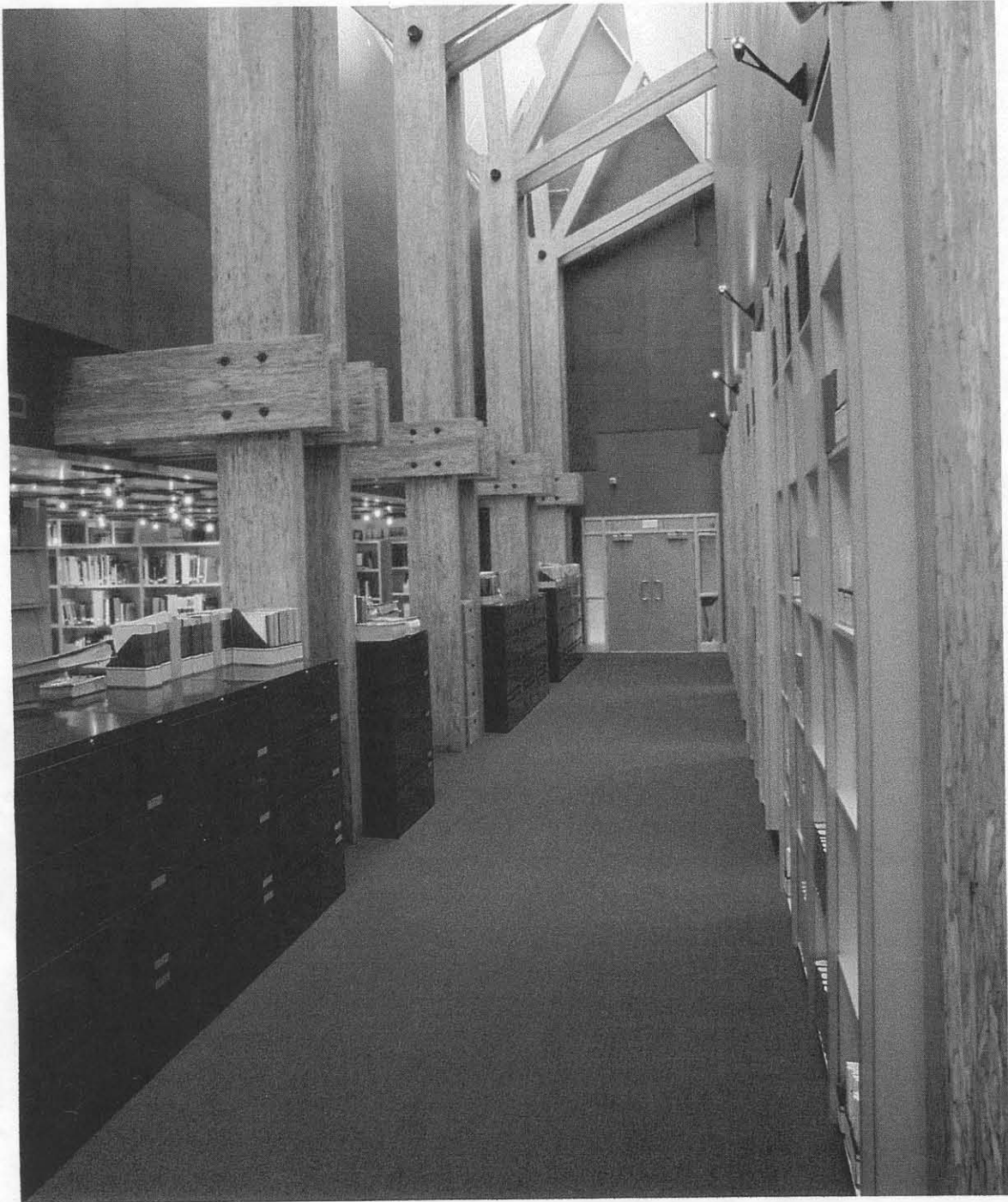


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# Resistance of borate-treated Douglas-fir to the Formosan subterranean termite

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## Abstract

Toxicity of disodium octaborate tetrahydrate (DOT as TIM-BOR®)<sup>1</sup> to Formosan subterranean termites and termite feeding on treated Douglas-fir heartwood were evaluated in laboratory and field tests. Feeding on filter papers impregnated with borate solutions reduced but did not eliminate termite gut protozoan populations. In a forced-feeding laboratory assay, Douglas-fir heartwood treated to retentions  $\geq 0.35$  percent boric acid equivalents (BAE) drastically reduced termite feeding and resulted in 100 percent termite mortality within 3 weeks. Gradual and significant mortality (49%) after 4 weeks of feeding at 0.16 percent BAE suggests that this or lesser concentrations may be useful in baits for remedial termite control. After 162 days of field exposure to an active termite colony, moderate feeding was noted at 0.65 percent BAE (13.6% weight loss) and 0.73 percent BAE (16.9% weight loss), and only slight damage (2.5% weight loss) at the highest retention field-tested of 1.02 percent BAE. These results indicate that treatment with DOT provides protection from Formosan subterranean termite attack, but that some cosmetic damage occurs even at high retentions. This cosmetic damage is unlikely to create a structural hazard, but additional field evaluations are needed to determine whether borate treatments will provide protection to visible timbers that will be acceptable to the consumer.

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The use of preservative-treated lumber in building construction is an important component of integrated pest management of termites (Isoptera) in Hawaii

<sup>1</sup> Mention of trade names is for informational purposes only and does not constitute an endorsement by funding agencies or by the University of Hawaii.

(21,23). The termite species involved are the highly destructive Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Family Rhinotermitidae), and the West Indian drywood termite, *Cryptotermes brevis* Walker (Family Kalotermitidae). Untreated or inadequately treated lumber can be quickly destroyed by these termites.

Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) heartwood is the principal construction lumber used in Hawaii (25). Unfortunately, Douglas-fir is both highly susceptible to Formosan subterranean termite attack (18) and resistant to preservative penetration. Treatment with ammoniacal-copper-zinc-arsenate (ACZA) has been demonstrated to provide protection against Formosan subterranean termites (22), but the required incisions and discoloration of the treated wood prevent the use of ACZA for exposed building timbers. Moreover, arsenical wood treatments in general have raised environmental and public health concerns (3).

Wood treatment with disodium octaborate tetrahydrate (DOT, as TIM-BOR®)<sup>1</sup> has no aesthetic drawbacks, low mammalian toxicity, can provide adequate penetration of Douglas-fir heartwood (2,12,26), and has been demonstrated to be toxic to termites (5,7,27-29),

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although leachability of borates from wood exposed to high moisture levels could be a problem. This study was performed to determine the effectiveness of DOT in protecting Douglas-fir heartwood from feeding by Formosan subterranean termites. This required 1) determining the toxicity of DOT to Formosan subterranean termites and their intestinal protozoa; and 2) evaluating its effectiveness as a treatment for Douglas-fir heartwood in laboratory and field studies.

### Experimental procedure

#### Toxicity test

Borates are considered stomach poisons, and toxicity was assessed by placing Formosan subterranean termites on treated filter paper (Whatman No. 2). A stock solution was made by dissolving 120 g DOT (TIM-BOR, United States Borax and Chemical Corp., Los Angeles, Calif.) in 1 liter of distilled water. Aliquots were further diluted to achieve test concentrations of 120, 12.0, and 1.2 g/l, or 14.4, 1.40, and 0.14 percent BAE (weight/volume).

Each filter paper was completely saturated by dipping in a solution of a particular concentration, and air-dried. It was then placed in a glass petri dish and 2 ml of distilled water was added to moisten the paper. Thirty termite workers, collected immediately before use from an active field colony (20), were introduced into each dish. The dishes were held in an unlighted incubator at 29°C and checked daily for mortality and symptoms of toxicity. Eight replicates were prepared of each solution concentration: five were monitored for termite mortality and the remaining three were used to determine the effect of DOT on the symbiotic gut protozoa *Pseudotriconympha grassi* Koidzumi, *Holomastigotoides hartmanni* Koidzumi, and *Spirotrichonympha leidyi* Koidzumi.

After 1, 2, 4, 7, 9, 11, 16, and 18 days of DOT exposure, three termites from each solution concentration were dissected and the protozoa in the hind gut identified by species and counted (10). Due to large individual and daily variations in protozoan numbers, the daily counts were grouped for analysis over days 1 to 11 and 16 to 18. Data within each of these groups were subjected to analysis of variance (ANOVA) blocked by day, and means were separated by the Ryan-Einot-Gabriel Welsch multiple F test (16).

#### Laboratory test of treated wood

Laboratory tests on the efficacy of DOT as a wood treatment for Douglas-fir heartwood were conducted using 1.9 by 1.9 by 1.9 cm cubes (approx. 2.5 g) pressure-impregnated with TIM-BOR by a modified full-cell process (13). Wood pieces were placed in a tray, weighted down, and immersed in the appropriate TIM-BOR solution. The trays were then placed in a pressure cylinder and subjected to 1 hour of vacuum (28 in. Hg) and 18 hours of pressure (140 to 150 psi). After treatment, the wood was dried (50°C) to constant weight, and selected pieces were assayed for boron content.

DOT retentions were determined by ashing selected

pieces, extracting the residue with dilute HCL, and analyzing the residue for boron content with a spectrophotometric method using the complexing agent azomethine-H (4,6). A small number of samples were also assayed using a hot water extraction method, and results were consistent with those obtained from ashing (13).

The Douglas-fir cubes were treated to 6 DOT retentions: 0.08, 0.13, 0.29, 0.45, 0.67, and 0.98 percent (weight/weight percentage). There were five replicates for retentions of 0.13 percent and higher, and four replicates for 0.08 percent and the untreated controls.

The force-feeding assay with Formosan subterranean termites was a modification of the ASTM D3345-74 (Reapproved 1980) (1) method (similar to AWWA M12-72). The method was modified as follows: 1) oven-dry weight loss instead of visual estimation was used to measure damage; and 2) more termites were used in the test and termite mortality was evaluated. The treated cubes were oven-dried (63°C for 7 days), weighed, placed individually in plastic containers (9.5 cm diameter by 3.5 cm high), and covered with 150 g coral sand (washed and oven-dried). This was a sufficient amount of sand to cover all but the upper surface of the test block. Thirty ml of distilled water was added to each container before adding the termites. Termites were collected as described previously, 400 workers were placed in each container, and the containers were held in an unlighted incubator at 29°C.

Containers were examined weekly to visually estimate termite mortality. Four weeks after the initiation of the test, the cubes were removed, cleaned, oven-dried, reweighed, and total termite mortality was determined. Data were subjected to ANOVA and means were separated by Duncan's multiple-range test (16).

#### Field test of treated wood

Nonleaching, aboveground field tests were conducted in a vigorous colony of Formosan subterranean termites located on the Manoa campus of the University of Hawaii. This colony is monitored on a regular basis and the foraging population is periodically assessed using mark-release-recapture methods (11,19).

Douglas-fir heartwood boards 2.5 by 10.2 by 20.4 cm (approx. 165 g) were pressure impregnated with DOT as described previously to four retentions: 0.18, 0.54, 0.61, and 0.85 percent (wt/wt). Although these boards were not assayed for boron content in a zonal fashion, analysis in three equal layers of similarly treated boards demonstrated essentially uniform retention for the three layers (13).

Rectangular traps or test boxes (10.2 by 10.2 by 20.4 cm) were constructed using two 2.5- by 10.2- by 20.4-cm boards treated to the same retention and two untreated 2.5- by 5.1- by 20.4-cm boards as sides, as described by Tamashiro et al. (22). Each test box was placed within a covered 5-gallon metal can (with the can bottom removed) on the soil surface. Termites had been actively foraging on untreated wood boxes placed within these cans for several years. To minimize leach-

ing of the preservative, a small hollow concrete block (5.1 cm high) was first placed on the soil surface within the can. A short wood stake was driven through the hollow center of the block into the soil (and the termite foraging galleries), and a 6-mil polyethylene sheet (with a hole for the stake) was laid over the top of the block. The test box was then placed on the plastic sheet, with

the wood stake allowing the termites direct access from the soil to the box.

The hollow interior of each test box was filled with paper toweling, and the top of each box was capped with an untreated 2.5- by 10.2- by 10.2-cm Douglas-fir heartwood board. Control test boxes were constructed in a similar manner with untreated wood. Each preservative retention was replicated with four test boxes, for a total of eight boards per treatment.

Traps were examined at weekly intervals to determine when the termites initially attacked each test box. Formosan subterranean termites are capricious in their pattern of attack, and termites were noted in some traps within a few days, while others were untouched for several months. In order to standardize the exposure period, each test box was removed 162 days (23 weeks) after the initial termite attack on the untreated wood in that particular test box was observed. Thus, each treated board was exposed for 162 days to actively foraging termites. After removal from the field, each test box was dismantled, cleaned, oven-dried, and the boards weighed to determine weight loss from termite feeding. Data were analyzed by ANOVA and Duncan's multiple-range test (16).

## Results and discussion

### Toxicity test

Forced feeding on DOT was toxic to Formosan subterranean termite workers in a concentration dependent manner (Table 1). At the highest DOT concentration (120 g/l), populations of the gut protozoa *P. grassi* and *H. hartmanni* were greatly reduced by the fourth day, but were not all killed or eliminated until the termite died. Populations of *S. leydeli* were not affected until the seventh day, when both termites and protozoa were dead.

Susceptibility of the protozoa appeared to be directly proportional to their size and location in the hindgut. *P. grassi*, the largest species, is predominant in the anterior part of the hindgut; *H. hartmanni*, the medium-sized species, is found in the middle; and *S. leydeli*, the smallest and least-susceptible species is predominantly found in the posterior part of the hindgut (10). Analysis of protozoan counts for the two

TABLE 1. — Mean cumulative percentage mortality of Formosan subterranean termite workers fed filter paper impregnated with DOT.<sup>a</sup>

Day	Solution concentration (g/l)			
	0.0	1.2	12.0	120.0
	----- (%) -----			
1	1.3	2.7	1.3	0.0
2	1.3	3.3	2.0	10.0
3	5.3	4.7	3.3	47.3
4	8.0	9.3	6.7	70.0
5	10.0	17.3	13.3	90.0
6	13.3	29.3	24.7	96.7
7	14.0	38.0	36.7	100.0
8	18.7	42.7	44.7	
9	20.7	46.7	55.0	
10	24.0	54.7	86.7	
11	25.3	64.7	95.3	
12	28.0	72.7	100.0	
13 <sup>b</sup>				
14 <sup>b</sup>				
15	36.6	88.7		
16	39.3	92.0		
17	44.0	95.3		
18	46.0	96.7		

<sup>a</sup> Mean of 5 groups of 30 termite workers.

<sup>b</sup> Data not collected on days 13 and 14.

TABLE 2. — Mean numbers of protozoa in individual Formosan subterranean workers over days 1 to 11 and 16 to 18 of feeding on filter paper impregnated with DOT.

Protozoa	Solution concentration (g/l)				
	Days 1 to 11			Days 16 to 18	
	0.0	1.2	12.0	0.0	1.2
<i>Pseudotrichonympha grassi</i>	442 A <sup>a</sup> (±65) <sup>b</sup>	493 A (±79)	200 B (±85)	207 A (±91)	0 B (±0)
<i>Holomastigotoides hartmanni</i>	851 A (±129)	687 A (±107)	331 B (±80)	133 A (±46)	80 A (±55)
<i>Spirotrichonympha leydeli</i>	1024 A (±177)	1087 A (±148)	858 A (±184)	353 A (±163)	233 A (±122)

<sup>a</sup> Mean count per termite. Three termites per treatment were dissected each day. Means within a row, within each day category, followed by different capital letters are significantly different at the  $p = 0.05$  level.

<sup>b</sup> Values in parentheses represent standard errors of the mean.

TABLE 3. — Estimated rate of termite mortality, final mean percent mortality, and mean amounts of DOT-treated Douglas-fir heartwood blocks (each approx. 2.5 g) eaten by Formosan subterranean termites in a 4-week laboratory test.

Percent retention		Mean percent mortality <sup>b</sup>				Wood weight loss	
%DOT <sup>a</sup>	%BAE <sup>a</sup>	Week 1	Week 2	Week 3	Week 4	Mean weight loss <sup>c</sup>	Percent weight loss
		----- (%) -----				(g)	(%)
0.00	0.00	0	0	0	18	1.231 A	53.4
0.08	0.10	0	0	0	23	1.339 A	47.8
0.13	0.16	0	0	0	49	0.784 B	33.4
0.29	0.35	0	39	100		0.211 C	8.4
0.45	0.54	0	73	100		0.141 C	5.4
0.67	0.80	0 <sup>d</sup>	94	100		0.091 C	3.6
0.98	1.18	0 <sup>d</sup>	99	100		0.074 C	2.9

<sup>a</sup> DOT = disodium octaborate tetrahydrate; BAE = boric acid equivalents.

<sup>b</sup> Mean of 5 replicates for 0.13 percent DOT and higher and 4 replicates for 0.08 percent and the untreated controls (400 termites per replicate).

<sup>c</sup> Mean weight losses followed by different capital letters are significantly different at the  $p = 0.05$  level.

<sup>d</sup> Termites affected; activities slowed but no mortality.

TABLE 4. — Mean amounts of DOT-treated Douglas-fir heartwood boards (each approx. 165 g) eaten by Formosan subterranean termites during 162 days of exposure to an active termite colony in a field test.

Percent retention		Weight retention <sup>b</sup>				Wood weight loss	
%DOT <sup>a</sup>	%BAE <sup>a</sup>	DOT	BAE	DOT	BAE	Mean weight loss <sup>b</sup>	Percent weight loss
		----- (pcf) -----		----- (kg/m <sup>2</sup> ) -----		(g)	(%)
0.00	0.00	0.00	0.00	0.00	0.00	115.9 A	70.0
0.18	0.22	0.05	0.06	0.80	0.96	105.5 A	60.2
0.54	0.65	0.15	0.18	2.40	2.88	24.1 B	13.6
0.61	0.73	0.17	0.20	2.72	3.27	26.9 B	16.9
0.85	1.02	0.24	0.29	3.84	4.61	3.7 C	2.5

<sup>a</sup> DOT - disodium octaborate tetrahydrate; BAE - boric acid equivalents.

<sup>b</sup> Mean weight losses followed by different capital letters are significantly different at the  $p = 0.05$  level. N = eight boards per treatment.

lowest concentrations (12.0 and 1.2 g/l) over the first 11 days, and over days 16 to 18 of exposure (i.e., sublethal effects) demonstrated reductions in *P. grassi* and *H. hartmanni* numbers, but no statistically significant change in *S. leydeli* relative to the control (Table 2). Termite mortality is not the direct result of starvation due to the reduction in protozoa numbers, since defaunated Formosan subterranean termites can survive as long as 30 days (9).

From this study, we could not determine whether DOT is directly toxic to the protozoa, or whether the protozoa are affected secondarily as a result of borate toxicity to the termite. Kard (8) also noted reductions in protozoan complement in eastern subterranean termite (*Reticulitermes flavipes*) workers exposed to soil treated with boric acid. However, frequent fluctuations in intestinal symbiont populations and the difficulty of defining primary effects on obligative symbionts have complicated other attempts to determine the mode of action of borate toxicity (29).

#### Laboratory test of treated wood

At the higher DOT retentions, termites feeding on treated Douglas-fir cubes were visibly affected (sluggish) after the first week, and high mortality was apparent at the end of the second week (Table 3). Termite feeding did not differ significantly from the controls at 0.10 percent BAE, but was significantly reduced at 0.16 percent BAE (Table 3). However, despite 49 percent termite mortality at 0.16 percent BAE, a mean 33.4 percent weight loss was observed. At retentions  $\geq 0.35$  percent BAE, all the termites died within 3 weeks, and wood weight losses did not exceed 10 percent. These results are in agreement with the conclusions from similar laboratory tests of Williams et al. (28) that *C. formosanus* failed to survive for 7 weeks on banak (*Virola* spp.) wood with  $\geq 0.125$  percent BAE, and of Williams and Amburgey (27) that retentions in banak  $> 0.17$  percent BAE were toxic to eastern subterranean termites (*R. flavipes*). Su and Scheffrahn (17) reported similar reductions in Formosan subterranean termite feeding, although less termite mortality in tests with DOT-treated pine blocks (DOT retentions were estimated from treating solution uptake).

#### Field test of treated wood

All of the boards, both treated and untreated, exposed to a field colony of Formosan subterranean

termites were attacked to some extent, with analysis indicating three damage levels (Table 4). The controls and those containing 0.22 percent BAE were essentially destroyed (range of 32.8% to 94.8% weight loss). Weight losses at 0.65 and 0.73 percent BAE ranged from 4.3 to 34.9 percent. At the highest retention of 1.02 percent BAE, an average 2.5 percent weight loss was recorded, with individual board weight losses ranging from 0.2 to 6.8 percent. Although the damage at 1.02 percent BAE was cosmetic and did not affect the structural integrity of the boards, this damage was easily noticeable.

This was a rigorous field test, since samples were placed directly into active termite feeding sites and then monitored to insure that foraging termite workers contacted them. During the 162 days of exposure, the untreated boards in each test box were almost completely consumed, with no statistical differences among the traps. This type of field test may be more applicable to the Formosan subterranean termite than more common "graveyard" style tests, since this species does not feed on all the available food items in its foraging territory in a homogenous fashion. For example, in tests with gravel barriers to foraging termites at a heavily infested location in Hawaii, only half of the control stakes have been attacked after 5 years (24).

There was a good relationship between results obtained with the highest borate retentions tested in the laboratory (1.18% BAE) and the field (1.02% BAE). These results also agreed with the report of Preston et al. (14,15) that 1.24 percent BAE was required to protect southern yellow pine from Formosan subterranean termite feeding. However, significant damage occurred with 0.65 and 0.73 percent BAE in the field test, even though comparable retentions in the laboratory killed all termites and prevented much feeding. Estimation of termite populations in the field colony before and after this study did not indicate a decline in numbers, and no reduction in feeding was noted that would indicate a decline in colony vigor. Termites feed alternately at many sites in the field (19), which reduces the frequency of exposure of individuals to a particular poisoned feeding site. Therefore, in order to prevent any damage to the treated wood, it may be necessary to use preservative retentions that are either toxic or repellent on the basis of a single exposure.

## Summary and conclusions

In the laboratory test, forced feeding by Formosan subterranean termites on DOT-treated Douglas-fir containing 0.16 percent BAE resulted in significant termite mortality (49%) within 4 weeks, while concentrations  $\geq 0.35$  percent BAE killed all termites within 3 weeks, and resulted in less than 10 percent weight loss in the treated blocks. However, laboratory tests alone, with small confined groups of termites, cannot accurately predict the retentions necessary for protection from termite feeding under field conditions.

In field tests, 1.02 percent BAE was required to limit termite feeding on the treated Douglas-fir to the status of cosmetic damage. These results indicate that DOT can protect Douglas-fir heartwood from Formosan subterranean termite attack, but it is not possible to predict a retention where absolutely no feeding would occur. Field evaluations of retentions  $> 1.02$  percent BAE are needed to determine the treatment requirements for visible timbers in areas of high Formosan subterranean termite hazard. But cosmetic damage to hidden structural timbers may not pose a problem, as long as repeated termite attacks on those timbers do not occur.

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