LABORATORY AND FIELD METHODS FOR INVESTIGATING SUBTERRANEAN TERMITE CONTROL

M. A. COFFELT[1], A. J. ADAMS[2] & J. L. LINDARS[2]
AgrEvo Environmental Health
[1]95 Chestnut Ridge Road, Montvale, NJ 07645, USA
[2]McIntyre House, High Street, Berkhamsted, Herts HP4 2DY, UK

Abstract—Determining termiticide performance has, historically, been conducted by assessing attack of wooden boards placed over treated soil. This method was developed by the USDA-FS Gulfport laboratory, and is referred to as the ground-board test. A modification of this involves pouring concrete over the treated soil to simulate a sub-slab treatment. Both tests are performed in 4 states and provide the basis of the data required for EPA registration which may, in turn, contribute to registration in other countries.

Several modifications of the ground board and concrete slab methods have been developed as increasing attention has been paid to the influence of factors such as soil type upon degradation rates, with the withdrawal of the persistent organochlorine soil termiticides such as chlordane. Vertical concrete square structures, centred in trenches, have been used that allow soil samples to be taken for the determination of residues in addition to the observable attack of termites on pine boards. Similarly, the termiticide may be mixed with soil, then either covered or left exposed for various periods before soil cores are removed for chemical analysis and bioassay. By using sites with different soil characteristics, it is possible to establish a comprehensive picture to help interpret termiticide performance, when used in the "traditional" pre- and post-construction barrier applications, and in less widely used applications such as direct injection into masonry, as occurs in France, for example.

The main laboratory tests that are used to evaluate the repellent and contact activity of termiticides feature the inclusion of a portion of treated soil in test apparatus where termites have the choice of tunnelling through that soil to a food source, or remaining in another part of the apparatus where food and moisture are present. Some of the more recently developed methods are aimed at examining alternative termite control methods, which require more subtle means of evaluation. Bait toxicants, or trap-treat-release systems require the use of markers or dyes to help estimate colony size and foraging behaviour. They have also required the development of laboratory systems that incorporate colony behaviours so that the potential to transfer dose via trophallaxis or grooming has been determined for slow-acting compounds with different modes of action.

INTRODUCTION

Subterranean termites are major pests of structures throughout the world. It has been estimated in the USA that subterranean termites are responsible for $250 million per year on direct damage for the cost of treatments and repairs (Gold, 1995). Chemical treatments to soil have traditionally been used to protect wooden structures from subterranean termite attack and the use of baits may be another tool to control termite populations. A variety of laboratory assays have been developed to aid the evaluation of candidate termiticides on the basis of their contact, repellency or bait activity and the relative merits of these methods are described and discussed below. Chemical controls, and determining their performance under field conditions, were first pioneered in the USA by the USDA Forest Service in Gulfport, MS. These data from Gulfport supported registration of termiticides, and contributed to registration in other countries. Within the last 5 years, several additional field studies have been designed that address the concerns of pest control operators and regulatory officials. A review of field evaluation methods, results with a synthetic pyrethroid, and influence of soil characteristics will be discussed.

MATERIALS AND METHODS

Laboratory assays

Laboratory repellency tests give a good indication of the activity of pyrethroids and other termiticides in the field. Of the many tests available the most widely used are the Japanese, CTBA and USA/Su methods.

Proceedings of the Second International Conference on Urban Pests.
K.B. Wildey (editor). 1996

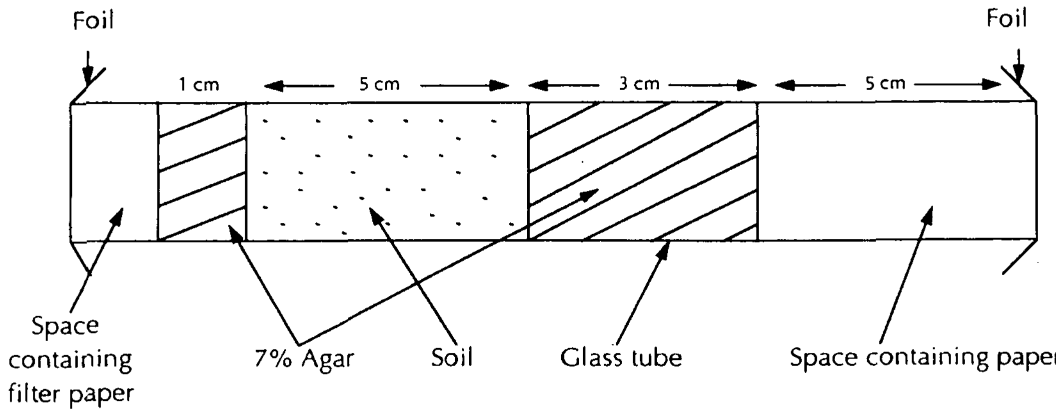

Figure 1. The USA/Su Repellency Test

The USA/Su technique (based on Su and Scheffrahn, 1990) uses a glass tube 25 mm diameter x 150 mm in length. Treated soil is present inside the tube sandwiched between two sections of 7 % agar (one measuring 10 mm and the other 30 mm). Filter paper is placed into voids in each end of the tube. Termites (80 workers and 1 soldier) are put into the tube adjacent to the 30 mm piece of agar (Figure 1). Penetration into the soil is monitored daily for 7 days, after this time the test is terminated and mortality is recorded.

The Japanese repellency test (JWPA Standard No. 13) is slightly more complicated than the USDA method and consists of two glass cylinders (120 mm high) joined at the bottom via a connecting tube (50 mm in length) which contains the treated soil. In one of the cylinders, where the termites (200 workers/20 soldiers) are placed, soil is present and the other cylinder contains pine tree chips. Penetration through the connecting tube and mortality (at 21 days) are the parameters that are assessed.

The CTBA (reference X-41540, Coudin, 1995) test works on the same principle as the USA/Su and Japanese Tests. It comprises 2 glass cylinders (50 mm x 50 mm in diameter) separated by a paper disc and a polyethylene film disc. The termites (150 workers/3 nymphs/2 soldiers) are placed in the top glass cylinder onto polyurethane foam which is 30 mm in depth. The bottom cylinder is filled with a standardised soil composition (Figure 2). The test is monitored over a 4 week period and the depth of penetration into the soil is assessed. When the test is terminated other indicators of activity such as galleries in the foam and holes made in the paper/film divider, as well as mortality, are recorded. The advantages of this test over the USA/Su test is that there are a larger body of soil and more parameters of termite activity are assessed. Although the monitoring period for the CTBA test is 3 weeks longer than with the USA/Su test, an indication of activity can be ascertained within the first few days post-infestation.

In some countries, termiticides are injected directly into masonry – presenting a very different environment to that encountered in soil. One standard test method has been developed by the CTBA in France, Norm X-41541. The experimental apparatus consists of concrete cylinders with 4 hollow tubes (3 mm diameter) through them, these are allowed to dry, weighed and then immersed in the chosen dilution of product. A vacuum of 40 mbar is applied for 20 minutes, prior to return to atmospheric pressure. The blocks are then left, immersed, for 2 hours prior to drying and re-weighing (to calculate the retention of product) and then allowed to acclimatise for a further 10 days (21^0 C, 70 % rh). A group of 150 workers, 2 soldiers and 3 nymphs (*Reticulitermes santonensis*) is transferred into the top portion of each apparatus (Figure 3). The experimental units are maintained in darkness at 25–28^0 C, 75 % rh for 4 weeks. Records are made of termite behaviour, distribution and mortality.

Newer technologies in termite control such as baiting and Trap-Treat-Release often lead to more complicated tests in the laboratory as they are based on the behaviour of the termites. Preliminary studies of slower acting compounds such as silafluofen or Insect Growth Regulators (IGRs) which can potentially be used in this way can be conducted in crude petri dish tests but these tests are not

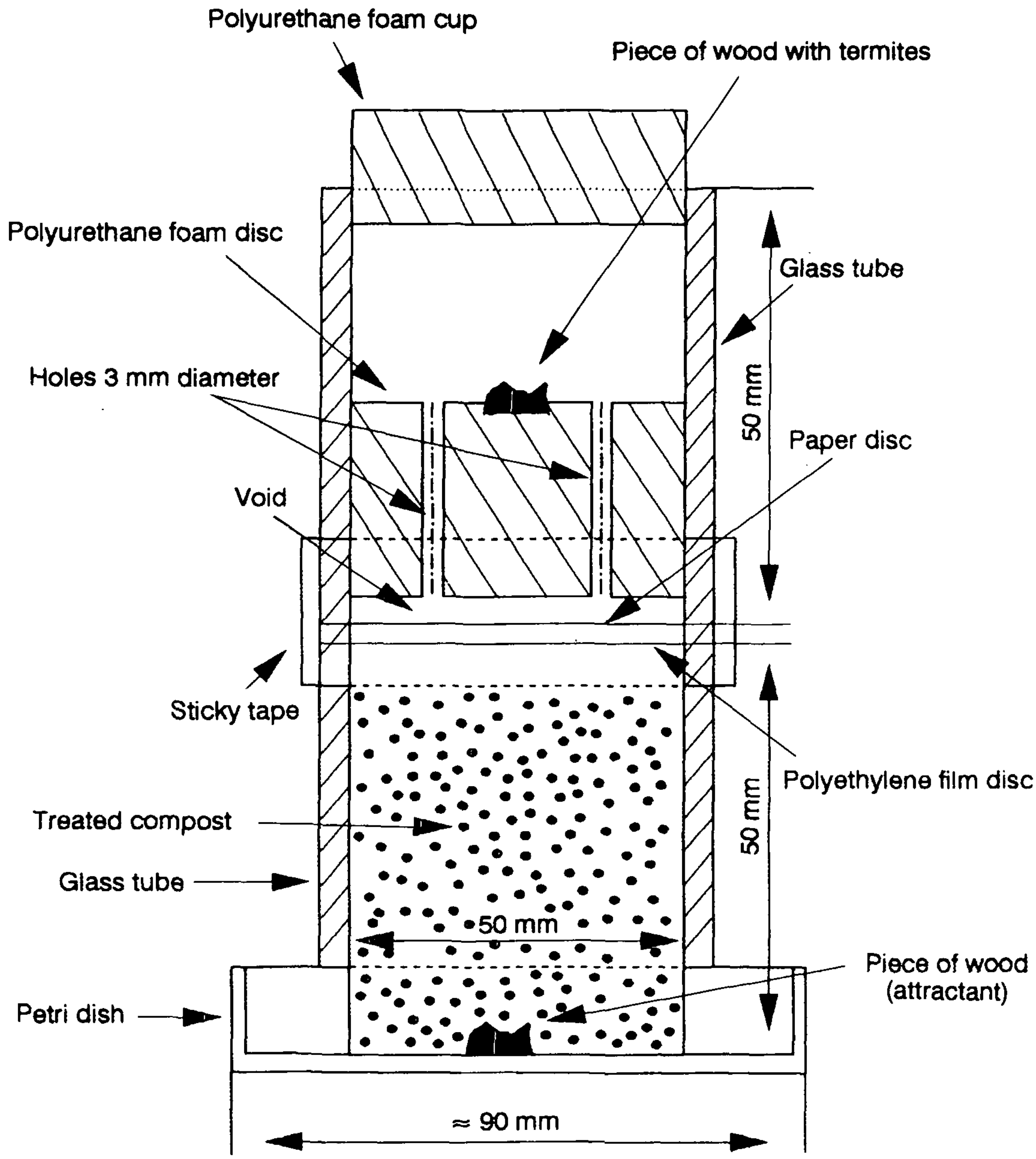

Figure 2. The CTBA Repellency Test

representative in any way of termite behaviour in the field. A more advanced techniques is a type of choice test (Figure 4).

Termites are placed into petri dish A and allowed to acclimatise for 24 hours pre-treatment. After this time the majority of termites will have become established in petri dish B or will be present in the adjoining tube (which is 170 mm in length). Baits, or termites taken from petri dish B where they have been treated (e.g. by direct dusting, or after ingesting a slow-acting bait), can then be placed into area A, the idea being that the termites will try to return to the rest of the group taking with them a lethal dose of insecticide (Figure 4). Again, this test can be scaled up with the use of established mini colonies containing Queens, workers, nymphs and eggs (requiring a longer acclimatisation period of a week, or more). Mini colonies have successfully been reared using *R. santonensis* bred on moistened wooden tongue depressors.

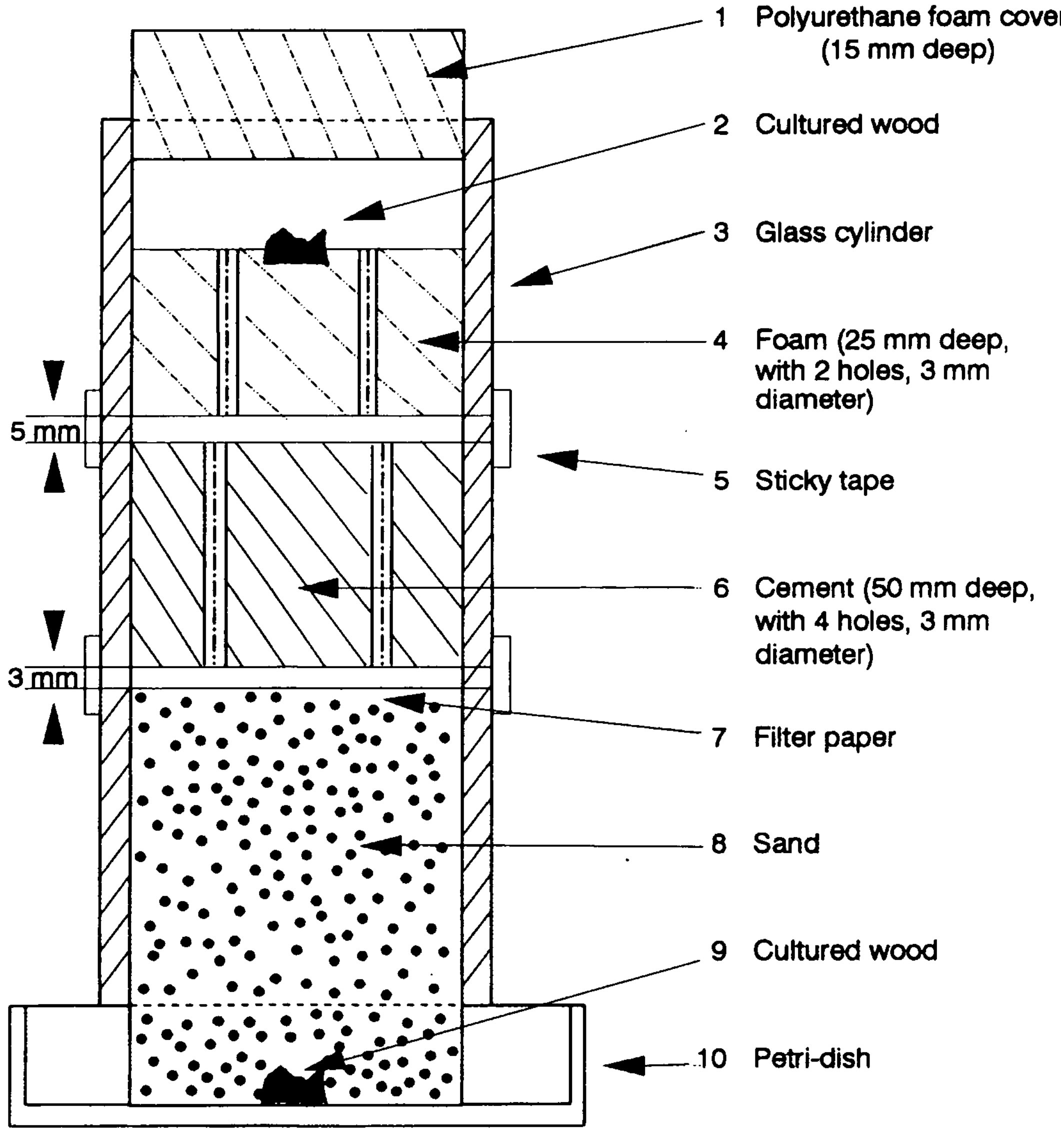

Figure 3. The CTBA Concrete Test

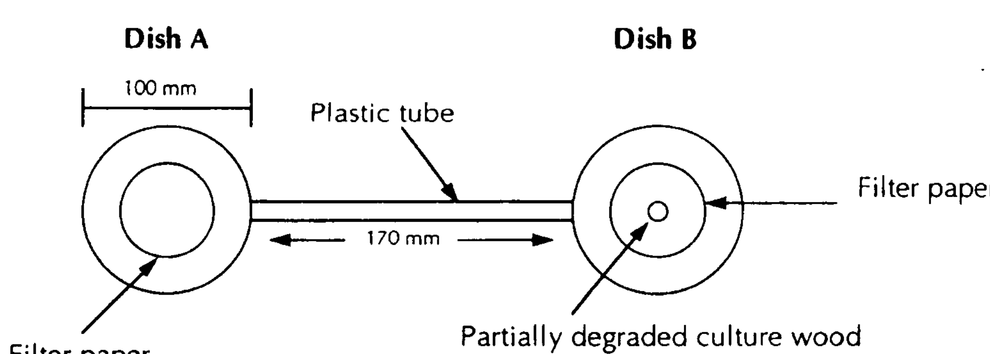

Figure 4. The Petri Dish Choice Test

Feeding *R. santonensis* on treated filter paper as the only source of nutrition has been shown to be a good technique for the evaluation of IGRs. In such a test the compounds have an effect via feeding and contact activity. Tests conducted using moistened filter paper discs treated with fenoxycarb at 50 ppm showed that 93.3 % of nymphs became pre-soldiers within 14 days post-exposure and 100 % mortality was achieved at 24 days. In the same test with diflubenzuron, also at 50 ppm, 100 % mortality was seen by day 53 post-exposure to the treated papers. Filter papers were re-moistened daily and changed weekly.

Field tests:

The USDA Forest Service ground board and concrete slab tests are described in Kard & Mauldin (1994). Replicated field plots are established in four southern states (Arizona, Florida, Mississippi and South Carolina). Several concentrations of termiticides are applied to bare ground plots using a sprinkler can. A pine board is placed in the middle of the plots (termed the ground board test), or a vapour barrier is placed over the treated ground and a concrete slab is poured over the barrier and around a centred plastic pipe (termed the concrete slab test). A pine board is placed inside the pipe and in contact with treated soil. This concrete slab test simulates pre-construction termiticide treatments to soil under a concrete slab. The pine boards are evaluated yearly for termite attack. There are several termite species at each site.

Concrete square structures were used in one Mississippi location to simulate vertical walls of crawl space building foundations (McDaniel & Kard, 1994). Structures were centred in trenches, and soil back-filled to form internal and external trenches around the vertical walls. A known portion of soil was sprayed with termiticide solutions as they rotated in a concrete mixer, and this treated soil was placed in the trenches. Pine blocks were placed on top of treated soil adjacent to internal and external walls. All structures were covered with plywood. Soil was sampled from trenches with a soil probe annually after the first year and soil residue analysis performed. Pine blocks were assessed for termite attack by *R. flavipes, R. virginicus,* and *R. mallettei.*

Another termiticide soil incorporation method is being used by Gold (1995) that simulates a trench and rod termiticide application by pest control operators. Five locations in Texas have been selected. This method involves auguring soil from a given area and placing the soil in a concrete mixer. The soil is mixed and termiticide is uniformly applied by spraying a known volume on a known weight of soil. The soil is placed back in the hole and plots are either covered with a concrete block or left exposed. Soil is sampled from plots with a soil probe annually after the first year. Soil residue analysis is performed and reported in ppm. Sub-samples of soil are used in a laboratory bioassay using test tubes where *R. flavipes* survival and distance tunnelled is measured.

In two Mississippi locations, concrete rectangular structures were constructed and placed 20 cm into the ground (Jarratt & Haskins, 1996). Trenches were made internally and externally from the structures and the soil removed. As the soil was replaced back in the trenches, termiticides were sprayed evenly on to the layered soil to ensure uniform distribution. The top of the structures was covered with plywood, and external trenches were left uncovered. Soil was sampled from trenches with a soil probe annually after the first year and soil residue analysis performed.

Termiticide field evaluations in Hawaii with Formosan termite (*Coptotermes formosanus* Shiraki) are conducted by Grace (1993). Six locations are used on four islands with clay and sandy soil. To simulate typical building construction, soils are sprayed with termiticides and treated soil is covered with a vapour barrier. Concrete slabs are placed over treated soil and sampled annually by lifting the concrete slabs and taking soil cores. Soil cores are used in a laboratory bioassay that determines termite mortality and distance tunnelled.

The Association of Structural Pest Control Regulatory Officials (ASPCRO) have established termiticide soil sampling guidelines based on field work with actual pest control operator applications. Operators applied termiticides to 21 homes in each of four states (Arizona, Georgia, Illinois and Oklahoma) (Mix, 1995). Soil samples were taken by regulatory inspectors at designated time intervals. Data are presented from all 4 states combined and ASPCRO was confident of data through 180 days.

RESULTS & DISCUSSION

The repellency tests all evaluate the same basic parameters, which are contact and repellent activity of the termiticide. The USA/Su test is, therefore, the preferable technique as it is easier to prepare and final results can be seen at 7 days: the threshold concentration for a repellent termiticide is that which totally prevents penetration of the soil column whereas a contact toxicant would cause 100 % kill (control mortality <20%). With the CTBA soil method (X-41540) it is harder to see tunnelling into the soil and sometimes termites tunnel through the middle of the soil mass making it impossible to evaluate penetration until the test is terminated. Uniform mixing of the termiticide into the soil is more difficult with a larger body of soil. The criteria for effective contact-acting termiticides are 100 % kill and <30 mm penetration of the soil. For repellent compounds the CTBA test requires that there is 0 mm penetration of the soil and >80 % kill. The requirement for kill in addition to repellency is largely a function of test duration because termites that do not enter the moist environment of the soil will die from dehydration by the end of the test. Consequently this feature is effectively an artifact of the test protocol rather than a feature of the termiticide and this criterion is now being relaxed by the CTBA. The field application rate that is derived from this standard is based upon a treatment at 5 litres/ m^2 evenly distributed in the top 5 cm of soil and features a safety factor of x4 the effective rate in the laboratory test. Although Norm X-41540 can provide additional information with respect to perforation of the filter paper and galleries formed in the foam, for establishing the basic activity of an insecticide the USA/Su method is both faster and simpler.

At present there is only one established standard procedure for evaluating termiticides that are intended for injection into walls – CTBA norm X-41541. This test requires that the product causes 100 % mortality or there is no perforation of the filter paper disc that separates the treated concrete block from the untreated soil. The effective concentration is subject to a safety factor of x1.5 (after leaching). Treated concrete blocks are buried in the field for upto 5 years and exhumed for test during this period as a means of establishing the longevity of the treatment. The test has only been developed recently and it remains to be seen how the effective rates obtained in the laboratory will compare with those that provide protection under actual use conditions.

The laboratory tests for the evaluation of slow acting termiticides are more complicated to perform. The superior techniques are those that are closer to a field population of termites such as techniques which use the mini colonies. This is because the latter have all stages of the termite lifecycle and so the social behaviours are more likely to represent those encountered in termite nests in the field.

Field trial results with a common synthetic pyrethroid (fenvalerate) are presented in Tables 1 & 2, and some important soil characteristics are listed. These soil characteristics have been identified as important contributing factors that influence termiticide residuals in soil. In general, the higher the clay content and soil pH, the less termiticide residual. Other contributing factors are numerous, such as environmental conditions (rainfall, drought, severe temperatures), physical features (rocks, limestone), termite species, and microbial activity.

The USDA-FS concrete slab data (Table 1), shows that fenvalerate gave 3–12 years of 100% control at the 0.5% rate, and 6–12 years at the 1.0% rate, depending on location and soil characteristics. Fenvalerate had a longer residual in highly alkaline sandy soils of AZ. These field trials rely on the assumption that termites will forage evenly throughout field plots and attack pine boards with equal probability. This has not been proven and termites may not be active in one particular area of the plots, giving false results for percentage control. Moreover, neither soil residue analysis nor bioassay are done to confirm pine board attack readings. These field methods could be improved by taking additional samples for such residue and bioassay analysis.

Termiticide soil incorporation methods simulate trench and rod applications and treatment of back-fill soil by pest control operators (Table 2). The percentage of fenvalerate in MS after 4 (26%) and 5 years (20%) and the corresponding ppm values (179 & 135), respectively, are shown in Table 2 (McDaniel *et al.*, 1996; McDaniel & Kard, 1994). This site has acidic soil and low clay content, more favourable conditions for many termiticides. Pine boards were attacked in all treated soils after 2 years at this site, and it was confirmed that termites were using untreated soil to construct tubes through the termiticide barrier and reaching pine blocks (McDaniel *et al.*, 1996). This finding may have profound effects on termiticide applications at some sites.

Table 1. Years of fenvalerate control in USDA – FS concrete slab tests.

Fenvalerate control, USDA-FS concrete slab test (Kard & Mauldin, 1994)						
	Years of 100% control		Soil characteristics			
USA state	0.5% rate	1.0% rate	Soil pH	% sand	% clay	% silt
Arizona	12	12	6.9	77	8	15
Florida	3	6	4.8	94	3	3
Mississippi	7	10	5.1	70	5	25
South Carolina	4	6	5.8	83	7	10

Table 2. Termiticide soil incorporation results with fenvalerate.

		% fenvalerate remaining after		Mean ppm fenvalerate after		Soil characteristics			
Author	Location	4 yrs.	5 yrs.	4 yrs.	5 yrs.	soil pH	% sand	% clay	% silt
McDaniel *et. al.* 1996	Harrison County, MS	26	20	179	135	5.1	70	5	25
Gold 1995	Corpus Christi, TX	15		114		7.8	38	47	15
	College Station, TX	12		104		7.1	54	12	34
	Dallas, TX	7		38		8.2	3	64	32
	Overton, TX	12		54		6.4	73	15	11
	Lubbock, TX	6		42		7.7	52	30	18
	All 5 TX sites	10.5		70					
Jarratt &	Gulfport, MS	73		417		4.9	73	4	22
Haskins 1996	Mississippi State, MS	60		436		4.5	10	26	64

– combined data from both covered and uncovered plots for all tests.
– tests have been completed for either 4 or 5 years.

The influence of soil characteristics on termiticide residuals is very evident from data generated by Gold (1995) (Table 2). The lowest amount of fenvalerate (38 ppm) was found at Dallas, TX, which has the highest soil pH and percentage clay compared to the remaining four locations. The highest amount of fenvalerate (114 ppm) was found at Corpus Christi, on the TX coast, with high soil pH and lower clay content compared to Dallas. These TX results confirm the importance of soil type on termiticide residuals. Bioassay results showed that fenvalerate was effective after 4 years in preventing termite tunnelling. This field test has all the necessary components for evaluating termiticide residual performance, including pest control operator type application, soil residue analysis, and bioassay.

In Mississippi, the clay site showed a lower percentage remaining of fenvalerate (51%) compared to a sandy soil, and both soils are acidic (Table 2) (Jarratt & Haskins, 1996). These two locations showed the highest amounts of fenvalerate compared to the other studies. This field method gave favourably results for most termiticides. Soil bioassay or evaluation of pine board attack would supplement this method.

In Hawaii, Grace (1993) has demonstrated a wide range in percent Formosan termite control, depending on the termiticide. The bioassay method is used, although soil residue data are not determined. Native soils are not used at each of the six locations, but sandy and clay soils are physically placed in each location. This method has been used successfully to determine residual termiticide performance under very wet and hot environmental conditions.

The ASCPRO data was statistically analysed and established a confidence level for soil residue amounts through 180 days (Mix, 1995). These residue data may be used as a regulatory tool. For fenvalerate, the mean ppm at 30, 90 and 180 days was 204, 180 and 150 ppm, respectively. These ppm data were higher than any other registered termiticide product, indicating that fenvalerate after 180 days degraded less and gave a longer residual. Soil characteristics were not used in this study because data from all four states were combined to establish an overall residue level for regulatory purposes. A regional approach may be needed to establish residue levels more closely tied to local soil conditions.

Field studies in the USA vary in their technique to evaluate termiticide residuals. Each method has advantages and disadvantages. Results with fenvalerate indicate that residuals vary depending on several factors, with soil charactieristics being among the more important. A prescription type approach to termiticide applications and field studies may be appropriate, especially with reference to current field data showing the influence of soil types on termiticide residual performance.

ACKNOWLEDGEMENTS

We would like to thank Dr. Jim Jarratt of Mississippi State University and Dr. Skip McDaniel of the USDA-FS, Gulfport, MS, for allowing us to use their unpublished data.

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