Efficacy and Speed of Kill of a Combination of Fipronil/(S)-Methoprene/ Pyriproxyfen Against *Ctenocephalides felis* Flea Infestations on Dogs from Day 2 to Day 30 Post-Treatment, Compared with a Combination of Fipronil/(S)-Methoprene

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ABSTRACT

Three studies were performed to assess the efficacy, speed of kill, and residual effects of FRONTLINE® Gold for Dogs (fipronil/(S)-methoprene/pyriproxyfen, Boehringer Ingelheim, Merial, Inc., Duluth, GA) against *Ctenocephalides felis* fleas. Although temporally separate, all three studies were performed at the same facility, each using eight dogs per treatment group, with two treatment groups in the first two studies and three treatment groups in the third study. In the first study, 16 dogs were infested with 100 unfed adult fleas on Day 2 following treatment, and the fleas were removed and counted 30 minutes later. In the second study, the same dogs were infested with 100 unfed adult fleas on Days 2, 9, 16, 23, and 30 following treatment, and the fleas were removed and counted 12 hours later. In the third study, a group of eight FRONTLINE Plus-treated dogs were included, so 24 total dogs were infested with 100 unfed adult fleas, and the fleas were removed and counted 6 hours later on Days 2, 9, 16, 23, and 30. In the first study, dogs treated with FRONTLINE Gold had significantly ($p=0.01$) fewer live fleas than the control dogs 30 minutes post-infestation on Day 2. Geometric mean efficacy was 64.8%. In the second study, dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live fleas than the control dogs 12 hours post-infestation on Days 2, 9, 16, 23, and 30. Geometric mean efficacy was 100% on Days 2, 9, 16, and

23, and 99.1% on Day 30. In the third study, dogs treated with FRONTLINE Plus had significantly ($p<0.01$) fewer live fleas than the control dogs at 6 hours post-infestation on all assessments through Day 23, and significantly ($p<0.05$) fewer live fleas than the control dogs at six hours post-infestation on Day 30. FRONTLINE Plus Geometric mean efficacy was 99.3%, 100%, 99.0%, 97.4%, and 74.4% on Days 2, 9, 16, 23, and 30, respectively. Dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live fleas than the control dogs at six hours post-infestation on all assessments through Day 30. Geometric mean efficacy was 100%, 100%, 99.5%, 99.9%, and 98.4% on Days 2, 9, 16, 23, and 30, respectively. In this study, dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live fleas than those treated with FRONTLINE Plus on Days 23 and 30. A single dose of FRONTLINE Gold for Dogs (1.34 ml) demonstrated excellent efficacy, speed of kill, and residual effects against *Ctenocephalides felis* fleas for a full 30 days, and demonstrated better persistent speed of kill and residual effects than FRONTLINE Plus at the six-hour count for a full 30 days.

INTRODUCTION

Ctenocephalides felis felis, known as the cat flea, is a common ectoparasitic insect. Cat fleas survive by feeding on the blood of mammals, most notably cats and dogs. Cat fleas are found all over the world, and are the most common flea species found in the United States.[1]

While a flea infestation may cause some pets to only experience irritation and discomfort, it may cause other pets to develop Flea Allergy Dermatitis (FAD), the most common dermatologic disease of domestic dogs in the United States.[2] FAD is an allergic reaction to flea saliva, and clinical signs are pruritus, swelling, welts, and dry skin. The physical trauma from scratching and allergic inflammations can cause lesions, scabs, scars, hair loss, and hot spots.[1]

Within the past several decades, many topical insecticidal formulations have been marketed in an effort to control flea populations and FAD in dogs, including insecticides such as avermectins, imidacloprid, pyrethroids/pyrethrins, fipronil, and various insect-growth regulators (IGRs) such as (S)-methoprene and pyriproxyfen.[4] The goals of a successful flea control program are to eliminate fleas quickly and continuously for the comfort of the dog and satisfaction of the pet owner, and to prevent fleas from producing viable eggs that contaminate the environment. In order to accomplish the latter goal, it is essential that the flea control product contain one or more IGRs. Given the prior success of the combination of fipronil/(S)-methoprene (FRONTLINE Plus) against fleas on dogs, and given the important role that IGRs play in successful flea control products, a new combination formulation of fipronil/(S)-methoprene/pyriproxyfen (FRONTLINE Gold) was developed.

The purpose of the three present studies was to assess the efficacy, speed of kill, and residual effects of FRONTLINE Gold for Dogs, and to compare these results with those of FRONTLINE Plus for Dogs.

MATERIALS AND METHODS

Animal Welfare

All three studies were conducted by the same experienced, independent contract research facility. Animals in all three studies were managed similarly and handled with due regard for their welfare. All animals were handled in compliance with the Merial (Merial is a part of Boehringer Ingelheim) Institutional Animal Care and Use Committee (IACUC) approvals. The trial facility used for all three studies meets USDA-APHIS animal welfare requirements. The Investigator ensured that all personnel were appropriately trained, and that procedures were in compliance with each protocol. Concomitant veterinary care and therapy, as well as any adverse events, were recorded.

All dogs were allowed to acclimate to the test facility for 10 days prior to the initiation of each study. All dogs were housed individually in accordance with the Animal Welfare Act. All dogs received 1-2 cups of

Table 1. *Individual dog information (as assessed on Day -5) and results of allocation to treatment groups (based on Day -4 counts)*

Study number	Animal ID	Sex	Weight (kg)	Live fleas	Replicate	Treatment group
1 & 2	1124	M	13.6	100	1	1 (Mineral oil-treated control dogs)
	MC0325	M	11.9	100	2	
	NE1216	M	12.6	98	3	
	F0088	M	15.5	96	4	
	NE1228	M	12.8	96	5	
	NE0910	M	15.2	93	6	
	6752	M	11.8	89	7	
	3087	M	13.3	87	8	
	596	M	15.1	100	1	2 (FRONTLINE Gold-treated dogs)
	519	M	12.1	100	2	
	MC9535	M	12.0	98	3	
	NE1217	M	16.1	97	4	
	NE1213	F	12.7	95	5	
	MC3194	F	13.2	93	6	
	MC3840	M	13.7	91	7	
	MC2139	M	13.3	86	8	
3	MC2507	M	15.1	100	1	1 (Mineral oil-treated control dogs)
	MC2647	F	25.4	96	2	
	MC7730	M	12.7	92	3	
	6752	M	13.6	91	4	
	NE1049	M	12.4	86	5	
	NE6733	F	10.0	79	6	
	MC9343	M	9.7	77	7	
	6934	F	9.2	71	8	
	MC3500	M	13.2	100	1	2 (FRONTLINE Plus-treated dogs)
	MC6148	M	13.2	93	2	
	MC1314	F	10.8	92	3	
	MC4690	F	11.3	90	4	
	FLX7	M	12.5	87	5	
	MC0782	M	11.5	82	6	
	F0202	M	13.3	77	7	
	1131	M	11.7	67	8	
	MC5605	F	17.7	100	1	3 (FRONTLINE Gold-treated dogs)
	1109	F	10.3	95	2	
	MC2026	M	12.8	92	3	
	MC7440	M	13.3	87	4	
	NE1228	M	13.4	85	5	
	NE1216	M	12.7	79	6	
	MC4107	M	12.4	78	7	
	MC9245	M	13.6	73	8	

commercial dry canine ration (Loyall, Adult Maintenance Formula, Nutrena) meeting their daily nutritional requirements, and fresh water from the city water supply was provided ad libitum.

Animal Management and Study Inclusion

For all three studies, dogs were ranked by decreasing pre-treatment flea counts, and eight replicates were formed utilizing the dogs with the highest pre-treatment flea counts. Within replicates, dogs in the first and second studies were randomly allocated to either the untreated control group, or to the FRONTLINE Gold-treated group, each group containing eight dogs. The first and second studies utilized the same 16 dogs, 14 of which were males, and 2 of which were females, and the dogs weighed between 11.8 and 16.1 kilograms (as weighed on Day -5).

In the third study, within replicates, dogs were randomly allocated to either the control group, the FRONTLINE Plus-treated group, or the FRONTLINE Gold-treated group, each group containing eight dogs. The third study utilized 17 males and 7 females weighing between 9.2 and 25.4 kilograms (as weighed on Day -5). For all three studies, no dogs younger than 8 weeks of age or under 2.3 kilograms were considered for use, which also applied to any dogs that may have been debilitated, suffering from disease or injury, fractious, presenting abnormalities at the application sites, or otherwise unsuitable for inclusion. Dogs in all three studies were in good health, and none had been treated with a monthly ectoparasiticide within three months prior to study initiation. Individual dog information, as assessed before inclusion in each study, is listed in Table 1.

Study Design

The studies were designed in accordance with the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines3 for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats. All three studies were controlled efficacy studies using a randomized block design based on dog pre-treatment infestation counts, and all evaluations of efficacy were performed by personnel in blinded conditions. Each dog was an experimental unit.

The first and second studies were conducted concurrently, using the same 16 dogs. These two studies continued together until the first study ended, and the second study proceeded. The third study was performed 15 months following the initial studies, and 24 dogs were utilized in this study.

The fleas used were from the BerTek, Inc. flea colony, maintained on cats, originating in 2004, with fleas purchased from Professional Laboratory and Research Services, Inc. (PLRS). The latest introduction of new genetics from local wild caught fleas (Greenbrier, AR) was on December 3, 2013.

Allocation

On Day -5, all dogs considered for qualification for each study were pre-infested with approximately 100 *Ctenocephalides felis* fleas, and were comb-counted approximately 24 hours later on Day -4. The dogs with the highest flea counts were selected and ranked based on pre-treatment flea infestation counts. Eight replicates of two dogs each were formed for Studies 1 and 2, and eight replicates of three dogs each were formed for Study 3. The dogs with the highest pre-treatment flea counts formed Replicate 1, the next highest formed Replicate 2, and so on, until all dogs were allocated.

For each of the three studies, within replicates, dogs were randomly allocated to one treatment group. In the first and second studies, Group 1 was designated as the control dogs, and Group 2 was designated as the dogs treated with FRONTLINE Gold. In the third study, Group 1 was designated as the control dogs, Group 2 was designated as the FRONTLINE Plus-treated dogs, and Group 3 was designated as the FRONTLINE Gold-treated dogs. The dogs remained in their assigned groups throughout the duration of each study. The results of the allocation processes are recorded in Table 1.

Treatment

Dogs in all three studies were weighed on Day -5, and the appropriate treatment size of Frontline Gold or FRONTLINE Plus was selected based on the animal's weight (1.34 ml for dogs weighing between 10.4 and 20.0 kilograms) for the FRONTINE Gold- and FRONTLINE Plus-treated groups, respectively, while each dog in the control group received one 0.5 ml dose of mineral oil, regardless of weight.

On Day 0 of each of the three studies, respective treatments were applied to each dog per the allocation. Both the FRONTLINE Gold and mineral oil treatments were applied according to FRONTLINE Gold label instructions: topically by parting the hair between the shoulder blades, and applying the formulation directly to the skin to form a stripe from the shoulder blades down the back, ending at the base of the tail. The FRONTLINE Plus treatment was applied according to FRONTLINE Plus label instructions: directly to the skin at the base of the neck, in one single spot. The dogs in all three studies were checked hourly for 4 hours post-treatment to ensure there were no adverse reactions to the applied treatment. The treatment records of FRONTLINE Gold, FRONTLINE Plus, and mineral oil are shown in Table 1.

Flea Counts

For all studies, each dog was infested with 100 unfed *Ctenocephalides felis* fleasthat were placed on the lateral aspect of the body to avoid direct contact with the product application site. In the first study, approximately 30 minutes post-infestation on Day 2, dogs in both groups were systematically combed with a standard flea comb, removing and counting all fleas. This was the only infestation and flea count conducted for the first study.

For the second study, the same dogs were re-infested with 100 unfed *Ctenocephalides felis* fleas on Day 2, and approximately 12 hours post-infestation, dogs in both groups were systematically combed with a standard flea comb, removing and counting all fleas. These processes of flea-infestation and comb-counting were repeated in the same way on Days 9, 16, 23, and 30.

In the third study, dogs in all three groups were infested with 100 unfed *Ctenocephalides felis* fleas on Days 2, 9, 16, 23, and 30, and approximately 6 hours post-infestation, all dogs were systematically combed with a standard flea comb, removing and counting all fleas.

STATISTICAL ANALYSIS

For all three studies, the statistician was responsible for the calculation of efficacy. The statistical unit was the individual animal, and the primary assessment variable in this study was the decrease in the number of live fleas. The average percent reduction in flea counts for each group in each study was calculated using geometric means:

Efficacy (%) against fleas = 100 x (GMC–GMT)/GMC, where GMC = geometric mean number of live fleas in the control group, and GMT = geometric mean number of live fleas in dogs in the FRONTLINE Gold- or FRONTLINE Plus-treated groups.

The transformed data of all three studies were analyzed using t-tests for means with poolable variances or for means with unequal variances, as appropriate. Variances were compared using the maximum-F test, and Sattertwaite's Approximation was used to determine the degrees of freedom for the unequal variance tests. When one group had zero variance, variances were declared unequal by definition. The t-tests are equivalent to one-way ANOVA when variances are poolable and are more appropriate when variances are found to be unequal. For the first and second studies, the FRONTLINE Gold-treated group was compared with the control group at each assessment. For the third study, the FRONTLINE Gold- and Plus-treated groups were compared with the control group, as well as with each other, at each assessment.

All analyses and calculations were performed using SAS Version 9.3 for Studies 1 and 2, and SAS Version 9.4 for Study 3. For all three studies, statistical significance

Table 2. *Summary of geometric mean1 Ctenocephalides felis flea counts (with percent efficacies and p-values) post-infestation on each study day, at each evaluation time, for dogs treated with FRONTLINE Plus, FRONTLINE Gold, or mineral oil (control)*

Study number	Day and time of flea counts	Geometric mean for control	Geometric mean for FRONTLINE Plus (% efficacies)	Geometric mean for FRONTLINE Gold (% efficacies)	P-value[U] for control vs. FRONTLINE Plus	P-value[U] for control vs. FRONTLINE Gold	P-value for FRONTLINE Plus vs. FRONTLINE Gold
1	Day 2, 30 m	94.4	--	33.3 A (64.8%)	--	0.0103	--
2	Day 2, 12 h	94.2	--	0.0B (100%)	--	<0.0001	--
	Day 9, 12 h	92.8	--	0.0B (100%)	--	<0.0001	--
	Day 16, 12 h	94.1	--	0.0B (100%)	--	<0.0001	--
	Day 23, 12 h	95.0	--	0.0B (100%)	--	<0.0001	--
	Day 30, 12 h	92.9	--	0.8B (99.1%)	--	0.0001	--
3	Day 2, 6 h	94.4	0.7B (99.3%)	0.0B (100%)	<0.0001	<0.0001	>0.10[U]
	Day 9, 6 h	94.7	0.0B (100%)	0.0B (100%)	<0.0001	<0.0001	--
	Day 16, 6 h	94.2	1.0B (99.0%)	0.5B (99.5%)	<0.0001	<0.0001	>0.10[E]
	Day 23, 6 h	95.5	2.5B (97.4%)	0.1B,D (99.9%)	<0.0001	<0.0001	0.0093[U]
	Day 30, 6 h	91.5	23.4B (74.4%)	1.4 B,D (98.4%)	0.0012	<0.0001	0.0012[E]

A Significantly different from control (p<0.05)
B Significantly different from control (p<0.01)
1 Based on transformation to natural logarithm of (count + 1)
U Results from t-test for means with unequal variance

was declared at a two-sided p-value of 0.05.

RESULTS

Adverse Reactions

No adverse events were reported in any dog at any time during any of the three studies.

Antiparasitic Efficacy

In the first study, dogs treated with FRONTLINE Gold had significantly ($p=0.01$) fewer live fleas than the control dogs 30 minutes post-infestation on Day 2. Efficacy was 64.8%.

In the second study, dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live fleas than the control dogs 12 hours post-infestation on Days 2, 9, 16, 23, and 30. Efficacy was 100% on Days 2, 9, 16, and 23, and 99.1% on Day 30.

In the third study, dogs treated with FRONTLINE Plus had significantly ($p<0.01$) fewer live fleas than the control dogs at 6 hours post-infestation on all assessments through Day 23, and significantly ($p<0.05$) fewer live fleas than the control dogs at 6 hours post-infestation on Day 30. FRONTLINE Plus efficacy was 99.3%, 100%, 99.0%, 97.4%, and 74.4% on Days 2, 9, 16, 23, and 30, respectively. Dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live fleas than the control dogs at 6 hours post-infestation on all assessments through Day 30. Efficacy was 100%, 100%, 99.5%, 99.9%, and 98.4% on Days 2, 9, 16, 23, and 30, respectively. In this study, dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live fleas than those treated with FRONTLINE Plus on Days 23 and 30.

All results (geometric means, arithmetic means, efficacies, and p-values) are listed in Table 2.

DISCUSSION AND CONCLUSIONS

Several past studies have shown that FRONTLINE Plus has demonstrated excellent efficacy against fleas. For instance, one study showed that a combination of fipronil/(S)-methoprene killed 100% of fleas on dogs by 24- and 48-hour assessments following each of Infestation Days 1, 7, 14, 21, 28, 35, and 42.[4]

A second study showed that this combination of fipronil/(S)-methoprene killed 100% of fleas on dogs by 24 hours post-infestation for up to 21 days, and 96.2% of fleas on dogs by 24 hours post-infestation on Day 28.[5]

A third study showed that the same combination of fipronil/(S)-methoprene killed 100% of fleas on dogs by 24 hours post-infestation on Days 2, 8, 15, 22, and 29, and >99.0% of fleas by 24 hours post-infestation on Day 36. In the same study, this combination killed 100% of fleas on dogs by 12 and 18 hours post-infestation on Days 7, 14, and 21, and >99.0% of fleas by 12 and 18 hours post-infestation on Day 28.[6]

The three studies reported here demonstrated that treatment with a single dose of FRONTLINE Gold (fipronil/(S)-methoprene/pyriproxyfen) resulted in a rapid reduction in live flea numbers in just 30 minutes after flea infestation 2 days after treatment, and that a high degree of efficacy was sustained at 6 and 12 hours post-infestation for a full 30 days. The present studies also demonstrated that FRONTLINE Gold efficacy was statistically superior ($p<0.001$ speed of kill against fleas than FRONTLINE Plus (fipronil/(S)-methoprene) at 6 hours post-infestation on Days 23 and 30 post-treatment.

Fipronil is the only active ingredient with efficacy against adult fleas in the products tested in this study, and the same dose of fipronil is delivered in both FRONTLINE Plus and FRONTLINE Gold. Differences in efficacy between the two products may be explained by differences in excipients. In particular, FRONTLINE Plus contains polyvinylpyrrolidone (PVP), whereas FRONTLINE Gold contains N-Octyl-2-pyrrolidone (NOP). These excipients differ in that NOP has a shorter alkyl group "tail" than PVP, and at room temperature, NOP is liquid, whereas PVP itself is a solid. Within the Frontline Gold formulation, these characteristics of NOP are thought to influence

solubility of fipronil and enhance exposure of fleas to fipronil, although the mechanism of action has not been elucidated.

The ability of FRONTLINE Gold to maintain a high degree of efficacy, by 6 hours after flea infestation, throughout the month provides several benefits for the dog and its owner. Fleas will have less time to bite and feed, providing rapid relief for the dog and greater satisfaction for the pet owner.

CONFLICT OF INTEREST

These clinical studies were funded by Boehringer Ingelheim, of which Doug Carithers is an employee, and Jordan Crawford is a contractor. BerTek, Inc., of which William Russell Everett is an employee, is an independent contract research facility contracted to conduct these studies. Sheila Gross is an independent statistician.

All authors voluntarily publish this article and have no personal interest in these studies, other than publishing the scientific findings in which they have been involved via planning, initiating, monitoring, and conducting the investigations, as well as analyzing the results.

DISCLAIMER

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