Descriptive Study of Non-Repellent Insecticide-Induced Behaviors in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

The effects of three slow acting non-repellent insecticides on the behavior of the eastern subterranean termite, *Reticulitermes flavipes* (Koller) was investigated in a continuous exposure study. Abnormal behaviors of fipronil and indoxicarb treated termites were similar; incipient intoxication (disorientation), followed by ataxia (uncoordinated movements), and morbundity (lack of ability to walk) leading to death. Reduction in walking speed (followed by morbundity and death) was the major behavioral symptom of chlorantraniliprole treated termites. The importance of behavior-based studies, particularly behavioral endpoints, to the development and use of non-repellent termiticides is discussed.

Keywords: *Reticulitermes flavipes,* behavior, non-repellent termiticides.

INTRODUCTION

Termites of the family Rhinotermitidae (Isoptera) are the most economically important species in the United States where damage and control efforts are estimated at $3 billion annually (Su 2002) and the subject of 90% of all treatments (Forschler & Lewis 1997). Chemical methods of termite control have evolved significantly, but the transition from repellent to non-repellent termiticides has been one of the most revolutionary changes. Avoidance is the only important behavioral element in repellent-based control of subterranean termites. The introduction of non-repellent compounds, however, brings other aspects of termite behavioral biology into sharper focus (Su *et al.* 1982). Non-repellent compounds circumvent the difficult task of creating a continuous chemical barrier around a structure to exclude termites (Hu &

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Hickman 2006). This is because broader aspects of termite behavior exert significant effects on the performance of non-repellent compounds. The non-repellent quality of these compounds allow termites to freely tunnel into treated soil and facilitate transfer between contaminated foraging termites to naïve nestmates through social interactions such as trophallaxis, grooming, and care-giving activities, resulting in so-called behavior-based termite control (Hu *et al.* 2005).

Behavior-based termite control needs to encompass the exploitation of the natural social behavior of termites as well as the use of well-defined and relevant behaviors for the assessment of insecticide performance. Behavior is a unique manifestation of the connection between the physiology and ecology of an organism and its environment (Little & Brewer 2001) and is a very important indicator of environmental changes such as the presence of toxicants. To be relevant to toxicological assessments, behavioral responses must be: well-defined, measurable, ecologically relevant, and sensitive to a range of toxicants; the mechanism of response must also be understood (Rand 1985). Behavioral endpoints have been used as indicators to distinguish between classes of insecticides with different modes of action and to distinguish different insect species (Kane *et al.* 2005).

The acceptance of behavioral endpoints as indicators of environmental toxicity in the United States began with the acceptance of avoidance behavior as legal evidence of injury to natural resources (NRDA 1986) and represents an important milestone in the development of behavioral toxicology. Well-defined behavioral endpoints have been used as early indicators of environmental pollution, but can be adapted for assessment of insecticide toxicity and performance (Duffard & Duffard 1996). This is particularly important given the long behavioral window between incipient intoxication and mortality of termites treated with non-repellent compounds.

Studies on non-repellent termiticides have generally concerned their laboratory and field efficacy, but little attention has been focused on the behavioral responses of termites (Henderson 2003). The purpose of this study is to describe and establish abnormal behavioral endpoints in termites exposed to three non-repellent termiticides (fipronil, indoxacarb, and chlorantraniliprole) to aid in the assessment of their toxicity against the eastern subterranean termite, *Reticulitermes flavipes* (Kollar).

MATERIALS AND METHODS

Termites.

Groups of termites were collected from field colonies in Auburn-Opelika (Lee County, Alabama) using open-bottom in-ground traps described by Hu & Appel (2004). Traps consisted of open-bottom plastic buckets (18 cm high, 13 cm internal diameter) provisioned with rolls of corrugated cardboard (15 cm high and 11 cm diameter). Termites were extracted by gentle tapping of rolls onto moist paper towel. Workers of 5th larval stage or older were selected for the study.

Chemicals.

Formulated indoxacarb 150 SC (15% AI) (E.I. DuPont, de Nemours and Company, Wilmington, DE, USA)], fipronil (9.1% AI, BASF Corp., Research Triangle Park, NC), and chlorantraniliprole (18.4% AI, E.I. DuPont, de Nemours and Company, Wilmington, DE, USA) were used to prepare 24-h LC_{90} concentrations of insecticide-treated Whatman No.1 filter paper (Whatman International Ltd., Maidstone, England). Treatments were 200 ppm indoxacarb, 50 ppm fipronil, and 300 ppm chlorantraniliprole, with distilled water-treated filter paper as control. These concentrations were selected based on previous unpublished studies by the authors. Filter paper disks (5.2 cm diameter) were treated in 5.2 cm diameter Petri dishes (1.5 cm in height) after which they were air-dried for 24 h in a hood.

Bioassay.

Groups of ten workers were introduced into each experimental unit (Petri dishes containing treated filter papers); each treatment was replicated five times. Experimental units were sealed with Parafilm® to maintain humidity and held at 25 ± 1°C. Termite behavior was observed continuously for the first 8 h and then at hourly intervals until 90% of termites died.

Data collected included descriptions of abnormal behaviors, incidence and nature of behaviors such as body movements, grooming/care-giving, and spatial distribution of termites. Termites were noted to be clustered if they remained in a stable group with individual distances of less than 0.4 cm. Similarities and differences in the behavioral response to the different insecticides and different termite species were noted and described.

RESULTS

Upon introduction into the test arenas, termites in both untreated and insecticide-treated arenas explored the arena and remained in motion; termites intermittently bit on the filter paper until those treated with insecticides became uncoordinated in their movements at which point some of them held onto the filter paper with their mandibles for support.

Individual termites exhibited a number of different behavioral symptoms as the exposure time increased until the termites died. Transition from each of the variant symptoms to more advanced stages occurred without intermediate symptoms/stages. The behavioral symptoms are classified exclusively into three major stages: incipient intoxication/state of disorientation, ataxia/lethargic behavior, and moribundity, each of which is defined by a range of behavioral symptoms. Termites exposed to untreated filter paper did not exhibit any unusual symptoms or behaviors indicative of a reduced state of well-being or increased level of disorientation.

The behavioral symptoms induced by fipronil and indoxacarb were largely similar with some slight differences, but chlorantraniliprole induced unique behaviors.

The following criteria are used to define the stages: nature of the behavior (e.g., sluggish movement, tremors), degree of mobility, and existence of alternative symptoms associated with a particular behavior.

Behavioral symptoms associated with fipronil and indoxacarb intoxication

Incipient intoxication (Disoriented state). The first sign of termite intoxication is disorientation. During the early parts (0-3 h post-exposure) of incipient intoxication, horizontal oscillatory movement also referred to as alarm reaction was exhibited by 10-20% of termites; allogrooming was observed in about 10% of termite, similar to the controls. Between 3 and the onset of Ataxia stage, the intoxication was characterized by a higher occurrence (>20%) of alarm reaction. Termites change directions frequently at walking speeds obviously faster than the control before showing body shook from side to side when walking at speeds slower than the controls and eventually becoming sluggish in movement. We were not able to quantitively measure the walking speeds because the unstable changes over times.

Ataxia/Uncoordinated Stage. Intoxicated termites circled around the same spot, walked in reverse, hunched their dorsum, fell on their backs and kicked their feet. Whole-body movements accompanied leg kicking. Termites at this stage usually righted themselves but lost their footing again in a cycle that continued until moribundity. During the early stages of ataxia, the antennae were less active, drooped slightly and termites appeared to be antennating the substrate floor. During the later stage of ataxia, fipronil-exposed termites often folded their antennae backward and appeared distorted but rarely kept a horizontal orientation, while the antennae of indoxicarb-exposed termites appeared collapsed or sometimes bent beneath the mouthparts and the termites appeared to be engaged in autogrooming.

We also observed the release of stomodeal fluid (liquid excrement) visible on the white filter paper as yellow sticky spots. Spotting of the substrate occurred more frequently among fipronil-exposed than indoxacarb-exposed termites. Termites treated with fipronil also spotted the substrate with proctodeal fluid, which was not observed in indoxacarb-treated termites. Another characteristic behavior of indoxacarb-exposed termites is that the termites often held onto the substrate with their mouthparts during the advanced stages of ataxia.

Moribundity. Termites at this stage were unable to move a distance of at least their body length (≈5 mm). They either remained on their feet or, more commonly, on their back until death. Leg kicking was weaker, sparser, and not accompanied with the vigorous whole-body movements associated with the uncoordinated stage. Movement of the antennae (if any) was weak. The antennae assumed a distorted shape i.e., they were either bent at the tip, or were usually motionless. Some termites spotted the substrate with liquid excrement. Termites were defined to be dead when they failed to exhibit any response when probed.

Another noteworthy behavior in fipronil-treated termites is that they tended to cluster together (about 65%) and generally engaged in more social interactions at any time during the study than indoxacarb-treated ones.

Behavioral symptoms associated with Chlorantraniliprole intoxication.

Termites treated with chlorantraniliprole displayed a range of behaviors distinct from those of fipronil and indoxacarb. The incipient stages of intoxication were characterized by sluggish movement; termites covered a distance of about 4 cm in about 10 s. This stage was followed by a characteristic lethargic/zombie-like movement (taking >30 s to walk 4-cm distance) that persisted until moribundity. There was a progressive reduction in walking speed until termites stopped moving (became moribund). At the advanced stage of lethargy, termites walked in slow-motion with raised head and thorax and assumed the take-off posture of a plane. This behavior replaced the ataxia observed in the fipronil and indoxacarb treatments. Moribundity, the penultimate stage, was characterized by the inability of termites to cover a distance of at least their body length. Lethargic up and down and/or side to side movement of the head and thorax were observed. Termites retained an amazing ability to roll/turn sideways in order to resume their upright position and thus remained on their feet until death. Another distinct feature of chlorantraniliprole is the high level of uniformity in the behavioral symptoms exhibited by treated termites; the same set of symptoms was observed in termites at each observation period.

In the chlorantraniliprole treatment, alarm behavior was exhibited by ≈14% of termites in the first 2 h after exposure. The frequency of alarm behavior declined progressively until complete cessation 2.5 h after treatment. Ataxia was not observed in the chlorantraniliprole treatment; termites in this treatment exhibited lethargic walking instead (Table 1).

Chlorantraniliprole also elicited the release of proctodeal, but not stomodeal, fluid. The antennae of chlorantraniliprole-treated termites were kept in

Table 1. Onset (h) and percentage of occurrence of abnormal behaviors in eastern subterranean termites exposed to three slow-acting insecticides.

	Indoxacarb		Fipronil		Chlorantraniliprole	
Behavior	Time (h)	% occurrence	Time (h)	% occurrence	Time (h)	% occurrence
Incipient Intoxication	1.5	50.0	2.0	40.0	1.5	90.0
Ataxia	2.5	80.0	4.0	80.0	NA[a]	NA
Lethargic Movement	NA	NA	NA	NA	2.5	50.0
Moribundity	3.5	70.0	4.5	50.0	6.0	100.0

% occurrence = Percentage of termites exhibiting behavior.
[a] Not applicable; did not occur.

contact with the substrate either in a splayed or forward pointing orientation; bending or distortion was limited to the tip of the antennae.

DISCUSSION

The quest for newer chemistries that are effective at low rates but with less adverse effects on the environment led to the recent development of chlorantraniliprole by DuPont (DuPont Crop Protection, Neward, DE, USA). Chlorantraniliprole has been registered as a "reduced risk" insecticide by the Environment Protection Agency in the US (EPA, 2008), which makes it an environmentally friendly option for termite control provided it performs well in field trials.

One of the most fundamental advantages of behavioral endpoints is that they are early indicators of intoxication when organisms are exposed to sublethal concentration of insecticides (Weis 1988). This has practical applications during inspection of structures after remedial treatment with insecticides. Recognition of behaviors induced by a specific insecticide helps to determine whether chemicals have been applied in the right places and also if termites have had contact with chemicals used to control them. Termiticide-induced behaviors such as ataxia, moribundity, release of fluid (proctodeal or stomodeal), reduced mobility, impaired tunneling (excavation), and avoidance (of repellent compounds) have significant impact on control efforts. Cessation of feeding as a direct consequence of neurotoxic or deterrent properties of insecticides aggravates the condition of treated termites through starvation. Reduced destruction of structures is an obvious advantage of this behavioral response. Chlorantraniliprole inhibits the normal contraction of the insect muscles (Cordova *et al.* 2006, Sattelle *et al.* 2008) whereas neurotoxic compounds cause nervous system disorders that seem to enhance the fluid release observed in the indoxacarb and fipronil treatments. Fluid release and trophallaxis are very significant in the successful horizontal transmission of insecticides. Trophallaxis could either enhance or reduce the performance of the insecticide depending on its mode of action and other characteristics peculiar to the compound. Insect-growth regulators such as noviflumuron perform better than hexaflumuron partly because of the slower rate of clearance of noviflumuron from the termite body (Karr *et al.* 2004). According to the authors, the greater half-life of noviflumuron increases the likelihood that sufficient quantities of the compound are available in the termite to inhibit the molting process. Previous work has shown that both fipronil and indoxacarb are capable of causing mortality in recipient termites when acquired by oral or dermal routes (Hu 2005, Hu *et al.* 2005, Song & Hu 2006, Bagneres *et al.* 2009). Indoxacarb, however, is metabolized into a more active form through the action of amidases and esterases (Silver & Soderlund 2005), which makes the metabolized compound more potent against subterranean termites. The tendency of chlorantraniliprole-treated termites to cluster could either be a positive or negative characteristic under field conditions. A reduction in horizontal transfer would be expected if clustering is limited to toxified termites. Cluster formation will be a drawback if this is restricted to exposed termites and occurs at the expense of contact with naïve termites; this will have adverse effects on the area of coverage, transfer, and general performance of chlorantraniliprole. In spite of the high concentration (300 ppm) of chlorantraniliprole used in this study, onset of toxicity symptoms lagged behind those treated with indoxacarb and fipronil. The combined duration of induced behaviors, prior to death, were also considerably longer in the chlorantraniliprole treatments relative to fipronil and indoxacarb. According to Henderson (2003) delayed and/or long durations of toxicity symptoms increase the likelihood of transmission of insecticides to untreated nestmates by increasing the transmission periods.

The behavioral end points described in this study meet the biological relevance requirement suggested by Kane *et al.* (2005). Incipient intoxication is a stage during which termites are still capable of moving and interacting with their nestmates in ways that can enhance the horizontal transfer of toxicants. When termites become ataxic, they release fluids (stomodeal or proctodeal) and elicit more care-giving behavior from relatively healthy nestmates. Evidence suggests that intake of these fluids facilitates the transmission of toxic compounds (Bagneres 2009). Moribundity is a stage during which termites are no longer capable of moving around; termites at this stage are less successful at eliciting care-giving behavior and can only contaminate their nestmates when touched or cannibalized. The significance of death as an endpoint cannot be overemphasized because it is the goal of all control efforts. Insecticide-induced behavioral symptoms serve as indicators of sublethal exposure and can complement traditional (mortality-based) indicators of toxicity that help to predict the performance of an insecticide more accurately. Information on insect-specific insecticide-induced behavioral symptoms in non-target (beneficial) insects can also serve as indicators of the environmental effects of toxicants (Kane *et al.* 2005).

Behavioral endpoints can also help to distinguish the relative performance of different classes of insecticides in situations in which retreatment is carried out with another compound or where different chemical compounds are applied to structures in close proximity. Analysis of behaviors will provide useful information on the relative performance of insecticides as well as resolve disputes concerning services provided by pest control firms.

The establishment of a link between biochemical and physiological processes and the provision of deeper insights both individual and community level effects of insecticides makes behavioral endpoints very important (Brewer *et al.* 2001, Vogl *et al.* 1999). Given the fact that behavior-based chemical control of subterranean termites relies heavily on termite behavior under both normal and intoxicated conditions, indicators such as those described in this study deserve more attention in the quest for more environmentally friendly and sustainable control measures against termites. The behavioral endpoints described in this study can complement mortality-based methods in predicting the performance of non-repellent termiticides; these endpoints provide information on the potential for horizontal transfer of toxicants. Practical applications of these insecticide-induced behaviors include determination of whether termites have had contact with the insecticide. Information on the prevalent insecticide-induced behavioral stage among treated-termites will be useful in estimating when termites will be eliminated from infested structure. Behavioral endpoints can also be used in identifying the active ingredients responsible for intoxication in termites; this will be particularly useful in cases where treatment failures necessitate retreatment by different pest control companies using different active ingredients.

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REFERENCES

Bagneres, A.-G., A. Pichon, J. Hope, R.W. Davis & J.-L. Clement 2009. Contact versus feeding intoxication by fipronil in *Reticulitermes* termites (Isoptera: Rhinotermitidae): laboratory evaluation of toxicity, uptake, clearance, and transfer among individuals. J. Econ. Entomol. 102: 347-356.

Brewer, S.K., E.E. Little, A.J. DeLonay, S.L. Beauvais, S.B. Jones & M.R. Ellersieck 2001. Behavioral dysfunctions correlate to altered physiology in rainbow trout (*Oncorynchus mykiss*) exposed to cholinesterase-inhibiting chemicals. Arch. Environ. Contam. Toxicol. 40: 70-76.

Cordova, D., E.A. Benner, M.D. Sacher, J.J. Rauh, J.S. Sopa, G.P. Lahm, T.P. Selby, T.M. Stevenson, L. Flexner, S. Gutteridge, D.F. Rhoades, L. Wu, R.M. Smith & Y. Tao 2006. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. Pestic. Biochem. Physiol. 84: 196-214.

Duffard, A.M.E. & R. Duffard 1996. Behavioral toxicology, risk assessment, and chlorinated hydrocarbons. Environ. Health Perspect. 104: 353-360.

EPA. 2008. Pesticide fact sheet (7505P). http://www.epa.gov/opprd001/factsheets/chloran.pdf.

Forschler, B.T. & V. Lewis. 1997. Why termites dodge your treatment. Pest Control 65: 42-53.

Henderson, G. 2003. Liquid learning. Pest Contr. Tech. 31: 48-59.

Hu, X. P. 2005. Evaluation of the efficacy and non-repellency of indoxacarb and fipronil treated soil at various concentrations and thicknesses against two subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 98: 509-517

Hu, X.P. & A.G. Appel 2004. Seasonal variation of critical thermal limits and temperature tolerance in two subterranean termites (Isoptera: Rhinotermitidae). Environ. Entomol. 33:197-205.

Hu, X.P. & B. Hickman 2006. Exterior perimeter plus limited interior treatments with fipronil as an IPM option for subterranean termite management. International Pest Control 48(4) 200-203.

Hu, X. P., D. Song & C. Scheler 2005. Transfer of indoxacarb among workers of Coptotermes formosanus Shiraki (Isoptera: Rhinotermitidae): effects of dose, donor/recipient ratio, and post-exposure time. Pest Management Science 61: 1209-1214.

Kane, A.S., J.D. Salierno & S.K. Brewer 2005. Fish models in behavioral toxicology: automated techniques, updates and perspectives, pp 559-590. In G.K. Ostrander (ed.). Methods in Aquatic Toxicology, Lewis Publishers, Boca Raton, FL.

Karr, L.L., J.J. Sheets, J.E. King & J.E. Dripps 2004. Laboratory performance and pharmacokinetics of the benzoylphenylurea noviflumuron in eastern subterranean termites (Isoptera: Rhintotermitidae). J. Econ. Entomol. 97: 593-600.

Little, E.E. & S.K. Brewer 2001. Neurobehavioral toxicity in fish, pp.139-174. In D. Schlenk, and W.H. Benson (eds.), Target Organ Toxicity in Marine and Freshwater teleosts Systems: New Perspectives. Toxicol. Environ. Vol. 2. Taylor and Francis, London and New York.

(NRDA) Natural Resource Damage Assessment 1986. Final rule. Federal Register 51: 27674-27753.

Rand, G.M. 1985. Behavior, pp 221-256. In G.M. Rand, and S.R. Petrocelli (eds.), Fundamentals of Aquatic Toxicology: Methods and Applications. Hemisphere Publishing, New York.

Sattelle, D.B., D. Cordova & T.R. Cheek 2008. Insect ryanodine receptors: molecular targets for novel pest control chemicals. Invert. Neurosci. 8: 107-119.

Silver, K.S. & D.M. Soderlund 2005. Action of pyrazoline-type insecticides at neuronal target sites. Pest. Biochem. Physiol. 81: 136-143.

Song, D. & X.P. Hu 2006. Effects of dose, donor-recipient interaction time and ratio on fipronil transmission among the Formosan subterranean termite nestmates (Isoptera: Rhinotermitidae). Sociobiology 48: 237-246.

Su, N.-Y. 2002. Novel technologies for subterranean termite control. Sociobiology: 40: 95-101.

Su, N.-Y. & R.H. Scheffrahn 1990. Economically important termites in the United States and their control. Sociobiology 17: 77-94.

Su, N-Y., M. Tamashiro, J.R. Yates & M.I. Haverty 1982. Effect of behavior on the evaluation of insecticide for prevention or remedial control of the Formosan subterranean termite. J. Econ. Entomol. 75: 188-193.

Vogl C., B. Grillitsch, R. Wytek, O. Hunrich Spieser & W. Scholz 1999. Qualification of spontaneous undirected locomotor behavior of fish for sublethal toxicity testing. Part I. Variability of measurement parameters under general test conditions. Environ. Toxicol. Chem. 18: 2736-2742.

Weis, B. 1988. Behavior as an early indicator of pesticide toxicity. Toxicol. Ind. Health 4: 351-360.