

# Immediate and Residual Speed of Kill of FRONTLINE® PLUS (Fipronil + (S)-Methoprene) Against *Rdl*-Homozygous Fleas on Dogs Assessed at Twelve, Eighteen, and Twenty Four Hours Post-treatment and Following Subsequent Weekly Infestations

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**KEY WORDS:** Dog, flea, *Ctenocephalides felis*, fipronil, efficacy, speed of kill, *Rdl*

## ABSTRACT

Flea efficacy of FRONTLINE® Plus (fipronil + (S)-methoprene) was evaluated at 12, 18, and, 24 hours following product application and subsequent infestations on days -1, 7, 14, 21, 28, and 35 after a single treatment. Twenty-four dogs were randomly allocated to groups based on 24 hour flea counts, following day -5 pretreatment flea infestations with 50 unfed fleas. Dogs were ranked by descending order flea counts within sex to treatment blocks. Each block was then randomly allocated to one of the following groups: Untreated control (24-hour count), FRONTLINE Plus (12-hour count), FRONTLINE Plus (18-hour count),

FRONTLINE Plus (24-hour count). All dogs but controls received a single treatment on day 0 with FRONTLINE Plus according to the manufacturer's instructions. Treated and control dogs were each infested with approximately 100 unfed fleas (*Ctenocephalides felis*) on days -1, 7, 14, 21, 28, and 35, and post-treatment and subsequent post-infestation flea counts were performed to determine efficacy over time. The flea strain used in this study was the BerTek strain, which was selected and established 3 years prior, and as of this publication, remains a closed colony. Genomic studies have determined that the BerTek strain is homozygous for the *Rdl* gene sequence (dieldrin resistant). In this study, dogs treated with FRONTLINE Plus had 100% efficacy at the 12, 18, and 24 hour assessments on day

1 post treatment, and on days 7, 14, and 21 post-infestation. On day 28, 100% efficacy was achieved by 24 hours post- infestation, and efficacy was >99% at the 12 and 18 hour time points. Efficacy continued against the day 35 infestation with >99% efficacy at 24 hours post infestation against this Rdl homozygous flea strain.

## INTRODUCTION

Numerous timed-exposure studies have investigated the speed of kill of FRONTLINE brand products on dogs and cats since its introduction on the U S market in the mid 1990's.<sup>1,2,3,4,5</sup> Such controlled studies provide an accurate indication of the on-animal activity of fipronil against flea populations from targeted flea strains. Finite time-based assessments using field studies are not possible, as they are subject to numerous variables (eg, environmental contamination by wildlife or feral animals) that diminish the value of such field studies for accurately analyzing flea adulticidal activity. Therefore, controlled laboratory studies using different strains of fleas offer the only means for such time-based efficacy assessments.

Previous work has shown that insecticide resistance can develop as a result of selection within a population for structural changes in a target protein, resulting in an insect less sensitive to an insecticide's effects.<sup>6</sup> An example of this is a result of substitution, at Alanine302, in the M2 region of a  $\gamma$ -aminobutyric acid (GABA)-gated chloride channel, which confers resistance to cyclodienes, described as the Rdl (resistance to dieldrin) gene.<sup>7</sup> Certain insects with this genetic makeup have been shown to be less susceptible to certain macrolides such as dieldrin.<sup>7,9</sup> Some researchers suggested that the presence of this same single-point mutation would lead to varying levels of cross-resistance to fipronil, a phenylpyrazole insecticide that also acts on the GABA-gated chloride channel.<sup>6,8,9</sup> Recent genomic research on available flea strains has identified the BerTek flea colony as a flea strain that is homozygous for the Rdl gene.<sup>10</sup> Thus, according to theories of Bass et al,<sup>6</sup> Cole et

al,<sup>8</sup> and Scott et al<sup>9</sup> The BerTek fleas would display cross-resistance to fipronil.

The present study was performed to assess the speed and duration of efficacy of fipronil in a timed-exposure challenge, testing these parameters of efficacy against Rdl homozygous fleas from the BerTek colony.

## MATERIALS AND METHODS

Thirty mixed-breed dogs were infested with 50 *Ctenocephalides felis* fleas, pre-allocation, on day -5. Twenty-four hours later, counts were performed, and the 24 dogs with the highest retained flea counts were selected for group assignment. These dogs were allocated within sex by pre-treatment flea counts to treatment blocks. Then, each block was randomly allocated by a draw to one of the following groups of six dogs each: Untreated control (24-hour count), FRONTLINE Plus (12-hour count), FRONTLINE Plus (18-hour count), FRONTLINE Plus (24-hour count), where they were assigned throughout the duration of the study.

All dogs were maintained and handled with due regard for their welfare. They were housed individually in chain-link runs with concrete floors with sufficient space for exercise requirements as specified by USDA-APHIS animal welfare requirements. Treatment groups were physically separated by space. Dogs were fed a commercial diet daily, and water was provided ad libitum. All animal care procedures conformed to guidelines established by the Institutional Animal Care and Use Committee (IACUC) at Merial and the local IACUC at the research facility.

On day 0, each treated dog received 1 pipette of FRONTLINE Plus that was appropriate for its weight, applied according to the manufacturer's recommendation. Because all dogs were similar in size, they all received 1.34 mL topically in a single application at the base of the neck, just anterior to the shoulder blades. Flea infestations were applied to the lateral aspect of each dog, using approximately 100 unfed

**Table 1:** Geometric mean flea counts from Untreated Control (24 hour) and fipronil/(S)-methoprene treated dogs (12, 18 & 24 hours) post-treatment (day 1) or post-infestation.

**Geometric Mean Flea Counts**

Group	D1	D7	D14	D21	D28	D35
Untreated control (24 hour count)	76.1	84.6	75.3	81.1	80.1	75.4
FRONTLINE Plus (12 hour count)	0	0	0	0	0.5	1.1
FRONTLINE Plus (18 hour count)	0	0	0	0	0.5	1.8
FRONTLINE Plus (24 hour count)	0	0	0	0	0	0.6

adult fleas per dog on days -1, 7, 14, 21, 28, and 35, and then the fleas were recovered from the appropriate treatment groups at 12, 18, and 24 hours post-infestation according to group allocation. The control dogs were not treated, and had a comb count and flea removal performed at 24 hours after infestations.

**RESULTS**

All control dogs retained flea infestations during the post-treatment study phase, with an average of 78.8 fleas per dog (geometric mean counts at 24 hours post-infestation).

Flea efficacy for FRONTLINE Plus treated dogs was 100% at all time points on days 7, 14, and 21. Efficacy of 100% was maintained on day 28 at the 24-hour count and was >99% at both 12 and 18 hours post-infestations on day 28. Flea kill at 24 hours was >99% for the day 35 count.

**DISCUSSION**

All dogs used in this study demonstrated the ability to support flea infestations prior to treatment (day -5 infestations), and following subsequent infestations, the untreated control dogs supported infestation rates greater than 75% at each 24-hour count throughout the study. The use of a single untreated control group, with a count at 24 hours, is an accepted practice for such speed of kill studies. Although, it is likely that had there also been 12 and 18 hour untreated control counts, the resulting efficacies for the 12 and 18 hour treated counts on days 28 and 35 could have been higher. On Untreated Controls dogs between 12 and 24 hours, the numbers of fleas could at best

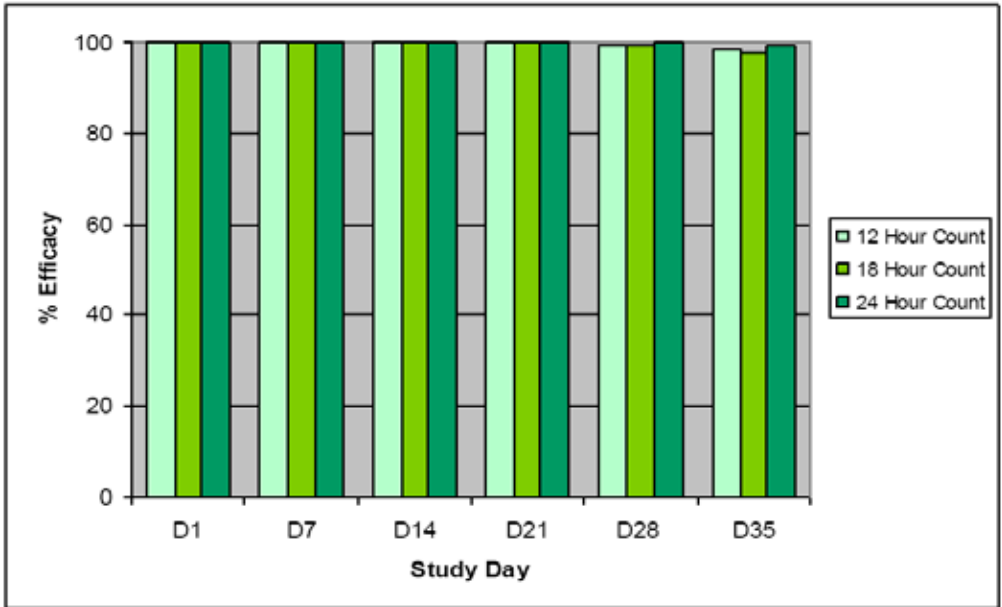
stay the same, but they would be more likely to decline (due to grooming, etc), and that would only decrease the potential percent efficacy. However, even when using 24-hour control counts to calculate 12 and 18 hour efficacies...the efficacy of FRONTLINE Plus against this flea strain at days 28 and 35 was 97.6 to 99.4%.

The BerTek strain of fleas was isolated, adapted, and had been raised as a closed colony under laboratory conditions for over 3 years prior to initiation of this study. After this strain was fully established and stabilized, it was analyzed for the presence of the Rdl gene sequence (resistant to dieldrin), and it was found to be homozygous for this mutation.<sup>10</sup> The genetic testing results of the study by Brunet et. al demonstrated that closed colonies of fleas that were fully susceptible to topically applied fipronil, were actually homozygous for the Rdl gene mutation. That paper used existing evidence to illustrate that level of clinical efficacy.

This study verifies that the efficacy, as determined by initial and residual speed of kill, was not affected in this flea strain. Thus, the presence of the Rdl gene mutation appears to not directly correlate to reduced clinical efficacy of fipronil against cat fleas. In fact, the Rdl gene SNP is widely distributed and has been demonstrated to be uniformly present in other closed strains of cat fleas that have been shown to be susceptible to topical fipronil products.

Further, this study demonstrated efficacy results that were consistent with those seen in studies performed in previous years demonstrating high efficacy. Dogs treated

**Figure 1: Immediate and Residual Efficacy of FRONTLINE® Plus (Fipronil + (S)-Methoprene) Against Rdl-Homozygous Fleas on Dogs Assessed at 12, 18, and 24 Hours Post-treatment/Post-infestation vs. Negative Controls assessed at 24 hours.**



with FRONTLINE Plus had significantly ( $p < 0.05$ ) fewer fleas than the controls at 12, 18, and 24 hours after infestation on all flea count dates after treatment. FRONTLINE Plus demonstrated rapid and complete elimination of existing flea infestations and residual activity for 5 weeks after treatment, killing all fleas within 12 hours of contact with treated dogs for the first 3 weeks (days 7, 24, and 21) and within 24 hours on day 28.

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