

Laboratory Evaluation of Two Slow-Acting Toxicants Against Formosan and Eastern Subterranean Termites (Isoptera: Rhinotermitidae)

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J. Econ. Entomol. 84(1): 170-175 (1991)

ABSTRACT Topical toxicity, lethal time, and bait acceptance of two slow-acting toxicants, mirex and sulfluramid, were determined for the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). When topically applied to *C. formosanus*, mirex was slightly less toxic ($LD_{50} = 9.14 \mu\text{g/g}$) than sulfluramid ($LD_{50} = 6.95 \mu\text{g/g}$), but mirex was approximately 34 times more potent ($LD_{50} = 1.78 \mu\text{g/g}$) against *R. flavipes* than sulfluramid ($LD_{50} = 60.64 \mu\text{g/g}$). Mortality of *R. flavipes* as a function of time was fastest for mirex and slowest for sulfluramid. Lethal time (time to kill 90% of test insects) was similar when *C. formosanus* was treated with either mirex or sulfluramid. Results of a choice bioassay indicated that concentration thresholds of 10 or 30 ppm in wood treated with sulfluramid were acceptable to *C. formosanus* and *R. flavipes*, respectively. These treatments also produced significant mortality ($\geq 68\%$ mortality at ≥ 4 ppm for *C. formosanus*, $\geq 80\%$ mortality at ≥ 18 ppm for *R. flavipes*) after an 8-wk exposure. Wood blocks treated with ≤ 90 ppm mirex were accepted by *C. formosanus*. Mirex concentrations of ≥ 10 ppm produced $\geq 68\%$ mortality. *R. flavipes* accepted blocks treated with up to 15 ppm of mirex and were killed at significantly higher rates ($\geq 80\%$) when exposed to blocks treated with ≥ 9 ppm of mirex.

KEY WORDS Insecta, subterranean termites, bioassays, bait-toxicants

FORMOSAN SUBTERRANEAN TERMITE, *Coptotermes formosanus* Shiraki, and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), are two of the most economically important termite species in the United States (Su & Scheffrahn in press). These subterranean termites construct underground foraging galleries extending up to 100 m and may include foraging populations of several million termites per colony (Su & Scheffrahn 1988a, Grace et al. 1989). The current control technique for subterranean termites is to form a termiticidal barrier that separates subterranean termite populations from structures to be protected. Applications of soil termiticides apparently would not affect the vast populations of subterranean termites around structures (Su & Scheffrahn 1988a). The failure of soil termiticide applications to affect these populations has contributed, in part, to the increasing problem of *C. formosanus* in some areas (Su & Tamashiro 1987).

Recently, interest in the use of slow-acting toxicants to suppress populations of subterranean termites has been renewed (Su et al. 1982a, Jones 1984). As suggested by Beard (1974), slow-acting toxicants could be introduced into the colony's gallery system and transferred to unexposed nestmates by social grooming or trophallaxis. Suppression of

subterranean termite populations reduces their damaging potential to nearby structures and may provide long-term control. Moreover, a successful bait-toxicant technique will drastically reduce insecticide application quantity in comparison with copious soil termiticide barrier applications.

Preliminary successes reported by Esenther & Beal (1974, 1978) were achieved when baits were treated with the pesticide mirex. Mirex also was used to control field colonies of an Australian subterranean termite, *Mastotermes darwiniensis* Froggatt (Paton & Miller 1980), and *C. formosanus* in China (Gao et al. 1985). Because mirex is no longer available for use in the United States, we have searched for alternatives and identified several candidate delayed toxicants. These include hydramethylnon (Su et al. 1982b), avermectin B₁ (Su et al. 1987), and a dihaloalkyl arylsulfone (Su & Scheffrahn 1988b). Although results of the preliminary field trial with hydramethylnon baits were inconclusive (Su et al. 1982b), similar field trials with A-9248 baits reduced foraging populations of *C. formosanus* in Florida by 33-98% (N.-Y. S. et al., unpublished data).

In our previous study (Su & Scheffrahn 1988c), we presented the toxicity data for a delayed toxicant, sulfluramid. Here, we compare sulfluramid

Table 1. Response of *C. formosanus* and *R. flavipes* to topically applied mirex and sulfluramid

Compound	Mean termite wt, mg	n	Slope \pm SE	LD ₅₀ (95% FL), (μ g/g body weight)
<i>C. formosanus</i>				
Sulfluramid	3.9	352	0.379 \pm 0.034	6.95 (6.49-7.44)
Mirex	3.9	263	0.289 \pm 0.032	9.14 (8.51-9.82)
<i>R. flavipes</i>				
Sulfluramid	2.4	446	0.040 \pm 0.003	60.6 (57.1-64.2)
Mirex	2.2	434	0.701 \pm 0.081	1.78 (1.48-2.02)

with the "proven" toxicant, mirex, for topical toxicity, time trends in mortality, and bait acceptance by *C. formosanus* and *R. flavipes*.

Materials and Methods

Two studies were done to establish the topical toxicity, lethal time, and the concentration-related bait acceptance by termites. *C. formosanus* was collected from three field colonies from Hallandale, Fla., as described by Su & Scheffrahn (1986); three colonies of *R. flavipes* were collected from fallen logs in Ft. Lauderdale, Delray Beach, and Gainesville, Fla. *R. flavipes* were extracted from logs by the method of La Fage et al. (1983). Technical grades (>99%) of mirex (Velsicol Chemical Corporation, Chicago) and sulfluramid (Griffin Corporation, Valdosta, Ga.) were used in this study. Mean body weight of termites was determined for all colony-species combinations by weighing five groups each of 10 individuals.

Topical Toxicity and Lethal Time. Twenty workers (undifferentiated larvae of at least third instar) per treatment were anesthetized with CO₂

for 20 s and inoculated with a 0.5- μ l acetone solution droplet or acetone droplet of toxicant. A microapplicator was used to administer the droplet onto the insect's abdomen. Concentration ranges of 10-200 ppm were tested for both toxicants against *C. formosanus*. *R. flavipes* were inoculated with acetone solutions at concentration ranges of 100-1,000 ppm and 5-50 ppm for sulfluramid and mirex, respectively. Ten to 15 concentrations were tested at equal intervals within the ranges. Workers' mean body weight was used to convert the concentration (ppm wt/vol) to the μ g/g ([AI] weight per body weight) dose.

Treated termites were transferred to a Petri dish (5.0 cm diameter, 1.5 cm deep) containing two moistened filter disks (Whatman No. 1). Two *C. formosanus* soldiers and one *R. flavipes* soldier were added to each dish for each species to approximate colony soldier proportions. Dishes containing termites were stored in an environmental chamber at $28 \pm 1^\circ\text{C}$. Dead or moribund workers were recorded and removed from each unit daily up to 14 d. Mortalities at day 14 were subjected to probit analysis (SAS Institute 1987) to estimate the topical LD₅₀. The effective lethal time (ELT₉₀), defined as the time required for a fixed dose of toxicant to kill 90% of the test insects (Su et al. 1987), was derived from the daily mortality.

Bait Acceptance. Concentration-dependent bait acceptance by termites was measured in a choice bioassay similar to that described by Su & Scheffrahn (1989). The experimental units consisted of screw-top jars (6.0 cm diameter, 6.5 cm high) in which two wooden (*Pinus* sp.) blocks (2 by 2 by 1.5 cm) were placed 1.5 cm apart. The blocks were covered with 75 ml sand (washed in acetone) mixed with 18 ml deionized water. One block was vacuum-impregnated (ASTM 1976) with an acetone solution of either sulfluramid or mirex, and the

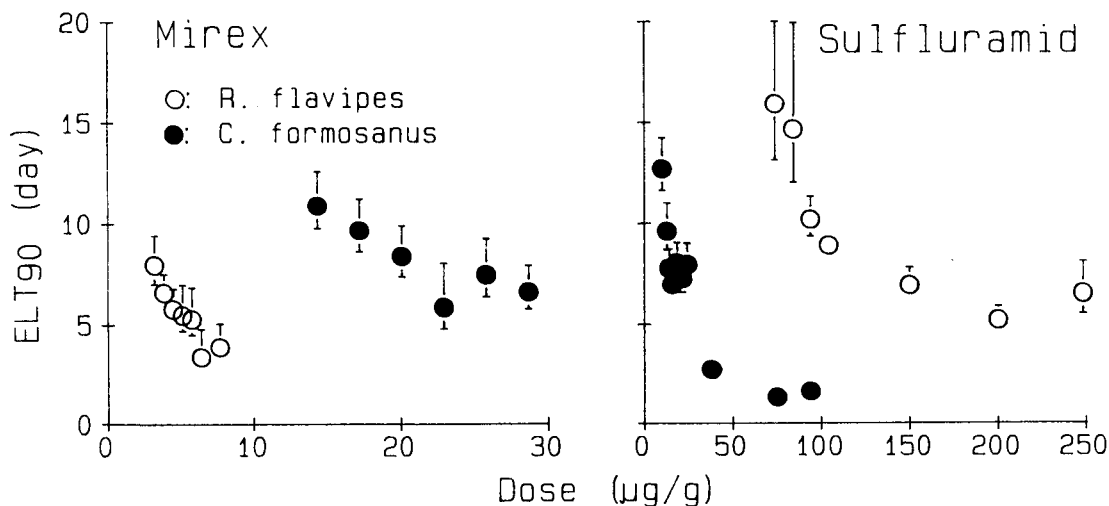


Fig. 1. Time (days) required for 90% mortality (ELT₉₀ \pm 95% FL) of the Formosan subterranean termite, *C. formosanus* (●), or the eastern subterranean termite, *R. flavipes* (○), topically treated with one of two slow-acting toxicants, mirex and sulfluramid.

Table 2. Comparison of weight loss between wood blocks treated with sulfluramid and untreated blocks 4, 6, and 8 wk after exposure to *C. formosanus* and *R. flavipes* in a choice bioassay

Concn., ppm	Wood wt loss, mg, and <i>t</i> statistics ^a											
	4 wk				6 wk				8 wk			
	T	U	<i>t</i>	<i>P</i>	T	U	<i>t</i>	<i>P</i>	T	U	<i>t</i>	<i>P</i>
<i>C. formosanus</i>												
0	233 ± 36	330 ± 45	1.39	0.22	322 ± 42	368 ± 45	0.63	0.56	369 ± 28	463 ± 38	1.69	0.15
2	187 ± 31	213 ± 29	0.58	0.59	239 ± 36	312 ± 49	0.94	0.39	327 ± 36	344 ± 23	0.32	0.76
4	165 ± 27	500 ± 351	0.96	0.38	189 ± 19	225 ± 20	1.50	0.19	211 ± 25	286 ± 45	1.20	0.28
6	122 ± 29	277 ± 110	1.13	0.31	192 ± 13	184 ± 50	-0.14	0.89	213 ± 30	212 ± 37	-0.01	0.99
8	129 ± 8	143 ± 25	0.50	0.64	148 ± 11	156 ± 34	0.20	0.85	137 ± 12	159 ± 19	1.04	0.34
10	99 ± 10	147 ± 21	2.00	0.10	133 ± 8	145 ± 12	0.77	0.48	150 ± 7	144 ± 10	-0.43	0.68
<i>R. flavipes</i>												
0	164 ± 26	173 ± 20	0.20	0.85	233 ± 58	261 ± 33	0.34	0.74	306 ± 57	367 ± 54	0.56	0.60
6	189 ± 19	225 ± 23	0.72	0.51	220 ± 65	272 ± 52	0.45	0.67	326 ± 51	328 ± 59	0.02	0.99
12	159 ± 34	162 ± 41	0.05	0.96	225 ± 29	292 ± 38	1.16	0.30	206 ± 23	415 ± 65	2.58	0.05
18	104 ± 34	201 ± 38	1.38	0.23	211 ± 67	316 ± 67	0.88	0.42	200 ± 32	299 ± 64	1.06	0.33
24	103 ± 25	184 ± 40	1.37	0.23	140 ± 27	295 ± 53	2.09	0.09	136 ± 15	365 ± 46	4.26	0.01
30	143 ± 44	261 ± 33	0.28	0.79	181 ± 12	129 ± 26	-2.31	0.07	191 ± 22	207 ± 29	0.31	0.77

^a T, treated with sulfluramid; U, untreated.

other was untreated. Before the impregnation, the blocks were dried at 80°C for 48 h and weighed (± 0.1 mg). Based on acetone volume absorbed by each block, the concentrations ([AI] weight per wood weight) tested for sulfluramid were 0, 2, 4, 6, 8, and 10 ppm for *C. formosanus* and 0, 6, 12, 18, 24, and 30 ppm for *R. flavipes*. Mirex was tested at concentrations of 0, 10, 30, 50, 70, and 90 ppm for *C. formosanus* and 0, 3, 6, 9, 12, and 15 ppm for *R. flavipes*. A preliminary choice bioassay with a broader range of concentrations (0, 0.1, 1, 10, 100, and 1,000 ppm for *C. formosanus*, 0, 1, 10, 20, 30, 40, and 50 ppm for *R. flavipes* with sulfluramid, and 0, 5, 10, 15, 20, 25, and 30 ppm for *R. flavipes* with mirex) indicated termites' preferences for untreated blocks compared with blocks treated with concentrations above the ranges selected for testing.

One hundred workers plus five soldiers of *C. formosanus* and one soldier of *R. flavipes* were placed on the moistened sand in the units. Six units were prepared for each concentration-by-species-by-colony-by-toxicant ($6 \times 2 \times 3 \times 2$) combination for a total of 432 units. Experimental units were held at $28 \pm 1^\circ\text{C}$. At 4, 6, and 8 wk, six units (two subsamples each of three colonies) were selected at random from each treatment combination (species-by-concentration-by-toxicant) and disassembled; the surviving termites were counted. Wood block remnants were rinsed under running water, oven-dried, and weighed as above. Mortality counts were transformed to $\log(X + 1)$ values and subjected to analysis of variance (ANOVA) for a randomized block design using colony origin as the blocking factor. Significant differences ($P = 0.05$) among concentrations of each biweekly observation for each toxicant-species combination were separated by the Student-Newman-Keuls test (SAS Institute 1987). Mean differences in wood weight loss (mg) between treated and untreated blocks for

each compound and concentration were compared by paired *t* tests (SAS Institute 1987).

Results and Discussion

Topical Toxicity and Lethal Time. Mortalities in control groups were 16.1 ± 7.0 and $7.8 \pm 3.7\%$ for *C. formosanus* and *R. flavipes*, respectively. As reported earlier (Su & Scheffrahn 1988c), *C. formosanus* was more susceptible to sulfluramid than *R. flavipes* (Table 1). The topical LD_{50} value for *R. flavipes* (60.6 $\mu\text{g/g}$) from this study was nearly the same as our previous result (68.1 $\mu\text{g/g}$). Our current data for *C. formosanus*, however, indicated that sulfluramid was slightly more toxic ($\text{LD}_{50} = 6.95 \mu\text{g/g}$) than previously reported ($\text{LD}_{50} = 9.94 \mu\text{g/g}$) (Su & Scheffrahn 1988c). Because termites

Table 3. Percentage of worker mortality of *C. formosanus* and *R. flavipes* exposed to sulfluramid for 4, 6, and 8 wk in a choice bioassay

Concn., ppm	% Worker mortality		
	4 wk	6 wk	8 wk
<i>C. formosanus</i>			
0	11.8 ± 0.8a	19.7 ± 2.3a	25.8 ± 2.6a
2	19.2 ± 2.1a	19.8 ± 2.6a	21.3 ± 3.1a
4	18.7 ± 2.4a	32.5 ± 1.5a	67.5 ± 12.6b
6	36.3 ± 13.0a	46.8 ± 8.7b	79.7 ± 9.8b
8	47.3 ± 14.8a	88.8 ± 6.8c	96.7 ± 2.1c
10	73.8 ± 11.0b	94.3 ± 4.9c	100.0 ± 0.0c
<i>R. flavipes</i>			
0	11.3 ± 1.1a	18.8 ± 1.4a	22.8 ± 2.3a
6	11.3 ± 1.8a	19.3 ± 2.5a	36.0 ± 7.6a
12	12.8 ± 3.4a	29.8 ± 13.3a	57.8 ± 17.0ab
18	16.5 ± 3.1a	27.7 ± 12.1a	82.2 ± 13.1bc
24	27.2 ± 14.0ab	66.3 ± 16.5b	84.2 ± 13.1bc
30	49.5 ± 17.5b	99.7 ± 0.3c	100.0 ± 0.0c

For each species, means followed by the same letter within a column are not significantly different ($P = 0.05$; Student-Newman-Keuls test [SAS Institute 1987]).

Table 4. Comparison of weight loss between wood blocks treated with mirex and untreated blocks 4, 6, and 8 wk after exposure to *C. formosanus* and *R. flavipes* in a choice bioassay

Concn., ppm	Wood wt loss, mg, and <i>t</i> statistics ^a											
	4 wk				6 wk				8 wk			
	T	U	<i>t</i>	<i>P</i>	T	U	<i>t</i>	<i>P</i>	T	U	<i>t</i>	<i>P</i>
	<i>C. formosanus</i>											
0	313 ± 56	340 ± 45	0.33	0.76	447 ± 58	363 ± 34	-1.04	0.34	436 ± 72	478 ± 44	0.63	0.55
10	245 ± 38	298 ± 55	0.72	0.50	188 ± 13	326 ± 87	1.51	0.19	263 ± 40	293 ± 60	0.72	0.50
30	158 ± 35	171 ± 36	0.28	0.79	156 ± 33	205 ± 29	1.39	0.22	167 ± 25	213 ± 35	1.24	0.27
50	140 ± 28	182 ± 38	1.30	0.25	132 ± 20	157 ± 20	1.27	0.26	105 ± 20	148 ± 15	1.48	0.20
70	95 ± 9	130 ± 10	4.21	0.01	161 ± 20	179 ± 42	0.53	0.62	192 ± 36	226 ± 20	0.65	0.54
90	183 ± 10	247 ± 50	1.60	0.17	201 ± 29	214 ± 25	0.27	0.80	228 ± 29	239 ± 29	0.34	0.74
	<i>R. flavipes</i>											
0	162 ± 33	211 ± 34	0.78	0.47	163 ± 41	308 ± 54	1.60	0.17	293 ± 75	270 ± 53	-0.19	0.86
3	158 ± 24	208 ± 39	0.80	0.45	202 ± 36	222 ± 47	0.27	0.80	158 ± 29	304 ± 58	1.82	0.13
6	85 ± 14	201 ± 33	2.66	0.04	110 ± 17	294 ± 52	2.66	0.04	122 ± 32	301 ± 56	2.18	0.08
9	90 ± 10	163 ± 24	2.26	0.07	132 ± 11	183 ± 41	0.99	0.37	91 ± 20	187 ± 42	1.60	0.17
12	95 ± 9	133 ± 29	1.03	0.35	205 ± 66	214 ± 26	0.17	0.87	119 ± 15	190 ± 71	0.83	0.44
15	96 ± 9	134 ± 27	1.10	0.32	147 ± 10	184 ± 41	0.75	0.49	110 ± 14	206 ± 62	1.27	0.26

^a T, treated with mirex; U, untreated.

that were tested originated from three colonies in this study, the value of 6.95 µg/g (Table 1) is more representative of topical toxicity of sulfluramid against *C. formosanus* than that in the previous study. Mirex was slightly less toxic against *C. formosanus* than sulfluramid (approximately 1.3-fold) but was approximately 34 times more toxic than sulfluramid against *R. flavipes* (Table 1).

Besides being more toxic, mirex also had the fastest kill (90% mortality in 3–8 d) against *R. flavipes* at the lowest lethal concentration range (3–8 µg/g) (Fig. 1). Conversely, sulfluramid caused 90% mortality of *R. flavipes* 5–16 d after treatment at the highest concentration range (70–250 µg/g). Effective lethal time (ELT₉₀) for *C. formosanus* was fairly similar for both toxicants (mirex, 6–11 d at 14–29 µg/g; sulfluramid, 2–13 d at 10–94 µg/g).

Table 5. Percentage of worker mortality of *C. formosanus* and *R. flavipes* exposed to mirex for 4, 6, and 8 wk in a choice bioassay

Concn., ppm	% Worker mortality		
	4 wk	6 wk	8 wk
	<i>C. formosanus</i>		
0	10.5 ± 2.3a	11.0 ± 2.5a	21.5 ± 5.4a
10	15.8 ± 3.4a	54.5 ± 12.6b	68.3 ± 13.8b
30	58.5 ± 13.1b	79.8 ± 9.3c	100.0 ± 0.0c
50	85.8 ± 8.3c	100.0 ± 0.0c	100.0 ± 0.0c
70	100.0 ± 0.0c	100.0 ± 0.0c	100.0 ± 0.0c
90	99.5 ± 0.3c	100.0 ± 0.0c	100.0 ± 0.0c
	<i>R. flavipes</i>		
0	26.8 ± 7.4a	33.7 ± 9.1a	27.0 ± 3.6a
3	36.8 ± 13.0ab	38.1 ± 10.9ab	51.2 ± 16.2ab
6	51.7 ± 16.2ab	57.3 ± 14.6ab	45.3 ± 12.6ab
9	61.7 ± 14.0ab	79.2 ± 13.3b	100.0 ± 0.0c
12	86.2 ± 13.6b	75.5 ± 13.5b	81.3 ± 14.3bc
15	85.3 ± 14.7b	87.2 ± 12.8b	79.3 ± 13.6bc

For each species, means followed by the same letter within a column are not significantly different (*P* = 0.05; Student-Newman-Keuls test [SAS Institute 1987]).

Bait Acceptance. Blocks treated with 10 ppm sulfluramid were slightly avoided by *C. formosanus*. In general, however, both species fed as much on blocks treated with sulfluramid as on the untreated ones for the first 4 wk (Table 2). In our preliminary test, wood impregnated with more sulfluramid than the concentrations shown in Table 2 were rejected by both termite species. At the fourth week, significantly higher mortality was observed in units containing the highest concentrations (10 and 30 ppm for *C. formosanus* and *R. flavipes*, respectively) (Table 3). Except for *R. flavipes* exposed to 24-ppm blocks at 6–8 wk, both termite species continued to feed on treated and untreated blocks at a similar rate. This feeding resulted in 80–100% mortality at higher concentrations (6–10 and 18–30 ppm for *C. formosanus* and *R. flavipes*, respectively) by 8 wk (Table 3).

The concentration range of blocks acceptable to *C. formosanus* after treatment with mirex was wider than sulfluramid (≤90 ppm versus ≤10 ppm) as shown in Table 4. Reflecting its slightly lower toxicity than sulfluramid (1.3-fold less, Table 1), 10 ppm mirex caused 68% mortality at 8 wk (Table 5), whereas blocks treated with 10 ppm sulfluramid produced 100% mortality of *C. formosanus* after the same period (Table 3). *C. formosanus* did not prefer untreated blocks compared with those treated with mirex at ≤90 ppm (Table 4), whereas wood treated with sulfluramid at >10 ppm was avoided by this species. Because of its 9-fold-higher avoidance threshold, mirex is probably a better toxicant than sulfluramid for control of *C. formosanus* with baits. This superiority also was demonstrated by the high mortality recorded from mirex treatments of ≥10 ppm (Table 5).

Preference of *R. flavipes* for wood treated with mirex was not concentration-dependent. Although our preliminary test suggested that wood treated with mirex at >15 ppm was avoided by *R. flavipes*,

preference compared with untreated blocks also was observed from units treated with 6 and 9 ppm at 4 wk (Table 4). This erratic behavioral response cannot be explained, but the resulting mortality from units containing blocks treated with ≥ 9 ppm mirex was significantly higher than those containing ≤ 6 ppm blocks (Table 5).

Esenther & Beal (1974, 1978), who reported suppression of field activity of *Reticulitermes* spp., used decayed wood baits treated with mirex at 4,000 and 13,000 ppm. Our study, however, indicated that such high concentrations (4,000–13,000 ppm) deterred feeding by *R. flavipes*. Because colony activity, foraging territory, and populations were not determined before the introduction of the mirex baits in the studies of Esenther & Beal (1974, 1978), termites might have been repelled (rather than killed) by eating blocks treated with mirex. If the *Reticulitermes* spp. populations were indeed suppressed by the mirex baits as claimed by Esenther & Beal (1974, 1978), use of decayed wood might have masked the deterrent concentrations of mirex. Finally, because of the high concentration of mirex in the baits, very little consumption would have resulted in a high relative intake and, therefore, ingestion of a lethal dose of mirex.

Su et al. (1982a) indicated that a bait toxicant must act slowly and not repel termites. These two characteristics are concentration-dependent (Su et al. 1987, Su & Scheffrahn 1988b). A desirable toxicant should be accepted by termites at concentrations sufficient to cause delayed mortality. In the choice bioassay, mirex bait was accepted by *C. formosanus* at ≤ 90 ppm (Table 4), whereas the 10-ppm bait produced significantly higher mortality (68%) than control at 8 wk (Table 5). Thus, the concentration range of 10–90 ppm of mirex can be incorporated into baits for control of *C. formosanus*. In contrast, sulfluramid deterred feeding of *C. formosanus* at > 10 ppm and caused significant mortality at > 4 ppm; thus, the useful concentration range was 4–10 ppm. Because mirex can be used in the baits at a wider concentration range (10–90 ppm, or 9-fold) than sulfluramid (4–10 ppm, or 2.5-fold), mirex provides more flexibility for use in field trials against *C. formosanus*. For *R. flavipes*, the possible concentration ranges were 18–30 ppm for sulfluramid and 9–15 ppm for mirex.

Acknowledgment

We thank T. Center (USDA-ARS) and F. W. Howard (University of Florida) for reviewing this manuscript, and P. M. Ban (University of Florida) for experimental assistance. Partial funding of this study was provided by the Griffin Corporation. This article is Florida Agricultural Experiment Station's Journal Series R-00490.

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Received for publication 27 February 1990; accepted 6 July 1990.
