Date of Approval: November 9, 2018

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-502

revolution® PLUS

(selamectin and sarolaner topical solution)

Cats

revolution® PLUS is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*. revolution® PLUS kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations, the treatment and control of tick infestations with *Ixodes scapularis* (black-legged tick), *Amblyomma maculatum* (Gulf Coast tick) and *Dermacentor variabilis* (American dog tick), the treatment and control of ear mite (*Otodectes cynotis*) infestations, and the treatment and control of roundworm (*Toxocara cati*) and intestinal hookworm (*Ancylostoma tubaeforme*) infections for one month in cats and kittens 8 weeks and older, and weighing 2.8 pounds or greater.

Sponsored by:

Zoetis Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-502

B. Sponsor

Zoetis Inc.,
333 Portage St.,
Kalamazoo, MI 49007

Drug Labeler Code: 54771

C. Proprietary Name

revolution® PLUS

D. Drug Product Established Name

selamectin and sarolaner topical solution

E. Pharmacological Category

Antiparasitic

F. Dosage Form

Topical solution

G. Amount of Active Ingredient

60 mg/mL selamectin
10 mg/mL sarolaner

H. How Supplied

revolution® PLUS is available in 0.25 mL, 0.5 mL, or 1 mL applicator tubes in cartons containing 1, 3, or 6 tubes.

I. Dispensing Status

Rx

J. Dosage Regimen

The recommended minimum dosage is 2.7 mg selamectin per pound (6.0 mg/kg) of body weight in combination with 0.45 mg sarolaner per pound (1.0 mg/kg) of body weight administered monthly.

K. Route of Administration

Topical

L. Species/Class

Cats

M. Indication

revolution® PLUS is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*. revolution® PLUS kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations, the treatment and control of tick infestations with *Ixodes scapularis* (black-legged tick), *Amblyomma maculatum* (Gulf Coast tick) and *Dermacentor variabilis* (American dog tick), the treatment and control of ear mite (*Otodectes cynotis*) infestations, and the treatment and control of roundworm (*Toxocara cati*) and intestinal hookworm (*Ancylostoma tubaeforme*) infections for one month in cats and kittens 8 weeks and older, and weighing 2.8 pounds or greater.

II. EFFECTIVENESS

The effectiveness of revolution® PLUS was demonstrated in seventeen well-controlled laboratory studies and three clinical field studies described below. These studies demonstrate that revolution® PLUS is effective against a variety of both internal and external parasites. revolution® PLUS was administered to 186 laboratory and 476 client-owned cats. The most common adverse reactions from the three clinical field studies were anorexia, lethargy, skin lesions, diarrhea, pruritus, vomiting, and alopecia at the application site.

A. Dosage Characterization

For the Treatment and Prevention of Flea Infestations:
The effectiveness of a minimum dose of 6.0 mg/kg selamectin against adult cat fleas (*Ctenocephalides felis*) is supported by data contained in the Freedom of Information summary for REVOLUTION® (selamectin) Topical Parasiticide for Dogs and Cats under NADA 141-152 (Original sponsor was Pfizer, Inc., now owned by Zoetis Inc.).

The effectiveness of sarolaner in revolution® PLUS was supported in a laboratory dose determination study evaluating the effectiveness of a single topical dose of 6.0 mg/kg selamectin + 1.0 mg/kg or 2.0 mg/kg sarolaner. In this study, both dosages provided greater than 90% effectiveness against fleas for at least 49 days.

For the Treatment and Control of Tick Infestations:
Laboratory dose determination studies evaluating the effectiveness of a single topical dose of 6.0 mg/kg selamectin + 0.5 mg/kg, 0.75 mg/kg, 1.0 mg/kg, or 2.0 mg/kg sarolaner against *Ixodes scapularis*, *Amblyomma maculatum,* and *Dermacentor variabilis* ticks support that 6.0 mg/kg selamectin + 1.0 mg/kg sarolaner was the minimum dosage required to provide greater than 90% effectiveness for one month. Therefore, a monthly topical dosage of 6.0 mg/kg selamectin + 1.0 mg/kg sarolaner was selected as the minimum dosage for the product.

For the Prevention of Heartworm Disease:
The effectiveness of a minimum dose of 6.0 mg/kg selamectin for the prevention of heartworm disease caused by *Dirofilaria immitis* is supported by data contained in the Freedom of Information summary for REVOLUTION® (selamectin) Topical Parasiticide for Dogs and Cats under NADA 141-152 (Original sponsor was Pfizer, Inc., now owned by Zoetis Inc.).

For the Treatment and Control of Ear Mite Infestations:
The effectiveness of a minimum dose of 6.0 mg/kg selamectin for the treatment and control of ear mite (*Otodectes cynotis*) infestations is supported by data contained in the Freedom of Information summary for REVOLUTION® (selamectin) Topical Parasiticide for Dogs and Cats under NADA 141-152 (Original sponsor was Pfizer, Inc., now owned by Zoetis Inc.).

For the Treatment and Control of Gastrointestinal Nematodes:
The effectiveness of a minimum dose of 6.0 mg/kg selamectin for the treatment and control of intestinal roundworm (*Toxocara cati*) and hookworm (*Ancylostoma tubaeforme*) infections is supported by data contained in the Freedom of Information summary for REVOLUTION® (selamectin) Topical Parasiticide for Dogs and Cats under NADA 141-152 (Original sponsor was Pfizer, Inc., now owned by Zoetis Inc.).

B. Substantial Evidence

For the Treatment and Prevention of Flea Infestations

1. **Type of Study:** Field Effectiveness and Safety Study A181C-US-13-084

   Title: Efficacy and Safety Study of Topically Administered Sarolaner + Selamectin in the Treatment and Prevention of Natural Flea Infestations on Cats Presented as Veterinary Patients.

   Study Dates: April 22, 2014 – June 15, 2017

   Study Locations:

   Austin, TX
   Baton Rouge, LA
   Battle Creek, MI
   Bogart, GA
   Farragut, TN
   Grand Rapids, MI
   Greenbrier, AR
   Harleysville, PA
   Lake Worth, FL
   Largo, FL
   Livonia, LA
   Manhattan Beach, CA
   Memphis, TN
   New Braunfels, TX
   Pensacola, FL
   Quakertown, PA

Savannah, GA
Springfield, MO
Terre Haute, IN
Tulsa, OK
Seguin, TX
West Palm Beach, FL
Zachary, LA

Of the 23 sites, six sites did not meet the enrollment criteria or met the enrollment criteria, but an insufficient number of cats completed the study; therefore, these sites were removed from the effectiveness evaluation. All sites were included in the safety database.

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
Evaluate the effectiveness and safety of revolution® PLUS in the treatment and prevention of natural infestations of fleas on cats under field conditions. The secondary objective was to evaluate the reduction of clinical signs associated with flea allergy dermatitis in cats.

Study Animals:
Two hundred eighty-one (281) revolution® PLUS-treated cats, 148 imidacloprid + moxidectin-treated cats, and one cat in an observational group (NT1), which received revolution® PLUS, were evaluated for safety. The study enrolled cats ranging in age from 9 weeks to 18 years of age, and 2.8 to 24 pounds of body weight. The effectiveness analysis included 151 revolution® PLUS-treated cats and 73 imidacloprid + moxidectin-treated cats.

Enrollment was limited to households with a maximum of three cats; there was no restriction on type or number of other pets in the household. There were no breed or gender restrictions, but cats intended for breeding, and pregnant and lactating cats were not eligible for enrollment.

Experimental Design:
For a household to be included, at least one cat (the primary cat) had to have clinical evidence of flea infestation with a flea count ≥5 viable adult fleas. In households where more than one cat met this requirement, the primary cat was selected randomly from all eligible cats in the household that harbored ≥5 viable adult fleas, and the other cats were designated as supplemental cats. All cats in the household received the same treatment as the primary cat and were included in safety evaluations. Only primary cats were included in effectiveness evaluations.

Veterinary personnel who performed Flea Allergy Dermatitis (FAD), safety assessments (physical examinations, clinical pathology result assessments, and adverse event assessments), and flea counts were masked to treatment. Owners and treatment dispensers at each study location were not masked.

Drug Administration:
On Day 0, owners administered revolution® PLUS or the active control (imidacloprid + moxidectin) to their cats in the presence of the treatment dispenser at the veterinary clinic. On Days 30 and 60, owners administered revolution® PLUS or the active control to their cats in the cats' home environment.

Measurements and Observations:
For primary cats only, post-treatment flea counts were performed on Days 30, 60, and 90. Primary cats were assessed for signs of flea allergy dermatitis on Day 0 and post treatment (on Days 30, 60, and 90).

Physical examinations, body weight, and clinical pathology (hematology, serum chemistry, and urinalysis) were performed on all cats at enrollment (Day 0) and at premature study exit or scheduled study completion (Day 90). Additionally, for all cats, dosing and any abnormal health events were recorded. Physical examinations and body weights were performed on all cats on Days 30 and 60.

Statistical Methods: The primary assessment of effectiveness was based on the reduction in mean live flea counts on primary cats on Days 30, 60, and 90 compared to the pre-treatment counts on Day 0. A ≥90% reduction in live flea counts in the revolution® PLUS-treated cats throughout the study was required in order for the product to be considered effective. For the flea counts, the percent effectiveness of each treated group with respect to the baseline was calculated at each time point using the formula [(C-T)/C] x 100, where C = pre-treatment geometric mean and T = post-treatment geometric mean. Changes in the severity of clinical signs related to flea allergy dermatitis were summarized.

Results: The revolution® PLUS-treated group had a 97.2%, 99.5%, and 99.8% reduction in live flea counts on Days 30, 60, and 90, respectively (Table II.1). The imidacloprid + moxidectin–treated group had a 79.7%, 91.4%, and 95.5% reduction in live flea counts on Days 30, 60, and 90, respectively (Table II.1).

Table II.1: Geometric Mean Live Flea Counts for Primary Cats (Percent Reductions Compared to Pre-Treatment) for Study A181C-US-13-084

Treatment Group	Treatment	Number of cats	Day 0 (pre-treatment) flea count	Day 30 flea count	Day 60 flea count	Day 90 flea count
T01	revolution® PLUS	151	24.7	0.7 (97.2%)	0.1 (99.5%)	0.1 (99.8%)
T02	imidacloprid+ moxidectin	73	24.4	5.0 (79.9%)	2.1 (91.4%)	1.1 (95.5%)

Of the revolution® PLUS-treated primary cats that had clinical signs of flea allergy dermatitis prior to treatment, at least 75.0% showed a decrease in the severity of the individual clinical signs by Day 90 (Table II.2). Of the imidacloprid + moxidectin-treated cats, at least 60.0% showed a decrease in the severity of the individual clinical signs of flea allergy dermatitis by Day 90.

Table II.2: Percentage of Primary Cats with Improvement in Individual Clinical Signs of Flea Allergy Dermatitis on Day 90 for Study A181C-US-13-084

Clinical Sign	revolution® PLUS	Imidacloprid+ Moxidectin
Alopecia	91.2% (31 of 34)	66.7% (4 of 6)
Dermatitis/Pyodermatitis	96.4% (27 of 28)	100% (6 of 6)
Erythema	100% (32 of 32)	100% (7 of 7)
Pruritus	100% (47 of 47)	89.5% (17 of 19)
Scaling	86.7% (26 of 30)	100% (6 of 6)
Papules	100% (16 of 16)	100% (4 of 4)
Other	75.0% (6 of 8)	60.0% (3 of 5)

Cats with signs of FAD showed improvement in pruritus, papules, erythema, alopecia, scaling, and dermatitis/pyodermatitis as a direct result of eliminating the fleas.

Adverse Reactions: Evaluation of safety was completed over the 90-day period through in-clinic physical examinations or through reporting abnormalities by the owner for both primary and supplemental cats (Table II.3). The safety database included 282 cats administered revolution® PLUS and 148 cats administered imidacloprid + moxidectin.

Table II.3: Adverse Reactions for Study A181C-US-13-084

Adverse Reaction	revolution® PLUS (n=282)	Imidacloprid + Moxidectin (n=148)
Lethargy	12 (4.3%)	1 (0.7%)
Skin lesions*	10 (3.5%)	3 (2.0%)
Anorexia	9 (3.2%)	3 (2.0%)
Pruritus	7 (2.5%)	3 (2.0%)
Hair changes at administration site (alopecia)	5 (1.8%)	0 (0.0%)
Lesions at administration site (scabbing)	2 (0.7%)	0 (0.0%)

* Lesions not associated with application site.

Conclusion: The results of this study demonstrate that revolution® PLUS, when used monthly at the minimum labeled dose of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, is safe and effective for the treatment and prevention of flea infestations in cats. The cats treated with revolution® PLUS showed a substantial improvement in clinical signs related to flea allergy dermatitis over the course of the study. Treatment with revolution® PLUS was associated with

lethargy, skin lesions, anorexia, pruritus, and hair and skin changes at the administration site.

2. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-108: Treatment and Prevention of Flea Infestations

Title: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Ctenocephalides felis* on Cats

Study Dates: May 22, 2014 – March 17, 2015

Study Location: Greenbrier, Arkansas

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at 1.0 mg/kg) in combination with selamectin (dosed at 6.0 mg/kg) applied topically against induced infestations of *C. felis* for up to 35 days on cats.

Study Animals:
16 domestic short hair cats (8 males and 8 females), 7 – 8 months of age, weighing between 2.8 – 5.5 kg.

Experimental Design:
Cats were combed on Day -9 to ensure they were free of fleas prior to the host suitability infestation. Prior to allocation to treatment groups, an initial flea infestation on Day -8 and a flea count on Day -7 was conducted to evaluate susceptibility of each cat to experimental infestation (host suitability). Cats were ranked by live flea count and randomly allocated within blocks to two groups. Flea infestations were conducted on Days -1, 6, 13, 20, 27, and 34. At each infestation, each cat was infested with approximately 100 (± 5) unfed, adult *C. felis* fleas.

Flea counts were performed at 24 (±2) hours after drug administration or flea infestation.

Table II.4: Treatment Groups for Study A186C-US-14-108

Treatment Group	Treatment	Day of Treatment	Number and Sex of Animals	Days of Flea Infestation	Days of Flea Count
T01	Vehicle Control	0	8 (3M, 5F)	Days -1, 6, 13, 20, 27, and 34	Days 1, 7, 14, 21, 28, and 35
T02	revolution® PLUS	0	8 (5M, 3F)	Days -1, 6, 13, 20, 27, and 34	Days 1, 7, 14, 21, 28, and 35

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
The primary variable for effectiveness was the live flea counts collected from the cats. At each flea count on Days 1, 7, 14, 21, 28, and 35, the number of live fleas were counted, and the fleas were removed from the cat. Clinical observations and administration site evaluations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. In addition, administration sites were evaluated for any abnormalities on Days 3 and 35. Health observations were conducted at least once daily. Flea counts and health observations were conducted by personnel who were masked to treatment.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean of live flea counts for the control group and T = geometric mean of live flea counts for the revolution® PLUS-treated group for each time point.

Comparisons of live flea counts between the treatment groups were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the natural logarithm-transformed counts + 1 by time point, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the treatment indication was determined on the basis of the percent reduction in live flea counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in live flea counts between the two groups.

Results: At each flea count, a minimum of six cats in each control group had an adequate flea infestation, defined as a retention rate of at least 50% (i.e. ≥ 50 live fleas).

The revolution® PLUS-treated group had a 100% reduction in the initial live flea counts 24 hours after treatment, and a 100% reduction in live flea counts 24 hours after weekly re-infestations for 35 days. Live flea counts for the revolution® PLUS-treated group were significantly lower than the control group ($P<0.0001$) on all post-treatment count days.

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *C. felis* when assessed 24 hours after treatment of an existing infestation and 24 hours after weekly re-infestation for 35 days.

3. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-130: Onset of Activity and Speed of Kill against Fleas

   Title: Speed of Kill of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Ctenocephalides felis* on Cats

   Study Dates: January 5, 2015 – July 27, 2015

   Study Location: Greenbrier, Arkansas

   Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

   Objective:
   To confirm the speed of kill of sarolaner (dosed at 1.0 mg/kg) in combination with selamectin (dosed at 6.0 mg/kg) applied topically against *C. felis* for a period of 35 days on cats. Flea counts were evaluated at 6, 12, 24, and 48 hours after treatment on Day 0 and at 3, 6, 12, and 24 hours after infestations on Days 7, 14, 21, 28, and 35.

   Study Animals:
   64 domestic short hair cats (31 males and 33 females), 7 – 46 months of age, and weighing between 2.1 – 6.7 kg.

   Experimental Design:
   Cats were combed on Day -8 to ensure they were free of fleas prior to the host suitability infestation. Each cat was subsequently infested with 100 (±5) viable, adult *C. felis* on Day -7, and live fleas were counted on Day -6 to determine flea host suitability. This study followed a randomized complete block design based on pre-treatment flea counts and cage location. Each cat was allocated randomly to one of the eight treatment groups in each block. Flea infestations were conducted on Days -1, 7, 14, 21, 28, and 35. At each infestation, each cat was infested with approximately 100 (± 5) unfed, adult *C. felis* fleas.

   Flea counts were performed on Days 0, 7, 14, 21, 28, and 35.

Table II.5: Treatment Groups for Study A186C-US-14-130

Treatment Group	Treatment	Day of Treatment	Cats per Group	Time of Flea Counts After Treatment on Day 0 (hours)	Time of Flea Counts After Infestations on Days 7, 14, 21, 28, and 35 (hours)
T01	Vehicle Control	Day 0	8	6	3
T02	revolution® PLUS	Day 0	8	6	3
T03	Vehicle Control	Day 0	8	12	6
T04	revolution® PLUS	Day 0	8	12	6
T05	Vehicle Control	Day 0	8	24	12
T06	revolution® PLUS	Day 0	8	24	12
T07	Vehicle Control	Day 0	8	48	24
T08	revolution® PLUS	Day 0	8	48	24

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
The primary variable for effectiveness was the live flea counts collected from the cats. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 35. Health observations were conducted twice daily. Flea counts and health observations were conducted by personnel who were masked to treatment.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using least squares means at each time point using the formula [(C-T)/C] x 100, where C = mean of live flea counts for the control group and T = mean of live flea counts for the revolution® PLUS-treated group for the same flea counting time point. The comparisons were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the flea counts at each time point, with treatment group as a fixed effect and the block and error as random effects.

For each infestation, the onset of effectiveness was identified by the time point at which counts for the revolution® PLUS-treated group were significantly lower than those for the respective control group.

Results: At each flea count, a minimum of six cats in the control group had an adequate flea infestation, defined as a retention rate of at least 50% (i.e. ≥ 50 live fleas).

Live flea counts for the revolution® PLUS-treated group were first noted to be significantly different from those for the control group at 12 hours after treatment (P=0.0002) when the reduction in flea count was 72.5% (Table II.6). The reduction in flea count was 98.1% by 24 hours after treatment (P<0.0001) and 100% by 48 hours after treatment (P<0.0001).

On Days 7, 14, 21, 28, and 35, flea counts for the revolution® PLUS-treated group were consistently noted to be significantly different from those for the control group at 6 hours after treatment (P≤0.0158); the reduction in flea count was 61.5%, 56.4%, 52.5%, 60.2%, and 26.0% on each day, respectively. The reduction in flea count for the revolution® PLUS-treated group at 12 hours was 98.6%, 97.7%, 97.1%, 93.8%, and 85.1% on Days 7, 14, 21, 28, and 35, respectively, and the differences in counts from the control group were significant at 12 hours on these days (P<0.0001). At 24 hours, the reduction in flea count for the revolution® PLUS-treated group was 100% on Days 7, 14, 21, 28, and 35, and the differences in counts from the control group were significant (P<0.0001).

Table II.6: Onset of Activity and Speed of Kill of revolution® PLUS against *Ctenocephalides felis* on Cats for A186C-US-14-130

Day of Flea Infestation	Time of Flea Count (hours)	Control Group Least Square Mean Live Flea Count	revolution® PLUS Least Square Mean Live Flea Count	Percent Effectiveness
Day 0	6	90.9	75.3	17.2%
Day 0	12	80.4	22.1	72.5%
Day 0	24	87.5	1.6	98.1%
Day 0	48	88.8	0.0	100%
Day 7	3	85.4	70.2	17.7%
Day 7	6	82.5	31.8	61.5%
Day 7	12	89.0	1.3	98.6%
Day 7	24	89.9	0.0	100%
Day 14	3	79.8	71.4	10.5%
Day 14	6	73.8	32.1	56.4%
Day 14	12	80.6	1.9	97.7%
Day 14	24	80.5	0.0	100%
Day 21	3	92.2	67.1	27.2%
Day 21	6	76.9	36.5	52.5%
Day 21	12	78.6	2.3	97.1%
Day 21	24	76.8	0.0	100%
Day 28	3	80.8	79.3	2.0%
Day 28	6	72.0	28.6	60.2%

Day of Flea Infestation	Time of Flea Count (hours)	Control Group Least Square Mean Live Flea Count	revolution® PLUS Least Square Mean Live Flea Count	Percent Effectiveness
Day 28	12	80.9	5.0	93.8%
Day 28	24	82.6	0.0	100%
Day 35	3	87.8	77.5	11.8%
Day 35	6	75.1	55.6	26.0%
Day 35	12	81.6	12.1	85.1%
Day 35	24	86.9	0.0	100%

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS started to kill adult fleas 12 hours after treatment of an existing infestation and demonstrated >90% effectiveness at 24 hours. Following subsequent infestations, revolution® PLUS started to kill the fleas within 6 hours and was >90% effective by 24 hours for 35 days.

4. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-132: Egg Production, Egg Hatch, and Adult Flea Emergence

   Title: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin against *Ctenocephalides felis* Egg Production, Egg Hatch, and Adult Flea Emergence

   Study Dates: April 6, 2015 – January 4, 2016

   Study Location: Greenbrier, Arkansas

   Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

   Objective:
   To confirm the effectiveness of sarolaner (dosed at 1.0 mg/kg) in combination with selamectin (dosed at 6.0 mg/kg) applied topically in the prevention of pre-adult stages of *C. felis* on cats.

   Study Animals:
   20 domestic short hair cats (10 males and 10 females), 12 – 35 months of age, and weighing between 2.1 – 7.0 kg.

Experimental Design:
On Day -9, 30 cats were combed to ensure they were free of fleas prior to the host suitability infestation. Each cat was subsequently infested with 100 (±5) viable, adult *C. felis* on Day -9, and live fleas were counted on Day -6 to determine flea host suitability. The 20 cats with the highest Day -6 flea counts were selected for the study. Cats were allocated to treatments and cages according to a randomization plan. Flea infestations were conducted on Days -1, 5, 12, 19, 26, and 33. At each infestation, each cat was infested with approximately 100 (± 5) unfed, adult *C. felis* fleas.

Table II. 7: Treatment Groups for Study A186C-US-14-132

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Flea Infestation	Days Cats Placed in Cages for Flea Egg Collection	Days of Flea Egg Collection, Flea Count, and Flea Removal
T01	Vehicle Control	Day 0	10	-1, 5, 12, 19, 26, and 33	1, 7, 14, 21, 28, and 35	2, 8, 15, 22, 29, and 36
T02	revolution® PLUS	Day 0	10	-1, 5, 12, 19, 26, and 33	1, 7, 14, 21, 28, and 35	2, 8, 15, 22, 29, and 36

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with approximately 100 unfed adult *C. felis* at each infestation. Flea eggs were collected for a 20-hour period beginning 48 hours after infestation. After each egg collection, cats were combed to remove adult fleas and fleas were counted. Up to 100 eggs from each cat were incubated for three days and evaluated for larval emergence, and up to 100 eggs from each cat were incubated for 35 days and evaluated for adult flea emergence. Administration sites were evaluated, and cats were assessed for overall health prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment. In addition, administration sites were evaluated for any abnormalities on Day 3, 5, and 36. Health observations were conducted at least once daily. Study participants making assessments of effectiveness and safety were masked to treatment allocation.

Statistical Methods: Success of the product was to be based on virtually zero egg production after the initial onset of effectiveness.

Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using least squares means at each time point using the formula [(C-T)/C] x 100, where C = mean of live flea counts for the control group and T = mean of live flea counts for the revolution® PLUS-treated group for each time point. The comparisons were tested using the (two-sided) 5% significance level. A mixed model analysis was used to

analyze the flea counts at each time point, with treatment group as a fixed effect and block and error as random effects.

The number of flea eggs collected, the number of eggs incubated, the number of larvae hatched, and the number of adult fleas emerged were summarized by animal.

Results: At each flea count, a minimum of six cats in the control group had an adequate flea infestation, defined as a retention rate of at least 50% (i.e. ≥50 live fleas).

The revolution® PLUS-treated group had a 100% reduction in flea counts on all count days. Least squares mean flea counts for the revolution® PLUS-treated group were significantly lower than the control group ($P<0.0001$) on all collection days.

Four flea eggs were collected from one revolution® PLUS-treated cat on Day 29. No flea eggs were collected from any other revolution® PLUS-treated cat on Day 29 or on any other post-treatment collection day. No flea egg hatch or adult flea emergence was observed from any revolution® PLUS-treated cat.

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, treatment with revolution® PLUS resulted in a 100% reduction in adult flea count against an existing infestation and against weekly re-infestations with *C. felis*, virtually zero flea egg production, and a significant reduction in flea egg hatch and adult flea emergence for 36 days after treatment.

For the Treatment and Control of Tick Infestations

5. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-098: *Ixodes scapularis* Ticks

   Title: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Ixodes scapularis* on Cats

   Study Dates: February 26, 2014 – March 17, 2015

   Study Location: Greenbrier, Arkansas

   Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

   Objective:
   To confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically against induced infestations of *I. scapularis* for up to 35 days on cats.

Experimental Design:

Table II.8: Treatment Groups for Study A186C-US-14-098

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35
T02	revolution® PLUS	Day 0	8[1]	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35

[1] One cat in the T02 group was removed from the study on Day 27. No tick count data were collected from this cat on Days 28 and 35.

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with an appropriate number of unfed adult *I. scapularis* ticks on Days -2, 5, 12, 19, 26, and 33. At each tick count on Days 2, 7, 14, 21, 28, and 35, the numbers of live and dead ticks were counted, and the ticks were removed from the cat. Clinical observations were conducted prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 35. Health observations were conducted at least once daily. Tick counts and health observations were conducted by personnel who were masked to treatment.

An adequate infestation was defined as the recovery from a control cat of a minimum of 25% of the original number of live ticks used to infest the animal. A minimum of six adequately infested control cats were required at each time point for a valid study.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = arithmetic mean of live tick counts for the control group and T = arithmetic mean of live tick counts for the revolution® PLUS-treated group for each time point.

Comparisons of live and dead tick counts between the treatment groups were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the tick counts by time point, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the control indication was determined on the basis of the percent reduction in live tick counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in live tick counts between the two treatment groups. Effectiveness for the treatment indication was determined on the basis of the significance of the difference in dead tick counts between the two treatment groups on each count day.

Results: The revolution® PLUS-treated group had an 82.3% reduction in the initial live tick counts 48 hours after treatment, and a 100% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.9).

Live tick counts for the revolution® PLUS-treated group were significantly different and numerically lower than the live tick counts for the control group following each of the infestation time points ($P \leq 0.0017$). Dead tick counts were significantly different and numerically higher in comparison with the control group following each tick infestation ($P \leq 0.0342$).

An adequate infestation was achieved for all time points evaluated.

Table II.9: Arithmetic mean live tick counts and percent effectiveness of revolution® PLUS for the control of induced *Ixodes scapularis* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations for Study A186C-US-14-098

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	revolution® PLUS Arithmetic Mean Live Tick Count	Percent Effectiveness
Day 2	19.8	3.5	82.3%
Day 7	21.4	0	100.0%
Day 14	26.1	0	100.0%
Day 21	27.5	0	100.0%
Day 28	23.9	0	100.0%
Day 35	7.4	0	100.0%

Table II.10: Study A186C-US-14-098: Arithmetic mean dead tick counts for revolution® PLUS-treated cats compared with placebo controls following induced *Ixodes scapularis* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	revolution® PLUS Arithmetic Mean Dead Tick Count
Day 2	0	5.5
Day 7	0	18.9
Day 14	0	16.8
Day 21	0	26.6
Day 28	0	15.6
Day 35	0	7.0

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *I. scapularis* within 7 days after first treatment administration and after weekly re-infestation for 28 days. When compared with the controls, the revolution® PLUS-treated group had significantly different and numerically higher numbers of dead ticks and significantly different and numerically lower numbers of live ticks, which supports the treatment and control indications for *I. scapularis*, respectively, for 35 days.

6. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-106: *Ixodes scapularis* Ticks

Title: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Ixodes scapularis* on Cats

Study Dates: May 16, 2014 – June 1, 2015

Study Location: Turlock, CA

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically against induced infestations of *I. scapularis* for up to 35 days on cats.

Experimental Design:

Table II.11: Treatment Groups for Study A186C-US-14-106

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35
T02	revolution® PLUS	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with an appropriate number of unfed adult *I. scapularis* ticks on Days -2, 5, 12, 19, 26, and 33. At each tick count on Days 2, 7, 14, 21, 28, and 35, the numbers of live and dead ticks were counted, and the ticks were removed from the cat. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 35. Health observations were conducted twice daily. Tick counts and health observations were conducted by personnel who were masked to treatment.

An adequate infestation was defined as the recovery from a control cat of a minimum of 25% of the original number of ticks used to infest the animal. A minimum of six adequately infested control cats were required at each time point for a valid study.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = arithmetic mean of live tick counts for the control group and T = arithmetic mean of live tick counts for the revolution® PLUS-treated group for each time point.

Comparisons of live and dead tick counts between the treatment groups were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the tick counts by time point, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the control indication was determined on the basis of the percent reduction in live tick counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in live tick counts between the two treatment groups. Effectiveness for the treatment indication was determined on the basis of the significance of the difference in dead tick counts between the two treatment groups on each count day.

Results: The revolution® PLUS-treated group had an 85.2% reduction in the initial live tick counts 48 hours after treatment, and ≥93.3% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.12).

Live tick counts for the revolution® PLUS-treated group were significantly different and numerically lower than the live tick counts for the control group following each of the infestation time points (P≤0.0001). Dead tick counts were significantly different and numerically higher in comparison with the control group following each tick infestation (P≤0.0012). An adequate infestation was achieved for all time points evaluated.

Table II.12: Arithmetic mean live tick counts and percent effectiveness of revolution® PLUS for the control of induced *Ixodes scapularis* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations; Study A186C-US-14-106

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	revolution® PLUS Arithmetic Mean Live Tick Count	Percent Effectiveness
Day 2	25.4	3.8	85.2%
Day 7	27.9	0.3	99.1%
Day 14	33.3	0.3	99.2%
Day 21	35.3	0.8	97.9%
Day 28	38.5	1.0	97.4%
Day 35	35.5	2.4	93.3%

Table II.13: Arithmetic mean dead tick counts for revolution® PLUS-treated cats compared with controls following induced *Ixodes scapularis* infestations of cats, 48 hours after treatment of the initial infestation and weekly re-infestations; Study A186C-US-14-106

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	revolution® PLUS Arithmetic Mean Dead Tick Count
Day 2	0.1	10.0
Day 7	0	9.9
Day 14	0	9.6
Day 21	0	13.9
Day 28	0	15.0
Day 35	0	12.8

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *I. scapularis* within 7 days after first treatment and after weekly re-infestation for 35 days. When compared with the controls, the revolution® PLUS-treated group had significantly different and numerically higher numbers of dead ticks and significantly different and numerically lower numbers of live ticks, which supports the treatment and control indications for *I. scapularis*, respectively, for 35 days.

7. **Type of Study:** Laboratory Non-Interference Study A182C-US-16-194: *Ixodes scapularis* Ticks

Title: Laboratory Non-Interference of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Ixodes scapularis* on Cats

Study Dates: February 5, 2016 – January 9, 2017

Study Location: Turlock, CA

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically against induced infestations of *I. scapularis* for up to 35 days on cats. To confirm the combination of the above compounds does not interfere with the effectiveness of sarolaner against *I. scapularis* on cats.

Experimental Design:

Table II.14. Treatment Groups for Study A182C-US-16-194

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35
T02	revolution® PLUS	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35
T03	Selamectin	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with an appropriate number of unfed adult *I. scapularis* ticks on Days -2, 5, 12, 19, 26, and 33. At each tick count on Days 2, 7, 14, 21, 28, and 35, the numbers of live and dead ticks were counted, and the ticks were removed from the cats. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 35. Health observations were conducted twice daily. Tick counts and health observations were conducted by personnel who were masked to treatment.

An adequate infestation was defined as the recovery from a control cat of a minimum of 25% of the original number of ticks used to infest the animal. A minimum of six adequately infested control cats were required at each time point for a valid study.

Statistical Methods: Percent effectiveness of the treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = least squares mean of live tick counts for the control group and T = least squares mean of live tick counts for the treated group for each time point.

Comparisons of live and dead tick counts were tested using the (two-sided) 5% significance level between the revolution® PLUS-treated group (Group T02) and the control group (T01) and between the selamectin-treated group (Group T03) and the control group (Group T01). A mixed model analysis was used to analyze the tick counts by time point, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the control indication was determined on the basis of the percent reduction in live tick counts in the treated group compared to the control group and the significance of the difference in live tick counts between the two treatment groups. Non-interference was determined on the basis of the revolution® PLUS-treated group meeting the requirement for effectiveness while the selamectin-treated group does not. Effectiveness for the treatment indication was determined on the basis of the significance of the difference in dead tick counts between the two treatment groups on each count day.

Results: The revolution® PLUS-treated group had a 91.1% reduction in least squares mean live tick count 48 hours after treatment, and ≥95.1% reduction in least squares mean live tick count 48 hours after weekly re-infestations for 35 days (Table II.15).

Live tick counts for the revolution® PLUS-treated group were significantly different and numerically lower than the live tick counts for the control group following each of the infestation time points ($p \leq 0.0005$). Dead tick counts were significantly different and numerically higher in comparison with the control group following each tick infestation ($p \leq 0.0008$).

The selamectin-treated group had an 11.3% reduction in least squares mean live tick count 48 hours after treatment, and the reduction in least squares mean live tick count 48 hours after weekly re-infestations was never greater than 12.6% for 35 days (Table II.15).

Live tick counts for the selamectin-treated group did not differ ($P \geq 0.2906$) from the control group following each of the infestation time points, except for Day 28, when the counts for the selamectin-treated group were significantly different and numerically higher than those for the control group ($P=0.0291$). Dead tick counts did not differ in comparison with the control group following each tick infestation ($P \geq 0.2047$).

An adequate infestation on six control cats was achieved for all time points except Day 14, when only five out of eight cats had an adequate infestation.

Table II.15: Least squares mean (LSM) live tick counts and percent effectiveness for revolution® PLUS and selamectin for the control of induced *Ixodes scapularis* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations; Study A182C-US-16-194

Day of Study	LSM Live Tick Count Control	LSM Live Tick Count revolution® PLUS	LSM Live Tick Count Selamectin	Percent Effectiveness* Based on LSM Tick Counts revolution® PLUS	Percent Effectiveness* Based on LSM Tick Count Selamectin
Day 2	15.5	1.4	13.8	91.1%	11.3%
Day 7	18.9	0.0	16.5	100%	12.6%
Day 21	15.4	0.0	17.6	100%	-14.6%
Day 28	22.9	1.1	33.5	95.1%	-46.4%
Day 35	15.8	0.5	15.5	96.8%	1.6%

* Effectiveness based on percent reduction in least squares means used as arithmetic means relative to Control.

Table II.16: Least squares mean (LSM) dead tick counts for revolution® PLUS and selamectin for the treatment of induced *Ixodes scapularis* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations; Study A182C-US-16-194

Day of Study	LSM Dead Tick Count Control	LSM Dead Tick Count revolution® PLUS	LSM Dead Tick Count Selamectin
Day 2	0.1	3.4	0.6
Day 7	1.0	6.8	0.3
Day 21	0.8	8.0	0.0
Day 28	3.1	19.4	0.0
Day 35	2.1	12.5	0.0

Adverse Reactions: No treatment-related adverse reactions were noted during the study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *I. scapularis* at 48 hours after treatment of an existing infestation and after weekly re-infestations for 35 days. When compared with the controls, the revolution® PLUS-treated group had significantly different and numerically higher numbers of dead ticks and significantly different and numerically lower numbers of live ticks, which supports the treatment and control indications for *I. scapularis*, respectively, for 35 days.

Treatment with selamectin alone was not effective against an existing infestation or subsequent infestations of adult *I. scapularis*, justifying the need for inclusion of sarolaner in the combination.

8. **Type of Study:** Onset of Activity and Speed of Kill Study A186C-US-14-131: *Ixodes scapularis* Ticks

Title: Speed of Kill of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Ixodes scapularis* on Cats

Study Dates: October 23, 2014 – November 12, 2015

Study Location: Greenbrier, Arkansas

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the speed of kill of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically against induced infestations of *I. scapularis* for up to 35 days on cats.

Experimental Design:

Table II.17: Treatment Groups for Study A186C-US-14-131

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Tick Infestation	Days of Tick Counts
T01	Vehicle Control	Day 0	8	Days -2, 7, 14, 21, 28, and 35	Post treatment[1] and post infestation on Days 7, 14, 21, 28, and 35[2]
T02	revolution® PLUS	Day 0	8	Days -2, 7, 14, 21, 28, and 35	Post treatment[1] and post infestation on Days 7, 14, 21, 28, and 35[2]

[1] Ticks were counted and categorized without removal 4, 8, 12, 24, and 48 hours after treatment on Day 0. Ticks were removed, counted and categorized 72 hours after treatment.
[2] Ticks were counted and categorized without removal 4, 8, and 12 hours after each weekly infestation on Days 7, 14, 21, 28, and 35. Ticks were removed, counted and categorized 24 hours after each weekly infestation.

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with an appropriate number of unfed adult *I. scapularis* ticks on Days -2, 7, 14, 21, 28, and 35. At each tick count on Days 0, 7, 14, 21, 28, and 35 (Table II.18), the numbers of live and dead ticks were

counted. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 36. Health observations were conducted twice daily. Tick counts and health observations were conducted by personnel who were masked to treatment.

An adequate infestation was defined as the recovery from a control cat of a minimum of 25% of the original number of ticks used to infest the animal. A minimum of six adequately infested control cats were required.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using least squares means at each time point using the formula [(C-T)/C] x 100, where C = mean of live tick counts for the control group and T = mean of live tick counts for the revolution® PLUS-treated group for each time point. The comparisons were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the tick counts at each time point, with treatment group as a fixed effect and block and error as random effects.

For each infestation, the onset of effectiveness was identified by the time point at which counts for the revolution® PLUS-treated group were significantly different and numerically lower than those for the respective controls.

Results: Live tick counts for the revolution® PLUS-treated group were first noted to be significantly different from those for the control group at 12 hours after treatment ($P=0.0151$) when the reduction in tick count was 50.9%. The reduction in tick count was 100% by 24 hours after treatment ($P<0.001$).

On Days 7, 14, 21, 28, and 35 counts for the revolution® PLUS-treated group were consistently noted to be significantly different from those for the control group at 24 hours after treatment ($P\leq0.0020$) when the reduction in tick count was 100%, 98.0%, 94.5%, 67.7%, and 56.6% on each day, respectively.

The control cats maintained adequate infestations at each time point on each day of counting.

Table II.18: Onset of Activity and Speed of Kill of revolution® PLUS against *Ixodes scapularis* on Cats for Study A186C-US-14-131

Day of Tick Infestation	Time of Tick Count	Control Group Least Squares Mean Live Tick Count	revolution® PLUS Least Squares Mean Live Tick Count	Percent Effectiveness
Day 0	4	23.6	24.4	-3.2%
Day 0	8	22.4	20.5	8.4%
Day 0	12	20.9	10.3	50.9%
Day 0	24	20.6	0.0	100%
Day 0	48	20.1	0.0	100%
Day 0	72	21.0	0.0	100%
Day 7	4	23.4	24.4	-4.3%
Day 7	8	21.6	17.8	17.9%
Day 7	12	18.5	10.8	41.9%
Day 7	24	31.9	0.0	100%
Day 14	4	27.6	23.5	14.9%
Day 14	8	24.3	20.6	14.9%
Day 14	12	20.4	18.4	9.8%
Day 14	24	31.6	0.6	98.0%
Day 21	4	26.5	21.6	18.4%
Day 21	8	27.4	25.0	8.7%
Day 21	12	26.1	21.7	16.7%
Day 21	24	36.4	2.0	94.5%
Day 28	4	21.7	22.9	-5.3%
Day 28	8	20.7	19.0	8.3%
Day 28	12	19.4	19.6	-1.0%
Day 28	24	31.7	10.3	67.7%
Day 35	4	27.0	24.1	10.6%
Day 35	8	31.9	21.8	31.7%
Day 35	12	27.9	23.8	14.7%
Day 35	24	36.9	16.0	56.6%

Adverse Reactions: No treatment-related adverse reactions were noted during the study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS started to kill adult *I. scapularis* ticks within 24 hours for 1 month. Treatment with revolution® PLUS was effective against an existing infestation of *I. scapularis* within 24 hours of administration and within 24 hours of subsequent infestations for at least 21 days. This study failed to demonstrate the effectiveness of revolution® PLUS for the control of *I. scapularis* on Days 28 or 35 when assessed 24 hours after infestation.

9. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-103: *Amblyomma maculatum* Ticks

Title: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Amblyomma maculatum* on Cats

Study Dates: March 12, 2014 – March 10, 2015

Study Location: Turlock, CA

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically against induced infestations of *A. maculatum* for 1 month on cats.

Experimental Design:

Table II.19: Treatment Groups for Study A186C-US-14-103

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35
T02	revolution® PLUS	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with an appropriate number of unfed adult *A. maculatum* ticks on Days -2, 5, 12, 19, 26, and 33. At each tick count on Days 2, 7, 14, 21, 28, and 35, the numbers of live and dead ticks were counted, and the ticks were removed from the cat. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 35. Health observations were conducted twice daily. Tick counts and health observations were conducted by personnel who were masked to treatment.

An adequate infestation was defined as the recovery from a control cat of a minimum of 25% of the original number of ticks used to infest the animal. A minimum of six adequately infested control cats were required at each time point for a valid study.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = arithmetic mean of live tick counts for the control group and T = arithmetic mean of live tick counts for the revolution® PLUS-treated group for each time point.

Comparisons of live and dead tick counts between the treatment groups were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the tick counts by time point, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the control indication was determined based on the percent reduction in live tick counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in live tick counts between the two treatment groups. Effectiveness for the treatment indication was determined on the basis of the significance of the difference in dead tick counts between the two treatment groups on each count day.

Results: The revolution® PLUS-treated group had a 92.3% reduction in the initial live tick counts 48 hours after treatment, and ≥91.6% reduction in live tick counts 48 hours after weekly re-infestations for 28 days (Table II.20).

Live tick counts for the revolution® PLUS-treated group were significantly different and numerically lower than the live tick counts for the control group following each of the infestation time points ($P \leq 0.0291$). Dead tick counts were significantly different and numerically higher in comparison with the control group following each tick infestation ($P \leq 0.0109$).

An adequate infestation was achieved for all time points evaluated.

Table II.20: Arithmetic mean live tick counts and percent effectiveness of revolution® PLUS for the control of induced *Amblyomma maculatum* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations; Study A186C-US-14-103

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	revolution® PLUS Arithmetic Mean Live Tick Count	Percent Effectiveness
Day 2	27.6	2.1	92.3%
Day 7	30.3	0.3	99.2%
Day 14	27.0	0.8	97.2%
Day 21	37.2	3.1	91.6%
Day 28	31.6	1.5	95.3%
Day 35	26.4	8.3	68.7%

Table II.21: Arithmetic mean dead tick counts for revolution® PLUS-treated cats compared with controls following induced *Amblyomma maculatum* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations; Study A186-US-14-103

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	revolution® PLUS Arithmetic Mean Dead Tick Count
Day 2	0.5	11.8
Day 7	0.0	16.5
Day 14	0.0	13.3
Day 21	0.0	18.0
Day 28	0.0	13.9
Day 35	0.1	9.1

Adverse Reactions: No treatment-related adverse reactions were noted during the study.

Conclusions: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *A. maculatum* at 48 hours after treatment of an existing infestation and after weekly re-infestation for 28 days. When compared with the control, the revolution® PLUS-treated group had significantly different and numerically higher numbers of dead ticks and significantly different and numerically lower numbers of live ticks, which supports the treatment and control indications for *A. maculatum*, respectively, for 35 days.

10. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-107: *Amblyomma maculatum* Ticks

Title: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Amblyomma maculatum* on Cats

Study Dates: July 28, 2014 – June 10, 2015

Study Location: Greenbrier, Arkansas

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically against induced infestations of *A. maculatum* for one month on cats.

Experimental Design:

Table II.22: Treatment Groups for Study A186C-US-14-107

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35
T02	revolution® PLUS	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with an appropriate number of unfed adult *A. maculatum* ticks on Days -2, 5, 12, 19, 26, and 33. At each tick count on Days 2, 7, 14, 21, 28, and 35, the numbers of live and dead ticks were counted, and the ticks were removed from the cat. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 35. Health observations were conducted twice daily. Tick counts and health observations were conducted by personnel who were masked to treatment.

An adequate infestation was defined as the recovery from a control cat of a minimum of 25% of the original number of ticks used to infest the animal. A minimum of six adequately infested control cats were required at each time point for a valid study.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = arithmetic mean of live tick counts for the control group and T = arithmetic mean of live tick counts for the revolution® PLUS-treated group for each time point.

Comparisons of live and dead tick counts between the treatment groups were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the tick counts by time point, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the control indication was determined on the basis of the percent reduction in live tick counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in live tick counts between the two treatment groups. Effectiveness for the treatment indication was determined on the basis of the significance of the difference in dead tick counts between the two treatment groups on each count day.

Results: The revolution® PLUS-treated group had a 100% reduction in the initial live tick counts 48 hours after treatment, and ≥95.0% reduction in live tick counts 48 hours after weekly re-infestations for 28 days (Table II.23).

Live tick counts for the revolution® PLUS-treated group were significantly different and numerically lower than the live tick counts for the control group following each of the infestation time points ($P \leq 0.0066$). Dead tick counts were significantly different and numerically higher in comparison with the control group following each tick infestation ($P \leq 0.0058$).

An adequate infestation was achieved for all time points evaluated.

Table II.23: Arithmetic mean live tick counts and percent effectiveness of revolution® PLUS for the control of induced *Amblyomma maculatum* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations; Study A186C-US-14-107

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	revolution® PLUS Arithmetic Mean Live Tick Count	Percent Effectiveness
Day 2	18.4	0.0	100.0%
Day 7	32.0	0.0	100.0%
Day 14	35.8	0.0	100.0%
Day 21	28.5	0.1	99.6%
Day 28	32.4	1.6	95.0%
Day 35	26.8	9.3	65.4%

Table II.24: Arithmetic mean dead tick counts for revolution® PLUS-treated cats compared with controls following induced *Amblyomma maculatum* infestations of cats, 48 hours after treatment of the initial infestation and after weekly re-infestations; Study A186C-US-14-107

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	revolution® PLUS Arithmetic Mean Dead Tick Count
Day 2	0.8	10.6
Day 7	0.0	17.6
Day 14	0.0	16.3
Day 21	0.0	15.4
Day 28	0.0	19.6
Day 35	0.0	9.0

Adverse Reactions: In the revolution® PLUS-treated group, one cat experienced a single incident of mild, transient salivation in the hour following dosing and one cat vomited once on the day following treatment. Both events resolved rapidly without treatment.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *A. maculatum* at 48 hours after treatment of an existing infestation and after weekly re-infestation for 28 days. When compared with the controls, the revolution® PLUS-treated group had significantly different and numerically higher numbers of dead ticks and significantly different and numerically lower numbers of live ticks, which supports the treatment and control indications for *A. maculatum*, respectively, for 35 days.

11. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-100: *Dermacentor variabilis* Ticks

Title: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Dermacentor variabilis* on Cats

Study Dates: June 27, 2014 – May 18, 2015

Study Location: Greenbrier, Arkansas

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically against induced infestations of *D. variabilis* for up to 35 days on cats.

Experimental Design:

Table II.25: Treatment Groups for Study A186C-US-14-100

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35
T02	revolution® PLUS	Day 0	8	Days -2, 5, 12, 19, 26, and 33	Days 2, 7, 14, 21, 28, and 35

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with 50 (±5) unfed adult *D. variabilis* ticks on Days -2, 5, 12, 19, 26, and 33. At each tick count on Days 2, 7, 14, 21, 28, and 35, the numbers of live and dead ticks were counted, and the ticks were removed from the cat. Clinical observations were conducted prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 35. Health observations were conducted at least once daily. Tick counts and health observations were conducted by personnel who were masked to treatment.

An adequate infestation was defined as the recovery from a control group cat of a minimum of 25% of the original number of live ticks used to infest the animal. A minimum of six adequately infested control cats were required at each time point for a valid study.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean of live tick counts for the control group and T = geometric mean of live tick counts for the revolution® PLUS-treated group for each time point.

Comparisons of live and dead tick counts between the treatment groups were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the natural logarithm-transformed-counts + 1 by time point, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the control indication was determined on the basis of the percent reduction in live tick counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in live tick counts between the two treatment groups. Effectiveness for the treatment indication was determined on the basis of the significance of the difference in dead tick counts between the two treatment groups on each count day.

Results: An adequate infestation was achieved for all time points on Days 7, 14, 21, 28, and 35. On Day 2, only four of the eight control cats were adequately infested. This was attributed to the grooming behavior of the cats, who had their Elizabethan collars removed following dosing and not replaced until the subsequent infestation. The day 2 tick counts were considered invalid and not included in the determination of effectiveness.

The revolution® PLUS-treated group had a 100% reduction in live tick counts 48 hours after weekly re-infestations for 35 days. Live tick counts for the revolution® PLUS-treated group were significantly reduced following each of the infestation time points in comparison to the control group ($P<0.0001$). Dead tick counts were significantly higher in comparison with the control group following each tick infestation ($P\leq0.0013$; Table II.26).

Table II.26: Study A186C-US-14-100: Geometric mean dead tick counts for revolution® PLUS-treated cats compared with controls following induced *Dermacentor variabilis* infestations of cats, 48 hours after weekly re-infestations

Day of Tick Count	Control Group Geometric Mean Dead Tick Count	revolution® PLUS Geometric Mean Dead Tick Count
Day 7	0	7.3
Day 14	0	6.6
Day 21	0	3.1
Day 28	0	1.8
Day 35	0	2.7

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *D. variabilis* after weekly re-infestation for 35 days.

When compared with the controls, the significantly higher number of dead ticks and the significant reduction of live ticks on the revolution® PLUS-treated cats support the treatment and control indications for *D. variabilis*, respectively, for 35 days.

12. **Type of Study:** Laboratory Non-Interference Study A186C-US-17-208: *Dermacentor variabilis* Ticks

Title: Laboratory Non-interference of a Combination Product Containing Sarolaner and Selamectin against Induced Infestations of *Dermacentor variabilis* on Cats

Study Dates: May 24, 2017 – September 12, 2017

Study Location: Turlock, California

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically against induced infestations of *D. variabilis* for up to 36 days on cats, and to justify the inclusion of sarolaner in the combination product.

Experimental Design:

Table II.27: Treatment Groups for Study A186C-US-17-208

Treatment Group	Treatment	Day of Treatment	Cats per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	Day 0	10	Days -2, 5, 12, 19, 26, and 33	Days 3, 8, 15, 22, 29, and 36
T02	revolution® PLUS	Day 0	10	Days -2, 5, 12, 19, 26, and 33	Days 3, 8, 15, 22, 29, and 36
T03	Selamectin	Day 0	10	Days -2, 5, 12, 19, 26, and 33	Days 3, 8, 15, 22, 29, and 36

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infested with 50 (±5) unfed adult *D. variabilis* ticks on Days -2, 5, 12, 19, 26, and 33. At each tick count on Days 3, 8, 15, 22, 29, and 36, the numbers of live and dead ticks were counted, and the ticks were removed from the cats. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment, as well as on Days 3, 5, and 36. Health observations were conducted at least once daily. Tick counts and health observations were conducted by personnel who were masked to treatment.

An adequate infestation was defined as the recovery from a control group cat of a minimum of 25% of the original number of ticks used to infest the animal. A minimum of six adequately infested control cats were required at each time point for a valid study.

Statistical Methods: Percent effectiveness of the treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = least squares mean of live tick counts for the control group and T = least squares mean of live tick counts for the treated group for each time point.

Comparisons of live tick counts were tested using the (two-sided) 5% significance level between the revolution® PLUS-treated group (Group T02) and the control group (Group T01; effectiveness indication) and between the selamectin-treated group (Group T03) and the control group (Group T01; non-interference indication). Comparisons of dead tick counts were also tested using the (two-sided) 5% significance level between the revolution® PLUS-treated group (Group T02) and the control group (Group T01). A mixed model analysis was used to analyze the tick counts by time point, with treatment group as a fixed effect and block and error as random effects. Tests for effectiveness and non-interference were performed in separate analyses.

Effectiveness for the control indication was determined on the basis of the percent reduction in live tick counts in the treated group compared to the control group and the significance of the difference in live tick counts between the two treatment groups. Effectiveness for the treatment indication was determined on the basis of the significance of the difference in dead tick counts between the treated group and the control group. Non-interference was determined on the basis of the revolution® PLUS-treated group meeting the requirement for effectiveness with the selamectin-treated group not meeting this requirement.

Results: An adequate infestation was achieved for all time points on Days 8, 15, 22, 29, and 36. On Day 3, only three of the ten cats were adequately infested. This was attributed to the grooming behavior of the cats, who had their Elizabethan collars removed following dosing and not replaced until the subsequent infestation. The Day 3 tick counts were considered invalid and not included in the determination of effectiveness.

The revolution® PLUS-treated group had a ≥93.5% reduction in least squares mean live tick counts 72 hours after weekly re-infestations for 36 days (Table II.28). Live tick counts for the revolution® PLUS-treated group were significantly different ($P < 0.0001$) and numerically reduced compared to the counts in the control group at each of the infestation time points.

Dead tick counts were significantly different from and numerically higher in comparison with the control group following each tick infestation (P≤0.0451; Table II.29).

The selamectin-treated group had a reduction in least squares mean live tick count 72 hours after weekly re-infestations that was never greater than 54.9%. Live tick counts for the selamectin-treated group were significantly different from the control group (P=0.0057) on Day 8, but did not differ from the control group (P≥0.0965) following each of the subsequent infestation time points.

Dead ticks were only recovered from a single animal in the selamectin-treated group—1 dead tick on Day 8 and 3 dead ticks on Day 29. There were no dead ticks recovered from the vehicle control cats. No comparisons between the dead tick counts in the selamectin-treated group and the control group were conducted.

Table II.28: Least squares mean (LSM) and geometric mean (GM) live tick counts and percent effectiveness for revolution® PLUS for the control of induced *Dermacentor variabilis* infestations of cats, 72 hours after weekly re-infestations; Study A186C-US-17-208

Day of Study	Control Group LSM (GM) Live Tick Count	revolution® PLUS LSM (GM) Live Tick Count	Percent Effectiveness*
Day 8	29.7 (27.2)	0.0 (0.0)	100% (100%)
Day 15	36.1 (33.5)	0.1 (0.1)	99.7% (99.8%)
Day 22	32.0 (27.2)	0.1 (0.1)	99.7% (99.7%)
Day 29	29.7 (26.3)	0.6 (0.3)	98.0% (98.7%)
Day 36	38.7 (38.3)	2.5 (0.8)	93.5% (97.9%)

* Based on percent reduction in least squares means (geometric means) of treated group compared with control group.

Table II.29: Study A186C-US-17-208: Least squares mean (LSM) and geometric mean (GM) dead tick counts for revolution® PLUS-treated cats compared with controls following induced *Dermacentor variabilis* infestations of cats, 72 hours after weekly re-infestations

Day of Study	Control Group LSM (GM) Dead Tick Count	revolution® PLUS LSM (GM) Dead Tick Count
Day 8	0.0 (0.0)	1.9 (1.3)
Day 15	0.0 (0.0)	2.5 (1.8)
Day 22	0.0 (0.0)	1.7 (1.0)
Day 29	0.0 (0.0)	0.9 (0.6)
Day 36	0.0 (0.0)	1.1 (0.9)

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *D. variabilis* at 72 hours after weekly re-infestations for 36 days.

When compared with the controls, the significantly higher number of dead ticks and the significant reduction of live ticks on the revolution® PLUS-treated cats support the treatment and control indications for *D. variabilis*, respectively, for 36 days.

Treatment with selamectin alone was not effective against infestations of adult *D. variabilis*, justifying the need for inclusion of sarolaner in the combination.

For the Prevention of Heartworm Disease

13. **Type of Study:** Laboratory Dose Confirmation Study A186C-US-14-116: Heartworm

    Title: Laboratory Dose Confirmation Study A186C-US-14-116: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin for Prophylaxis against *Dirofilaria immitis* in Cats

    Study Dates: June 30, 2014 – June 3, 2015

    Study Location: Richland, MI

    Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

    Objective:
    Confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically as a single treatment or at monthly intervals for three consecutive months for the prevention of heartworm disease caused by *D. immitis* in cats.

    Experimental Design:

Table II.30: Treatment Groups for Study A186C-US-14-116

Treatment Group	Treatment	Days of Treatment	Days of Placebo Treatment	Cats per Group	Day of L3 Inoculation	Day of Adult *D. immitis* Count
T01	Vehicle Control	N/A	Days 0, 28, and 56	10	Day -30	Day 145
T02	revolution® PLUS	Day 0	Days 28 and 56	10	Day -30	Day 145
T03	revolution® PLUS	Days 0, 28, and 56	N/A	10	Day -30	Day 145

N/A: Not applicable. L3: Third-stage larvae of *D. immitis*.

Drug Administration:
All treatments were administered topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Thirty days prior to the first treatment, each cat was inoculated with 100 third-stage larvae of *D. immitis*. Clinical observations were conducted prior to and 1, 3, 6, and 24 hours after each treatment. Administration sites were evaluated for any abnormalities prior to and 1, 3, 6, and 24 hours after each treatment, 3 and 5 days after each treatment, and on Day 84. General health observations were conducted once daily. Each cat was necropsied for recovery of adult heartworms 145 days after the first treatment. Worm counts and health observations were conducted masked to treatment.

Statistical Methods: For the live, adult heartworm counts, percent effectiveness of the treated groups with respect to the vehicle control group were calculated using the formula [(C-T) / C] x 100, where C = geometric mean of worm counts for the control group and T = geometric mean of worm counts for the revolution® PLUS-treated groups. The comparisons between the revolution® PLUS-treated groups and the vehicle control group were tested using the (two-sided) 5% significance level. A mixed linear model analysis was used to analyze log-counts, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the prevention indication was determined on the basis of the percent reduction in live, adult heartworm counts in the revolution® PLUS-treated group which received a single treatment (Group T02) compared to the placebo control group (Group T01).

Results: There was 100% prevention of development of *D. immitis* in both groups of cats treated with revolution® PLUS. The difference in the numbers of live worms counted in the revolution® PLUS-treated and the vehicle control groups was significant ($P<0.0001$). Nine of the 10 vehicle control cats had live, adult heartworms at necropsy. Six of the cats in the vehicle control group each had at least 5 live, adult heartworms at necropsy. The average number of heartworms found in the placebo control group was 5.4 (geometric mean) with a range of 1-21 worms in the nine infected cats.

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusions: revolution® PLUS applied topically as a single minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin was 100% effective in preventing the development of heartworms in cats inoculated with infective larvae of *D. immitis* 30 days prior to treatment.

14. **Type of Study:** Laboratory Dose Confirmation and Non-Interference Study A186C-US-16-193: Heartworm

    Study Title: Laboratory Dose Confirmation and Non-interference Study A186C-US-16-193: Laboratory Non-interference of a Combination Product Containing Sarolaner and Selamectin for Prophylaxis against *Dirofilaria immitis* in Cats

Study Dates: March 11, 2016 – April 10, 2017

Study Location: Athens, GA

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Study Objective:
Confirm the effectiveness of sarolaner (dosed at a minimum dosage of 1.0 mg/kg) in combination with selamectin (dosed at a minimum dosage of 6.0 mg/kg) applied topically as a single treatment or at monthly intervals for three consecutive months for the prevention of heartworm disease caused by *D. immitis* in cats. Confirm the combination of the above compounds does not interfere with the effectiveness of selamectin for the prevention of heartworm disease caused by *D. immitis* in cats.

Experimental Design:

Table II.31: Treatment Groups for Study A186C-US-16-193

Treatment Group	Treatment	Days of Treatment	Days of Vehicle Treatment	Cats per Group	Day of L3 Inoculation	Day of Adult *D. immitis* Count
T01	Vehicle Control	N/A	Days 0, 28, and 56	10	Day -30	Day 146
T02	revolution® PLUS	Day 0	Days 28 and 56	10	Day -30	Day 146
T03	Sarolaner	Day 0	Days 28 and 56	10	Day -30	Day 146
T04	revolution® PLUS	Days 0, 28, 56	N/A	10	Day -30	Day 146

N/A: Not applicable. L3: Third-stage larvae of *D. immitis*.

Drug Administration:
All treatments were administered topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Thirty days prior to the first treatment, each cat was inoculated with 100 third-stage larvae of *D. immitis*. Clinical observations were conducted prior to and 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to and 1, 3, 6, and 24 hours after each treatment, 3 and 5 days after each treatment, and on Days 77 and 84. General health observations were conducted once daily. Each cat was necropsied for recovery of adult heartworms 146 days after the first treatment. Worm counts and health observations were conducted masked to treatment.

Statistical Methods: For the live, adult heartworm counts, percent effectiveness of the treated groups with respect to the vehicle control group were calculated using the formula [(C-T) / C] x 100, where C = geometric mean of worm counts for the control group and T = geometric mean of worm

counts for the revolution® PLUS-treated groups. The comparisons between the revolution® PLUS-treated groups and the vehicle control group were tested using the (two-sided) 5% significance level. A mixed linear model analysis was used to analyze log-counts, with treatment group as a fixed effect and block and error as random effects.

Effectiveness for the prevention indication was determined on the basis of the percent reduction in live, adult heartworm counts in the revolution® PLUS-treated group which received a single treatment (Group T02) compared to the vehicle control group (Group T01).

Non-interference for the prevention indication was determined on the basis of whether the inclusion of selamectin in the combination was justified. The inclusion was justified if the single treatment with sarolaner (Group T03) did not meet the criteria for effectiveness while the single treatment with revolution® PLUS (Group T02) did.

Results: There was 100% prevention of development of *D. immitis* in both groups of cats treated with revolution® PLUS. The difference in the numbers of live worms counted in the revolution® PLUS-treated and the vehicle control groups was significant ($P<0.0001$). Six of the cats in the vehicle control group each had at least 3 live, adult heartworms at necropsy. The remaining three cats in this group had 1 worm each (one cat was excluded from analysis due to a positive heartworm test during the study). The average number of heartworms found in the vehicle control group was 3.2 (geometric mean) with a range of 1-20 worms.

The sarolaner treatment was not effective in preventing the development of *D. immitis*. The difference in worm counts between the sarolaner-treated and the vehicle control groups was not significantly different ($P=0.4374$). Live, adult heartworms were recovered from eight of the cats in the sarolaner group. No worms were recovered from the remaining two cats in this group. The average number of heartworms found in this group was 2.4 (geometric mean) with a range of 1-10 worms in the eight infected cats.

Adverse Reactions: One case of regurgitation post dosing was recorded.

Conclusions: revolution® PLUS applied topically as a single minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin was 100% effective in preventing the development of heartworms in cats inoculated with infective larvae of *D. immitis* 30 days prior to treatment.

For the Treatment and Control of Ear Mites Infestations

15. **Type of Study:** Field Effectiveness and Safety Study Number A181C-US-13-093: Ear Mites

    Title: Efficacy and Safety of Topically Administered Sarolaner + Selamectin in the Treatment and Control of Natural Infestations of *Otodectes cynotis* in Cats Presented as Veterinary Patients

Study Dates: June 13, 2014 – April 26, 2017

Study Locations:

Albemarle, NC
Bartlesville, OK
Battle Creek, MI
Everett, WA
Knoxville, IA
Livonia, LA
Locust, NC
Montgomery, AL
New Braunfels, TX
Portage, MI
Quakertown, PA
Seguin, TX
Springfield, MO

Of the 13 sites, six sites did not meet the enrollment criteria or met the enrollment criteria, but an insufficient number of cats completed the study; therefore, these sites were removed from the effectiveness evaluation. All sites were included in the safety database.

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
Evaluate the effectiveness and safety of sarolaner and selamectin against natural infestations of ear mites in cats under field conditions.

Study Animals:
One hundred twenty-four (124) revolution® PLUS-treated cats and 63 imidacloprid + moxidectin-treated cats were evaluated for safety. The effectiveness analysis for Day 30 was performed on 54 revolution® PLUS-treated cats and 25 imidacloprid + moxidectin-treated cats. Enrollment was limited to those households with a maximum of three cats; there was no restriction on the type or number of other pets in the household. There were no breed or gender restrictions, but cats intended for breeding, and pregnant and lactating cats were not eligible for enrollment.

Experimental Design:
For a household to be included, at least one cat (the primary cat) had to have clinical evidence of ear mite infestation with a mite count of ≥5 live mites. In households where more than one cat met this requirement, the primary cat was selected randomly from all eligible cats in the household that harbored ≥5 live mites, and the other cats were designated as supplemental cats. All cats in the household received the same treatment as the primary cat and were included in safety evaluations. Only primary cats were included in effectiveness evaluations.

Drug Administration:
All treatments were administered topically. The owner administered the revolution® PLUS or the imidacloprid + moxidectin in the presence of the dispenser at the veterinary clinic on Day 0.

Measurements and Observations:
For primary cats only, post-treatment bilateral otoscopic examinations for ear mites and microscopic examinations of aural material from both ears were performed on Days 14 and 30. The primary requirement for effectiveness was absence of ear mites in ≥90% of the treated cats at Day 30. For all cats, physical examination, clinical pathology, dosing, and any abnormal health events were recorded.

On or within one day prior to Day 0, each cat was weighed and given a physical examination, otoscopic examination was performed for ear mites, and blood and urine were collected for clinical pathology. All cats were weighed and given a post-treatment physical examination on Day 14 and Day 30, and blood and urine were collected for clinical pathology on Day 30. Data for Day 14 procedures included in the evaluation were collected within a window of Day 11 to Day 19, and data for Day 30 procedures within a window of Day 25 to Day 35.

Statistical Methods: Data for the presence or absence of live ear mites were summarized using frequency distributions to show the number of animals in each group that had ear mites present or absent on Days 14 and 30.

Results: In the revolution® PLUS-treated group, 48 of 55 cats (87.3%) did not have mites present on examination on Day 14, and 51 of 54 cats (94.4%) did not have mites present on Day 30. In the imidacloprid + moxidectin-treated group, 16 of 25 cats (64.0%) did not have mites present on Day 14, and 18 of 25 cats (72.0%) did not have mites present on Day 30 (Table II.32).

Table II.32: Effectiveness of revolution® PLUS against *Otodectes cynotis* for Study A181C-US-13-093

Treatment Group	Treatment	Day 14 Mite-free cats	Day 14 Percent Effectiveness	Day 30 Mite-free cats	Day 30 Percent Effectiveness
T01	revolution® PLUS	48 of 55	87.3	51 of 54	94.4
T02	imidacloprid + moxidectin	16 of 25	64.0	18 of 25	72.0

Adverse Reactions: Evaluation of safety was completed over the 30-day period through in-clinic physical examinations or through reporting of abnormalities by the owner for both primary and supplemental cats. The safety database included 124 cats administered revolution® PLUS and 63 cats administered imidacloprid + moxidectin.

Table II.33: Adverse Reactions for Study A181C-US-13-093

Adverse Reaction	revolution® PLUS (n=124)	Imidacloprid + Moxidectin (n=63)
Vomiting	4 (3.2%)	0 (0.0%)
Pruritus	4 (3.2%)	2 (3.2%)
Diarrhea	2 (1.6%)	0 (0.0%)
Anorexia	2 (1.6%)	0 (0.0%)
Anemia	2 (1.6%)	0 (0.0%)

Conclusion: The results of this study demonstrate that revolution® PLUS, when used at the minimum labeled dose of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, is safe and effective for the treatment and control of ear mite infestations in cats for one month. Treatment with revolution® PLUS was associated with vomiting, pruritus, diarrhea, anorexia, and anemia.

16. **Type of Study**: Laboratory Dose Confirmation Study A186C-US-14-110: Ear Mites

    Title: Dose Confirmation of a Combination Product Containing Sarolaner and Selamectin against infestations of *Otodectes cynotis* in Cats

    Study Dates: March 31, 2015 – September 11, 2015

    Study Location: Rockwood, Tennessee

    Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

    Objective:
    Confirm the effectiveness of sarolaner (dosed at 1.0 mg/kg) in combination with selamectin (dosed at 6.0 mg/kg) applied topically as a single treatment against infestations of *O. cynotis* in cats.

    Study Animals:
    14 domestic short hair cats (8 male and 6 female), 4 to 12 months of age, weighing between 2.2-5.7 kg.

    Experimental Design:
    Enrolled cats had established ear mite infestations, defined as a minimum of 5 live mites in at least one ear. Infestations were induced 5 to 8 weeks prior to Day 0 by interaural transfer of aural material. The cats were treated on Day 0. Bilateral otoscopic examinations were performed on all cats on Day 14 and total live ear mite counts were conducted on Day 30.

Table II.34: Treatment Groups for Study A186C-US-14-110

Treatment Groups	Treatment	Day of Treatment	Cats per Group	Day of Otoscopic Exam	Day of *O. cynotis* Counts
T01	Vehicle Control	Day 0	7	Day 14	Day 30
T02	revolution® PLUS	Day 0	7	Day 14	Day 30

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
The primary variable for the determination of effectiveness was live ear mite counts (larvae, nymphs and adults) on Day 30. While sedated, each cat's ear was flushed. The flushings were collected separately per ear and examined for live mites, which were then counted. Clinical observations were conducted prior to, and 1, 3, 6, and 24 hours after treatment. Administration sites were evaluated for any abnormalities prior to, and 1, 3, 6 and 24 hours after treatment, as well as on Days 3, 5, and 30. General health observations were conducted twice daily. Ear mite counts and health observations were conducted masked to treatment group.

Statistical Methods: For the live ear mite counts, percent effectiveness of the treated group with respect to the vehicle control group was calculated using the formula [(C-T) / C] x 100, where C = geometric mean of mite counts for the vehicle control group and T = geometric mean of mite counts for the revolution® PLUS-treated groups. The comparisons were tested using the (two-sided) 5% significance level. A mixed linear model analysis was used to analyze log-counts, with treatment group as a fixed effect and batch and block within batch as random effects.

Effectiveness for the treatment and control indication was determined on the basis of the percent reduction in geometric mean live mite counts in the revolution® PLUS-treated group on Day 30 compared to the vehicle control group.

Results: On otoscopic examination on Day 14, all control cats had mites in at least one ear while no mites were noted in any of the revolution® PLUS-treated cats. On Day 30, all seven of the control cats had ≥5 live mites in at least one ear while only one revolution® PLUS-treated cat had ≥5 live mites. The geometric mean live mite counts were reduced by 99.6% in the revolution® PLUS-treated group compared with the control group (Table II.35). The difference in geometric mean mite count between the revolution® PLUS-treated group and the control group was significantly different (P=0.0003).

Table II.35: Effectiveness of revolution® PLUS against *Otodectes cynotis* for Study A186C-US-14-110

Treatment Group	Treatment	Day 30 Mite Count (Range)*	Day 30 Percent Effectiveness
T01	Vehicle Control	301.6 (9-1176)	Not Applicable
T02	revolution® PLUS	1.3 (0-28)	99.6%

*Mite counts are geometric means.

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusions: revolution® PLUS applied topically as a single minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin in cats was effective in reducing live ear mite counts when assessed 30 days after treatment.

For the Treatment and Control of Gastrointestinal Nematodes

17. **Type of Study:** Field Effectiveness and Safety Study A181C-US-15-166: *Ancylostoma tubaeforme*

    Title: Efficacy and Safety Study of Topically Administered Sarolaner + Selamectin in the Treatment and Control of Natural Infections of *Ancylostoma tubaeforme* in Cats Presented as Veterinary Patients

    Study Dates: July 10, 2015 – June 1, 2017

    Study Locations:

    Athens, AL
    Bay Springs, MS
    Boca Raton, FL
    Grand Rapids, MI
    Kamuela, HI
    Lake Charles, LA
    Largo, FL
    Lawrence, KS
    Lumberton, TX
    Memphis, TN
    New Braunfels, TX
    Saucier, MS
    Seguin, TX
    Terre Haute, IN
    Waianae, HI
    Waipahu, HI

    Of the 16 sites, seven sites were removed from the effectiveness evaluation because the site did not enroll an adequate number of cases, or an adequate number of cases from the site did not complete the study. All sites were included in the safety database

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To evaluate the effectiveness and safety of revolution® PLUS in the treatment and control of natural infections of *A. tubaeforme* in cats under field conditions. The secondary objective was to evaluate the effectiveness in the treatment and control of *Toxocara cati*.

Study Animals:
Seventy (70) revolution® PLUS-treated cats and 65 selamectin-treated cats were evaluated for safety. The effectiveness analysis for Days 30 and 60 was based on data for 40 revolution® PLUS-treated cats.

Only one cat was enrolled per household, but there was no restriction on type or number of other pets in the household. There were no breed or gender restrictions, but cats intended for breeding, and pregnant and lactating cats were not eligible for enrollment. The use of medications or products with anthelmintic activity against hookworms and/or roundworms in enrolled cats prior to or during the study period was not permitted. Treatment of any other pets in the household with a topical product with anthelmintic activity against gastrointestinal nematodes was not allowed, however, treatment with oral anthelmintic products was allowed for other pets.

Experimental Design:

Table II.36. Treatment Groups for Study for Study A181C-US-15-166

Treatment Group	Treatment	Days of Treatment	Days of Fecal Egg Count	Total Cats Enrolled per Group	Cats Evaluable for Effectiveness for *A. tubaeforme* for Day 30*	Cats Evaluable for Effectiveness for *A. tubaeforme* for Day 60*
T01	revolution® PLUS	Days 0 and 30	Days 0, 30, and 60	70	40	40
T02	Selamectin	Days 0 and 30	Days 0, 30, and 60	65	44	40

* Only data for Group T01 cats were evaluated for effectiveness.

Drug Administration:
On Day 0, owners administered revolution® PLUS or the active control (selamectin) to their cats in the presence of the dispenser at the veterinary clinic. On Day 30, owners administered revolution® PLUS or the active control (selamectin) to their cats in the home environment.

Measurements and Observations:
Post-treatment fecal egg counts were performed on Days 30 and 60 for comparison with the Day 0 fecal egg count. Findings on physical examination, clinical pathology results, dosing, and any abnormal health events were recorded.

Statistical Methods: The primary assessment of effectiveness for revolution® PLUS was based on the reduction in geometric mean *A. tubaeforme* fecal egg counts on Days 30 and 60 compared with the pre-treatment count on Day 0, and the calculation of the 95% confidence intervals for the percentage reductions for Day 30 and 60 post treatment. To be considered effective, the values for the lower bounds of the confidence intervals were required to be ≥92.4% and ≥91.7% on Day 30 and Day 60, respectively. These values were based on those calculated in a field study conducted in support of the original approval of revolution® for the treatment and control of *A. tubaeforme*. For the fecal egg counts, the percent effectiveness of the revolution® PLUS-treated group with respect to the baseline was calculated at each time point using the formula [(C-T)/C] x 100, where C = pre-treatment geometric mean fecal egg count and T = post-treatment geometric mean fecal egg count. A bootstrap method was used to calculate the 95% confidence intervals of the percentage reductions. Comparisons between Day 0 and Day 30 and between Day 0 and Day 60 were conducted separately. The inclusion of the selamectin-treated group in the study served to maintain masking of investigators, but the fecal egg count reduction for this group post-treatment was not evaluated. Effectiveness for *T. cati* and/or other gastrointestinal nematode species was assessed using the same procedure as for *A. tubaeforme* provided there were a sufficient number of evaluable cases in each treatment group.

Results: For the revolution® PLUS-treated group, fecal egg count data for *A. tubaeforme* for 40 cats on Day 30 and for 40 cats on Day 60 were available for comparison with the Day 0 counts. The effectiveness of revolution® PLUS against *A. tubaeforme* was 99.4% on Day 30 and 99.7% on Day 60 based on a reduction in fecal egg count. The lower bounds of the confidence intervals for the reductions were 98.0% and 95.2% on Day 30 and Day 60, respectively. These values were greater than the required values for the lower bounds of the confidence intervals. The *A. tubaeforme* fecal egg counts for Day 0 differed significantly from those for Day 30 and for Day 60 ($P<0.0001$).

For the revolution® PLUS-treated group, fecal egg count data for *T. cati* were available for 14 cats on Day 30 and 14 cats on Day 60 for comparison with the Day 0 counts. The effectiveness of revolution® PLUS against *T. cati* was 100% on Day 30 and 100% on Day 60 based on a reduction in fecal egg count. The *T. cati* fecal egg counts for Day 0 differed significantly from those for Day 30 and for Day 60 ($P<0.0001$).

Table II.37: Study A181C-US-15-166: Field Effectiveness of revolution® PLUS against *Ancylostoma tubaeforme* and *Toxocara cati*—Geometric Mean Fecal Egg Counts, Percentage Reductions Compared to Pre-Treatment (Day 0), and 95% Confidence Intervals (CI)

N/A	Geometric Mean Fecal Egg Count (Eggs per Gram of Feces)			Day 30 Percentage Reduction	95% CI Day 30 Percentage Reduction	Day 60 Percentage Reduction	95% CI Day 60 Percentage Reduction
	Day 0	Day 30	Day 60				
A. tubaeforme	439.4	2.6	1.3	99.4	98.0 – 99.9	99.7	95.2 - 100
T. cati	541.4	0.0	0.0	100	100 - 100	100	100 - 100

The mean values (or, in the case of categorical variables, the majority of animals) for all hematology and serum chemistry variables for both treatment groups were within the normal reference ranges on Day 0 and Day 60. Overall, the urinalysis results were unremarkable for the cats in both treatment groups at both sampling days.

Adverse Reactions: Evaluation of safety was completed over the 60-day period through in-clinic physical examinations or through reporting of abnormalities by the owner (Table II.38). The safety database included 70 cats administered revolution® PLUS and 65 cats administered selamectin.

Table II.38: Adverse Reactions with Study A181C-US-15-166

Adverse Reaction	revolution® PLUS (n=70)	Selamectin (n=65)
Diarrhea	9 (12.9%)	3 (4.6%)
Anorexia	6 (8.6%)	0 (0.0%)
Vomiting	4 (5.7%)	1 (1.5%)
Lethargy	3 (4.3%)	2 (3.1%)

There were no serious treatment-related adverse events noted during the study.

Conclusion: The results of this study demonstrate that revolution® PLUS, when used monthly at the minimum labeled dose of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, is safe and effective for the treatment and control of *A. tubaeforme* and *T. cati* infections in cats.

18. **Type of Study:** Laboratory Non-Interference and Dose Confirmation Study A186C-US-15-167: *Ancylostoma tubaeforme*

Title: Laboratory Non-interference of a Combination Product Containing Sarolaner and Selamectin against Induced Infections of *Ancylostoma tubaeforme* in Cats

Study Dates: May 12, 2015 – December 7, 2015

Study Location: Rockwood, Tennessee

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at 1.0 mg/kg) in combination with selamectin (dosed at 6.0 mg/kg) applied topically against induced infections of *A. tubaeforme* in cats.

Study Animals:
32 domestic short hair cats (16 males and 16 females), 13–14 weeks of age, weighing between 1.8–3.0 kg.

Experimental Design:

Table II.39: Treatment Groups for Study A186C-US-15-167

Treatment Group	Treatment	Day of Treatment	Cats per Group	Day of *Ancylostoma tubaeforme* Inoculation	Day of Necropsy and Worm Recovery
T01	Vehicle Control	Day 0	8	Day -31	Day 10
T02	revolution® PLUS	Day 0	8	Day -31	Day 10
T03	Sarolaner	Day 0	8	Day -31	Day 10
T04	Selamectin	Day 0	8	Day -31	Day 10

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infected with approximately 150 (±50) infective larvae of *A. tubaeforme* on Day -31. On Day 10, the cats were humanely euthanized and a necropsy performed for the recovery of adult and immature *A. tubaeforme*. Clinical observations and administration site evaluations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment.
In addition, administration sites were evaluated for any abnormalities on Days

3, 5, and 10. Health observations were conducted at least once daily. Worm counts and health observations were conducted by personnel who were masked to treatment.

A minimum of six control cats with at least 10 *A. tubaeforme* worms was required for a valid study.

Statistical Methods: Percent effectiveness of the treated groups with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean worm count for the control group and T = geometric mean worm count for the treated groups.

Comparisons of worm counts between the control and treated groups were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the natural logarithm-transformed counts +1, with treatment group as a fixed effect and block and error as random effects.

Effectiveness was determined on the basis of the percent reduction in worm counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in worm counts between the two groups. Non-interference was determined on the basis of whether the inclusion of selamectin in the combination was justified. The inclusion was justified if the single treatment with revolution® PLUS (Group T02) met the criteria for effectiveness while the single treatment with sarolaner (Group T03) did not.

Results: The revolution® PLUS-treated group had a 99.2% reduction in adult *A. tubaeforme* counts compared with controls (Table II.40). The selamectin-treated group had a 98.6% reduction in worm counts. Worm counts in each of these treated groups were significantly lower than in the control group (P<0.0001).

The sarolaner-treated group was not effective in reducing *A. tubaeforme* counts. The sarolaner-treated group did not have a reduction in worm counts compared with the control group. The difference in worm counts between the sarolaner-treated group and the control group was not significant (P=0.6799). An adequate infection was achieved in that each control cat had ≥24 worms (range: 24-136 worms) at necropsy.

Table II. 40: Effectiveness of revolution® PLUS against Adult *Ancylostoma tubaeforme* Based on Geometric Mean Worm Counts; Study A186C-US-15-167

Treatment Group	Treatment	*A. tubaeforme* counts	Percent Reduction
T01	Vehicle Control	79.7	Not Applicable
T02	revolution® PLUS	0.6	99.2
T03	Sarolaner	92.6	-16.1
T04	Selamectin	1.1	98.6

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusions: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *A. tubaeforme* worms from an induced infection.

19. **Type of Study:** Laboratory Non-Interference and Dose Confirmation Study A186C-US-14-117: *Ancylostoma tubaeforme*

Title: Laboratory Non-interference of a Combination Product Containing Sarolaner and Selamectin against Induced Infections of *Ancylostoma tubaeforme* in Cats

Study Dates: November 20, 2014 – November 4, 2015

Study Location: Rockwood, Tennessee

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at 1.0 mg/kg) in combination with selamectin (dosed at 6.0 mg/kg) applied topically against induced infections of *A. tubaeforme* in cats.

Study Animals:
32 domestic short hair cats (16 males and 16 females), 14–15 weeks of age, weighing between 1.8–3.5 kg.

Experimental Design:

Table II.41: Treatment Groups for Study A186C-US-14-117

Treatment Group	Treatment	Day of Treatment	Cats per Group	Day of *Ancylostoma tubaeforme* Inoculation	Day of Necropsy and Worm Recovery
T01	Vehicle Control	Day 0	8	Day -33	Day 7
T02	revolution® PLUS	Day 0	8	Day -33	Day 7
T03	Sarolaner	Day 0	8	Day -33	Day 7
T04	Selamectin	Day 0	8	Day -33	Day 7

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infected with approximately 200 (±50) infective larvae of *A. tubaeforme* on Day -33. Any cats that vomited following inoculation were re-inoculated with a full fraction of larvae. On Day 7, the cats were humanely euthanized and a necropsy performed for the recovery of adult and immature *A. tubaeforme*. Clinical observations and administration site evaluations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. In addition, administration sites were evaluated for any abnormalities on Days 3, 5, and 7. Health observations were conducted at least once daily. Worm counts and health observations were conducted by personnel who were masked to treatment.

A minimum of six control cats with at least 10 *A. tubaeforme* worms was required for a valid study.

Statistical Methods: Percent effectiveness of the treated groups with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean worm count for the control group and T = geometric mean worm count for the treated groups.

Comparisons of worm counts between the control and treated groups were tested using the (two sided) 5% significance level. A mixed model analysis was used to analyze the natural logarithm-transformed counts +1, with treatment group as a fixed effect and block and error as random effects.

Effectiveness was determined on the basis of the percent reduction in worm counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in worm counts between the two groups. Non-interference was determined on the basis of whether the inclusion of selamectin in the combination was justified. The inclusion was justified if the single treatment with revolution® PLUS (Group T02) met the criteria for effectiveness while the single treatment with sarolaner (Group T03) did not.

Results: The revolution® PLUS-treated group had an 84.1% reduction in adult *A. tubaeforme* counts compared with controls (Table II.42). The selamectin-treated group had a 90.3% reduction in worm counts. Worm counts in each of these treated groups were significantly lower than in the control group ($P \leq 0.0020$).

The sarolaner-treated group was not effective in reducing *A. tubaeforme* counts. The sarolaner-treated group had a 9.8% reduction in worm counts compared with the control group. The difference in worm counts between the sarolaner-treated group and the control group was not significant ($P=0.6053$). An adequate infection was achieved in that each control cat had ≥43 worms (range: 43-171 worms) at necropsy.

Table II.42: Study A186C-US-14-117: Effectiveness of revolution® PLUS against Adult *Ancylostoma tubaeforme* Based on Geometric Mean Worm Counts

Treatment Group	Treatment	*A. tubaeforme* counts	Percent Reduction
T01	Vehicle Control	105.0	Not Applicable
T02	revolution® PLUS	16.7	84.1
T03	Sarolaner	94.7	9.8
T04	Selamectin	10.1	90.3

Adverse Reactions: One revolution® PLUS-treated cat vomited post dosing on the day of treatment after being fed, but showed no further clinical signs. A second cat in this group had diarrhea on Day 1 which had resolved within a day.

Conclusions: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was less than 90% effective against adult *A. tubaeforme* worms from an induced infection.

20. **Type of Study:** Dose Confirmation Study A186C-IE-15-185: *Ancylostoma tubaeforme*

Title: Dose Confirmation of Sarolaner in Combination with Selamectin Administered Topically against Induced Infections of *Ancylostoma tubaeforme* in Cats

Study Dates: October 15, 2015 – January 14, 2016

Study Location: Glenamoy, County Mayo, Ireland

Study Design: The study was conducted in accordance with Good Clinical Practice guidelines.

Objective:
To confirm the effectiveness of sarolaner (dosed at 1.0 mg/kg) in combination with selamectin (dosed at 6.0 mg/kg) applied topically against induced infections of *A. tubaeforme* in cats.

Study Animals:
14 domestic short hair cats (10 males and 4 females), 3–5 months of age, weighing between 2.3-3.8 kg.

Experimental Design:

Table II.43: Treatment Groups for Study A186C-IE-15-185

Treatment Group	Treatment	Day of Treatment	Cats per Group	Day of *Ancylostoma tubaeforme* Inoculation	Day of Necropsy and Worm Recovery
T01	Vehicle Control	Day 0	7	Day -42	Day 10
T02	revolution® PLUS	Day 0	7	Day -42	Day 10

Drug Administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Measurements and Observations:
Each cat was infected with approximately 150 (±50) infective larvae of *A. tubaeforme* on Day -42. On Day 10, the cats were humanely euthanized and a necropsy performed for the recovery of adult *A. tubaeforme*. Clinical observations and administration site evaluations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. In addition, administration sites were evaluated for any abnormalities on Days 3, 5, and 10. Health observations were conducted at least once daily. Worm counts and health observations were conducted by personnel who were masked to treatment.

Statistical Methods: Percent effectiveness of the revolution® PLUS-treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean worm count for the control group and T = geometric mean worm count for the treated group.

Comparisons of worm counts between the control and treated groups were tested using the (two-sided) 5% significance level. A mixed model analysis was used to analyze the natural logarithm-transformed counts +1, with treatment group as a fixed effect and block and error as random effects.

Effectiveness was determined on the basis of the percent reduction in worm counts in the revolution® PLUS-treated group compared to the control group and the significance of the difference in worm counts between the two groups.

Results: The revolution® PLUS-treated group had a 94.3% reduction in adult *A. tubaeforme* counts compared with controls (Table II.44). The worm count in the revolution® PLUS-treated group was significantly lower than in the control group ($P<0.0001$). An adequate infection was achieved in that there were six control cats that each had ≥5 worms (range: 5-8 worms) at necropsy.

Table II.44: Study A186C-IE-15-185: Effectiveness of revolution® PLUS against Adult *Ancylostoma tubaeforme* Based on Geometric Mean Worm Counts

Treatment Group	Treatment	*A. tubaeforme* counts	Percent Reduction
T01	Vehicle Control	5.1	Not Applicable
T02	revolution® PLUS	0.3	94.3

Adverse Reactions: No treatment-related adverse reactions were noted in this study.

Conclusion: When applied topically at the minimum dosage of 1.0 mg/kg sarolaner + 6.0 mg/kg selamectin, revolution® PLUS was effective against adult *A. tubaeforme* worms from an induced infection.

Note: Although Study A186C-US-14-117 failed to demonstrate ≥90% effectiveness, when combined with Study A186C-US-15-167 and Study A186C-IE-15-185, the average effectiveness is ≥90% (92.5%). Therefore, the combined data demonstrate that revolution® PLUS is effective for the treatment of *A. tubaeforme* infections.

Studies to confirm the dose necessary for the treatment and control of roundworm (*T. cati*) and hookworm (*A. tubaeforme*) infections were conducted for the approval of REVOLUTION® (selamectin) Topical Parasiticide For Dogs and Cats under NADA 141-152 (Zoetis Inc.). Therefore, only dose confirmation studies (laboratory studies and one field study) using the dose-limiting parasite (*A. tubaeforme*) were necessary to demonstrate effectiveness against both roundworms and hookworms. Effectiveness against roundworms was evaluated in the field study as part of a secondary effectiveness analysis.

III. TARGET ANIMAL SAFETY

The safety of revolution® PLUS was demonstrated in 3 well-controlled laboratory studies described below. The purpose of these studies was to provide information on the safety of revolution® PLUS when used according to the label in cats. Two margin of safety studies were conducted, one in 8 week old kittens and one in 9-10 month old cats. Both studies included groups of cats administered escalating doses of the drug. In one of the studies, a cat in the 3.75X treatment group was found dead. The cause of death was found to be hemorrhage in multiple tissues, likely attributable to a markedly decreased platelet count (measured on day 111). The cause of the decreased platelet count is unknown. This finding has not been recorded in any other study and there were no other treatment-related effects in any of the other cats in either margin of safety study.

Oral tolerance was evaluated to assess the effects of accidental oral ingestion in cats, including from licking or grooming the application site. After oral administration, vomiting, soft feces, and salivation were noted. In one cat, transient mild tremor and decreased activity were observed. Correct application of revolution® PLUS will minimize the occurrence of such events. Finally, three well-controlled field studies

were conducted in client-owned cats. These studies included 476 cats administered the labeled dose. Adverse reactions from the three field studies included anorexia, lethargy, skin lesions, diarrhea, pruritus, vomiting, and alopecia at the application site. These safety studies, in combination with the safety information collected in the effectiveness studies, demonstrate the safety of revolution® PLUS when used according to the label.

In an exploratory tolerance study, one female cat receiving a 5X dose (10/60 mg/kg sarolaner/selamectin) experienced neurologic signs that included piloerection, tremors, and mydriasis one day after the third treatment. These signs resolved without treatment within 2 hours and did not recur with administration of 3 subsequent 5X doses.

A. Type of Study: Margin of Safety Study in Kittens, Study A386N-US-13-083

Title: A Tolerance Study of a Repeat Topical Dose of Sarolaner and Selamectin in Domestic Cats

Study Dates: November 4, 2013 – August 17, 2015

Study Location: Ashland, Ohio

Study Design: This study was conducted in accordance with Good Laboratory Practice Regulations (21 CFR Part 58).

Objective:
To evaluate the safety of the sarolaner and selamectin combination at one (1X), three (3X), three and three quarters (3.75X), and five times (5X) the highest recommended dose administered 8 times at 28-day intervals in kittens beginning at 8 weeks of age

Study Animals:
40 domestic shorthair kittens (20 male, 20 female), 8 weeks of age and 0.63 to 1.03 kg body weight.

Experimental Design:
Cats were randomly allocated to one of five treatment groups of eight cats per group (4 per sex) according to a split-plot design with sex as the whole plot factor and treatment as the sub-plot factor. Blocking was based on pre-treatment body weight and cage location with the individual animal as the experimental unit for treatment. Cats were administered sarolaner and selamectin in fixed combination eight times at 28-day intervals (Days 0, 28, 56, 84, 112, 140, 168, and 196) or were administered saline.

Table III.1: Dose Levels for Study A386N-US-13-083

Group Number	Treatment	Relative dose	Dosage Level (mg/kg)	Number Males	Number Females
T01	Saline	control	0	4	4
T02	Sarolaner and Selamectin	1x	2/12 [a]	4	4
T03	Sarolaner and Selamectin	3x	6/36 [a]	4	4
T04	Sarolaner and Selamectin	3.75x	7.5/45 [a]	4	4
T05	Sarolaner and Selamectin	5x	10/60 [a]	4	4

a = Dosage levels presented as Sarolaner (mg/kg)/Selamectin (mg/kg).

Drug administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Duration of the Study: 155 days

Parameters Measured:
Clinical Observations were made before dosing, 6 hours (± 30 minutes) post-dosing, and daily for 7 days after dosing. The general health of each cat was evaluated twice daily throughout the study. Blood was collected for clinical pathology evaluation (hematology, coagulation, and serum chemistry) on Days -2, 27, 55, 83, 111, 139, 167, 195, and 210. Serial blood samples for pharmacokinetics (PK) were collected from all animals prior to the first dose and after each dose with three to five time-points per dosing interval. Blood was collected for PK evaluation via a jugular vein into chilled tubes containing potassium (K3) EDTA. The plasma sarolaner and selamectin concentrations were determined using validated LC-MS/MS procedure. The calibration range used for both the analytes was 1 to 5000 ng/mL with lower limit of quantitation (LLOQ) of 1 ng/mL. The PK parameters for both sarolaner and selamectin were calculated using non-compartmental methods.

The following sarolaner and selamectin PK parameters were determined using a non-compartmental analysis for each dose for each animal in T02 (1x), T03 (3x), T04 (3.75x), and T05 (5x):

- AUC24-t (the area under the drug concentration-time curve from time 24 hours to the last time-point in the dosing interval)
- C_{max} (the observed peak plasma concentration)
- T_{max} (the time of C_{max})

The AUC for each dosing interval was calculated with the available PK time-points resulting in an AUC from 24 hours post dose to the last time-point of the interval, which was day 27 (648 hours) for doses one to seven and day 14 (336 hours) for dose number eight.

Individual body weights were recorded twice weekly during acclimation, on the first day of dosing, and once weekly throughout the study. Individual food consumption was recorded daily, beginning 6 days prior to dosing. Ophthalmic examinations

were conducted on all animals during acclimation and on the day prior to study completion. A veterinarian performed a complete physical examination on all animals once during acclimation and at the end of the study. A complete necropsy with organ weights and microscopic examination was completed at the end of the study.

Statistical Methods: Post-treatment body weight, average weekly food consumption, and numerical clinical pathology data were analyzed by using a mixed linear model of variance for repeated measures. Where appropriate, a baseline covariate was included in the model. General health observation, veterinary physical examination, veterinary clinical observation, and unscheduled observation findings were summarized with a data listing and frequency distributions by treatment and time point. For organs collected for both sexes, organ weight, organ weight relative to final body weight, and organ weight relative to brain weight were analyzed using a general linear mixed model analysis of variance.

Results: Three unexpected deaths (or morbidities resulting in euthanasia) occurred during this study. Two were determined to be unrelated to the drug product (one in a control animal and one with a diagnosis of infectious pneumonia). The third death had unknown relationship to the drug product. On study day 115, a cat in the 3.75X treatment group was found dead. A post-mortem examination revealed the cause of death to be hemorrhage in multiple tissues. This hemorrhage was likely secondary to a markedly decreased platelet count (as measured on day 111). The cause of the decreased platelet count is unknown.

There were no other treatment-related effects in any of the cats in the study. There were no other treatment-related alterations in any clinical pathology parameter during the study. No test article-related macroscopic or microscopic findings or organ weight alterations were noted.

Plasma concentration of selamectin and sarolaner confirmed systemic exposure in all kittens administered the combination drug product. There were no gender differences in the pharmacokinetics of sarolaner and selamectin. The cats achieved steady state-plasma concentrations following the third dose for selamectin and the sixth dose for sarolaner. The dose proportionality assessment of exposure was influenced by high variability in the estimated PK parameters and therefore, the dose proportionality cannot be concluded definitively. The data suggested that systemic exposure increased less than dose proportionally for both selamectin and sarolaner. It was noted that the control animals showed detectable levels for both sarolaner and selamectin in samples collected after each administration (1-8). This suggests that there was contamination in either dosing of animals or due to unintended exposure in control animals by coming in contact with active treated animals and their cages. These concentrations were low and not thought to contribute to any adverse events in the study.

Conclusions: The study supports the safe use of the selamectin and sarolaner topical solution in cats 8 weeks of age and older when used at the labeled dose and duration.

B. Type of Study: Margin of Safety Study A382N-US-15-161

Title: Margin of Safety Study of a Repeat Topical Dose of Sarolaner and Selamectin in Domestic Cats

Study Dates: April 16, 2015 - July 6, 2016

Study Location: Ashland, Ohio

Study Design: This study was conducted in accordance with Good Laboratory Practice Regulations (21 CFR Part 58).

Objective:
To evaluate the safety of the sarolaner and selamectin combination at one (1X), three (3X), and five times (5X) the highest recommended dose in cats beginning at 9-10 months of age.

Study Animals:
32 domestic shorthair cats (16 male, 16 female), 9-10 months of age and 2.40 to 5.93 kg in body weight.

Experimental Design:
Cats were randomly allocated to one of four treatment groups of eight cats per group (4 per sex) according to a split-plot design with sex as the whole plot factor and treatment as the sub-plot factor. The whole plot design had a completely randomized design with one-way treatment structure, and the sub-plot design was a completely randomized design with one-way treatment structure. Animals were assigned to pens completely at random. Within sex, animals were assigned to treatments completely at random. The experimental unit for treatment was animal. Cats were administered sarolaner and selamectin in fixed combination six times at 28-day intervals (Days 0, 28, 56, 84, 112, and 140) or were administered saline.

Table III.2: Dose levels for Study A382N-US-15-161

Group Number	Treatment	Relative dose	Dosage Level (mg/kg)	Number Males	Number Females
T01	Saline	control	0	4	4
T02	Sarolaner and Selamectin	1x	2/12 [a]	4	4
T03	Sarolaner and Selamectin	3x	6/36 [a]	4	4
T04	Sarolaner and Selamectin	5x	10/60 [a]	4	4

a = Dosage levels presented as Sarolaner (mg/kg)/Selamectin (mg/kg).

Drug administration:
All treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades.

Duration of the Study: 155 days

Parameters Measured:
Clinical Observations were made before dosing, 6 hours (± 30 minutes) post-dosing, and daily for 6 days after dosing. The general health of each cat was evaluated twice daily throughout the study. Blood was collected for clinical pathology evaluation (hematology, coagulation, and serum chemistry) on days -2, 27, 55, 83, 111, 139, and 154/155. Urine was collected for urinalysis on days 27, 55, 83, 111, 139, and 154/155. Individual body weights were recorded twice during acclimation, on the first day of dosing, and once weekly throughout the study. Individual food consumption was recorded daily, beginning 27 days prior to dosing. Ophthalmic examinations were conducted on all animals during acclimation and on the day prior to study completion. A veterinarian performed a complete physical examination on all animals once during acclimation and at the end of the study. Serial blood samples for pharmacokinetics were collected from all animals prior to the first dose and after each dose with five time-points per dosing interval for all doses except the last which had four. The plasma sarolaner and selamectin concentrations were determined using validated LC-MS/MS. A complete necropsy with organ weight and microscopic examination was completed at the end of the study.

Results: There were no treatment-related alterations in any clinical pathology parameter during the study. No test article-related macroscopic or microscopic findings or organ weight alterations were noted. Cosmetic changes included wet appearance and dried white material on the dose site sporadically following dose administration. This was attributed to the test article formulation and route of administration and was not considered an indication of toxicity. Hair loss at the dose site was also noted in two cats in the 1X group and one cat in the 5X group within 1-8 days after the fourth dose administration on day 84.

Plasma concentrations of selamectin and sarolaner confirmed systemic exposure in all cats administered the combination drug product. The cats achieved steady state- plasma concentrations following the second dose for selamectin and the fourth dose for sarolaner. Systemic exposure increased dose proportionally. It was noted that the control animals showed detectable levels for both sarolaner and selamectin in samples collected after each administration (1-6). This suggests that there was contamination in either dosing of animals or due to unintended exposure in control animals by coming in contact with active treated animals and their cages.

Conclusions: The study supports the safe use of the selamectin and sarolaner topical solution in cats when used at the labeled dose and duration.

C. Type of Study: Oral Tolerance Study A386N-US-14-099

Title: An Oral Tolerance Study of Sarolaner and Selamectin in Domestic Cats

Study Dates: April 2, 2014 to April 29, 2015

Study Location: Ashland, Ohio

Study Design: This study was conducted in accordance with Good Laboratory Practice Regulations (21 CFR Part 58).

Objective:
To evaluate the safety of a topical combination of sarolaner and selamectin when administered orally via syringe once to 12-week old domestic shorthair cats followed by a 7-day observation period.

Study Animals:
16 domestic shorthair cats (8 male, 8 female), 12 weeks of age and 1.17 and 1.70 kg body weight.

Experimental Design:
Animals were randomly allocated to treatments and pens (4 cats per sex per treatment group) according to a split-plot design with sex as the whole plot factor and treatment as the sub-plot factor. The whole plot design was a completely randomized design with one-way structure, and the sub-plot design was a randomized complete block design with one-way treatment structure. The selamectin and sarolaner topical solution was administered orally by syringe once to domestic shorthair cats. A concurrent control group received deionized water on a comparable regimen.

Table III.3: Dose levels for Study A386N-US-14-099

Group Number	Treatment	Relative dose	Dosage Level (mg/kg)	Number Males	Number Females
T01	Deionized Water	control	0	4	4
T02	Sarolaner and Selamectin	1x	2/12 [a]	4	4

a = Dosage levels presented as Sarolaner (mg/kg)/Selamectin (mg/kg).

Route of Administration: Oral administration by syringe.

Parameters Measured:
Clinical observations were made prior to dosing, at 6 hours post-dosing, and daily during the 7-day observation period. The general health of each cat was evaluated twice daily throughout the study. Body weights were recorded on days 0 and 7 and food weights were recorded daily. Veterinary physical examinations were performed once during acclimation and at study completion.

Results: There were no test article-related veterinary physical examination findings or effects on survival or body weights. Test article-related general health observations and veterinary clinical observations were noted for the group administered sarolaner and selamectin (T02) and consisted of emesis, soft feces, and salivation. In one male, mild tremor was observed and resolved within 3 hours after dosing; the same cat demonstrated reduced activity approximately 6 hours after dosing. Test article-related lower food consumption was noted for the sarolaner and selamectin (T02) group during study days 0 and 1.

Conclusions: Oral administration of the highest recommended dose of the selamectin and sarolaner topical solution for cats resulted in transient lower food consumption and clinical findings of emesis, soft feces, and salivation. In one male,

mild tremor was observed and resolved within 3 hours after dosing; the same cat demonstrated reduced activity approximately 6 hours after dosing.

D. Preliminary Safety Studies:

In study A386W-US-13-064, an exploratory tolerance study, one adverse event possibly related to the test drug was reported. On study day 59, approximately 1 day after the third treatment administration, one female cat in the 5X (10mg/kg sarolaner and 60 mg/kg selamectin) group experienced neurologic signs that included piloerection, tremors, and mydriasis. These signs resolved without treatment within 2 hours and are consistent with the known toxic effects of this drug combination. This cat completed the study, receiving 3 subsequent 5X doses with no abnormal observations.

IV. HUMAN FOOD SAFETY

This drug is intended for use in cats. Because this new animal drug is not intended for use in food producing animals, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this NADA.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to revolution® PLUS:

Not for human use. Keep this and all drugs out of the reach of children. In humans, revolution® PLUS may be irritating to skin and eyes. revolution® PLUS and selamectin topical solution contain isopropyl alcohol and the preservative butylated hydroxytoluene (BHT). Reactions such as hives, itching, and skin redness have been reported in humans in rare instances after exposure to selamectin topical solution. Individuals with known hypersensitivity to selamectin topical solution should use this product with caution or consult a health care professional. Wash hands after use and wash off any product in contact with the skin immediately with soap and water. If contact with eyes occurs, then flush eyes copiously with water. In case of ingestion by a human, contact a physician immediately. The safety data sheet (SDS) provides more detailed occupational safety information. For a copy of the SDS or to report a suspected adverse reaction, call Zoetis at 1-888-963-8471.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that revolution® PLUS when used according to the label, is safe and effective for the prevention of heartworm disease caused by *Dirofilaria immitis*. revolution® PLUS is also effective for killing adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations, the treatment and control of tick infestations with *Ixodes scapularis* (black-legged tick), *Amblyomma maculatum* (Gulf Coast tick) and *Dermacentor variabilis* (American dog tick), the treatment and control of ear mite (*Otodectes cynotis*) infestations, and the treatment and control of roundworm (*Toxocara cati*) and intestinal hookworm (*Ancylostoma tubaeforme*) infections for one month in cats and kittens 8 weeks and older, and weighing 2.8 pounds or greater.

A. Marketing Status

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is required to monitor the safe use of the product, including treatment of any adverse reactions.

B. Exclusivity

revolution® PLUS, as approved in our approval letter, qualifies for THREE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act because the sponsor submitted an original NADA that contains new studies that demonstrate the safety and effectiveness of revolution® PLUS.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.