

Toxicity and Feeding Deterreny of a Dihaloalkyl Arylsulfone Biocide, A-9248, Against the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

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ABSTRACT The topical LD₅₀ of A-9248 (diiodomethyl para-tolyl sulfone) against the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was estimated at 141.7 µg/g with 95% fiducial limits of 110.4-168.3 µg/g. A-9248 showed protracted activity against this termite. Time required to kill 90% of *C. formosanus* (ELT₉₀) was 8.4-18.9 d when administered topically, 21-26 d after 24-h forced feeding, and 19-22 d when *C. formosanus* were confined continuously on treated feeding substrate. Results of a choice test revealed that A-9248 is a feeding deterrent at concentrations ≥8,000 ppm. Initially, *C. formosanus* fed on wood treated with 1,000-6,000 ppm A-9248 but learned to avoid the treatment as a result of ingesting sublethal doses of A-9248. Only those groups exposed to wood treated with <1,000 ppm continued feeding on the treated substrate; ingestion of these concentrations resulted in 85-100% mortality at the end of the 4-wk experiment.

KEY WORDS Insecta, slow-acting insecticides, diiodomethyl para-tolyl sulfone, feeding deterreny

A MATURE COLONY of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, may contain 2-3 million individuals, with foraging galleries extending 100 m from end to end (King & Spink 1969, Li et al. 1976, Lai 1977). The use of a slow-acting termiticide was suggested as a possible strategy to eliminate established colonies of subterranean termites, *Reticulitermes* spp. (Beard 1974) and *C. formosanus* (Su et al. 1982b). When introduced into a part of the gallery system as a bait or tracking powder, such a compound could be transferred to nestmates via social grooming or trophallaxis.

Laboratory studies indicated that hydramethylnon (Amdror), avermectin B₁ (Su et al. 1987), and insect growth regulators (IGRs) such as methoprene and fenoxycarb (Jones 1984, Su et al. 1985) showed delayed toxicity against *C. formosanus*. A field trial with hydramethylnon baits, however, did not result in successful control of *C. formosanus* colonies (Su et al. 1982a).

Dechlorane (Mirex) baits suppressed field activities of *Reticulitermes* colonies (Esenther & Beal 1974, 1978) and killed field colonies of the Australian subterranean termite, *Mastotermes darwiniensis* Froggatt. (Paton & Miller 1980). Gao et al. (1985) also reported successful field control of termite infestations with Mirex baits in China. This slow-acting insecticide is no longer available in the United States.

In a search for alternative slow-acting and non-repellent toxicants, we tested the effects of a dihaloalkyl arylsulfone biocide, A-9248 (diiodomethyl para-tolyl sulfone), against *C. formosanus*.

Materials and Methods

A series of tests was conducted to investigate the topical and oral toxicity, lethal time, and the inter-relationships among concentration, feeding deterreny, and mortality. Termites were collected from a field colony by the method of Su & Scheffrahn (1986). The mean (±SE) worker weight of this colony was 5.33 ± 1.51 mg ($n = 50$), with a soldier proportion of ca. 10% and a foraging population of ca. 6.8 million. Individuals of this colony have been used in other laboratory studies to represent *C. formosanus* in our area. The termites were undifferentiated larvae (workers) of at least the third instar. Technical grade (>95%) A-9248 (Abbott Laboratories, N. Chicago, Ill.) was used in all tests.

Topical Toxicity and Lethal Time. Thirty workers per treatment were anesthetized with gaseous CO₂ for 20 sec before inoculation with a 0.5-µl droplet of acetone solution of A-9248 at AI concentrations of 0-4,000 ppm in 400-ppm increments and 6,000-10,000 ppm in 2,000-ppm increments. A microapplicator (Model M, Instrumentation Specialities, Calif.) was used to administer the solution onto the abdomen. The resultant concentrations

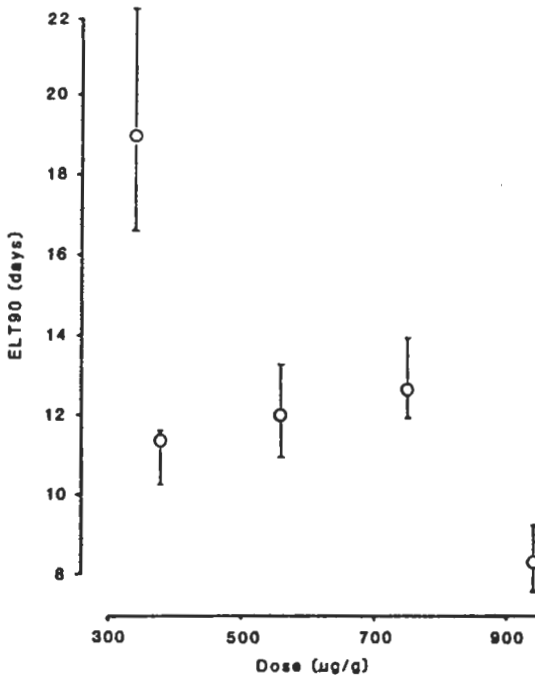


Fig. 1. Topical $ELT_{90} \pm 95\%$ FL of A-9248 against *C. formosanus*.

were 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 3.0, 4.0, and 5.0 μg per termite. Treatments were replicated three times. The concentration (μg per termite) was converted to dose ($\mu\text{g}/\text{g}$) using the mean worker weight. Treated termites were transferred to a Petri dish (5.0 cm diameter by 1.5 cm high) provisioned with two filter disks (Whatman No. 1) moistened with deionized water. Three soldiers were added to each unit to approximate soldier proportions of the colony. The experimental units were stored in an environmental chamber at $29 \pm 1^\circ\text{C}$. Dead or moribund workers were recorded and removed from each unit daily up to 14 d. Mortality at 14 d was corrected by Abbott's (1925) formula, and the topical LD_{50} was estimated by probit analysis (SAS Institute 1985). The effective lethal time (ELT_{90}), defined as the time required for a fixed dosage of toxicant to cause 90% mortality of termites (Su et al. 1987), was used to estimate lethal time.

Oral Toxicity and Lethal Time. A temporary and a continuous forced-feeding test were performed. Eighty workers and five soldiers were placed in a Petri dish provisioned with an absorbant cellulose pad (4.7 cm diameter; Gelman Instrument, Ann Arbor, Mich.) impregnated with A-9248 at concentrations of 0, 500, 1,000, 2,000, 4,000, and 8,000 ppm (wt/wt), and moistened with deionized water. All of the treatments were replicated three times. Temporary forced-feeding groups were exposed to treatments for 24 h at $29 \pm 1^\circ\text{C}$ and transferred to similar Petri dishes containing un-

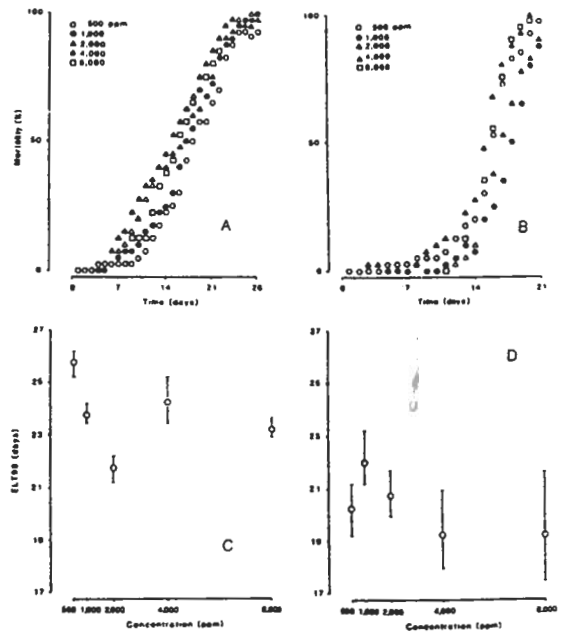


Fig. 2. Accumulative mortalities (A and B) and $ELT_{90} \pm 95\%$ FL (C and D) of *C. formosanus* for 24-h forced-feeding (A and C), and for continuous feeding (B and D) on A-9248 treated cellulose pads at concentrations of 500–8,000 ppm.

treated filter paper. Observations after exposure were made up to 28 d for temporary forced-feeding groups. The continuous groups were confined to the treated pads for 21 d.

Choice Test. The experimental units comprised screw-top jars (6.0 cm diameter by 6.5 cm high) in which two slices of commercial-grade baldcypress (*Taxodium distichum* (L.) Rich), measuring 3.0 by 3.5 by 0.2 cm, were placed vertically about 1.5 cm apart and covered with 75 ml acetone-washed sand and 18 ml deionized water. One slice of the wood was previously treated with an acetone solution of A-9248 to yield concentrations of 0, 200, 400, 600, 800, 1,000, 2,000, 4,000, 6,000, 8,000, and 10,000 ppm (wt/wt). The other slice was not treated. Dry weights of the slices were determined before use. Twelve units were prepared for each concentration (132 total). Eighty workers and five soldiers of *C. formosanus* were placed in each unit and held at $29 \pm 1^\circ\text{C}$. Three units per treatment were disassembled weekly for 4 wk, and the surviving termites were counted. Wood remnants were washed under running water to remove sand, oven-dried at 80°C for 48 h, and cooled in a desiccator before weighing. Differences in wood weight loss between treated and untreated slices were compared by paired *t* tests (SAS Institute 1986). The weekly mortality (%) of workers was transformed to logarithms and subjected to analyses of variance. Significant differences among 11 concentrations were separated by Student-Newman-Keuls test at $P = 0.05$ (SAS Institute 1986).

Table 1. Differences in wood consumption by *C. formosanus* between the untreated wood slices and those treated with A-9248 at concentrations of 0-10,000 ppm during a 4-wk period

Concn (ppm)	Treatment ^a and <i>t</i> statistics	Wood consumption (g) ($\bar{x} \pm SE$)			
		Week			
		1	2	3	4
0	T	32.9 ± 14.6	57.3 ± 10.2	86.9 ± 15.3	143.5 ± 35.3
	U	52.4 ± 17.6	95.0 ± 16.6	97.5 ± 10.8	77.4 ± 41.2
	<i>t</i> (P)	0.61 (0.61)	1.47 (0.28)	0.62 (0.60)	-0.89 (0.47)
200	T	64.2 ± 8.6	76.3 ± 7.4	98.6 ± 15.2	91.3 ± 8.9
	U	68.9 ± 6.8	87.2 ± 9.1	105.5 ± 12.6	85.0 ± 11.0
	<i>t</i> (P)	0.37 (0.75)	0.71 (0.55)	0.27 (0.81)	1.96 (0.19)
400	T	39.9 ± 5.4	69.5 ± 10.1	65.2 ± 7.7	53.9 ± 4.0
	U	36.9 ± 11.8	95.5 ± 18.6	74.5 ± 13.9	104.3 ± 13.2
	<i>t</i> (P)	-0.19 (0.87)	2.68 (0.12)	0.63 (0.60)	3.06 (0.09)
600	T	39.2 ± 11.5	46.4 ± 18.8	42.6 ± 9.2	46.0 ± 13.8
	U	56.4 ± 8.9	67.8 ± 3.1	76.9 ± 14.9	62.8 ± 8.2
	<i>t</i> (P)	0.98 (0.43)	1.38 (0.08)	1.50 (0.27)	0.76 (0.52)
800	T	33.5 ± 4.7	16.6 ± 4.5	25.8 ± 4.5	33.8 ± 11.5
	U	51.8 ± 15.2	71.9 ± 12.2	94.9 ± 16.9	94.2 ± 10.6
	<i>t</i> (P)	1.03 (0.41)	3.31 (0.08)	4.85 (0.04)	2.91 (0.10)
1,000	T	29.2 ± 21.5	20.4 ± 4.5	10.0 ± 3.2	14.4 ± 1.4
	U	19.1 ± 2.4	98.4 ± 13.3	104.5 ± 24.1	97.8 ± 8.8
	<i>t</i> (P)	-0.53 (0.65)	4.58 (0.04)	3.59 (0.07)	9.49 (0.01)
2,000	T	26.3 ± 5.6	24.1 ± 11.3	21.8 ± 3.0	54.0 ± 27.2
	U	46.6 ± 2.1	269.2 ± 40.7	98.1 ± 6.3	215.6 ± 57.5
	<i>t</i> (P)	2.96 (0.10)	6.90 (0.02)	15.8 (0.004)	5.29 (0.03)
4,000	T	47.8 ± 27.6	2.0 ± 1.1	59.2 ± 4.1	22.6 ± 18.6
	U	59.5 ± 22.6	142.8 ± 8.0	179.5 ± 23.4	179.2 ± 11.8
	<i>t</i> (P)	0.49 (0.67)	15.7 (0.004)	4.50 (0.05)	6.94 (0.02)
6,000	T	51.3 ± 22.8	15.2 ± 5.3	14.1 ± 3.7	47.2 ± 20.6
	U	78.9 ± 3.0	124.5 ± 17.4	153.3 ± 14.1	167.2 ± 21.4
	<i>t</i> (P)	1.23 (0.34)	9.1 (0.01)	11.5 (0.008)	5.17 (0.04)
8,000	T	8.4 ± 2.7	7.0 ± 6.5	9.8 ± 3.7	23.1 ± 13.8
	U	77.7 ± 5.4	123.1 ± 18.0	165.3 ± 7.8	168.9 ± 21.5
	<i>t</i> (P)	14.4 (0.005)	6.4 (0.02)	21.1 (0.002)	4.86 (0.04)
10,000	T	13.2 ± 3.1	10.4 ± 3.3	10.4 ± 1.4	32.2 ± 17.1
	U	86.9 ± 11.6	172.9 ± 24.9	139.2 ± 26.4	169.5 ± 25.3
	<i>t</i> (P)	5.59 (0.03)	6.01 (0.03)	4.80 (0.04)	8.46 (0.01)

^a T, treated; U, untreated.

Results and Discussion

Topical Toxicity and Lethal Time. The topical LD₅₀ of A-9248 against *C. formosanus* was estimated at 141.7 µg/g (*n* = 373; 95% fiducial limits 110.4-168.3 µg/g). The regression equation was $Y = 4.1641 + 0.0060X (\pm 0.0007 \text{ slope SE})$.

When administered topically, effects of A-9248 were fully expressed 14 d after the inoculation. At that time, only groups receiving >1.8 µg/termite (ca. 337.7 µg/g) exhibited mortality ≥90%. The ELT₉₀'s ranged over 8.4-18.9 d (Fig. 1), indicating delayed activity of A-9248 against *C. formosanus*.

Oral Toxicity and Lethal Time. When *C. formosanus* workers were exposed to A-9248-treated substrates for 24 h, few died <10 days after exposure (Fig. 2A). Subsequently, mortality increased almost linearly until the end of the experiment. The ELT₉₀'s derived from data up to 28 d indicated that 21-26 d were required for A-9248 to kill 90% of the termites (Fig. 2C).

In the continuous feeding test, mortality increased exponentially after 10 d (Fig. 2B). Termites died faster than in the 24-h forced-feeding tests because of the daily accumulated intake of A-9248.

ELT₉₀'s derived from the 21-d exposure ranged from 19-22 d (Fig. 2D). A previous study (Su et al. 1987) showed the ELT₉₀'s of 1-12 d for hydramethylnon and 2-14 d for avermectin B₁ against *C. formosanus* after 24-h forced-feeding tests. Thus, A-9248 elicits even more protracted activity than these two slow-acting compounds.

In both forced-feeding tests, only minor differences in mortality or ELT₉₀ were observed among concentrations. Because results of topical tests indicate that A-9248 toxicity is dose-dependent (µg/g), similar mortality or ELT₉₀ among concentration: observed in the feeding tests is probably due to the concentration-dependent feeding deterrence of A-9248 against *C. formosanus*. Groups force-fed on higher concentrations apparently consumed less treated substrate, resulting in toxicant uptake similar to that of termites exposed to lower concentrations.

Choice Test. The concentration-dependent feeding deterrence of A-9248 was further demonstrated in the choice test results (Table 1). Termites avoided feeding on wood treated with ≥8,000 ppm at all weekly determinations, indicating

Table 2. Mortality of *C. formosanus* workers placed in choice test jars with A-9248-treated wood at 0–10,000 ppm during a 4-wk period

Concn (ppm)	Mortality (%) ($\bar{x} \pm \text{SEM}$)			
	Week			
	1	2	3	4
0	4.3 \pm 1.5a	10.0 \pm 0.6a	14.7 \pm 1.7a	16.0 \pm 4.6a
200	15.8 \pm 3.3b	29.2 \pm 2.2b	60.8 \pm 2.9bc	86.3 \pm 9.5b
400	17.1 \pm 1.1b	40.8 \pm 4.4b	91.3 \pm 8.8c	93.3 \pm 6.7b
600	15.8 \pm 1.7b	67.1 \pm 7.0b	87.1 \pm 6.5c	99.6 \pm 0.4b
800	8.8 \pm 1.9ab	53.3 \pm 20.1b	85.0 \pm 13.8c	100.0 \pm 0.0b
1,000	11.3 \pm 1.9ab	62.1 \pm 14.3b	96.3 \pm 3.8c	100.0 \pm 0.0b
2,000	12.0 \pm 2.0ab	36.3 \pm 8.9b	59.3 \pm 5.2bc	64.0 \pm 4.4bc
4,000	9.3 \pm 0.7ab	32.7 \pm 6.6b	53.7 \pm 14.3bc	43.0 \pm 14.6c
6,000	5.3 \pm 2.7ab	39.7 \pm 9.5b	43.0 \pm 8.3b	66.7 \pm 6.6bc
8,000	11.7 \pm 5.0ab	39.0 \pm 10.0b	43.7 \pm 5.5b	57.3 \pm 8.7bc
10,000	8.0 \pm 3.2ab	41.3 \pm 5.9b	62.7 \pm 8.3bc	61.3 \pm 1.9bc

Within a column, means followed by the same letter are not significantly different ($P > 0.05$; Student-Newman-Keuls test [SAS Institute 1986]).

strong antifeedant property of A-9248 at this concentration range. Results from the first week suggest that *C. formosanus* generally (except at 1,000 ppm) consumed less wood treated with A-9248 at 600–6,000 ppm, although the differences were not significant ($P > 0.05$). Termites apparently did not detect the presence of ≤ 400 ppm A-9248 in wood.

One week after the exposure to A-9248, when approximately 10% of the individuals had died (Table 2), termites fed less on the slices treated at $\geq 1,000$ ppm than on the untreated control (Table 1). This trend continued until the end of the experiment. Slight avoidance to the treatment was also observed in the 800-ppm group (Table 1).

Groups exposed to $\geq 8,000$ ppm exhibited lower mortality than was observed at lower concentrations because of the feeding deterrent effect at these rates (Table 2). Termites apparently also became more sensitive to the treatment after initial ingestion of or contact with sublethal doses (1,000–6,000 ppm) of A-9248 during the first week. As a result of the learned avoidance and the sublethal encounters, mortalities for groups exposed to $\geq 2,000$ ppm were 40–60%, while those exposed to 200–1,000 ppm were 85–100% at the end of the experiment.

Similar results were observed with *C. formosanus* workers exposed to hydramethylon under laboratory conditions (Su et al. 1982b). Moreover, when hydramethylon baits were placed in field colonies, *C. formosanus* fed on the baits for several days, then abandoned them after some individuals showed evident symptoms of intoxication (Su et al. 1982a).

The results indicate that the feeding deterrence of this compound is concentration-dependent and that there are at least two levels by which *C. formosanus* responds to the toxicant. Termites avoided feeding toxicants of higher concentrations ($\geq 8,000$ ppm for A-9248) but fed on those treated with lower concentrations. As a result of ingestion or contact with sublethal doses of A-9248, termites avoided feeding on wood treated with intermediate

concentrations (1,000–6,000 ppm for A-9248). This avoidance suggests an associative learning response by a negatively reinforced feedback (Thorpe 1963). Only at even lower concentrations did termites continue feeding on treated substrate. The results of the fourth week indicated that only those groups exposed to concentrations of $\leq 1,000$ ppm exhibited 85–100% mortality (Table 2). Thus, the threshold concentration for a candidate bait with A-9248 appears to be ca. 800–1,000 ppm.

This study has shown that, at the higher concentrations ($\geq 8,000$ ppm), A-9248 may be used as a soil termiticide or wood preservative (or both) because of its toxic and deterrent properties. When used as a bait or tracking powder to eliminate the entire colony of *C. formosanus*, concentration ranges of 200–800 ppm may prove effective.

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