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Degradation of Fipronil (Termidor®) in Aqueous Solution Stored in Polyethylene Tanks Exposed to Sunlight or Shade

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Abstract Pest Management Professionals commonly use polyethylene or fiberglass tanks for mixing and applying termiticides. We investigated the stability of fipronil (Termidor® SC) (0.06%, 0.09% and 0.125% active ingredient) in aqueous solutions stored in polyethylene tanks under sun and shade for 2 week. Chemical analysis of tank-mixed solutions sampled at 0, 24, 48, 72, 168 and 336 h indicated that fipronil remained stable. Our data indicated that polyethylene tanks prevent fipronil from photodegradation and these tanks can be safely used for short term storage (up to 2 week) of liquid termiticides.

Keywords Fipronil · Stability · Polyethylene tanks · Photolysis

Subterranean termites are commonly controlled and excluded from structures by creating a subsoil chemical barrier adjacent to the exterior and/or interior of the structural foundation (Kamble 2006). In the past, control of subterranean termites relied on using chlorinated hydrocarbon, organophosphate, carbamate, and pyrethroid soil termiticides which persisted in the environment and had moderate to high mammalian toxicities (Gold et al. 1996). Currently, non-repellent termiticides with novel modes of action and considerably low mammalian toxicity are being used for termite control. These termiticide AIs and their classes include: imidacloprid and acetamiprid (neonicotinoids), fipronil (phenyl pyrazole), chlorfenapyr (pyrazole), and indoxacarb (oxadiazine). Termidor® (fipronil) Soluble Concentrate (SC) was registered in 2000 for termite control and it continues to be the most effective and widely used termiticide today.

Fipronil [(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethyl sulfinyl-pyrazole-3-carbonitrile], a broad-spectrum, interferes with the passage of chloride ions through gamma-aminobutyric acid (GABA) regulated chloride ionophore thereby disrupting inhibitory post synaptic neuron activity, resulting in uncontrolled central nervous system function, and eventually causing death (Cole et al. 1993; Tingle et al. 2003). Fipronil is slightly water soluble with solubility ranges from 1.9–2.4 mg/L at 20°C, very low vapor pressure (3.7×10^{-4} mPa @ 25°C), and a low Henry's law constant (3.7×10^{-5} Pa m^3/mol) (Hainzl and Casida 1996; Connelly 2006; Tingle et al. 2003). Fipronil degrades to its major metabolites by reduction to sulfide, oxidation to sulfone, hydrolysis to amide, and photolysis to des-sulfinyl (Hainzl and Casida 1996). The des-sulfinyl photodegradate is extremely stable and actually more toxic than the parent compound (USEPA 1998). Fipronil, in aqueous solution, is stable to hydrolysis in mildly acidic to neutral water under dark conditions and at a constant ambient temperature (USEPA 1996).

It is common for Pest Management Professionals (PMPs) conducting termite treatments to mix large volumes of aqueous insecticide solutions in a polyethylene tank. These tanks are often mounted on an open pick-up truck without protection from sunlight or within a van (shaded from sunlight). At times, termiticides mixed with water in tanks remain stored for extended periods of time due to inclement weather or sudden medical emergencies. PMPs have always asked questions about how long a termiticide will remain stable in aqueous solution in a polyethylene tank? These solutions are subject to a wide range of environmental conditions such as temperature and

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differing degrees of UV light exposure which could lead to increased degradation rates of the insecticide AI.

Our research objectives were to (1) determine concentration-dependent degradation of fipronil in tank mixed solutions (2) evaluate degradation profiles of fipronil in tanks stored in sunlight or shade, and (3) ascertain loss of fipronil due to adsorption on tank surface.

Materials and Methods

Termidor® SC was obtained from BASF (Florham Park, NJ), batch number 60828004. The 23.67, 35.59 and 47.34 mL of Termidor SC were individually mixed with 3.785 L of tap water to acquire 0.06%, 0.09% and 0.125% AI application rates, respectively. These three aqueous solutions ranged from lowest to highest rates allowed by Termidor® SC label. The diluted Termidor® SC aqueous solutions were stored in individual 3.785 L polyethylene tanks (Hudson Quick and Easy Deck Sprayer, Hastings, MN). An extra 3.785 L of each concentration was prepared and used to fill eight 0.95 L clear glass jars (Ball mason jars, Allrista Consumer Products Co., Muncie, IN) to quantify fipronil adsorption to the polyethylene tank surfaces. Each Termidor concentration was subject to four storage regimes as follows: (1) within polyethylene tanks exposed to sun (sun polyethylene tank); (2) within glass jars held in a polyethylene tank (132.5 L cylindrical polyethylene tank) exposed to sun (sun glass jar); (3) within polyethylene tanks in shaded conditions (shade polyethylene tank); and (4) within glass jars held in shade conditions (shade glass jar). The experimental setup was a 2 × 3 × 2 factorial with four replications at each concentration for each tank/glass jar.

The experimental location was at the University of Nebraska-Agricultural Research and Development Center (ARDC) near Mead, NE. Ambient air temperature was recorded every hour by a weather station at the ARDC. The sun location was within a field devoid of trees and buildings. Individual tanks/glass jars within tank were placed on a 1.83 m by 3.66 m metal trailer (30.5 cm of ground clearance) with flat metal mesh bottom over the course of the 2 week study. The shade location was in a metal storage building (4.57 m wide by 12.19 long by 4.27 m high) with no windows approximately 100 m from the sun plot location. Individual tanks/glass jars were placed in shade on a 1.22 by 2.44 m piece of 1.91 cm plywood lying flat on a concrete floor.

Aqueous solution was sampled from each polyethylene tank and glass jar at 0, 24, 48, 72, 168, and 336 h intervals. Prior to sampling, all polyethylene tanks and glass jars were thoroughly shaken by hand for 2 min to ensure uniform agitation/mixing and to remove residue from sides and bottom of holding containers. Samples of fipronil were placed in 120 mL amber bottles (The Glass Group Inc., Millville, NJ) and bottles were transported to the urban entomology laboratory. Extraction of fipronil from the tank/glass jar samples began by removing 75 mL of the tank/jar solution and mixing with 25 mL of 100% HPLC grade acetonitrile (CAN) in a 120 mL amber bottle. Subsequently, sample bottles were shaken for 10 min on a Wrist Action® mechanical shaker (Burrell Corp., Pittsburg, PA). After shaking, 10 mL were pipetted into a preweighed 100 mL volumetric flask (pre-weighed to determine the weight of the 10 mL sample). This 10 mL sample was diluted to 100 mL with 75% acetonitrile: 25% deionized water (v:v). This solution was mixed by inversion and then pipetted into a 1.5 mL microcentrifuge tube. The sample was centrifuged in an Eppendorf-5415C centrifuge (Eppendorf Int., Hamburg, Germany) at 10,000 cycles per min for 5 min. The supernatant from this solution was placed into a sterile 3 cc syringe (Becton-Dickinson, Franklin Lakes, NJ) with an Acrodisc CR 4 mm syringe filter (0.45 μm PTFE membrane-HPLC Certified) and filtered into a 1.8 mL amber sample injection vial (VWR International, West Chester, PA). The samples were then run on an HPLC with a detection sensitivity of 0.5 μg/mL and a characteristic retention time of 7.1 min.

Fipronil from all samples was analyzed using high performance liquid chromatography (HPLC) (Varian® 9012 pump solvent delivery system equipped with 9050 variable length UV/VIS detector and 9100 autosampler). Data collection and peak analyses were performed using the Varian® Star Chromatography workstation. A reverse phase C-18 column (250 mm × 4.6 mm ID, 5 μ particle size, Luna®-Phenomenex) was used to conduct analysis. The same HPLC-UV method was used to determine fipronil concentration for each sample. The parameters of this method were: a mobile phase consisting of HPLC grade methanol and deionized water, 80:20/v:v, the UV detector set to a wavelength of 280 nm, a mobile phase flow rate of 1 mL per min, 10 μL injection volume, and a total run time of 12 min for each sample. All reagents and solvents used were HPLC grade. Technical grade fipronil standard (98%, ChemService, Lot # 313-60B) was used to create a standard line on the HPLC using the same method that was used for each sample. This line contained data points at six concentration levels (0.2, 0.16, 0.1, 0.06, 0.03, and 0.003 mg/mL). These data points encompassed all points expected to be seen in the experiment.

Using linear regression from the standard curve, the fipronil concentration of the sample injected into the HPLC system was determined using the equation:

$$[\text{Fipronil}]\ \text{mg/mL} = \frac{(Y - b)}{m}$$

where Y = peak area; b = y intercept; m = slope of the line.

Using the concentration from the above equation, the true percent composition AI of the original tank mix was determined with the following equation:

$$\%\mathrm{AI} = \left[\frac{\mathrm{mg}}{\mathrm{L}}\right] \times \left(\frac{1000\,\mathrm{mL}}{1\,\mathrm{L}}\right) \times \left(\frac{V_s}{W_s}\right) \times \left(\frac{V_t}{V_{\mathrm{tmused}}}\right) \times (100)$$

where Concentration = mg/L found using the standard curve; V_s = sample volume that has been diluted in the volumetric in liters; W_s = weight of the sample volume in milligrams; V_t = Total volume of diluted insecticide in liters; V_{tmused} = volume of tank mixed used in the dilution in liters; Conversion factor = 0.1% AI = 1,000 ppm AI.

Concentration (ppm) of fipronil tank mixes were analyzed by analysis of variance using the PROC MIXED program (SAS Institute 2002) to test for differences within treatment means Fisher's LSD procedure ($p \leq 0.05$).

Results and Discussion

The weather data during this field study indicated that the highest and the lowest temperatures ranged from 33.66 to 13.13°C, respectively. No significant differences were detected in amount of fipronil recovered from 0.06% AI samples held in sunlight versus in shade and polyethylene versus glass jar surfaces at 0 h ($F = 0.78$; $df = 3, 12$; $p > 0.5284$), 24 h ($F = 0.12$; $df = 3, 12$; $p > 0.9446$), 48 h ($F = 1.06$; $df = 3, 12$; $p > 0.4008$), 72 h ($F = 0.20$; $df = 3, 12$; $p > 0.8954$), and 168 h ($F = 1.23$; $df = 3, 12$; $p > 0.34$) (Fig. 1). A difference was detected at the 336 h sampling interval ($F = 3.32$; $df = 3, 12$; $p > 0.057$). Within the 336 h sampling interval, significant differences were found between the sun glass jar samples versus the shade polyethylene tank samples ($t = -2.64$; $df = 12$; $p > 0.0217$) and the sun glass jar samples versus the shade glass jar samples ($t = -2.65$; $df = 12$; $p > 0.0213$) (Fig. 1).

No significant differences were detected in concentration of fipronil extracted from the 0.09% solutions in sun vs shade and tank surface type at 0 h ($F = 0.27$; $df = 3, 12$; $p > 0.8437$), 24 h ($F = 0.39$; $df = 3, 12$; $p > 0.7592$), 48 h ($F = 2.00$; $df = 3, 12$; $p > 0.1677$), 72 h ($F = 1.64$; $df = 3, 12$; $p > 0.2315$), 168 h ($F = 1.62$; $df = 3, 12$; $p > 0.2366$), and 336 h ($F = 1.97$; $df = 3, 12$; $p > 0.1718$) (Fig. 2). However, within the 48 h sampling interval there was a significant difference between the sun polyethylene tank and the shade polyethylene tank treatments ($t = -2.42$; $df = 12$; $p > 0.0322$) (Fig. 2).

In tank solutions containing 0.125% fipronil, significant differences were detected in sunlight versus shade conditions and polyethylene versus glass jar surfaces at 0 h ($F = 9.66$; $df = 3, 12$; $p > 0.0016$) (Fig. 3). Within the 0 h sampling interval, significant differences were detected between the sun polyethylene tank samples versus the sun glass jar samples ($t = 3.99$; $df = 12$; $p > 0.0018$), the sun polyethylene tank samples versus the shade glass jar samples ($t = 4.67$; $df = 12$; $p > 0.0005$), the shade polyethylene tank samples versus the sun glass jar samples ($t = -2.63$; $df = 12$; $p > 0.0219$), and the shade polyethylene tank

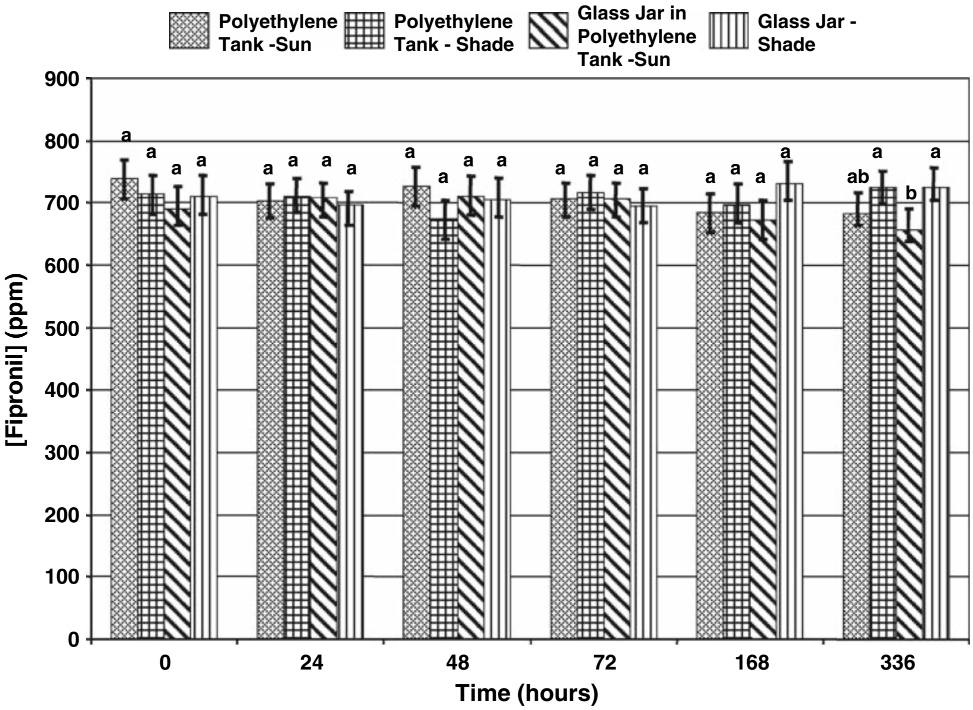

Fig. 1 Degradation of 0.06% fipronil in aqueous solutions stored in polyethylene and glass tanks exposed to sun or shade conditions. *Letters above* SEM bars represent significant differences and are sampling interval specific

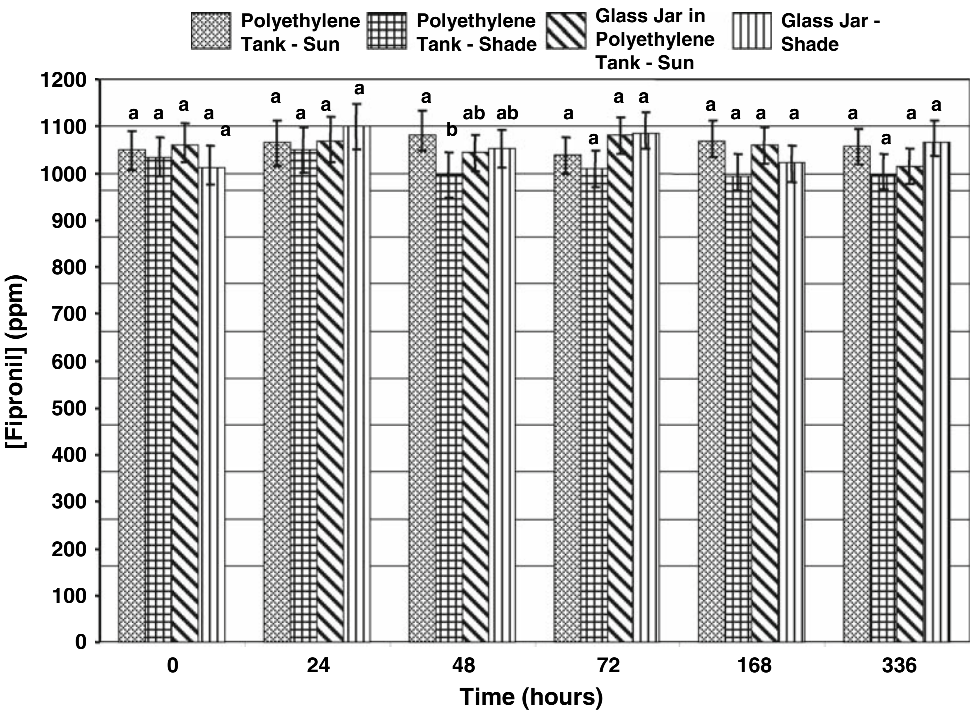

Fig. 2 Degradation of 0.09% fipronil in aqueous solutions stored in polyethylene and glass tanks exposed to sun or shade conditions. Letters above SEM bars represent significant differences and are sampling interval specific

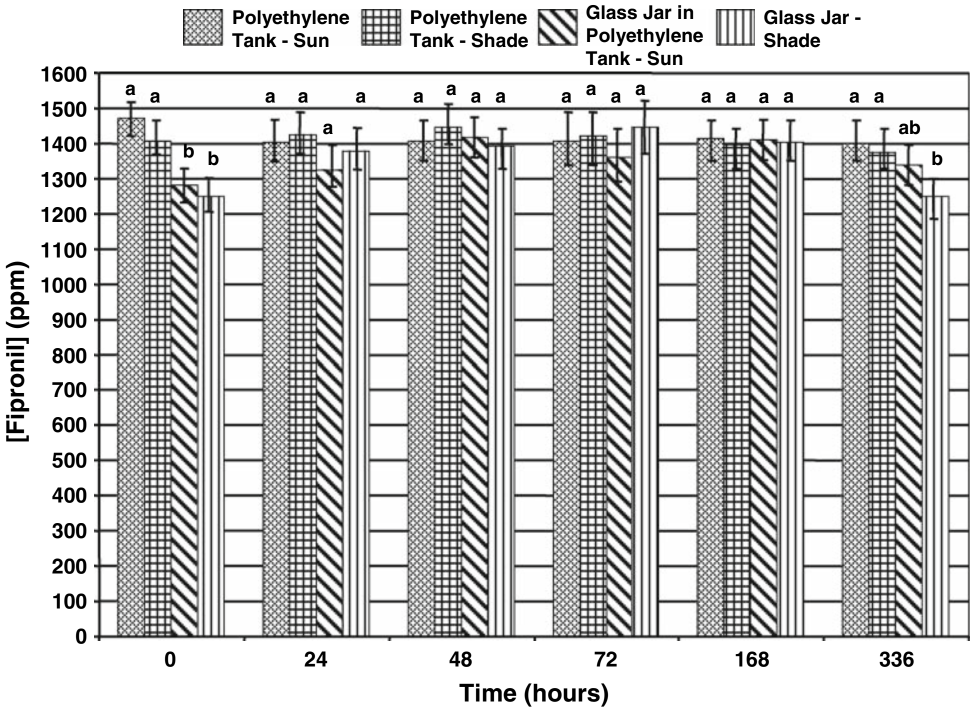

Fig. 3 Degradation of 0.125% fipronil in aqueous solutions stored in polyethylene and glass tanks exposed to sun or shade conditions. Letters above SEM bars represent significant differences and are sampling interval specific

samples versus the shade glass jar samples ($t = 3.32$; $df = 12$; $p > 0.0061$) (Fig. 3). No differences between light and surface treatments were detected at the 24 h ($F = 1.04$; $df = 3,\ 12$; $p > 0.4099$), 48 h ($F = 0.33$; $df = 3,\ 12$; $p > 0.8032$), 72 h ($F = 0.45$; $df = 3,\ 12$; $p > 0.7219$), 168 h ($F = 0.04$; $df = 3,\ 12$; $p > 0.987$), and 336 h ($F = 2.92$; $df = 3,\ 12$; $p > 0.0775$) sampling intervals (Fig. 3). However, within the 336 h sampling interval there were significant differences between the shade glass jar treatment when compared to the sun polyethylene tank ($t = 2.78$; $df = 12$; $p > 0.0167$) and the shade polyethylene tank ($t = 2.27$; $df = 12$; $p > 0.0421$) (Fig. 3).

All data gathered in this experiment reveal that fipronil, if kept in a shaded area or proper holding container, is extremely photo-stable for one to two weeks after mixing with water. For fipronil at 0.06% in tank mixes, there were mean degradations of 6.27% and 1.21% for all sun and shade experimental units, respectively. For the 0.09% tank mixes, there were mean degradations of 2.62% and 1.88% for all sun and shade experimental units, respectively. Lastly, for the 0.125% tank mixes, there were mean degradations of 2.35% and 5.82% for all sun and shade experimental units, respectively. However, there were no significant differences in concentration-dependent degradation based on light treatment or holding container surface over all sampling intervals (Figs. 1, 2, and 3). Within the 0.06% and 0.09% AI solutions, there was more degradation in the tank and glass jar samples in the sun treatment opposed to the shade treatment. However, in the 0.125% AI solutions, there was more degradation in the shade than in the sun treatment.

An unexplained finding was the increase in fipronil concentration observed primarily in the glass jar samples (shade and sun) at 0.09% and 0.125% starting concentrations from 0 to 24 h. One possible explanation for this trend is due to the low Henry's constant of fipronil. A low Henry's constant means a chemical has a tendency to remain in solution rather than volatilize into air (Connelly 2006). This could possibly result in water volatilization and in increase in fipronil concentration or at least a counteraction of initial fipronil degradation. Another possible explanation of this trend is due to the settling of the Termidor® SC solution within the 3.785 L tank while it was transferred to individual glass jars. The filling tank was thoroughly agitated prior to samples being poured into the glass jars, but some settling still may have occurred.

The results of this study indicate that the polyethylene tanks commonly used by PMPs protect fipronil from photodegradation and that a minimal amount of hydrolytic degradation occurs over a 2 week time period. High temperatures did not cause significant degradation of fipronil. The highest and the lowest temperatures experienced during this field study are typical in various states during peak termiticide application periods. In conclusion, our results indicate that fipronil (Termidor® SC) solutions, if properly stored in polyethylene tanks, resuspended and thoroughly stirred, may be usable for termiticide applications for up to 2 week after their initial mixing.

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