

Efficacy of Fipronil–(S)-Methoprene on Fleas, Flea Egg Collection, and Flea Egg Development Following Transplantation of Gravid Fleas onto Treated Cats*

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CLINICAL RELEVANCE

The goal of this study was to assess the insect growth regulator activity of the combination product fipronil–(S)-methoprene under a severe challenge model. Gravid fleas were allowed to feed on untreated donor cats for 48 hours before being transplanted onto untreated control cats and treated cats (treated once on day 0); 24 hours later, adult fleas were collected from all cats and counted to assess the 24-hour kill efficacy against the transplanted fleas, and flea eggs were collected and incubated to assess viability. The process was repeated weekly for 11 weeks. The 24-hour efficacy against transplanted adult fleas in the treated group was about 100% for the first 3 weeks and gradually declined to 93.4% by week 6. Egg production numbers were reduced on the treated cats compared with controls, with geometric mean egg counts on treated cats reduced from 76.9% to 96.3% during the initial 6 weeks of the study. The combination product was 100% ovicidal through day 56 and was still about 98% effective against eggs at the end of the study (day 76).

INTRODUCTION

It has long been acknowledged that an integrated approach provides the best direct control of flea infestations on domestic animals.^{1–3} Such an approach not only targets activity against adult fleas but also affects other stages

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of the flea life cycle. Most flea control products that offer integrated control combine an adulticide with an insect growth regulator (IGR).⁴ IGRs affect eggs and subsequent juvenile development. They can affect eggs while they are still inside the adult flea or by direct penetration of eggs when they come in contact with treated skin and hair.^{5,6} Environmental infesta-

tion by multiple life stages necessitates several treatments at regular intervals to resolve an existing flea problem.^{2,6,7} However, combining an adulticide with an IGR may help speed the rate of control.⁸ These same combinations rarely allow the opportunity to critically assess IGR activity alone because the adulticide typically eliminates or negatively affects fleas before any eggs are produced throughout most of the monthly treatment interval.

Essentially all naturally occurring infestations involve fleas that are newly emerged from cocoons in the environment. When these newly emerged fleas come in contact with animals treated with the combination of fipronil-(S)-

felt that this scenario offered the most severe challenge possible.

■ MATERIALS AND METHODS

Nineteen shorthaired cats were included in this trial. Nine cats were selected to be used only as flea-donor cats, providing engorged, gravid fleas that had the opportunity to feed for 48 hours. The remaining 10 cats were randomly allocated, first by sex and then by body weight and receptivity to flea infestations, to form two groups of five cats each. By random draw, Group 1 was selected as the control group and Group 2 as the treatment group. Each of the five cats in Group 2 was treated once on day

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methoprene, there is little chance they will survive long enough to produce any significant numbers of eggs during the normal monthly treatment interval.^{4,9} Thus, a typical weekly post-treatment challenge or infestation study design would not readily offer the opportunity for critical assessment of the IGR activity of the (S)-methoprene component in a combination product. Therefore, a different infestation model was deemed necessary for accurate assessment of IGR activity. Although relocation of actively reproducing female fleas from one host to another is possible,¹⁰ it is understood that this is likely rare in a natural setting. However, to fully assess the ovicidal activity of an adulticide-IGR combination product, it was decided that the transfer of actively reproducing fleas from untreated to treated cats would allow production of sufficient numbers of eggs for analysis of the product's ovicidal activity. Because the primary goal of this study was to critically assess the IGR activity, we

0 with a single dose of the commercially available combination of fipronil-(S)-methoprene (Frontline Combo [Frontline Plus in the United States], Merial). Per the manufacturer's instructions, the 0.5-ml dose (9.8% fipronil [7.5–13 mg/kg] and 11.8% (S)-methoprene [10–20 mg/kg]) was applied directly to the skin on the dorsal aspect of the base of the neck just anterior to the shoulder blades.

Throughout the study, cats were maintained in individual stainless steel cages with trays beneath each cage to collect flea eggs. The cats were managed similarly and with due regard for their safety and well-being. They were handled in compliance with and with the approvals of the National Veterinary School of Toulouse (France) Institutional Animal Care and Use Committee (IACUC) and any applicable local regulations, as well as requirements of any local IACUC, which may have exceeded the university IACUC. Cats were acclimated to their en-

vironment 14 days before treatment. All cats received food and drinking water ad libitum.

Pretrial experiments confirmed the success of the transplant methodology of this protocol and helped to determine the appropriate quantity of fleas necessary to achieve the targeted infestation rate of 50 fleas/cat. On day -1 and then approximately weekly throughout the 11-week study, each of the nine cats in the dedicated donor group was infested with 200 unfed fleas. Forty-eight hours later, the fleas were carefully combed off the donor cats, assessed for mobility and viability, and then pooled together. Healthy-appearing fleas with normal mobility were collected from the pool in lots of at least 50 fleas (approximately 50% female and 50% male), and each lot was transferred to individual cats in Groups 1 and 2 on days 1, 7, 14, 21, 28, 35, 42, 49, 56, 62, 69, and 76.

Twenty-four hours after each infestation, cats from Groups 1 and 2 were combed to collect fleas. To perform flea counts, two technicians simultaneously combed each cat to collect fleas. All cats were combed for 7 to 12 minutes; however, if no fleas had been obtained after 7 minutes, combing was discontinued at that time. If either technician discovered fleas, combing continued until no fleas were found for a period of 1 minute (e.g., if no fleas were obtained between 10 and 11 minutes, combing was discontinued at 11 minutes). For each flea count day, geometric mean flea counts were calculated using the transformation to the natural logarithm of (count + 1). Comparisons of treatment means were performed using *t*-tests for means with poolable variances or for means with unequal variances, as appropriate. Variances were then compared using the maximum *F*-test; if variances were found to be unequal, degrees of freedom were estimated using Satterthwaite's approximation.

At the same time flea comb counts were performed, flea eggs retrieved from the collecting

TABLE 1. Geometric Mean 24-Hour Flea Counts from Treated and Control Cats and Percent Efficacy Following Transplantation of Fleas Previously Fed for 48 Hours

Study Day	Geometric Mean Counts		% Efficacy
	<i>Fipronil</i> -(S)- <i>Methoprene</i>	Control	
1	0.25	33.93	99.30 ^a
7	0.00	35.86	100 ^a
14	0.00	37.87	100 ^a
21	0.32	39.85	99.20 ^a
28	1.35	39.69	96.60 ^a
35	2.44	36.12	93.30 ^a
42	2.59	39.27	93.40 ^a
49	4.43	35.15	87.40 ^b
56	16.78	37.39	55.10 ^b
62	16.63	35.76	53.50
69	25.67	35.86	28.40
76	35.47	39.43	10.10

^aDenotes value is statistically significant ($P < .0001$).
^bDenotes value is statistically significant ($P < .02$).

trays were counted. For each egg count day, geometric mean flea egg counts were determined for eggs collected using the transformation to the natural logarithm of (count + 1).

After egg counts were recorded, eggs from each cage were placed in individual vials containing sand and nutritive medium. The vials were individually identified by date and cat number and placed in an incubation unit that maintained temperature at 27°C and relative humidity at 75% to 80%.

After 1 month of incubation, any emerged fleas in each individual vial were counted. As with adult efficacy assessment, for each count day, geometric mean counts of emerging fleas

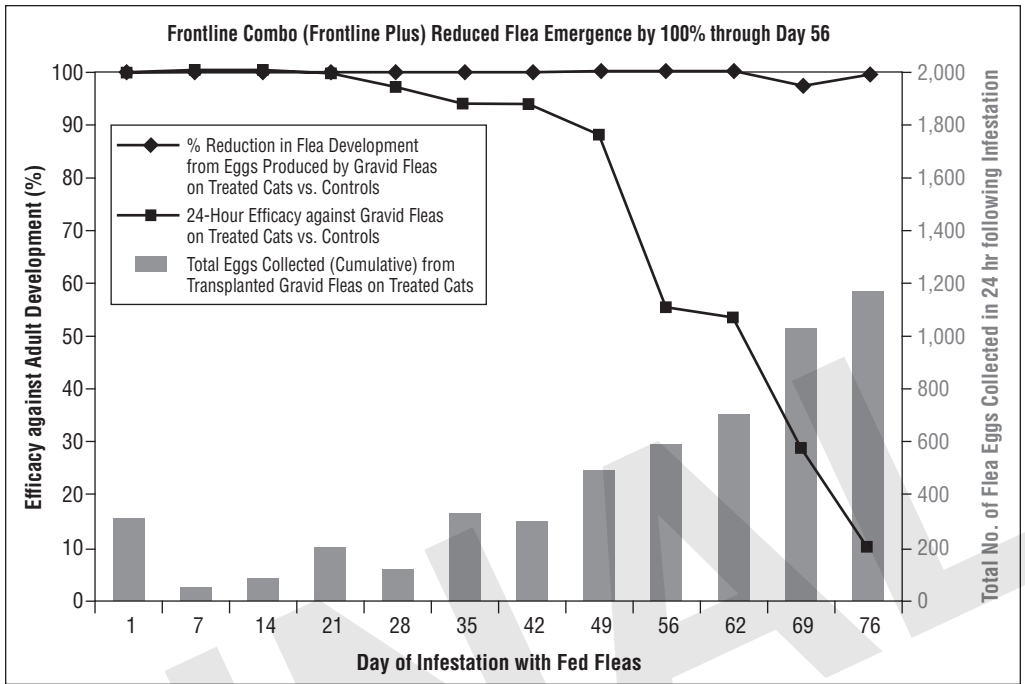


Figure 1. Frontline Combo (Frontline Plus) IGR activity, 24-hour adulticidal efficacy, and total eggs produced following each infestation with fleas fed for 48 hours prior. Treated cats received a single dose of the combination product on day 0.

in each vial were calculated using the transformation to the natural logarithm of (count + 1). Comparisons of treatment means were performed using *t*-tests for means with poolable variances or for means with unequal variances, as appropriate. Variances were then compared using the maximum *F*-test; if variances were found to be unequal, degrees of freedom were estimated using Satterthwaite's approximation.

RESULTS

No adverse effects were observed in any cats during the trial. Three variables were assessed during this 11-week trial: adulticidal efficacy, production of flea eggs, and viability of flea eggs (i.e., the development and emergence of fleas from the collected eggs).

Adulticidal Activity

The 24-hour adulticidal activity against actively reproducing transplanted fleas ranged between 100% and 93.3% during the first 6 weeks (day 42) after the treatment of the cats, and the difference was statistically (*P* < .02) significant through day 56 (Table 1 and Figure 1).

Production of Flea Eggs

The geometric mean number of eggs collected from the control group throughout the study ranged from 192.71 to 337.09 during each 24-hour collection period. Geometric mean number of eggs/cat produced from fleas transferred to treated cats for each count period ranged from 9.45 to 219.03 during the study. Total egg collec-

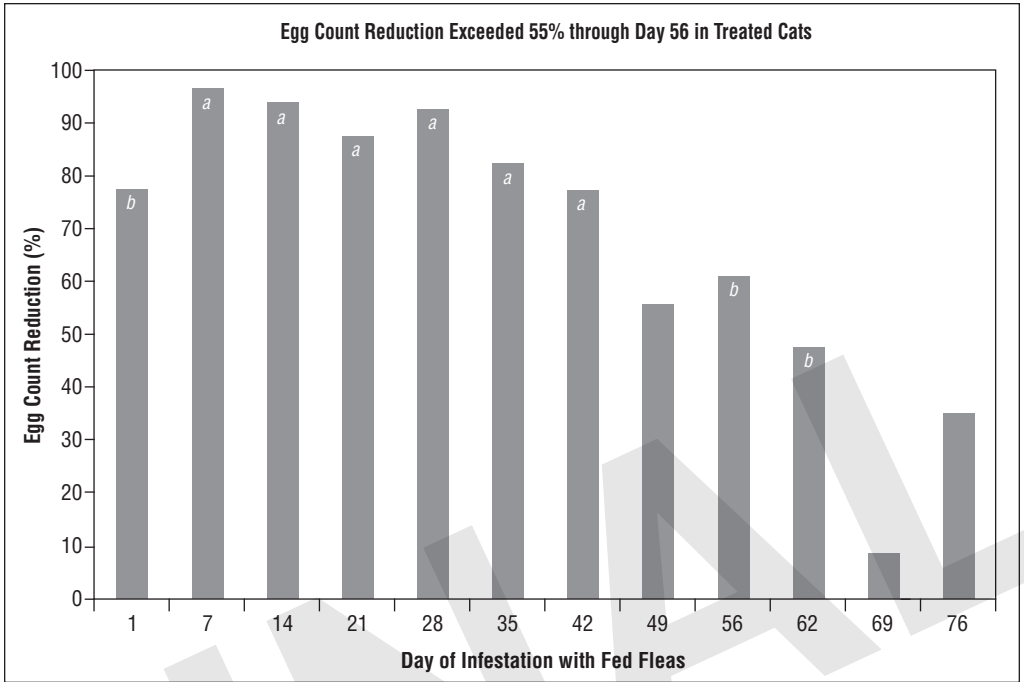


Figure 2. Egg count reduction provided by a single treatment (day 0) of Frontline Combo (Frontline Plus) against weekly infestations of transplanted engorged fleas fed for 48 hours prior. ^aDenotes value is statistically significant ($P < .01$). ^bDenotes value is statistically significant ($P < .05$).

tion (cumulative) from the treated group stayed below 200 until day 35 and below 600 through day 56. When viewed as percent egg count reduction, based on geometric means, the treated cat group reached levels as high as 96% reduction versus controls and maintained reduction exceeding 55% through day 56 (Figures 1 and 2).

Viability of Flea Eggs

When assessing flea development from eggs on cats treated with fipronil-(S)-methoprene versus the control group, the reduction of adult flea emergence was 100% from day 1 to day 56 (week 8) and was still at 98.7% at the end of the study (day 76 [week 11]). The geometric mean counts for adult emergence from control cats ranged from 113.1 to 208.35 new fleas,

with an average emergence count of 160.98 throughout the study (Figures 1 and 3).

DISCUSSION

This unique experimental design was developed to allow a high number of eggs to be placed on treated cats, thereby providing a severe challenge to assess the effect of fipronil-(S)-methoprene on egg viability. The model was successful in that an average geometric mean of more than 200 eggs was collected from each control cat in the weekly 24-hour collection periods. Additionally, the incubation parameters were validated in that 30-day flea production from the collected eggs had an average geometric mean of more than 160 adult fleas/collection.

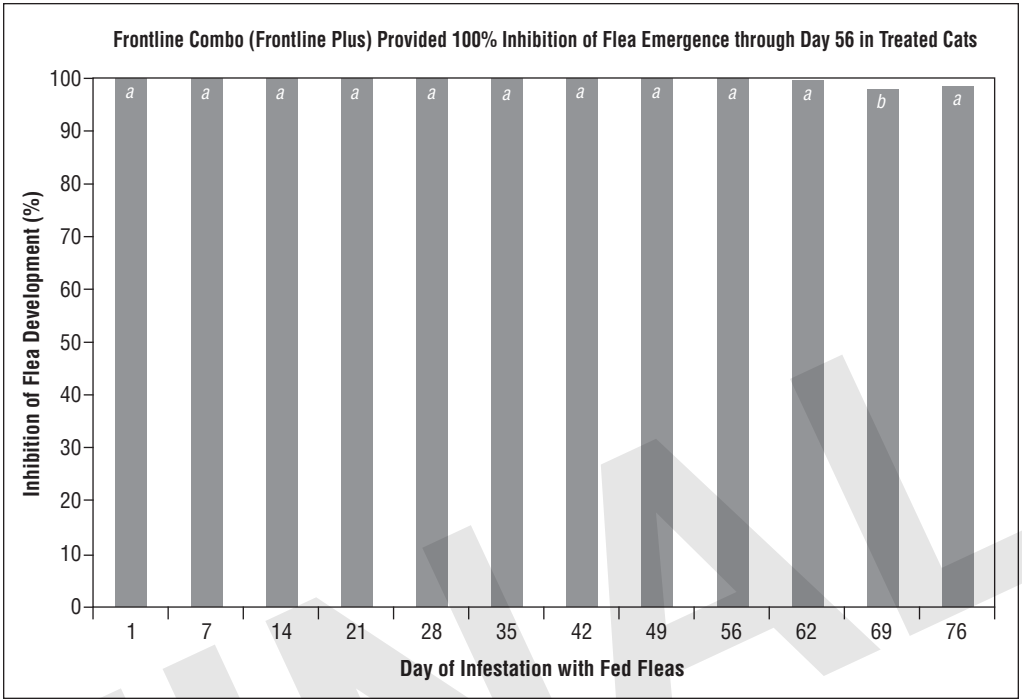


Figure 3. Inhibition of flea development from eggs collected following weekly infestations of transplanted engorged fleas (fed for 48 hours prior) on cats receiving a single treatment (day 0) of Frontline Combo (Frontline Plus). ^aDenotes value is statistically significant ($P < .0001$). ^bDenotes value is statistically significant ($P < .01$).

When assessing adulticidal activity, the efficacy of the combination fipronil-(S)-methoprene product ranged from 100% to 93.30% during the first 6 weeks after application of a single treatment to cats on day 0. These efficacies are comparable to the results of other studies in which unfed, nongravid fleas were used for animal infestations.^{4,9} Typically, flea studies are performed using fleas that have just emerged from their cocoons (newly emerged fleas).^{4,8,9} In this study, however, female fleas were essentially double the size of newly emerged fleas, as they had had the opportunity to feed for 48 hours.¹¹

By using gravid fleas for infestations, egg collection counts from control cats were con-

sistently high. However, a dramatic decrease in overall egg collection was seen on treated cats, even though the female fleas were already producing eggs before being transplanted to the cats. This suppression of egg production from the gravid fleas in the treated group was likely due to the rapid mortality of the fleas and by a direct action of the fipronil-(S)-methoprene on their capacity to produce and lay viable eggs.^{5,6}

The design of the study and laboratory technique used to assess flea emergence allowed collection of a geometric mean average of 160.98 newly emerged adult fleas from each cat in the control group at each assessment. These numbers indicate that the methodology used pro-

vided an excellent opportunity for ova to survive and develop into young adult fleas—the geometric mean average for egg production throughout the study was 262.24, for a 61.4% average rate of completed development in the eggs collected from control cats. Even with the study design allowing for sufficient egg production from treated cats and an excellent immature life stage development, none of the eggs collected from cats treated with fipronil-(S)-methoprene developed into adult fleas until after the ninth week posttreatment (day 62). To further illustrate this point, at the last collection time (day 76), 1,898 eggs were collected from the control group and 1,174 from the treated group; 1 month later, 53.8% of the eggs from the control group completed development, whereas only 2.0% of the eggs from the treated cats ultimately produced adult fleas. These data are a clear indication that (S)-methoprene has prolonged (lasting at least 76 days) and potent lethal activity against flea eggs laid on cats treated with a commercial spot-on formulation of fipronil-(S)-methoprene.

This study clearly illustrates a couple of points that should be considered when working to control flea infestations. As demonstrated in previous studies, adulticidal products may be able to reduce, but not completely eliminate, egg production; therefore, a high level of efficacy against flea eggs and ultimately adult flea development is critical. In addition, in the rare cases of transfer of mature fleas in a multiple-pet household or through other close contact between pets, the speed of kill of an adulticidal product is not always sufficient to avoid the production of eggs and their development in the environment.^{12,13} A formulation that provides both adulticidal and ovicidal activity, such as the combination of fipronil-(S)-methoprene found in Frontline Combo and Frontline Plus,

should provide long-lasting integrated flea control.

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