



## Efficacy of a novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel against adult and immature stages of the cat flea (*Ctenocephalides felis*) on cats

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### ABSTRACT

The efficacy of a novel topical combination of fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v) and praziquantel 8.3% (w/v) (BROADLINE<sup>®</sup>) was tested against adult and immature stages of *Ctenocephalides felis* fleas in six studies. For that purpose, fleas from different colonies from North America, Germany and South Africa were used to induce infestations in cats under laboratory conditions. In each study, between 12 and 16 cats were allocated randomly to 2 groups. Cats in Group 1 were not treated and served as controls. Cats in Group 2 were treated once on Day 0 with BROADLINE<sup>®</sup> at the minimum recommended dosage of 0.12 mg/kg body weight. In 4 studies, all animals were infested experimentally with unfed *C. felis* (100 ± 5) on Days 2 (or 1), 7, 14, 21, 28 and 35. Live fleas were counted 24 h post-treatment or infestation. In 2 additional studies, animals were infested at the same frequency with gravid *C. felis* fleas (100 ± 5) that were fed previously on an untreated host. Forty-eight hours post-infestation, flea eggs were collected, counted and incubated for the evaluation of the reduction of emergence of adults. The combined curative efficacy against adult fleas at 24 h after treatment was 94.3% and the combined preventive efficacy values remained greater than 95.9% at 24 h after 5 subsequent weekly infestations. In addition, the product reduced dramatically the emergence of new adult fleas for at least 5 weeks (>98.1% for one month and 93.2% at 5 weeks after infestation), demonstrating its efficiency in preventing environmental contamination by immature stages.

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### 1. Introduction

The cat flea, *Ctenocephalides felis*, is the most common ectoparasite found on cats and many other animal species worldwide (Rust and Dryden, 1997). In addition to

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causing annoyance and discomfort to pets and their owners, cat fleas are associated with several diseases. *C. felis* is primarily responsible for flea bite allergy dermatitis (FAD) in dogs and cats (Dryden and Blakemore, 1989; Plant, 1991; Carlotti and Costargent, 1994) as a result of hypersensitivity to components of flea saliva (Dryden and Rust, 1994; Stopler, 1994). The cat flea is also the primary intermediate host of the tapeworm *Dipylidium caninum*, the common intestinal cestode of dogs and cats (Dunn, 1978; Pugh, 1987). In addition, *C. felis* can transmit the agent of flea-borne spotted fever, *Rickettsia felis*, and it has been implicated in the transmission of some *Bartonella* species, such as *B. henselae*, the agent of Cat Scratch Disease (Azad et al., 1997; Orloski and Lathrop, 2003; Just et al., 2008; Dryden and Hodgkins, 2010).

Although the use of highly effective and practical insecticides such as fipronil, imidacloprid or selamectin have revolutionized flea control, treatment and prevention of cat flea infestations remain a major concern for pet owners and veterinarians (Rust, 2005; Dryden and Hodgkins, 2010; Beugnet and Franc, 2012; Siak and Burrows, 2013). The most difficult component of flea control is related to an aspect that the majority of pet owners are unaware of, namely controlling the pre-existing environmental infestation by immature stages (Rust, 2005; Beugnet and Franc 2012; Beugnet and Fourie, 2013). As a result, pet owners only treat their animals once when they see fleas, an approach that allows for continuous re-infestations by new emerging fleas. In order to improve owner compliance, there is a need for efficient and easy to administrate solutions allowing integrated control.

Broadline<sup>®</sup> (Merial) is a new topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel developed for cats with the aim to offer a wide spectrum of antiparasitic activity. The association of the phenylpyrazole fipronil with the insect growth regulator, (S)-methoprene is well known for its efficacy against adult and immature flea stages and for its ability to break the flea life cycle on treated animals and in their environment (Ritzhaupt et al., 2000; Young et al., 2004; Franc and Yao, 2007; Bonneau et al., 2010; Cadiergues et al., 2011; Everett et al., 2011). The objective of the studies presented in this paper was to examine the efficacy of Broadline<sup>®</sup> against adult and immature stages of several strains of *C. felis* fleas. For this six controlled, blinded and randomized laboratory studies were conducted in four different laboratories located in Germany, South Africa, and the United States.

## 2. Materials and methods

### 2.1. Animals

All animals were healthy, purpose-bred laboratory short hair cats. Each study was conducted under a controlled and blinded design, with cats randomly allocated to two groups (treated and control), each with eight (studies 1–5), or six cats (Study 6). Before treatment, the good health of each cat was confirmed by a physical examination conducted by a veterinarian, and by daily health observations by trained personnel. The protocol of the studies was reviewed and approved by the Merial Institutional

Animal Care and Use Committee. Cats were handled with due regard for their welfare. To detect the presence or absence of any treatment-related or unrelated health abnormality or adverse event, health observations were conducted at hourly intervals for four hours after treatment and daily thereafter throughout all studies.

### 2.2. Study design

The studies were designed in accordance with the “World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats” (Marchiondo et al., 2007), and were conducted in accordance with Good Clinical Practices as described in International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guideline GL9 (EMA, 2000). All animals were managed similarly, with due regard for their well-being and in compliance with Merial Ethics Committee, other local applicable regulations and requirements, and International laws and ethics.

### 2.3. Flea strains

The six studies were conducted by four different investigators in four different laboratories. Each study used a distinct flea colony for infestations, sourced from North America, Germany, or from Germany and South Africa. All fleas were laboratory-maintained *C. felis*.

### 2.4. Treatment

Cats assigned to the control groups were not treated. On Study Day 0, each cat in the treated groups received a topical application of BROADLINE<sup>®</sup> at the minimum recommended dose of 0.12 mL/kg body weight (bw), delivering 10 mg/kg bw fipronil, 12 mg/kg bw (S)-methoprene, 0.5 mg/kg bw eprinomectin and 10 mg/kg bw praziquantel. The treatments were applied directly onto the skin, after parting the hair, in one spot on the midline of the neck between the base of the skull and the shoulder blades.

### 2.5. Flea infestations and adult flea counts

Each cat was infested with 100 ( $\pm 5$ ) unfed adult fleas on Days 2, 7, 14, 21 and 28. An additional flea infestation was performed on Day 35 for 2 studies. All live fleas remaining on the cats were removed and counted via thorough combing of all body areas with a fine-tooth flea comb on Day 1 at 24 h after treatment (72 h after pre-treatment infestation), and at 24 h after each of the subsequent weekly flea infestations.

### 2.6. Evaluation of the adult emergence from flea eggs

The adult emergence after incubation of the flea eggs collected during the month after treatment was evaluated in two studies using the study design described by Franc et al. (2007). Briefly, each cat was infested with

**Table 1**  
Efficacy of BROADLINE against adult fleas at 24 h after treatment or infestations.

Study and origin of the flea strain		Flea count time-point					
		Day 1	Day 8	Day 15	Day 22	Day 29	Day 36
Study 1 Northern California, USA	GeoMean control cats	83.0	81.8	85.3	84.3	89.7	90.5
	GeoMean treated cats	3.9	0.5	0.3	2.4	5.8	4.1
	Efficacy against Fleas <sup>a</sup>	95.3%	99.4%	99.6%	97.1%	93.5%	95.4%
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Study 2 South Germany	GeoMean control cats	60.8	61.8	64.5	60.8	68.4	67.8
	GeoMean treated cats	1.8	0.0	0.0	0.0	0.6	2.4
	Efficacy against fleas	97.0%	100%	100%	100%	99.1%	96.4%
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Study 3 Central California, USA	GeoMean control cats	56.5	73.1	73.9	68.9	71.5	ND
	GeoMean treated cats	6.4	0	0	0	1.7	ND
	Efficacy against fleas	88.7%	100%	100%	100%	97.6%	ND
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Study 4 Germany mixed with South Africa	GeoMean control cats	74.4	67.0	62.3	62.3	72.9	ND
	GeoMean treated cats	3.0	0.0	0.2	0.0	0.0	ND
	Efficacy against fleas	96.0%	100%	99.7%	100%	100%	ND
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Combined percent efficacy at 24 h		94.3%	99.9%	99.8%	99.3%	97.6%	95.9%

<sup>a</sup> Efficacy = percent reduction of live adult flea counts in treated compared to control cats.

ND: not done.

100 ( $\pm 5$ ) gravid fleas that had previously fed and matured for two days on untreated donor cats. This was done to ensure that the fleas used for infestation produced eggs immediately after being placed on the cats and prior to being incapacitated or killed by the fipronil component of the combination product, to allow for evaluation of (S)-methoprene ovicidal and larvicidal activity. Each cat was infested on Days 1, 7, 14, 21, 28 and 35. The flea eggs produced on each cat were collected for two days after each infestation and incubated in Petri dishes containing sand and nutritive medium at 21.0–29.5 °C and 61.4–86.1% relative humidity. After five weeks of incubation, all emerged adult fleas were killed via freezing and counted, pupae were dissected and any normal encased adults were included in the counts.

### 2.7. Data analysis

For the evaluation of efficacy against adult fleas, the flea counts were transformed to the natural logarithm of (count +1) for calculation of geometric means for each treatment group. The percent efficacy was calculated as  $100 \times [(C - T)/C]$ , where *C* is the geometric mean of the flea counts among the untreated cats and *T* is the geometric mean among the treated cats. The log counts of the treated groups among were compared to the log counts of the untreated groups using an *F*-test adjusted for the allocation blocks used to randomize the cats to treatment group. The mixed procedure in SAS<sup>®</sup> version 9.1.3 was used for the analysis, with the treatment groups listed as a fixed effect and the allocation blocks listed as a random effect. All testing was two-sided at the significance level of *P* = 0.05.

For the evaluation of efficacy against adult emergence (studies 5 and 6), the rate of adult flea emergence was assessed as the mean number of adult fleas emerged (after five weeks of egg incubation) divided by the mean number of eggs collected and incubated at each time point for each

cat. Arithmetic means were used because the emergence of an adult flea from an incubated flea egg is a binomial response. At each time point, the percent reduction of adult emergence from the eggs collected from the treated compared to the control cats was calculated as  $100 \times [1 - T/C]$ , where *T* and *C* are the proportion of emerged adults compared to the number of eggs collected for the treated and control groups respectively. The results were analyzed as a repeated ANOVA using the GLIMMIX procedure in SAS. Time point was the repeated measure and cat-within-treatment group was the subject. For this analysis, the treatment, time point, and treatment-by-time point interaction were listed as the fixed effects, while allocation blocks were listed as the only random effects.

## 3. Results

### 3.1. Flea counts

The adult flea counts throughout the studies in the treated and untreated groups are summarized in Table 1. In all studies, the geometric means of the flea counts of untreated cats ranged from 56.5 to 90. As the design of the studies was similar, a percent efficacy combining the results of the 4 studies was calculated. At each time point in all studies, the number of live fleas was significantly lower on treated animals versus control (*P* < 0.001). At 24 h after treatment the pooled percent curative efficacy was 94.3%. The preventive efficacy was above 99% for the 3 following weeks and it was still at 95.9% 5 weeks after treatment.

### 3.2. Adult emergence

The numbers, proportions and percent reduction of adult flea emergence are presented in Table 2. In the two egg hatch inhibition studies, a sufficient number of eggs were obtained from control and treated animals (minimum

**Table 2**  
Percent reduction of adult emergence from eggs produced on cats treated with BROADLINE.

Study and origin of the flea strain	Egg collection week <sup>a</sup>	Proportion of emerged adult fleas <sup>b</sup>		Percent reduction of adult flea emergence <sup>c</sup>	P value
		Control cats	Cats treated with BROADLINE		
Study 5 North Germany	1	0.573 (57.3/100)	0.000 (0/75.7)	100.0	<.001
	2	0.641 (64.1/100)	0.000 (0/33)	100.0	<.001
	3	0.643 (64.3/100)	0.000 (0/40.7)	100.0	<.001
	4	0.636 (63.6/100)	0.006 (0.3/50.7)	99.1	<.001
	5	0.679 (67.9/100)	0.003 (0.3/77.1)	99.5	<.001
	6	0.629 (62.9/100)	0.043 (4.3/100)	93.2	<.001
Study 6 North Carolina, USA	1	0.682 (68.2/100)	0.000 (0/45.4)	100.0	<.001
	2	0.755 (75.5/100)	0.012 (0.2/16.2)	98.4	<.001
	3	0.605 (60.5/100)	0.010 (0.2/16)	98.3	<.001
	4	0.632 (63.2/100)	0.000 (0/59.5)	100.0	<.001
	5	0.558 (55.8/100)	0.011 (0.8/77.3)	98.1	<.001
	6	0.388 (38.8/100)	0.018 (1.5/83.3)	95.4	<.001

<sup>a</sup> Eggs collected for 2 days after weekly infestation of each cat with ~100 *C. felis* previously matured on untreated donor cats for 48 h.

<sup>b</sup> Proportion of emerged adult fleas = arithmetic mean of the number of emerged adults/arithmetic mean of the number of eggs collected and incubated (details in parentheses).

<sup>c</sup> Percent reduction =  $100 \times [1 - T/C]$ , where *T* and *C* are the proportion of emerged adults compared to the number of eggs collected for the treated and control groups, respectively

16 eggs collected) to demonstrate a significantly lower adult emergence in treated than control animals at each time point in weeks 1–6 ( $P < 0.001$ ). After each weekly infestation, the efficacy of Broadline against adult emergence from incubated flea eggs was greater than 98% through week 5 and greater than 93% in week 6 in both studies.

#### 4. Discussion

As natural variation in the susceptibility of flea strains to insecticide compounds has been described (Rust, 2005), the efficacy of the novel topical combination was tested against 6 strains of fleas of various origins including Africa, Europe and North America. The results illustrate the overall high level of efficacy of Broadline against these flea strains, with slight variations in the natural susceptibility of fleas to this new product.

The results of studies 1–4 demonstrate the efficacy of the new formulation against adult fleas, as previously reported for fipronil based products used on cats or dogs (Ritzhaupt et al., 2000; Franc and Yao, 2007; Franc and Beugnet, 2008; Bonneau et al., 2010; Boushira et al., 2011; Cadiergues et al., 2011). The results of studies 5 and 6 confirm the inhibition of egg hatching, as published previously for the combination of fipronil and (S)-methoprene (Young et al., 2004).

The association of fipronil and (S)-methoprene with eprinomectin and praziquantel in the Broadline formulation offers a wide spectrum of efficacy against the main parasites of cats including ecto- and endoparasites. The control of multiple concurrent parasitic infestations by a range of cat parasites is important for animal and public health. Decisions on the need for such treatments will be determined by the practicing veterinarian, based on diagnosis and risk assessment according to the region and the cat's environment. The use of an integrated approach for cats presenting multiple parasitic infestations, or

presenting risks of such infestation, this new combination product will be a distinct advantage. Its safety and efficacy as a combination spot-on product has been demonstrated here and its ease of application may improve owner compliance.

#### Conflict of interest

The work reported herein was funded by Merial Limited, GA, USA. All authors are current employees of Merial or contractors.

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