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Toxicity and horizontal transfer of chlorantraniliprole against the Asian subterranean termite *Coptotermes gestroi* (Wasmann): effects of donor : recipient ratio, exposure duration and soil type

Kok-Boon Neoh,[a†] Jian Hu,[a†] Boon-Hoi Yeoh[a,b] and Chow-Yang Lee[a*]

Abstract

BACKGROUND: The effectiveness of chlorantraniliprole and other insecticides (bifenthrin, fipronil, indoxacarb, imidacloprid and chlorfenapyr) were tested against *Coptotermes gestroi* (Wasmann). Four experiments were conducted: a topical bioassay, a horizontal transfer study, an insecticide bioavailability test and a feeding bioassay.

RESULTS: The topical bioassay showed that chlorantraniliprole was significantly less active to *C. gestroi* at 24 h post-treatment compared with the other insecticides tested. Nevertheless, it is likely that a lesser amount of chlorantraniliprole was required to cause 50% mortality of *C. gestroi* at 7 and 14 days post-treatment. The exposure duration and donor : recipient ratio affect the mortality of recipient termites. Mortality after exposure to chlorantraniliprole in sandy clay was significantly lower than in sand; however, by 14 days, >90% of donor and recipient termites died in both substrates, irrespective of concentration. Fipronil and imidacloprid showed faster action, and high to moderate toxicity to *C. gestroi*. Termite workers also ceased to feed after exposure for 1 h to 50 mg kg^{-1} chlorantraniliprole-treated sandy clay.

CONCLUSION: Chlorantraniliprole demonstrated delayed toxicity at the lowest label rate (50 mg kg^{-1}) in sandy clay. Its slow action will enable greater transfer of toxicant between nestmates, while feeding cessation will promote greater social interaction between healthy and exposed termites.

Keywords: fipronil; imidacloprid; feeding cessation; colony elimination; slow-acting activity; non-repellent insecticide

1 INTRODUCTION

Soil treatment has been used for termite control since 1920.[1,2] Today, non-repellent liquid insecticides such as fipronil (Termidor®), imidacloprid (Premise®), chlorfenapyr (Phantom®) and indoxacarb, which have a delayed mode of action, are gaining popularity. In soils treated with these products, the insecticides are not detectable by termite foragers, so they will forage freely through the treated soil. Most importantly, the exposed termite workers are not killed immediately but instead are able to transfer a lethal dose to other healthy nestmates before they die.[3] This may impact upon the entire termite colony. However, such effects can be highly variable, depending on the physical and chemical properties of the soil and the termiticide used. Termite colony elimination using soil termiticides may be difficult to achieve without precise knowledge about the time required between insecticide exposure and the onset of termite mortality or abnormal insecticide-induced behavioural responses. These factors may depend on insecticide toxicity, horizontal transfer efficiency and bioavailability of the termiticide in various soil types.

Chlorantraniliprole is a novel anthranilic diamide insecticide with the International Union of Pure and Applied Chemistry name 3-Bromo-*N*-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloro-2-pyridine-2-yl)-1H-pyrazole-5-carboxamide. It was developed by DuPont™ Crop Protection and is registered as a reduced-risk pesticide. It has high specific selectivity for insect ryanodine receptors, causing impairment of normal muscle contraction, paralysis and death.[4] This insecticide has been widely used against pests in crop[5,6] and turf grass[7] owing to its excellent non-target organism safety.[8–10] Chlorantraniliprole is a timely candidate to be incorporated into integrated pest management programmes to alleviate development of insecticide resistance.[11,12]

* *Correspondence to: Chow-Yang Lee, Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia. E-mail: chowyang@usm.my*

† *These authors contributed equally to this work.*

a *Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia*

b *Bentz Jaz Singapore Pte Ltd, Singapore*

Chlorantraniliprole is also registered as Altriset® (EPA No. 352–829) for soil treatment to control termites. Many commonly used termiticide treatments induce similar behavioural symptoms: disorientation → ataxia → moribundity.[13] In contrast, termites exposed to chlorantraniliprole become lethargic and walk in slow motion until they become moribund. Prior to death, poisoned termites were found to release proctodeal fluid.[3,13] Hence, chlorantraniliprole might also be available to nestmates via proctodeal trophallaxis, besides incidental contact and grooming.

The focus of this study was *Coptotermes gestroi* (Wasmann) (Blattodea: Rhinotermitidae), which is a highly destructive termite species in South-east Asia. Approximately $US 400 million are spent annually in the region owing to termite damage, most of which is caused by *C. gestroi*.[14] In this study, a topical bioassay using five commercially available insecticides, including chlorantraniliprole, was conducted to determine their toxicity to *C. gestroi*. The efficiency of horizontal transfer of chlorantraniliprole among nestmates under different donor : recipient ratios and exposure times was also evaluated. In addition, the bioavailability of chlorantraniliprole in different soil types was compared with that of fipronil and imidacloprid, which currently are widely used in South-east Asia for termite control. Finally, the feeding activity of termite workers was assessed after they had been exposed to chlorantraniliprole-treated soil at various exposure durations.

2 MATERIALS AND METHODS

2.1 Termites

Termites were collected from in-ground termite monitoring stations at Universiti Sains Malaysia, Minden Campus, Penang, Malaysia (5″ 21°N, 100″ 18°E). Specimens (at least at the third-instar stage) from two field colonies were used in these studies.

2.2 Chemicals and soil types

For the topical bioassay, the following five technical-grade insecticides were used: chlorantraniliprole (96.45%), fipronil (97.7%), indoxacarb (54.8%), bifenthrin (98%) and chlorfenapyr (99.2%). For the other experiments, commercial formulations of chlorantraniliprole (Altriset® 20 SC; DuPont Crop Protection, Wilmington, DE), imidacloprid (Premise® 20 SC; Bayer Environmental Service, Montvale, NJ) and fipronil (Termidor® 2. 5 EC; BASF Corp., Research Triangle Park, NC) were used.

Two substrates were tested in this study. Sandy clay [pH ± SD = 6.04 ± 0.36; % organic matter (OM) ± SD = 6.94 ± 1.01; % sand : silt : clay = 51.37 : 12.68 : 35.95] was obtained from the immediate surroundings of the termite monitoring stations, and sea sand (pH ± SD = 7.21 ± 0.17; % OM ± SD = 0.54 ± 0.04; % sand : silt : clay = 99.99: <0.01: <0.01). Samples were sieved through a 40 mesh and then sterilised in an oven (Heraeus, Hanau, Germany) at 105 °C for 24 h.

2.3 Topical bioassay

For each insecticide tested, a series of 5–10 concentrations diluted with acetone were used; these dilutions caused between 5 and 95% mortality for each insecticide tested. Prior to the bioassay, five sets of 20 worker termites were preweighed to obtain the mean body weight of an individual termite.

For each insecticide tested, the following procedure was used. Twenty-five worker termites were anaesthetised with carbon dioxide (pressure 20 kPa) for 10 s. Each worker was gently handled with soft forceps and was topically treated with 0.5 µL of a given insecticide concentration on the pronotum using a Burkard microapplicator (Burkard Scientific Ltd, Uxbridge, UK). Each treatment was replicated 4 times. The treated termites were kept in a clean disposable petri dish provisioned with moist Whatman No. 1 filter paper under laboratory conditions (26.4 ± 0.2 °C, 63.2 ± 0.6% RH). Mortality was recorded at 24 h post-treatment. The data for each insecticide were pooled and subjected to probit analysis using POLO-PC (LeOra Software, Menlo Park, CA).

2.4 Horizontal transfer study

The effects of donor exposure duration and donor : recipient ratio on toxicant transfer of chlorantraniliprole to untreated *C. gestroi* workers were studied. Prior to the experiment, untreated workers (recipients) were marked by feeding them with filter papers dyed with 0.5% Nile blue A (Aldrich, Milwaukee, WI) for 7 days. For donors, worker termites were allowed to forage freely in a petri dish (15 cm diameter) containing 100 g of freshly prepared chlorantraniliprole-treated sand. The sand was treated with a chlorantraniliprole concentration (100 mg kg^{-1}) that was twofold higher than the label rate, and donor termites were exposed for three different exposure durations (5, 10 and 30 min). The treated workers were added to a petri dish provisioned with a 3.0 × 3.0 cm filter paper that served as the food source at the following five donor : recipient ratios: 1 : 10, 1 : 5, 3 : 10, 2 : 5 and 1 : 2, with 20 termites $group^{-1}$. The termites were kept in polyethylene containers (30 × 37 × 16 cm) and held at 26.4 ± 0.2 °C and >90% RH. Mortality of recipients was recorded daily for up to 21 days. Each test combination was replicated 5 times. Kaplan–Meier analysis was used to estimate the mean survival time of recipient termites. Percentage mortality was subjected to arcsine square-root transformation. A two-way between-groups ANOVA was used to evaluate the effects of the two main factors (i.e. exposure duration and donor : recipient ratio) on recipient mortality. All analyses were performed using SPSS, v.11.0 (SPSS Inc., Chicago, IL). All statistical analyses were performed at $\alpha = 0.05$.

2.5 Bioavailability in two soil types

To evaluate the bioavailability of chlorantraniliprole in various soil types, a simple donor–recipient model was used. Mortality recorded in donors and recipients was used as an indicator of termiticide bioavailability. Concentrations of chlorantraniliprole tested were 5 and 50 mg kg^{-1} for both substrates (i.e. sandy clay and sand). The bioavailability of two other commercial insecticides in the sandy clay substrate was also evaluated: 50 mg kg^{-1} imidacloprid and 60 mg kg^{-1} fipronil (these concentrations were based on the label rate for field application). Control soils were treated with water only. All substrates were kept at 10% moisture content.

Approximately 350 donor termites (~1 g) were allowed to forage freely for 6 h in a petri dish (9.0 cm diameter) containing 25 g of a given freshly prepared treated substrate. The termites were then transferred to clean petri dishes for 30 min to dislodge any soil debris from the termites' body. Recipient termites were dyed as described above.

Twenty donor termites and 20 recipient termites (ratio = 1 : 1) were mixed and introduced into petri dishes (9.0 cm diameter) provisioned with a 3.0 × 3.0 cm filter paper that served as the food source. Mortalities of donors and recipients were recorded at 1, 3, 7, 14 and 21 days post-mixing. Dead termites were not removed from the petri dishes during the experiment. The tests

were replicated 10 times. The percentages of termite mortality in the respective treatments were compared between substrate types on the observation days by Student's *t*-test.

2.6 Feeding bioassay

Worker termites were exposed to termiticide by allowing them to tunnel through Tygon tubing[15] containing 50 mg kg^{-1} chlorantraniliprole-treated sandy clay for various periods of time (i.e. 1, 2, 4 and 8 h). The Tygon tubing (inner diameter 1.0 cm) contained 1 cm of untreated sand (10% moisture content) and 2 cm (for 1 and 2 h tunnelling) or 6 cm (for 4 and 8 h tunnelling) of chlorantraniliprole-treated sandy clay (10% moisture content). For the control, only deionised water was mixed with the sandy clay. Both ends of the Tygon tubing were sealed with rubber stoppers. The tubes were stored under laboratory conditions for 24 h to allow the moisture to become uniformly distributed in the substrate prior to introduction of the termites.

For each experiment, 20 workers were introduced into the top of the tube containing the untreated sand. The tube was held vertically to allow termites to tunnel through the treated sandy clay to reach the bottom of the tube. Exposure times were recorded when termites contacted the layer of treated sandy clay. After a given exposure duration, the termites were tapped out of the Tygon tubing and transferred into a clean petri dish (9.0 cm diameter). The termites were provided with a moist filter paper (9 cm diameter) as the food source. Additionally, 20 termites were directly introduced into a petri dish as the non-tunnel control. All petri dishes were kept in a dark chamber and maintained at 26.4 ± 0.2 °C and >90% RH. At day 3, the experiment was terminated. The filter papers were cleaned and dried at 60 °C for 48 h. The filter papers were scanned (Canon CanoScan LiDE20; Canon Inc., Beijing, China), and the consumed areas were evaluated using AutoCAD v.2006 software (Autodesk Inc., San Rafael, CA). Each treatment (i.e. various exposure times) was replicated 7 times. The percentage of the surface areas of the filter paper that was consumed was calculated and used for statistical analysis.

3 RESULTS

3.1 Topical bioassay

Among the five termiticides tested, chlorantraniliprole was significantly the least active against *C. gestroi* at 24 h post-treatment (Table 1). The LD_{50} value (27.225–88.184 ng termite^{-1}) of chlorantraniliprole at 24 h post-treatment was 327-fold higher than that of bifenthrin and 48-fold higher than that of fipronil. The insecticidal activity of chlorantraniliprole also was significantly lower than that of the other slow-acting insecticides tested (i.e. chlorfenapyr and indoxacarb). The toxicity of the termiticides tested decreased in the following order: bifenthrin > fipronil > chlorfenapyr > chlorantraniliprole. On the other hand, indoxacarb showed equal toxicity to bifenthrin, fipronil and chlorfenapyr, as evident from the overlapping of the fiducial limits.

3.2 Horizontal transfer study

As exposure duration increased, the mean survival times of the recipients grew significantly shorter (Table 2). This was particularly true when recipients were introduced to 30 min chlorantraniliprole-exposed donors with donor : recipient ratios of 3 : 10, 2 : 5 and 1 : 2 (range 9–10 days). Exposure duration ($F_{2,72}$ = 21.988, $P < 0.05$) and donor : recipient ratio ($F_{5,72}$ = 12.028, $P < 0.05$) affected the mortality of recipient termites. However, no evidence indicated that the interaction between the exposure duration and the donor : recipient ratio affected recipient mortality ($F_{8,72}$ = 1.265, $P > 0.05$).

3.3 Bioavailability in two soil types

Sand treated with chlorantraniliprole at 5 and 50 mg kg^{-1} caused significant mortality ($P < 0.05$) to donor and recipient termites compared with treated sandy clay at days 3 and 7 (Fig. 1). At 50 mg kg^{-1}, exposure to chlorantraniliprole-treated sand resulted in mean high mortality (± SE) values of 76.0±4.5% and 54.5±6.1% for donor and recipient termites respectively during the first 3 days. Low mortality (<32.5% mortality) was recorded with sandy clay at doses of 5 and 50 mg kg^{-1} chlorantraniliprole for both donor and recipient termites after 7 days of holding them together. However, by day 14, more than 90% of donor and recipient termites had died, irrespective of concentration and soil type. As predicted, the rise in termite mortality rate was paralleled by increments in concentrations tested. However, mortality among donors and recipients exposed to treated sandy clay did not differ significantly when compared within a given observation day ($P > 0.05$) (Fig. 1).

For the other termiticides tested, sandy clay treated with fipronil provided 100 and 73.5% mortality of donors and recipients respectively at day 1 (Fig. 2). Sandy clay treated with imidacloprid killed approximately 50% of both donors and recipients at day 1, followed by more than 70 and 90% mortality at days 3 and 7 respectively.

3.4 Feeding bioassay

Areas of filter papers consumed by treated termites for the four exposure durations tested were negligible (mean consumption areas ± SE = 0.4 ± 0.4 mm^2, n = 28) and significantly lower by comparison with pooled data from the controls. The mean consumption area of the 28 treatment controls was 30.1±6.0 mm^2 (T = 6.009, df = 54, $P < 0.001$), and that of the non-tunnel control was 27.6 ± 4.1 mm^2 (n = 7, T = 14.084, df = 33, $P < 0.001$).

Table 1. Toxicity of five termiticides against *Coptotermes gestroi* at 24 h post-treatment

Insecticide	n	LD_{50} (ng g^{-1}) (95% FL)	LD_{50} (ng insect^{-1}) (95% FL)	LD_{50} (ng mg^{-1} body weight) (95% FL)	Slope (± SE)	χ^2 (df)
Chlorantraniliprole	900	83.837 (54.509–76.368)	41.919 (27.255–88.184)	17.650 (11.476–37.130)	0.930(±0.105)	18.361 (7)
Bifenthrin	500	0.256 (0.096–0.356)	0.128 (0.048–0.178)	0.054 (0.020–0.075)	3.086(±0.820)	0.5424 (2)
Chlorfenapyr	500	20.020 (11.834–30.518)	10.010 (5.917–15.259)	4.215 (2.491–6.425)	2.039(±0.142)	5.622 (3)
Fipronil	500	1.730 (1.570–1.934)	0.865 (0.758–0.967)	0.364 (0.319–0.407)	4.633(±0.536)	0.5684 (1)
Indoxacarb	500	8.084 (0.232–19.186)	4.042 (0.116–9.593)	1.702 (0.049–4.039)	1.319(±0.492)	0.4116 (3)

Table 2. Mean survival time of recipient *Coptotermes gestroi* workers after being exposed to chlorantraniliprole-treated donor workers at various exposure durations and donor : recipient ratios

Donor: recipient ratio ($n = 20$)	Mean survival time (h) (95% CL)		
	5 min exposure	10 min exposure	30 min exposure
Control	437.880 (411.353–464.407)		
1 : 10	366.360 (336.901–395.819)	344.880 (319.051–370.709)	323.640 (295.498–351.782)
1 : 5	367.560 (377.162–397.958)	368.280 (339.857–396.703)	318.720 (297.952–339.488)
3 : 10	340.560 (309.467–371.652)	350.195 (317.060–383.333)	250.680 (225.313–276.047)
2 : 5	324.600 (290.160–359.040)	341.520 (309.837–373.203)	253.320 (232.997–273.643)
1 : 2	361.200 (329.316–393.084)	345.240 (310.132–380.348)	226.200 (205.947–246.453)

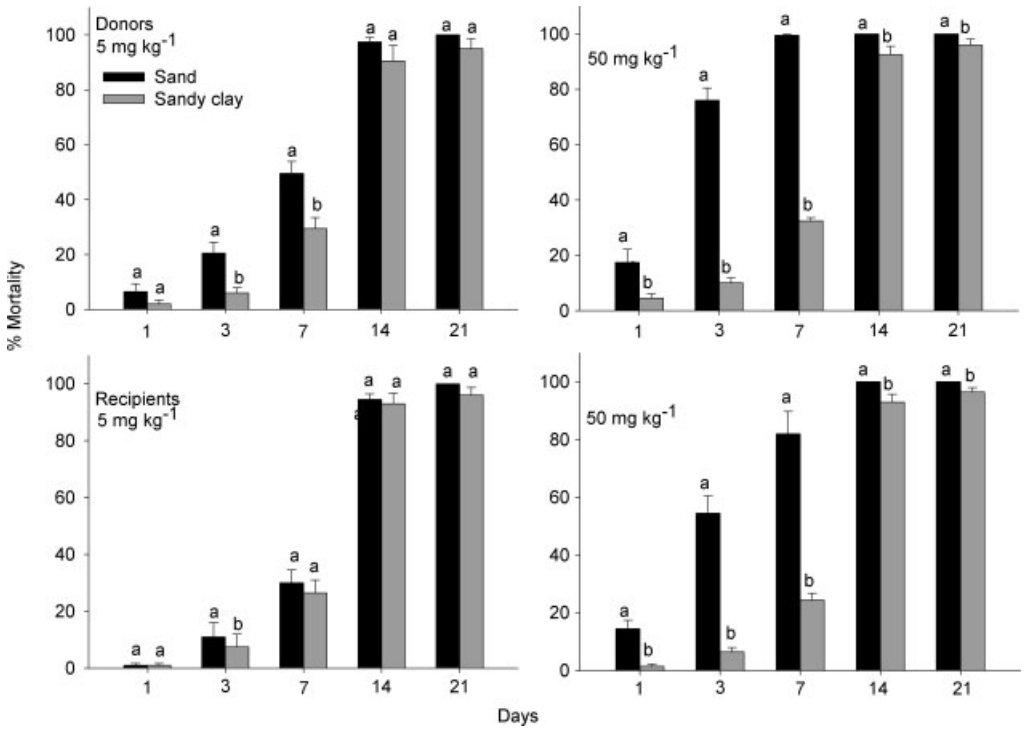

Figure 1. Mean (± SEM) mortality of donor and recipient *Coptotermes gestroi* for sand and soil treated with 5 and 50 mg kg^{-1} chlorantraniliprole. Means followed by a different letter within the same day indicate a significantly difference between substrates ($P < 0.05$, Student's *t*-test).

4 DISCUSSION AND CONCLUSION

It is claimed that several termiticides (e.g. fipronil, imidacloprid and chlorfenapyr) are slow-acting insecticides, and that only small amounts are required to kill both donor and potential recipients at 5–7 days post-treatment, which therefore increases the likelihood of horizontal transfer among termites.[16–18] However, this scenario may not always be true if termites are highly susceptible to a termiticide with low LD_{50} and early intoxication at early treatment. In the present topical bioassay it was found that approximately 42 ng chlorantraniliprole termite^{-1} is required to kill 50% of test insects at day 1, which indicates that *C. gestroi* is less susceptible to chlorantraniliprole by comparison with the other tested termiticides (Table 1). Mao *et al.*[19] reported LD_{50} values of 0.98 and 0.81 ng chlorantraniliprole termite^{-1} for *Coptotermes formosanus* Shiraki at days 7 and 14 respectively. Thus, clearly there is a delayed action of this chemical. Considering that *C. gestroi* workers are about 30% smaller than *C. formosanus* workers, the amount of chlorantraniliprole required to cause 50% mortality of *C. gestroi* at days 7 and 14 would have been much lower compared with *C. formosanus*. The present hypothesis that the likelihood of horizontal transfer might be higher for chlorantraniliprole than for the other insecticides tested is corroborated by the fact that *C. gestroi* is less susceptible to chlorantraniliprole than the other insecticides for at least the first day of post-treatment, which would allow the poisoned termites to return to their colony and distribute the lethal dose to other nestmates.

The degree of toxicity of insecticides to termites varies considerably among species. For example, Spomer *et al.*[20] reported that chlorantraniliprole was topically more toxic to *Reticulitermes flavipes* (Kollar) workers than indoxacarb. In contrast, it was found that a mere 4 ng of indoxacarb was sufficient to cause 50% mortality at 24 h, which was tenfold lower than the amount of chlorantraniliprole required to achieve the same effect. In another study, fipronil was about 187 times more toxic to *Reticulitermes hesperus* Banks than chlorfenapyr,[17] whereas the present authors found that fipronil was only 11.6 times more toxic to *C. gestroi* than chlorfenapyr. Ibrahim *et al.*[21] reported that 2.93 ng of fipronil was required to cause 50% mortality of *C. formosanus* at 24 h, whereas

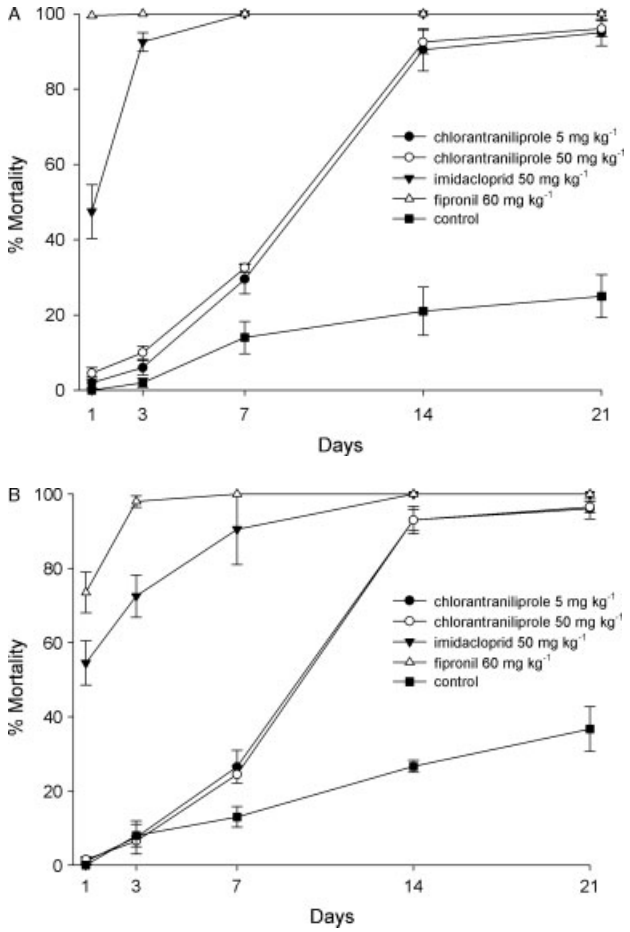

Figure 2. Mean (± SEM) mortality of donor (A) and recipient (B) *Coptotermes gestroi* for soil treated with 5 and 50 mg kg^{-1} chlorantraniliprole, 50 mg kg^{-1} imidacloprid, 60 mg kg^{-1} fipronil and water (control).

the present authors found that only 0.9 ng of fipronil was required to achieve the same effect for *C. gestroi*.

The reason for these differences is largely unknown, but they may be due to size differences and differences in the insecticide susceptibility of the termites. Valles and Woodson[22] detected an inverse correlation between the weight of *C. formosanus* and chlorpyrifos tolerance. *C. gestroi* (2.2–2.5 mg) weighs less than *C. formosanus* (2.69–3.66 mg),[22] thus, it is likely that *C. gestroi* is more susceptible to insecticides than *C. formosanus*. Valles and Woodson attributed the correlation between termite size and insecticide tolerance to the increased activity of the gut symbiont in larger individuals that may, to a certain extent, be involved in insecticide detoxification.[22] Several studies have documented varying levels of insecticide metabolism (detoxification enzyme activities) in different termite species. Cytochrome P450 content and aldrin expoxidase and methoxyresorufin *O*-demethylase activities in *R. flavipes* were lower than those in *C. formosanus*.[22,23] Similarly, the cytochrome P450 content of *Coptotermes acinaciformis* (Froggatt) was 2–4-fold lower than that of *C. formosanus*.[24] These differences in detoxification enzyme activities likely cause different susceptibility of termites to certain insecticides.

In the present study, all the substrates were prepared at 10% moisture content, whereas previous studies used 20% moisture.[18] This difference may have affected the tunnelling activity of worker termites as well as the amount of insecticide that was picked up and carried by the workers.[25]

The timing of when mortality and abnormal behaviour followed by insecticide exposure begin is key to achieving termite colony elimination using soil treatment with termiticides.[3] It is important that the treated termites (donors) pick up the lethal dose from treated sites and transfer it to other nestmates before dying or before abnormal behaviour that may deter termite foraging activities. The transfer efficiency of termiticide among nestmates is associated with the insecticide exposure duration, the donor : recipient ratio and bioavailability of the termiticide in the soil. The present data suggest that *C. gestroi* mortality is dependent on exposure duration, as a significantly shorter mean survival time is observed in the termites exposed to chlorantraniliprole for 30 min versus 5 and 10 min in the horizontal transfer test. The result agrees with most previous studies. For example, Rust and Saran[17] reported that the amount of chlorfenapyr taken up by donors and transferred to recipients was significantly greater when the donors were exposed for 4 h as opposed to 1 h. Saran and Rust[18] also documented a direct linear relationship between fipronil uptake and exposure time when *R. hesperus* workers were exposed to sand treated with 0.5, 1.0 and 5.0 ppm of fipronil. Quarcoo *et al*.[3] found that in *R. flavipes* the onset of termite mortality appeared faster when termites were exposed to indoxacarb for longer durations. Although substantial data support this relationship, Shelton and Grace[26] reported inconsistent results for mortality of *C. formosanus* exposed to 1 ppm fipronil and imidacloprid at 1, 3, 6, 12 and 24 h.

The present statistical analysis suggested that the donor : recipient ratio is one of the main factors affecting the mortality of recipient termites. Significant differences in mean survival time were observed when the recipient termites were exposed to 3 : 10, 2 : 5 and 1 : 2 donor : recipient ratios at 30 min exposure. Hu *et al*.[27] reported that doses of >20 ng indoxacarb termite^{-1} at donor : recipient ratios of 1 : 1 and 1 : 4 caused significant mortality in *C. formosanus*. However, the correlation between mortality and ratio was weak in their study. For fipronil, a donor : recipient ratio of at least 2 : 5 was needed for *C. formosanus* either topically treated with 2.5 ng fipronil worker^{-1} or exposed to sand treated with 0.5–10 ppm (wt : wt) in order to achieve >90% mortality.[21,28]

In the present study, the simple donor : recipient bioavailability test showed that the mortality of both donor and recipient termites increased in parallel with the termiticide concentration present in the treated sand. Rust and Saran[17] and Shelton *et al*.[29] also reported that the amount of insecticide taken up by donor termites and transferred to recipient termites is concentration dependent. Hu *et al*.[27] observed a similar phenomenon when *C. formosanus* workers were exposed to 0, 10, 20, 50, 100 or 200 ng indoxacarb donor^{-1}. However, this could not be shown in the present bioavailability test. Substantial variation in the biological availability of chlorantraniliprole was observed for the substrates tested. High mortality was recorded at days 3 and 7 for the sand substrate, whereas the opposite was true for the sandy clay substrate. Spomer *et al*.[20] used the ratios obtained by dividing LD_{50} values by LC_{50} values of each termiticide mixed with a particular soil type to determine the bioavailability of termiticide. In the study of *R. flavipes*, chlorantraniliprole was less bioavailable to the termites in the soils tested (the bioavailability ratio at 48 h was 0.018–0.049) compared with indoxacarb (0.117–0.352).[20] Soil contains a high colloidal fraction that greatly increases hydrogen bonding sites to hydrophobic insecticides.[30] The present results correspond well to other studies showing that the bioavailability of insecticides in sand (low clay soil) generally is higher and inversely

Table 3. Characteristics of termiticides used in the bioavailability test

Chemicals	Water solubility (mg L^{-1})	Octanol/water partition coefficient (K_{ow})	Organic carbon partitioning coefficient (K_{oc})	Henry's law constant (atm m^3 mol^{-1})
Chlorantraniliprole[52]	1.023 (deionised water) at 20 °C	589	328	3.1×10^{-15}
Fipronil[53]	1.9 (pH 5)–2.4 (pH 9) at 20 °C	825	1.00×10^{4}	3.7×10^{-5}
Imidacloprid[54]	610 (deionised water) at 20 °C	3.715	249–336	1.7×10^{-15}

associated with the percentage of organic matter present.[20,31–33] In the present study, the mortality of termites did not appear to be concentration dependent, at least between 5 and 50 mg kg^{-1}, for chlorantraniliprole-treated sandy clay.

Insecticide properties also play a pronounced role in determining the bioavailability of termiticides. Of the three insecticides tested (Table 3), chlorantraniliprole and fipronil have lower water solubility and a higher octanol/water partition coefficient (K_{ow}) than imidacloprid. This suggests that chlorantraniliprole and fipronil are more hydrophobic (or lipophilic) than imidacloprid and thus are more likely to be adsorbed to soil particles. A field simulation trial showed that fipronil had slow dissipation and low mobility in red earth loam.[34] In contrast, imidacloprid is highly soluble in water and has a low K_{ow}, suggesting that it may be less tightly bound to soil compared with chlorantraniliprole and fipronil but precipitate at the soil surface. Although imidacloprid has very little intrinsic toxicity against termites,[19] continuous uptake of imidacloprid from soil by termites may result in significant mortality. Fipronil-treated soil, on the other hand, resulted in high termite mortality beginning on day 1 in the present study. Fipronil was highly toxic to *C. gestroi*, where uptake of a mere 0.9 ng was sufficient to kill 50% of test insects in 24 h. Thus, fipronil in the treated soil might be the most active against *C. gestroi*, in spite of its high affinity to soil.

One of the most intriguing aspects of this study is the finding of slow activity of chlorantraniliprole in sandy clay at the lowest label rate (50 mg kg^{-1}). Quantifying insecticide efficacies is always difficult owing to varying methods and concentrations employed. Nevertheless, in the present bioavailability study, delayed mortality (<35%) of both donors and recipients was observed in the chlorantraniliprole-treated sandy clay even at 7 days post-exposure. Chlorantraniliprole is a delayed toxicant, with the likelihood that an increasingly lower amount of chlorantraniliprole will be required to kill termites as days after treatment increase. Thus, chlorantraniliprole might accumulate, and a cascading effect may occur owing to repetitive transfer.[27,35,36] Furthermore, extensive tunnelling activities were observed in *C. gestroi* workers, with 40% mortality recorded at day 7 in sand treated with 10 ppm chlorantraniliprole.[37] These observations, together with the results of the present study, suggest that *C. gestroi* workers can transfer the lethal dose of chlorantraniliprole to nestmates, which in turn can lead to colony elimination in due course.

The antifeedant activity of chlorantraniliprole is well documented against agricultural insect pests.[38] Hannig *et al.*[38] reported that feeding cessation occurred in *Plutella xylostella* (Linn.), *Trichoplusia ni* (Hübner), *Spodoptera exigua* (Hübner) and *Helicoverpa zea* (Boddie) larvae in less than 25 min after being exposed to 167 ppm chlorantranilirpole. In this study, *C. gestroi* workers ceased to feed after exposure to 50 mg kg^{-1} chlorantraniliprole-treated sandy clay for a minimum of 1 h. The present results are consistent with those of Yeoh and Lee,[37] who reported low wood consumption by chlorantraniliprole-treated termites. In this situation, these termites likely were starved before dying. These starved treated termites would probably be attended by healthy workers and might undergo active trophallaxis and grooming from healthy nestmates. This would indirectly help spread chlorantraniliprole via grooming, casual contact or oral ingestion [trophallaxis (proctodeal) and cannibalism].[27] Such phenomena are not uncommon. Hu *et al.*[39] reported that fipronil-treated *R. flavipes* workers were taken care of by nestmates instead of being isolated. If this scenario occurs in the field, a colony-level impact would be expected. Undoubtedly, this warrants further investigation. This behavioural response may also reduce the damage that occurs to termite-infested wooden structures.

The present study indicated that, unlike chlorantraniliprole, the two commercially available termiticides, fipronil and imidacloprid, exhibited high to moderate toxicity to *C. gestroi* in a short amount of time. They killed >50% of test insects at 24 h post-exposure. In another study, *C. gestroi* completed only 20% of cumulative tunnelling distance after 7 days of exposure to 10 ppm fipronil in the glass tube test.[37] *C. formosanus* workers were significantly less mobile an hour after being exposed to 1 ppm treated sand and were only able to tunnel approximately 0.3 cm.[18] This fast action probably explains why fipronil managed to provide a reduction in *R. flavipes* activity for only 2 m from the treated zone in a field evaluation.[40] Saran and Rust[18] suggested that the horizontal transfer effect among termites treated with fipronil is limited to the vicinity of the treated sites. Similarly, the fast rate of intoxication of termites and the limited tunnelling activity observed for imidacloprid[16,41,42] may reduce the transfer efficiency of this termiticide among non-exposed nestmates outside the treated zone.[43–45] Thus, imidacloprid treatment is unlikely to cause colony elimination. Coupled with the rapid rate of loss of imidacloprid activity under field conditions,[46–48] these traits might lead to termite re-infestations.[49,50] Conversely, the opposite was found by Parman and Vargo,[51] who successfully eliminated or suppressed colonies of *Reticulitermes* spp. using soil treatments with imidacloprid.

In summary, this study demonstrated that chlorantraniliprole may work effectively against *C. gestroi* to achieve colony elimination, as termites may have sufficient time to pick up the lethal dose and transfer it among nestmates before they die. Furthermore, chlorantraniliprole is selective for insect ryanodine receptors and causes feeding cessation in termites. This effect would allow social interactions to occur between starved poisoned termites and healthy termites, which may facilitate the spread of the toxicant among colony members.

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