Efficacy of Pyriprole Topical Solution against the Cat Flea, *Ctenocephalides felis*, on Dogs

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Three studies evaluating various aspects of the performance of pyriprole against the cat flea, *Ctenocephalides felis*, on dogs demonstrated that 12.5% pyriprole applied as a spot-on provides rapid, long-lasting efficacy against adult cat fleas, even under severe flea challenge. Speed of kill data indicate treatment with this product can interrupt an already established adult flea infestation, whereas monthly treatment can prevent reinestation. Pyriprole disrupts the flea life cycle by killing adult fleas before they lay eggs for at least 30 days after treatment. The residual effect of pyriprole on debris from treated dogs (dander, hair, scales, and flea feces) resulted in a decreased ability of cat flea larvae to complete development to the adult stage for 2 weeks after application. Based on the results of these studies, 12.5% pyriprole represents a valuable new tool in the control of the cat flea, *C. felis*, on dogs.

INTRODUCTION

*Ctenocephalides felis*, the cat flea, is a major ectoparasite of dogs throughout the world. Infestation by fleas may cause severe inflammation of the skin and intense itching, resulting in such chronic conditions as pruritis and flea allergy dermatitis.¹ In addition, fleas are known to be responsible for the transmission of various parasites and diseases.²-⁴

Pyriprole belongs to the phenylpyrazole class of insecticides. The phenylpyrazoles are effective against a broad spectrum of chewing and sucking insects. Their activity is attributed to

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their action at the \( \gamma \)-aminobutyric acid (GABA) receptor as noncompetitive blockers of the GABA-gated chloride channel. Pyriproxyfen is currently approved in some countries for use on dogs for the control of ticks and fleas.

Three separate studies were conducted to evaluate the efficacy of a spot-on solution containing 12.5% (w/v) pyriproxyfen against induced infestations of the cat flea, *C. felis*, on dogs. Study 1 was designed to demonstrate pyriproxyfen’s speed of kill of adult cat fleas on dogs. Study 2 investigated the effect of (1) speed of kill on the flea’s ability to lay eggs and (2) pyriproxyfen on immature flea stages. Study 3 was designed to show that the rapid and complete kill provided by received fixed daily rations of standard commercial food and water ad libitum.

This study was approved by the Charles River Laboratories BioLabs Europe Animal Care and Use Committee. Masking was implemented by separation of function of study personnel. Individuals collecting pivotal data were not responsible for dosing. An individual who had no further responsibilities for data gathering performed the dosing. In this manner, the association of the treatment groups was masked from individuals collecting pivotal data.

To test the dogs’ ability to host fleas, 100 adult fleas were placed on the dogs on day –8; on day –6, fleas were combed from all dogs and the number of live fleas was recorded. All fleas were discarded. Only dogs shown to be good flea hosts, defined as those retaining \( \geq 50\% \) of fleas 48 hours after infestation, were used. On day –4, dogs were randomly assigned to each group using a computer-generated random assignment output.

Each dog was infested with 100 unfed, healthy, 1- to 2-week old adult *C. felis* on day –1. Flea infestations were accomplished by releasing fleas from vials and placing them along the thoracolumbar region of the dogs. Dogs in the treatment group \((n = 32)\) were dosed with a 12.5% pyriproxyfen topical solution at 0.1 ml/kg once on day 0. The volume of the test article to be administered was determined based on each animal’s body weight on day –1. The application site was between the shoulder blades; the hair was parted to reveal the skin, and the tip of a disposable plastic syringe containing the test article was then placed on the skin. The full content of the syringe was applied directly

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**Ctenocephalides felis, the cat flea, is a major ectoparasite of dogs throughout the world.**

**Materials and Methods**

**Study 1: Speed of Kill of Adult Cat Fleas on Dogs**

Sixty-four dogs (32 female and 32 male beagles; weight range, 8.7 to 17.9 kg; obtained from Charles River Laboratories BioLabs Europe) were placed on acclimatization on day –8. Animals were divided into two equal groups: one treated with a 12.5% (w/v) pyriproxyfen topical solution and one left untreated. Each group was divided into four subgroups of eight dogs each to be assessed for efficacy at either 12, 24, 36, or 48 hours after treatment. Animals were housed in pens \((1.7 \times 1.4 \text{ m})\) for the duration of the study. All dogs were healthy based on physical examination performed by a veterinarian and had not been treated with a product having an insecticidal effect within the 6 months before study initiation. The animals and the number of live fleas was recorded. All fleas were discarded. Only dogs shown to be good flea hosts, defined as those retaining \( \geq 50\% \) of fleas 48 hours after infestation, were used. On day –4, dogs were randomly assigned to each group using a computer-generated random assignment output.

Each dog was infested with 100 unfed, healthy, 1- to 2-week old adult *C. felis* on day –1. Flea infestations were accomplished by releasing fleas from vials and placing them along the thoracolumbar region of the dogs. Dogs in the treatment group \((n = 32)\) were dosed with a 12.5% pyriproxyfen topical solution at 0.1 ml/kg once on day 0. The volume of the test article to be administered was determined based on each animal’s body weight on day –1. The application site was between the shoulder blades; the hair was parted to reveal the skin, and the tip of a disposable plastic syringe containing the test article was then placed on the skin. The full content of the syringe was applied directly

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to the skin. The syringe was examined after administra-
tion to ensure successful application of the test article. Dogs in the control group (n = 32) were left untreated.

The speed of kill provided by pyriprole was assessed by performing flea counts 12, 24, 36, and 48 hours after treatment. Dogs were sedated (using xylazine and ketamine at dose rates of approximately 0.1 ml/kg) to facilitate counting of fleas. Each dog was combed for a minimum of 15 minutes using a fine-toothed flea comb. Any dog on which live, viable fleas were found in the last 5 minutes (of the 15-minute combing) was combed for an additional 5 minutes (for a total of 20 minutes). Live, viable fleas were defined as those that could maintain an upright posture and cling to animal hair. The number of live fleas was recorded, and fleas were then transferred to a glass container to be discarded.

Geometric means were calculated at each combing time point, and percent efficacy against adult fleas was calculated using the following equation, in which $C =$ geometric mean number of live viable fleas in the control group and $T =$ geometric mean number of live viable fleas in the treatment group:

$$\text{% Effectiveness} = 100 \times \left[ \frac{(C - T)}{C} \right]$$

Separate efficacy calculations were conducted for each flea-combing time point, and the number of live fleas present on control versus treated groups at each point was then compared using Kruskal–Wallis and Mann–Whitney U tests. All comparisons were tested at a $P \leq .05$ level of significance.

**Study 2: Efficacy of Pyriprole against Immature Life Stages of the Flea**

Twenty-four dogs (9 female and 15 male beagles obtained from the Stillmeadow Inc. colony) were placed on acclimatization on study day –14. Dogs weighed between 6.5 and 15.3 kg on day –1. Animals were housed in individual cages (1.0 × 1.2 m) for the duration of the study. All dogs were healthy based on physical examination performed by a veterinarian and had not been treated with a product having insecticidal properties within the 6 months before study initiation. The animals received fixed daily rations of standard commercial food and water ad libitum.

This study was approved by the Stillmeadow Animal Care and Use Committee. Masking was implemented by separation of function of study personnel. Individuals collecting pivotal data were not responsible for dosing. An individual who had no further responsibilities for data gathering performed the dosing. In this manner, the association of the treatment groups was masked from individuals collecting pivotal data.

Animals were tested for their ability to host fleas on day –13, when 100 cat fleas were placed on each dog. Dogs were combed for fleas on day –11, and the number of live fleas was recorded. Each dog that did not have 50% retention was reinfested with approximately 100 fleas on day –8 and combed on day –6. Only dogs shown to be good flea hosts, defined as those retaining ≥50% of fleas 48 hours after infestation or reinfestation, were used. On day –1, dogs were weighed, ranked by weight within gender, and randomly assigned to one of two treatment groups using a computer-generated random assignment output.

On day 0, dogs in Group 1 (n = 18) were treated with a 12.5% (w/v) pyriprole topical solution at 0.1 ml/kg and dogs in Group 2 (n = 6) were treated with tap water. The volume of the test item administered was determined based on each animal’s body weight on day –1. For dogs weighing ≤22 kg, the site of application was the base of the skull. Dogs weighing >22 kg received the test item in three equal aliquots, administered to the base of the skull,
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between the shoulder blades, and at the midpoint of the back. The test article was administered with a disposable plastic syringe using the procedure outlined for Study 1. Syringes were weighed before and after administration to check dosing accuracy.

**Adult Fleas**

On days 11, 18, 26, 39, 46, and 53, each dog was infested in the lumbosacral area with 1,000 newly emerged, unfed cat fleas obtained from the Stillmeadow laboratory colony. Flea infestation rates were high because collection of flea eggs from the pyriprole-treated group was desired and a high level of flea mortality was expected in this group. On days 14, 21, 29, 42, 49, and 56, dogs were combed as outlined for Study 1 (except dogs were not sedated) and adult live fleas were counted and returned to each dog. On days 15, 22, 30, 43, 50, and 57, live adult fleas were combed, counted, and discarded as described in Study 1.

The efficacy of pyriprole against adult fleas was determined on the basis of the percent reduction in live fleas in the treated group compared with the control group. Geometric mean flea counts were calculated, and efficacy was evaluated using the following equation, in which $C =$ geometric mean number of live fleas in the control group and $T =$ geometric mean number of live fleas in the treatment group:

$$\text{% Effectiveness} = 100 \times \frac{[C - T]}{C}$$

Separate efficacy calculations were conducted for flea counts recorded at each combing. To test the sensitivity of the model regarding pyriprole’s effect on percent reduction in live fleas, an analysis of variance using $\ln(flea\ count + 1)$ was performed between the treated group and the control group. All hypotheses were tested at a $P \leq 0.05$ level of significance.

**Flea Eggs**

To determine the effect of pyriprole on the ability of flea eggs collected from treated dogs to successfully complete development, up to 300 flea eggs were collected from each dog either by combing the eggs from the fur or by using a collection device placed under the perforated floor of each cage for 15 hours. Eggs were scheduled to be collected on days 14, 15, 21, 22, 29, 30, 42, 43, 49, 50, 56, and 57, but no eggs were produced from fleas on treated dogs until day 42. Subsequently, eggs collected from control dogs were discarded during this time. Eggs collected from treated and control dogs from day 42 to day 57 were immediately transferred to larval-rearing medium and incubated for 30 days. Emerged adult fleas were counted at the end of the incubation period, and the number was recorded. Pupae that had not released adult fleas at this time were dissected and counted. Fully developed encased adults were recorded and included with the number of emerged adult fleas. The effect of pyriprole on the ability of flea eggs collected from treated dogs to successfully complete development was determined on the basis of the percent developmental success (PDS) of the incubated eggs collected from dogs in the treatment

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**Live viable flea counts were significantly lower in the pyriprole-treated dogs than in the untreated control dogs at all combing time points.**
group compared with the PDS of incubated eggs collected from control dogs. Efficacy was calculated using the following equation, in which \( C \) = the PDS in the control group, \( T \) = the PDS in the treated group, and \( PDS = \frac{\text{number of developed adult fleas}}{\text{number of collected eggs}} \times 100 \).

\[
\% \text{ Effectiveness} = 100 \times \frac{(C - T)}{C}
\]

Separate efficacy calculations were conducted for eggs collected at each time point, where available. To test the sensitivity of the model regarding pyriprole’s effect on the PDS, an analysis of variance using \( \ln(\text{developmental success count} + 1) \) was performed between the treated group and the control group. All hypotheses were tested at a \( P \leq .05 \) level of significance.

**Larval and Pupal Stages**

To determine the effect of exposure of larval and pupal fleas to debris collected from treated dogs, approximately 100 mg of debris (dander, flea feces, hair, and scales) was collected from each dog either by combing the dog or by sweeping the collection device on days 14, 15, 21, 22, 29, and 30 and placed in a Petri dish. Fifty healthy, newly emerged flea larvae from the laboratory colony were placed in each dish. After 30 days of incubation, the number of emerged adult fleas, including fully developed encased adults, was recorded. The effect of the product on larval development after exposure to debris was determined on the basis of the number of emerged adult fleas from larvae exposed to debris from the treatment group compared with the number of emerged adult fleas from larvae exposed to debris collected from the control group. Geometric mean counts of emerged adult fleas were calculated, and efficacy was determined using the following equation, in which \( C \) = geometric mean number of emerged adult fleas after exposure of larvae to debris collected from control group dogs and \( T \) = geometric mean number of emerged adults fleas after exposure of larvae to debris collected from pyriprole-treated dogs:

\[
\% \text{ Efficacy} = 100 \times \frac{(C - T)}{C}
\]

Separate efficacy calculations were conducted for each collection time point. To test the sensitivity of the model regarding the product’s effect on the development of normal flea larvae exposed to debris for 30 days, an analysis of variance using \( \ln(\text{emerged adult flea count} + 1) \) was performed between flea larvae exposed to debris from the pyriprole-treated group and the control group. All hypotheses were tested at a \( P \leq .05 \) level of significance.

**Study 3: Efficacy against Fleas on Dogs in a Simulated Home Environment**

Sixteen dogs (eight female and eight male beagles; weight range, 6.4 to 11.2 kg; obtained from Stillmeadow dog colony) were placed on acclimatization on study day –14. All dogs were healthy based on physical examinations conducted by a veterinarian and had not been treated with a product having an insecticidal effect within the 6 months before study initiation. The animals received fixed daily rations of standard commercial food and water ad libitum.

This study was approved by the Stillmeadow Animal Care and Use Committee. Masking was implemented by separation of function of study personnel. Individuals collecting pivotal data were not responsible for dosing. An individual who had no further responsibilities for data gathering performed the dosing. In this manner, the association of the treatment groups was masked from individuals collecting pivotal data.

Animals were housed in individual pens (1.2 \( \times \) 1.2 m), with half of the pen floor covered in carpet to simulate a home environment. Environmental conditions conducive to flea reproduction were maintained. Animals were tested for their ability to host fleas on day –4, when 50
newly emerged, unfed cat fleas from a laboratory colony were placed on each dog. On day –2, fleas were combed from all dogs and the number of live fleas was recorded. All fleas were discarded. Only dogs shown to be good flea hosts, retaining ≥50% of fleas 48 hours after infestation, were used. On day –1, dogs were ranked by weight within gender and were randomly assigned to one of two groups using a computer-generated random assignment output.

Dogs in the control group (n = 8) were treated with mineral oil, while dogs in the treatment group (n = 8) were treated with a 12.5% pyriprole topical solution at a dose of 0.1 ml/kg. Dogs were treated on days 0 and 30. The volume of the test item to be administered was determined based on each animal’s body weight on day –1 and day 29. Dogs weighing <20 kg received the test article applied in a single spot between the shoulder blades. Dogs weighing ≥20 kg received the test article in three spots: at the base of the skull, between the shoulder blades, and at the midpoint of the back. Application was made with a disposable, plastic syringe using the procedure as described for Study 1.

Each dog was infested with 50 newly emerged unfed cat fleas of mixed sex, obtained from the Stillmeadow flea colony, on days 1, 7, and 14. Flea infestation was accomplished by releasing fleas from vials and placing them along the lumbosacral region of the dog. On days 7, 14, 21, 30, and 45, fleas were combed from each dog and counted using the procedures outlined for Study 1 (except dogs were not sedated). Up to 200 live fleas were then returned to the dog. On day 60, fleas were combed out, counted, and discarded, and the number of live fleas was recorded.

The adulticidal effects of the pyriprole solution were determined based on the comparative numbers of live adult fleas combed from the fur of the treated and control animals. Efficacy was calculated using the following equation, in which \( C \) = geometric mean number of live viable fleas in the control group and \( T \) = geometric mean number of live viable fleas in the treatment group:

\[
\% \text{ Efficacy} = 100 \times \frac{(C - T)}{C}
\]

Separate efficacy calculations were conducted for each treatment group at each flea combing time point. To test the sensitivity of the model regarding the product’s efficacy as an adulticide, an analysis of variance using ln(cumulative flea count + 1) was performed between the two treatment groups versus the control group. All hypotheses were tested at a \( P \leq .05 \) level of significance.

**RESULTS**

**Study 1: Knockdown Efficacy of Pyriprole against Adult Fleas**

Table 1 summarizes the mean flea counts from placebo- and pyriprole-treated dogs up to 48 hours after treatment. The efficacy of pyriprole was >90% at all time points and >99% at 24, 36, and 48 hours after treatment. Live viable flea counts were significantly lower in the pyriprole-treated dogs than the untreated control dogs at all combing time points (\( P \leq .0003 \).
in the Kruskal–Wallis test and \( P \leq 0.0009 \) in the Mann–Whitney U test).

**Study 2: Efficacy of Pyriprole against Immature Life Stages of the Flea**

Study 2 investigated the effect of pyriprole on the development of immature life stages of the flea and also confirmed the adulticidal effect of pyriprole and its effect on egg production (Table 2).

**Adult Fleas**

Numbers of live adult fleas were recorded at all time points except days 14 and 15, when flea numbers were mistakenly not recorded because of an oversight at the laboratory; therefore, data from these days were not included in the efficacy calculations and Table 2. The geometric mean number of adult fleas combed from placebo-treated dogs ranged from 199.72 (day 57) to 524.89 (day 21), whereas the geometric mean number of adult fleas on pyriprole-treated dogs ranged from 0.00 (days 22, 29, and 30) to 40.91 (day 56). Pyriprole-treated dogs showed statistically significantly fewer fleas than control animals at all counts except day 57 (\( P = .059 \)). Pyriprole provided flea control > 90% for all time points up to day 50.

**Flea Eggs**

To evaluate the impact of pyriprole on the ability of flea eggs collected from treated dogs to develop to the adult stage, up to 300 eggs were combed from dogs and/or collected from collection devices on days 14, 15, 21, 22, 29, 30, 42, 43, 49, 50, 56, and 57. Whereas flea eggs were collected from control dogs throughout the study, no eggs were available from pyriprole-treated dogs until day 42. Subsequently, eggs collected from control dogs before day 42 were discarded, and only data from eggs collected between days 42 and 57 are presented in Table 2. Pyriprole continued to reduce the numbers of eggs produced on treated dogs by >90% up to day 56 after treatment. However, although pyriprole had a significant effect on the number of eggs produced, once eggs were laid, pyriprole did not affect the ability of those eggs to mature to the adult stage. The efficacy of pyriprole in the inhibition of development to the adult stage ranged from -15.62% to 37.04%.

**Larval and Pupal Stages**

The effect of pyriprole-laden debris on the ability of healthy flea larvae to develop to adults is shown in Table 3. The geometric mean number of adult fleas developing from 50 healthy larvae exposed to debris from dogs
in the treatment group ranged from 2.34 (day 15) to 14.27 (day 29). The geometric mean number of adult fleas developing from larvae exposed to control group debris ranged from 35.85 (day 15) to 46.27 (day 30). Healthy larvae exposed to debris collected from pyriprole-treated dogs showed a significant reduction in the ability to develop to the adult stage compared with healthy larvae exposed to debris collected from placebo-treated dogs at all time points through day 30 after treatment. However, the percent efficacy was >90% only for days 14 and 15 after treatment.

**Study 3: Efficacy against Fleas on Dogs in a Simulated Home Environment**

The data collected demonstrated that pyriprole topical solution was effective in preventing the establishment of cat flea infestations on dogs held under simulated home environments (Table 4). The geometric mean number of living viable fleas on control group dogs ranged from 34.30 (day 7) to 83.29 (day 45). No live fleas were recovered from dogs treated with pyriprole at any time point; therefore, the calculated effectiveness of the pyriprole topical solution was 100% at each time point. An analy-

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**TABLE 2. Effect of a Single Pyriprole Treatment on Egg Production from 1,000 Cat Fleas Placed on Dogs on Posttreatment Days 11, 18, 26, 39, 46, and 53**

<table>
<thead>
<tr>
<th>Study Day*</th>
<th>Geometric Mean No. of Adult Fleas/Dog</th>
<th>% of Adult Fleas Killed</th>
<th>Geometric Mean No. of Eggs Collected/Dog</th>
<th>% Reduction in Flea Development†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pyriprole (n = 18)</td>
<td>Control (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>NA</td>
<td>NA</td>
<td>0.00</td>
<td>300.00</td>
</tr>
<tr>
<td>15</td>
<td>NA</td>
<td>NA</td>
<td>0.00</td>
<td>300.00</td>
</tr>
<tr>
<td>21</td>
<td>0.04</td>
<td>524.89</td>
<td>99.99a</td>
<td>0.00</td>
</tr>
<tr>
<td>22</td>
<td>0.00</td>
<td>406.57</td>
<td>100.00a</td>
<td>0.00</td>
</tr>
<tr>
<td>29</td>
<td>0.00</td>
<td>395.39</td>
<td>100.00a</td>
<td>0.00</td>
</tr>
<tr>
<td>30</td>
<td>0.00</td>
<td>312.26</td>
<td>100.00a</td>
<td>0.00</td>
</tr>
<tr>
<td>42</td>
<td>0.04</td>
<td>431.84</td>
<td>99.99a</td>
<td>1.08</td>
</tr>
<tr>
<td>43</td>
<td>0.09</td>
<td>363.10</td>
<td>99.97a</td>
<td>1.08</td>
</tr>
<tr>
<td>49</td>
<td>9.53</td>
<td>352.21</td>
<td>97.29a</td>
<td>20.21</td>
</tr>
<tr>
<td>50</td>
<td>6.28</td>
<td>232.27</td>
<td>97.30a</td>
<td>17.64</td>
</tr>
<tr>
<td>56</td>
<td>40.91</td>
<td>301.86</td>
<td>86.45a</td>
<td>88.20</td>
</tr>
<tr>
<td>57</td>
<td>30.66</td>
<td>199.72</td>
<td>84.65</td>
<td>66.82</td>
</tr>
</tbody>
</table>

*Day fleas were counted and eggs collected.
†% Reduction in Flea Development = \([100 \times (C - T)/C]\), where \(C\) = PDS in the control group and \(T\) = PDS in the treated group.

Statistically significant (\(P \leq .05\)).
— = not calculated (no eggs were produced by fleas on treated dogs); NA = data unavailable; PDS (percent developmental success) = [(no. of developed adults/no. of collected eggs) × 100].
sis of variance confirmed that the differences between the treatment group and the control group were statistically significant \((P < .05)\) at all time points. Pyriprole was thus confirmed to be effective in preventing \(C.\ felis\) infestation in dogs held in a simulated home environment.

**DISCUSSION**

These studies were conducted to investigate various facets of the efficacy of 12.5% pyriprole spot-on formulation against the cat flea, \(C.\ felis\), on dogs. The flea recovery rates in all groups before treatment ranged from 30% to 99% and confirmed the vitality of the fleas used and the suitability of the study dogs as flea hosts.

Study 1 showed that the speed of kill provided by pyriprole is very fast for a topical solution. After 12 hours exposure, 96.5% of fleas on dogs were killed by pyriprole. This increased to >99% at 24, 36, and 48 hours. The

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**TABLE 3. Effect of Exposure to Debris from Pyriprole-Treated Dogs (Single Treatment on Day 0; \(n = 18\)) vs. Debris from Untreated Control Dogs (\(n = 6\)) on the Ability of Healthy Flea Larvae to Complete Development to the Adult Stage**

<table>
<thead>
<tr>
<th>Study Day*</th>
<th>Geometric Mean No. of Adults Produced from 50 Healthy Larvae Exposed to Debris</th>
<th>% Reduction in Flea Development†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pyriprole-Laden Debris</td>
<td>Control Debris</td>
</tr>
<tr>
<td>14</td>
<td>3.31</td>
<td>44.54</td>
</tr>
<tr>
<td>15</td>
<td>2.34</td>
<td>35.85</td>
</tr>
<tr>
<td>21</td>
<td>9.27</td>
<td>44.34</td>
</tr>
<tr>
<td>22</td>
<td>8.38</td>
<td>41.90</td>
</tr>
<tr>
<td>29</td>
<td>14.27</td>
<td>45.85</td>
</tr>
<tr>
<td>30</td>
<td>11.86</td>
<td>46.27</td>
</tr>
</tbody>
</table>

*Days debris collected.
†% Reduction in Flea Development = \([100 \times (C – T)/C]\), where \(C =\) geometric mean no. of emerged adult fleas after exposure of larvae to debris from untreated dogs and \(T =\) geometric mean no. of emerged adult fleas after exposure of larvae to debris from pyriprole-treated dogs.
‡Statistically significant \((P \leq .05)\).

**TABLE 4. Efficacy of Two Pyriprole Treatments (Days 0 and 30) against Adult Fleas on Dogs Housed in Simulated Home Environments**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Geometric Mean No. of Adult Fleas Combed from Dogs</th>
<th>% Efficacy*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>Placebo ((n = 8))</td>
<td>34.3</td>
<td>54.32</td>
</tr>
<tr>
<td>Pyriprole ((n = 8))</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*% Efficacy = \([100 \times (C – T)/C]\), where \(C =\) geometric mean no. of live viable fleas in the control group and \(T =\) geometric mean no. of live viable fleas in the treatment group.
‡Statistically significant \((P \leq .05)\).
speed of kill of pyriprole is comparable to that reported in similar experiments with a fipronil spot-on (a phenylpyrazole)\textsuperscript{3} and an imidacloprid spot-on (a neonicotinoid).\textsuperscript{5,6} For a selamectin spot-on (a macrocyclic lactone), it took 36 hours after the first treatment to achieve an efficacy (>98%) similar to that seen with other commercial spot-ons, and selamectin spot-on was practically inefficacious (<15% control) at 12 and 24 hours.\textsuperscript{6}

The effect of pyriprole’s speed of kill on the ability of adult fleas to lay eggs is clearly shown in Study 2. In the face of severe flea challenge (1,000 fleas/dog at each infestation), pyriprole provided ≥99% kill for 43 days and >97% kill of adult fleas for 50 days following a single treatment. In addition, the speed with which pyriprole killed adult fleas resulted in a complete lack of egg production for at least 30 days after treatment. These data further substantiate the value of pyriprole in the control of adult flea populations. However, once fleas lived long enough to lay eggs (day 42), a significant inhibitory effect on the ability of those eggs to develop to the adult stage was not observed in this study. A similar absence of a significant effect on the development of eggs from surviving fleas has been reported after treatment with a fipronil spot-on solution.\textsuperscript{7}

Exposure to pyriprole-laden debris (dander, scales, hair, and flea feces) collected from treated dogs significantly reduced the number of healthy larvae that developed to the adult stage at all time points for 30 days after treatment. These results support a possible role for pyriprole in the prevention of flea infestation by disrupting the flea life cycle when larvae are exposed to pyriprole-laden debris, as well as by targeting incoming adult fleas. Other flea adulticides have also shown activity against flea larvae, both when cultivated in rearing medium previously treated with the corresponding active ingredients\textsuperscript{8} and after mixing the rearing medium with debris collected from dogs previously treated with the commercial products.\textsuperscript{9,10} However, these studies did not investigate the quantitative effect of the adulticides on a given larval population; thus, a comparison of specific results is not possible.

All facets of pyriprole’s effect on flea mortality were evaluated in Study 3; pyriprole demonstrated prevention of the establishment of a flea infestation in a simulated home environment in which conditions were maintained for optimal flea reproduction. When pyriprole was used once every 30 days, no live viable fleas were recovered from pyriprole-treated dogs throughout the duration of the study (60 days), and the calculated efficacy of pyriprole against \textit{C. felis} was 100% at each time point. This level of control was achieved under significant flea challenge, in that three infestations of 50 fleas each (for a total of 150 fleas) were placed on each dog and environmental conditions were optimal for the development of any flea eggs produced. A similar level of flea control on dogs held in simulated home environments has also been reported for various other flea adulticidal spot-ons (selamectin, fipronil, imidacloprid) after monthly treatments.\textsuperscript{11–13}

\section*{CONCLUSIONS}

The data collected from these studies show that 12.5% pyriprole provided rapid, long-lasting efficacy against adult cat fleas even under severe flea challenge (up to 1,000 fleas/dog at each infestation). Speed of kill data indicate that treatment with this product can interrupt an already established adult flea infestation, and monthly treatment can prevent reinfestation. Pyriprole affects the immature stages of the flea by killing adult fleas before they lay eggs for at least 30 days after treatment. The residual effect of pyriprole on debris (dander, scales, hair, and flea feces) significantly decreased the ability of flea larvae to complete de-
velopment to the adult stage for 30 days after application. Based on the results of these studies, 12.5% pyriprole represents a valuable tool in the control of the cat flea, *C. felis*, on dogs.

**REFERENCES**