DRAFT TERMITE BAIT TESTING GUIDELINE DATED 7/01/2002
DRAFT - OPPTS 810.3800. Product Performance Guideline - Termite Baits - 7/01/2002
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(a) Scope.

(1) Applicability. This guideline describes test protocols that EPA believes will generally satisfy product performance testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq*.) for termite bait products. As explained in 40 CFR section 158.70(a), applicants for pesticide registration may utilize any appropriate protocol provided that it is of suitable quality and completeness to provide EPA with sufficient information to adequately assess the product. Accordingly, instructions in this protocol directing applicants to conduct testing in a specific manner do not and are not intended to establish a regulatory requirement, but are intended simply to instruct applicants what they must do should they choose to follow this particular bait testing protocol.

(2) Background. The published literature on termiticide bait research was the source material for issues not addressed in the above guideline. A list of references is attached.

(b) Definitions. The following definitions are of special importance in understanding this guideline:

The term *structural protection* refers to the elimination or prevention of termite activity in a structure as a result of a preventive or curative application of a pesticide product. For a preventive application, termites cannot infest a structure and no damage to the structure can be caused by termites. For a curative or remedial application, structural damage by termites ceases.

The terms *protect(s) a structure, protection, eliminate(s)* and *control(s)* have the same meaning as the term *structural protection.*

The term(s) *termite infestation* or *termite activity* refer to the presence of live termites in a structure.

The term *kills termites* refers to termites dying as result of feeding or contacting a pesticide. The affected termite life stage should be referenced.

The term *termite colony* refers to a group of termites of the same species which constructs a nest (may consist of dispersed galleries and chambers), rears offspring in a cooperative manner, and shares an interconnected gallery system (Wilson 1971, Su and Scheffrahn 1998b).

The terms *termiticide bait* or *termite bait* refer to any pesticide product that kills or controls termites and has at least two-principal components: (1) a bait matrix, equal to or preferred to other available sources of food; and (2) a pesticide incorporated into the bait matrix that kills termites.

The term *pre-construction* refers to and includes all phases of a structure's construction up to and

including installation of the final grade.

The term *pre-construction treatment* or *application* refers to and includes all pesticide treatments made to kill and/or control termites during all phases of a structures's construction up to and including installation of the final grade.

The term *post-construction treatment* refers to all pesticide treatments made to kill and/or control termites after the installation of the final grade.

The terms *remedial* or *curative treatment/application* refer to and include all pre-construction or post-construction pesticide treatments made to kill and control a termite infestation when present.

The term *preventive* or *preventative treatment* refers to all pre-construction or post-construction pesticide treatments made to provide structural protection before a termite infestation is present.

The term *stand alone* refers to a pesticide product that provides structural protection when applied without other pesticide products for the same purpose.

Method modification is an alternative way to perform the described test.

(c) Overview.

(1) Purpose

(A) This guideline concerns the product performance testing for evaluation of pesticides used as baits to kill and control termites. Good Laboratory Practice Standards (GLP) apply to these laboratory and field studies as defined in 40 CFR Part 160.1 to 160.195. Studies which do not comply with GLP standards may be considered at the option of the Agency, based on its assessment of the scientific reliability of the study. All testing is to be done with the end-use product formulation or treated article.

(B) This guideline describes specific methods for conducting product performance testing of termite baits which reflect the Agency's considered recommendations for minimum steps necessary to develop reliable data on termite bait product performance. Deviations from this guideline should be fully explained and justified.

(C) A general discussion of criteria for assessing termite bait success can be found in Thorne and Forschler 1999, Thorne and Traniello 1994, Forschler 1998, Su 1991, Esenther and Beal 1974, Pawson and Gold 1996, Su 1994 a,b,c; Su et al. 1997, Su and Scheffrahn 1996 a,b,c, Su 1998, Scheffrahn and Su 1997, Su 1999, Jones 1989, 1991, Lenz 1996, Robson 1996, Rust 1996, Su 1994, Su 1996a,b, Su 1995, Traniello 1994, Grace 1996, Sornnuwat et al 1996a, b; Tsunoda 1998, 1999, and Rust 1996. Other references are cited throughout this guideline.

(2) General considerations. As a general matter, a termite bait does not become a candidate for field testing until it has demonstrated success in laboratory tests similar to those conducted by Jones 1984, or Su and Scheffrahn 1989, 1991a,b, 1993a, 1996c or equivalent methods. In laboratory testing, characteristics of a candidate bait are to be: it should readily be fed upon by termites, exhibit slow-acting delayed toxicity (presumably to aid in secondary kill), be effective over a wide range of concentrations with a palatable alternative food source concurrently available, and cause 100 percent mortality in greater than two weeks but less than ten weeks post-treatment at the dose selected for field testing. Laboratory data should be provided to the field test personnel before inclusion of a bait product in field tests.

If a termite bait is successful in laboratory studies, it will be a candidate for small and large scale field testing to include structures as described in this guideline. An Experimental Use Permit (EUP) may be required as set forth in 40CFR 172.3. The Agency recommends that each termite bait formulation with its associated application materials be placed in small-scale field tests to include the following United States Department of Agriculture - Forest Service (USDA-FS) field test sites located in southern Arizona, southern Mississippi, and either the northern panhandle of Florida or South Carolina. These sites represent varying climatic and soil conditions, and include several subterranean termite species. However, since these sites do not have populations of *Coptotermes formosanus*, a field site for testing against this species shall be added (recommendations include Hawaii and/or Louisiana). Field tests not performed at the USDA-FS field sites should be conducted as described in this guideline.

(d) General considerations for LABORATORY tests.

(1) **Species.** Identify test termites as to genus and species and by subspecies or strain when possible. Laboratory termite populations should be colonized from field collected cultures of four subterranean termite species from the United States to include: *Coptotermes formosanus;* two species of *Reticulitermes* from among *R. flavipes, R. virginicus, R. hesperus*; and *Heterotermes aureus.* Collect field cultures according to the method of Su and Scheffrahn (1986) or by an equivalent method. Optional evaluations against the dampwood termites, *Zootermopis* spp., and/or drywood termites, *Cryptotermes* and *Incistermes* spp. should be performed only if specific label claims are intended to be made for the control of these termite species. All laboratory bioassays should be made using triplicate colonies of the test species.

(2) **Stage, caste, and age**. Test laboratory termite populations cultured from the field no later than 90 days after field collection. The selection of the life stage to test will be dependent upon the pesticidal action of the active ingredient, but at a minimum should include 100 worker termites [undifferentiated nymphs of at least the third instar in the genus *Reticulitermes* (Thorne 1996) and fifth or sixth instars of true workers in the genus *Coptotermes* (Roisin and Lenz 1999, Forschler and Jenkins 1999)]. This determination should be made where possible for U.S. species) from the same field collection site. In addition, bioassays may include soldier caste members collected from the same site as the worker termites. Caste proportion in the test population should reflect the optimum for

the test species (Haverty 1977).

(3) **Rearing techniques**. Rear termites according to species specific requirements. A description of the rearing methods should accompany laboratory test results.

(4) **Bioassay design**. The method described for subterranean termites by Su and Scheffrahn (1989) should be used. This method uses no-choice and choice bioassays to assess active ingredient efficacy. Bioassays with drywood termites may be done as described by Scheffrahn and Su (1997). Use of other methods should be justified.

(5) **Feeding Preferences and palatability testing.** Testing to show that a candidate bait is preferred more than other food sources may be needed. For subterranean termites, it is suggested that feeding preferences be tested by the method of Oi et al.(1996). In addition, data must be submitted that show that the bait is palatable to termites at the active ingredient concentration(s) to be tested in the field and for commercial distribution as a registered product.

(6) **Test arenas and testing conditions**. See paragraph (d)(4) of this guideline.

(7) **Data reporting**. See paragraph (c)(1)(A) of this guideline.

(8) **Data evaluation and analyses**. See paragraphs (d)(4) and (d)(5) of this guideline.

(e) General considerations for SMALL-SCALE FIELD TESTS against subterranean termites

Background and overview. Two differing approaches to small-scale field testing are proposed to test whether or not a termite bait works. The first approach is based on a concrete block or concrete slab test that is similar to those conducted for soil applied termiticides. In preventive treatments, the bait must prevent termite attack on wood in the unit (100% success) for a minimum of five years. For remedial treatments, the infestation must be eliminated within 12 months of bait installation and not be re-infested for another 12 months. Success must be 100%. The second approach is based on bait's ability to exterminate termite populations/colonies on an area-wide basis and has been widely used in termite bait product development. Each test replicate consists of one termite population/colony. The performance standard is 80% (4 of 5 treated colonies exterminated 12 months after treatment)over a testing period of three years. The Agency expects the SAP to review, evaluate, and comment on each method and to recommend the best method for testing termite bait efficacy under conditions that mirror actual use.

#1 and #2 - Concrete-block/slab Test Methods

(1) **Site selection.** The Agency recommends that each termite bait product with its associated application materials be placed in pilot field tests to include the USDA-FS field test sites located in southern Arizona, southern Mississippi, and the northern panhandle of Florida or South Carolina. These sites represent varying climatic and soil conditions, and several subterranean termite species. However, since these sites do not have populations of *Coptotermes formosanus,* a field site shall be selected for testing against this species. Recommendations include Hawaii and/or Louisiana. Field tests not performed at the USDA-FS field sites should be conducted as described in this section. At a minimum, sites shall be located in EPA Regions 4, 6, and 9.

(2) **Termite species.** The subterranean termite species that must be tested at the field sites include species from three genera*, Reticulitermes, Heterotermes* and *Coptotermes.* Data collected with any of the following *Reticulitermes* species are acceptable*: Reticulitermes flavipes, R. virginicus,* and *R. tibalis.* For data collected from termites from the genera *Heterotermes* and *Coptotermes,* only *Heterotermes aureus* and *Coptotermes formosanus* are acceptable.

(3) **Method #1- Concrete-Block Test Method**. The USDA-FS Termite Bait Concrete-Block Field Test protocol as described by Kard 1999 (unpublished) provides the basis for the standardized test method presented below. This test is designed to test termite baits under simulated crawl space construction methods currently used in the United States. This protocol is also applicable to concrete slab, basement, and plenum construction. The bait(s) will be evaluated for preventing subterranean termites from feeding on wood in a

non-infested simulated structure and for eliminating subterranean termites from a termite infested simulated structure. Products intended to be labeled with preventive claims are subject to remedial and preventive testing.

(I) CONCRETE FIELD TEST UNITS

(i) **Concrete-Block Field Test Unit**. The following procedure is performed with a square open precast concrete building construction block, [41cm (16in) outside measurement, 30cm (12in) inside measurement, with 5cm (2in)-thick by 20cm (8in)-high walls]. To establish a concrete-block test unit in a field plot (Figure 1), leaves and debris are removed to expose soil in a square, level area 62cm (24in) on a side. Excavate and remove soil in this area to the depth of 2.5cm (1in). Drill a 4cm (1.6in) diameter hole through two opposite sides of the block, 2.5cm (1in) below the top edge and centered on the side of the block. A 7.5cm x 7.5cm (3in x 3in) square piece of window screen (metal or synthetic) glued (caulked; epoxied) over each hole to allow for ventilation and to exclude insects, rodents, or other invaders from entering the block through the hole. The ventilation holes will reduce the humidity inside the block, thereby simulating crawl space building construction ventilation and reducing the rate of wood decay. Place the pre-drilled concrete block into the 2.5cm(1in)-deep excavation in the soil. On top of the soil in the center of the excavation, place a six-board prefabricated southern yellow pine sapwood sandwich (see (e) (3)(ii) of this guideline) (use of other preferred wood should be supported by published methods and palatability test data) inside the concrete block. Place a 30cm x 43cm x 1cm (12in x 17in x 3/8in)-thick opaque white plexiglass lid on top of the block and hold it in place with a common building brick on top. Humidity and temperature measurements should be made to insure that termite activity or fungal growth are not affected by high temperature or excessive condensation.

Method Modification (Alternative Method) Number 1

Add a buried wood block to the test unit below the six-board sandwich. Soil beneath the concrete block should be excavated to eliminate live termites from the soil beneath the concrete block. A 60cm x 60cm (24in x 24in)-square by 15cm (6in) deep hole should be excavated and the soil screened through a 6.4mm (1/4in) square opening steel mesh screen to remove debris and kill soil arthropods, including termites. Back-fill the excavated, screened soil into the hole in 5cm (2in) layers, compacting each layer vigorously to kill any remaining termites. During this procedure, carefully inspect the soil and kill live termites when found. As a modification, remove all the soil and replace it with heated treated soil. This would insure that there are no foraging termites in the excavated soil. Fill the soil to the original surface and dig a 20cm(8in) square by 7.5cm (3in) deep hole in the center of the excavated 41cm (16in) square area. Place a 1in x 6in x 6-inch (2.5cm x 15cm x15cm) southern yellow pine sapwood board horizontally in the bottom of

the hole. Place the six-board sandwich on top in the center of the wood block.

(ib) **Method Number 2 - MODIFIED CONCRETE SLAB METHOD FOR PREVENTIVE AND REMEDIAL TREATMENTS**

(ib) **Field Test Unit II - CONCRETE SLAB TEST UNIT**. Establish a concrete slab test unit as in the USDA-FS Modified ground board test for liquid termiticides. Bait station placement and six-board sandwich construction are the same as described in this guideline except: (1) exclude the buried board; (2) place the six-board sandwich over the polyvinylchloride (PVC) pipe opening; and (3) place the concrete block on the slab. The termites must tunnel underneath or over the slab to reach the six-board sandwich, located on the open PVC pipe above grade instead of directly on the soil (a condition that does not normally exist in most structures). The presence of the slab may also help to reduce humidity in the test unit.

(ia and ib continued) **Bait Installation** Two bait stations should be placed outside each concrete block/slab. Each station should be located 50cm (20in) or less (as directed by the label) perpendicularly from opposite sides of the block (Figure 2). Negative control replicates are configured the same as the two treatments (preventive and remedial) except that the bait matrix without the active ingredient is placed in each bait station.

If any active bait materials or monitoring strips or paper matrices in the bait stations become severely decayed, completely soaked with water, or disintegrate during the test, they will be replaced with similar fresh material. The date of replacement and condition of the matrix will be recorded. Bait stations will receive fresh bait or monitoring materials at least once a year.

Method Modification (Alternative Method) Number 2
Four (4) bait stations may be installed instead of two. Place a bait station on each of four sides of the test unit.

(ii) **Six-board sandwich construction (prefabricated) and termite marking techniques.**

The prefabricated six-board sandwich is constructed of 2.5cm x 15cm x 15cm (1in x 6in x 6in) southern yellow pine sapwood boards separated by spacers made of thin strips of wood (cut from wooden tongue depressors, popsicle sticks, or other palatable wood), or strips of heavy twine or other functional spacer material, that are 6mm (1/4in) wide and 3mm (1/8in) thick. Spacers should be placed along two opposite sides of the boards (Figure 1). Spaces between sandwich boards allow termites to forage freely between the boards, and they facilitate collection and assessment of dyed termites. Four layers (5in x 5in; equivalent

to12.7cm x l2.7cm squares) of Whatman No.1 filter paper, previously dyed with Sudan Red 7B dye (Lai et al. 1983) or other acceptable dye, are placed between the two bottom boards of each sandwich where the dyed paper will likely be fed on by foraging termites. The six-board sandwich should then be taped together with at least three complete 360 degree vertical wrappings of 1in-wide masking tape around the two opposite sides only or joined by screw or bolt.

The six-board sandwich is then centered on top of the soil inside the concrete block (and directly over the buried pine board, if used) or on top of the PVC pipe if the concrete slab method is used. An indelible mark is made on the ordinal direction top surface of each six-board sandwich and on the adjacent concrete to ensure the sandwich is replaced in its original orientation during inspections. A 30cm x 43 cm (12in x 17in) by 1cm(3/8in) thick opaque white plexiglass lid is placed on top of the concrete unit and held in place with a common building brick.

During evaluations for termite presence, sandwiches are dismantled and re-taped as needed. Care should be taken to ensure that the six-board sandwich is replaced on the soil/sod in the exact orientation and position from which it was removed. Sandwiches that become severely decayed (decay rating of 4 to 0; [ASTM 1986]) will be replaced during inspections. Termites recovered from sandwiches requiring replacement can be returned to the soil at the base of the new sandwich.

(iii) **Dye and Marking Techniques.** The preferred method is to dye filter paper with a known concentration of Sudan Red 7B dye (Lai et al. 1983). Nile Blue A or Neutral Red dyes (Su et al.1991c) may also be used (see also Delaplane et al. 1989, and Evans 1997 and 2000). The use of other dyes should be justified. The procedure consists of applying red dye dissolved in an appropriate solvent (usually acetone) to filter paper and then allowing the dyed paper to air dry in a fume hood. Layers of dyed paper are compressed between the bottom two boards of the pine sandwich. The purpose of the dye is to verify that the termites feeding on the pine sandwich and in the bait stations are from the same foraging group. Similar procedures are described by Atkinson (2000). If this procedure does not produce enough dyed termites to verify identity of the foraging group, then other techniques may be employed as mentioned in paragraph (e)(3)(iv) of this guideline.

(iv) **Other marking techniques** such as using fluorescent spray paint as described by Forschler (1994), or mark-release-recapture techniques as described by Jones (1990), may also be employed provided there are laboratory and field data for the termite species under investigation. If the dyed paper in the six-board sandwich does not result in an adequate number of dyed foraging termites, it will be necessary to collect and spray paint, or feed large numbers (thousands) of termites dyed paper in the laboratory, then return these marked termites to the sandwich or bait stations from which they were collected. However, it must be clearly proven

that the termites feeding in the pine board sandwiches and adjacent bait stations are from the same foraging group. Depending on the species, such data may include agonistic behavior, average dry weight of worker termites (minimum of 5 replicates, 10 workers each), cuticular hydrocarbon profiles, and molecular DNA techniques (Haverty et al. 1996, 1999a,b and Getty et al. 2000).

(v) **Configuration and plot design of field test unit** At each test site, remedial and preventive treatments for each bait active ingredient will be evaluated using a minimum of ten (10) field test units per concentration, plus an additional ten "control" test units. Individual concrete-block field test units should be placed a minimum of 23m (75 ft) apart and randomly assigned to test plots. Field plots may be arranged in a grid or other pattern as determined by the scientist conducting the tests, but all test units must be placed in locations of known, consistent termite foraging activity.

(4) Preventive Treatment Testing

For testing performance of a bait as a preventive treatment, the bait matrix with active ingredient (treated) or an untreated monitoring device is placed in the bait stations according to label directions at the time of installation of the concrete-block field test unit(s). Evaluations should be accomplished at 30 $\pm$ 10 day intervals post-installation and should determine the presence or absence of termites in the bait stations and inside each concrete block field test unit. At each inspection, baits should only be visually observed for mud tubing and should not be disturbed or dismantled until obvious extensive termite feeding has occurred. In stations where monitoring devices have been attacked, bait stations must be dismantled before all the bait has been consumed, at which time fresh baits will be placed into the station(s). When all termite activity ceases in a treated bait station after two inspections, the bait matrix should be removed to determine the amount of bait consumed but may be returned to the bait station or replaced with a monitoring matrix (per label directives). Fresh bait should be replaced in the station when needed since the active ingredient in the bait is to be present in all stations with termite activity in the preventive installations at all times. Test duration is a minimum of five years.

It is unlikely, given the test design, that sufficient numbers of termites will be available at treated sites to verify colony associations. However, the bait matrix and/or monitoring devices of one station should contain a dye. Stations at each test unit replicate should be examined for dyed termites to verify that the termites comprise the same colony. Termites will be returned to the bait stations from which they were removed so as to remain with their foraging population. **(NOTE: If termites do not survive after feeding on the bait, the dye cannot be evaluated).**

NOTE*:* if population level suppression or colony elimination claims are not intended to be listed on the label, the dyed matrix is not necessary. However, the study director must show that the wood is protected.

NOTE**:** In the method described, use of a "monitoring device" as part of the bait system is based on the possible product label directions in a preventive treatment.

Method Modification (Alternative Method) Number 3

The six-block pine sandwich should be installed after termites have been observed in a bait station to enable colony associations to be made with a monitoring device containing dye. Once this association is established, a six-board sandwich without dye can be installed, active bait with dye added to the stations, and the sandwich can be observed for termite attack by dyed termites.

(5) **Remedial Treatment Testing**

Remedial treatment evaluations should be conducted at 30 ± 10 day intervals, and will determine presence or absence of termites in the wood monitoring strips (or paper matrix or other monitoring matrix intended for commercial applications) in the external bait stations and in the six- board sandwich inside the concrete block field test units. Initially, there is no active ingredient in the bait stations, and the six-board sandwiches are visually observed for mud tubing but are neither disturbed, nor the number of termites estimated until termites have infested at least the lower four boards of the six-board sandwich as evidenced by mud tubing on the boards and in the spaces between boards. When a sandwich is found by foraging termites and the red dyed filter paper is fed upon, wooden monitoring strips or other monitoring matrix in bait stations should be examined for red dyed termites to verify that the termites in the six-board sandwiches and the bait stations are from the same attacking colony. The duration of this test is a minimum of three years.

If the dyed filter paper has been fed upon but no red termites are found in bait stations, then a second dye method should be employed as follows. **(NOTE: the use and continued presence of only one dye in the test unit is a factor that could confound results if dye were to be employed at the stations and the six-board sandwich. At the sandwich, there is no way to determine if dyed termites came from the bait site versus the sandwich. Therefore, a different color dye must be used at the bait station so termites originating at the station can be detected in the pine-board sandwich and vis versa.).** The dyed filter paper will be temporarily removed from the six-board sandwich. At the following inspection, wooden monitoring strips or matrices will be removed from the bait stations and termites extracted from this monitoring material. At least 1,000 foraging worker termites (more if possible) should be collected and removed to the laboratory, where they will be fed dyed filter paper for at least seven days and until they turn noticeably red (Lai et al. 1983) or blue. Red or blue termites should then be returned to their original bait station, and the adjacent pine sandwich (still devoid of dyed filter paper) should be monitored weekly or biweekly for 4 weeks for the presence of dyed termites. If red termites are not readily obvious on the wood or soil immediately beneath the sandwich, the pine sandwiches should be picked up and termites on its bottom surface

and on the soil/sod surface should be collected with an aspirator and placed in a petri dish on moist, white filter paper where they can be better evaluated for the presence of red color termites. Presence or absence of red termites should be recorded. The pine sandwich is not dismantled during these inspections. If red termites are found, then dyed filter paper should once again be inserted into the six-board sandwich and further "same colony" evaluations of the sandwich will consist of notes regarding the presence of dyed termites.

Once the six-board sandwich is thoroughly infested, the bait active ingredient in its intended commercial configuration (matrix, shredded paper, wooden strips, etc.) is pre-weighed and placed into the bait station. The weight of the bait matrix at ambient laboratory temperature and humidity is determined.

After installation of the bait active ingredient and as long as termites are active at test units or bait stations, evaluations should be conducted at 30 ± 10 day intervals. If termite activity has not ceased in a partially consumed bait, the bait is left in place and evaluations continued. If a sandwich is totally destroyed and termites remain active in the baited stations or under what remains of the six-board sandwich, the sandwich is replaced and evaluations continued until termite activity ceases. If bait matrix is entirely consumed in any bait station, and active termites are still found in a station or pine sandwich, the bait station will be refilled with pre-weighed active bait. Baits should continue to be replaced as needed until all termite activity in both bait stations and the pine sandwich has ceased and no live termites are found. The stations then receive the non-treated monitoring wooden strips or other non-treated monitoring matrix, with subsequent examinations at 60 ± 10 day intervals.

When a matrix is only partially consumed by termites and termite activity has ceased in both the matrix and the pine sandwich, the remaining matrix will be removed and cleaned of soil and debris as best as possible, allowed to dry for seven days at ambient laboratory temperature and humidity, and re-weighed to determine the amount of bait consumed. Monitoring wooden strips or other monitoring matrix should be replaced in the bait stations and the stations monitored at 30 ± 10 day intervals for return of termites. The period that termites remain absent from bait stations and six-board sandwiches following cessation of termite activity in the previously baited plot should be recorded.

When termite activity has ceased due to baiting, a new pine sandwich is placed into the concrete block (and buried board is also replaced, if used). Damage to the wood is rated according to ASTM standards (ASTM 1986). The wood is cleaned in the laboratory, allowed to air dry for seven days, and re-weighed to determine loss due to termite feeding.

(6) **Product performance standards for field tests using concrete test units.**

References pertaining to evaluation of bait field plots can be found in Jones 1989, 1991, and Lenz et al. 1996.

(i) **Preventive treatment test**. Termites must feed in at least one of the bait stations and the six-board sandwich must be 100 percent protected (no feeding on the wood) from termite attack as long as the bait is installed, but for a minimum of five years. If the six- board sandwich is attacked by termites, the bait has not prevented damage within the simulated structure, and that specific replicate should be evaluated as a failure. The control plots should serve as the untreated monitoring sites.

(ii) **Remedial treatment test**. The bait should eliminate a termite infestation within twelve months of bait station application using the methods described in this guideline in 100% of the field plots. In addition, each replicate should be termite free for12 months following cessation of termite activity.

Method Number 3, Small-Scale Field Tests - Evaluation of Termite Population Management Using Bait Technology

(1) **Site selection.** Each termite bait product with its associated application materials should be placed in field tests to include at least EPA Office Regions 4, 6 and 9 as described in paragraph (f)(2) of this guideline. These sites represent varying climatic and soil conditions, and are habitats for several subterranean termite species (see paragraph (e)(2)). Field tests in addition to those performed at these field sites may be conducted according to the protocol described below.

(2) **Termite species.** The subterranean termite species that shall be tested at the field sites include species from three genera, *Reticulitermes, Heterotermes* and *Coptotermes.* Data collected with any of the following *Reticulitermes* species are acceptable*: Reticulitermes flavipes, R. virginicus, R. hesperus,* and *R. tibalis.* For data collected from termites in the genera *Heterotermes* and *Coptotermes,* only *Heterotermes aureus* and *Coptotermes formosanus* are acceptable. For each species tested, a minimum of 5 colonies will serve as the treated colonies and 5 colonies should serve as the untreated controls.

(3) **Method Number 3 Termite Population Management**. The protocols described by various authors (Su 1994, Grace et al. 1996, Forschler and Ryder 1996, Haverty et al. 2000, Getty et al. 2000) provide the basis for the standardized test method presented below. This test is designed to evaluate termite baits at sites of vigorous termite activity in the United States. The bait(s) should be evaluated for preventing subterranean termites from feeding on wood in monitoring stations and for eliminating or suppressing subterranean termite populations from termite-infested monitoring devices. Such tests will simulate remedial control of subterranean termites and apply to all bait products. Once termites are eliminated from the monitoring stations, the test can be continued to demonstrate preventive claims.

(I) Test Unit

(i) The protocol for establishing each field site has been derived from a combination of research studies (Su 1994, Grace et al. 1996, Forschler and Ryder 1996, Su et al. 1997, Getty et al. 2000). The process involves the following steps: 1) identify the species at the site, 2) establish or install monitoring devices and bait delivery devices, 3) associate termites in each monitoring device with termites in other monitoring devices and bait stations to establish colony foraging areas by mark-recapture or mark-release-recapture, 4) assess wood consumption patterns to evaluate cessation of feeding, 5) randomly assign colonies to treated or control (untreated), and 6) initiate baiting and subsequent evaluation process.

Once a site with existing termite colonies has been selected, wooden survey stakes should be driven into the ground to encourage termites to feed at specific sites (Su and Scheffrahn 1986, Haverty et al. 2000). [*Heterotermes aureus* may require a different approach (see Jones 1990)] When the survey stakes have been fed upon by the termites, an independent monitoring device (monitoring station) can be placed adjacent to it or can replace the stake.

(ii) **Bait Delivery Devices and Monitoring Stations**. It is critical to associate the termites feeding at the bait stations with those occupying the monitoring stations. Two types of stations should be used: 1) commercial bait stations are used to deliver the active ingredient, and 2) independent monitoring stations or devices (Tamashiro et al. 1973, Su and Scheffrahn 1986, Grace 1990) are used for observing termite foraging activity. Bait stations should be installed in the vicinity of independent monitoring stations with termite activity (see paragraph (f) (4)). For a colony to be included in the evaluation, either as a treated or untreated colony, at least three of the independent monitoring stations must be connected (see above). Prior to the evaluation of the bait, these monitoring stations should be used to determine the dispersion and the seasonal foraging and feeding of the termite colonies (Su and Scheffrahn 1988, Su et al. 1993b, Haverty et al. 1999b, 2000). These same monitoring stations can then be used to measure feeding of the termite colonies after baiting. Bait stations must be placed within the foraging range of the colony, i.e., inside a polygon connecting all of the occupied independent monitoring stations, or the interconnection of the bait stations and independent monitoring stations need to be established by the marking techniques or other appropriate methods (see also Evans 2001, Evans et al. 1998, 1999, and Forschler and Jenkins 2000)

(iii) **Dye and Marking Techniques.** The preferred method is to dye filter paper with a known concentration of Sudan Red 7B (Lai et al. 1983), Nile Blue A and Neutral Red (Su et al.1991) or other acceptable dye (see also Delaplane et al. 1989, and Evans 1997, 2000). The procedure consists of applying dye dissolved in an appropriate solvent (usually acetone) to filter paper and then allowing the dyed paper to air dry in a fume hood. Layers of dyed paper can be compressed between the boards of the monitoring device . The purpose of the dye is to verify that the termites feeding in the monitoring device and in the bait stations are from the same colony . A similar procedure is described by Atkinson (2000). If this procedure does not produce enough marked termites to verify identity of the foraging group, then other techniques may be employed as mentioned in paragraph (e)(3)(iv) of this guideline.

(iv) **Other marking techniques** such as using fluorescent spray paint as described by Forschler (1994), or mark-release-recapture techniques as described by Jones (1990), may also be employed provided there are laboratory and field data for the

termite species under investigation. If the dyed paper in the monitoring device does not result in an adequate number of marked foraging termites, it will be necessary to collect and spray paint, or feed large numbers (thousands) of termites dyed paper in the laboratory, then return these marked termites to the monitoring device or bait stations from which they were collected. However, it must be clearly proven that the termites feeding in the monitoring device and adjacent bait stations are from the same foraging group. Depending on the species, such data may include agonistic behavior, average dry weight of worker termites (minimum of 5 replicates, 10 workers each), cuticular hydrocarbon profiles, and molecular techniques (Haverty et al. 1996, 1999a,b, and Getty et al. 2000).

(6) **Product performance standards for small-scale field tests**
References pertaining to evaluation of bait field plots can be found in Jones 1989, 1991, Forschler and Ryder 1996, Grace 1990, Haverty 1999b, Lenz et al. 1996, Pawson and Gold 1996, Su 1991a, 1994, 1996a, 1996c, and Thorne and Forschler 2000.

(i) **Remedial treatment test**. The bait should eliminate a termite infestation from the wood in the monitoring devices within twelve months of bait station application with the pesticidal active ingredient using the methods described in this guideline in 80% (4 of the 5 colonies of each species in the field plots). In addition, each replicate should be termite-free for 12 months following cessation of
termite activity.

(ii) **Preventive treatment test**. Termites must feed in at least one of the bait stations and the wood in the monitoring devices/six-board sandwich must be 80% (4 or the 5 colonies of each species) protected (no-feeding on the wood) from termite attack as long as the bait is installed, for a minimum of three years. If the wood in the monitoring devices is attacked by termites, it can be assumed that the bait will not prevent damage within a structure, and that specific replicate can be evaluated as a failure.

(7) **Termite Species Identification**. Termites should be collected and identified as to species from at least one bait station or six-board sandwich from each concrete-block field test unit that contains termites. Appropriate termite identification keys such as those published by Weesner 1965, Nutting 1990, and Scheffrahn and Su 1994, should be used to identify termites. Identification using DNA techniques or cuticular hydrocarbon profiles (Forschler and Jenkins 1999, Haverty et al. 1999, Haverty et al. 1996, 1999) can be used to supplement morphological keys, but should not replace morphological keys. Termite samples should be collected and identified annually to note the possible arrival of a different termite species. Voucher specimens (soldiers, workers, and alates [if available] in 80% ethanol, workers and soldiers frozen at –30° C; or dried workers for

characterization of cuticular hydrocarbons) should be kept for each treated and untreated colony. See also Jenkins et al. 1998, 1999, and 2001 for a discussion of methodologies on use of DNA sequencing and genetic markers for phylogenetic analyses and genetic structure determination in subterranean termite populations.

(8) **Data Collection**. Record the following information: location and test unit designation; date field test unit installed (note preventive or remedial test); date of evaluation; termite species present; presence or absence of termites in the six-board sandwich and bait stations; presence of dyed termites in the six-board sandwich and bait stations; amount of bait consumed (estimated percent) in six-board station and bait stations; bait and sandwich weight(s); date termite activity ceased in the six-board sandwich and bait stations; and date termite activity resumed in the six-board sandwich and bait stations; ASTM damage rating to each pine board following cessation of termite activity in the six-board sandwich and bait stations; date dyed paper first fed upon; date of release of laboratory dyed or marked termites into the six-board sandwich or bait station(s); date termites found in six-board sandwich bait station(s); and date and location of mud tubing observed on or in test unit.

(9) **Colony level control tests**. Colony level control and elimination testing is optional for termite bait registration unless the registrant makes termite colony elimination or control claims on the bait product label. In addition to the tests described above, a similar experimental design should be established following characterization of the subterranean termite colony's activity, population, and territory. Su 1996a, Grace et al. 1989 and Forschler and Ryder 1996 present procedures for making these determinations. Colony level control and elimination tests are supplemental to the field test for simulated structures and can not be considered a substitute.

(**F) General considerations for FIELD TESTS USING EXISTING STRUCTURES and BUILDINGS.** Nationwide field tests using existing structures to evaluate the performance of a termite bait product should be conducted under an Experimental Use Permit after the candidate termite bait product is successful in laboratory testing and/or field testing with simulated structures. Nationwide field tests should be conducted as described in this guideline. Field tests with infested structures (remedial installations) are necessary to show that the bait controls termites. Testing is also described for preventive treatments.

(1) **Number and selection of homes/structures**. Field tests must use the bait product and its associated application materials as intended for registration and sale. Testing should be conducted at a minimum of 500 existing wooden buildings/structures in the United States each for preventive and remedial installations. The selected structures/buildings should be evenly distributed (minimum of 80 homes per region) in six of the EPA Office Regions as described in paragraph (f) (2) of this guideline. Additional testing (80 structures) should be done in the applicable EPA Region if claims for dampwood termites are intended to be listed on the proposed product label.

NOTE: A modification to this approach is to allow homes to serve as sites for preventive treatments a year after they were treated successfully in remedial treatment installations. This approach reflects the preventive post-construction bait treatment market as it currently exists. However, it is not indicative of bait treatments in new construction.

(2) **EPA Office Regions:** At a minimum, testing should be conducted in the following EPA Office Regions: Region III (DE, DC, MD, PA, VA, WV), Region IV (AL, FL, GA, KY, MS, NC, SC, TN), Region V(IL, IN, MI, MN, OH, WI), Region VI (AR, LA, NM, OK and TX), Region VII (IA, KS, MO, NE) and Region IX (AZ, CA, HI, NV, AS, GU). Optional testing for dampwood termites should be conducted in Region X (restricted to WA and OR) if they are intended to be listed on the proposed product label. Additional testing in additional regions is permitted.

(3) **Termite species**. Termite species to be tested are based on label claims. If prevention or control of subterranean termites is listed on the proposed product label, the subterranean termite species that must be tested are *Reticulitermes* spp., *Heterotermes aureus*, and *Coptotermes formosanus.* Evaluations against the dampwood termites, *Zootermopis* spp., and/or the drywood termites, *Cryptotermes* and *Incistermes* spp., should be performed only if specific label claims are made for the control of these termite species.

(i) **Termite Species Identification**. Termites should be collected and identified as to species from at least one bait station or six-board sandwich from each structure that contains termites. Appropriate termite identification keys such as those published by Weesner 1965, Nutting 1990, and Scheffrahn and Su 1994, should be used to identify termites. Identification using DNA techniques or cuticular hydrocarbon profiles (Forschler and Jenkins 1999, Haverty et al. 1999, Haverty et al. 1996, 1999) can be used to supplement morphological keys, but should not replace morphological keys. Termite samples should be collected and identified annually to note the possible arrival of a different termite species. Voucher specimens (soldiers, workers, and alates [if available] in 80% ethanol, workers and soldiers frozen at –30° C; or dried workers for characterization of cuticular hydrocarbons) will be kept for each treated and untreated colony. See also Jenkins et al. 1998, 1999, and 2001 for a discussion of methodologies on use of DNA sequencing and genetic markers for phylogenetic analyses and genetic structure determination in subterranean termite populations.

(4) **Monitoring for subterranean termite presence and activity**. Termite presence and activity must be monitored during the entire field test using methods described by Su and Scheffrahn 1986, Pawson and Gold 1996, Haverty et al. 1975, and Jones 1990, or equivalent methods. A combination of the methods listed below should be used to assess the presence and extent of the foraging termite population in the vicinity of the foundation for preventive and remedial treatment bait installations. It is recommended that the monitoring devices/bait stations be placed within one meter of the foundation but their

exact placement should conform with pesticide label and the final experimental design should be determined by the study director. Additional monitoring stations can be installed farther away from the structures and used for assessing termite activity provided that the interconnection of these stations and those within one meter of the structure can be established using techniques such as mark-recapture method. Generally, monitoring stations should be checked at a minimum every 30 $\pm$ 10 days during the course of the study.

(i) **Placement of untreated wood stakes/modified wood stakes/bait matrix stations as independent monitoring devices** Wood stakes, preferably southern yellow pine sapwood stakes or an equally attractive wood, should be placed into the soil to a depth of 15cm (6in) or deeper and spaced 2m (6ft) or less apart around the perimeter of the structure. Modified wood stakes are stakes cut to the size of the proposed commercial monitoring station and applied to the soil as directed by the bait product label. The bait matrix, less the active ingredient, can be used in the proposed commercial bait station as an additional means of monitoring termite activity. Wood stakes, or modified wood stakes, and bait matrix should be used in combination to monitor activity. They can be moistened prior to installation to make them more attractive to termites.

(ii) **"Bucket Trap" installations**. Bucket traps should be used in addition to the above independent monitoring tools and should be installed every 10m (30ft) or less (These traps can also serve as sites to "mark" termites during bait evaluation). To establish a bucket trap installation at an existing structure (Pawson and Gold 1996), excavate the soil to form a circular hole 25cm (10in) deep x 20cm (8in) in diameter. Cut and discard the bottom of a small plastic bucket that measures approximately 17.5cm in height x 20cm in diameter (7in x 8in). Drill fifteen 6mm (1/4in) holes into the top 7.5cm (3 in) of the bucket and place the bucket in the excavated hole. Into the bucket place a prefabricated pine six-board sandwich prepared as discussed in paragraph (e) (1) (ii) of this guideline or prepared according to the method of Su and Scheffrahn (1986). Do not add the dyed filter paper when monitoring, it should be used for "marking" termites during bait evaluation as described in this guideline. The trap should be covered with a tight fitting plastic or metal lid and covered with 5cm (2in) of soil. A flag or other means of identification should be used to mark the location of the trap.

(5) **Monitoring/Inspection methods for drywood termites.** Acoustical emission devices, microwave devices, and visual inspection can be used to monitor wood in a structure for drywood termites.

(6) **Monitoring methods for dampwood termites**. See paragraph F (4) on subterranean termites.

(7) **Preventive treatment test installation**. Selected structures should represent the wide

range of construction types existing in the United States. Subterranean termites must be present adjacent to the buildings/structures used in these field tests as described below. Conditions favoring infestation by drywood or dampwood termites should exist if baits are tested against these species, however, justification must be presented to show that preventive bait treatments can protect a structure from drywood termites. These tests should be conducted for a minimum of five years from the time of active ingredient installation.

(i) **Inclusion of a building/structure in a preventive treatment field test.** The following conditions must be met: (1) the building/structure should be deemed termite free following a complete structural inspection; (2) foraging worker termites should be present within two meters of the building/structure foundation as indicated from use of methods described by Su and Scheffrahn 1986, Grace et al.1989, and Pawson and Gold 1996; and (3) the history of termiticide application to the structure is known for five years preceding the start of the bait evaluation. In addition, one or both of the following conditions should disqualify a building/structure from inclusion in a preventive field test: application of a repellent (pyrethroid) termiticide within the last five years and/or application of any termiticide control product (excluding fumigants) within 24 months prior to the start of the field test.

(ii) **Number of bait stations applied**. A minimum of 10 bait stations must be applied as directed by the proposed label to each structure/building.

(iii) **Bait station placement - subterranean termites** (including dampwood termites). Place bait stations in the soil according to label directions but at least 10 bait stations should be within one meter or less of the foundation. Additional baits can be placed at distances exceeding one meter from the foundation.

(iv) **Bait station placement - drywood termites**. Placement should be made according to label directions. Since these termite species do not require soil contact to survive, baits should be placed in areas where these species are likely to invade the test structure. At a minimum, applications to prevent drywood termites should be made to attics and eaves.

(v) **Bait Station Placement - dampwood termites** See paragraph (F)(7)(iii) of this guideline.

(vi) **Data collection**. Data collection sheets should record the following information at a minimum. Location (address), construction type (split foyer etc.), square footage, age, foundation type (slab, basement, crawl space etc.), siding type (brick, block, foam board/stucco, etc.), termiticide treatment history of the structure; date of termite inspection; date and location of placement of monitoring devices and bait stations; soil type; soil pH; percent soil moisture and soil surface

temperature at each monthly evaluation; climatic data - temperature and rainfall; date of each monthly evaluation; termite species present; presence or absence of termites in the six-board sandwich for each bucket trap and for other monitoring stakes/stations; if applicable, presence of dyed termites in the six-board sandwich and bait stations; amount of wood/bait matrix consumed (estimated percent) in six-board station and each bait station, respectively; weight of bait(s) and sandwich consumed (see paragraph (e) (5) of this guideline); number and percent of bait stations fed upon; date of bait station replacement; date termite activity ceased in the six-board sandwich and/or bait stations; and date termite activity resumed in the six-board sandwich and/or bait stations; ASTM damage rating to each pine board following cessation of termite activity in the six-board sandwich, wood stakes, and bait stations; date dyed paper first fed upon; date of release of laboratory dyed or marked termites into the six board sandwich or bait station(s); date termites found in six-board sandwich, wood stakes, and/or bait station(s); and date and location of mud tubing observed in bait station, monitoring station and structure (if infested).

(vii) **Evaluation of a preventive treatment.** The bait matrix with active ingredient is placed in bait stations as directed by the proposed label or stations containing active ingredient can be used from the beginning of the preventive installation if specified by the proposed product label. The first evaluation after placing test units in the field should be accomplished at 60 $\pm$ 10 days post-installation to determine the presence or absence of termites in the bait stations, bait monitoring stations, the six-board pine sandwich inside each bucket trap and at wood stakes. However, six-board sandwiches and baits should only be visually observed for mud tubing and should not be disturbed or dismantled until extensive termite feeding has occurred. Bait stations should be dismantled according to the proposed label directions at which time fresh baits should be placed into the station (s) or stations replaced. Subsequent evaluations should be conducted at 30 $\pm$ 10 day intervals during each year of testing for the duration of the test. If all termite activity ceases in a bait station, the bait matrix should be removed and the amount of bait consumed determined. Fresh bait should be replaced in the station(s) or a new station installed as active bait should be present in preventive installations at all times. If any monitoring strips or bait matrices, or any active bait materials become severely decayed, completely soaked with water, or disintegrate during the test, they should be replaced with the same fresh material. The date of replacement and condition of the matrix should be recorded.

Bait station wooden monitoring strips or matrices should be removed from the bait stations and at the direction of the study director. Termites may be removed from bait stations containing active ingredient (record procedure and justify timing). At least 1,000 or more foraging worker termites should be collected and removed to a laboratory. In the laboratory they should be fed dyed filter paper for seven days or more until they turn the color of the dye (Lai et al. 1983), Nile Blue A, Neutral

Red (Su et al. 1991a), or by other methods described in (e) (3) (iv) of this guideline. Dyed termites should then be returned to their original bait station, and the adjacent six-board sandwich should be monitored weekly for four weeks for the presence of dyed termites. To do this, the pine board sandwich should be picked up and termites on its bottom surface and on the soil surface should be collected with an aspirator and placed in a petri dish on moist white filter paper and evaluated for dyed termites unless the dyed termite are observed on the six-board sandwich or the soil immediately beneath the sandwich. The presence or absence of dyed termites should be recorded together with the date the inspection is made. The six-board sandwich should not be dismantled during these inspections.

A preventive bait test should result in termites feeding on the bait stations, and no (0 percent) termites detected within the building/structure for five years. In addition, the termite population feeding on the six-board sandwich in the bucket traps and at all other monitoring stations must be eliminated within twelve (12) months of the bait application and termite activity must not resume for 12 months thereafter. When termite activity has ceased in the six board sandwich due to baiting, a new sandwich is placed into the bucket trap. Damage to the wood stakes and pine boards in the sandwich is rated according to ASTM standards (ASTM 1986). The remaining wood is cleaned in the laboratory, allowed to air dry for seven days, and re-weighed to determine feeding loss. Once all termite activity ceases in a bucket trap test unit, the longer termites remain absent, the more successful the bait application is, provided termites do not infest the structure.

Note: If the six-board sandwich is continually fed upon by large numbers of termites for more than twelve months after initial bait application, the bait has not suppressed the foraging termite population adjacent to the structure. This result shows that the bait application has not protected the structure because termites are present within one meter of the foundation (Termite activity in close proximity to the structure increases the likelihood of an attack on the structure. Baits do not perform like barriers and in order to protect the structure, the bait must eliminate population activity and foraging adjacent to the structure.). Furthermore, if termites infest the structure at any time following bait application as determined by a structural inspection, the bait application has failed to protect the structure and that replicate can be evaluated as a failure.

(8) **Remedial treatment bait test.** Selected structures should represent the wide range of construction types existing in the United States. Subterranean termites should be present adjacent to the buildings/structures and the structure must be infested with termites. Infestation by drywood or dampwood termites should be present if baits are tested against these species. These tests should be conducted in a minimum of 500 infested structures and the structures should be monitored for a period of three years from the time bait stations are installed. This includes post-application monitoring inside and outside the

structure.

(i) **Inclusion of a building/structure in a remedial installation field test.** The building/structure must be infested with termites feeding on wood in the structure.

(ii) **Number of commercial bait stations applied**. Application of bait stations should be according to proposed label directions. The number of stations applied will vary with the structure, termite species to eliminated, extent and location of infestation, size of bait station, and action of the active ingredient. Either below ground or above ground stations can be used.

(iii) **Bait station placement**. Placement of baits should be done according to proposed label directions to eliminate an infestation.

(iv) **Data collection**. In addition to the description of paragraph (f)(7)(v) above, record: the date the termite infestation was eliminated; the length of time required to achieve control; and date of bait station replacement. Data should be collected for a minimum of five years in a structure.

(v) **Evaluation of remedial installations.** The first evaluation after installation of the test units should be accomplished before 60 $\pm$ 10 days post-installation. Subsequent evaluations should be conducted at 30 $\pm$ 10 days intervals during the duration of testing, and will determine presence or absence of termites (all life stages considered including alates) in the structure, monitoring stations, and bait stations. The six-board sandwiches should visually observed for mud tubing but should not be disturbed or dismantled nor the number of termites estimated until termites have infested at least the lower four boards of the six-board sandwich as evidenced by mud tubing on the boards and in the spaces between boards. Bait station monitoring and replacement should be done according to the proposed label directions.

(9) **Product performance standards**

Alate swarms, mud tubing, and presence of worker or soldier termites in the structure are indications of a termite infestation and can indicate the failure of a bait product to protect a structure.

(i) **Preventive treatment** tests must prevent structural infestation by termites in 100 percent of the structures for the duration of the field test, a minimum of five years, as measured by 60 months of termite activity at the monitoring stations/bucket traps/wood stakes. Alate swarms, mud tubing, and presence of worker or soldier termites in or on the structure are indications of a termite infestation and can indicate the failure of a bait product to protect a structure. Note: a bait must be successful in remedial treatment testing before it can

considered successful as a preventive treatment. Published articles from scientific journals are not a substitute for submission of these required data.

(ii) **Remedial treatments**. As described above in paragraph (f)(8)(v) of this guideline, remedial treatments should eliminate an existing termite infestation in 12 months or less in 100% of the structures treated and the treated structure must remain termite free for 12 months following treatment. Alate swarms, mud tubing, and presence of worker or soldier termites in or on the structure are indications of a termite infestation and can indicate the failure of a bait product to protect a structure. If termites are not detected from structures but remained active in the monitoring station or independent monitor (IM), then structural inspections every month are needed during the 12 month period. After baiting eliminates the infestation in the structure based on a structural inspection but the activity resumes during the 12-month post-baiting observation period at the monitoring station, additional bait application has to eliminate termite activity within 12 months at the station and IM following the re-application of baits without causing >10 percent of the maximum wood consumption as recorded in the IMs during the pre-baiting period. After the reapplication of baits, termites should remain absent for another 12 months in the structure and IMs.

REFERENCES

1. American Society for Testing and Materials (ASTM). 1986. Standard method for evaluating wood preservatives by field tests with stakes. In: Designation D-1758-86, wood, book of ASTM standards, Vol.04.09. ASTM, Philadelphia.

2. Atkinson, T. H. 2000. Use of dyed matrix in bait stations for determining foraging territories of subterranean termites (Isoptera: Rhinotermitidae: *Reticulitermes* spp. and Termitidae: *Amitermes wheeleri*). Sociobiology 36: 149-167.

3. Beard, R. L. 1974. Termite biology and bait-block method of control. Conn. Agric. Exp. Stn. Bull. 748.

4. Curtis, A.D. and D.A. Walker. 1997. Problems with the interpretation of mark-release data in subterranean termites (Isoptera: Rhinotermitidae) Sociobiology 30:233-241.

5. Delaplane, K.S., and J.P.La Fage. 1989. Supression of termite feeding amd symbiotic protozoans by the dye, Sudan 7B. Entomol.Exp. Appl. 50: 265-270.

6. Esenther, G. R., and R. H. Beal. 1974. Attractant-mirex bait suppresses activity of *Reticulitermes* spp. J. Econ. Entomol. 67: 85-88.

7. Esenther, G. R., and R. H. Beal. 1978. Insecticidal baits on field plot perimeters suppress *Reticulitermes*. J. Econ. Entomol. 71: 604-607.

8. Evans, T.A. 1997. Evaluation of marks for Australian subterranean termites (Isoptera: Rhinotermitidae and Termitidae) Sociobiology 29: 277-292.

9. Evans, T.A., M.. Lenz, and P.V. Gleeson. 1998. Testing assumptions of mark-recapture protocols for estimating population size using Australian mound-building, subterranean termites. Eco. Entomol. 23: 139-159.

10. Evans, T.A., M.Lenz, and P.V. Gleeson. 1999. Estimating population size and forager movement in a tropical subterranean termite (Isoptera; Rhinotermitidae) J. Environ. Entomol. 28 (5): 823-830.

11. Evans, T.A. 2000a. Fast marking termites (Isoptera: Rhinotermitidae) Sociobiology 36: 517-523.

12. Evans, T.A. 2001. Estimating relative decline in populations of subterranean termites (Isoptera: Rhinotermitidae) due to baiting. J. Econ. Entomol. 94 (6): 1602-1609.

13. Forschler, B.T. 1994. Fluorescent spray paint as a topical marker on subterranean termites (Isoptera: Rhinotermitidae). Sociobiology 24(1): 27-37.

14. Forschler, B.T. and J.C. Ryder. 1996. Subterranean termite, Reticulitermes spp. (Isoptera: Rhinotermitidae), colony response to baiting with hexaflumuron using a prototype commercial system. J. Entomol. Sci. 31: 143-151.

15. Forschler, B.T. and T.M. Jenkins. 1999. Evaluation of subterranean termite biology using genetic, chemotaxonomic, and morphometric markers and ecological data: a testimonial for multi-disciplinary efforts. Trends in Entomol. 2: 71-80.

16. Forschler, B.T. and T.M. Jenkins. 2000. Subterranean termites in the urban landscape: understanding their social structure is the key to successfully implementing population management using bait technology. Urban Ecosystems (4): 231-251.

17. Getty, G. M., M. I. Haverty, K. A. Copren, & V. R. Lewis. 2000. Response of Reticulitermes sp. (Isoptera: Rhinotermitidae) in north California to baiting with hexaflumuron with Sentricon termite colony elimination system. J. Econ. Entomol. 93(5): 1498-1507.

18. Getty, G. M., M. I. Haverty, and V. R. Lewis. 2000. Agonistic behavior between recent collections and laboratory cultures of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California. Pan-Pacific Entomol. (In press)

19. Grace, J.K., A. Abdallay, and K.R. Farr. 1989. Eastern subterranean termite (Isoptera: Rhinotermitidae) foraging territories and populations in Toronto. Can. Entomol. 121: 551-

556.

20. Grace, J. K. 1990. Mark-recapture studies with *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 16: 297-303.

21. Grace, J. K., J. R. Yates III, and C.H.M. Tome. 1995. Modification of a commercial bait station to collect large numbers of subterranean termites (Isoptera: Rhinotermitidae). Sociobiology 26: 259-268.

22. Grace, J.K., C.H.M. Tome, T.G. Shelton, R.J. Oshuro and J.R. Yates III. 1996. Baiting studies and considerations with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Hawaii. Sociobiology 28(3): 511-520.

23. Hagsma, K., and J. Bean. 1998. Evaluation of a Hexaflumuron-based bait to control subterranean termites in Southern California (Isoptera: Rhinotermitidae) Sociobiology 31 (3): 363-369.

24. Haverty, M.I., W.L. Nutting and J.P. LaFage. 1975. Density of colonies and spatial distribution of foraging territories of the desert termite, *Heterotermes aureus* (Snyder). J. Environ. Entomol. 4: 105-109.

25. Haverty, M.I. 1977. The proportion of soldiers in termite colonies: a list and bibliography (Isoptera). Sociobiology 2: 199-216.

26. Haverty, M.I. and B.T. Thorne. 1989. Agonistic behavior correlated with hydrocarbon phenotypes in dampwood termites, Zootermopsis (Isoptera: Termopsidae). J. Insect. Behav. 2: 523-543.

27. Haverty, M.I., B.T. Forschler, and L.J. Nelson. 1996. An assessment of the taxonomy of *Reticulitermes* (Isoptera: Rhinotermitidae) from the southeastern United States based on cuticular hydrocarbons. Sociobiology 28: 287-318.

28. Haverty, M. I., and L. J. Nelson. 1997. Cuticular hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California indicate undescribed species. Comp. Biochem. Physiol. 118B: 869-880.

29. Haverty, M.I., K.A. Copren, G.M. Getty and V.R. Lewis. 1999. Agonistic behavior and cuticular hydrocarbon phenotypes of colonies of *Reticulitermes* (Isoptera: Rhinotermitidae) from Northern California. Ann. Ent. Soc. Amer. 92(2): 269-277.

30. Haverty, M. I., G. M. Getty, K. A. Copren, and V. R. Lewis. 1999b. Seasonal foraging and feeding behavior of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a wildland and a residential location in northern California. Environ. Entom. 28: 1077-1084.

31. Haverty, M. I., G. M. Getty, K. A. Copren, and V. R. Lewis. 2000. Size and dispersion of colonies of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a wildland and a residential location in northern California. Environ. Entomol. 29(2): 241-249.

32. Howard, R.W., C.A. McDaniel, D.R. Nelson, G.J. Blomquist, L.T. Gelbaum, and L.H. Zalkow. 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species and caste-recognition cues. J. Chem. Ecol. 8: 1227-1239.

33. Jenkins, T.M., C.J. Basten, S. Krescovich and B.T. Forschler. 1998. Matriarchal genetics structure of *Reticulitermes* (Isoptera: Rhinotermitidae). Sociobiology 33: 239-263.

34. Jenkins, T.M., C.J. Basten, S. Krescovich and B.T. Forschler. 1999. Mitochondrial gene sequence questions: *Reticulitermes* sp. social structure (Isoptera: Rhinotermitidae). Sociobiology 34: 161-172.

35. Jenkins, T.M., R. Vertok, R. Dean, and B.T. Forschler. 2001. Phylogenetic analyses of two mitochondrial and one nuclear intron region illuminate European subterranean termite (Isoptera: Rhinotermitidae) taxonomy and gene flow. Mol. Phlgenet. Evol. 20: 286-293.

36. Jones, S.C. 1984. Evaluation of two insect growth regulators for the bait-block method of subterranean termite (Isoptera: Rhinotermitidae) control. J. Econ. Entomol. 77 (5): 1086-1091.

37. Jones, S.C. 1989. Field evaluation of fenoxycarb as a bait toxicant for subterranean termite control. Sociobiology 15(1): 33-41.

38. Jones, S.C. 1990. Delineation of *Heterotermes aureus* (Isoptera: Rhinotermitidae) foraging territories in a Sonoran Desert grassland. Environ. Entomol. 19(4): 1047-1054.

39. Jones, S.C. 1991. Field evaluation of boron as a bait toxicant of Heterotermes aureus (Isoptera: Rhinotermitidae). Sociobiology 19 (1): 187-209.

40. Kard, B. 1999. Field testing protocol and product performance guidelines for subterranean termite baits. (USDA-FS unpublished)

41. Lai, P.Y., M. Tamashiro, J.K Fujii, J.R. Yates and N-Y. Su. 1983. Sudan Red 7B, a dye marker for *Coptotermes formosanus*. Proc. Hawaiian Entomol. Soc. 24(2,3): 277-282.

42. Lenz, M., P.V. Gleeson, L.R. Miller and H.M. Abbey. 1996. How predictive are laboratory experiments for assessing the effects of chitin inhibitors (CSI) on field colonies for termites? - A comparison of laboratory and field data from Australian mound-building species of termite. International Research Group on Wood Preservation. 27th Annual Meeting 1-10.

43. Lewis, V. R., M. I. Haverty, G. M. Getty, K. A. Copren, and C. Fouche. 1998. Monitoring station for studying populations of *Reticulitermes* (Isoptera: Rhinotermitidae) in California. Pan-Pac. Entomol. 74: 121-133.

44. Nutting 1990. Insecta Isoptera, pp.997-1032 in D.L. Dindal [ed.] , Soil Biology Guide, Wiley, New York.

45. Oi, F., N-Y. Su, P.G. Koehler, and F. Slansky. 1996. Laboratory evaluation of food placement and food-types on the feeding preference of Reticulitermes virginicus (Isoptera: Rhinotermitidae). J. Econ. Entomol. 89(4): 915-921.

46. Pawson, B.M. and R.E. Gold. 1996. Evaluation of baits for termites (Isoptera: Rhinotermitidae) in Texas. Sociobiology 28 (3): 485-510.

47. Randall, M., and T. C. Doody. 1934. Poison dusts. I. treatments with poisonous dusts, pp. 463-476. *In* C.A. Kofoid [ed.], Termites and termite control. Univ. Calif. Press, Berkeley.

48. Robson, S.K. , M.G. Lesniak, R.V. Kothandapani, J.F.A., Traniell, B.L.Thorne and V. Fourcassie. 1996. Nonrandom search geometry in subterranean termites. Naturwissenschaften 82: 526-528.

49. Rosin, Y. and M. Lenz. 1999. Caste developmental pathways in colonies of Coptotermes lacteus (Frogett) headed by primary reproductives (Isoptera, Rhinotermitidae) Insectes Sociaux 46: 273-280.

50. Rust, M.K. , K. Haagsma and J. Nyugen. 1996. Enhancing foraging of Western subterranean termites (Isoptera: Rhinotermitidae) in arid environments. Sociobiology 28: 275-286.

51. Scheffrahn, R.H. and N-Y. Su. 1994. Keys to soldier and winged adult termites of Florida. Florida Entomologist 77(4): 460-474.

52. Scheffrahn, R.H. and N-Y. Su and P. Busey. 1997. Laboratory and field evaluations of selected chemical treatments for control of drywood termites (Isoptera: Kalotermitidae). J. Econ. Entomol. 90: 492-502.

53. Shelton, T.G. and J.K. Grace. 1996. Rev. of agonistic behaviors in the Isoptera. Sociobiology 28: 155-176.

54. Sorrnuwat, Y., C. Vongkaluang, M. Takahashi, K. Tsunoda, and T. Yoshimura. 1996. Foraging territory of subterranean termites, *Coptotermes gestroi* Wasmann. Journ. J. Soc. Environ. Entomol. & Zool. 7 (4): 201-210.

55. Sorrnuwat, Y., K. Tsunoda, T. Yoshimura, M. Takahashi, and C. Vongkaluang. 1996. Foraging populations of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) in an urban area.

J. Econ. Entomol. 89 (6): 1485-1490.

56. Su, N-Y., and R.H. Scheffrahn. 1986. A method to access, trap, and monitor field populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment. Sociobiology 27: 253-275.

57. Su, N.-Y., and R. H. Scheffrahn. 1988. Foraging population and territory of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment. Sociobiology 14: 353-359

58. Su, N-Y. and R.H. Scheffrahn. 1989. Comparative effects of an insect growth regulator, S-1183, against the Formosan subterranean termite and eastern subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 82(4): 1125-1129.

59. Su, N. -Y. 1991a. Evaluation of bait-toxicants for suppression of subterranean termite populations. Sociobiology 19: 211-220.

60. Su, N.-Y., and R. H. Scheffrahn. 1991b. Laboratory evaluation of two slow-acting toxicants against Formosan and eastern subterranean termites. (Isoptera: Rhinotermitidae). J. Econ. Entomol. 84(1): 170-175.

61. Su, N.-Y., P. M. Ban and R. H. Scheffrahn. 1991c. Evaluation of twelve dye markers for population studies of the eastern and Formosan subterranean termites (Isoptera: Rhinotermitidae). Sociobiology 19: 349-362.

62. Su, N.-Y. and R. H. Scheffrahn. 1993a. Laboratory evaluation of two chitin synthesis inhibitors, hexaflumuron and diflubenzuron, as bait toxicants against Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 86(5): 1453-1457.

63. Su, N.-Y., P. M. Ban, and R. H. Scheffrahn. 1993b. Foraging populations and territories of the eastern subterranean termite (Isoptera: Rhinotermitidae) in southeastern Florida. Environ. Entomol. 22(5): 1113-1117.

64. Su, N.-Y. 1994. Field evaluation of a hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 87 (2): 389-397.

65. Su, N.-Y., R.H. Scheffrahn and P.M. Ban. 1995. Effects of sulfluramid-treated bait blocks on field colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae) J. Econ. Entomol. 88 (5): 1343-1348.

66. Su, N.-Y. and R.H. Scheffrahn. 1996a. Fate of subterranean termite colonies (Isoptera) after bait applications - an update and review. Sociobiology 27 (3): 253-275.

67. Su, N-Y. and R.H. Scheffrahn. 1996b. A review of the evaluation criteria for bait-toxicant efficacy against field colonies of subterranean termites (Isoptera). Sociobiology 28(3): 521-530.

68. Su, N.-Y., and R. H. Scheffrahn. 1996c. Comparative effects of two chitin synthesis inhibitors, hexaflumuron and lufenuron, in a bait matrix against subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 89(5): 1156-1160.

69. Su, N.-Y., P.M. Ban, and R.H. Scheffrahn. 1997. Remedial baiting with hexaflumuron in above ground stations to control structure-infesting populations of the Formosan subterranean termite. (Isoptera: Rhinotermitidae). J. Econ. Entomol. 90 (3): 809-817.

70. Su, N-Y., J.D. Thomas, and R.H. Scheffrahn. 1998. Elimination of subterranean termite populations from the Statue of Liberty National Monument using a bait matrix containing an insect growth regulator, Hexaflumuron. JAIC 37: 282-92.

71. Su, N.-Y. and R.H. Scheffrahn. 1998b. A review of subterranean termite control practices and prospects for integrated pest management. Integrate Pest Management Reviews 3: 1-13.

72. Tamashiro, M., J. K. Fujii, and P.-Y. Lai. 1973. A simple method to observe, trap, and prepare large numbers of subterranean termites for laboratory and field experiments. Environ. Entomol. 2: 721-722.

73. Thorne, B.T. and M..I. Haverty. 1991. A review of intra-colony, intraspecific, and interspecific agonistism in termites. Sociobiology 19: 115-145.

74. Thorne, B.L. 1996. Termite terminology. Sociobiology 28: 253-261.

75. Thorne, B.L. 1998. Biology of the subterranean termites of the Genus *Reticulitermes*. Part I, Research Report on Subterranean Termites. pp. 1-30. National Pest Control Association, Dunn Loring, Virginia.

76. Thorne, B.L. and B.T. Forschler. 2000. Criteria for assessing efficacy of stand-alone termite bait treatments at structures. Sociobiology 36 (1): 245-255.

77. Traniello, J.F.A. and B.L. Thorne. 1994. Termite baits in theory and practice. Proceedings of the National Conference on Urban Entomology (1994): 28-40.

78. Tsunoda, K., H. Matsuoka, T. Yoshimura, and M. Tokoro. 1999. Foraging populations and territories of *Reticulitermes speratus* (Ispotera: Rhinotermitidae). J. Econ. Entomol. 92(3):604 -609.

79. Tsunoda, K., H. Matsuoka, and T. Yoshimura.. 1999. Colony elimination of *Reticulitermes*

speratus (Isoptera: Rhinotermitidae) by bait application and the effect of foraging territory. J. Econ. Entomol. 91 (6): 1383-1386.

80. Weesner, F.M. 1965. The Termites of the United States. The National Pest Control Association. Elizabeth, New Jersey.

81. Wilson, E.O 1971. The Insect Societies. The Belknap Press of Harvard University Press, Cambridge, MA.