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Efficacy of a novel formulation of metaflumizone for the control of fleas (*Ctenocephalides felis*) on cats

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Abstract

A novel spot-on formulation containing metaflumizone (ProMeris[®] for Cats, Fort Dodge Animal Health, Overland Park, KS) was evaluated in five laboratory studies to determine the duration of residual efficacy in cats against fleas after a single spot treatment. In each study, eight domestic shorthair cats were randomly allocated to each treatment group and individually housed. One group in each study remained non-treated. In one study, an additional group of eight cats was treated with a placebo formulation. Cats were treated topically with metaflumizone formulation to provide a dose of at least 40 mg metaflumizone/kg. Cats were infested with 100 cat fleas (*Ctenocephalides felis felis*) once per week for approximately 8 weeks. Cats were comb counted 48 h after treatment and each infestation to determine the number of viable fleas present. There were no significant differences in flea counts between the non-treated control and the placebo-treated control ($P > 0.05$) other than a 26% reduction at week 1, demonstrating that the formulation excipients had no activity. Metaflumizone treatment resulted in significantly lower flea numbers relative to non-treated controls on all post-treatment count days ($P < 0.05$). Metaflumizone provided >90% control of flea infestations up to 7 weeks following a single treatment. © 2007 Published by Elsevier B.V.

Keywords: ProMeris[®]; *Ctenocephalides felis felis*; Metaflumizone; Flea; Cat

1. Introduction

The cat flea, *Ctenocephalides felis felis*, is endemic worldwide and considered to be the most important ectoparasite of companion animals (Rust and Dryden, 1997). Adult fleas are blood feeders, penetrating the skin with their sucking mouthparts and injecting salivary antigens as they feed (Dryden, 1989; Dryden and Rust, 1993). They are recognized as a major cause of allergic skin disease in companion animals and are capable, when

present in sufficient numbers, of causing anemia. They are intermediate hosts for the dog tapeworm, *Dipylidium caninum*, and can transmit a number of pathogens including *Bartonella henselae* (Kwochka, 1987; Foil et al., 1998; Krämer and Menke, 2001).

Control of fleas is primarily based on chemical means and recently, convenient on-animal treatments have become the standard accepted method (Dryden and Payne, 2004; Rust, 2005). The most widely used products are generally applied as spot-on applications on a monthly schedule. The spot-on treatments include active ingredients from a number of chemical classes, including phenyl pyrazoles, fipronil; neonicotinoids, imidacloprid; the pyrethroids, permethrin and phenothrin; and selamectin, an avermectin (Rust, 2005).

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These chemistries have direct insecticidal activity and control fleas on the animal. In addition, there are products such as the insect growth regulators, lufenuron and methoprene, that may be applied or ingested and control fleas by disrupting the development of eggs and larvae. Despite the variety of available products and different application methods, fleas remain an ongoing problem for many pet owners. Fleas have developed resistance to a number of insecticides and pest management strategies to reduce the development of resistance are needed (Bossard et al., 1998; Ross et al., 1998; Rust, 2005), especially if we want to conserve these new active ingredients.

Metaflumizone is a novel insecticide belonging to the semicarbazones with no known cross-resistance to other chemistries (Salgado and Hayashi, this volume). Metaflumizone has been found to have potent activity against fleas *in vivo* by a single topical application to cats (Takagi et al., this volume). The following studies were designed to evaluate the residual efficacy of a single spot-on dose of metaflumizone (ProMeris[®] for Cats, Fort Dodge Animal Health, Overland Park, KS) for the control of fleas on cats.

2. Materials and methods

Five studies were conducted at four laboratories in different geographical regions of the US (Table 1). All studies were conducted according to good laboratory practices as outlined in US EPA 40CFR160 (http://www.access.gpo.gov/nara/cfr/waisidx_00/40cfr160_00.html), and followed the basic methodology of US EPA Guideline, OPPTS 810.3300

Table 1
Design and locations of five studies conducted to evaluate the efficacy of a single topical dose of ≥ 40 mg metaflumizone/kg against *Ctenocephalides felis felis* in cats

Study	Treatment	No. of cats	Study location
A	Metaflumizone	8	Kansas
	Non-treated	8	
B	Metaflumizone	8	Texas
	Non-treated	8	
	Placebo ^a	8	
C	Metaflumizone	8	California
	Non-treated	8	
D	Metaflumizone	8	Oklahoma
	Non-treated	8	
E	Metaflumizone	16	Texas
	Non-treated	8	

^a Placebo consisted of inert ingredients from the commercial formulation of metaflumizone.

(http://www.epa.gov/opptsfrs/publications/OPPT-S_Harmonized/810_Product_Performance_Test_Guidelines/Series/index.html).

2.1. Animals

Domestic shorthair cats obtained from commercial sources were used in these studies. Ninety-six cats (46 males and 50 females) were selected to enter the treatment phase of these studies. The cats ranged from 6 months to 9 years of age and weighed between 2.1 and 6.2 kg at the time of treatment. These animals were selected from larger groups of cats based on pretreatment flea counts.

Cats were housed individually in indoor cages that conformed to accepted guidelines for floor area and type, lighting, temperature and welfare (including environmental enrichment) as required by local and national animal welfare legislation. They were housed in such a manner as to avoid contamination with fleas or transfer of test materials between animals. Water was available *ad libitum* and an appropriate quantity of commercial dry feline ration was provided daily throughout the study. Animals had not been treated with any ectoparasiticide for at least 60 days and were in good health when enrolled in the study and at treatment. Cats were grouped by treatment and were individually identified by numbered or lettered ear tattoos and/or cage identification cards. Each individual cage was labeled with the cat identification number only and was not identified by treatment. Cats from different treatment groups were physically separated by space equivalent to at least one empty cage.

2.2. Experimental design and methods

Efficacy of metaflumizone against adult fleas on cats following administration of a single topical dose unit was evaluated in five controlled studies (Table 1).

Cats were acclimated to the study conditions for 14 days prior to treatment. The cats were observed for general health once daily during the preconditioning period. Prior to treatment, a physical exam was performed on each cat by a veterinarian to determine health and suitability for inclusion in the trial. Cats were infested prior to treatment to determine if they were good flea hosts. Approximately 7 days prior to treatment, each cat was infested with approximately 100 unfed cat fleas. Twenty-four hours following infestation, each cat was thoroughly examined and combed to remove and count fleas. Using the pretreatment flea counts, cats with the highest flea

counts were selected for inclusion in the study. Cats which were difficult to handle or that retained less than 30 fleas for the pretreatment comb counts were excluded from the study.

Each study was conducted as a randomized complete block design. The cats were ranked in descending order by flea count; any cats with equal flea counts were ranked by ascending identification number. Cats were then assigned block numbers and were randomly allocated to treatment groups within these blocks. Each animal was allocated randomly to treatment with the test material or to the non-treated group. In Study C, one additional group of cats was randomly allocated to a group treated with placebo (formulation without metaflumizone). In Study E, one additional group of cats was randomly allocated to a group treated with an alternative formulation of metaflumizone.

On Day 1, the cats were infested with approximately 100 adult fleas each. On Day 0, each animal was treated as follows: control, animals were not treated; placebo control, vehicle was applied (Study B only) or metaflumizone at a dose providing at least 40 mg metaflumizone/kg.

Following the application of the test materials, cats were observed for general health and any reaction to treatment approximately 1–4 h after treatment on Day 0, then once daily for the remainder of the study.

All study personnel who were involved in clinical observations and flea comb counts were blinded to treatment groups. On Day 1 (approximately 24 h after treatment), all cats were combed and the number of live fleas was counted. Commercial fine-toothed flea combs were used. Cats were combed using repeated strokes starting at the head and neck, then posteriorly along the dorsum and sides. Cats were repeatedly combed over these areas until no fleas were recovered for about 5 min. Each animal was examined for a minimum of 10 min. Fleas able to stand upright and/or move in a coordinated manner were considered live. Live fleas were replaced on the animal after combing was completed. On Day 2, each cat was again combed to remove and count fleas. All fleas were then immersed in soapy water and discarded. Subsequently, the animals were reinfested with new fleas once each week for 7 weeks (Study D) or 8 weeks (Studies A–C and E). The cats were comb counted for fleas 48 h after each infestation; live fleas were not replaced on the cats.

Flea counts were performed by personnel trained in the standard procedures in use at the test facility. Protective gloves and clothing were changed before handling cats from different groups.

2.3. Cat fleas

Cat fleas were reared and maintained on laboratory cats at the study locations (Table 1). For Study A, fleas were from a flea colony (“KSU Wildcat Strain”), which was initiated with fleas originally collected from a dog at the Kansas State University teaching hospital in 2001. No new fleas have been added to the colony since its inception. For Studies B and E, the flea colony was started in 1993 with fleas obtained from Kansas State University. Approximately once per year, additional fleas from EL Labs in Soquel, CA were introduced into the colony. For Study C, the flea colony was established in 1997 from fleas collected in Stanislaus County, CA; field-collected fleas are added to the colony once per year. For Study D, fleas were obtained from shelter animals in 2003 in Payne County, OK.

At each infestation, fleas were applied to the lateral midline of each cat, which was restrained by hand for sufficient time to allow the parasites to penetrate the hair coat.

2.4. Treatment

The animals receiving metaflumizone were treated with a formulation containing 200 mg metaflumizone/ml. The placebo-treated cats were treated with a solvent formulation without the active ingredient. Animals were dosed according to pretreatment body weight. Cats weighing ≤ 4 kg received 0.8 ml of metaflumizone or vehicle, and cats weighing > 4 kg received 1.6 ml metaflumizone or vehicle. All applications were administered using disposable syringes. The dose was applied to the skin at a single spot at the base of the skull.

2.5. Data analysis

Statistical analyses were performed separately for flea counts for each examination day. Flea counts were transformed by the $\log_{10}(\text{count} + 1)$ transformation prior to analysis in order to stabilize the variance and normalize the data. Using the PROC MIXED procedure (SAS 8.2, Cary, NC), transformed counts were analyzed using analysis of variance (ANOVA) with a model that considered treatment as a fixed effect and replicate as a random effect. Each treatment groups' least square means were compared using the two-sided student's *t*-test at the 5% level of significance. Percent efficacy, relative to the non-treated control group and based on geometric means

Table 2
Flea counts after a single topical dose of 40 mg kg⁻¹ metaflumizone against *C. felis felis* in cats

Study	Treatment	Geometric mean flea counts									
		Day 1	Day 2	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
A	Metaflumizone	1.0* (98.5)	0.0* (100)	0.0* (100)	0.0* (100)	0.01* (100)	0.1* (99.8)	0.0* (100)	0.3* (99.5)	1.8* (95.5)	7.8* (83.6)
	Non-treated	68.6	53.7	79.8	61.8	50.7	56.8	56.6	53.3	40.4	47.7
B	Metaflumizone	2.2* (96.4)	0.0* (100)	0.1* (99.9)	0.0* (100)	0.0* (100)	0.0* (100)	0.2* (99.6)	1.1* (97.8)	5.1* (90.8)	15.6* (64.5)
	Placebo ^a	50.7 (15.6)	54.3 (9.0)	47.3* (26.0)	62.4 (9.6)	59.8 (5.4)	47.8 (15.6)	37.7 (24.5)	34.8 (30.1)	37.9 (32.1)	38.4 (12.7)
	Non-treated	60.0	59.6	63.8	69.0	63.3	56.6	50.0	49.9	55.9	44.0
C	Metaflumizone	4.3* (93.7)	0.0* (100)	0.0* (100)	0.4* (99.3)	0.0* (100)	0.8* (98.8)	1.0* (98.2)	4.6* (92.3)	6.2* (91.2)	17.3* (74.2)
	Non-treated	68.6	53.2	62.0	63.2	58.4	62.9	53.2	59.9	70.3	67.1
D	Metaflumizone	7.2* (79.0)	1.7* (94.4)	0.3* (99.4)	0.5* (99.1)	0.9* (98.2)	1.4* (97.5)	2.9* (94.4)	4.9* (92.8)	10.7* (82.2)	N/A
	Non-treated	34.1	29.5	49.8	48.4	51.0	57.5	51.3	67.4	59.9	N/A
E	Metaflumizone	4.0* (94.0)	0.0* (100)	0.0* (100)	0.0* (100)	0.0* (100)	0.1* (99.8)	0.0* (100)	2.1* (96.1)	6.4* (84.3)	10.3* (76.5)
	Metaflumizone (A) ^b	15.4* (76.8)	0.0* (100)	0.1* (99.8)	0.0* (100)	0.0* (100)	0.0* (100)	0.0* (100)	0.7* (98.7)	1.6* (96.0)	7.0* (84.0)
	Non-treated	66.3	53.1	59.7	47.7	41.3	53.9	54.5	54.1	40.7	43.9

^a Placebo consisted of inert ingredients from the commercial formulation of metaflumizone.

^b Alternate formulation of metaflumizone in which an alternate minor surfactant component was evaluated.

* Geometric mean counts significantly less than the respective non-treated controls $\alpha = 0.05$. Percent efficacy compared with non-treated controls is given in parentheses.

(G_{mean}), was calculated as follows:

$$\% \text{ Efficacy} = \frac{G_{\text{mean control}} - G_{\text{mean treated}}}{G_{\text{mean control}}} \times 100$$

3. Results

3.1. Flea counts

All cats included in these studies demonstrated good pretreatment flea holding ability; counts ranged from 30 to 107. Non-treated animals maintained good flea infestations throughout all studies (Table 2).

The placebo treatment in Study B appeared to have little if any effect on flea numbers. There was no significant difference between non-treated and placebo-treated mean counts ($P > 0.05$) on any count other than Week 1 (Table 2). Even at Week 1 the counts for the placebo-treated animals were only 26% lower than non-treated animals. The maximum reduction seen in placebo-treated animals was 32.1% at Week 7. All of the metaflumizone treatments resulted in significantly lower flea counts relative to non-treated controls on all post-treatment count days ($P < 0.05$, Table 2). Metaflumizone provided >90% control of fleas from Day 1 (Studies A–C, E—formulation 1), or Day 2 (Studies D and E—formulation 2) up to Week 7 (Studies A–C and E—formulation 2) or Week 6 (Studies D and E—formulation 1). Efficacy at Day 2 was 100% for four of the five studies and 94.4% for the fifth (Study D). Generally very high efficacy (>99–100%) was achieved for 4–6 weeks after treatment (Table 2).

3.2. Health observations

In Study A, one non-treated cat vomited twice on Day 4. On Day 7, another non-treated cat had diarrhea. On Days 11–33, one cat (treated with metaflumizone) was observed to have alopecia on the dorsal midline. On Day 35, one cat (treated with metaflumizone) was observed to have multiple severe excoriations and scab formations. On Days 53–55, one cat (treated with metaflumizone) was observed to have a sore behind the left ear.

In Study B, one cat (treated with placebo) had a small area of alopecia on the neck on Days 19, 20 and 26–56. One cat (treated with metaflumizone) had moderate soft stool on Day 26. One non-treated cat had a sub-mandibular lesion on Days 38–56 and alopecia on the left leg on Days 44–50.

In Study C, one cat (treated with metaflumizone) salivated briefly after licking some of the formulation that had run from the application site.

In Study D, one cat (treated with metaflumizone) was observed to have mild flea allergy dermatitis on Days 36–66. One cat (treated with metaflumizone) was observed to be “moderately thin” on Day 52.

There were no adverse health observations in Study E.

4. Discussion

There were no significant differences in the flea counts between the non-treated control and the placebo-treated control groups, except for a very slight reduction on Day 7. Thus the formulation excipients had no antiparasitic or repellent activity as demonstrated by the generally equivalent flea infestations of the placebo-treated animals to the untreated animals.

The experimental design used in the studies discussed here, in which animals were challenged repeatedly over the course of approximately 8 weeks, mimics the situation that exists when animals live in a flea-infested environment and are repeatedly exposed to fleas. Metaflumizone provided effective flea control (>90%) within 24–48 h post-treatment and controlled subsequent flea infestations for up to 7 weeks after treatment, indicating that metaflumizone will be a useful and effective product for the treatment of existing flea infestation and also as a once-monthly application for prophylactic control of fleas in cats. There were no adverse reactions in any treated cat that could be attributed to treatment other than one cat which experienced transient salivation due to inadvertent oral ingestion of a small amount of the test material, indicating that metaflumizone is well tolerated. The novel mode of action of metaflumizone will provide a new tool for veterinarians in a product with no known cross-resistance to existing flea products, thus could be an important aid in managing and preventing resistance in fleas.

5. Conclusions

The results from these studies demonstrate that a single dose of metaflumizone is highly effective against adult *C. felis* for up to 7 weeks after treatment

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