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Toxicity of Etofenprox to the Formosan Subterranean Termite, *Coptotermes formosanus*

by

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Toxicity of Etofenprox to the Formosan Subterranean Termite, *Coptotermes formosanus*[1]

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ABSTRACT
Etofenprox is an insecticide with exceptionally low mammalian toxicity. When applied topically to workers of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, the LD_{50} of etofenprox of 4.78 to 6.59 was in the same range as termiticides currently in use. Etofenprox was a relatively quick acting termiticide, with most mortality occurring within 24 hours. In both vertical and horizontal laboratory tunneling assays, etofenprox concentrations as low as 10 ppm reduced termite penetration of crushed coral and silica sand, and consistent protection was achieved with concentrations of 100 ppm or greater. Termites avoided contact with the treated substrate and mortality was generally low, indicating that most termites did not contact the substrate often or long enough to acquire a lethal dose. These results, and the low mammalian toxicity of etofenprox, indicate that it may be useful both as a contact insecticide for injection into active termite infestations within structures, and as a soil insecticide to prevent termites from entering structures. Although more research is required to establish the longevity of etofenprox in the soil under field conditions, our laboratory results indicate that 100 ppm in the soil is a reasonable minimum target concentration for field application.

KEYWORDS: Soil insecticide, termite control, Rhinotermitidae, Isoptera

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1 INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) is generally the most damaging termite wherever it occurs. In Hawaii, it not only causes more economic losses than any other termite but is the most damaging pest in the State (Tamashiro et al. 1990). The cost of preventing and controlling infestations and repairing the damage caused by this pest is conservatively estimated at more than $100 million a year.

Over and above the economic damage, however, additional hazards to people and the environment are presented by attempts to stop this termite. At present, the remedial control of these pests involves injecting or spraying relatively toxic chemicals directly into and/or around infested buildings. This has the potential of exposing both applicators and building occupants to significant hazards and posing a threat to the environment.

Unfortunately, at present, there are no effective alternatives to chemical insecticides for remedial termite control. Since chemicals must be used, it is desirable to those which are least toxic to humans and the environment.

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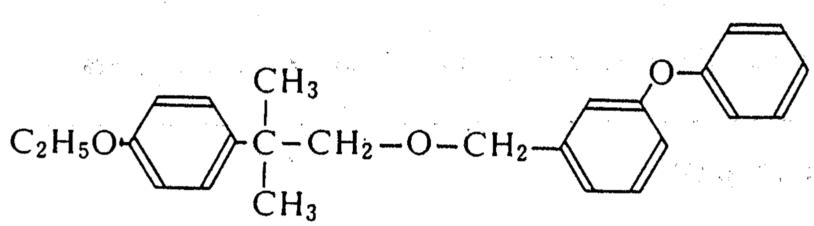
[1]Research results reported here have been submitted for publication in a refereed journal.

In the search for alternatives to the currently used termiticides, etofenprox (2-[4-ethoxyphenyl]-2-methylpropyl 3-phenoxybenzyl ether) (Mitsui Toatsu Chemicals, Inc., Tokyo, Japan) was found to have desirable characteristics. Etofenprox is related to synthetic pyrethroids but is composed of only carbon, hydrogen, and oxygen. It does not have the halogen and/or CN group common to the pyrethroids (Figure 1).

The mammalian toxicity of etofenprox is very low. In fact, the acute LD_{50} of etofenprox to mice, rats and dogs, was lower than the tolerable limits using oral, dermal, subcutaneous, or intraperitoneal routes of administration, and could therefore not be determined for these animals (Udagawa 1988). However, etofenprox is highly toxic to many insects.

Our study was initiated to determine (i) the toxicity of etofenprox to the Formosan subterranean termite (ii) its potential as a soil termiticide and (iii) its potential for use in remedial termite control.

Figure 1. Structure of the insecticide etofenprox.



2 TOPICAL TOXICITY

Toxicity tests in which technical etofenprox was dissolved in acetone and topically applied to the dorsum of *C. formosanus* workers were conducted in the laboratory.

2.1 Materials and methods

Technical etofenprox (334.7 mg) (2-[4-ethoxyphenyl]-2-methylpropyl 3-phenoxybenzyl ether) (Mitsui Toatsu Chemicals, Inc., Tokyo, Japan) was dissolved in 100 ml of acetone to make a stock solution containing 3.347 mg etofenprox per ml. This stock solution was refrigerated, and aliquots were used for all dosage-mortality tests.

Three field colonies of *C. formosanus* located on the Manoa campus of the University of Hawaii were used in the tests. All three colonies have been monitored for at least 15 years, and all were considered very active. Termites were collected immediately before their use in laboratory assays to ensure that they were healthy and vigorous.

Termites were collected and extracted from wooden boxes set on the soil surface in a trapping technique described by Tamashiro et al. (1973). Five groups of 10 workers (pseudergates, or undifferentiated individuals older than the third instar) from each colony were weighed to obtain the average worker weight for each colony. Insecticide dosages were calculated on the basis of amount of toxicant per unit weight of termite.

Before treatment, the termites were anesthetized by a brief exposure to carbon dioxide. The anesthetized termite was carefully held by the head capsule with a soft tweezer and 0.5 microliters of the test solution was placed on the dorsal part of the termite. Topical applications were performed with a Model 1002 Micro-jector (Houston Atlas Inc., Houston, Texas).

After topical application of insecticide, each termite was held for a short period until the acetone evaporated. It was then placed in a Petri dish containing a Whatman No. 1 filter paper moistened with distilled water to provide moisture and food. Treated termites were held in an incubator at 29° C, and checked daily for mortality. All dead termites were removed from each dish at each mortality count.

Tests were repeated at least twice, with five concentrations of etofenprox and acetone controls. Three replicates of 10 termites each were treated with each concentration of etofenprox for each test. Observations were terminated after 4 days, and data subjected to probit analysis (Finney 1962) using a commercial computer program (SAS Institute 1987).

2.2 Results and discussion

Although the topical toxicity of etofenprox to workers from each termite colony (Table 1, LD_{50} = 6.59, 5.38, 4.78 µg/g) was slightly less than that described by Khoo and Sherman (1979) for chlorpyrifos (LD_{50} = 2.33, 3.18), or by Su and Scheffrahn (1990) for chlorpyrifos (3.39), fenvalerate (2.14) and permethrin (2.03) , it is in the same range as these insecticides. Thus, etofenprox is approximately as toxic to the Formosan subterranean termite as termiticides currently in use.

However, termites from the three colonies differed slightly in their susceptibility to etofenprox. Individuals from the Miller colony had the highest LD_{50} (Table 1) and were significantly less susceptible to etofenprox ($P = 0.05$) than workers from the other two colonies. The basis of this differential susceptibility, which was not suggested by our preliminary tests, is not known, although colony differences have been noted in other studies (Su and LaFage 1984). Termites from the Miller colony were larger than those of the other two colonies, and size may be correlated with insecticide susceptibility, although dosages in this study were calculated on the basis of body weight. A more important variable than mass alone may be the smaller surface-to-volume ratio of larger individuals, which could reduce insecticide distribution over the cuticle following topical application and result in less, or slower penetration of the cuticle.

Countering any reduced susceptibility due to larger body size is the possible correlation of large worker size in a termite colony with reduced vigor due to colony senescence, as noted by Shimizu (1962) and Nakajima et al. (1964). In fact, increasing average body weight in the Pope colony over the past 15 years has been associated with an apparent slow reduction in the colony population size, generally supporting this correlation (Grace, Tamashiro and Yamamoto, unpublished observation). This could explain why workers from the Pope colony, although equivalent in size to those from the Miller colony, were as susceptible to etofenprox as the smaller individuals found in the Andrews colony. However, differences in body size which do not appear to be attributable to senescence, but rather to some intrinsic colony characteristic, are also frequently observed among *C. formosanus* field colonies. In our 15-20 years of observations, individuals from the Miller and Andrews colonies have differed in size fairly consistently, with no significant increase in the size of the Miller colony workers over this period. At the time of this study, workers collected from all three colonies appeared active and vigorous, with none of the mottled white appearance that indicates urate retention and is often seen in sluggish laboratory termite colonies.

Table 1. Toxicity of etofenprox topically applied to Formosan subterranean termite workers from three field colonies on the Manoa campus of the University of Hawaii.[a]

Colony	Mean worker wt. (mg)	LD_{50} (µg/g)	95% fiducial limits	LD_{95} (µg/g)	95% fiducial limits	Slope
Miller	4.51	6.59	6.10-7.13	11.06	9.71-13.68	7.31
Pope	4.48	5.38	4.93-5.81	8.83	7.87-10.68	7.65
Andrews	3.11	4.78	4.29-5.21	8.63	7.52-10.94	6.52

[a]Colony names refer to adjacent buildings on the Manoa campus.

The small difference in susceptibility among the three colonies was significant only with respect to their LD_{50} values, and was not observed with the LD_{95}, dosages. There were also no significant differences in the slopes of the dosage-mortality curves among the colonies. However, the slopes for etofenprox obtained in our experiments (Table 1), were steeper than those for chlorpyrifos, permethrin and fenvalerate obtained by Su and Scheffrahn (1990). The slopes observed by Su and Scheffrahn (1990) tended to be flat, probably reflecting the heterogeneity of their test population, which consisted of termites from several colonies. These authors' slope for chlorpyrifos was 0.613, while Khoo and Sherman (1979) obtained slopes of 9.979 to 11.571 in their determinations.

Table 2. Daily percentage mortality of *Coptotermes formosanus* workers from the Miller colony (mean individual weight of 4.51 mg) after topical treatment with etofenprox.

Dosage µg/g	Daily percentage mortality Day 1	Day 2	Day 3	Day 4	Total % mortality
10.2	80.0	13.3	3.3	0.0	96.7
8.2	50.0	23.3	0.0	6.7	76.7
6.6	23.3	6.7	0.0	6.7	36.7
5.1	16.7	6.7	3.3	0.0	23.3
4.1	6.7	0.0	3.3	0.0	10.1
0.0	0.0	3.3	3.3	6.7	13.3

Table 3. Daily percentage mortality of *Coptotermes formosanus* workers from the Pope colony (mean individual weight of 4.48 mg) after topical treatment with etofenprox.

Dosage µg/g	Daily percentage mortality Day 1	Day 2	Day 3	Day 4	Total % mortality
10.3	76.7	20.0	3.3	0.0	100.0
8.3	86.7	3.3	0.0	0.0	90.0
6.7	66.7	6.7	0.0	0.0	73.3
5.1	33.3	13.3	3.3	0.0	50.0
4.1	3.3	10.0	3.3	0.0	16.7
0.0	0.0	0.0	0.0	0.0	0.0

Table 4. Daily percentage mortality of *Coptotermes formosanus* workers from the Andrews colony (mean individual weight of 3.11 mg) after topical treatment with etofenprox.

Dosage µg/g	Daily percentage mortality Day 1	Day 2	Day 3	Day 4	Total % mortality
9.6	90.0	6.7	0.0	0.0	96.7
7.4	86.7	0.0	0.0	3.3	93.3
6.0	56.7	10.0	0.0	3.3	70.0
4.8	46.6	0.0	0.0	0.0	46.7
3.7	20.0	3.3	3.3	0.0	26.7
0.0	0.0	0.0	0.0	0.0	0.0

The steeper slope means that etofenprox may require a lower dosage to attain the LD_{95} and LD_{99} levels than termiticides with flat slopes. This is significant in field use, since LD_{99} or better is the goal of such applications. As previously mentioned, etofenprox also has the advantage of reduced acute toxicity to mammals and fish, in comparison to termiticides in current usage.

As with currently used termiticides, etofenprox was a relatively fast acting toxicant. As is apparent from the daily mortality patterns for individuals from each colony (Table 2, 3, and 4), most termite mortality occurred within the first 24 hours after treatment. Overall, the results of our topical toxicity tests indicate that etofenprox is similar in both speed of action and toxicity to currently used termiticides, and sufficiently toxic for use against the Formosan subterranean termite.

3 TUNNELING ASSAYS

Tests were conducted to determine the concentration of etofenprox (ppm) required to stop termites from penetrating treated substrates, and to determine whether etofenprox prevented penetration through toxicity or repellency, or a combination of both factors. Thus, we assessed the potential of etofenprox as a barrier in the soil to prevent termite infestation. These assays also provided information on the potential of etofenprox for remedial termite control.

3.1 Materials and methods

Tunneling assays were conducted using two substrates, crushed coral sand and silica sand, and two different methods of exposure. Locally-purchased crushed coral sand was washed, oven dried and sifted to pass a U.S. 14-mesh (1.4 mm) sieve, with pH = 9.45 as determined by the method of Chapman and Pratt (1961). Silica sand (Silica S151 [Fine Granular Silicon Dioxide], Fisher Scientific, Fair Lawn, New York), with pH = 7.34, was sifted to pass a U.S. 40-mesh (0.425 mm) sieve and was stopped by a U.S. 100-mesh (0.15 mm) sieve. Termites were exposed to the substrates in tunneling assays consisting of either (i) a glass tube (Tamashiro et al. 1987, 1990a; Grace et al. 1993), or (ii) a "sandwich" formed by two microscope slides (Grace 1991; Grace et al. 1992). The tube test mimics vertical penetration by termites from beneath a building, while the sandwich test mimics horizontal tunneling by termites from the outside perimeter of the building.

In the tube test, (Figure 2) 4 cm of the test substrate was sandwiched between two pieces of 8% agar in an upright glass tube with an internal diameter of 13 mm. The tube, which had an ID of 16 mm, was capped top and bottom with metal faucet buttons as described by Tamashiro et al. (1987). Crumpled paper toweling was provided at either end of the tube as food, and 150 termites (131 workers and 19 soldiers) were placed in the bottom of the tube, and the ends of the tube sealed with metal caps. Termite mortality and the total vertical distance tunneled upward through the test substrate were recorded after four days of incubation in an unlighted temperature cabinet (29 ± 0.5 °C). Percentage mortality and penetration data were transformed by the arcsine of the square root and subjected to analysis of variance (ANOVA), and means significantly different at the 0.05 level were separated by Duncan's multiple range test (SAS Institute 1987).

Figure 2. Tube test for measuring termite penetration vertically through insecticide-treated sand. Termite mortality and distance penetrated through 4 cm of substrate were recorded after 4 days exposure.

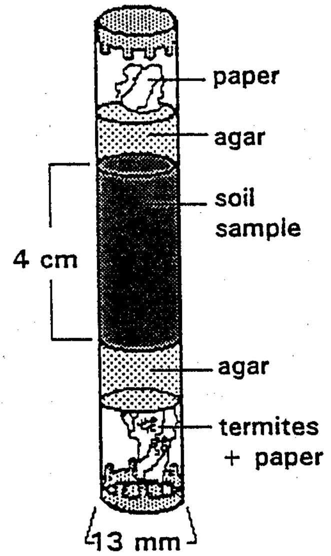
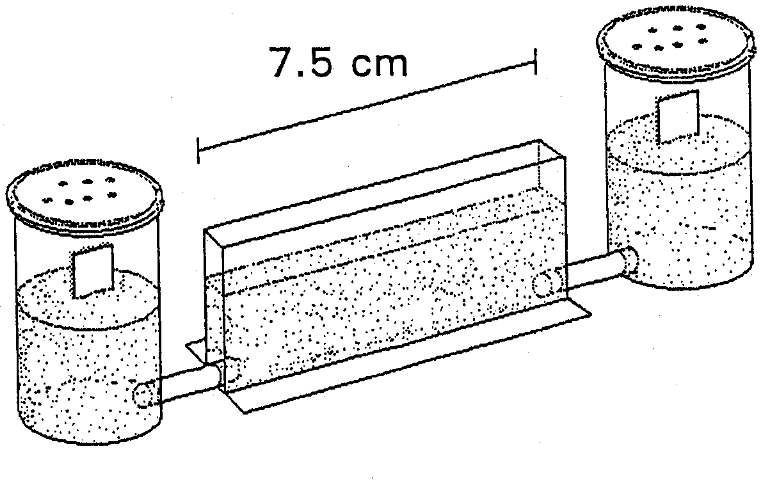


Figure 3. Sandwich test for measuring termite penetration horizontally through insecticide-treated sand. Termite mortality and distance penetrated through 7.5 cm of substrate were recorded after 4 days exposure.



In the sandwich test (Figure 3), the test substrate was placed in a horizontal tunneling arena consisting of two glass microscope slides (2.5 X 7.5 cm) spaced 3-4 mm apart and secured in a horizontal upright position along one edge by silicone rubber sealant to a base consisting of a third flat glass microscope slide. The ends of the tunneling arena were sealed with plastic spacers and silicone caulking, with a 1.5 cm long Tygon tube at the base of each end of the sandwich leading into the base of one of two 55 ml polystyrene vials, each containing ca 15 g untreated sand, 3 ml water, and a 1.5 X 2.5 cm length of wooden tongue depressor as food. After ca 7 g of the test sand and 1.5 ml distilled water was poured into the top of the tunneling arena, the top was sealed with plaster of paris, 100 termites (87 workers and 13 soldiers) were placed into one of the two vials, and the vials were sealed with plastic vials containing small air holes. Termite mortality and the total horizontal distance tunneled through the test substrate were recorded after four days of incubation in an unlighted temperature cabinet (29 ± 0.5 °C), and analyzed in the same manner as the tube test.In our first test, coral sand was treated with technical etofenprox to provide information on the repellency and toxicity of the active ingredient (a.i.) without emulsifying agents or carriers. Aliquots of a stock solution of technical etofenprox in acetone were diluted as necessary and 15 ml of solution applied to 50 g of coral sand to achieve a.i. concentrations of 0 (acetone controls), 1, 10, 100, or 1000 ppm (weight of a.i. to weight of oven-dry sand) in the test substrates. After evaporation of the acetone in a fume hood, the treated sand was tested against *C. formosanus* using the tube test, as described above. As a follow-up to this test, termites were also placed directly on sand containing 100 or 1000 ppm etofenprox in Petri dishes in a forced-contact toxicity test.

In all of our subsequent tests, a formulated emulsifiable concentrate of etofenprox (Trebon 30EC) was diluted in distilled water as necessary to achieve the desired a.i. concentrations on a ppm basis (weight a.i. / weight sand). For initial bracketing of the concentration needed to prevent termite penetration, 80 g of coral sand and 100 g of silica sand were treated with 22 and 23 ml of emulsion respectively to achieve etofenprox concentrations of 1, 10, 100, or 1000 ppm. In tests to refine the necessary concentration, 150 g of coral or silica sand were treated with 30 ml or 29 ml respectively of the etofenprox emulsion to achieve a.i. concentrations of 10, 20, 50, 100 or 200 ppm.

3.2 Results and Discussion

Although treatment of coral sand with concentrations of technical etofenprox as low as 10 ppm stopped termites from completely penetrating 4 cm of coral sand in four days, concentrations of 100 ppm or greater were required to statistically differentiate the mean termite penetration from that observed in the controls (Table 5). Mortality was low at 100 ppm (28%) and at 1000 ppm (58%), indicating that termites avoided contact with the treated sand. In contrast, the untreated (acetone only) controls were all completely penetrated within a few hours.

Repellency of the treated sand was confirmed by placing termites directly on etofenprox-treated sand in Petri dishes. When forced to remain in contact with sand containing 1000 ppm etofenprox, all termites died within 24 hours, while those exposed to 100 ppm were all dead or moribund within four days.

Table 5. Mean percent penetration and mortality of Formosan subterranean termites tunneling through 4 cm of coral sand treated with an acetone solution of technical etofenprox in a vertical tube test.

Substrate	Concentration ppm	Percent Penetration[1]	Percent Mortality[1]
Coral	1000	12.5a	58.4a
	100	49.2ab	28.0ab
	10	75.0bc	13.6b
	1	100.0c	5.6b
	0	100.0c	6.2b

[1]Three replicates per concentration. Means within each column followed by the same letter are not significantly different at the 0.05 level.

Table 6. Mean percent penetration and mortality of Formosan subterranean termites tunneling through 4 cm of coral or silica sand treated with an emulsifiable formulation of etofenprox in a vertical tube test.

Substrate	Concentration ppm	Percent Penetration[1]	Percent Mortality[1]
Coral	1000	14.2a	45.1a
	100	21.7a	6.7b
	10	87.5b	3.6b
	1	100.0c	4.2b
	0	100.0c	3.8b
Silica	1000	1.7a	60.9a
	100	4.2a	33.1ab
	10	26.7a	2.7b
	1	79.2b	3.6b
	0	100.0b	2.8b

[1]Three replicates per concentration. Means within each column and within each substrate followed by the same letter are not significantly different at the 0.05 level.

Table 7. Mean percent penetration and mortality of Formosan subterranean termites tunneling through 7.5 cm of coral or silica sand treated with an emulsifiable formulation of etofenprox in a horizontal sandwich test.

Substrate	Concentration ppm	Percent Penetration[1]	Percent Mortality[1]
Coral	1000	1.4a	18.3a
	100	16.7a	7.3ab
	10	90.5b	8.0ab
	1	100.0b	3.3b
	0	100.0b	3.7b
Silica	1000	1.9a	25.0a
	100	2.8a	17.3a
	10	79.0b	8.0b
	1	100.0b	4.0bc
	0	100.0b	2.0c

[1]Three replicates per concentration. Means within each column and within each substrate followed by the same letter are not significantly different at the 0.05 level.

Termites generally penetrated further in etofenprox-treated coral than in silica sand, suggesting that the higher pH and/or larger particle size of coral sand were detrimental to insecticide efficacy. However, the results obtained with both substrates indicated that the minimum concentration of etofenprox necessary to reduce to termite penetration to 1 cm or less lay between 10 and 1000 ppm, and probably close to 100 ppm. Thus, the range of concentrations for further tests was narrowed to 10 to 200 ppm.

Termite penetration of coral or silica sand did not differ significantly at 100 and 200 ppm etofenprox (Table 8 and 9), indicating that concentrations above 100 ppm provide maximum protection. Although concentrations as low as 10 ppm prevented complete termite penetration of the assay apparatus in some cases, termite penetration increased to unacceptable levels at etofenprox concentrations below 100 ppm.

Table 8. Mean percent penetration and mortality of Formosan subterranean termites tunneling through 4 cm of coral or silica sand treated with an emulsifiable formulation of etofenprox in a vertical tube test.

Substrate	Concentration ppm	Percent Penetration[1]	Percent Mortality[1]
Coral	200	20.0a	28.0a
	100	19.5a	22.8ab
	50	44.5b	18.8abc
	20	67.5bc	7.9bc
	10	76.0c	9.5bc
	0	100.0d	4.3c
Silica	200	14.0a	53.1a
	100	20.5a	58.0a
	50	44.5b	24.4b
	20	78.5c	10.0bc
	10	97.0d	4.8c
	0	100.0d	4.8c

[1]Five replicates per concentration. Means within each column and within each substrate followed by the same letter are not significantly different at the 0.05 level.

Table 9. Mean percent penetration and mortality of Formosan subterranean termites tunneling through 7.5 cm of coral or silica sand treated with an emulsifiable formulation of etofenprox in a horizontal sandwich test.

Substrate	Concentration ppm	Percent Penetration[1]	Percent Mortality[1]
Coral	200	22.9a	8.4a
	100	50.6a	9.2a
	50	43.1a	9.6a
	20	78.6b	4.8a
	10	86.9b	3.4a
	0	100.0b	4.2a
Silica	200	2.9a	14.8a
	100	4.6a	10.6abc
	50	29.1b	8.2abc
	20	93.4c	7.6bc
	10	100.0c	12.2ab
	0	100.0c	5.6c

[1]Five replicates per concentration. Means within each column and within each substrate followed by the same letter are not significantly different at the 0.05 level.

In both vertical tube (Table 6) and horizontal sandwich (Table 7) tunneling assays with the emulsifiable formulation, termites reacted similarly as in tests with technical etofenprox. Thus, solvent and emulsifiers in the Trebon 30EC formulation did not affect the behavior of termites nor the toxicity of the active ingredient.

The vertical tube and horizontal sandwich tunneling assays yielded similar results, although termites generally tunneled further into the 7.5 cm horizontal sandwich than the 4 cm vertical tube at low etofenprox concentrations. Grace et al. (1992) hypothesized, from horizontal sandwich assays with silafluofen, that termites were able to minimize contact with the insecticide-treated sand by lining their tunnels with untreated sand imported from the adjacent vial. This defensive behavior was not possible in the vertical tube assay, where only treated sand was available, but is very likely to occur in the field, and may well explain apparent contradictions between the results of laboratory screening trials and observed termite penetration of insecticide-treated soil under field conditions.

Both tunneling assay methods have their respective advantages. The horizontal sandwich test may mimic field conditions somewhat better than the vertical tube test, and allow more critical observations of tunneling behavior due to the narrow gap between the sides of the tunneling arena. However, it is a more difficult test to initiate than the tube test and more subject to minor design variations among the individual replicates. The tube test is more standardized and simple to initiate with a large number of treatments or replicates. Termites occasionally tunneled directly through the center of the substrate within the tube, hiding the tunnel from view; but, their thigmotropic behavior imparts a strong tendency to initiate tunnels between the substrate and the glass, where they are readily visible and easily measured.

4 CONCLUSIONS

Etofenprox is an insecticide with exceptionally low mammalian toxicity, but high toxicity to the Formosan subterranean termite. When applied topically to termite workers, the LD_{50} of 4.78 to 6.59 was in the same range as termiticides currently in use for soil treatment and injection into termite galleries within structural lumber. Etofenprox was a relatively quick acting termiticide, with most mortality occurring within 24 hours.

Although etofenprox concentrations as low as 10 ppm reduced termite penetration of crushed coral and silica sand, consistent protection was achieved with concentrations of 100 ppm or greater in both vertical tube tests and horizontal sandwich tunneling tests. Termites avoided contact with the treated substrate and mortality was generally low, indicating that most termites did not contact the substrate often or long enough to acquire a lethal dose of etofenprox.

The low mammalian toxicity and high contact toxicity to termites of etofenprox suggest that it may be useful in remedial termite control as a contact toxicant, where direct injections of insecticide into active infestations in structural lumber are required. Resmethrin is applied in this manner in Hawaii for both Formosan subterranean termite and drywood termite (Kalotermitidae) control.

Etofenprox also appears to be a promising soil insecticide to prevent termites from entering structures. Although more research is required to establish the longevity of etofenprox in the soil under field conditions, our laboratory results indicate that such studies are warranted. To provide the necessary safety margin, field application rates should be designed to maintain a minimum of 100 ppm in the soil for the required period of longevity, which is generally at least five years under most soil types and environmental conditions.

5 ACKNOWLEDGMENTS

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