various times with three different donor-to-recipient ratios is shown in Table 2. Levels of hexaflumuron in donors significantly increased as feeding period increased ($F = 79.9$; $P < 0.0001$, $df = 3$). Association time with recipient termites

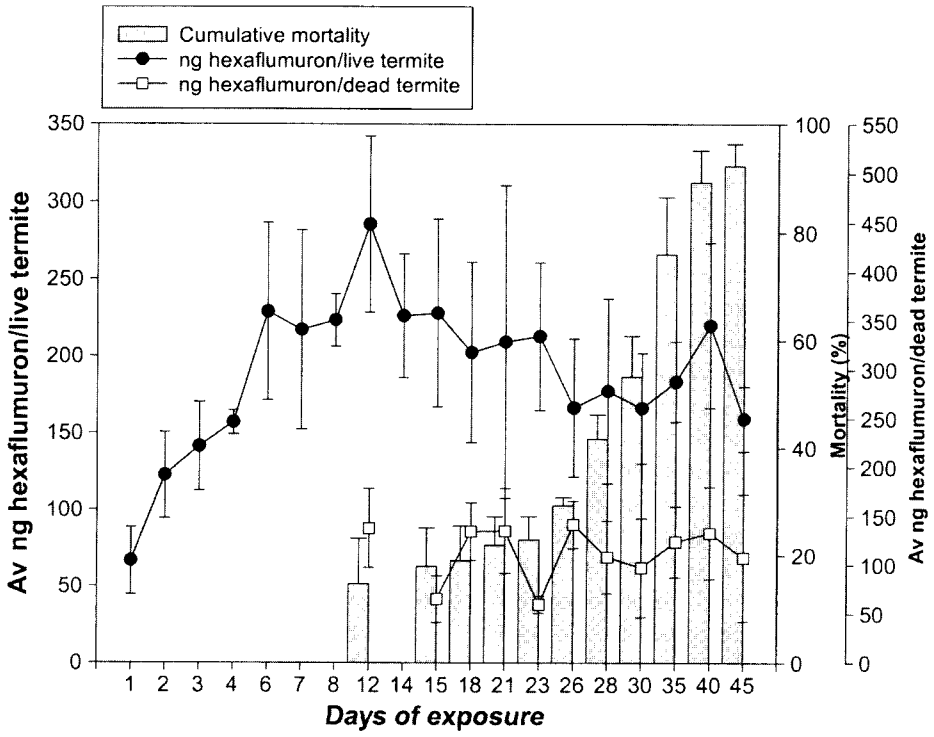

Figure 2. Uptake of hexaflumuron in live termites, cumulative mortality and amount of hexaflumuron present in dead termites held continuously on filter paper treated with 5 g kg^{-1} ^{14}C-labeled hexaflumuron for up to 45 days. Bars indicate standard deviations.

Table 1. Hexaflumuron clearance by worker termites at 1, 3, 5, 9, 15 and 30 days following feeding on hexaflumuron-treated filter paper for similar time periods, and cumulative mortality of termites at 7, 14 and 30 days following each exposure period

Feeding period (days)	Post-feeding sampling (days)	Hexaflumuron (ng/live termite) (±SD)	Regression statistics[a]	Half-life (days)[b]	Post-exposure mortality (days)	Cumulative mortality (±SD)[c]
1	1	177.8 (±107.9)		4.07		
	3	129.8 (±35.9)	$R^2 = 0.61$		7	0.66 (±0.57) a
	5	152.4 (±46.9)	$F = 35.04$		14	1.33 (±0.57) a
	9	83.9 (±21.3)	$P < 0.0001$		30	2.0 (±1.00) a
	15	68.8 (±14.1)	$b = -0.017$			
	30	49.8 (±6.9)				
3	1	194.3 (±28.0)		4.65		
	3	156.5 (±20.8)	$R^2 = 0.70$		7	0.0 (±0.00) a
	5	121.2 (±9.7)	$F = 53.75$		14	0.0 (±0.00) a
	9	101.0 (±19.6)	$P < 0.0001$		30	2.66 (±1.52) b
	15	82.0 (±21.5)	$b = -0.015$			
	30	66.1 (±13.1)				
5	1	229.1 (±45.3)		3.91		
	3	183.7 (±25.0)	$R^2 = 0.67$		7	0.0 (±0.0) a
	5	132.0 (±15.0)	$F = 45.52$		14	5.66 (±1.52) a
	9	103.0 (±19.6)	$P < 0.0001$		30	8.0 (±1.0) b
	15	71.2 (±12.4)	$b = -0.017$			
	30	67.6 (±22.2)				
9	1	257.6 (±43.8)		2.77		
	3	157.5 (±44.5)	$R^2 = 0.84$		7	11.66 (±5.50) a
	5	155.5 (±48.3)	$F = 121.05$		14	14.0 (±3.0) a
	9	131.3 (±29.6)	$P < 0.0001$		30	17.0 (±4.0) a
	15	100.5 (±18.0)	$b = -0.025$			
	30	38.8 (±10.2)				
15	1	306.8 (±65.2)		2.10		
	3	196.7 (±63.0)	$R^2 = 0.85$		7	14.33 (±9.6) a
	5	145.6 (±38.6)	$F = 129.68$		14	17.0 (±8.54) a
	9	143.6 (±26.0)	$P < 0.0001$		30	27.33 (±5.50) b
	15	85.5 (±7.7)	$b = -0.030$			
	30	26.6 (±15.0)				
30	1	307.2 (±57.1)		2.60		
	3	142.6 (±31.4)	$R^2 = 0.82$		7	55.33 (±7.50) a
	5	138.3 (±12.5)	$F = 104.88$		14	60.33 (±9.71) a
	9	110.6 (±13.2)	$P < 0.0001$		30	63.33 (±10.1) a
	15	78.5 (±21.6)	$b = -0.026$			
	30	37.7 (±7.9)				

[a] Regression analysis performed on log-transformed data.
[b] Half-life calculated by $-\ln(2)$/slope.
[c] One-way ANOVA performed within initial exposure treatments on arcsin (sqrt(p)) transformed data; means followed by the same letter indicate no significant difference (Tukey's HSD).

also affected hexaflumuron load in donor termites, with significantly less hexaflumuron present in donor termites 7 days after mixing with recipients than it was at 1 or 3 days ($F = 11.4$; $P < 0.0001$; $df = 2$). Donor-to-recipient ratio did not significantly reduce hexaflumuron levels present in donors ($F = 0.1$; $P < 0.90$; $df = 2$). There was a significant difference in recipient termite uptake due to initial donor exposure, even though it was not directly related to donor exposure time ($F = 5.35$; $P < 0.002$; $df = 3$). Recipient uptake was greatest from donors confined to treated paper for 12 days, followed by 1- and 3-day donor exposures. Hexaflumuron uptake by recipients was least from donors fed for 7 days. Significantly more hexaflumuron was taken up by recipients as the mixing time of donors and recipients increased ($F = 21.1$; $P < 0.0001$; $df = 2$). There was no significant difference in recipient uptake based on donor to recipient ratios ($F = 2.12$; $P < 0.12$; $df = 2$).

Figure 3 shows hexaflumuron uptake by recipient termites held on and off a non-treated feeding substrate and mixed with donor termites which continually fed on hexaflumuron-treated filter paper. Hexaflumuron content in donor termites ranged from 265 to 369 ng, and there was no significant difference in hexaflumuron levels within donor termites ($F = 1.74$; $P < 0.10$; $df = 11$). Hexaflumuron levels in recipient termites increased linearly over time for those held on ($R^2 = 0.81$; $F = 94.84$; $P < 0.0001$; $b = 0.059$), and off untreated filter paper($R^2 = 0.67$; $F = 44.91$; $P < 0.0001$; $b = 0.06$), but at all sampling periods there was no significant difference in hexaflumuron levels attributed to the feeding substrate. By day 20, hexaflumuron levels of recipients

Table 2. Hexaflumuron uptake by donors feeding on treated filter paper for 1, 3, 7 and 12 days and the amount of transfer to recipient termites mixed with donors for 1, 3 and 7 days at 1:1, 1:2 and 1:4 donor-to-recipient ratios

Donor confinement (days)	Recipient mixing (days)	Donor-to-recipient ratio	Hexaflumuron: donors (ng) (±SD)	Hexaflumuron: recipients (ng) (±SD)	Transfer (%)[a]
1	1	1:1	61.06 (±24.6)	6.52 (±3.2)	9.2
		1:2	71.21 (±25.9)	6.88 (±3.5)	8.8
		1:4	44.00 (±23.4)	5.99 (±2.5)	11.9
	3	1:1	58.68 (±20.1)	17.52 (±2.1)	23.6
		1:2	62.48 (±23.2)	15.45 (±3.9)	19.8
		1:4	55.77 (±20.2)	14.42 (±6.5)	20.5
	7	1:1	49.11 (±13.7)	18.45 (±7.9)	27.3
		1:2	31.52 (±10.6)	13.77 (±5.2)	30.4
		1:4	49.86 (±8.0)	9.99 (±3.9)	16.7
3	1	1:1	93.19 (±11.4)	9.15 (±7.3)	8.9
		1:2	104.51 (±20.4)	7.27 (±3.4)	6.5
		1:4	105.57 (±23.3)	9.99 (±1.5)	8.6
	3	1:1	65.47 (±18.3)	12.94 (±7.4)	16.5
		1:2	59.84 (±13.4)	13.22 (±0.3)	18.1
		1:4	69.49 (±19.4)	8.36 (±1.8)	10.7
	7	1:1	80.79 (±9.0)	20.59 (±4.6)	20.3
		1:2	60.07 (±18.7)	14.71 (±5.7)	19.7
		1:4	42.98 (±11.4)	11.19 (±4.7)	20.7
7	1	1:1	110.42 (±42.0)	6.86 (±2.5)	5.8
		1:2	130.94 (±20.9)	9.09 (±4.0)	6.5
		1:4	149.93 (±36.2)	5.01 (±1.2)	3.2
	3	1:1	152.15 (±37.2)	13.70 (±6.8)	8.3
		1:2	139.29 (±30.5)	7.80 (±3.3)	5.3
		1:4	129.93 (±23.8)	11.21 (±7.2)	7.9
	7	1:1	113.71 (±20.9)	13.47 (±4.7)	10.6
		1:2	94.22 (±39.9)	7.85 (±2.0)	7.7
		1:4	102.01 (±15.5)	10.72 (±2.4)	9.5
12	1	1:1	135.35 (±42.5)	10.82 (±3.7)	7.4
		1:2	174.38 (±22.2)	9.79 (±3.3)	5.3
		1:4	181.16 (±24.3)	14.82 (±5.3)	7.6
	3	1:1	161.93 (±46.8)	15.26 (±4.6)	8.6
		1:2	175.72 (±55.9)	14.60 (±5.7)	7.7
		1:4	162.23 (±28.8)	15.36 (±5.9)	8.6
	7	1:1	128.48 (±57.2)	12.67 (±2.8)	9.0
		1:2	140.08 (±59.5)	12.63 (±3.2)	8.3
		1:4	138.44 (±27.9)	13.83 (±4.4)	9.1

[a] Percentage transfer calculated as [ng recipients/(ng donors + ng recipients)] * 100).

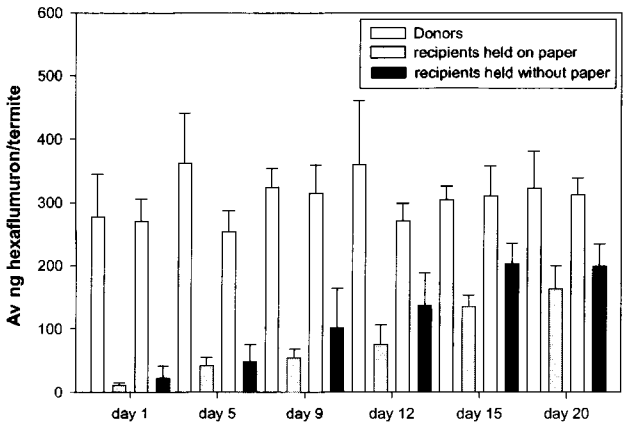

Figure 3. Hexaflumuron uptake by recipient termites held with donors continuously feeding on filter paper treated with 5 g kg^{-1} hexaflumuron for 1, 5, 9, 12, 15 and 20 days. Bars indicate standard deviations.

held without food leveled out at approximately 200 ng per termite, or approximately two-thirds the level of donor termites.

3.3 Multiple transfers

Figure 4 indicates the transfer of hexaflumuron by successive trophallactic exchanges for various feeding times. Initial levels of hexaflumuron in donors ranged from approximately 137 to 168 ng per termite. The amount of hexaflumuron in the recipients after the first exchange increased with exposure period of 1 to 7 days, from approximately 17 ng to 40 ng. Recipient termites acting as donor termites for the second iteration had significantly different levels of hexaflumuron, with uptake by the second recipients increasing with increased exposure time ($F = 5.08$; $P < 0.0006$; $df = 8$). Second iteration levels of hexaflumuron dropped in donors with increased exposure time with recipients

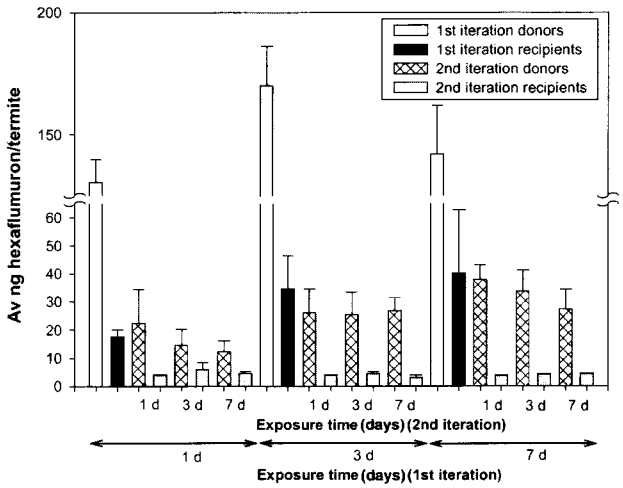

Figure 4. Transmission of hexaflumuron by successive trophallactic exchanges at exposure times of 1, 3 and 7 days (d) between donor termites and recipient termite over two iterations where recipient termites from the previous iteration serve as donor termites for the following iteration. Bars indicate standard deviation.

except for the 3-day exposure. Within exposure times, there was no significant difference in hexaflumuron content within donors. Second iteration recipient hexaflumuron levels ranged from approximately 4 to 8 ng and there were significant differences ($F = 3.33$; $P < 0.008$; $df = 8$). Only at 1 day (first iteration) and 3 days (second iteration) were recipients significantly different than the rest of the recipients in other treatment regimens. No data were available for the third and fourth iterations because hexaflumuron present in individual termites was below detectable levels (approximately 50 cpm).

3.4 Distance and rate of transfer of hexaflumuron

Table 3 shows hexaflumuron uptake during and after exposure to a 5 g kg^{-1} hexaflumuron-treated food source in various sizes of laboratory groups provisioned with or without an additional food source. At day 3 for all size groups, there were significant differences in the amount of hexaflumuron per termite due to

Table 3. Hexaflumuron uptake by worker termites at 3, 7 and 14 days following feeding on a food source treated with 5 g kg^{-1} hexaflumuron in laboratory groups of various sizes and at 7, 14 and 30 days following removal from treatment

			Hexaflumuron/termite (ng) (± SD)					
			Days during exposure			Days post-exposure		
Colony size	Condition	Section[a]	3	7	14	7	14	30[b]
Small	With food	a	47.3 (±20.4)	112.3 (±21.5)	251.0 (±28.2)	96.0 (±8.0)	11.3 (±3.5)	ND
		b	31.3 (±11.3)	129.0 (±20.2)	207.3 (±30.5)	78.3 (±18.7)	32.3 (±6.1)	ND
		c	7.6 (±3.2)	95.0 (±18.6)	220.3 (±20.3)	61.6 (±7.0)	15.0 (±11.2)	ND
	Without food	a	65.0 (±8.1)	222.3 (±12.6)	307.3 (±23.5)	124.0 (±29.6)	13.3 (±5.1)	ND
		b	52.0 (±8.8)	195.6 (±28.5)	284.0 (±8.7)	97.0 (±18.0)	25.3 (±11.2)	ND
		c	28.3 (±11.2)	200.0 (±42.3)	251.6 (±31.5)	83.3 (±14.0)	6.6 (±8.0)	ND
Medium	With food	a	56.0 (±13.5)	143.6 (±9.0)	227.0 (±23.0)	72.6 (±16.5)	13.6 (±11.5)	ND
		b	62.6 (±9.6)	104.0 (±41.5)	204.0 (±30.0)	51.6 (±6.0)	9.0 (±6.5)	ND
		c	22.0 (±14.0)	135.0 (±7.0)	162.3 (±28.0)	47.0 (±21.6)	26.6 (±7.0)	ND
		d	24.0 (±6.2)	40.0 (±9.6)	184.6 (±24.7)	67.0 (±29.8)	5.6 (±5.5)	ND
		e	15.6 (±5.5)	62.0 (±16.0)	230.6 (±38.6)	14.3 (±11.1)	30.3 (±9.2)	ND
	Without food	a	76.6 (±19.1)	197.6 (±21.0)	270.3 (±31.0)	34.3 (±9.2)	8.3 (±4.9)	ND
		b	88.0 (±13.5)	114.6 (±23.1)	201.0 (±33.5)	45.0 (±14.7)	11.3 (±7.2)	ND
		c	33.3 (±5.1)	182.0 (±31.1)	218.0 (±18.6)	21.0 (±8.8)	14.0 (±4.0)	ND
		d	27.6 (±16.0)	103.6 (±15.5)	274.0 (±16.5)	20.0 (±3.6)	2.0 (±2.6)	ND
		e	31.6 (±8.3)	90.3 (±15.8)	155.6 (±30.2)	34.6 (±9.0)	5.0 (±3.6)	ND
Large	With food	a	60.3 (±10.0)	109.0 (±28.8)	268.6 (±31.5)	63.6 (±15.0)	5.0 (±3.6)	ND
		b	32.0 (±11.7)	94.0 (±6.5)	204.0 (±29.8)	52.3 (±31.5)	15.6 (±10.5)	ND
		c	58.0 (±10.5)	120.3 (±22.5)	202.3 (±32.7)	22.6 (±5.0)	2.0 (±1.9)	ND
		d	15.6 (±8.5)	62.6 (±8.6)	276.6 (±33.0)	34.6 (±9.2)	6.6 (±7.2)	ND
		e	2.0 (±2.0)	74.0 (±12.7)	180.3 (±47.2)	5.0 (±4.0)	1.0 (±1.7)	ND
		f	4.6 (±3.2)	26.3 (±10.2)	250.6 (±17.0)	19.6 (±6.8)	4.0 (±3.0)	ND
		g	ND	43.3 (±12.0)	225.3 (±37.2)	74.0 (±22.3)	8.6 (±5.5)	ND
	Without food	a	115.0 (±32.3)	187.3 (±28.0)	312.6 (±44.8)	42.6 (±14.2)	17.3 (±6.1)	ND
		b	103.3 (±11.3)	182.3 (±43.0)	281.6 (±24.7)	11.6 (±11.5)	4.3 (±2.0)	ND
		c	53.3 (±14.5)	216.6 (±41.7)	329.6 (±23.7)	11.0 (±1.7)	22.0 (±14.0)	ND
		d	25.3 (±7.5)	130.3 (±17.2)	273.3 (±39.0)	27.0 (±9.5)	3.0 (±2.6)	ND
		e	8.6 (±6.5)	116.6 (±20.2)	206.3 (±31.9)	15.6 (±14.1)	15.6 (±11.5)	ND
		f	ND	90.6 (±31.2)	287.6 (±52.1)	40.0 (±17.4)	2.3 (±3.2)	ND
		g	ND	75.6 (±13.0)	257.3 (±66.9)	35.3 (±17.2)	17.6 (±9.2)	ND

[a] Hexaflumuron was introduced into section 'a' in all cases.
[b] ND indicates hexaflumuron was not detectable in samples.

Table 4. Hexaflumuron uptake of worker termites following 7 days of feeding on filter paper treated with 5 g kg^{-1} hexaflumuron, and subsequent distribution of treated termites and transfer of hexaflumuron to non-treated workers at 7 and 14 days post-introduction in laboratory groups of various sizes[a]

	Hexaflumuron on marked termites (ng) (±SD)	Distribution of marked termites (%) (±SD)	Hexaflumuron on unmarked termites (ng) (±SD)	
	7-day exposure		7-day exposure[b]	14-day exposure[b]
Section a	115.3 (±19.0)	25.0 (±6.0)	2.3 (±3.2)	ND
Section b	124.3 (±27.5)	35.0 (±7.0)	3.3 (±2.0)	ND
Section c	177.3 (±24.0)	40.0 (±12.7)	ND	ND
Section a	156.0 (±43.5)	11.3 (±4.1)	4.3 (±3.5)	ND
Section b	84.0 (±33.1)	8.0 (±3.6)	11.6 (±8.3)	ND
Section c	107.0 (±34.3)	31.3 (±12.3)	3.6 (±4.7)	ND
Section d	140.0 (±43.0)	29.6 (±2.3)	5.6 (±4.7)	ND
Section e	155.3 (±53.8)	19.6 (±12.0)	11.3 (±9.5)	ND
Section a	109.0 (±23.6)	7.6 (±2.5)	2.0 (±2.6)	ND
Section b	120.6 (±35.2)	19.3 (±9.0)	8.0 (±7.8)	ND
Section c	126.0 (±36.5)	14.0 (±10.8)	10.6 (±7.3)	ND
Section d	91.0 (±18.7)	25.6 (±5.8)	3.3 (±3.0)	ND
Section e	105.6 (±40.2)	18.6 (±7.0)	1.3 (±2.3)	ND
Section f	133.3 (±44.7)	7.0 (±1.0)	11.0 (±9.5)	ND
Section g	161.6 (±28.5)	7.3 (±4.6)	6.0 (±7.8)	ND

[a] Hexaflumuron-treated termites introduced into section 'a'.
[b] ND indicates hexaflumuron levels were not detectable.

distance from the source (section 'a'), and due to the presence of an additional food source (Table 4). In all cases, hexaflumuron levels were highest in sections in which the treatment had been placed (section 'a'), and decreased with increasing distance from the source. Furthermore, hexaflumuron levels were higher per termite in groups that were not provisioned with additional food. A similar trend occurred at day 7, with the exception that there was no significant difference in hexaflumuron levels in termites between sections 'a' through 'c' in the small laboratory groups. Hexaflumuron levels increased dramatically from day 3, levels ranging from approximately 155 to 329 ng per termite throughout all the sections ('a' through 'g'). By 7 days post-exposure, hexaflumuron levels had decreased dramatically to between 5 and 124 ng hexaflumuron per termite. Levels of hexaflumuron were randomly distributed through sections 'a' through 'c'. However, there were significant differences in hexaflumuron presence in the small and large group apparatus. The presence of an additional food source was only significant on hexaflumuron levels in the small laboratory colonies. At day 14 post-exposure, hexaflumuron levels had again dropped in each treatment situation. Again, there was no significant trend in hexaflumuron levels per termite based on sampling location, although there was a significant difference in the small and medium sized groups. Presence of an additional food source was significant only in the medium and large groups, though there was significantly less hexaflumuron per termite in medium-sized groups held without food, while there was significantly more hexaflumuron per termite in large groups held with food.

Hexaflumuron uptake by larval termites and distribution at 7 and 14 days following introduction into laboratory groups are shown in Table 4. Hexaflumuron levels in donor termites were not significantly different for any of the group sizes ($F = 5.12$; $P < 0.51$; $df = 2$; small group) ($F = 1.95$; $P < 0.17$; $df = 4$; medium group) ($F = 1.37$; $P < 0.29$; $df = 6$; large group). At 7 days post exposure, hexaflumuron levels in recipient termites were low compared with donor termites, ranging from 1.3 to 11.6 ng per termite. There was no significant difference in hexaflumuron levels in recipient termites within any size groups ($F = 3.26$; $P < 0.11$; $df = 2$; small) ($F = 1.04$; $P < 0.43$; $df = 4$; medium) ($F = 1.66$; $P < 0.20$; $df = 6$; large). Donor termite distribution was not significantly different at the 7-day sampling period for small and large groups ($\chi^2 = 2.04$; $P < 0.36$; $df = 2$, $\chi^2 = 10.05$; $P < 0.12$; $df = 6$ for small and large groups, respectively). Donor termite distribution was significantly different in medium size groups ($\chi^2 = 11.13$; $P < 0.03$; $df = 4$), with a larger proportion of termites being in sections 'c' and 'd'.

3.5 Movement of hexaflumuron by cannibalism

Table 5 shows hexaflumuron uptake by recipient termites by cannibalism. Hexaflumuron content in dead labeled termites was significantly different, with increasing hexaflumuron levels as the feeding time for donors on treated filter paper increased ($F = 100.81$; $P < 0.0001$; $df = 2$). Within treatments, there was no significant difference in hexaflumuron levels between donor termites presented to starved and non-starved recipients with the exception of the 12-day donor feeding period and the 3-day recipient exposure. Hexaflumuron uptake was significantly different in

Table 5. Hexaflumuron uptake by cannibalism from termites held on a substrate treated with 5 g kg^{-1} hexaflumuron 3, 7 and 12 days and killed. Live termites which were either starved, non-starved, or had sealed mouthparts were provisioned with dead labeled termites and were held for 1, 3 and 7 days

Feeding period (days)	Recipient exposure (days)	Termite status	Hexaflumuron: donor (ng) (±SD)[a]	Hexaflumuron: recipient (ng) (±SD)[b]
3	1	Starved	112.56 (±22.1) c	7.30 (±1.0) a
		Not-starved	165.65 (±35.1) bc	4.50 (±1.2) b
		Closed mouthparts		4.54 (±1.0) b
	3	Starved	113.08 (±34.7) c	8.47 (±1.6) a
		Not-starved	101.32 (±19.2) c	5.25 (±0.9) b
		Closed mouthparts		4.32 (±1.4) b
	7	Starved	104.08 (±34.8) c	10.28 (±1.7) a
		Not-starved	100.89 (±12.6) c	6.08 (±1.3) b
		Closed mouthparts		4.85 (±1.6) b
7	1	Starved	275.01 (±20.2) ab	8.58 (±1.9) a
		Not-starved	232.00 (±92.1) ab	4.16 (±1.0) b
		Closed mouthparts		3.70 (±0.6) b
	3	Starved	253.97 (±70.9) ab	7.21 (±1.1) a
		Not-starved	271.61 (±8.1) ab	3.88 (±0.1) b
		Closed mouthparts		3.48 (±0.9) b
	7	Starved	236.24 (±71.0) ab	13.39 (±3.0) a
		Not-starved	253.70 (±73.1) ab	3.98 (±0.3) b
		Closed mouthparts		4.01 (±1.5) b
12	1	Starved	281.08 (±37.7) ab	8.50 (±1.0) a
		Not-starved	287.47 (±70.7) ab	6.09 (±0.7) ab
		Closed mouthparts		4.78 (±1.3) b
	3	Starved	346.40 (±77.4) a	9.24 (±1.3) a
		Not-starved	289.79 (±58.2) ab	6.44 (±2.0) ab
		Closed mouthparts		5.22 (±0.9) b
	7	Starved	332.60 (±70.1) a	11.36 (±2.0) a
		Not-starved	278.37 (±81.2) ab	6.74 (±2.8) ab
		Closed mouthparts		4.27 (±1.2) b

[a] Means followed by a similar letter within donors indicate no significant difference.
[b] Means followed by a similar letter within treatment for recipients indicate no significant difference. All ANOVAs were performed on log($Y + 1$) transformed data.

recipient termites due to treatment. Recipient uptake was affected by donor termite feeding times ($F = 7.76$; $P < 0.0007$; $df = 2$), with the greatest uptake in recipients held with donors that had fed for 12 days. The amount of hexaflumuron in recipients significantly increased as the time they mixed with dead termites increased ($F = 4.27$; $P < 0.01$; $df = 2$). Termite status was also a significant factor in hexaflumuron uptake ($F = 94.8$; $P < 0.0001$; $df = 2$), in that levels of hexaflumuron were significantly higher in termites that had previously been starved as opposed to those that had not been starved or those with non-functional mouthparts.

3.8 Movement of hexaflumuron by fecal ingestion

Hexaflumuron transfer from fecal material produced by termites held on hexaflumuron-treated filter paper to starved and non-starved termites and termites with non-functional mouthparts is shown in Table 6. Pre-exposure weight of fecal material was 240.1, 232.8 and 137.1 mg for 1-, 3- and 7-day feeding periods, respectively. Average hexaflumuron content was 1.99, 3.03 and 6.0 ng per mg of feces, respectively, for the same feeding periods. Uptake of hexaflumuron by recipients ranged from 0.71 to 7.5 ng per termite. There was a significant difference in values due to exposure time of donors ($F = 15.85$; $P < 0.0001$; $df = 2$), with the greatest hexaflumuron uptake in recipients held with fecal material produced by donors held for 14 days of treatment. Exposure time to fecal material by recipients had no significant effect on uptake ($F = 1.8$; $P < 0.17$; $df = 2$), but the feeding status of the recipient termites did ($F = 73.3$; $P < 0.0001$; $df = 2$). In most cases, uptake of hexaflumuron in fecal material was not significantly different between starved and non-starved termites, but was greater than that of termites having non-functional mouthparts.

3.6 Transfer to reproductives and eggs

Table 7 shows egg production, hexaflumuron levels and successful development of eggs produced by secondary reproductives held on hexaflumuron-treated filter paper. After 8 days on treatment, reproductives produced 13 eggs, seven of which were held. None of these eggs developed into larvae, in contrast with controls in which both eggs collected successfully developed. At day 15, a similar trend was noted. Of 17 eggs produced and 10 held, there was no egg development. In the

Table 6. Hexaflumuron uptake from material from feces produced for 3, 7 and 14 days and exposed to starved termites, non-starved termites and termites with sealed mouthparts for 1, 3 and 7 days

Fecal deposition time (days)	Termite exposure (days)	Termite status	Hexaflumuron/termite (ng) (±SD)[a]
3	1	Starved	4.23 (±0.19) a
		Not starved	4.24 (±0.28) a
		Closed mouthparts	0.71 (±0.23) b
	3	Starved	6.13 (±2.08) a
		Not starved	4.33 (±0.41) a
		Closed mouthparts	0.50 (±0.58) b
	7	Starved	5.45 (±1.21) a
		Not starved	4.84 (±0.72) a
		Closed mouthparts	2.10 (±1.27) b
7	1	Starved	6.78 (±2.68) a
		Not starved	4.73 (±1.34) ab
		Closed mouthparts	2.57 (±0.87) b
	3	Starved	4.46 (±0.19) a
		Not starved	4.04 (±0.21) a
		Closed mouthparts	2.53 (±0.88) b
	7	Starved	4.60 (±0.58) a
		Not starved	4.08 (±0.14) a
		Closed mouthparts	1.92 (±1.62) b
14	1	Starved	5.22 (±0.61) ab
		Not starved	6.21 (±2.92) a
		Closed mouthparts	3.08 (±0.44) b
	3	Starved	5.43 (±0.71) a
		Not starved	5.17 (±1.50) a
		Closed mouthparts	3.36 (±2.03) a
	7	Starved	7.50 (±0.86) a
		Not starved	6.83 (±1.07) a
		Closed mouthparts	3.93 (±1.38) b

[a] Means followed by the same letter do not differ significantly (see text). Factorial and one-way ANOVAs within treatment performed on $\log(Y + 1)$ transformed data. Pre-exposure wt of fecal material was 240.1, 232.8 and 137.1 mg for 1-, 3- and 7-day exposure periods, respectively. Average hexaflumuron content was 1.99, 3.03 and 6.0 ng mg^{-1}, respectively, for treatment periods mentioned above.

Table 7. Egg production, hexaflumuron levels and successful egg development from eggs produced by secondary reproductives held on paper treated with 5 g kg^{-1} hexaflumuron

		Eggs produced	Hexaflumuron/egg (ng)	Eggs held for development	Successfully developed eggs
Day 8	Rep 1	2	9.40	1	0
	Rep 2	6	7.87	3	0
	Rep 3	5	11.15	3	0
	Control	2	N/A	2	2
Day 15	Rep 1	7	7.2	4	0
	Rep 2	7	9.53	4	0
	Rep 3	3	13.0	2	0
	Control	5	N/A	5	3

control, three of five eggs developed into larvae. Hexaflumuron levels ranged from 7.2 to 10.0 ng per egg.

Secondary reproductives showed no mortality over the exposure periods, and no data regarding hexaflumuron uptake by secondary reproductives were collected.

4 DISCUSSION

The successful use of bait requires that a large proportion of the target population receives a toxic dose. Because lethality to target populations from insect growth regulators such as hexaflumuron is more dependent on time and less dependent on dose as opposed to conventional termiticides such as organophosphates or pyrethroids, our hexaflumuron uptake and mortality data suggest that hexaflumuron might indeed be an appropriate but not ideal bait material for the Western subterranean termite. Termites readily consumed the filter paper treated with hexaflumuron, and after 45 days of continuous feeding the mortality approached 100% (Fig 1). Our data were consistent with data published for *R flavipes* (Kollar).[17]

Onset of mortality in that study was more protracted than in our data, but peak levels of hexaflumuron were approximately equal between species. Dose independence was also shown when mortality was observed over several concentrations from 125 to 16 000 mg kg^{-1} for *R flavipes* and for *Coptotermes formosanus* Shiraki.[18] Continuous exposure of termites to serial dilutions of hexaflumuron indicated that, above a minimum threshold exposure (approximately 100 ppm), mortality essentially remained unchanged by virtue of concentration at 9 days post exposure.

Hexaflumuron levels in dead termites may be an indication of a minimum threshold dose necessary to kill *R hesperus*. There was relatively little variation in the amount of hexaflumuron in dead termites (113.2 ±43.2 ng per termite), but levels were always significantly less than those of live termites actively feeding on a treated substrate (Fig 1). Of course, this may be explained because termites stopped eating before death and the active ingredient was subsequently excreted, as was speculated in a similar study.[17] However, in our situation, mortality was not observed unless the minimum dose was maintained until the next molt (8–14 days), and hexaflumuron levels in groups removed from treatment were consistent with the levels in dead termites feeding constantly on a hexaflumuron-treated food source, as shown in Table 1. Uptake and excretion of hexaflumuron just prior to death were not addressed by this study.

Trophallaxis plays a significant role in the movement of hexaflumuron between members of termite colonies. Our data indicated that a significant amount of hexaflumuron is transferred from donor to recipient termites. The time that recipient termites are mixed with donor termites actively feeding on hexaflumuron-treated filter paper is important. In our study, recipients accumulated almost two-thirds of donor hexaflumuron levels after 20 days of donors and recipients being mixed together (Fig 3). If this exposure level is maintained for enough time, mortality of recipient termites is assured (Fig 1). Sheets *et al*[17] observed similar transfer of hexaflumuron by trophallaxis in *R flavipes*. Their data indicated a significant difference in hexaflumuron transfer based on donor to recipient ratios. Our data did not show this (Table 2), however the ratios at which donors and recipients were held together for our transfer experiments ranged from 1:1 to 1:4, whereas their recipient ratios ranged from 1:5 to 1:20. In other studies, trophallaxis has also been shown to be relatively rapid and efficient. Suarez and Thorne[19] found that transfer of a ^{60}Co-labeled feeding substrate ranged from 5 to 30% of the isotope initially acquired by donor termites within 6–12 h for *R flavipes, R virginicus* (Banks) and *Zootermopsis nevadensis nevadensis* (Hagen). Rapid movement of a ^{140}La-labeled bait through colonies of *Mastotermes darwiniensis* (Froggatt) has also been observed.[20] Our data also indicated that transfer from donors to recipients was not a one-time event (Fig 4), but rather a cascading event of repetitive transfers as suggested by others.[19]

Of greater interest is the clearance of hexaflumuron in surviving members of the termite population following treatment and the cumulative mortality post-treatment. We found that clearance rates (half lives) of hexaflumuron ranged from 4.7 to 2.1 days, generally with increased clearance rates being related to increased feeding times on treated substrates. Differences in half-life of hexaflumuron were mostly due to degrees of uptake from increasing the initial exposure. These data are in marked contrast to those presented by Sheets *et al*,[17] who found, for groups of *R flavipes* exposed to 5 mg kg^{-1} hexaflumuron, an average half-life of approximately 9 days. Excretion rates in their study were only based on hexaflumuron clearance following 7 days of contact and feeding. They speculated that the 9-day half-life might be ideal for the movement of active ingredient throughout a colony, because the clearance of hexaflumuron might be slower than the rate at which termites take up hexaflumuron in constant-feeding situations. However, in our situation the relatively short half-life (<5 days) might provide an opportunity for a significant proportion of termites in a colony to successfully clear hexaflumuron before the molt if continuous feeding on a hexaflumuron-treated food source is not maintained or termites not feeding on treated material do not receive regular doses of hexaflumuron by trophallactic exchange. This observation is reinforced by consideration of mortality data during constant feeding and cumulative mortality data post-feeding. In our situation, cumulative mortality at 7 days post-exposure was consistent with that during continuous feeding. Mortality of termites at 14 and 30 days after feeding was not significantly different in most cases. The exceptions were at 3, 5 and 15 days where mortality at 30 days post-exposure was significantly greater, but the mortality rates at these exposure times were probably not biologically significant, as artificial variation in data was added to groups exhibiting no mortality. These data would suggest that consistent feeding on a bait source for a minimum time period of at least 9 days is necessary to achieve significant mortalities in field populations of *R hesperus* because of the relatively rapid rates of hexaflumuron clearance. Unfortunately, hexaflumuron levels in dead termites following mixing and after removal from treatment was not quantified, so absolute minimum threshold levels of hexaflumuron needed to affect mortality post-exposure are not known, although post-mortality levels of hexaflumuron on continuously feeding individuals was approximately 113 ng per termite. as was mentioned previously.

Despite a multitude of field studies, a clear picture of the physical network of subterranean termite galleries and nesting systems remains elusive. The typical perception of a termite colony is often a centralized nest with foraging runways extending

outward, with each colony being a discrete unit in time and space. Early work (primarily on *Coptotermes* spp) suggested colony size might be quite variable, probably as a result of colony age. If fact, destructive sampling of galleries and runways showed that some colonies might cover areas measured in hectares and contain foraging runways exceeding 50 m.[21,22] More recent investigations of termite colony size in North America have been conducted using a non-destructive mark-and-release technique to define termite colonies,[23,24] and variations of this technique have been adopted to determine the effectiveness of baits against subterranean termite in a variety of locations and situations.[25–29] In many cases, gallery and foraging systems have been extensive. In a previous study of *R hesperus* in southern California, marked termites presumed to be from the same colony were found in termite monitoring stations over 170 m apart, implying an extensive subterranean foraging and gallery system.[13] In the light of these findings, our data on movement of hexaflumuron in laboratory colonies become relevant. We have shown that it takes a significant amount of time (up to 14 days) for hexaflumuron to fully diffuse through colonies, and that the spatial distances involved were a significant factor in the amount of hexaflumuron reaching termites furthest removed from treatment. The rate of movement was also hindered by the presence of alternative food sources (Table 3). Hedlund and Henderson[30] found that, as food size and availability increased in laboratory colonies of *C formosanus*, search tunnel volume decreased and food consumption increased, implying more concentrated attack on more abundant food sources. We may be observing the same phenomenon here, in that the presence of additional food sources slows movement of hexaflumuron. Furthermore, from a behavioral point of view, that is the movement of hexaflumuron by trophallaxis from donor termites fed on hexaflumuron filter paper to a naïve colony, hexaflumuron levels were negligible after 7 days of mixing and not detectable 14 days following initial feeding, despite the reasonably good mixing of donor termites throughout the various sized group apparatus (Table 4). Although exogenous variables were kept relatively constant, our laboratory groups might be a poor model for field colonies, as most field situations are probably not strictly linear in nature. It has been noted that there is a wide variety in tunneling geometry among various termite species, with many bifurcations in foraging tunnels and multi-dimensional dispersion of foraging termites.[20] Our data might represent a best-case scenario of hexaflumuron movement by time and distance. A multi-dimensional array of foraging tubes and galleries might further dilute hexaflumuron or slow its rate of movement.

As suggested previously, there have been many reports of successful suppression or elimination of termite colonies. However, most of these studies took place in tropical or southern locations where termites tend to be active year round and foraging is intense.[31] In contrast, relatively few studies have evaluated effectiveness in the northern, more temperate climes or arid environments, where they suggest 'foraging and bait acquisition may be more seasonal and moderate'.[31] Collins[32] suggested that the primary physical factors affecting termite distribution are temperature and moisture. Coastal southern California has a Mediterranean desert climate with summer ground temperatures in some areas exceeding 40 °C and average rainfall less than 36 cm per year. Smith and Rust[33] found survival of *R hesperus* was best in cool temperatures at relatively high humidity and that *R hesperus* avoided temperatures above 40 °C. Presumably, in these temperature extremes, termites would avoid foraging at or near the surface, instead remaining deeper underground. As might be expected, Haagsma and Rust[13] found peak surface foraging times in ground monitoring stations in spring and late summer months. Similar results were experienced in a northern California study.[9] Other authors have also noted seasonal changes in termite foraging in locales with more variable climates.[31] DeMark and Thomas[34] noted variable foraging in bait/monitoring stations outside structures in Pennsylvania and Wisconsin, but noted little change in activity when termites were presented baits in temperature-controlled situations inside structures. Obviously, seasonal patterns in foraging might affect delivery of baits to termite colonies in effect creating a seasonal 'window of opportunity' probability of getting the necessary amount of active ingredient into the colony to suppress or eliminate it. This may be compounded by the relatively low rates of attack on stations or feeding sources in western arid climates. Another factor impeding bait uptake by colonies of *R hesperus* in southern California is fidelity to baits once presented in the field. The Sentricon baiting protocol prescribes removal of monitoring devices (wood stakes) and replacement with bait material once termite activity is detected. Field trial experience has show that very few stations (<21%) are attacked again within two months of bait placement, and of these, only 38% have continued feeding on the bait the following month (Haagsma, unpublished data). In these situations, if the bait is not attacked, bait devices are replaced with monitoring devices and the process begins anew. Given such situations, it is conceivable that, because of poor bait consumption and the relatively quick clearance rates of hexaflumuron, impact on termite colonies is negligible.

In both the cases of cannibalism and consumption of fecal material, there was a significant difference between termites that were starved and those fed *ad libitum*. However, our data showed that hexaflumuron uptake by cannibalism and fecal feeding activities was nominal (Tables 5, 6) and for the exposure times tested would not result in significant mortality of termites so engaged, particularly if said events were relatively random. Admittedly, our studies were

limited in scope as feeding times and exposure times may not be realistic in field colony situations where termites might continuously interact. Interestingly, a small amount of hexaflumuron was transferred to the recipient termites by contact. This mode of transfer should be investigated in greater detail. Bell[35] suggested that organisms enlist various survival strategies when subject to limiting resources such as a lack of normal feeding sources. Survival might enlist an increase in cannibalism or consumption of fecal material.[36] Considering seasonal variation in foraging discussed earlier, available resources might be limited due to extreme abiotic conditions, and subsequent dependence on alternative sources of nutrients such as cannibalism and fecal material consumption may become important. Certainly, if hexaflumuron was significantly incorporated into dead termites or fecal material, some mortality of live termites feeding consistently on these resources might be expected and also affect colony viability. Clearly, these and aforementioned lethal dosages may be important and may explain the slow and final death of a baited colony.

Hexaflumuron is taken up by secondary reproductives as a result of feeding or trophallactic activities, and may be transovarially transmitted to eggs, preventing egg hatch. This phenomenon has been noted in a variety of different insect models. Marco *et al*[37] found topical application of hexaflumuron to the curculionid *Aubeonymus mariaefranciscae* Roudier resulted in significant inhibition of egg hatch. Similar effects of hexaflumuron on egg hatch were noted for the codling moth feeding on treated apples.[38] Other benzyl-ureas seem to have similar effects. Meola *et al*[39] also found significant decrease in cat flea egg hatch when adults were supplied blood treated with several concentrations of lufenuron. Apparent pre-hatch larvicidal effects included deposition of several layers of cuticle as well as effects on the vitelline membrane of the egg itself. Ultrastructure of unhatched larvae and of eggs was not considered in our study. Obviously, successful inhibition of egg viability by sub-lethal doses of hexaflumuron in closed termite colonies might contribute to colony elimination in that increased termite numbers in populations might result increased time necessary for a hexaflumuron treatment to take effect, or an increase in the amount of active ingredient necessary to achieve control. In addition, these termites might exploit alternative, non-treated food sources, allowing colony populations to rebound.

In conclusion, hexaflumuron effectively suppresses or eliminates *R hesperus* when populations actively feed on hexaflumuron-treated baits for an adequate amount of time. Possible solutions to increasing attack rates and bait fidelity might include the use of semiochemical attractants and/or arrestments as suggested by Rust *et al*,[12] a baiting station design maximizing termite return and subsequent foraging, or a bait station design or program to minimize exposure of bait or termites to predators or non-target organisms.

Movement of hexaflumuron via trophallaxis is relatively rapid and efficient. However, as evidenced by our hexaflumuron-movement studies, significant titres of hexaflumuron which might cause mortality take time to diffuse to areas of the colony that are distant from the treatment source. Sub-lethal effects on egg viability are probably important in termite colony control. Clearance of hexaflumuron in *R hesperus* is rapid, and may affect colony survival in situations where bait take in field situations is variable due to the effects of seasonal foraging and where attack rates and bait fidelity are low, particularly in southern California.

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