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ABSTRACT A laboratory study was conducted to determine the toxicity of indoxacarb and chlorantraniliprole to Eastern subterranean termites, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) resulting from topical applications and exposure to treated soil. Soils with varying organic matter (0.57–3.64%) and chemical characteristics were used in termiticide bioassays. Lethal dose resulting from topical application indicated that chlorantraniliprole was two- to 11-fold more toxic than indoxacarb. Lethal concentration assays yielded opposite results where concentrations of indoxacarb in soil that caused either 50 or 90% mortality of *R. flavipes* workers at 48 and 144 h were two- to six-fold lower than chlorantraniliprole. The bioavailability of indoxacarb and chlorantraniliprole was negatively correlated with soil organic matter. Our results suggest that indoxacarb is more bioavailable to termites in soil than chlorantraniliprole based on calculated bioavailability ratios. However, how these laboratory results correlate to actual field application data and termite efficacy is unknown, and more research is needed. These compounds seem to have excellent activity on termites and have potential to provide new modes of action and new chemistry as liquid termiticides.

KEY WORDS bioavailability, termiticide, termites, toxicity, *Reticulitermes flavipes*

The use of soil termiticides is a common remedial and preventative control measure to protect structures from termite damage. These treatments typically involve creating a termiticide barrier in soil around and under buildings. Currently registered termiticides such as Termidor (fipronil), Premise (imidacloprid), and Phantom (chlorfenapyr) are nonrepellent and thus allow termites to forage through treated soils and acquire exposure to the toxicant.

The environmental fate and bioavailability of termiticides in a particular soil are important considerations when deciding if a high or low label application rates is appropriate. Soil properties including pH, clay, sand, and organic matter (OM) are key factors affecting the fate and biological availability of insecticides. Harris and Bowman (1981) reported a significant negative correlation between toxicity of treated soil to insects and insecticide solubility in water. Harris (1966) reported that organic matter content of soil generally has an inverse relationship with insecticide toxicity. Several studies report the performance of insecticides in soils with varying soil properties, including OM content (Getzin and Chapman 1960; Harris 1966, 1970; Harris and Mazurek 1966; Whitney 1967; Harris and Hitchon 1970; Campbell et al. 1971; Felsot and Dahm 1979; Felsot and Lew 1989; Forschler and Townsend 1996; Gold et al. 1996). Gold et al. (1996) evaluated bioavailability of organophosphate and pyrethroid termiticides in five different soil types. In terms of efficacy and activity, the termiticides performed the best in slightly acidic soils (pH 6.4) with low organic content (0.8% OM).

Chlorantraniliprole and indoxacarb are two of the newest termiticides currently being developed. Chlorantraniliprole is a new insecticide from the anthranilic diamide class of insecticides with a novel mode of action that targets and activates the ryanodine receptor in insects. Chlorantraniliprole binds to the ryanodine receptor causing the release of internal stored calcium. The release of stored calcium causes insects to loose control of muscle regulation. The resulting muscle contractions can lead to rapid feeding cessation, lethargy, partial paralysis, cardiac muscle failure, and regurgitation (Cordova et al. 2006). Chlorantraniliprole has a water solubility of 1.023 mg liter^{-1} (deionized [DI] water, 20°C), octanol/water partitioning coefficient (K_{ow}) of 589 (DI water, 20°C), average organic carbon partitioning coefficient (K_{oc}) of 328, and Henry's law constant of 3.1×10^{-15} atm m^3 mol^{-1} (USEPA 2008).

Indoxacarb is an oxadiazine proinsecticide that is metabolically activated after entering the insect. The activated decarbomethoxylated indoxacarb metabolite acts by blocking the sodium channel of the insect nervous system (Wing et al. 2000). Although the sodium channel is a common target of other chemical classes (i.e., DDT and pyrethroids), it seems indoxacarb acts on a novel binding site of the receptor because there is no cross-resistance between classes (Nauen and Bretschneider 2002). Indoxacarb has a

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Table 1. Soil characteristics

Soil[a]	pH ± SEM	Particle size analysis (%)			CEC (cmol kg^{-1})	%OM ± SEM
		Sand	Silt	Clay		
SL	6.94 ± 0.04	75.40	7.45	17.15	6.93	0.57 ± 0.04
SCL-1	7.08 ± 0.04	15.85	46.95	37.20	25.54	2.58 ± 0.03
SCL-2	6.98 ± 0.03	15.15	48.95	35.90	35.48	3.64 ± 0.03

[a] SL, sandy loam; SCL, sandy clay loam.

solubility of 0.2 mg $liter^{-1}$ (25°C), K_{ow} of 45,000, K_{oc} range of 2,200–9,400, and Henry's law constant of $<6.1 \times 10^{-10}$ atm m^3 mol^{-1} (USEPA 2000).

The objectives of this study were to 1) determine LD_{50} and LD_{90} values of indoxacarb and chlorantraniliprole to Eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae); 2) establish LC_{50} and LC_{90} values of indoxacarb and chlorantraniliprole for *R. flavipes* in three different soil types; and 3) ascertain whether bioavailability of chlorantraniliprole and indoxacarb is influenced by soil organic matter.

Materials and Methods

Termiticides. Technical grade indoxacarb, (*S*)-methyl 7-chloro-2, 5-dihydro-2-[[(methoxy-carbonyl)[4(trifluoromethoxy)phenyl]amino]-carbonyl]indeno-[1,2-*e*][1,3,4]oxadiazine-4*a*-(^{3}H)-carboxylate, was obtained from Chem Service (West Chester, PA) [98% pure (*S*)-isomer, lot 352-67A). Technical grade chlorantraniliprole (DPX E2Y45-30), 3-bromo-*N*-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloro-2-pyridine-2-yl)-1*H*-pyrazole-5-carboxamide, was obtained from DuPont Corp., Wilmington, DE (>93% purity).

Termite Collection and Rearing. Subterranean termites were collected from fallen logs in Wilderness Park Recreation area in Lancaster Co., NE, on 23 August 2008. Termites were extracted from the logs and maintained in Plexiglas containers (35 by 25 by 10 cm) provisioned with moistened sand and corrugated cardboard and held in complete darkness at 23°C. Termites were allowed to acclimate for 1 wk before using in bioassays. The termites were confirmed to be *R. flavipes* by using soldier morphology (Weesner 1965, Nutting 1990). The average weight of each termite worker was 3.97 ± 0.11 mg ($n = 240$).

Soil Collection and Preparation. Soils were collected from Cumming and Lancaster counties in Nebraska and were analyzed by the Soil and Plant Analytical Laboratory at the University of Nebraska, Lincoln, NE. Soils were characterized for %OM, pH, cation exchange capacity, and particle size (Table 1). The three soils were identified and designated sandy loam (SL), silty clay loam (SCL)-1, and SCL-2 (USDA 1993) were autoclaved, air-dried, and sieved (2 mm) before use in experiments.

Lethal Dose Based on Topical Application Bioassay. Dilutions of chlorantraniliprole (11 concentrations ranging from 0.196 to 200.8 $\mu g\,g^{-1}$) and indoxacarb (11 concentrations ranging from 0.196 to 200.9 $\mu g\,g^{-1}$) in acetone (99.9%; Sigma, St. Louis, MO) were prepared. Termites were chilled in a cooler (≈4°C) for 5 min before topical application of insecticide. Diluted chlorantraniliprole and indoxacarb (0.2 μl) was topically applied to the dorsal portion of the third to fourth abdominal segments of each *R. flavipes* worker (fourth to fifth instar) by using a microapplicator (Hamilton Co., Reno, NV) fitted with a 10-μl syringe. Termites in controls were treated with 0.2 μl of acetone. Groups of 15 termite workers were placed in a Plexiglas container (35 mm in diameter by 10 mm in depth) provisioned with untreated sand (20% moisture content) and white pine as a food source. Termites were monitored for mortality at 48 and 144 h.

Lethal Concentration Based on Soil Contact Bioassay. Air-dried soils were treated with either chlorantraniliprole (10 concentrations ranging from 0.088 to 45.0 $\mu g\,g^{-1}$) or indoxacarb (10 concentrations ranging from 0.098 to 50.0 $\mu g\,g^{-1}$) in acetone. After insecticide application, the soils were agitated to ensure uniform treatment. Control samples from each soil type were treated with acetone only. Soils were then air-dried under a fume hood for 15 h to allow for acetone evaporation. Three grams of each soil and active ingredient (AI) concentration was weighed into Plexiglas containers (35 mm in diameter by 10 mm in depth) moistened with 0.75 ml of distilled water and provisioned with a piece of white pine (2 by 1 by 0.5 cm). Control containers (units) containing soil treated with acetone were prepared in a similar manor. All containers (units) were acclimated for 24 h in an environmental growth chamber at 23°C in complete darkness. In total, four replicates were constructed for each soil by termiticide by concentration combination for a total of 252 individual containers (units). Fifteen *R. flavipes* workers (fourth to fifth instar) were added to each unit, and mortality was assessed at 48 and 144 h. Termites were recorded as dead if they could not right themselves within 10 s of being flipped onto their back.

Bioavailability Ratios. Bioavailability ratios were calculated by dividing LD_{50} values by LC_{50} values of each termiticide for a particular soil type and time interval (see Table 4). LD_{50} (nanograms of insecticide per termite) values were converted to nanograms of insecticide per gram of termite. LC_{50} values also were converted from micrograms of insecticide per gram of soil to nanograms of insecticide per gram of soil. The purpose of the bioavailability ratio is to compare LC values taking into account the LD values for each termiticide for a specific time period. Evaluating termiticides based on this ratio may be extremely valu-

Table 2. Lethal doses of indoxacarb and chlorantraniliprole to *R. flavipes* workers resulting from topical application

Chemical	Time (h)	n	Slope (± SE)	LD_{50} (ng per termite) (95% CI)[a]	LD_{90} (ng per termite) (95% CI)[a]	χ^2
Indoxacarb	48	480	1.52 (0.14)	8.22 (5.93–12.17)	57.18 (32.49–138.17)	7.38
	144	420	1.71 (0.13)	2.50 (1.39–4.84)	13.98 (6.79–48.00)	15.24
Chlorantraniliprole	48	480	1.07 (0.09)	2.13 (1.61–2.84)	34.02 (21.08–64.47)	5.83
	144	420	2.37 (0.21)	0.37 (0.26–0.52)	1.28 (0.88–2.15)	5.82

[a] LD values with overlapping CIs are not significantly different.

able to determine actual bioavailability in different soil types.

Statistical Analysis. Lethal doses and concentrations and 95% fiducial limits were calculated using PoloPlus (LeOra Software 2003; LeOra Software, Petaluma, CA), a probit and logit analysis software program. Regression analysis of bioavailability factor and organic matter correlation was conducted using SigmaPlot 9.0 (Systat Software, Inc. 2004; Systat Software, Inc., Point Richmond, CA).

Results and Discussion

Lethal Dose Topical Application Bioassay. The LD_{50} and LD_{90} data of topically applied indoxacarb and chlorantraniliprole to *R. flavipes* workers are presented in Table 2. The LD values decreased with time (48 versus 144 h) for both indoxacarb and chlorantraniliprole. LD values were generally higher for indoxacarb compared with chlorantraniliprole at both 48 and 144 h. Chlorantraniliprole LD_{50} values were 3.9- and 6.8-fold lower than indoxacarb at 48 and 144 h, respectively, whereas LD_{90} values were 1.7- and 10.9-fold lower at 48 and 144 h. LD_{50} values were all significantly different with the exception of LD_{50} indoxacarb at 144 h and chlorantraniliprole at 48 h based on nonoverlapping fiducial limits. These results reflect the generally higher intrinsic toxicity of chlorantraniliprole relative to indoxacarb.

Lethal Concentration Soil Contact Bioassay. The LC_{50} and LC_{90} data are presented in Table 3. Although all not statistically different, numerical LC values differed based on soil type (SL < SCL-1 < SCL-2). As expected, LC_{50}s decreased with time for both indoxacarb and chlorantraniliprole, with 48-h values being significantly higher based on nonoverlapping fiducial limits. This trend was also observed for LC_{90} values; however, the wide 95% limits indicate that not all differences were significant. Interestingly, LC values were higher for chlorantraniliprole than indoxacarb, which is opposite of the topical toxicity data. Indoxacarb LC_{50} values were 1.7–2.6- (48 h) and 2.7–5.9 (144 h)-fold lower than chlorantraniliprole values in the various soils. Indoxacarb LC_{90} values were 1.8–4.3- (48 h) and 1.7–3.7 (144 h)-fold lower than LC_{90} chlorantraniliprole values.

Bioavailability Ratios. LD values determined by topical applications should be consistent for a particular colony of termites at a particular point in time. In contrast, LC values of insecticide treated soils will probably fluctuate based on soil conditions and properties such as type, and percentage of organic matter, among others. The bioavailability ratio as calculated in the current study is based on the proportion of insecticide required to contact the termite cuticle as determined by topical application to the soil concentration that causes 50% population mortality based on termite weight at a particular time interval. Lethal doses determined by topical application indicates the amount of AI required to contact the termite cuticle and kill a certain percentage of the test population at a particular time interval. These data are specific for an AI and generally reflect the toxicokinetics (e.g., penetration, biotransformation and target site interaction) of the AI. Lethal concentrations as determined by exposure to treated soil reflect the propensity of an AI to be taken up from soil by an organism in spite of environmental factors such as soil properties and moisture content. LC values are also specific for a particular time interval and reflect the AI concentra-

Table 3. Lethal concentrations of indoxacarb or chlorantraniliprole to *R. flavipes* workers after contact exposure to treated soils

Chemical	Soil	Time (h)	*n*	Slope (± SE)	LC_{50} (μg per g soil) (95% CI)[a]	LC_{90} (μg per g soil) (95% CI)[a]	χ^2
Indoxacarb	Sl	48	360	1.34 ± 0.14	5.88 (3.64–10.94)	50.91 (22.56–254.60)	6.34
		144	360	1.33 ± 0.13	0.34 (0.15–0.64)	3.12 (1.57–9.91)	7.75
Indoxacarb	Scl-1	48	360	1.35 ± 0.13	8.14 (4.56–14.05)	71.96 (35.53–257.36)	7.01
		144	360	1.27 ± 0.12	0.52 (0.22–0.97)	5.29 (2.60–17.18)	12.09
Indoxacarb	Scl-2	48	420	1.92 ± 0.23	17.67 (14.07–23.89)	82.32 (52.37–165.92)	2.04
		144	540	1.57 ± 0.11	1.29 (1.06–1.56)	8.40 (6.34–11.88)	5.23
Chlorantraniliprole	SL	48	420	1.27 ± 0.14	11.03 (8.25–15.80)	111.77 (61.85–270.05)	2.27
		144	360	1.89 ± 0.16	1.12 (0.71–1.81)	5.34 (3.05–13.10)	7.16
Chlorantraniliprole	Scl-1	48	420	1.62 ± 0.18	20.85 (15.59–29.76)	129.51 (77.62–276.86)	1.89
		144	360	1.79 ± 0.13	3.07 (1.45–6.46)	15.94 (7.41–59.58)	13.54
Chlorantraniliprole	SCL-2	48	480	1.20 ± 0.13	30.22 (18.99–62.63)	354.46 (137.64–647.37)	2.04
		144	360	1.36 ± 0.12	3.51 (1.50–7.09)	31.00 (13.79–155.17)	5.23

[a] LC values with overlapping CIs are not significantly different.

Table 4. Bioavailability ratios derived from LD_{50} and LC_{50} values of indoxacarb and chlorantraniliprole from three soils

Chemical	Soil	Time (h)	LD_{50} (ng per g termite)[a]	LC_{50} (ng per g soil)[a]	Bioavailability ratio, LD_{50}/LC_{50}
Indoxacarb	SL	48	2,070.5	5,880	0.352
		144	629.7	340	1.852
Indoxacarb	SCL-1	48	2,070.5	8,140	0.254
		144	629.7	520	1.211
Indoxacarb	SCL-2	48	2,070.5	17,670	0.117
		144	629.7	1,290	0.488
Chlorantraniliprole	SL	48	536.5	11,030	0.049
		144	93.2	1,120	0.083
Chlorantraniliprole	SCL-1	48	536.5	20,850	0.026
		144	93.2	3,070	0.030
Chlorantraniliprole	SCL-2	48	536.5	30,220	0.018
		144	93.2	3,510	0.027

[a] LD_{50} and LC_{50} values converted from data presented in Tables 2 and 3.

tion in the soil. Establishing both LD and LC toxicity data for the same time interval and evaluating toxicity on the same percentage basis (i.e., LD_{50} and LC_{50}) the ratio can be determined. For an example, the 48-h LD_{50} of indoxacarb was 2,070 ng indoxacarb per g termite and the 48-h LC_{50} from the SL soil was 5,880 ng indoxacarb per g soil. By comparison, the 48-h LD_{50} was 536.5 ng chlorantraniliprole per g termite and the LC_{50} from the SL soil was 11,030 ng chlorantraniliprole per g soil. Although indoxacarb has lower toxicity when topically applied, it seems to be taken up from the soil more readily by the termites than chlorantraniliprole; thus, lower concentrations in the soil are required for the termite to receive an equivalent dose. Therefore, a bioavailability ratio as defined in this study could be especially useful when comparing the bioavailability of two different insecticides with known LD and LC data.

The larger the bioavailability ratio the more insecticide is "available" to foraging termites. This is reflected in data presented (Table 4) as the SL soil had the largest bioavailability ratios followed by the decreasing values for SCL-1 and SCL-2 soils, respectively. This trend held for both indoxacarb and chlorantraniliprole at 48 and 144 h. Indoxacarb was more bioavailable to termites compared with chlorantraniliprole, indicating that indoxacarb was more easily acquired from the soil by the termites. It is important to note that this does not reflect the inherent toxicity of the insecticide to termites but only the likelihood of termiticide uptake from the soil. Topical application LD data revealed that chlorantraniliprole is actually more toxic at a lower dose than indoxacarb. The calculation of bioavailability ratios for the two compounds suggest that at 48 h, indoxacarb is 7.18-, 9.77-, and 9.83-fold more biologically available than chlorantraniliprole in SL, SCL-1, and SCL-2 soils, respectively. These ratios were even greater at 144 h and reflect a 22.31- (SL), 40.37- (SCL-1), and 18.07 (SCL-2)-fold greater bioavailability for indoxacarb than chlorantraniliprole.

Insecticide factors that can contribute to differences in bioavailability include solubility, K_{oc}, and K_{ow} (values reported above). These factors are reflective of the hydrophobicity of a particular chemical. Indoxacarb has a lower water solubility and a much higher K_{ow} and K_{oc} value compared with chlorantraniliprole. These differences reflect the higher hydrophobicity of indoxacarb compared with chlorantraniliprole and thus make it more lipophilic. The higher lipophilicity suggests that indoxacarb would have a greater affinity for the waxy cuticular layer of the termite. The comparatively high K_{oc} value of indoxacarb means that it will be more tightly bound in soil, suggesting that it would be harder for that chemical to move from the soil to the termite. However, if indoxacarb is applied at a rate higher than its water solubility the AI may precipitate, thereby remaining available to foraging termites. Kamble and Saran (2005) reported K_{oc} values of 3,787.34–11,934.68 for fipronil at termiticide application concentrations, which are much higher than the averaged 727 K_{oc} value reported by Tingle et al. (2003). The higher K_{oc} value reported by Kamble and Saran (2005) may be due to precipitation of AI during adsorption assays, resulting in a higher apparent K_{oc}. High rate application of AIs with low solubility, such as fipronil, indoxacarb, and chlorantraniliprole, may result in precipitation of the compound in the soil. As a result, compounds that demonstrate higher lipophilicity may increase bioavailability to termites.

Correlation of bioavailability factors and %OM are presented in Fig. 1. Regression equations for each plot are as follows: indoxacarb, 48 h ($y = 0.4092 - 0.0718x$; $R^2 = 0.9469$); indoxacarb, 144 h ($y = 2.1674 - 0.4204x$; $R^2 = 0.9713$); chlorantraniliprole, 48 h ($y = 0.0539 - 0.0099x$; $R^2 = 0.9810$); and chlorantraniliprole, 144 h ($y = 0.0905 - 0.0187x$; $R^2 = 0.8964$). Analysis indicated that bioavailability factors and OM were negatively correlated, suggesting that as OM increased there was an apparent decrease in AI availability.

The term "bioavailability" can have different meanings. One of the uses may refer to AI residues that are present and thus available to termites and measuring that by a particular response variable (i.e., mortality, LC, or lethal time [LT] data). Another use of the term could be more specific to the availability or tendency of an AI to be taken up from a particular soil by termites. Saran and Kamble (2008) reported bioavailability of fipronil, imidacloprid, and bifenthrin by us-

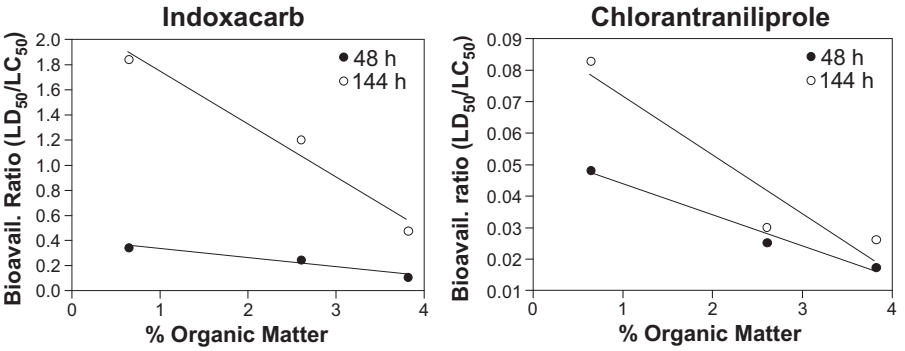

Fig. 1. Correlation between bioavailability ratios (LD_{50}/LC_{50}) of indoxacarb and chlorantraniliprole to *R. flavipes* workers, and percentage of organic matter of three soils.

ing soil residue and LT toxicity data. This method is illustrative but does not take into account toxicokinetics; so, it is unknown whether longer reported LT values for different AIs are a result of internal and integumental factors or reduced uptake from soil. In addition, other researchers have used treated soil penetration bioassays reporting bioavailability as percentage of mortality of exposed termites (Gold et al. 1996; French and Ahmed 2005; Baker and Bellamy 2006). Although none of these methods for determining bioavailability are wrong, and in fact can be very illustrative, using a bioavailability ratio of LD_{50} data from topical application and LC_{50} data from treated soil exposure can provide more detail regarding the tendency of an insecticide to move from various soils to a termite. We propose that using bioavailability ratios may provide a more accurate assessment of the biological availability of a particular insecticide in soils to soil dwelling insects. Simply using a single descriptive parameter, such as soil exposure toxicity data or topical toxicity, may not fully represent the complex interaction of toxicokinetics and insecticide bioavailability.

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