Efficacy of Two 65% Permethrin Spot-on Formulations Against Canine Infestations of Ctenocephalides felis and Rhipicephalus sanguineus*

Richard G. Endris, PhD\textsuperscript{a}
Ronald Everett, PhD\textsuperscript{b}
Jerry Cunningham, MS\textsuperscript{b,c}
Terry L. Katz, MS\textsuperscript{a}
Kenneth Thompson, DVM, PhD\textsuperscript{a}

\textsuperscript{a}Schering Plough Animal Health Corp.
1095 Morris Avenue
Union, NJ 07083

\textsuperscript{b}Ag Research Consultants, Inc.
P.O. Box 606
Greenbrier, AR 72438

\textsuperscript{c}Current Address:
PRA International
4 Industrial Way West
Eatontown, NJ 07724

\section*{ABSTRACT}

The efficacy of two formulations of a topically applied 65\% permethrin spot-on for dogs (Defend\textsuperscript{®} EXspot\textsuperscript{®} Treatment for Dogs, Schering-Plough Animal Health Corp.) was evaluated against experimental infestations of the cat flea, Ctenocephalides felis, and the brown dog tick, Rhipicephalus sanguineus. Thirty dogs were randomly allocated to treatment with 65\% permethrin in diethylene glycol monomethyl ether (original formulation), 65\% permethrin in propylene glycol monomethyl ether (test formulation), or to an untreated control group. Dogs assigned to treatment with a permethrin formulation received either 1 or 2 ml of the formulation in accordance with label directions on Day 0. One hundred unfed, adult cat fleas and 50 unfed, adult ticks were placed on each dog on Days –1, 5, 12, 19, 26, 33, and 40. Live fleas and ticks were counted on each dog on Days 2, 7, 14, 21, 28, 35, and 42. Treatment of dogs with either formulation of 65\% permethrin significantly ($P < .05$) reduced the number of live fleas and ticks from Days 2 through 42. No statistical differences were noted between the formulations regarding efficacy against \textit{C. felis} or \textit{R. sanguineus}.

\section*{INTRODUCTION}

Topically applied insecticide formulations for the treatment and control of flea and tick infestations on dogs have evolved over the past 4 to 5 decades from relatively high-volume sprays, dips, and powders applied over the entire body surface to relatively low-volume ‘spot-on’ formulations applied to one or a few locations on the dog’s back. Defend\textsuperscript{®} EXspot\textsuperscript{®} Treatment for Dogs (Schering-Plough Animal Health Corp.), a 65\% permethrin in diethylene glycol monomethyl ether, was the first high-concentration, low-volume, spot-on product introduced into global markets. Introduced in the United States in 1991, the 65\% permethrin
formulation has demonstrated efficacy in laboratory challenge studies against fleas (*Ctenocephalides felis*), ticks (*Ixodes scapularis, Ixodes ricinus, Ixodes holocyclus, Dermacentor variabilis, Amblyomma americanum, Rhipicephalus sanguineus*), mosquitoes (*Aedes aegypti*), lice (*Trichodectes canis*), mites (*Cheyletiella yasguri*), and sand flies (several species, including *Phlebotomus perniciosus*). The duration of activity (efficacy $\geq$ 90%) observed for each ectoparasite species ranged from 3 to 6 weeks. In addition, the 65% permethrin formulation has been shown to prevent flea-bite dermatitis and has exhibited high-order repellency against ticks, mosquitoes, and sand flies.

Criteria for selection of excipients for low-volume (1 to 2 ml) spot-on formulations include nontoxicity, high-order solubility for the active ingredient (to decrease the dose volume), compatibility with direct application to the hair coat to maintain aesthetic characteristics, ability to facilitate translocation of the active ingredient from the site of application over the entire body surface, and ability to aid in retention of the active ingredient on the skin and hair coat to improve the duration of efficacy. Low-volume, high-concentration, topically applied formulations for control of ectoparasites, such as the patented 65% permethrin in a glycol ether formulation, offer the advantages of ease of application and reduced or eliminated drying time after treatment. However, there is little published information regarding the effect of different inert excipients on the level and duration of efficacy of low-volume spot-on formulations. Therefore, a study was designed to test whether changes in excipients within the same chemical class could improve the overall performance of the product. The study described here was designed to investigate the effect of two different solvents in the glycol ether class on the duration of efficacy of 65% permethrin against flea and tick infestations on dogs.

**MATERIALS AND METHODS**

**Dogs**

Forty dogs of mixed breeds, including both sexes, ranging from 2.2 to 31.5 kg in weight and from 1 to 9 years in age, were washed with a noninsecticidal soap on Day −9. Dogs were initially infested on Day −2 with 100 unfed adult fleas and 50 unfed adult ticks. Thirty of the dogs that were in good health and that retained at least 34 fleas and nine ticks per dog 3 days after the initial infestation were included in the study. All dogs were individually housed indoors in heated, chain-link–fenced runs with forced-air ventilation and concrete floors that were cleaned daily. Dogs of different treatment groups were separated either by an empty run or by a solid barrier between cages. Fluorescent lighting was provided for approximately 10 hours per day. Commercial dog food was provided to each dog daily, and water was available ad libitum.

**Allocation and Treatments**

The 30 selected dogs were ranked in descending order according to flea counts determined on Day −2. From this ranking order, dogs were stratified into 10 groups of three dogs each. Within each stratum thus formed, dogs were randomly allocated to three treatment groups, as follows: 65% permethrin in diethylene glycol monomethyl ether (Methyl Carbitol, Comet Chemical Company Ltd, Innisfil, Ontario, Canada) (original formulation), 65% permethrin in propylene glycol monomethyl ether (Dowanol® PM, The Dow Chemical Company) (test formulation), and untreated control. Location assignments of treatment groups within the testing facility were made randomly.

Dogs were reinfested with fleas and ticks on Day −1, and a single application of the appropriate 65% permethrin formulation was made with a graduated syringe on Day 0. For dogs that weighed less than 15 kg, 1 ml was applied...
to the skin at the shoulder blades. For dogs that weighed 15 kg or more, 1 ml was applied to the skin at the shoulder blades and 1 ml was applied to the skin on the hindquarters.

Flea and Tick Challenges
Unfed, adult cat fleas (*C. felis*) were from a laboratory colony maintained at the test facility and unfed, adult brown dog ticks (*R. sanguineus*) were from EL Labs, Soquel, CA. After the pretreatment infestation on Day –1, repeat infestations were made on Days 5, 12, 19, 26, 33, and 40.

Flea and Tick Counting
The number of fleas and ticks on each dog was determined on Days 2, 7, 14, 21, 28, 35, and 42. The individuals performing flea and tick counting were blinded regarding the treatment group to which each dog was assigned. Blinding was assured by having one person retrieve the dog from the kennel room for inspection by a different person who performed the flea and tick counts. Additionally, dogs of each treatment group were brought to the observation room in random order. On each day of parasite evaluations, ticks were located, counted, and removed. Dogs were combed for at least 5 minutes (or until no more fleas were found, whichever was longer) with an extra-fine flea comb, and live fleas were removed and counted. Laboratory personnel and animal handlers wore impermeable gloves and aprons, which were changed after each group of animals was combed. Counting tables were covered with disposable plastic, which was changed between treatment groups. Flea combs were rinsed with alcohol between dogs. Dogs were observed daily to assess their general health and any signs of adverse reactions to treatment. Treatment sites on the animals were observed for signs of irritation at each infestation and count.

Statistical Analysis
Geometric mean flea and tick counts were calculated for each treatment group on each observation day. Counts were transformed by $\log_{10}(\text{count} + 1)$ transformation prior to analysis to stabilize the variance. Efficacy for each parasite was determined for each treatment group at each evaluation by comparison of counts at that evaluation versus the count on Day –2. Percent efficacy for each 65% permethrin formulation versus the nontreated group was calculated by Abbott’s formula,

$$\% \text{ Efficacy} = \frac{\text{Geometric mean count/dog (treated)} - \text{Geometric mean count/dog (control)}}{\text{Geometric mean count/dog (control)}} \times 100$$

The logarithmically transformed counts were analyzed by a one-way analysis of variance (ANOVA). The treatment effect was tested against the residual error at the 5% level. Using the SAS Proc Mixed procedure, the differences between the least squares means (LSM) and the $P$ values associated with these differences were computed. Means were compared using a two-sided (original formulation versus new formulation) or one-sided (control versus treated groups) $t$-statistic at the 5% level.

RESULTS
Geometric mean live fleas and ticks per dog and the percent efficacy (% killed) are shown in Tables 1 and 2. Treatment of dogs with the 65% permethrin in diethylene glycol monomethyl ether reduced flea numbers on dogs by at least 91.1% from Days 2 through 35, compared with 96.2% for dogs treated with 65% permethrin in propylene glycol monomethyl ether. The reduction in flea numbers on Day 42 was 80.0% with the diethylene glycol monomethyl ether formulation and 88.7% with the propylene glycol
monomethyl ether formulation. Both formulations significantly \((P < .05)\) reduced the number of live fleas recovered from dogs throughout the 42-day study, and the difference between the two formulations was not significantly different \((P \leq .05)\).

Treatment of dogs with the 65% permethrin in diethylene glycol monomethyl ether reduced

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**TABLE I. Efficacy of Two Glycol Ether Formulations of 65% Permethrin Against the Cat Flea, *Ctenocephalides felis*, After Placement of 100 Adult Fleas 48 Hours Before Counting**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day –2</th>
<th>Day 2</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>69.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>65% Permethrin in diethylene glycol monomethyl ether</td>
<td>66.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>65% Permethrin in propylene glycol monomethyl ether</td>
<td>68.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

\[ \% \text{ Efficacy} = \frac{\text{Geometric mean fleas/dog (control)} - \text{Geometric mean fleas/dog (treated)}}{\text{Geometric mean fleas/dog (control)}} \times 100 \]

<sup>a,b</sup>Means in the same column with different letter superscripts are significantly different \((P < .05)\).

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**TABLE II. Efficacy of Two Glycol Ether Formulations of 65% Permethrin Against the Brown Dog Tick, *Rhipicephalus sanguineus*, After Placement of 50 Unfed Ticks 48 Hours Before Counting**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day –2</th>
<th>Day 2</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>24.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>65% Permethrin in diethylene glycol monomethyl ether</td>
<td>24.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>65% Permethrin in propylene glycol monomethyl ether</td>
<td>31.0&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

\[ \% \text{ Efficacy} = \frac{\text{Geometric mean ticks/dog (control)} - \text{Geometric mean ticks/dog (treated)}}{\text{Geometric mean ticks/dog (control)}} \times 100 \]

<sup>a,b</sup>Means in the same column with different letter superscripts are significantly different \((P < .05)\).
brown dog tick numbers by 93.8% to 100% between Days 2 and 42, compared with 89.3% to 99.4% for the propylene glycol monomethyl ether formulation. Mean tick recovery from dogs treated with either formulation was significantly ($P = .0001$) different from untreated controls, but no significant differences ($P < .05$) were noted between the formulations.

### DISCUSSION

Efficacy against fleas was first assessed in this study after fleas had been exposed to 65% permethrin on the dogs for 48 hours. Under these conditions, the efficacy for both formulations was approximately 95%. Previous studies have shown that 98% or more of the fleas were killed within 24 hours and nearly 80% were killed within 8 hours of exposure to a 65% permethrin formulation (Table 3). The data from the present study and results previously reported (Table 3) show that there is rapid elimination of fleas on dogs following treatment with a 65% permethrin spot-on. The data also indicate that nearly 95% of fleas infesting dogs at 3 weeks after treatment were killed within 24 hours. A higher level of efficacy was observed for both formulations in the present study 4 weeks after treatment when compared with results shown in Table 3. This improved efficacy may be due to the increased period of flea exposure (48 hours) to permethrin. From the report of Liebisch and Reimann, it is unclear why the efficacy declined from 24 to 48 hours (Days 22 to 23 and 29 to 30) when fleas were combed from the dogs and counted and live surviving fleas were replaced on the dogs for a further 24-hour exposure followed by removal by combing and counting. Efficacy after the second counting for each exposure period would be expected to increase. The efficacy of both formulations in the present study exceeded 90% 5 weeks after treatment, and the propylene glycol formulation demonstrated greater than 88% efficacy 6 weeks after treatment. It is likely that the rapid onset and duration of flea killing by the 65% permethrin spot-on could enhance control of dog tapeworms, *Dipylidium caninum*, for which fleas serve as the intermediate host.

The brown dog tick, *R. sanguineus*, serves as

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**TABLE 3. Efficacy of a 65% Permethrin Spot-on Against Cat Fleas, *Ctenocephalides felis*, Determined in Studies Conducted in Dogs**

<table>
<thead>
<tr>
<th>Time After Infestation</th>
<th>Duration of Flea Exposure to Permethrin on Dogs</th>
<th>Efficacy Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liebisch and Reimann</td>
</tr>
<tr>
<td>8 hours</td>
<td>8 hours</td>
<td>Not done</td>
</tr>
<tr>
<td>24 hours</td>
<td>24 hours</td>
<td>100%</td>
</tr>
<tr>
<td>48 hours</td>
<td>48 hours</td>
<td>100%</td>
</tr>
<tr>
<td>8 days</td>
<td>24 hours</td>
<td>100%</td>
</tr>
<tr>
<td>9 days</td>
<td>48 hours</td>
<td>No surviving fleas</td>
</tr>
<tr>
<td>15 days</td>
<td>24 hours</td>
<td>100%</td>
</tr>
<tr>
<td>16 days</td>
<td>48 hours</td>
<td>No surviving fleas</td>
</tr>
<tr>
<td>22 days</td>
<td>24 hours</td>
<td>96.2%</td>
</tr>
<tr>
<td>23 days</td>
<td>48 hours</td>
<td>94.9%</td>
</tr>
<tr>
<td>29 days</td>
<td>24 hours</td>
<td>73.8%</td>
</tr>
<tr>
<td>30 days</td>
<td>48 hours</td>
<td>57.3%</td>
</tr>
</tbody>
</table>
vector, intermediate host, or definitive host of the canine pathogens shown in Table 4. The duration of efficacy observed for both formulations of 65% permethrin in the present study as well as the repellent properties previously reported suggest that treatment of dogs with 65% permethrin will aid in prevention of these diseases. In the case of the spotted fever group rickettsiae, and *Ehrlichia canis* effective tick control on dogs in close proximity to hu-
mans may help to reduce human infection rates of these pathogens.

**CONCLUSIONS**

Two formulations of 65% permethrin (diethylene glycol monomethyl ether and propylene glycol monomethyl ether) were compared for efficacy against the cat flea, *C. felis*, and the brown dog tick, *R. sanguineus*. Both formulations performed well and provided excellent long-term efficacy against these parasites of dogs.

**REFERENCES**


