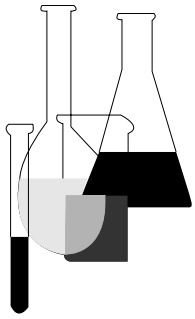
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–04–371
August 2004

Product Performance Test Guidelines

OPPTS 810.3800 Methods for Efficacy Testing of Termite Baits



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/opptsfrs/home/guidelin.htm) under the ''Harmonized Test Guidelines.''

OPPTS 810.3800 Methods for Efficacy Testing of Termite Baits

(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 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 on what they must do should they choose to follow these particular bait testing protocols. This guideline is not intended to address labeling for termite bait products. EPA intends to address labeling guidance in a pesticide registration notice or equivalent document.

(2) **Background**. The published literature on termiticide bait research is the source material for issues addressed in this guideline. Paragraph (g) of this guideline contains a complete list of references cited in this guideline.

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

Kills termites. The term *kills termites* refers to termites dying as result of feeding or contacting a pesticide.

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

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

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

Remedial or curative treatment/application. 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.

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

Structural protection. 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.

Termite colony. 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) (see paragraphs (g)(81) and (g)(71) of this guideline, respectively).

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

Termiticide bait or *termite bait.* The terms *termiticide bait* or *termite bait* refer to any pesticide product that kills or controls termites and has at least two principal components: A bait matrix, intended to be the equal of or preferred to other available sources of food and a pesticide incorporated into the bait matrix that is intended to kill termites.

(c) **Overview**. (1) 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 160.1 to 160.195. Studies which do not comply with GLP standards may nonetheless be considered if, in the Agency's judgment, the design and conduct of the study are sufficient to demonstrate that the results are scientifically reliable. All testing should be done with the product intended for registration unless EPA approves testing of the termite bait with bait station prototypes.

(2) 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. Data should be collected from the laboratory and both field experiments as described in this guideline to demonstrate the efficacy of a termite bait product.

(3) 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 1991 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 (see respective references in paragraph (g) of this guideline). Other references are cited throughout this guideline.

(d) **General considerations for laboratory tests**. EPA believes that laboratory testing for bait products bearing termite control claims should be designed to evaluate characteristics that are critical to the success of

the bait. Specifically, such testing should assess whether the bait: Is readily fed upon by termites, exhibits slow-acting delayed toxicity, is effective at the proposed product label concentration with a palatable alternative food source concurrently available, and causes 100 percent mortality in greater than two weeks but less than ten weeks post-treatment at the tested dose. Additional or alternative assessments may be considered depending on the nature of the active ingredient and bait product.

(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 three subterranean termite species from the United States to include *Coptotermes formosanus; Reticulitermes flavipes*, and *Heterotermes aureus*. Collect field cultures according to the method of Su and Scheffrahn (1986) (see paragraph (g)(56) of this guideline) or by an equivalent method. Evaluations against the dampwood termites, *Zootermopis* spp., and/or drywood termites, *Cryptotermes* and *Incistermes* spp. should be performed if the bait is intended to control these termite species. Testing with other species may be considered.

(2) **Stage, caste, and age**. Test laboratory termite populations cultured from the field no later than 90 days after field collection. Data collected with laboratory populations older than 90 days may be considered if survivorship in the control treatments is greater than 85 percent. The selection of the life stage to test will be dependent upon the pesticidal action of the active ingredient, but should include 100 worker termites (undifferentiated nymphs of at least the third instar in the genus *Reticulitermes* (Thorne 1996) (see paragraph (g)(74) of this guideline) and fifth or sixth instars of true workers in the genus *Coptotermes* (Roisin and Lenz 1999, Forschler and Jenkins 1999) (see paragraphs (g)(49) and (g)(15) of this guideline, respectively)). 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) (see paragraph (g)(25) of this guideline).

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

(4) **Bioassay design**. All laboratory bioassays should be made using three colonies of the test species. The selected method(s) should include no-choice and choice bioassays to assess active ingredient efficacy. Testing should be done under circumstances in which the termites can forage for food in a manner similar to their natural behavior. Bioassays with drywood termites may be done as described by Scheffrahn and Su (1997) (see paragraph (g)(52) of this guideline).

(5) **Feeding preferences and palatability testing**. Testing should be conducted to show that the bait product is palatable to termites at the active ingredient concentration(s) on the product label.

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

(e) **General considerations for control of subterranean termites using termite baits in area-wide field tests not using existing structures and buildings**—(1) **Geographic areas**. Each termite bait product with its associated application materials should be, at a minimum, placed in field sites located in EPA Regions 4, 6 and 9 (EPA Regions are 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) of this guideline). Other Regional locations may be added but should not substitute for testing in Regions 4, 6, and 9.

(2) **Termite species**. The subterranean termite species that should be considered as the test subjects for field site testing include species from the genus *Reticulitermes.* Data collected with any of the following *Reticulitermes* species should be acceptable: *Reticulitermes flavipes, R. virginicus, R. hesperus, R. hageni,* and *R. tibalis. R. flavipes* is preferred because this species is the principal structural pest in the U.S. Data collected from termites in the genera *Heterotermes* and *Coptotermes* should include *Heterotermes aureus* and *Coptotermes formosanus*.

(3) **Test design**. This test is designed to evaluate baits as termite toxicants at sites of vigorous termite activity in the United States. Impact on termite populations should be readily demonstrated. See paragraphs (e)(3)(viii) and (e)(4) of this guideline for types of data to collect and recommendations for evaluating baiting success. The bait should be evaluated for eliminating subterranean termite populations from termite-infested monitoring devices/stations. Post-elimination monitoring should continue at these devices/bait stations for at least one year. For each species tested in each EPA Region, one colony should be exposed to a location at the test site with treated baits and one colony should serve as the untreated control colony and therefore should be in a location at the site without treated baits. Each test site should include at least one ‘‘treated’’ colony and one ‘‘untreated’’ control colony. Treated and untreated colonies should be differentiated and separation demonstrated by the method specified in paragraph (e)(3)(i) of this guideline. Testing and monitoring should be conducted for up to three years.

(i) **Establishing the field site**. The protocol for establishing each field site where the tests will be conducted has been derived from a combination of research studies (Su 1994 (see paragraph (g)(64) of this guideline), Grace *et al.* 1996 (see paragraph (g)(22) of this guideline), Forschler and Ryder 1996 (see paragraph (g)(14) of this guideline), Su *et al.* 1997 (see paragraph (g)(69) of this guideline), Getty *et al.* 2000a,b (see paragraphs

(g)(17) and (g)(18) of this guideline)). Site size and location can vary depending on the termite species present and the size of their respective populations. The site selection process should involve the following steps: Identify the termite species at the site, establish or install monitoring devices (wood stakes, bucket traps, or untreated bait stations), determine which termites are infesting the monitoring devices, establish colony foraging areas (using mark-recapture, mark-release-recapture, DNA analyses or equivalent methods), assess wood consumption patterns, and assign colonies to bait treatments or controls (untreated).

(ii) **Installation of untreated wood stakes or cellulose material**. Once a site with at least two existing termite colonies has been selected, wooden survey stakes or other cellulose material should be driven into the ground to encourage termites to feed at specific sites (Su and Scheffrahn 1986 (see paragraph (g)(56) of this guideline), Haverty et al. 2000 (see paragraph (g)(31) of this guideline). (*Heterotermes aureus* may require a different approach (Jones 1990) (see paragraph (g)(38) of this guideline)). Wood stakes, preferably southern yellow pine sapwood stakes or an equally attractive cellulose material, should be arranged in a grid pattern across the selected site. This should be done for untreated control and bait treatment sites. It is recommended that wood stakes or other cellulose materials should be spaced 2m (6ft) to 3m (9ft) apart and placed into the soil to a depth of 15cm (6in) or deeper. Other spacing and soil depths may be considered by EPA for treated and untreated colonies. The bait matrix, less the active ingredient, or an untreated wood stake, or other cellulosic material can be used in the proposed commercial bait station or a prototype as an additional means of monitoring termite activity. The wood stakes and other untreated bait matrix can be moistened prior to installation to make them more attractive to termites. When the wooden stakes or untreated bait matrix have been fed upon by the termites, an independent monitoring device (bucket trap) should be placed adjacent to it or can replace the stake. Two to three foot quartered wood log sections held together with a strap can also be placed approximately six inches to one foot deep to recruit termites to these locations and make the observation of termite activity easier.

(iii) **‘‘Bucket Trap’’ installations**. Bucket traps should be used as independent monitoring tools and should be installed where termites are actively feeding. These traps can also serve as sites to ‘‘mark’’ termites during bait evaluation (see paragraph (e)(3)(vi) of this guideline). To construct and establish a bucket trap installation see Pawson and Gold 1996 (see paragraph (g)(46) of this guideline). Into the bucket place a prefabricated pine sandwich prepared according to the method of Su and Scheffrahn 1986 (see paragraph (g)(56) of this guideline) or equivalent method. 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. Dyed untreated bait

matrix can be used in addition to bucket traps to ‘‘mark’’ termites. Tamashiro *et al.* 1973 (see paragraph (g)(72) of this guideline), Su and Scheffrahn 1986 (see paragraph (g)(56) of this guideline), and Grace 1990 (see paragraph (g)(20) of this guideline) provide additional information and procedures for field detection and monitoring.

(A) Prior to baiting, these monitoring stations should be used to determine the dispersion and the seasonal foraging and feeding by the termite colonies (Su and Scheffrahn 1988 (see paragraph (g)(57) of this guideline), Su *et al.* 1993b (see paragraph (g)(63) of this guideline), Haverty *et al.* 1999b, 2000 (see paragraphs (g)(30) and (g)(31) of this guideline)). These same monitoring stations should be used to measure feeding of the termite colonies after baiting. For a termite colony to be included in the evaluation, either as a treated or untreated colony, the foraging area of the target termite colony should be determined in the study. This is usually done by documenting that at least three of the monitoring stations must be ‘‘connected,’’ i.e., fed upon by termites from the same colony, in order to define the colony foraging area. However, other methods may be used at the digression of the study director.

(B) Measure wood consumption and damage at the bucket traps in the control colonies in order to have a standard of comparison for feeding cessation at the bucket traps for the baited colonies.

(iv) **Bait product installation**. Install the bait formulation intended for registration, sale and distribution to deliver the active ingredient. The bait station used should be the station to be used for sale and distribution of the product but a prototype may be considered by the Agency. A minimum of five bait stations should be placed within the foraging range of the colony, i.e., at locations within the perimeter of the ‘‘polygon’’ described by the termite occupied independent monitoring stations that are utilized by or ‘‘connected to’’ that colony or within the area established by the interconnection of the bait product stations and the bucket traps (same termite colony foraging at both devices; see also Evans 2001, Evans *et al.* 1998, 1999, and Forschler and Jenkins 2000 (see paragraphs (g)(12), (g)(9), (g)(10) and (g)(16) of this guideline, respectively)). It should be clearly shown that the termites feeding in the monitoring devices and treated bait stations are from the same colony.

(v) **Termite species identification**. Termites should be collected and identified as to species from at least one bait station or independent monitoring device from each test plot that contains termites. Appropriate termite identification keys such as those published by Weesner 1965 (see paragraph (g)(80) of this guideline), Nutting 1990 (see paragraph (g)(44) of this guideline), and Scheffrahn and Su 1994 (see paragraph (g)(51) of this guideline), should be used to identify termites. Identification using DNA techniques or cuticular hydrocarbon profiles (Forschler and Jenkins 1999, (see paragraph (g)(15) of this guideline)), Haverty *et al.* 1996, 1999) (see

paragraphs (g)(29), (g)(30), and (g)(27) of this guideline, respectively) 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 percent 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 (see paragraphs (g)(33), (g)(34) and (g)(35) of this guideline) for a discussion of methodologies on use of DNA sequencing and genetic markers for phylogenetic analyses and genetic structure determination in subterranean termite populations.

(vi) **Colony identification**. Identification of termite colonies foraging in the treated and control plots should be at least once per year. The purpose of marking termites is to verify that the termites feeding in the wood stakes/bucket traps and in the bait product stations are from the same colony. The preferred method is to dye filter paper with a known concentration of dye such as Sudan Red 7B (Lai *et al.* 1983 (see paragraph (g)(41) of this guideline), Nile Blue A and Neutral Red (Su *et al.* 1991 a, b, c (see paragraphs (g)(59), (g)(60) and (g)(61) of this guideline) or other acceptable dye (see also Delaplane *et al.* 1989 (see paragraph (g)(5) of this guideline)), and Evans 1997, 2000 (see paragraphs (g)(8) and (g)(11) of this guideline) for a discussion of dyes). 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 wooden boards or incorporated into the bait matrix in a bait station. A similar procedure is described by Atkinson 2000 (see paragraph (g)(2) of this guideline). 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)(vii) of this guideline.

(vii) **Other marking techniques**. Other marking techniques such as using fluorescent spray paint as described by Forschler 1994 (see paragraph (g)(13) of this guideline), mark-release-recapture techniques as described by Jones 1990 (see paragraph (g)(38) of this guideline), or molecular marking techniques (Forschler and Jenkins 1999 (see paragraph (g)(15) of this guideline); see also Forschler and Jenkins 2000 (see paragraph (g)(16) of this guideline) for related approaches may also be employed but there should be laboratory and field data for the termite species under investigation. If a method using 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. It should be clearly shown that the termites feeding in the moni-

toring device and adjacent bait stations are from the same foraging group or colony. Depending on the species, such data may also include agonistic behavior, average dry weight of worker termites (minimum of 5 replicates, 10 workers each), and cuticular hydrocarbon profiles (Haverty *et al.* 1996, 1999a,b (see paragraphs (g)(27), (g)(29) and (g)(30) of this guideline)).

(viii) **Data collection and reporting**. The following information should be recorded and reported during the course of these tests in order to provide the data necessary to evaluate the impact of the bait treatment on termite populations and their foraging activity:

(A) Purpose of study;

(B) Location and test unit designation;

(C) Date field test unit installed and duration and intervals of monitoring and data reporting;

(D) Date of evaluation;

(E) Termite species present;

(F) Termite colony designations, presence or absence of termites in the wood stakes, bucket traps, split logs (if used) and bait stations;

(G) Presence of dyed termites in the independent monitors and bait stations;

(H) Amount of wood/bait consumed (estimated percent) in the bucket traps and bait stations;

(I) Bait and wood weights;

(J) Date termite activity ceased in the bucket traps and bait stations;

(K) Date termite activity resumed in the bucket traps and bait stations;

(L) ASTM damage rating to each pine board/bait matrix following cessation of termite activity in the bucket traps and bait stations;

(M) Date dyed paper/matrix is first fed upon;

(N) Date of release of laboratory dyed or marked termites into the bucket trap or bait stations;

(O) Date and location of mud tubing observed on or in a test unit;

(P) Results and discussion;

(Q) Conclusions;

(R) Certification;

(S) References;

(T) Appendices.

(4) **Evaluating baiting success**. These recommendations are derived from recognized pest management practices and bait field test evaluations 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 (see respective references in paragraph (g) of this guideline). The study should describe how bait success will be measured. For a bait to be effective, it should control termite populations on an area-wide basis. At a minimum, five bait product stations and their interconnected bucket traps should be active for each colony tested and evaluated. Generally, treated baits should be evaluated to determine whether they demonstrate efficacy (i.e., colony extermination; population suppression) within 12 months after initiation of feeding on the bait treatment and during the 12-month period following the cessation of termite activity at the monitoring devices. Su 1996a, Grace *et al.* 1989 and Forschler and Ryder 1996 (see paragraphs (g)(66), (g)(19) and (g)(14) of this guideline, respectively) present procedures for making these determinations. The amount of bait eaten/used at each location for each colony should be documented in the study and effects determined by comparison with control colonies.

(f) **General considerations for field tests using existing structures and buildings**. Field tests at existing structures should use the bait product and its associated application materials as intended for registration. The test period under this protocol should be three years from the time that the bait product stations are installed. See paragraphs (f)(4)(vi) and (f)(5) of this guideline for types of data to collect and recommendations for evaluating baiting success.

(1) **Number of homes/structures**. Testing should be conducted in the United States at 100 or more subterranean termite infested wooden buildings/structures. Testing with drywood termites should be performed if the product is intended to control these termite species. In this case, testing should be conducted in 100 drywood termite infested structures.

(2) **EPA Regions**: The selected structures/buildings for subterranean termite testing should be distributed (approximately 15 homes per region) in the following EPA 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).

(3) **Site selection and inclusion of a building/structure in a field test**. Selected structures should represent the wide range of construction types existing in the United States and the testing should include post-application monitoring inside and outside the structure. In-ground and

above-ground baits should not be tested in the same structure unless they are to applied as a co-pack.

(i) A wooden frame building must be present. A building can be residential or commercial and may also include sheds, barns, or garages.

(ii) Based on the results of a thorough inspection, the building should be infested with one or more of the following subterranean termite species *Reticulitermes* spp., *Heterotermes aureus*, and *Coptotermes formosanus* per one or more of the following infestation conditions: Either alates have swarmed inside the structure or live termites are found to be active within the building; or there is clear evidence of termite activity or damage on or in the building (mud tubes, galleries in wood) and live termites or evidence of recent termite activity (recently formed mud tubes and/or galleries in wood) are found in outside wood attached or immediately adjacent to the building, i.e., attached fences, wood decks, landscape timbers, etc. When assessing structures infested with desert termites (*Heterotermes*only), an additional condition should be considered: There is clear evidence of termite activity or damage on or in the building (mud tubes, galleries in wood) and the presence of active, live termites found in bait stations placed in the ground or around the perimeter of the structure according to label directions.

(iii) The termiticide applications made to the structure should be known for five years preceding the start of the bait evaluation. Structures that have been treated within the last five years with a liquid soil termiticide should not be included in the test because these treatments could affect bait success.

(iv) Termite species and their identification. See paragraph (e)(3)(v) of this guideline.

(v) Detection of termites in an existing structure. Acoustical emission devices, microwave devices, and/or visual inspection should be used to monitor wood in a structure for termites. Termite ‘‘sniffing’’ dogs may be considered for detecting termites but should not substitute for the other methods.

(4) **Test design, monitoring termite activity and bait product installation**. A combination of the methods in paragraphs (e)(4)(i), (ii), and (iii) of this guideline should be used to assess the presence and extent of the foraging termite population in the vicinity of the foundation for 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 the proposed bait product label’s directions for use. 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 (or as directed by the label) can be established using techniques such as the mark-recapture, mark-release recapture method, and/or DNA analyses. Generally, monitoring stations should be checked at a minimum every 30 days during the course of the study.

(i) **Installation of untreated wooden stakes or other cellulose materials**. Wood stakes or other cellulose materials should be spaced 2m (6ft) to 3m (9ft) apart and placed into the soil to a depth of 15cm (6in) or deeper around the perimeter of the structure. Other spacing and soil depths may be considered by EPA. The bait matrix, less the active ingredient, and/or wood stakes cut to the required size, can be used in the proposed commercial bait station as an additional means of monitoring termite activity. Wood stakes or cellulose materials, 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-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, see Pawson and Gold 1996 (see paragraph (g)(46) of this guideline). Into the bucket place a prefabricated pine board sandwich prepared according to the method of Su and Scheffrahn 1986 (see paragraph (g)(56) of this guideline). 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.

(iii) **Dyed untreated and treated bait matrix**. Dyed untreated and treated bait matrix can used in addition to bucket traps to ‘‘mark’’ termites.

(iv) **Dye and Marking**. See (e)(3)(vi) of this guideline.

(v) **Termite bait product application and placement**. Application of bait product stations should be done 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. It is recommended that 10 or more bait stations be applied per structure to ensure baiting success and sufficient information to adequately assess the efficacy of the bait product.

(vi) **Data collection and reporting**. Data from all structures included at any time during the course of the study should be reported. The following information should be recorded and reported during the course of these tests in order to provide the data necessary to evaluate the impact of the bait treatment on existing structures:

(A) Purpose of study;

(B) Location (address);

(C) Date test monitoring units and bait stations installed and duration and intervals of monitoring and data reporting;

(D) Construction type (split foyer, etc.), square footage, age, foundation type (slab, basement, crawl space, etc.), siding type (brick, block, foam board/stucco, etc.);

(E) Termiticide treatment history of the structure;

(F) Date of termite inspection;

(G) Termite species present, termite species colony designations, date and location of placement of monitoring devices and bait stations;

(H) Soil type, soil pH, percent soil moisture and soil surface temperature at each monthly evaluation, climatic data - temperature and rainfall;

(I) Date of each monthly evaluation;

(J) Termite species present;

(K) Presence or absence of termites in each bucket trap and for other monitoring stakes/stations;

(L) If applicable, presence of dyed termites in the bucket trap and bait stations;

(M) Amount of wood/bait matrix consumed (estimated percent) in bucket trap and each bait station, respectively;

(N) Weight of bait(s) and wood consumed;

(O) Number and percent of bait product stations fed upon;

(P) Date of bait station replacement;

(Q) Date termite activity ceased in the bucket trap and or bait stations and date termite activity resumed in the bucket trap and or bait stations;

(R) ASTM damage rating to each pine board following cessation of termite activity in the bucket trap, wood stakes, and bait station(s);

(S) Date dyed paper first fed upon;

(T) Date of release of laboratory dyed or marked termites into the bucket trap or bait station(s);

(U) Date termites found in the bucket trap, wood stakes, and or bait station(s);

(V) Date and location of mud tubing observed in the bait stations, bucket traps and structure

(W) Conclusions;

(X) Certification;

(Y) References;

(Z) Appendices.

(5) **Evaluating bait success**. (i) The first evaluation after installation of the test units should be accomplished by 60 days post-installation. Subsequent evaluations should be conducted at 30-day intervals during the duration of testing, and should determine presence or absence of termites (all life stages considered, including alates) in the structure, monitoring stations, and bait stations. The monitoring devices should be visually observed for mud tubing but should not be disturbed or dismantled. The number of termites present should not be estimated until they have infested most of the monitoring device as evidenced by mud tubing in the wood boards or bait matrix. Bait product station replacement should be done according to the proposed label directions. Termite presence and activity must be monitored during the entire field test using methods described by Su and Scheffrahn 1986 (see paragraph (g)(56) of this guideline), Pawson and Gold 1996 (see paragraph (g)(46) of this guideline), Haverty *et al.* 1975 (see paragraph (g)(24) of this guideline), and Jones 1990 (see paragraph (g)(38) of this guideline), or equivalent methods. Inspections of treated infested structures should take place within 12 months of bait installation and during the times of the year when termites are active, generally from May to September in much of the United States.

(ii) The bait product treatment should eliminate an existing termite infestation in 12 months or less following installation of the commercial product (with or without active ingredient) in the infested structures and these structures should remain termite free for 12 months following elimination of the termite infestation. Alate swarms, mud tubing, and presence of worker or soldier termites in or on the structure are indications of a termite infestation and may indicate the failure of a bait product to control termites. Generally, baiting should cease as directed by the product label but monitoring for termites should continue for an additional 12 months. If termite activity resumes during the 12-month post-observation period at the bait product station, bucket trap, or wood stake, additional bait application can be made according to the product label directions. Claims of preventive treatment are generally satisfied when a structure is not infested with termites for 12 months or more following elimination of a structural infestation.

(g) **References**. The following references should be consulted for additional background material on this test guideline:

(1) American Society for Testing and Materials (ASTM). 1996. Standard method for evaluating wood preservatives by field tests with stakes. In: Designation D–1758–96, wood, book of ASTM standards, 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. Suppression of termite feeding and 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. 2000. 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 *Riculitermes 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. 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. 1999a. 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 laboratoryexperiments 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. 1996a. 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. 1996b. 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. 1998a. 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 speratusT1 (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.