STATEMENTS FOR FIFRA SAP ON TERMITE BAIT PRODUCT PERFOMANCE TESTING GUIDELINES

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The following comments are mine presented at the request of the Association of Structural Pest Control Regulatory Officials (ASPCRO) as a member of their Termiticide Label Review Committee. John McCauley and Jim Wright of ASPCRO regret that scheduling conflicts prevent them from being present at this meeting but express agreement with the statements made in this report that includes a brief, and by no means complete, list of the scientific literature cited along with a biosassy protocol.

INTRODUCTION

Funding for scientific research has increased since the regulatory climate-induced removal of the chlorinated hydrocarbon insecticides from use in termite control. This funding boast has lead to a better appreciation for the complex nature of termite biology. It is important to realize that our knowledge base on these cryptic, eusocial insects is driven by assumption and inference (**5,6**). Despite the strides made in the past 10 years, our understanding of many of the important underlying processes that drive termite biology is still lacking. Some of the critical unknowns include the forces behind the complexities of caste development from a physiological basis; the chemical communication cues used by individual termites and how these cues bind termite societies; the inheritance patterns of termite DNA and measures of population size, social organization and vigor (**2, 4, 6, 9, 10, 11, 15, 18, 19, 20**). The complexities of termite foraging behavior - location, identification and recruitment to a food resource - are driven by various factors that have not been fully elucidated (**2, 16, 21**). In addition a better understanding of food exploitation and dissemination is also needed to predict termite bait technology impacts (**18, 19, 20, 22**). Regardless of what we do not know it must be understood that most scientists will agree that termite societies have limited dispersal abilities (when unassisted by factors such as transport of infested materials) and therefore their developmental options and social organizations are, by necessity, dynamic and opportunistic in order to take advantage of prevailing conditions (**14, 23**). The state of the scientific art for examining termite societies is changing rapidly and every year new techniques replace time-tested methods for studying their biology (**6**).

The Industry's perception of termite control has also changed along with this increased scientific knowledge base. Fifteen years ago control was centered on an event – the termite treatment. That mentality, although still with us, is evolving toward the realization that termite control should be a process built around an inspection program. I believe that this trend will continue until termite control is 'traditionally' practiced as a

set or series of treatment events specifically tailored to each structure while regularly scheduled inspections are the keystone of the control effort.

Termite baits have a place in termite control and actually have been the driving force toward the realization that the process of termite control must replace the treatment/event mentality. The efficacy of termite baits – as far as this SAP exercise is concerned – revolves around the claims made on termite bait products. The question then becomes; what can termite bait technology deliver to the consumer? Termite baits are, to use the vernacular of insect pest management, a population management tool. From a scientific point of view, population impacts must, therefore, be the measure of termite bait efficacy. As a result, the definition of "works" (as in "does this termite bait work?") depends on what measures are used to determine population impacts.

In order to impact a termite population an efficacious bait must, first and foremost, kill termites. Yet, termites are social insects, not solitary pests. As a result, an efficacious bait product should impact groups of termites - preferably using their social organization to effectively deliver a toxicant. The degree or extent that a termite bait product will impact a group (population, colony or whatever term is agree upon) of termites is dependent on several dynamics. At the simplest level, the amount of termites that a given bait product will kill is dependent on the active ingredient (AI) - essentially the mode of action of the AI - and the size of the targeted termite population. In other words, the amount of AI delivered to a particular termite population. As a result, the efficacy of a termite bait product must be defined within the context of how many termites a given amount of product can kill with one application. Yet from a consumer perspective, an answer to the question "does this termite bait work?" depends on the claims that accompany the product and the diligent application of the technology (competence of the applicator).

Aside from structural protection claims, the question of how best to measure termite bait efficacy must revolve around the definition of a termite population and the means used to measure that population. Unfortunately there are no sound, repeatable measures or indices that the entire scientific community can agree on – at this time (I believe that one day we will have these measures). The measures of termite population parameters available today are founded in assumptions based on our current knowledge of the biology of these cryptic insects and data must be interpreted within the limits imposed by the technique used to obtain a particular measure (**4, 5, 6, 9, 10,14**). The current state of the science is that termite population structure can only be accurately measured by employing multiple scientific techniques at a single site over time (**5**).

I firmly believe that most termite baits will continue to be used in conjunction with other, additional, treatment tactics until reliable population measures are adapted for commercial use. The practical experience that most Pest Management Professionals (PMP's) have gained while using termite baits over the past 5 years highlights the problems associated with our current lack of usable termite population indices. Simply relying on a "monitoring" program that records the presence/absence of termites at stations around a structure has confronted the industry with having to explain the

continued presence of termites following baiting at many of their accounts. Without recording information that can be explained at the population level the industry will forever be faced with defending assumptions rather than providing a level of comfort to the consumer that their efforts were successful (**4**). The intuitive association of bait consumption with population impacts is and should never be allowed with a population management tool like termite baits. Only when population indices are developed that the industry can use - at the consumer level - will claims of population impacts be appropriately included in consumer claims. Such industry-friendly/consumer-ready termite population measures are currently not available but I believe they will be found with continued research.

THE CURRENT STATE OF THE SCIENCE OF TERMITE POPULATION MEASUREMENTS

What are the measures of termite populations currently available to the termite research community? To date, there are four broad categories of techniques that can be used in research aimed at measuring termite populations in the field.

Species determination.
Morphological descriptions of the soldier or alate castes.
Genetic differentiation using length polymorphism (fingerprinting) or gene sequence data. (**1, 7, 10, 12, 13**)

Utility in determining population measures: These techniques can be used to determine if termites found at disparate sites have the potential for interaction at the population level. Simply put, if they are the same species they have the potential to interact and if they are not the same species they probably don't interact.

Limitations: The taxonomy of the subterranean termites found in the US is in desperate need of revision. We routinely collect termites where the soldiers and alates collected at the same site key to different species. Research by various groups is continuing in this area using chemotaxonomic characters, traditional morphology and genetic techniques to provide an updated opinion on termite speciation.

Agonism bioassay.
Placement of termites from two different collection points in a single arena and recording the mortality after a prescribed period of time – usually 24 hours. (**1, 17**)

Utility in determining population measures: This technique will determine if termites of the same species are capable of interacting on a population level. Again, if they fight they probably are not interacting as a population but if they don't fight all that can be said is that they have the potential to interact.

Limitations: Science does not know what drives the process of kin recognition (the term used to describe how termites distinguish between nestmates and non-nestmates). Agonism bioassay results are impacted by the experimental design. Factors such as arena

size and numbers of termites per arena, among other variables, can affect the outcome of agonism bioassays. The data reported in the scientific literature has been equivocal: for example it is not uncommon to record results from three replicates using termites from the same collection site where two replicates fight and the third replicates remains passive.

Mark/recapture – single and multiple release protocols.
These techniques consist of collecting termites from a single site, coloring them with either an internal or external mark and then releasing the marked termites back into the original collection site. After a prescribed period of time all collection sites in the area are examined for the presence of marked termites. (**5, 6**)

Utility in determining population measures: This technique will provide information on the movement of termites between known feeding sites. The recovery of a marked termite at a point other than the release site can only be related to the potential the termites at those two sites (the release point and the collection point) have for interaction indicative of a single population.

Limitations: The percentage of marked termites recaptured is often less than one percent. Mark-release-recapture (MRR) is therefore dependent on marking a large proportion of the targeted population. Studies using a single mark-release cycle followed by multiple recaptures over time have shown that the distribution of marked termites is clumped and non-random (random redistribution of marked individuals is a prerequisite for population estimates using this technique) (**5, 6**). The use of multiple MRR cycles at a site runs the risk of overestimating population interactions. The appearance of one or a few marked termites at a feeding site some distance from the release point could be an anomaly associated with the disturbance that accompanies the act of marking and releasing termites. This is especially true if separate termite populations share galleries - over time - as has been proposed with the open and closed social systems thought to be common with certain subterranean termite species.

Molecular genetics – several techniques including gene sequence and multi-locus fingerprinting. (**1, 5, 6, 9, 10, 11, 12, 13, 14**)

Collecting termites from a site and comparing genetic markers between sites.
There are numerous techniques available and comparisons or interpretation of the data are contingent on the technique and the question being asked. These measures are indicators of relatedness and the potential for interaction at the population level can therefore be inferred based on the molecular technique utilized.

Utility in determining population measures: These techniques can be conducted following a single collection of a few termites – obviating the need to collect large numbers of particular castes (as is required by MRR and agonism bioassay). Termites collected at a site and properly stored can be re-examined years later as new techniques become available to verify results. The molecular techniques published in the scientific

literature allow the use of commercial DNA processing laboratories so that any research group can take advantage of the technology.

Limitations: This is a technology that is relatively new to the termite research community. Because of our lack of understanding of termite reproductive strategies and social structure, current data analysis is dependent on untested assumptions. However, as research in this area increases reliable population markers will be found. Relatedness has not been reliably correlated with population structure.

Summary of the termite population indices available to researchers. Each of the aforementioned techniques can provide valuable information on the potential that termites - found at disparate sites - have for interacting on the level of a single population. Unfortunately none of the population indices available today, when used alone, can provide the answer to 'what is a termite population or colony?'. The current state of scientific art requires use of several, if not all, of the aforementioned techniques, over time, to provide the most reliable information on the dynamics of termite social interactions. The latest research indicates that termite social structure is an amorphous, ever-changing entity that depends on caste proportions, available food resources (competition), chemical communication cues (hydrocarbons or other smells), the reproductive status of a group, and the termite species involved to name a few of the factors affecting the potential for groups of termites to function cooperatively in rearing young and sharing resources (the most common definition of a social insect colony)(**5, 6**). All of the factors that may influence the cooperation between termites at the population level change over time and therefore a timeline of information is needed to properly assess the data within the context of a given site. As research efforts continue to examine the social organization of termite societies I am confident that a reliable set or single measure of termite populations will eventually be developed and adapted but at this point in time there is no single, reliable consensus index. The use of molecular markers holds the most promise for providing a reliable population index yet more research is needed in this important scientific quest.

TERMITE BAIT PRODUCT CLAIMS

This SAP faces the challenge of determining the most equitable measures to use today, knowing the (limited) state of the current scientific knowledge base, while keeping an open mind for future scientific breakthroughs. The guidelines must allow for the inclusion of new technologies – specifically molecular techniques that are currently being researched around the country – that will increase our powers of resolution for questions such as species determination and population/colony affiliations.

It is my opinion that there are three basic claims that a termite bait product can make:
1). Kills termites; 2). Manages populations or 3). Protects structures.
Each claim should have a separate set of evaluation criteria.

Kills Termites - This claim can be satisfied by laboratory bioassay alone. The tests outlined in this draft will provide sufficient proof of such a claim.

Manage Termite Populations – This claim can be made in conjunction with the Kills Termites claim but laboratory tests should include using a microcosm bioassay such as the one outlined later in this report.

Field tests must include use of control (untreated) populations in the same area (within 0.5 km) as the baited population. At this time it is not advisable to use a single population index or measure in place of multiple techniques. In addition, any population parameter must also be measured over time to assess interactions with nearby populations and the impacts of weather, 'normal' movement, and disturbance on the presence/absence of termites at baited or untreated stations. Field trial results from both simulated field (small scale) and EUP-style trials (around structures) must only use reductions in population parameters as a measure of efficacy. Whatever population parameters are measured they must justify the conclusion that the targeted termite population was impacted by the treatment and only through the use of untreated, control populations can that conclusion be satisfied.

Protects Structures – This claim can be tested in simulated field trials and there is no need to verify termite population parameters because the Protect Structures claim assumes that any termite population in the vicinity will be detected, intercepted and controlled prior to infestation. The simulated field trials outlined in this draft are sufficient to prove such a claim. I believe data from EUP-style trials can add to this information base but from a scientific standpoint it is redundant and unnecessary. However, from a consumer and regulatory standpoint it is important to have EUP-style trials to test the end-user efficacy of a termite bait product.

COMMENTS ON THE METHODS DESCRIBED IN THE TERMITE BAIT PRODUCT PERFORMANCE TESTING GUIDELINES

Laboratory trials -Worker termites should be used in all laboratory experiments related to killing termites - not nymphs. The age limits listed in the guidelines are sufficient for the bioassays outlined for the kills termite claims. However, the in-culture age of termites used in the microcosm bioassays must, by necessity, be older than three months. Placing a group of 10,000 workers in bioassay requires 4-6 weeks for the neotenic reproductives, eggs and larvae to appear. These castes are necessary to provide the overlapping generations and full complement of castes needed to simulate a field population. It will then take up to one week for the termites to colonize all available feeding sites in the arena and an additional 4-8 weeks to verify impacts once the bait is introduced. The microcosm bioassay has several advantages including the ability to observe activity and mimic the field situation. For each replicate at least two microcosms must be set up with termites from the same population so that one can be used as a control. Choice tests for bait materials should be conducted using separate feeding arenas with some distance separating the choices rather than the single arena with no real distance between choices as in the Oi et al. citation provided in the guidelines (**3, 18, 20**).

Field trials – Control sites should be established whenever possible to provide information on the seasonal, weather, and disturbance related movement of termites (**6**). Without controls the natural rhythms of termite activity at feeding sites (monitoring stations) such as disappearance due to dry or cold conditions may be interpreted as a bait-impact effect. In population control studies independent inspection ports (bucket traps or monitoring stations) must be established and the populations delimited prior to bait placement. The requirement of using MRR to delineate termite populations in field trials is inappropriate given the state of the science involved in termite research today. Sole use of MRR as a population index is problematic because this approach will provide too many false positive *and* false negative results with most of the endemic U.S. termite species and therefore across most of the regions required for efficacy testing. For example, in most of the southeastern United States with *Reticulitermes* spp. and with *Heterotermes* spp. in Arizona (**5, 6, 8**). As a result it is imperative that these guidelines provide for use of techniques (some yet to be developed) that are appropriate for the diverse species and regions listed. The use of molecular techniques will increase in acceptance and provide a higher resolution of population parameters than MRR with less labor and in a shorter time frame. These guidelines must allow efficacy testing using population indices other than MRR alone and should recommend the use of multiple scientific techniques until a single, reliable technique is developed. Because the scientific community has yet to agree on the definition of a functional termite colony (from field studies), this panel should not accept any single technique that the current state of the science has proven inappropriate – for example using MRR alone or agonism bioassay alone as the standard population measure - as the bench mark for satisfying consumer protection.

Conclusion

The Guidelines for termite bait product performance testing provided to this SAP outlines a rigorous standard that will meet the needs of consumer protection. The following responses to the questions posed in the FIFRA SAP Charge will serve as our concluding statement.

1. (a) The draft Guidelines produce sufficient data to determine if a bait product kills termites.
   (b) The laboratory data should include microcosm tests to infer termite bait efficacy before small-scale field tests are initiated. The attached microcosm bioassay protocol is provided as an example of such a test.

2. (a) The small-scale field tests outlined in the Guidelines are scientifically sound. The feasibility of those tests however depends on allowing the use of other techniques (i.e. molecular markers) as a measure of termite populations. The reliance placed on MRR in this draft will require establishing at least three times the number of intended replicates to provide sufficient data across most of the country.

(b) These small-scale tests provide sufficient data.
(c) Small-scale field tests will never mirror actual use conditions. The concept of small-scale field tests allows for control of variables such as applicator error that have no bearing on product efficacy. Therefore these tests should be an essential part of product testing.
(d) Establish plots, identify populations, apply baits, and record impacts.
(e) Test sites should be placed in areas where termite populations are known to exist. The Guidelines provide for sufficient coverage of the important regions of the United States. The number of replicates should be at least 20 identified termite populations per product per site. Baits should be placed in small-scale test plots per manufacturer label directions. The methods outlined in the Guidelines are appropriate only if MRR is not the standard measure of termite populations. As stated previously, *Reticulitermes* in the southeastern United States and *Heterotermes* in Arizona will only accept paper stained with a fat-soluble dye in sufficient numbers to make connections away from that site when their populations are large and alternate food resources scarce. Meeting those criteria will require enormous effort for any meaningful data.

3. (a) Field tests around existing structures and buildings relate more to applicator competence than product efficacy. However, these tests should be considered an informative addition to the product efficacy database.
(b) The state of the science requires use of multiple techniques over time to identify and follow termite populations. The Guidelines should provide room for the use of other, proven, measures as independent research in the area of termite social organization progresses. The most promising measures will probably come from molecular genetics research because these measures do not require collections of large numbers of termites and if stored properly (100% ETOH or frozen) the samples can be re-visited (and the data from old test sites are still useful) as new techniques are developed.
(c) The product performance standards are appropriate.
(d) Homeowner bait efficacy tests should be measured in the small-scale field trials to record termite population impacts and in large-scale trials using homeowners as the applicators. The use of a product has more to do with applicator competence than product efficacy yet product claims must be measured within the context of the end-use pattern.

PROTOCOL FOR LABORATORY MICROCOSM TESTING OF BAIT PRODUCTS AGAINST SUBTERRANEAN TERMITES

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Termites: *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks) collected from infested logs or other infested materials be used for bioassay. Only those termites that have been maintained in the laboratory for at least 2 months will be used. The intent is to use groups of termites that closely mimic field populations. Therefore the termites used in these bioassays will only be those groups that contain, at least, reproductives, eggs, larvae, soldier and worker caste members. Termites collected from logs will be maintained in clear plastic containers measuring 31.5 X 25.6 X 9.8-cm (L:W:H) containing pieces of pine wood, pine sawdust and filter paper. Containers with termites will be kept in an environmental chamber at 27^{o} C in complete darkness. Each box will contain between approximately 10,000 termites.

Bioassay arenas: The main chamber (or nest chamber) will consist of the aforementioned clear plastic container. The main chamber will be connected to a series of smaller chambers (feeding chambers) by 2-mm diameter tygon tubing. The length of tubing between chambers will be 1 meter. Feeding chambers will measure 15-cm in circumference by 4-cm high. There will be four feeding chambers per main chamber arranged so that there are two tubes each leading from the main chamber with two feeding chambers connected in succession separated by a 1-m length of tubing. Feeding chambers will be lined with a 2-mm layer of moistened play sand and contain pieces of pine wood and pine sawdust as a feeding substrates.

Bioassay: Termites will be allowed 1-2 weeks to find and "colonize" each feeding chamber. Test solutions, suspensions, blocks or feeding substrates will be prepared according to manufacturer directions and applied to one of the two most distant feeding chambers, selected at random. The data recorded will include daily observations of the activity of termites in each chamber and the position of dead termites. Test duration will be 4-8 weeks at 27^{o} C after treatment (bait) is administered. At the end of the test, each arena will be disassembled and the number and position of termites recorded.
There will be at least 1 treatment replicate and 1 control from each group of termites from a single field collection site. Although the number of termites in each arena may not be known before each test we will have an idea of the starting number based on the weight of termites added when the arena was established several weeks previous. The number of surviving termites will be compared to the estimated starting number for the controls to provide an indication of the efficacy within this system. The observations on activity within each chamber will give an indication of the relative repellence of the treatments throughout the duration of the test. The final measure of efficacy will be the mortality within the treatment group compared to the control.

Photo of the microcosm bioassay arena arrangement described in the accompanying protocol

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