Final report for CDPR Contract #14-C0102

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During 2015 and 2016 we conducted insecticide field trials at the University of California, Riverside (UCR) campus and at homes in Riverside, CA, to compare different bandwidth applications of fipronil on runoff concentrations and efficacy to Argentine ants (*Linepithema humile*). Runoff studies were conducted both on a constructed wall on campus and around private houses in Riverside. For the house trials, efficacy studies were simultaneously conducted around the same residences used for runoff studies. We have summarized the studies by year.

Summer 2015

Treatments on a constructed wall and concrete pad

For a previous contract (11-C0086; http://cdpr.ca.gov/docs/emon/surfwtr/contracts.htm) we had constructed on the UCR campus a wall and concrete pad to simulate a wall/driveway interface. The wall is 3 ft high and 36 ft wide (Figure 1). The concrete pad narrows down to 10.4 ft from the wall to a spout where water samples are collected (Figure 2). There are 12 3-ft wide segments on each side of the wall for a total of 24 segments that can be used for treatments. Sprinklers on both sides of the wall are adjusted to give an equal distribution of simulated rain on both the wall and pad. Depending on the width of the treatment band, the area to be treated is masked with paper and cardboard (Figure 3). The water samples were analyzed for fipronil, fipronil desulfinyl, fipronil sulfone, and fipronil sulfide.

Methods. Table 1 shows the three 2015 wall treatments. The standard treatment (labeled rate) for homes used 2 quarts of 0.06% fipronil per 160 linear feet, while each of the other treatments used 1 quart, one-half of the fipronil mass of the standard treatment. All treatments were done with a 1-gal B&G tank sprayer with a 4-Way Multeejet Tip. We attached a pressure gauge to keep the tank pressure at 20 psi. The standard and the reduced swath treatments were done with a coarse fan spray setting whereas the pin stream setting provided a very narrow but concentrated spray. To apply the standard treatment we did two passes of 2.5 secs each (7 ml of 0.006% solution/sec), one pass 1 ft up the vertical wall and the other pass 1 ft out on the pad. For the 6 in x 6 in band we did one pass at 2.5 secs (also 7 ml/sec). And for the pin stream we did one pass at 3.5 secs (5 ml/sec).

Each side of the wall with its 12 segments was considered a block in a randomized block design. There were 3 treatments, with 8 replicates of each treatment, for a total of 24 treated segments on the wall. On each side of the wall, segments were randomly assigned 1 of the 3 treatments. The two sides were treated one week apart. Prior to application, the wall was washed with Liquinox and water before use. Due to constraints on resources, no pre-treatment samples were collected (the wall had last received treatments 1 year earlier). It was assumed that after cleaning the constructed wall there would not be any fipronil residues remaining (although 2016 data shows some residues exist after one year). Samples were collected 1 day and 30 days post-treatment. Concentrations in the runoff were compared to US EPA aquatic life benchmarks for fipronil, and the desulfinyl, sulfone, and sulfide degradates (USEPA, 2017).

Results. At one day after treatment (Figure 4), the 1 quart pin stream application had significantly less runoff than either the 2 quart standard treatment or the 1 quart 6 in x 6 in band treatment (for fipronil and each degradate; $p < 0.05$). The pin stream application had greater than 80% reduction of fipronil and fipronil desulfinyl and greater than 70% reduction of the sulfide and sulfone degradates (Table 2). There were no significant differences between the 6 in x 6 in band and the 1 ft x 1 ft band for fipronil or its degradates. All day 1 runoff amounts were above fipronil's acute benchmark of 110 ppt (mean = 5743 ppt; median = 3156 ppt). At 30 days post-treatment there were no statistically significant differences in runoff between the treatments (Figure 5). However, runoff amounts from the pin stream applications were numerically lower. Table 3 shows 30-day post-treatment runoff means and medians. Most values are below fipronil's chronic aquatic benchmark value of 11 ppt, and all are below its acute benchmark of 110 ppt. Within each treatment, the reduction in runoff at 30 days post-treatment was > 99% for all chemicals.

At 1 day post-treatment, fipronil desulfinyl was a major contributor to runoff concentration, followed by the parent fipronil. By 30 days post-treatment, there was a significant conversion from parent to the sulfone and desulfinyl degradates in all treatments (Figure 6).

Discussion. The large reduction in the runoff of fipronil and its degradates at 30 days post-treatment suggests that fipronil should not be applied prior to rain. Although we did not look at other intervals, 30 days post-treatment gives sufficient time for breakdown of fipronil and its degradates to concentrations below US EPA benchmarks. Furthermore, a 1 in x 1 in (1 quart) pin stream application gives reduced runoff compared to a 1 ft x1 ft (2 quart) and a 6 in x 6 in (1 quart) application. Reducing the band width from 1 ft to 6 in does not reduce runoff. However, reducing mass (to 1 quart) with a pin stream application reduces initial fipronil runoff. Band width appears to be a bigger factor in initial runoff than volume of fipronil applied. Additional research is needed to determine how band width influences runoff (investigated in 2016 trials).

Summer 2016

Treatments on a constructed wall and concrete pad

Objective. To determine the effect of band width and mass of fipronil applied on runoff of fipronil and its degradates. Expanded from 2015 trials by looking at different mass of fipronil applied by a pin stream application.

Methods. Table 4 describes the fipronil wall treatments applied in 2016. The general methods were the same as described for 2015. For 2016, three treatments were applied, each replicated six times. Samples were collected 1 and 30 days post-treatment. Treatment A1 is the standard label application of one foot up and one foot out from the foundation using 2 quarts of 0.06% Termidor. Treatment B was applied as a pin stream application (1 in x 1 in band) at 1/12 the mass of the standard label rate per linear foot. Treatment C2 was applied as a pin stream application using 2 quarts of 0.06% solution per linear foot, identical (except for spray swath) to the label treatment A1. Prior to application, the walls were washed with Liquinox® and water and pre-treatment values were obtained from the three wall segments that had the highest fipronil runoff the previous year.

Results. Figure 7 shows pre-treatment values (n=3) from the wall. Fipronil sulfide was the major component with median runoff of 62 ppt, followed by fipronil sulfone, fipronil, and fipronil desulfinyl with median concentrations of 10, 5, and 1 ppt, respectively. All values were below US EPA aquatic acute benchmarks. However, some of the fipronil and fipronil sulfide concentrations were higher than US EPA aquatic chronic benchmarks. Figures 8-11 summarize the results of the 2016 wall trials. The day 1 post-treatment levels detected were lower than previous years. The data (including transformed data) was highly skewed (non-normal distribution), so log transformed data or nonparametric statistics were used to evaluate the data. At day 1 post-treatment, the standard treatment had a numerically higher median runoff than other treatments for all fiproles (fipronil and degradates), but there were no statistically differences between the treatment medians (Figure 8). This was true when looking at total fiproles concentration (Figure 9).

At 30-days post-treatment, the pin stream application with 5.3 oz of Termidor (treatment B) had lowest runoff (Figure 10). Although the sample size is low (n=6 replicates), the results for fipronil 30 days post-treatment are close to statistically significant (ANOVA using log transformed data, p=0.066). And runoff of the sulfide and desulfinyl degradates were significantly reduced with treatment B (p=0.046 and 0.018, respectively), as was the combined data (fiproles, p=0.047) (Figure 11).

Discussion. The pre-treatment residues show that fipronil and its degrades can persist longer than one year at concentrations above US EPA chronic benchmarks. For example, one of the pre-treatment samples contained 74 ppt fipronil and 207 ppt fipronil sulfone. These persisted through the hot summers in Riverside and from rainy season wash-off, as well as the pre-treatment wash. This was observed in a previous study (Greenberg et al., 2016). The constructed wall is on UC Riverside's Agricultural Operations on campus and not near any urban structures that could have received fipronil treatments other than what we applied.

There were no significant differences in the runoff between the treatments at 1 post-treatment, but the data show pretty strongly that the pin stream application with 5.3 oz Termidor (treatment B) reduces runoff at 30 post-treatment. This is likely because this treatment had less mass applied than the standard treatment or the pin stream applied at 2 quarts. The pin stream application with 5.3 oz Termidor likely had mostly washed off at 1 post-treatment, and insufficient fipronil remained to wash off by 30 post-treatment. This treatment would allow for reduced runoff for fipronil treatments applied in September, closer to California's rainy season. This finding is significant for justification of the use of pin stream application as a mitigation method to reduce fipronil runoff if the actual mass of fipronil is reduced. However, if fipronil mass is not reduced, using a pin stream application will not reduce fipronil runoff (Greenberg et al., 2016). This is the concern with using pin stream application as a mitigation method; actual fipronil mass may not be reduced (Budd et al., 2017)

House treatments

Objectives. There were two main objectives. First, we wanted to know how different bandwidths of fipronil sprayed around house perimeters affect ant efficacy and fipronil runoff. Second, we wanted to know the effect of not spraying fipronil on the driveway/garage door interface on ant control and fipronil runoff.

Methods. Before treating the houses, the driveways and the driveway/garage door interface were thoroughly hosed down. After they had dried, pre-treatment water samples were collected at 3 of the 5 houses for each treatment, for a total of 12 samples. For the house trials, fipronil treatments consisted of the labeled rate applied the house perimeter, labeled rate around the perimeter but with a pin stream application at the driveway/garage door interface, the labeled rate applied as a pin stream application around the house perimeter, or labeled rate around the perimeter but with no fipronil application to this area (Table 5). Fipronil treatments were applied within a few days of collecting the pre-treatment samples. After application, water samples were collected at 1 and 30 days post-treatment. The houses used for water sampling were also used simultaneously to measure Argentine ant numbers (*L. humile*), previously described (Greenberg et al., 2016). Briefly, we put out sucrose water monitors and measured its consumption to give an estimate of ant numbers. For this project we measured ant numbers before application (pre-treatment) and at 1, 2, 4, 6, and 8 weeks post-treatment.

Results and Discussion

Efficacy of treatments. There were no significant differences between ant numbers of any of the four treatments regardless of post-treatment timing. The data was highly skewed, so analysis was conducted with Kruskal-Wallis nonparametric statistical test. Pin stream application or no application to the driveway/garage door interface area had control of ants similar to the standard treatment (Figure 12).

Runoff concentration. Pre-treatment runoff of fipronil and its degradates ranged from 2.8 to 323 ng/L (Figure 13). These houses had a fipronil treatment one year previous and were exposed to rain during the rainy season, in addition to the pre-treatment wash down of the driveway. Despite these conditions many of the results were above the chronic and acute US EPA benchmarks.

At 1 day post-treatment, there were high concentrations of fipronil and degradates in the runoff (Figure 14). For fipronil, concentrations were above US EPA acute benchmarks for all treatments. Many of the degradates were also detected above acute or chronic benchmarks. Interestingly, Treatment G (no application to the driveway/garage door area) had a different runoff profile of parent to degradate than treatments with applications to the garage door. This treatment had a higher percentage of the sulfone degradate (Figure 15).

Probability plots show the data was highly skewed, so the nonparametric Kruskal-Wallis test was used to determine if fipronil runoff was reduced by any of the treatments. Treatment G (no application to the driveway/garage door area) was the only treatment that had significantly reduced runoff compared to the other treatments at 1 day post-treatment ($p=0.019$-0.039; sulfone was close to significant, $p=0.089$) (Figure 14). For example, Treatment G had an 88% reduction in fipronil runoff and 93% reduction in runoff of all three degradates.

At 30 days post-treatment, there were no significant differences between the treatments (Figure 16). Treatment G had a 35% reduction in fipronil runoff and a 52% reduction in degradate runoff compared to the labeled treatment, although these differences were not significant. At 30 days post-treatment, runoff was severely reduced and matched background levels (Figure 17; data not shown for degradates). As in our previous work (Greenberg et al., 2016) this suggest that professional applicators should not apply fipronil with 30 days before the rainy season, or during the rainy season.

Discussion. The pre-treatment samples show that traces of fipronil and its degradates are still present one year after application, suggesting that it is hard to completely remove these residues. As perhaps expected, Treatment G, which had no driveway treatment, showed the lowest runoff of all treatments); significantly so at 1 day post-treatment. On day 30, although we cannot show statistically significant differences with n=5 replicates, Treatment G showed reductions with respect to the standard application. Treatment G gave ant efficacy equal to the standard treatment. There was no loss of ant control when fipronil was not applied to the driveway/garage door interface. These results suggest that a mitigation measure to reduce fipronil runoff is to not allow fipronil applications to the garage door/driveway interface. These results also suggest that fipronil treatments should not be done within one month of the rainy season in California (or during the rainy season), as also shown in our previous work (Greenberg et al., 2016).

Significant findings.

1. Fipronil and degradates can persist in the environment for more than one year at concentrations above US EPA chronic benchmarks;
2. Pin stream applications which reduce the fipronil mass have lower fipronil runoff than the labeled fipronil rate;
3. Ant efficacy can be maintained around the house perimeter if fipronil is not applied to the driveway/garage door interface;
4. When fipronil is applied to the house perimeter but not to the driveway/garage door interface, fipronil runoff concentrations are similar to background levels.
5. Fipronil should not be applied 30 days prior to the rainy season, or during California's rainy season.

References

Budd, R., Y. Luo, N. Singhasemanon. 2017. Addendum: Evaluation of alternative fipronil use scenarios: Modeling results, runoff trials, and product efficacy. http://cdpr.ca.gov/docs/emon/pubs/ehapreps/analysis_memos/fipronil_addendum_6-26-17_update.pdf.

Greenberg, L., M. K. Rust, and M. P. Ensminger. 2016. Final report CDPR Contract 13-C0031. http://cdpr.ca.gov/docs/emon/surfwtr/contracts/ucriverside_13-C0031_final_rpt.pdf.

US EPA. 2017. Aquatic life benchmarks for pesticide regulation (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration).

Table 1. Treatments on the constructed wall during 2015.

Treatment (Contract Treatment ID)	Treatment swath at base of wall	Projected treatment area	Actual amount of Termidor SC used/area
Labeled rate (standard treatment) (A)	1 foot up, 1 foot out band application	2 quarts of a 0.06% Termidor SC solution per 160 linear feet (320 square feet) (0.2 fl. oz/ft^2)	3.8 μg/cm^2
Pin Stream (C1)	1 inch up, 1 inch out pin stream application	1 quart of a 0.06% Termidor SC solution per 160 linear feet (26.67 square feet) (1.2 fl. oz/ft^2)	22.9 μg/cm^2
Reduced swath (D)	6 inches up, 6 inches out band application	1 quart a 0.06% Termidor SC solution per 160 linear feet (160 square feet) (0.2 fl. oz/ft^2)	3.8 μg/cm^2

Table 2. Day 1 percent reduction between a standard (2 quart) 1 ft x 1 ft treatment (treatment A) and a pin stream (1 quart) 1 in x 1 in treatment (treatment C1) in 2015. For the 1 ft x1 ft and 1 in x1 in treatment n=8. The table shows fipronil and its 3 most common degradates. *= $p < 0.05$; **= $p < 0.01$. See Table 1 for treatment list.

Compound	Mean % reduction with pin stream treatment ("A")	Median % reduction with pin stream treatment ("C1")
Fipronil	80.2**	92.9**
Fipronil desulfinyl	84.2**	94.2**
Fipronil sulfide	71.6*	87.8**
Fipronil sulfone	71.6*	87.3**

Table 3. 2015 -day 30 post-treatment wall runoff medians and means in ppt. Most values are below the fipronil chronic aquatic benchmark of 11 ppt, whereas all are below the fipronil acute benchmark of 110 ppt. For the 1 ft x 1 ft and 1 in x 1 in treatments n=8; for the 6 in x 6 in treatment n=7. See Table 1 for treatment list.

	Treatment		
	1 ft x1 ft (A)	6 in x 6 in (D)	1 in x 1 in (C1)
Chemical	Median concentrations		
Fipronil	0.01	0.01	0.01
Fipronil desulfinyl	12.96	13.13	6.28
Fipronil sulfide	5.01	6.02	2.09
Fipronil sulfone	6.75	5.87	4.76
	Mean concentrations		
Fipronil	5.15	7.33	3.69
Fipronil desulfinyl	23.97	13.75	8.58
Fipronil sulfide	5.53	6.63	2.57
Fipronil sulfone	10.46	9.28	4.88

Table 4. Wall treatments during 2016. Treatment A1 is the standard treatment (labeled rate) and the other two treatments are pin stream applications (1 in x 1 in pin stream) with either the same mass or 1/12 the mass of the standard treatment.

Treatment ID	Application spray swath	Spray Volume	Fraction of fipronil mass compared to label (label = 1)
A1	band spray, labeled rate (1 foot up/1 foot out)	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet	1
B	pin stream (1 inch up/1 inch out)	5.3 fl oz. of a 0.06% Termidor SC finished dilution per 160 linear feet	1/12
C2	pin stream (1 inch up/1 inch out)	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet	1

Table 5. Home treatments done in 2016. A1 is the standard label treatment to which the others are compared.

Treatment ID	Area	Application spray swath	Spray volume	Fraction of fipronil mass compared to label (label = 1)
A1*	Garage door	band spray, labeled rate (1 foot up/1 foot out)	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet (labeled rate)	1
	House Perimeter	band spray, labeled rate (1 foot up/1 foot out	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet (labeled rate)	1
A3*	Garage door	pin stream (1inch up/1 inch out)	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet	1
	House Perimeter	band spray, labeled rate (1 foot up/1 foot out)	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet	1
C2	Garage door	pin stream (1inch up/1 inch out)	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet	1
	House Perimeter	pin stream (1inch up/1 inch out)	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet	1
G	Garage door	No application of any pesticide or insect control method	None at the driveway/garage door interface	0 (none)
	House Perimeter	band spray, labeled rate (1 foot up/1 foot out)	2 quarts of a 0.06% Termidor SC finished dilution per 160 linear feet	1

Figure 1. One side of constructed wall showing 12 3-ft wide segments.

Figure 2. Collecting a 1-L water sample for analysis.

Figure 3. Masking an area to be treated.

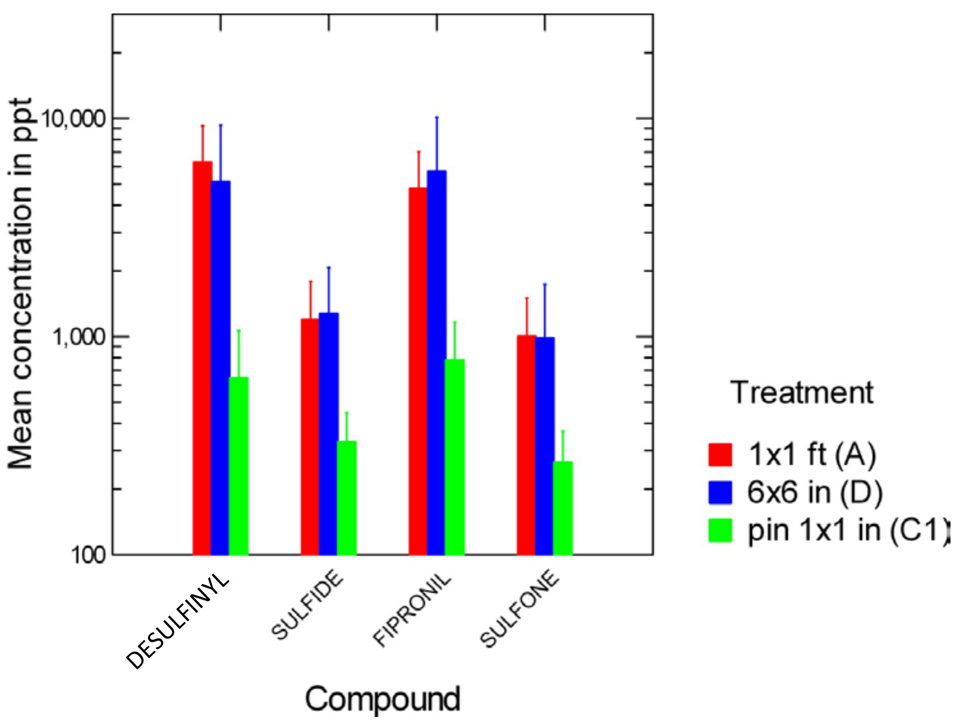

Figure 4. Concentration of fipronil and degradates in runoff from 1-L water samples collected 1 day post-treatment from the constructed wall located on the UC Riverside campus (2015).

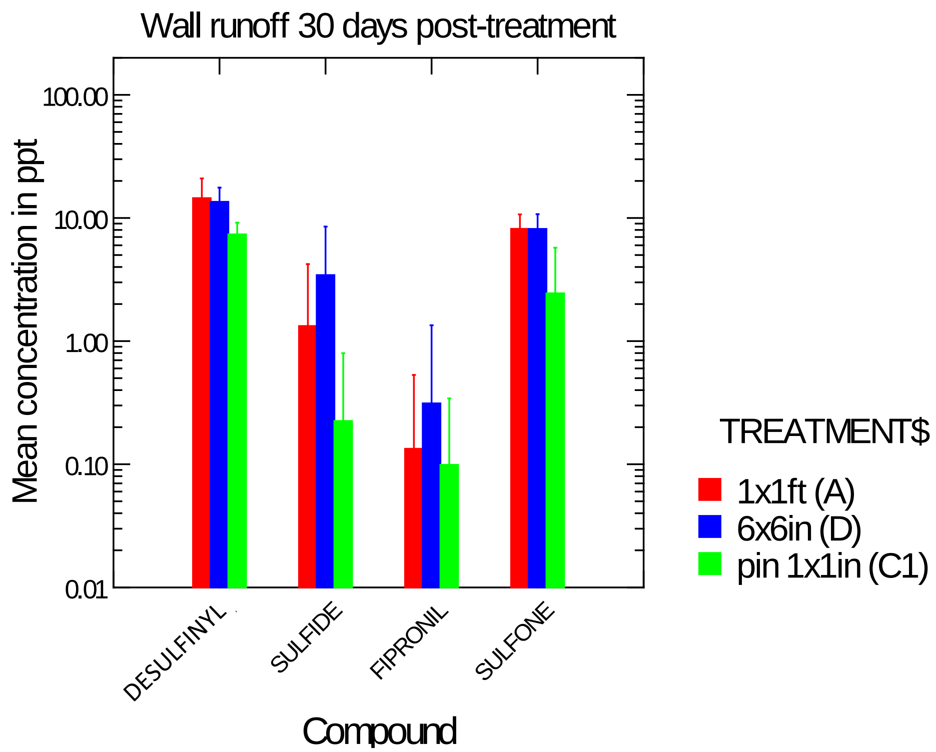

Figure 5. Concentration of fipronil and degradates in runoff from 1-L water samples collected 30 days post-treatment from the constructed wall trials located on the UC Riverside campus (2015).

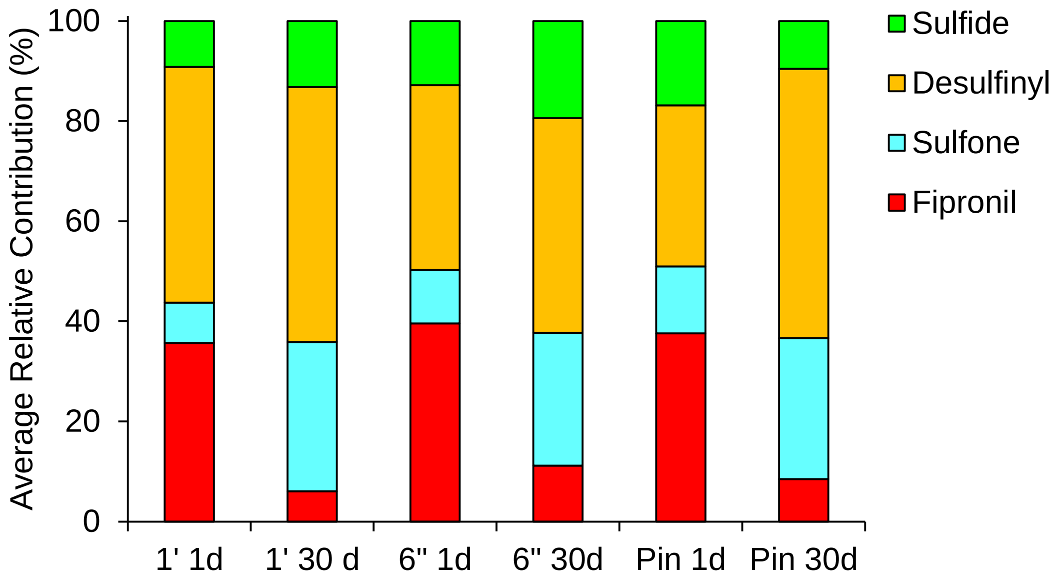

Figure 6. Proportion of fipronil and its degradates from 1-L water samples collected 1 and 30 days post-treatment from constructed wall trials (2015; all treatments combined).

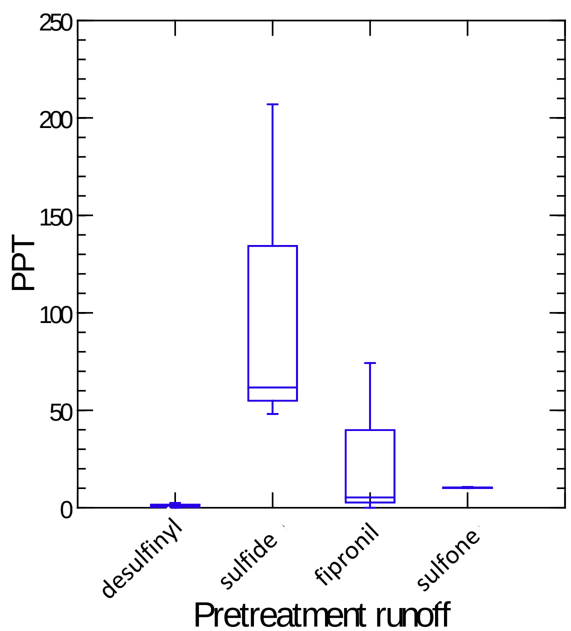

Figure 7. Pre-treatment samples from the wall in 2016 showing the parent compound fipronil and three of its breakdown products. The samples were taken from the three wall segments that had the highest fipronil runoff in 2015. The line inside the box is the median of the three measurements and the whiskers show the minimum and maximum values.

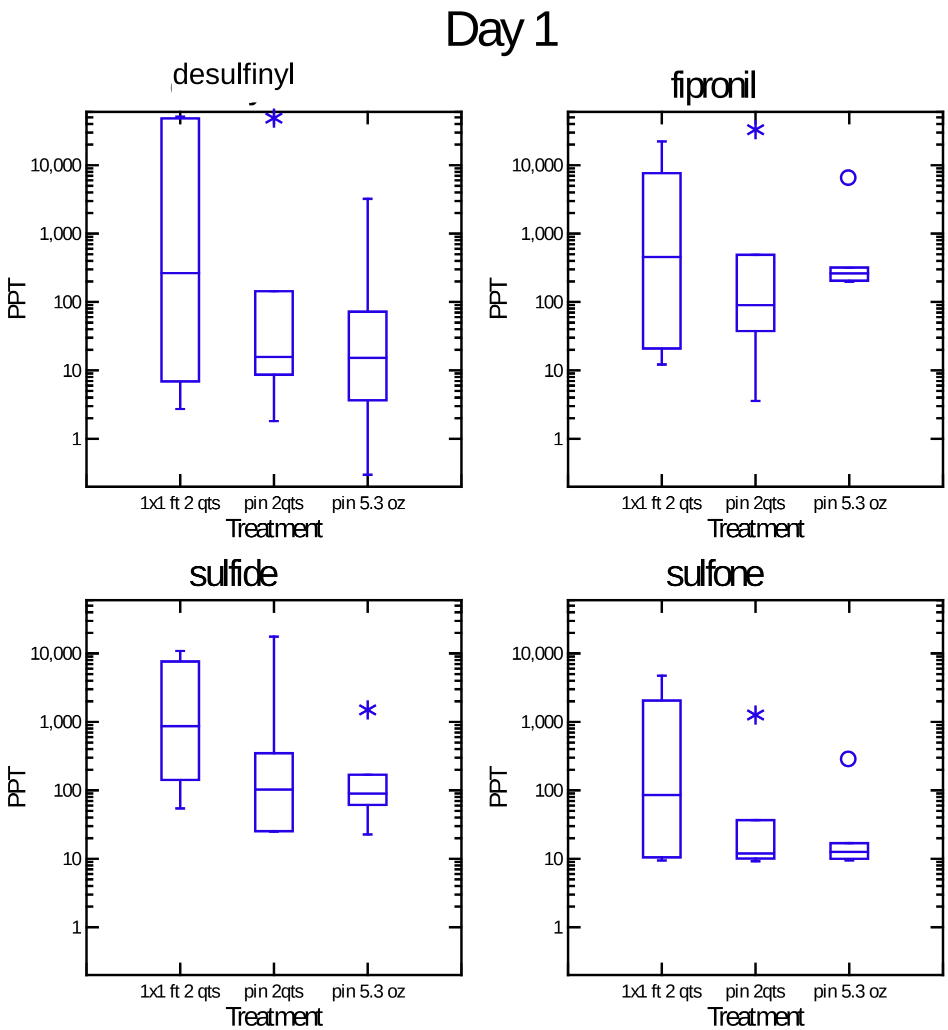

Figure 8. Concentration of fipronil and degradates in runoff from 1-L water samples collected 1 day post-treatment from the constructed wall trials (2016). The line in the middle of each box shows the median value, while the whiskers show the ranges of values. *= an outlier; O = a far outlier. See Table 4 for a detailed treatment list.

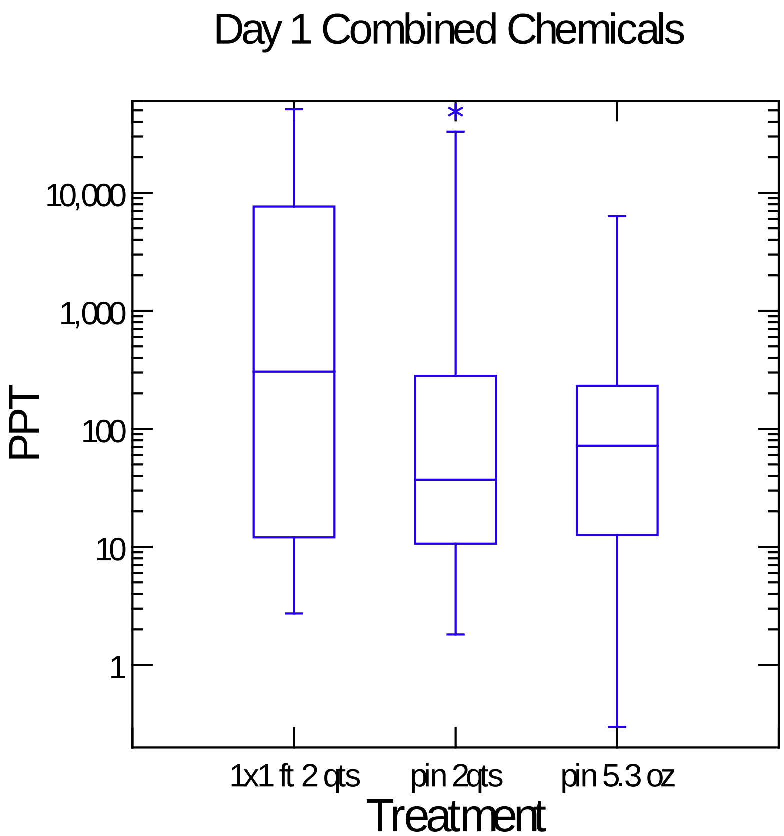

Figure 9. Concentration of fipronil combined with its three main degradates in runoff from 1-L water samples collected 1 day post-treatment from the constructed wall trials (2016). The line in the middle of each box shows the median value, while the whiskers show the ranges of values. *= an outlier; O = a far outlier. See Table 4 for a detailed treatment list.

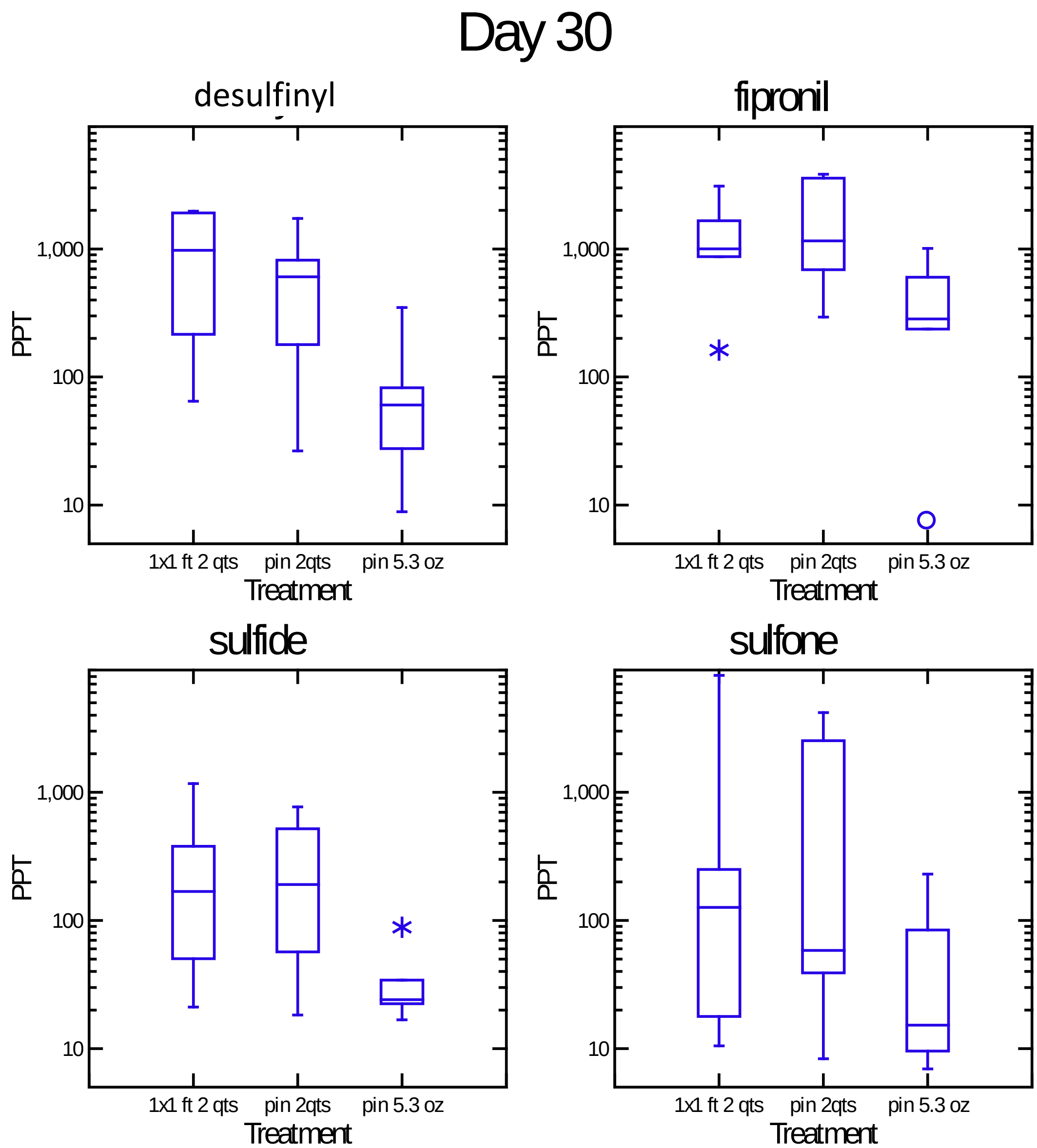

Figure 10. Concentration of fipronil and degradates in runoff from 1-L water samples collected 30 days post-treatment from the constructed wall trials (2016). The line in the middle of each box shows the median value, while the whiskers show the ranges of values. *= an outlier; O = a far outlier. See Table 4 for a detailed treatment list.

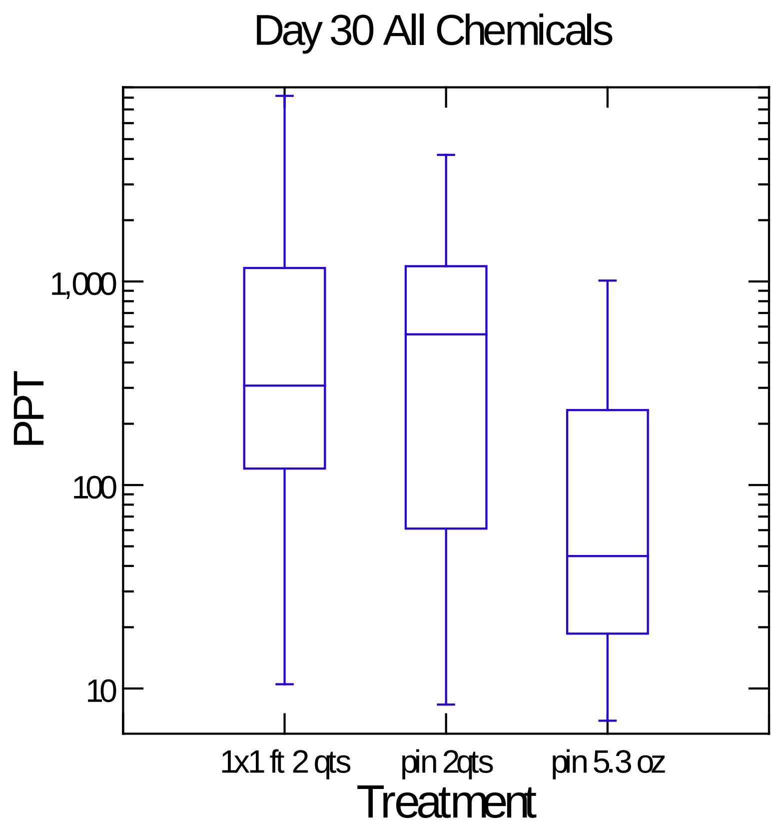

Figure 11. Concentration of fipronil combined with its three main degradates in runoff from 1-L water samples collected 30 days post treatment from the constructed wall trials (2016). The line in the middle of each box shows the median value, while the whiskers show the ranges of values. See Table 4 for a detailed treatment list.

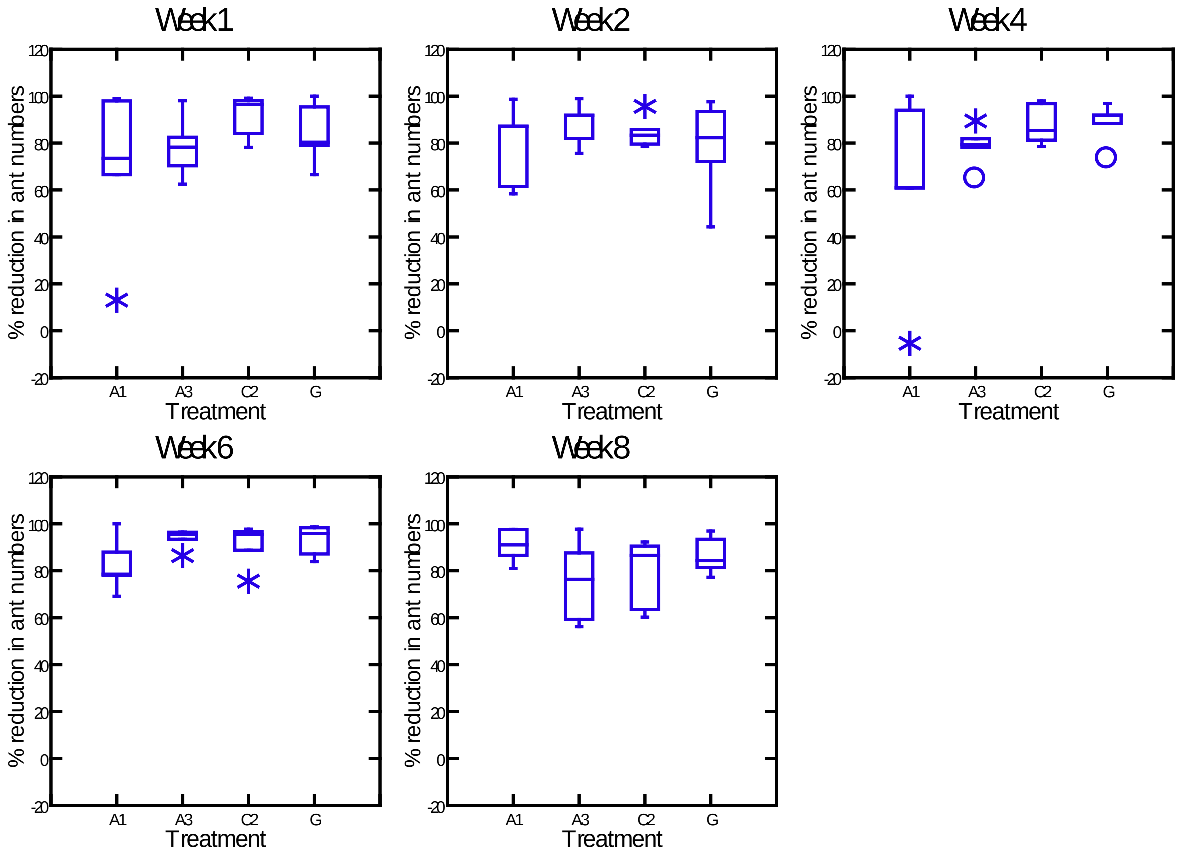

Figure 12. Ant reductions from house runoff trials (2016). The line inside each box shows the median value. See Table 5 for treatment list (Treatment A1 is the standard treatment). Asterisks are near outliers, circles are far outliers.

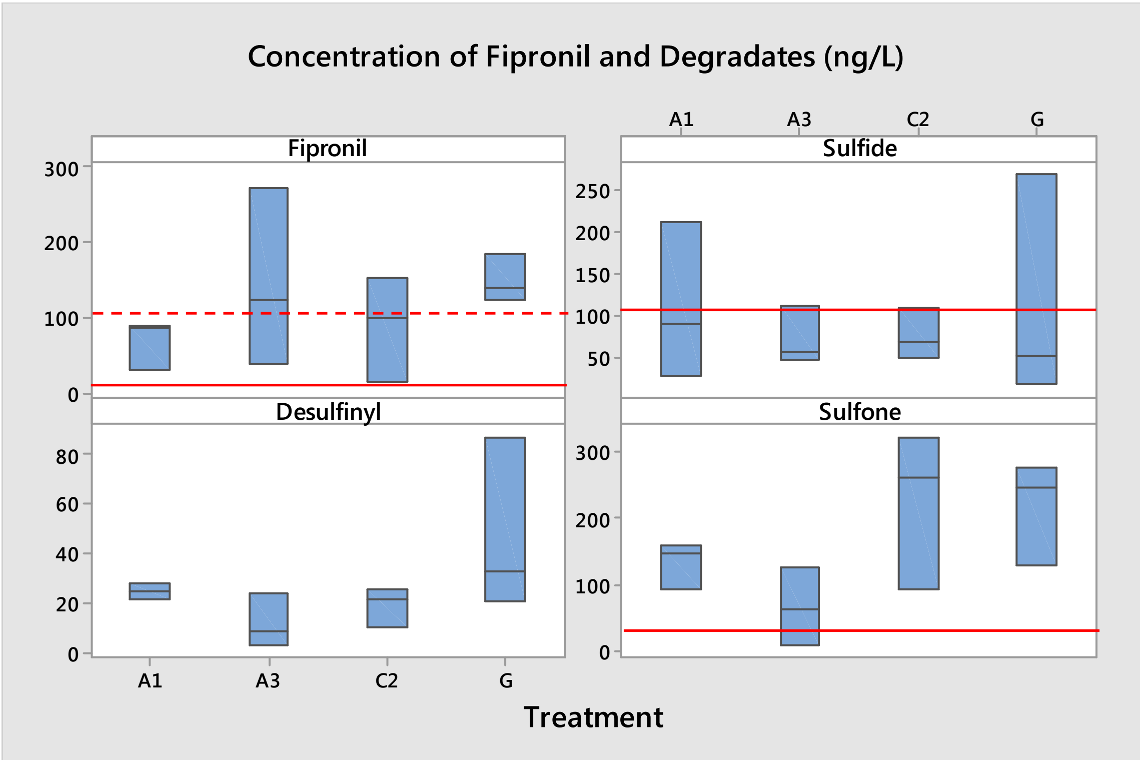

Figure 13. Concentration of fipronil and its three main degradates in runoff from 1-L water samples prior to application (pre-treatment) from the houses in the Riverside area (2016). See Table 5 for treatment list. Upper dotted red line is the EPA invertebrate acute benchmark and the lower red solid is the chronic benchmark (no dotted line, benchmark is above y-axis scale; if there no solid line, the chronic benchmark is lower than the y-axis scale). The line in the middle of each box is the median value.

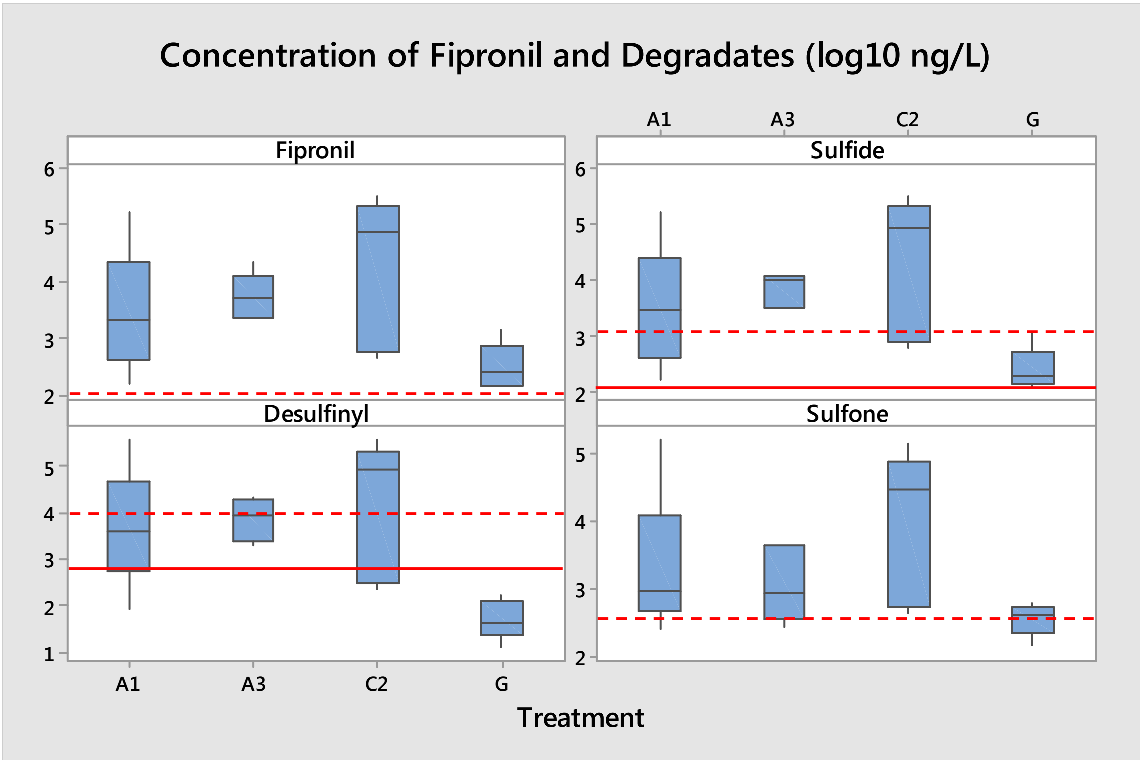

Figure 14. Concentration of fipronil and its three main degradates in runoff from 1-L water samples taken 1 day post-treatment. See Table 5 for treatment list. Upper dotted red line is the EPA invertebrate acute benchmark and the lower red solid is the chronic benchmark (no dotted line, benchmark is above y-axis scale; if there no solid line, the chronic benchmark is lower than the y-axis scale). For the desulfinyl degradate, fish acute and chronic benchmarks are shown. The line in the middle of each box is the median value.

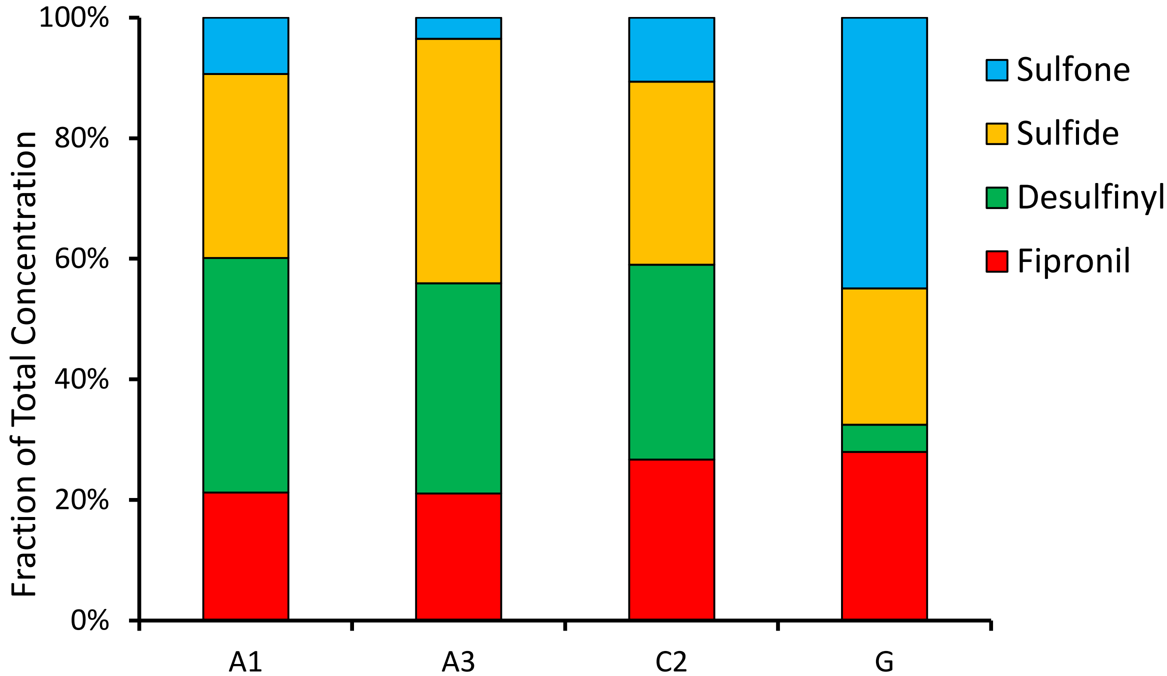

Figure 15. Proportion of fipronil and its degradates from 1-L water samples collected 1 day post-treatment from house trials (2016). See Table 5 for treatment list.

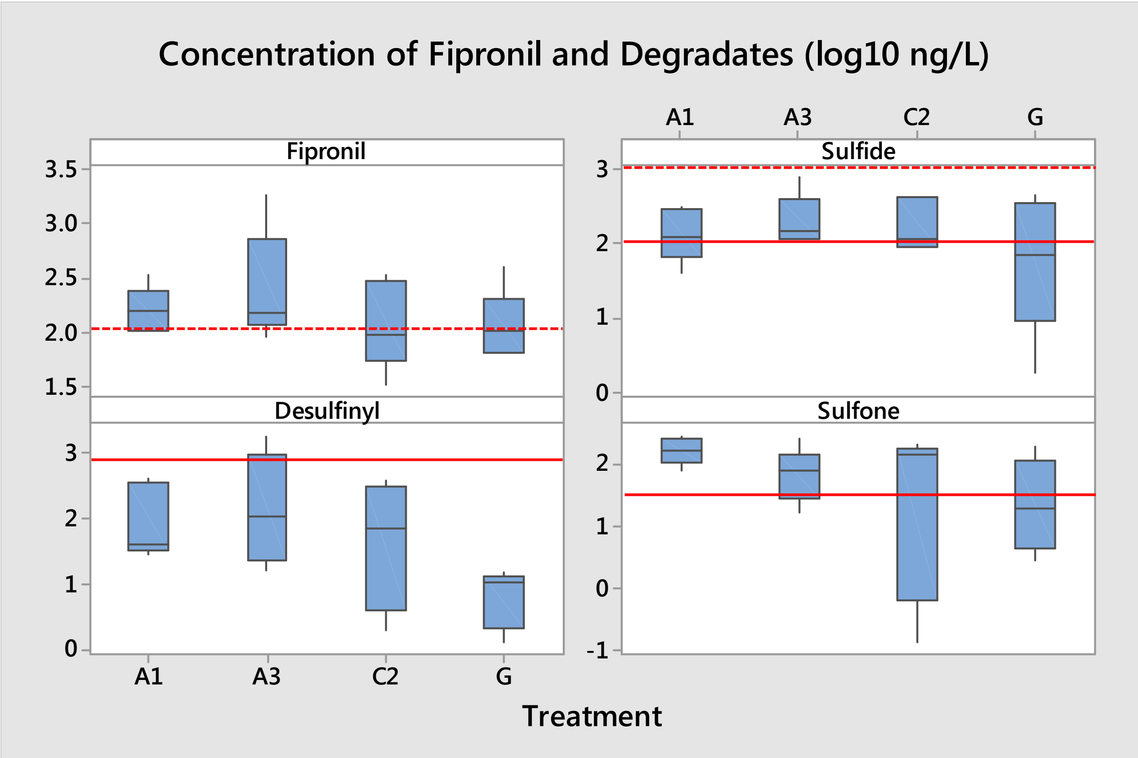

Figure 16. Concentration of fipronil and its three main degradates in runoff from 1-L water samples taken 30 days post-treatment. See Table 5 for treatment list. Upper dotted red line is the EPA invertebrate acute benchmark and the lower red solid is the chronic benchmark (no dotted line, benchmark is above y-axis scale; if there is no solid line, the chronic benchmark is lower than the y-axis scale). For desulfinyl degradate, fish acute and chronic benchmarks are shown. The line in the middle of each box is the median value.

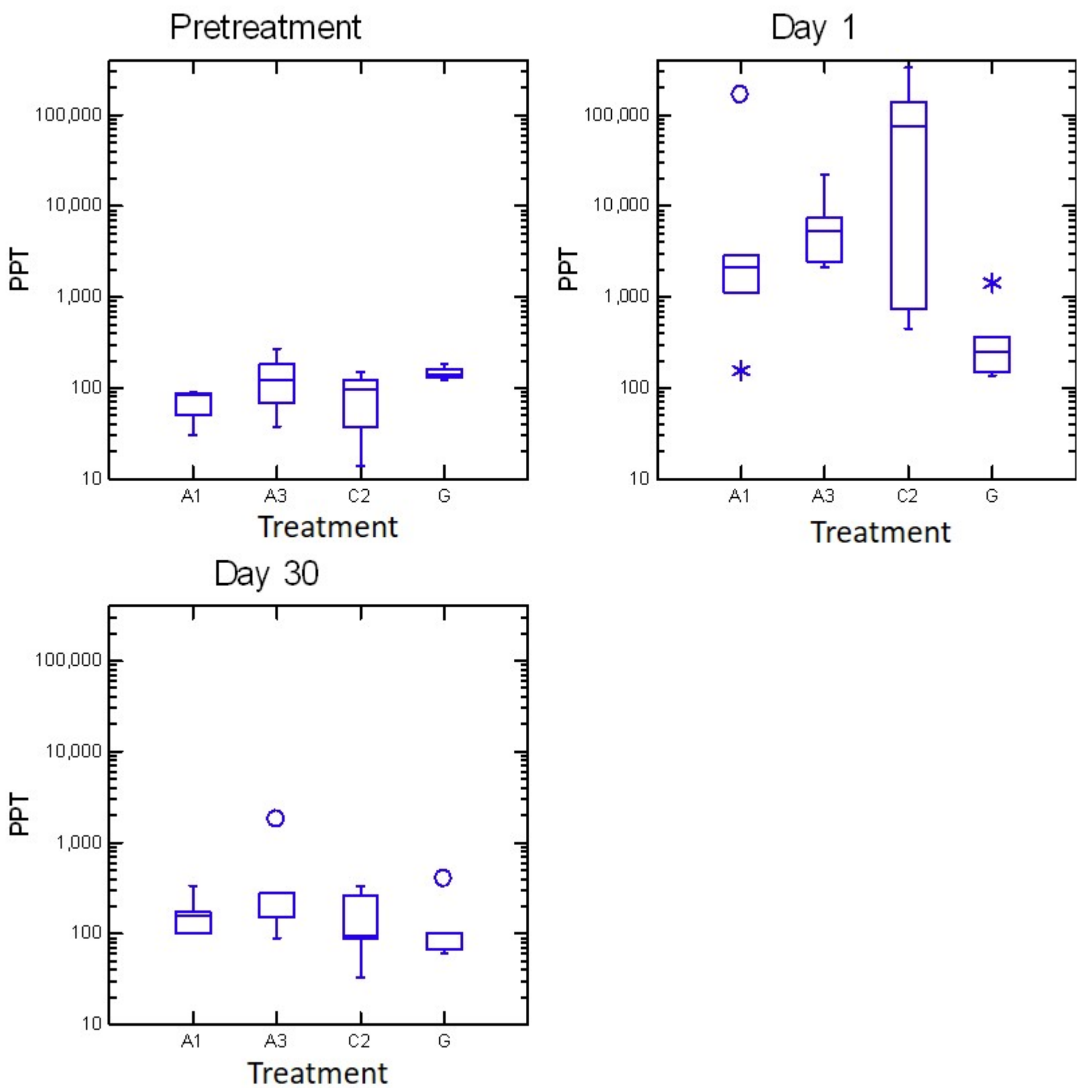

Figure 17. Boxplots of fipronil runoff around homes in 2016. For pre-treatment, n=3; for other graphs n=5. Note that day 30 runoff is similar to the pre-treatment values. See Table 5 for treatment list.