Date of Approval: February 27, 2020

CORRECTED FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-521

Simparica Trio™

(sarolaner, moxidectin, and pyrantel chewable tablets)

Chewable Tablet

Dogs

Simparica Trio™ is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (immature adult and adult *Toxocara canis* and adult *Toxascaris leonina*) and adult hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) infections. Simparica TRIO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations, and the treatment and control of tick infestations with *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *Ixodes scapularis* (black-legged tick), and *Rhipicephalus sanguineus* (brown dog tick) for one month in dogs and puppies 8 weeks of age and older, and weighing 2.8 pounds or greater.

Sponsored by:

Zoetis Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-521

B. Sponsor

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

Drug Labeler Code: 54771

C. Proprietary Name

Simparica Trio™

D. Drug Product Established Name

Sarolaner, moxidectin, and pyrantel chewable tablets

E. Pharmacological Category

Antiparasitic

F. Dosage Form

Chewable Tablet

G. Amount of Active Ingredient

Each chewable tablet contains:
3.0 mg sarolaner / 0.06 mg moxidectin / 12.5 mg pyrantel (as pamoate salt)
6.0 mg sarolaner / 0.12 mg moxidectin / 25.0 mg pyrantel (as pamoate salt)
12.0 mg sarolaner / 0.24 mg moxidectin / 50.0 mg pyrantel (as pamoate salt)
24.0 mg sarolaner / 0.48 mg moxidectin / 100 mg pyrantel (as pamoate salt)
48.0 mg sarolaner / 0.96 mg moxidectin / 200 mg pyrantel (as pamoate salt)
72.0 mg sarolaner / 1.44 mg moxidectin / 300 mg pyrantel (as pamoate salt)

H. How Supplied

Simparica Trio™ is available in six sizes, in color-coded packages of 1, 3, or 6 flavored chewable tablets.

I. Dispensing Status

Rx

J. Dosage Regimen

Simparica Trio™ is given orally, once a month, at the recommended minimum dose of 0.54 mg/lb (1.2 mg/kg) sarolaner, 0.011 mg/lb (24 µg/kg) moxidectin, and 2.27 mg/lb (5 mg/kg) pyrantel (as pamoate salt).

K. Dosage Schedule

Body Weight (lbs)	Sarolaner per Tablet (mg)	Moxidectin per Tablet (mg)	Pyrantel per Tablet (mg)	Number of Tablets Administered	Color Coding on Carton
2.8 to 5.5	3.0	0.06	12.5	One	Gold
5.6 to 11.0	6.0	0.12	25.0	One	Purple
11.1 to 22.0	12.0	0.24	50.0	One	Caramel
22.1 to 44.0	24.0	0.48	100.0	One	Blue
44.1 to 88.0	48.0	0.96	200.0	One	Green
88.1 to 132.0	72.0	1.44	300.0	One	Dark Brown
>132.0	Administer the appropriate combination of tablets				

L. Route of Administration

Oral

M. Species/Class:

Dogs

N. Indications:

Simparica Trio™ is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (immature adult and adult *Toxocara canis* and adult *Toxascaris leonina)* and adult hookworm *(Ancylostoma caninum* and *Uncinaria stenocephala)* infections. Simparica Trio kills adult fleas *(Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations, and the treatment and control of tick infestations with *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *Ixodes scapularis* (black-legged tick), and *Rhipicephalus sanguineus* (brown dog tick) for one month in dogs and puppies 8 weeks of age and older, and weighing 2.8 pounds or greater.

II. EFFECTIVENESS

A. Dosage Characterization

For *the Prevention of Heartworm Disease:*
Three laboratory dose determination studies were conducted to evaluate the effectiveness of one or multiple oral doses between 3 and 60 µg moxidectin/kg body weight against heartworm isolates with reduced susceptibility to oral macrocyclic lactone products. These studies demonstrated that the effectiveness of moxidectin improved by increasing the dosage and with repeated monthly

dosing. The results of these dose determination studies and pre-clinical safety data support the selection of a minimum monthly oral dosage of 24 µg/kg for further evaluation for the prevention of canine heartworm disease caused by *Dirofilaria immitis*.

For the Prevention and Treatment of Flea and Tick Infestations:
Laboratory effectiveness studies demonstrated that fleas were more susceptible to sarolaner than ticks, and that *Amblyomma spp*. were the dose limiting tick species. A laboratory effectiveness study demonstrated that a dose of 1.2 mg/kg sarolaner in combination with 24 µg/kg moxidectin and 5 mg/kg pyrantel (as pamoate salt) provided greater than 90% effectiveness through Day 35 against *Amblyomma maculatum*. A laboratory effectiveness study demonstrated that a dose of 1.2 mg/kg sarolaner in combination with 24 µg/kg moxidectin and 5 mg/kg pyrantel (as pamoate salt) provided greater than 90% effectiveness at 48 hours after treatment of an existing infestation and after weekly re-infestation through Day 35 against one isolate of *Amblyomma americanum*. However, in a study against a second *Amblyomma americanum* isolate, a single dose achieved greater than 90% effectiveness 48 hours after treatment of an existing infestation and after weekly re-infestations only through Day 21. In a separate study against the second *Amblyomma americanum* isolate, a single dose achieved greater than 90% effectiveness 72 hours after treatment of an existing infestation and after weekly re-infestations through Day 36. These studies support the selection of 1.2 mg/kg sarolaner in combination with 24 µg/kg moxidectin and 5 mg/kg pyrantel (as pamoate salt), with an assessment 72 hours after dosing, for the treatment and prevention of flea and tick infestations.

For the Treatment and Control of Roundworms and Hookworms:
The dosage of pyrantel (as pamoate salt) necessary for the treatment and control of adult roundworms (*Toxocara canis* and *Toxascaris leonina*) and adult hookworms (*Ancylostoma caninum* and *Uncinaria stenocephala*) in dogs was determined to be 5 mg/kg under Nemex® Tablets (NADA 100-237).

B. Substantial Evidence

The effectiveness of Simparica Trio™ was demonstrated in twenty well-controlled laboratory studies and three clinical field studies described below. These studies demonstrate that Simparica Trio™ is effective against a wide variety of both internal and external parasites. Simparica Trio™ was administered to 227 laboratory and 670 client-owned dogs. The most common adverse reactions reported in the three clinical field studies were vomiting, diarrhea, lethargy, anorexia, otitis externa, pruritus, polyuria, hyperactivity, and polydipsia.

For the Prevention of Heartworm Disease:

1. Field Safety and Effectiveness Study A161C-US-13-211: Prevention of Heartworm Disease

Title: Efficacy and Safety of Sarolaner + Moxidectin + Pyrantel Pamoate in the Prevention of Heartworm Disease caused by *Dirofilaria immitis* in Dogs Presented as Veterinary Patients

Study Dates*:* May 8, 2015 – January 22, 2018

Study Locations*:*

Bartlesville, OK
Baton Rouge, LA
Boca Raton, FL
Bogart, GA
Caledonia, MI
Chester, CT
Farragut, TN
Fort Collins, CO
Grand Rapids, MI
Grapevine, TX
Lake Worth, FL
Lawrence, KS
Livonia, LA
Metairie, LA
Quakertown, PA
Raleigh, NC
Springfield, MO
Terre Haute, IN
West Palm Beach, FL
Wichita Falls, TX
Zachary, LA

Study Design: The study was conducted in accordance with Good Clinical Practice (GCP) guidelines. A masked, multi-center, field safety and effectiveness study was conducted using a generalized randomized block design based on order of presentation of the dogs to the clinic, comparing a combination product containing sarolaner, moxidectin, and pyrantel (non-final formulation #1; see section II.C. Bioequivalence Studies) to an active control, containing both ivermectin and pyrantel. Dogs were confirmed negative of heartworm infection (by *D. immitis* antigen and blood microfilariae testing) prior to enrollment and treatment on Day -1 or 0. Dogs that were less than six months of age were not required to be *D. immitis* antigen or blood microfilariae tested. Dogs were enrolled from 23 veterinary practices located in heartworm endemic regions of the United States. Owners administered the sarolaner, moxidectin, pyrantel pamoate tablet or the active control to the dog in the dog's home environment on Days 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300. Treatments could be administered with or without food. Except for Day -1 or 0, study visit procedures could occur within ± 3 days of the intended day. Physical examination and body weight measurement were performed prior to treatment on Day 0, and on Days 30, 60, 90, 120, 150, 180, 210, 240, and 330. Blood and urine were collected for clinical pathology testing prior to treatment on Day -1 or 0, and on Day 330. Blood was collected on Days 120, 240, and 330 for *D. immiti*s antigen and blood microfilariae testing.

Objective: Evaluate the effectiveness and safety of the sarolaner, moxidectin, pyrantel pamoate tablet administered at monthly intervals for the prevention of heartworm disease caused by *Dirofilaria immitis* in dogs presented as veterinary patients.

Study Animals: Two hundred seventy-two (272) dogs administered the sarolaner, moxidectin, pyrantel pamoate tablet and 138 dogs administered active control were evaluated for safety.

Two hundred forty-six (246) dogs administered the sarolaner, moxidectin, pyrantel pamoate tablet and 119 dogs administered active control were included in the effectiveness evaluation. Only one dog per household could enroll in the study. The use of other heartworm preventatives within 30 days prior to or during the study period was not permitted. The use of ProHeart® 6 within 12 months prior to or during the study period was not permitted. There were no breed or gender restrictions, but dogs intended for breeding, and pregnant and lactating dogs were not eligible for enrollment.

Treatment Groups:

Table II.1. Treatment Groups for study A161C-US-13-211

Treatment Group	Treatment	Minimum Dose	Days of Treatment[i]	Total Dogs per Group (Evaluable Dogs)[ii]	Days of Blood Microfilaria and Heartworm Antigen Assessment
T01	Sarolaner, moxidectin, pyrantel pamoate tablet	2.0 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300	272 (246)	Day -1 or 0, 120, 240, and 330
T02	Active control	6 µg/kg Ivermectin + 5 mg/kg Pyrantel	Day 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300	138 (119)	Day -1 or 0, 120, 240, and 330

[i] With the exception of Day 0, procedures could occur within ±3 days of the Schedule Day.
[ii] Cases evaluable for effectiveness.

Drug Administration: Owners administered the sarolaner, moxidectin, pyrantel pamoate tablet or the active control orally to their dogs monthly in the dog's home environment with or without food.

Measurements and Observations: Post-treatment heartworm antigen and blood microfilariae testing were performed on Days 120, 240, and 330. Findings on physical examination, clinical pathology, palatability assessments, and any abnormal health events were recorded.

Statistical Methods: Heartworm antigen and microfilariae test results were summarized in two-way frequency tables to show the number of animals in each combination of the test results.

The sarolaner, moxidectin, pyrantel pamoate tablet was to be considered effective if all dogs in treatment group T01 had negative heartworm results on Day 330. If any dog in treatment group T01 had at least one positive heartworm test at Day 330, binomial data (negative/positive heartworm test) were to be analyzed using a non-inferiority test with a margin of 5% conducted at the one-sided 0.025.

Frequency distributions for method of dosing and for complete dosing were calculated for treatment groups for each day of dosing for the acceptability assessment.

Results: None of the 246 dogs administered the sarolaner, moxidectin, pyrantel pamoate tablet that completed the study tested positive for adult heartworms on Days 120, 240, or 330.

Adverse Reactions: Evaluation of safety was completed over the 330-day period through in-clinic physical examinations and through reporting of abnormalities by the owner (Table II.2). The safety database included 272 dogs administered the sarolaner, moxidectin, pyrantel pamoate tablet and 138 dogs administered the active control.

Table II.2. Study A161C-US-13-211: Field Effectiveness of Simparica Trio™ for prevention of heartworm disease –Adverse Reactions

Clinical Sign	Sarolaner, moxidectin, pyrantel pamoate tablet n = 272	Active control n = 138
Vomiting	14.3%	10.9%
Diarrhea	13.2%	8.0%
Lethargy	8.5%	6.5%
Anorexia	5.1%	5.8%
Polyuria	3.7%	3.6%
Hyperactivity	2.2%	0.7%
Polydipsia	2.2%	2.9%

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

Conclusions: This study demonstrated that Simparica Trio™ is safe and effective for the prevention of heartworm disease in dogs under field conditions.

2. Laboratory Dose Confirmation study A162C-US-13-220: Prevention of Heartworm Disease

Title: Dose Confirmation of the Effectiveness of a Combination of Sarolaner, Moxidectin and Pyrantel Pamoate for Prophylaxis against *Dirofilaria immitis* Infections in Dogs

Study Dates: June 25, 2015 – June 24, 2016

Study Location: Stanwood, MI

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

Objective: To confirm the effectiveness of a combination product containing sarolaner, moxidectin, and pyrantel pamoate in the prevention of adult *Dirofilaria immitis* infections in dogs.

Study Animals: Sixteen (16) Beagle dogs (8 male and 8 female), 7 months of age and 8.2 - 10.8 kg body weight.

Treatment Groups:

Table II.3. Treatment Groups for Study A162C-US-13-220

Group	Treatment	Dosage	Days of Treatment	Dogs per Group	Day of L_3 Inoculation	Necropsy Day
T01	Vehicle control	NA	Day 0	8	Day -30	Day 122
T02	Sarolaner, moxidectin, pyrantel pamoate tablet*	2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	Day -30	Day 122

L_3: *D. immitis* third-stage larvae.
* Non-final formulation #1; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Thirty days prior to treatment, each dog was inoculated with 50 third-stage larvae of *D. immitis*. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily. Knott's and antigen tests were conducted on Day -32 and Day 90. Each dog was necropsied for recovery of adult heartworms 122 days after treatment.

Statistical Methods: For the log-transformed live, adult heartworm counts, percent effectiveness of the treated group with respect to the control group was 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 sarolaner, moxidectin, pyrantel tablet-treated groups. 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. A 5% level of significance was used to determine whether the treatment effect was significant.

Effectiveness for the prevention indication was determined by the percentage of treated animals without heartworms from the necropsy on Day 122.

Results: There was 100% prevention of development of *D. immitis* in the dogs treated with the sarolaner, moxidectin, pyrantel tablet. The difference in geometric mean worm count between the treated group and the control group was significant ($P<0.0001$). Adult *D. immitis* were recovered from all eight control dogs. Counts ranged from 20 to 37 worms per dog, and geometric mean counts were 30.7.

Adverse Reactions: No treatment-related adverse events were recorded during the study.

Conclusions: A single oral dose of Simparica Trio™ was 100% effective in preventing development of *D. immitis* in dogs inoculated with third-stage larvae 30 days before treatment.

3. Laboratory Dose Confirmation and Non-Interference study A162C-US-13-221: Prevention of Heartworm Disease

Title: Non-interference of Moxidectin in a Combination of Sarolaner, Moxidectin and Pyrantel Pamoate for Prophylaxis against *Dirofilaria immitis* Infections in Dogs.

Study Dates: June 25, 2015 - January 25, 2017

Study Location: Athens, GA

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

Objectives: To confirm the effectiveness of moxidectin, in combination with sarolaner and pyrantel pamoate, for the prevention of *Dirofilaria immitis* infections in dogs.

Study Animals: Thirty-two (32) Beagle dogs (16 male and 16 female), 4 to 5 months of age and 6.1 to 9.8 kg body weight.

Treatment Groups:

Table II.4. Treatment Groups for Study A162C-US-13-221

Group	Treatment	Dosage	Days of Treatment	Dogs per Group	Day of L_3 Inoculation	Day of Adult *Dirofilaria immitis* Count
T01	Vehicle control	NA	Day 0	8	Day -30	Day 118
T02	Sarolaner, moxidectin, pyrantel pamoate tablet*	2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	Day -30	Day 118
T03	Sarolaner	2 mg/kg	Day 0	8	Day -30	Day 118
T04	Moxidectin	24 µg/kg	Days 0	8	Day -30	Day 118

L_3: *D. immitis* third-stage larvae.
* Non-final formulation #1; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Thirty days prior to treatment, each dog was inoculated with 50 third-stage larvae of *D. immitis*. Clinical observations were conducted prior to treatment on Day 0 and at 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily. Knott's and antigen tests were conducted on Day -32 and Day 90. Each dog was necropsied for recovery of adult heartworms 118 days after treatment.

Statistical Methods: For the log-transformed live, adult heartworm counts, percent effectiveness of the sarolaner, moxidectin, pyrantel tablet-treated group with respect to the control group was 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 sarolaner, moxidectin, pyrantel tablet-treated groups. 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. Treatment differences were assessed at the two-tailed 5% level of significance.

Effectiveness for the prevention indication was determined by the percentage of treated animals without heartworms from the necropsy on Day 118.

Non-interference for the prevention of heartworm disease caused by *Dirofilaria immitis* was determined on the basis of whether the inclusion of

moxidectin in the combination was justified. The inclusion was justified if the sarolaner, moxidectin, pyrantel tablet (Group T02) met the criteria for effectiveness while the single treatment with sarolaner (Group T03) did not.

Results: There was 100% prevention of development of *D. immitis* in dogs treated with sarolaner, moxidectin, pyrantel tablets or moxidectin tablets. The difference in worm counts compared with the control group was significant ($P<0.0001$) for both treatment groups. Adult *D. immitis* were recovered from all eight control dogs. Counts ranged from 35 to 44 worms per dog, and geometric mean counts were 39.9.

Adult *D. immitis* were recovered from all eight sarolaner–treated dogs. Counts ranged from 32 to 40 worms per dogs, and geometric mean counts were 36.7. Percent reduction in geometric mean count compared to control dogs was 8.2%.

Adverse Reactions: No treatment-related adverse events were recorded during the study.

Conclusions: Both sarolaner, moxidectin, pyrantel tablets and moxidectin only tablets administered as a single oral dose were 100% effective in preventing development of *D. immitis* in dogs inoculated with third-stage larvae 30 days before treatment. The moxidectin in Simparica Trio™ tablets is as effective as moxidectin alone in preventing heartworm disease.

Treatment with sarolaner alone was not effective in preventing development of heartworm disease, justifying the need for inclusion of moxidectin in the combination.

For the Treatment and Prevention of Flea Infestations

4. Field Safety and Effectiveness study A161C-US-15-634: Treatment and Prevention of Flea Infestations

Title: Field Effectiveness and Safety Study A161C-US-15-634: Efficacy and Safety of Orally Administered Sarolaner + Moxidectin + Pyrantel Pamoate in the Treatment and Control of Natural Flea Infestations on Dogs Presented as Veterinary Patients

Study Dates: June 17, 2016 – January 29, 2018

Study Locations:

Bartlesville, OK
Bogart, GA
Caledonia, MI
Farragut, TN
Gainesville, FL
Lake Worth, FL
Lumberton, TX
Metairie, LA
Memphis, TN

Pensacola, FL
Quakertown, PA
Raleigh, NC
Riverside, CA
San Diego, CA
Savannah, GA
Seguin, TX
Springfield, MO
Wichita Falls, TX

Of the 18 sites, thirteen (13) sites met the case number enrollment criteria or met the enrollment criteria with the required number of complete cases and were included in the effectiveness evaluation. Cases from all 18 sites were included in the safety database.

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

A masked, multi-center, field safety and effectiveness study was conducted using a generalized randomized block design based on order of enrollment of households comparing Simparica Trio™ to an active control, afoxolaner. Dogs were enrolled from 18 sites located in five distinct geographic regions of the United States. The use of medications or products with flea treatment or control activity in any household dogs or household premises prior to or during the study period was not permitted.

Objective: Evaluate the effectiveness and safety of Simparica Trio™ against natural infestations of fleas on dogs under field conditions. Secondary objectives were to evaluate improvement in the clinical signs of flea allergy dermatitis, tick counts, and oral acceptability of the product.

Study Animals: Two hundred seventy-eight (278) Simparica Trio™-treated dogs and 144 active control-treated dogs were evaluated for safety.

One hundred thirty-nine (139) Simparica Trio™-treated dogs and 69 active control-treated dogs were included in the effectiveness evaluation. The effectiveness analysis for Day 30 was performed on 139 Simparica Trio™-treated dogs and 69 active control-treated dogs. The effectiveness analysis for Day 60 was performed on 136 Simparica Trio™-treated dogs and 68 active control-treated dogs.

Enrollment was limited to those households with a maximum of three dogs; there was no restriction on the type or number of other pets in the household. There were no breed or gender restrictions, but dogs intended for breeding, pregnant or lactating dogs were not eligible for enrollment. Dogs were a minimum of 8 weeks old at the start of the study.

For a household to be included, at least one dog (the primary dog) had to have at least 10 or more live fleas. In households where more than one dog met this requirement, the primary dog was the dog whose name began with the letter that comes first in the alphabet from among all dogs in the household that harbored ≥10 live fleas, and the other dogs were designated

as supplementary dogs. All dogs in a household received the same treatment as the primary dog and were included in safety evaluations. Only primary dogs were included in effectiveness evaluation.

Treatment Groups:

Table II.5. Treatment Groups for Study A161C-US-15-634: Field Effectiveness against Fleas

Treatment Group	Treatment	Minimum Dosage	Days of Treatment	Total Dogs per Group (Primary Dogs)	Days of Flea Counts and Clinical Assessments
T01	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0 and 30	278 (167)	Day -1 or 0, 30, and 60
T02	Active Control	2.5 mg/kg Afoxolaner	Day 0 and 30	144 (84)	Day -1 or 0, 30, and 60

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: On Days 0 and 30 owners administered Simparica Trio™ or the active control orally to their dogs at home with or without food. Owners recorded palatability at each dosing.

Measurements and Observations: For primary dogs, post-treatment flea counts were performed on Days 30 and 60. Primary dogs were assessed for signs of flea allergy dermatitis on Day 0 or -1 and post-treatment (on Days 30 and 60).

On or within one day prior to Day 0, each dog underwent a physical examination, flea counts were performed, body weight was measured, and blood and urine were collected for clinical pathology.

For all dogs, post-treatment physical examination and body weight measurement were performed on Days 30 and 60, and blood and urine were collected for clinical pathology on Day 60.

Any ticks present on the dog during flea counts were counted, removed, and stored for later speciation.

Statistical Methods: The primary assessment of effectiveness was based on the reduction in mean live flea counts on primary dogs on Days 30 and 60 compared to the pre-treatment counts on Day 0 or -1. A ≥90% reduction in live flea counts throughout the study and a statistically significant difference

between the live flea pre-treatment counts on Day -1 or 0 and the Day 30 and Day 60 live flea counts at a two-sided α = 0.05 in the Simparica Trio™-treated dogs 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. Log transformed live flea counts $\{\log_e(x+1)\}$ from each treatment group were analyzed separately at each post-treatment time point using a linear mixed model with repeated measures including time as a fixed effect, clinic, the clinic by time interaction, animal within clinic and error as the random effects. Least squares means were back transformed to obtain the estimates for geometric mean flea counts at each post-treatment time point. Testing was two-sided at the significance level α=0.05.

Severity of clinical signs of flea allergy dermatitis (FAD), physical examination, clinical pathology, dosing, and abnormal health data were summarized.

Results: The Simparica Trio™-treated group had a 99.0% and 99.7% reduction in live flea counts on Days 30 and 60, respectively (Table II.6). The active control–treated group had a 98.3% and 99.6% reduction in live flea counts on Days 30 and 60, respectively (Table II.6). Post-treatment geometric mean live flea counts on Days 30 and 60 were significantly lower than pre-treatment for both groups (P<0.0001).

Table II.6. Study A161C-US-15-634: Field Effectiveness against Fleas – Geometric Mean Live Flea Count (Percent Reduction Compared to Pre-Treatment)

Treatment Group	Treatment	Day 0 or -1 Flea Count	Day 30 Flea Count (Percent Reduction)	Day 60 Flea Count (Percent Reduction)
T01	Simparica Trio™	42.4	0.4 (99.0%)	0.1 (99.7%)
T02	Active control	34.8	0.6 (98.3%)	0.1 (99.6%)

Of the treated dogs that had clinical signs of flea allergy dermatitis prior to treatment, at least 78% had improvement of the clinical signs of flea allergy dermatitis by Day 60 (Table II.7).

Table II.7. Study A161C-US-15-634: Field Effectiveness against Fleas-Improvement in Clinical Signs of Flea Allergy Dermatitis - Percentage of Primary Dogs with Improvement in Individual Clinical Signs of Flea Allergy Dermatitis on Day 60

Clinical Sign	Simparica Trio™	Active control
Alopecia from Self Trauma	86.8% (33 of 38)	85.7% (12 of 14)
Dermatitis / Pyodermatitis	90.3% (28 of 31)	84.2% (16 of 19)
Erythema	87.2% (34 of 39)	88.9% (16 of 18)
Papules	90.0% (18 of 20)	100.0% (12 of 12)
Pruritus	94.2% (49 of 52)	93.3% (28 of 30)
Scaling	78.6% (22 of 28)	88.2% (15 of 17)

Owners recorded palatability information for 517 doses of Simparica Trio™ administered. They first offered Simparica Trio™ without food (free choice). If not consumed within five minutes, they offered Simparica Trio™ in food, and if not consumed they administered Simparica Trio™ by placement of the chewable tablet in the back of the dog's mouth. Dogs voluntarily consumed 74.5% of the doses without food, an additional 17.4% consumed it when offered in food, and 8.1% required placement of the chewable tablet in the back of the dog's mouth.

Adverse Reactions: Evaluation of safety was completed over the 60-day period through in-clinic physical examinations and through reporting of abnormalities by the owner (Table II.8). The safety database included 278 dogs administered Simparica Trio™ and 144 dogs administered the active control.

Table II.8. Adverse Reactions

Clinical Sign	Simparica Trio™ n (%)	Active control n (%)
Otitis Externa	12 (4.3%)	5 (3.5%)
Pruritus	8 (2.9%)	3 (2.1%)
Diarrhea	6 (2.2%)	4 (2.8%)
Emesis	4 (1.4%)	6 (4.2%)
Otitis (not specified as externa)	4 (1.4%)	0 (0.0%)
Weight loss	2 (0.7%)	0 (0.0%)

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

Conclusions: The results of this study demonstrate that Simparica Trio™, when used monthly at the minimum labeled dose of 1.2 mg/kg sarolaner, 24 µg/kg moxidectin, and 5 mg/kg pyrantel (as pamoate salt), is safe and effective for the treatment and prevention of flea infestations in dogs under field conditions. The dogs treated with Simparica Trio™ showed an improvement in clinical signs related to flea allergy dermatitis over the course of the study as a direct result of eliminating fleas.

5. Laboratory Dose Confirmation and Non-Interference Study A162C-US-13-214: Treatment and Prevention of Flea Infestations

Title: Laboratory Dose Confirmation and Non-Interference of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Ctenocephalides felis* on Dogs

Study Dates: July 7, 2016 – August 16, 2017

Study Location: Sugar Land, TX

Study Design: The study was conducted in accordance with Good Clinical Practice (GCP) guidelines

Objectives: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations of *C. felis* for up to 35 days on dogs; and confirm that the combination product formulation does not interfere with the effectiveness of sarolaner against *C. felis*.

Study Animals: Forty (40) Beagle and mixed-breed dogs (20 male and 20 female), 6 to 46 months of age, and 6.1 to 12.3 kg body weight.

Treatment Groups:

Table II.9. Treatment groups for Study A162C-US-13-214

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Flea Infestation	Days of Flea Count
T01	Vehicle control	NA	Day 0	8	-1, 6, 13, 20, 27, and 34	1, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	-1, 6, 13, 20, 27, and 34	1, 7, 14, 21, 28, and 35
T03	Sarolaner	1.2 mg/kg	Day 0	8	-1, 6, 13, 20, 27, and 34	1, 7, 14, 21, 28, and 35
T04	Moxidectin	24 µg/kg	Day 0	8	-1, 6, 13, 20, 27, and 34	1, 7, 14, 21, 28, and 35
T05	Pyrantel	5 mg/kg	Day 0	8	-1, 6, 13, 20, 27, and 34	1, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 100 unfed adult *C. felis* fleas at each infestation. At each flea count the numbers of live fleas were counted, and the fleas were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

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

Flea counts at each time point were analyzed using a general mixed linear model with fixed effect of treatment and random effects of block and error. Testing was two-sided at the significance level $\alpha=0.05$.

Non-interference for the treatment and control of *C. felis* was determined on the basis of whether the inclusion of sarolaner in the combination was justified. The inclusion was justified if neither the single treatment with moxidectin (T04) nor pyrantel (T05) met the criteria for effectiveness while the single treatment with Simparica Trio™ (T02) did.

Results: Control dogs maintained adequate flea infestations on Days 1, 7, 14, 28, and 35, with at least six of the eight dogs having 50 or more live fleas at each flea count.

On Day 1, there were no live fleas on any Simparica Trio™-treated dogs. The Simparica Trio™-treated group had a 100% reduction in live flea counts 24 hours after treatment of the existing infestation, and ≥99.7% reduction in live flea counts 24 hours after weekly re-infestations for 35 days (Table II.10). Live flea counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

The sarolaner-treated group had a 100% reduction in live flea counts 24 hours after treatment of the existing infestation, and ≥97.6% reduction in live flea counts 24 hours after weekly re-infestations for 35 days. Live flea counts for the sarolaner group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

The moxidectin-treated group had a 6.6% reduction in live flea counts 24 hours after treatment of the existing infestation, and ≤15.2% reduction in live flea counts 24 hours after weekly re-infestations for 35 days. Live flea counts for the moxidectin group were not significantly different than the control group ($P\geq0.0791$) on any post-treatment count day.

The pyrantel-treated group had a 5.0% reduction in live flea counts 24 hours after treatment of the existing infestation, and ≤26.7% reduction in live flea counts 24 hours after weekly re-infestations for 35 days. Live flea counts for the pyrantel group were not significantly different than the control group on Days 1, 7, 14, and 35 ($P\geq0.2018$). Live flea counts for the pyrantel group were significantly different ($P=0.0087$) and numerically lower than control on Day 28 and were significantly different ($P=0.0050$) and numerically higher than control on Day 21.

Table II.10. Effectiveness Against Adult *C. felis* by Treatment Group; Arithmetic Mean Live Flea Count (Percent Effectiveness)

Day of Flea Count	Control	Simparica Trio™	Sarolaner	Moxidectin	Pyrantel
1	70.1	0.0 (100%)	0.0 (100%)	65.5 (6.6%)	66.6 (5.0%)
7	78.3	0.0 (100%)	1.9 (97.6%)	66.4 (15.2%)	73.8 (5.8%)
14	65.8	0.0 (100%)	0.0 (100%)	70.6 (0.0%)	76.8 (0.0%)
21	54.1	0.1 (99.8%)	0.4 (99.3%)	64.8 (0.0%)	71.8 (0.0%)
28	86.6	0.3 (99.7%)	0.0 (100%)	78.9 (8.9%)	63.5 (26.7%)
35	79.6	0.0 (100%)	0.4 (99.5%)	79.4 (0.3%)	87.8 (0.0%)

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the treatment and control of adult *C. felis* when assessed 24 hours after treatment of an existing infestation, and 24 hours after weekly re-infestation for 35 days.

Treatment with moxidectin alone or pyrantel alone was not effective against adult *C. felis*, justifying the need for inclusion of sarolaner in the combination.

6. Laboratory Dose Confirmation A166C-US-16-650: Egg Production, Egg Hatch, and Adult Flea Emergence

Title: Laboratory Dose Confirmation Study A166C-US-16-650: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against *Ctenocephalides felis* Egg Production, Egg Hatch, and Adult Flea Emergence

Study Dates: June 6, 2016 – September 18, 2017.

Study Location: Turlock, CA

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

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ in the prevention of pre-adult stages of *C. felis.*

Study Animals: Twenty (20) Beagle dogs (9 male and 11 female), 44 to 48 months of age, and 9.0 to 13.5 kg body weight.

Treatment Groups:

Table II.11. Treatment groups for Study A166C-US-16-650

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Flea Infestation	Days of Flea Egg Collection	Days of Flea Comb and Removal
T01	Vehicle control	NA	Day 0	10	-1, 5, 12, 19, 26, 33	1, 7, 14, 21, 28, 35	2, 8, 15, 22, 29, 36
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-1, 5, 12, 19, 26, 33	1, 7, 14, 21, 28, 35	2, 8, 15, 22, 29, 36

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 100 unfed adult *C. felis* fleas at each infestation. Flea eggs were collected for a 20-hour period beginning 48 hours after each infestation. After each egg collection, dogs were combed to count and remove adult fleas. Up to 100 eggs from each dog per collection were incubated for five days and evaluated for larval emergence, and up to 100 eggs from each dog were incubated for 35 days and evaluated for adult flea emergence.

Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General 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.

For live flea counts, percent effectiveness of the treated group with respect to the control group was calculated using arithmetic means at each time point using the formula [(C-T)/C] x 100, where C = arithmetic mean of live flea counts for the control group and T = arithmetic mean of live flea counts for the treated group.

A general mixed linear 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 mean comparisons were tested using the (two-sided) 5% significance level.

Results: Control dogs maintained adequate flea infestations with at least six of the ten dogs having 50 or more live fleas at each flea count.

On Day 1, there were no live fleas on any Simparica Trio™-treated dogs. The Simparica Trio™-treated group had a 100% reduction in live flea counts on all count days (Table II.12). Live flea counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.12. Effectiveness Against Adult *C. felis*

Day of Flea Count	Control Group Arithmetic Mean Adult Flea Count	Simparica Trio™ Arithmetic Mean Adult Flea Count	Percent Effectiveness
2	73.8	0.0	100%
8	71.7	0.0	100%
15	67.0	0.0	100%
22	61.5	0.0	100%
29	60.8	0.0	100%
36	63.6	0.0	100%

No flea eggs were collected from any Simparica Trio™-treated dog on any post-treatment collection day (Table II.13). Flea egg counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment egg collection days.

Table II.13. Effectiveness Against *C. felis* Egg Production

Day of Flea Egg Collection	Control Group Arithmetic Mean Flea Egg Count	Simparica Trio™ Arithmetic Mean Flea Egg Count	Percent Effectiveness
1	364.0	0.0	100%
7	444.5	0.0	100%
14	496.2	0.0	100%
21	488.5	0.0	100%
28	440.6	0.0	100%
35	366.8	0.0	100%

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusion: A single oral dose of Simparica Trio™ resulted in 100% reduction in adult flea count against an existing infestation and against weekly re-infestations with *C. felis* and zero flea egg production for 35 days.

7. Laboratory Dose Confirmation Study A166C-US-16-649: Onset of Activity and Speed of Kill Against Fleas

Title: Laboratory Dose Confirmation Study A166C-US-16-649: Speed of Kill of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Ctenocephalides felis* on Dogs

Study Dates: September 28, 2016 – August 9, 2017

Study Location: Greenbrier, AR

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

Objective: Confirm the speed of kill of a single oral administration of Simparica Trio™ against *Ctenocephalides felis* on dogs.

Study Animals: Sixty-four (64) Beagle and mixed-breed dogs (39 male and 25 female), 10 to 92 months of age, and 6.5 to 15.7 kg body weight.

Treatment Groups:

Table II.14. Treatment groups for Study A166C-US-16-649

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Time of Flea Count After Treatment/ Infestation
T01	Vehicle control	NA	Day 0	8	3 Hours
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	3 Hours
T03	Vehicle control	NA	Day 0	8	4 Hours

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Time of Flea Count After Treatment/ Infestation
T04	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	4 Hours
T05	Vehicle control	NA	Day 0	8	8 Hours
T06	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	8 Hours
T07	Vehicle control	NA	Day 0	8	12 Hours
T08	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	12 Hours

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 100 unfed adult *C. felis* fleas at each infestation on Days -1, 7, 14, 21, 28, and 35. At each flea count the numbers of live fleas were counted, and the fleas were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For flea counts, percent effectiveness against control was calculated based on arithmetic means using the formula [(C-T)/C] x 100, where C = arithmetic mean of live flea counts for the control group and T = arithmetic mean of live flea counts for the treated group. When observations were missing at a given time point, least squares means and corresponding percent effectiveness estimates were used.

Flea counts at each time point were analyzed using a general mixed linear model with fixed effect of treatment and random effects of block and error. Testing was two-sided at the significance level α=0.05.

Results: Control dogs maintained adequate flea infestations throughout the study with at least six of the eight dogs having 50 or more live fleas at each flea count.

Simparica Trio™ provided 0.0%, 40.9%, 100%, and 100% reduction in live flea counts at 3, 4, 8, and 12 hours after treatment of an existing infestation (Table II.15). Live flea counts in the Simparica Trio™-treated group were statistically different ($P \leq 0.0001$) and numerically lower than in the control group at the 4, 8, and 12-hour time points.

Effectiveness after weekly re-infestation ranged from 0.0 to 43.7% at 3 hours, from 1.5 to 65.1% at 4 hours, and 25.2 to 100% at 8 hours for 35 days. Live flea counts for the Simparica Trio™-treated group were significantly different and numerically lower than control group at the 3-hour counts on Days 7 and 14 ($P < 0.0001$), at the 4-hour count on Days 0, 7, 14, and 21 ($P \leq 0.0001$), and at the 8-hour count on all days ($P \leq 0.0138$).

Table II.15. Onset of Activity and Speed of Kill of Simparica Trio™ Against *C. felis*

Day of Flea Infestation	Time of Flea Count	Control Group Arithmetic Mean Live Flea Count	Simparica Trio™ Arithmetic Mean Live Flea Count	Percent Effectiveness
-1	3 Hours	77.1	77.8	0.0%
-1	4 Hours	72.4	42.8	40.9%
-1	8 Hours	84.0	0.0	100%
-1	12 Hours	85.0	0.0	100%
7	3 Hours	95.0	61.6	35.1%
7	4 Hours	94.1	43.6	53.7%
7	8 Hours	88.9	0.4	99.6%
7	12 Hours	93.1	0.0	100%
14	3 Hours	89.0	50.1	43.7%
14	4 Hours	89.5	31.3	65.1%
14	8 Hours	89.1	6.9	92.3%
14	12 Hours	86.6	0.0	100%
21	3 Hours	93.8	88.3	5.9%
21	4 Hours	93.1	72.1	22.6%
21	8 Hours	87.8	3.6	95.9%

Day of Flea Infestation	Time of Flea Count	Control Group Arithmetic Mean Live Flea Count	Simparica Trio™ Arithmetic Mean Live Flea Count	Percent Effectiveness
21	12 Hours	92.0	0.0	100%
28	3 Hours*	90.4	92.8	0.0%
28	4 Hours	95.9	85.9	10.4%
28	8 Hours	94.0	26.6	71.7%
28	12 Hours	96.0	2.1	97.8%
35	3 Hours	98.8	93.0	5.8%
35	4 Hours	97.4	95.9	1.5%
35	8 Hours	94.1	70.4	25.2%
35	12 Hours	95.3	13.8	85.6%

* Time point with missing observations, least squares means and corresponding percent effectiveness estimates were used.

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

Conclusion: This study demonstrated that against an existing infestation, Simparica Trio™ begins to kill *C. felis* within 4 hours after treatment and is effective against the flea infestation by 8 hours. For subsequent re-infestations, Simparica Trio™ was >90% effective within 12 hours for 28 days.

For the Treatment and Control of Tick Infestations

8. Laboratory Dose Confirmation Study A166C-US-16-652: *Amblyomma americanum* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-652: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Amblyomma americanum* on Dogs

Study Dates: July 29, 2016 – September 24, 2017

Study Location: Greenbrier, AR

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

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations *of Amblyomma americanum* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (10 male and 10 female), 37 to 69 months of age, and 6.4 to 11.2 kg body weight.
Treatment Groups:

Table II.16. Treatment groups for Study A166C-US-16-652

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *A. americanum* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and room, block within room, and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

On Day 2, there were no live ticks on any Simparica Trio™-treated dogs. The Simparica Trio™-treated group had a 100% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥99.5% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.17).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.17. *A. americanum* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	19.0	0.0	100%
7	23.1	0.0	100%
14	20.7	0.0	100%
21	23.1	0.0	100%
28	20.9	0.1	99.5%
35	29.7	0.1	99.7%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P\leq0.0011$) and numerically higher than the control group on all post-treatment count days.

Table II.18. *A. americanum* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	0.3	14.7
7	0.0	9.5
14	0.0	7.1
21	0.0	14.1
28	0.0	13.3
35	0.0	11.9

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

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of

A. americanum when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 35 days.

9. Laboratory Dose Confirmation Study A166C-US-17-812: *Amblyomma americanum* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-17-812: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Amblyomma americanum* on Dogs

Study Dates: February 27, 2017 – October 9, 2017

Study Location: Nowata, OK

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

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations *of Amblyomma americanum* for up to 36 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (11 male and 9 female), 32 to 126 months of age, and 6.8 to 13.2 kg body weight.

Treatment Groups:

Table II.19. Treatment groups for Study A166C-US-17-812

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle Control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	3, 8, 15, 22, 29, and 36
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	3, 8, 15, 22, 29, and 36

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *A. americanum* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and

dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and block and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

The Simparica Trio™-treated group had a 99.4% reduction in live tick counts 72 hours after treatment of the existing infestation, and ≥98.4% reduction in live tick counts 72 hours after weekly re-infestations for 36 days (Table II.20).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.20. *A. americanum* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
3	17.7	0.1	99.4%
8	23.7	0.1	99.6%
15	16.1	0.0	100%
22	13.9	0.0	100%
29	12.4	0.2	98.4%
36	14.4	0.1	99.3%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P \leq 0.0021$) and numerically higher than the control group on all post-treatment count days.

Table II.21. *A. americanum* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
3	1.1	10.0
8	1.0	5.6
15	1.1	9.5
22	0.6	7.9
29	0.6	6.2
36	0.7	7.5

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

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *A. americanum* when assessed 72 hours after treatment of an existing infestation and 72 hours after weekly re-infestation for 36 days.

10. Laboratory Dose Confirmation and Non-Interference Study A166C-US-13-216: *Amblyomma maculatum* Ticks

Title: Laboratory Dose Confirmation / Non-Interference Study A162C-US-13-216: Laboratory Non-Interference of a Combination Product Containing Sarolaner, Moxidectin and Pyrantel Pamoate against Induced Infestations of *Amblyomma maculatum* on Dogs

Study Dates: June 1, 2015 – November 20, 2017

Study Location: Turlock, CA

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

Objectives: Confirm that the combination of moxidectin and pyrantel with sarolaner does not interfere with the effectiveness of sarolaner against *A. maculatum*.

Study Animals: Fifty (50) mixed breed dogs (33 male and 17 female), 15 to 122 months of age, and 7.4 to 33.1 kg body weight.

Treatment Groups:

Table II.22. Treatment groups for Study A162C-US-13-216

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	sarolaner, moxidectin, pyrantel*	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T03	Sarolaner	2.0 mg/kg	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T04	Moxidectin	24 µg/kg	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T05	Pyrantel	5 mg/kg	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #1; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *A. maculatum* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Log-transformed tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect and block and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Non-interference for the treatment and control of *A. maculatum* was determined on the basis of whether the inclusion of sarolaner in the combination was justified. The inclusion was justified if neither the single treatment with moxidectin (T04) nor pyrantel (T05) met the criteria for effectiveness while the single treatment with sarolaner, moxidectin, pyrantel (T02) did.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

On Day 2, there were no live ticks on any sarolaner, moxidectin, pyrantel-treated dogs. The sarolaner, moxidectin, pyrantel-treated group had a 100% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥95.3% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.23). Live tick counts for the sarolaner, moxidectin, pyrantel-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

The sarolaner-treated group had a 100% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥97.8% reduction in live tick counts 48 hours after weekly re-infestations for 35 days. Live tick counts for the sarolaner group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

The moxidectin-treated group had a 0.0% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≤8.8% reduction in live tick counts 48 hours after weekly re-infestations for 35 days. Live tick counts for the moxidectin group were not significantly different than the control group ($P\geq0.3484$) on any post-treatment count day.

The pyrantel-treated group had a 1.6% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≤9.7% reduction in live tick counts 48 hours after weekly re-infestations for 35 days. Live tick counts for the pyrantel group were not significantly different than the control group ($P\geq0.2257$) on any post-treatment count day.

Table II.23. *A. maculatum* Live Tick Effectiveness; Arithmetic Mean Live Tick Count (Percent Effectiveness)

Day of Tick Count	Control Group	Sarolaner, moxidectin, pyrantel	Sarolaner	Moxidectin	Pyrantel
2	38.7	0.0 (100%)	0.0 (100%)	39.1 (0.0%)	38.1 (1.6%)
7	41.1	0.1 (99.8%)	0.2 (99.5%)	42.4 (0.0%)	41.0 (0.2%)
14	34.1	0.2 (99.4%)	0.1 (99.7%)	36.3 (0.0%)	37.9 (0.0%)
21	43.3	0.7 (98.4%)	0.4 (99.1%)	39.5 (8.8%)	39.1 (9.7%)
28	38.6	1.8 (95.3%)	0.6 (98.4%)	35.9 (7.0%)	42.9 (0.0%)
35	40.5	1.2 (97.0%)	0.9 (97.8%)	37.7 (6.9%)	37.7 (6.9%)

Dead ticks found on sarolaner, moxidectin, pyrantel-treated and sarolaner-treated dogs were significantly different ($P \leq 0.0001$) and numerically higher than on control dogs at all time-points. Mean dead tick counts for the moxidectin and pyrantel-treated groups were not significantly different than control group at any time point ($P \geq 0.1657$) except Day 14 where the control mean was significantly different ($P = 0.0380$) and numerically higher.

Table II.24. *A. maculatum* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group	Sarolaner, moxidectin, pyrantel	Sarolaner	Moxidectin	Pyrantel
2	1.0	14.0	16.4	0.0	0.0
7	0.2	11.8	10.4	0.0	0.4
14	2.3	9.4	10.5	0.0	0.0
21	0.4	9.8	8.0	0.0	0.2
28	0.3	9.6	11.5	0.2	0.1
35	0.0	10.7	13.8	0.1	0.0

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

Conclusions: Treatment with moxidectin alone or pyrantel alone was not effective against adult *A. maculatum*, justifying the need for inclusion of sarolaner in Simparica Trio™.

11. Laboratory Dose Confirmation Study A166C-US-16-654: *Amblyomma maculatum* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-654: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Amblyomma maculatum* on Dogs

Study Dates: August 2, 2016 – November 14, 2017

Study Location: Sugar Land, TX

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

Objective: Confirm the effectiveness of Simparica Trio™ against induced infestations *of Amblyomma maculatum* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (11 male and 9 female), 7 to 76 months of age, and 7.6 to 31.5 kg body weight.

Treatment Groups:

Table II.25: Treatment groups for Study A166C-US-16-654

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *A. maculatum* ticks (approximately equal numbers of males

and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, block and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

On Day 2, there were no live ticks on any Simparica Trio™-treated dogs. The Simparica Trio™-treated group had a 100% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥97.0% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.26).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P \leq 0.0010$) and numerically lower than the control group on all post-treatment count days.

Table II.26. *A. maculatum* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	30.0	0.0	100%
7	33.8	0.1	99.7%
14	30.1	0.1	99.7%
21	32.6	0.2	99.4%
28	23.6	0.3	98.7%
35	23.1	0.7	97.0%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P \leq 0.0033$) and numerically higher than the control group on all post-treatment count days.

Table II.27. *A. maculatum* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	0.6	11.0
7	0.0	7.8
14	0.0	9.0
21	0.3	14.9
28	0.3	8.6
35	0.1	7.9

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

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *A. maculatum* when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 35 days.

12. Laboratory Dose Confirmation Study A166C-US-16-655: *Amblyomma maculatum* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-655: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Amblyomma maculatum* on Dogs

Study Dates: August 30, 2016 – August 21, 2017

Study Location: Greenbrier, AR

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

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations of *Amblyomma maculatum* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (10 male and 10 female), 8 to 35 months of age, and 5.3 to 14.6 kg body weight.

Treatment Groups:

Table II.28. Treatment groups for Study A166C-US-16-655

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *A. maculatum* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and room, block within room and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

The Simparica Trio™-treated group had a 99.1% reduction in live tick counts 48 hours after treatment of the existing infestation, ≥90.4% reduction in live tick counts 48 hours after weekly re-infestations for 28 days, and a 76.3% reduction in live tick counts on Day 35 (Table II.29).

Live tick counts for Simparica Trio™-treated group were significantly different ($P \leq 0.0005$) and numerically lower than the control group on all post-treatment count days.

Table II.29. *A. maculatum* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	35.1	0.3	99.1%
7	26.3	0.3	98.9%
14	33.9	0.0	100%
21	28.9	0.7	97.6%
28	25.0	2.4	90.4%
35	25.7	6.1	76.3%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P \leq 0.0362$) and numerically higher than the control group on all post-treatment count days.

Table II.30. *A. maculatum* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	0.0	17.3
7	2.3	12.3
14	0.0	14.8
21	0.0	9.6
28	0.0	9.3
35	1.6	6.7

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

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *A. maculatum* when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 28 days.

13. Laboratory Dose Confirmation Study A166C-US-16-657: *Dermacentor variabilis* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-657: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Dermacentor variabilis* on Dogs

Study Dates: August 23, 2016 – September 24, 2017

Study Location: Turlock, CA

Study Design: This study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations of *Dermacentor variabilis* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (10 male and 10 female), 30 to 139 months of age, and 13.3 to 29.2 kg body weight.

Treatment Groups:

Table II.31. Treatment groups for Study A166C-US-16-657

Group	Treatment	Dosage	Days of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *D. variabilis* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and block and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

When observations were missing at a given time point, least squares means and corresponding percent effectiveness estimates were used.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the nine to ten dogs having 12 or more live ticks at each tick count.

The Simparica Trio™-treated group had a 99.7% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥92.6% reduction in live tick counts 48 hours after weekly re-infestations for 28 days (Table II.32).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.32. *D. variabilis* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	34.0	0.1	99.7%
7	39.8	0.4	99.0%
14*	39.1	0.1	99.7%
21*	30.4	1.1	96.4%
28*	31.2	2.3	92.6%
35*	31.6	4.2	86.7%

* Time point with missing data, least squares means and corresponding percent effectiveness estimates were used.

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P\leq0.0018$) and numerically higher than the control group on all post-treatment count days except Day 35 ($P=0.2118$).

Table II.33. *D. variabilis* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	0.0	19.8
7	0.0	17.3
14*	0.0	13.8
21*	0.0	11.1
28*	0.0	9.7
35*	0.0	6.0

* Time point with missing data, least squares means estimates were used.

Adverse Reactions: No treatment–related adverse reactions were reported in this study.

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *D. variabilis* when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 28 days.

14. Laboratory Dose Confirmation Study A166C-US-16-658: *Dermacentor variabilis* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-658: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Dermacentor variabilis* on Dogs

Study Dates: September 19, 2016 – November 21, 2017

Study Location: Sugar Land, TX

Study Design: This study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations of *Dermacentor variabilis* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (9 male and 11 female), 8 to 73 months of age, and 7.4 to 34.4 kg body weight.

Treatment Groups:

Table II.34. Treatment groups for Study A166C-US-16-658

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *D. variabilis* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and block and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

The Simparica Trio™-treated group had a 98.9% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥98.3% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.35).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.35. *D. variabilis* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	26.8	0.3	98.9%
7	19.7	0.2	99.0%
14	28.7	0.5	98.3%
21	30.4	0.0	100%
28	31.1	0.0	100%
35	28.0	0.4	98.6%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P\leq0.0480$) and numerically higher than the control group on all post-treatment count days.

Table II.36. *D. variabilis* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	1.0	11.0
7	0.2	3.4
14	0.4	5.9
21	0.1	4.1
28	0.0	4.6
35	0.6	5.5

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

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *D. variabilis* when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 35 days.

15. Laboratory Dose Confirmation Study A166C-US-16-741: *Ixodes scapularis* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-741: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Ixodes scapularis* on Dogs

Study Dates: August 15, 2016 – November 20, 2017

Study Location: Greenbrier, AR

Study Design: This study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations of *Ixodes scapularis* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (6 male and 14 female), 13 to 38 months of age, and 5.9 to 12.2 kg body weight.

Treatment Groups:

Table II.37. Treatment groups for Study A166C-US-16-741

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *I. scapularis* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and room, block within room and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

On Day 2, there were no live ticks on any Simparica Trio™-treated dogs. The Simparica Trio™-treated group had a 100% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥95.1% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.38).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.38. *I. scapularis* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	21.9	0.0	100%
7	13.8	0.0	100%
14	19.6	0.0	100%
21	20.6	1.0	95.1%
28	21.6	0.0	100%
35	23.2	0.0	100%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P\leq0.0004$) and numerically higher than the control group on all post-treatment count days.

Table II.39. *I. scapularis* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	0.0	11.3
7	0.0	9.8
14	0.0	8.3
21	0.0	8.2
28	0.0	11.1
35	0.0	13.9

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

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *I. scapularis* when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 35 days.

16. Laboratory Dose Confirmation Study A166C-US-16-660: *Ixodes scapularis* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-660: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Ixodes scapularis* on Dogs

Study Dates: December 13, 2016 – August 18, 2017

Study Location: Turlock, CA

Study Design: This study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations of *Ixodes scapularis* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (9 male and 11 female), 15 to 89 months of age, and 7.5 to 35.1 kg body weight.

Treatment Groups:

Table II.40. Treatment groups for Study A166C-US-16-660

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *I. scapularis* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and block and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

On Day 2, there were no live ticks on any Simparica Trio™-treated dogs. The Simparica Trio™-treated group had a 100% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥99.6% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.41).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.41. *I. scapularis* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	26.7	0.0	100%
7	27.2	0.0	100%
14	25.5	0.0	100%
21	26.6	0.1	99.6%
28	24.8	0.0	100%
35	26.8	0.0	100%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P\leq0.0009$) and numerically higher than the control group on all post-treatment count days.

Table II.42. *I. scapularis* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	0.0	8.4
7	0.0	6.0
14	0.0	9.8
21	0.0	9.3
28	0.0	10.7
35	0.0	9.6

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *I.*

scapularis when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 35 days.

17. Laboratory Dose Confirmation Study A166C-US-17-808: *Rhipicephalus sanguineus* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-17-808: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Rhipicephalus sanguineus* on Dogs.

Study Dates: February 27, 2017 to November 30, 2017

Study Location: Turlock, CA

Study Design: This study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations of *Rhipicephalus sanguineus* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle dogs (12 male and 8 female), 92 to 155 months of age, and 8.0 to 19.5 kg body weight.

Treatment Groups:

Table II.43. Treatment groups for Study A166C-US-17-808

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *R. sanguineus* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and block and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count.

The Simparica Trio™-treated group had a 99.7% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥94.0% reduction in live tick counts 48 hours after weekly re-infestations for 35 days (Table II.44).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.44. *R. sanguineus* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	34.7	0.1	99.7%
7	29.4	0.1	99.7%
14	21.1	0.1	99.5%
21	23.1	0.7	97.0%
28	24.6	0.2	99.2%
35	24.9	1.5	94.0%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P\leq0.0065$) and numerically higher than the control group on all post-treatment count days.

Table II.45. *R. sanguineus* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	0.0	5.9
7	0.0	4.8
14	0.0	4.2
21	0.0	3.8
28	0.0	5.2
35	0.0	2.8

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

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *R. sanguineus* when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 35 days.

18. Laboratory Dose Confirmation Study A166C-US-16-663: *Rhipicephalus sanguineus* Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-663: Dose Confirmation of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Rhipicephalus sanguineus* on Dogs

Study Dates: June 14, 2016 to August 10, 2017

Study Location: Greenbrier, AR

Study Design: This study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infestations of *Rhipicephalus sanguineus* for up to 35 days on dogs.

Study Animals: Twenty (20) Beagle and mixed-breed dogs (4 male and 16 female), 11 to 68 months of age, and 6.6 to 11.3 kg body weight.

Treatment Groups:

Table II.46. Treatment groups for study A166C-US-16-663

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Days of Tick Infestation	Days of Tick Count
T01	Vehicle control	NA	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	10	-2, 5, 12, 19, 26, and 33	2, 7, 14, 21, 28, and 35

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *R. sanguineus* ticks (approximately equal numbers of males and females) at each infestation. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group.

For dead tick counts, percent effectiveness of treatment was calculated based on arithmetic means using the formula [(T-C)/T] x 100, where C = arithmetic mean of dead tick counts for the control group and T = arithmetic mean of dead tick counts for the treated group.

Tick counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and room, block within room, and error as random effects at each time point for live tick counts and dead tick counts separately. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the ten dogs having 12 or more live ticks at each tick count through Day 28.

The Simparica Trio™-treated group had a 99.6% reduction in live tick counts 48 hours after treatment of the existing infestation, and ≥94.2% reduction in live tick counts 48 hours after weekly re-infestations for 28 days, except for Day 14 when the effectiveness was 89.7% (Table II.47).

Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than the control group on all post-treatment count days.

Table II.47. *R. sanguineus* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness

Day of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
2	23.8	0.1	99.6%
7	17.3	1.0	94.2%
14	11.7	1.2	89.7%
21	15.1	0.0	100%
28	14.6	0.0	100%

Dead tick counts for the Simparica Trio™-treated group were significantly different ($P\leq0.0398$) and numerically higher than the control group on all post-treatment count days except Day 35 ($P=0.0604$).

Table II.48. *R. sanguineus* Dead Tick Effectiveness; Arithmetic Mean Dead Tick Count

Day of Tick Count	Control Group Arithmetic Mean Dead Tick Count	Simparica Trio™ Arithmetic Mean Dead Tick Count
2	0.0	2.5
7	0.5	5.0
14	0.1	3.3
21	0.0	2.5
28	0.2	3.4

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

Conclusion: This study demonstrated the effectiveness of Simparica Trio™ for the control (reduced live ticks) and treatment (increased dead ticks) of *R. sanguineus* when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for 28 days.

19. Laboratory Dose Confirmation Study A166C-US-16-661: Onset of Activity and Speed of Kill Against Ticks

Title: Laboratory Dose Confirmation Study A166C-US-16-661: Speed of Kill of a Combination Product Containing Sarolaner, Moxidectin, and Pyrantel Pamoate Against Induced Infestations of *Ixodes scapularis* on Dogs

Study Dates: June 7, 2016 – November 28, 2017

Study Location: Greenbrier, AR

Study Design: This study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Study Objective: Confirm the speed of kill of a single oral administration of Simparica Trio™ against *Ixodes scapularis* on dogs.

Study Animals: Fifty-four (54) Beagles and mixed-breed dogs (28 male and 26 female), 7 months to 42 months of age, and 5.5 to 13.6 kg body weight.

Treatment Groups:

Table II.49. Treatment groups for Study A166C-US-16-661

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Time of Tick Count After Treatment/ Infestation
T01	Vehicle control	NA	0	9	8 Hours
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	0	9	8 Hours
T03	Vehicle control	NA	0	9	12 Hours
T04	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	0	9	12 Hours
T05	Vehicle control	NA	0	9	24 Hours

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Time of Tick Count After Treatment/ Infestation
T06	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	0	9	24 Hours

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *I. scapularis* ticks (approximately equal numbers of males and females) at each infestation on Days -2, 7, 14, 21, 28, and 35. At each tick count the numbers of live and dead ticks were counted, and the ticks were removed from the dog. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily.

Statistical Methods: For live tick counts, percent effectiveness against control was calculated based on arithmetic means 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 treated group. When observations were missing at a given time point, back-transformed least squares means (model based geometric mean estimates) and corresponding percent effectiveness estimates were used.

Tick log-counts for treated and control dogs were compared using a mixed linear model with treatment group as a fixed effect, and room, block within room, and error as random effects at each time point for live tick counts. Testing was two-sided at the 5% significance level.

Results: Control dogs maintained adequate tick infestations throughout the study with at least six of the nine dogs having 12 or more live ticks at each tick count on all assessment days.

Simparica Trio™ provided 67.5%, 98.4%, and 99.4% reduction in live tick counts at 8, 12, and 24 hours after treatment of an existing infestation (Table II.42). Live tick counts for the Simparica Trio™-treated group were significantly different ($P<0.0001$) and numerically lower than control group at 12 and 24 hours after treatment of existing infestation.

Effectiveness at 8 and 12 hours after weekly re-infestation ranged from 3.1 to 61.5%, and 44.3 to 84.9%, respectively, for 35 days. Live tick counts for the Simparica Trio™-treated group were significantly different and numerically

lower than control at the 8-hour counts on Day 7 (P=0.0013), and at the 12-hour counts on all Days (P≤0.0404) except Day 28 (P=0.0957). Effectiveness at 24 hours post-infestation was ≥94.2% for 28 days, and live tick counts for the Simparica Trio™-treated group were significantly different (P≤0.0022) and numerically lower than control group on all Days.

Table II.50. *I. scapularis* Onset of Activity and Speed of Kill

Day of Tick Infestation	Time of Tick Count	Control Group Arithmetic Mean Live Tick Count	Simparica Trio™ Arithmetic Mean Live Tick Count	Percent Effectiveness
-2	8 Hours[a]	18.1	5.9	67.5%
-2	12 Hours[a]	21.3	0.3	98.4%
-2	24 Hours[a]	17.4	0.1	99.4%
7	8 Hours*	20.7	8.0	61.5%
7	12 Hours	23.6	3.6	84.9%
7	24 Hours	17.2	0.1	99.4%
14	8 Hours	21.6	17.1	20.6%
14	12 Hours	18.9	7.7	59.4%
14	24 Hours	24.1	0.0	100%
21	8 Hours	19.1	13.9	27.3%
21	12 Hours*	21.2	8.0	62.2%
21	24 Hours	16.8	0.3	98.0%
28	8 Hours	17.7	17.1	3.1%
28	12 Hours	24.9	11.9	52.2%
28	24 Hours	23.1	1.3	94.2%
35	8 Hours	17.7	16.8	5.0%
35	12 Hours	19.6	10.9	44.3%
35	24 Hours	14.8	4.8	67.7%

[a] Time after treatment administration
* Time point with missing observations, back-transformed least squares means and corresponding percent effectiveness estimates were used.

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusion: This study demonstrated that against an existing *I. scapularis* infestation, Simparica Trio™ began to kill ticks within 8 hours after treatment and provided 98.4% effectiveness by 12 hours. For subsequent re-

infestations, Simparica Trio™ achieved ≥94.2% effectiveness within 24 hours for 28 days.

For the Treatment and Control of Roundworm and Hookworm Infections

Studies to confirm the effective pyrantel dose for the treatment and control of adult roundworm (*Toxocara canis* and *Toxascaris leonina*) and adult hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) were previously conducted with Nemex®-2 (pyrantel pamoate) Suspension under NADA 100-237. Therefore, only two studies (one field study and one laboratory study) were conducted against the dose-limiting parasite for pyrantel (*Toxocara canis*) to demonstrate effectiveness of Simparica Trio™ against both adult roundworms and hookworms. Two additional laboratory studies were conducted to demonstrate effectiveness of Simparica Trio™ against immature adult *T. canis*.

20. Field Effectiveness and Safety Study A161C-US-15-530: Treatment of Natural Infestations of *Toxocara canis*

Study Title: Efficacy and Safety of Sarolaner + Moxidectin + Pyrantel Pamoate in the Treatment of Natural Infestations of *Toxocara canis* in Dogs Presented as Veterinary Patients

Study Dates: October 6, 2016 – November 3, 2017

Study Locations:
Bartlesville, OK
Canton, MO
Catonsville, MD
Decatur, IL
Downingtown, PA
Fort Collins, CO
Grand Rapids, MI
Great Falls, VA
Lumberton, TX
Memphis, TN
New Preston, CT
Portland, OR
Quakertown, PA
Rochester, NY
Seguin, TX
Springfield, MO
Wichita Falls, TX
Zachary, LA

Of the 18 sites, 10 sites met the case number enrollment criteria or met the enrollment criteria with the required number of complete cases and were included in the effectiveness evaluation. Cases from all 18 sites were included in the safety database.

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

A masked, multi-center, field safety and effectiveness study using a generalized randomized block design based on order of dogs' presentation to the clinic, comparing Simparica Trio™ to an active control (ivermectin, pyrantel). Dogs were screened by fecal examination for *T. canis* eggs. Dogs positive for *T. canis* were enrolled from 18 different sites located in geographically diverse regions across the United States. On Day 0, each dog was weighed, given a physical examination, and a fecal sample of at least 1.0 gram of feces was collected and submitted to an independent parasitology laboratory for verification of the infection and for fecal *T. canis* egg count. Treatments were administered on Day 0. For all dogs, post-treatment fecal egg count, physical examination, and body weight measurement were performed on Day 10.

Objective: Evaluate the effectiveness and safety of Simparica Trio™ administered once orally in the treatment of natural infections of *Toxocara canis* in dogs presented as veterinary patients.

Study Animals: One-hundred twenty (120) Simparica Trio™-treated dogs, 41 active control-treated dogs, and one dog who inadvertently received milbemycin oxime and lufenuron (randomized and counted in with the active control dogs), were evaluated for safety.

The effectiveness analysis for Day 10 was based on data for 83 Simparica Trio™-treated dogs and 31 active control-treated dogs.

Treatment Groups:

Table II.51. Treatment Groups for Study A161C-US-15-530

Treatment Group	Treatment	Minimum Dosage	Day of Treatment	Total Dogs per Group (Evaluable Dogs[1])	Days of Fecal Egg Count
T01	Simparica Trio™ *	1.2 mg/kg sarolaner + 24 µg/kg moxidectin + 5 mg/kg pyrantel	Day 0	120 (83)	0, 10
T02	Active control	6 µg/kg Ivermectin + 5 mg/kg Pyrantel	Day 0	42† (31)	0, 10

*non-final formulation #2; see section II.C. Bioequivalence Studies
[1] Cases evaluable for effectiveness
† One dog assigned to T02 was administered a combination tablet containing milbemycin oxime and lufenuron instead of ivermectin and pyrantel.

Drug Administration: On Day 0, owners administered Simparica Trio™ or the active control orally to their dogs in the presence of the dispenser at the veterinary clinic.

Measurements and Observations: Post-treatment fecal egg counts were performed on Day 10 for comparison with the Day 0 fecal egg count. Findings on physical examination and any abnormal health events were recorded.

Statistical Methods: Percent effectiveness at Day 10 for each treatment group with respect to the baseline (Day 0) was calculated using the formula [(C–T)/C] x 100, where C = pre-treatment (Day 0) geometric mean and T = post-treatment (Day 10) geometric mean.

Based on results of modeling simulations comparing fecal egg counts and adult worm count effectiveness data, it was determined that a fecal egg count reduction effectiveness of 98% would provide adequate assurance of product effectiveness. Therefore, the treatment was considered successful if the Simparica Trio™-treated group achieved at least 98% reduction in fecal egg count and there was a statistically significant difference between baseline (Day 0) and the final (Day 10) *T. canis* egg counts at a two-sided $\alpha = 0.05$ level of significance.

The paired difference between Day 0 and Day 10 *T. canis* egg counts for Simparica Trio™-treated dogs was analyzed using a mixed linear model with fixed effect of the overall mean, and random effects for clinic and error.

Geometric means for each treatment at each time point (Day 0 and Day 10) were estimated using the back-transformed least squares means from a mixed linear model for repeated measures for log transformed egg counts {loge (x+1)} with fixed effects of treatment, time and the treatment by time interaction, and random effects of clinic, block within clinic, the clinic by treatment interaction, animal within clinic, block and treatment, the interaction of clinic, treatment and time, and error.

Results: For the Simparica Trio™-treated group, fecal egg count data for *T. canis* for 83 dogs on Day 10 were available for comparison with the Day 0 counts. The Simparica Trio™-treated group had a 99.2% reduction in *T. canis* fecal egg count on Day 10 compared to Day 0. The *T. canis* fecal egg counts for Day 0 differed significantly from those for Day 10 ($P<0.0001$).

For the active control group, fecal egg count data for *T. canis* for 31 dogs on Day 10 were available for comparison with the Day 0 counts. The active control group had a 98.6% reduction in *T. canis* fecal egg count on Day 10 compared to Day 0.

Table II.52. Study A161C-US-15-530: Field Effectiveness of Simparica Trio™ against *T. canis*—Geometric Mean *T. canis* Egg Counts and Percent Reductions Compared to Pre-Treatment

Treatment Group	Treatment	Day 0 (Pre-Treatment) Geometric LS* Mean	Day 10 Geometric LS* Mean	Percent Reduction in Fecal Egg count
T01	Simparica Trio™	272.98	2.06	99.2%
T02	Active control	137.65	1.89	98.6%

*LS=Least Squares

Adverse Reactions: Evaluation of safety was completed over the 10-day period through in-clinic physical examinations and through reporting of abnormalities by the owner (Table II.53). The safety database included 120 dogs administered Simparica Trio™ and 42 dogs administered an active control.

Table II.53. Adverse events occurring in Study A161C-US-15-530: Field Effectiveness of Simparica Trio™ against *T. canis*

Clinical Sign	Simparica Trio™* n (%)	Active Control† n (%)
Diarrhea	7 (5.8%)	4 (9.5%)
Vomiting	3 (2.5%)	5 (11.9%)
Alopecia	2 (1.7%)	1 (2.4%)
Anorexia	1 (0.8%)	2 (4.8%)

*A total of 120 dogs were evaluated.
† A total of 42 dogs were evaluated.

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

Conclusions: The results of this study demonstrate that Simparica Trio™, when given at the minimum labeled dose of 0.54 mg/lb (1.2 mg/kg) sarolaner, 0.011 mg/lb (24 µg/kg) moxidectin, and 2.27 mg/lb (5 mg/kg) pyrantel (as pamoate salt), is safe and effective for the treatment of *T. canis* in dogs under field conditions.

21. Laboratory Dose Confirmation Study A162C-US-13-218: Adult *Toxocara canis*

Title: Laboratory Dose Confirmation of Simparica Trio™ Against Induced Infections of *Toxocara canis* in Dogs.

Study Dates: August 16, 2016 – February 28, 2018

Study Location: Rockwood, TN

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

Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infections of *Toxocara canis* in dogs.

Study Animals: Sixteen (16) Beagle dogs (10 male and 6 female), 11 to 12 weeks of age, and 3.7 to 5.6 kg bodyweight.

Treatment Groups:

Table II.54. Treatment Groups for Study A162C-US-13-218

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Day of *Toxocara canis* Inoculation	Day of Necropsy and Adult *Toxocara canis* Count
T01	Vehicle Control	NA	Day 0	8	-49	7
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	-49	7

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was inoculated orally with 300 (±50) infective *Toxocara canis* L_3 larvated eggs 49 days prior to treatment administration. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily. On Day 7 post treatment all dogs were humanely euthanized and necropsied for recovery of adult *Toxocara canis*.

Statistical Methods: Effectiveness was determined on the basis of the percentage reduction in *Toxocara canis* worm counts in the treated group compared to the control group.

For the log-transformed *Toxocara canis* worm counts, percent effectiveness of the treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean (back-transformed mean) of worm counts for the control group and T = geometric mean (back-transformed mean) of worm counts for the treated group. A mixed linear model analysis was used to analyze log-counts, with treatment group as a fixed effect and room, block within room and error as random effects. Treatment differences were assessed at the two-tailed 5% level of significance.

Results: Control dogs had adequate *T. canis* infections.

Effectiveness of Simparica Trio™ against adult *Toxocara canis* is shown in Table II.55.

Table II.55. Effectiveness Against Adult *T. canis* (Study A162C-US-13-218)

Treatment	Adult *Toxocara canis* Worm Counts: Range	Adult *Toxocara canis* Worm Counts: Geometric Mean	Adult *Toxocara canis* Worm Counts: Percentage Reduction
Control	3 to 28	11.5	NA
Simparica Trio™	0 to 1	0.1[1]	99.2%

[1] The geometric mean worm count for the Simparica Trio™-treated group was significantly lower than those from the control group (P<0.0001).

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusions: A single oral dose of Simparica Trio™ is effective in the treatment and control of adult *Toxocara canis* infection in dogs.

22. Laboratory Dose Confirmation/ Non-Interference Study A166C-US-17-821: Immature Adult *T. canis*

Title: Laboratory Dose Confirmation / Non-Interference of Simparica Trio™ Against Induced Infections of Immature Adult *Toxocara canis* in Dogs.

Study Dates: August 10, 2017 – March 02, 2018

Study Location: Rockwood, TN

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

Objectives: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infections of immature adult *Toxocara canis* in dogs.

Confirm that moxidectin in combination with sarolaner does not provide effectiveness against immature adult *Toxocara canis* and that pyrantel is required in the combination to be effective for this indication.

Study Animals: Twenty-four (24) Beagle dogs (12 female and 12 male), 11 weeks of age, and 2.4 to 4.7 kg bodyweight.

Treatment Groups:

Table II.56. Treatment Groups for Study A166C-US-17-821

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Day of *Toxocara canis* Inoculation	Day of Necropsy and *Toxocara canis* Count
T01	Vehicle Control	NA	Day 0	8	-24	7
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	-24	7
T03	Sarolaner, Moxidectin	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin	Day 0	8	-24	7

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was inoculated orally with 300 (±50) infective *Toxocara canis* L_3 larvated eggs 24 days prior to treatment administration. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily. On Day 7 post treatment all dogs were humanely euthanized and necropsied for recovery of *Toxocara canis*.

Statistical Methods: Effectiveness was determined on the basis of the percentage reduction in *Toxocara canis* worm counts in the treated group compared to the control group.

For the log-transformed *Toxocara canis* worm counts, percent effectiveness of the treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean (back-transformed mean) of worm counts for the control group and T = geometric mean (back-transformed mean) of worm counts for the treated group. 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. Treatment differences were assessed at the 5% level of significance.

Results: Control dogs had adequate *T. canis* infections.

Effectiveness of Simparica Trio™ or sarolaner and moxidectin against immature adult *Toxocara canis* is shown in Table II.57.

Table II.57. Effectiveness Against Immature Adult *T. canis* (Study A166C-US-17-821)

Treatment	*T. canis* Worm Counts: Range	*T. canis* Worm counts: Geometric Mean	*T. canis* Worm Counts: Percentage Reduction
Control	11 to 70	33.3	NA
Simparica Trio™	0 to 20	1.6[1]	95.2%
Sarolaner, Moxidectin	0 to 31	8.4[2]	74.7%

[1] The geometric worm count for the Simparica Trio™-treated group was significantly lower than those from the control group ($P \leq 0.0001$).
[2] The geometric mean worm count for the sarolaner, moxidectin-treated group was significantly lower than those from the control group ($P=0.0070$).

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusions: This study confirms that a single oral dose of Simparica Trio™ is effective in the treatment and control of immature adult *Toxocara canis* and that pyrantel pamoate is required in Simparica Trio™ to provide effectiveness against immature adult *Toxocara canis*.

23. Laboratory Dose Confirmation Study A166C-ZA-16-749: Immature Adult *T. canis*

Study Title: Laboratory Dose Confirmation of Simparica Trio™ Against Induced Infections of Immature Adult *Toxocara canis* in Dogs

Study Dates: July 20, 2017 – December 04, 2017

Study Location: Bloemfontein, South Africa

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

Study Objective: Confirm the effectiveness of a single oral administration of Simparica Trio™ against induced infections of immature adult *Toxocara canis* in dogs.

Study Animals: Sixteen (16) Beagle and mixed-breed dogs (8 male and 8 female), 7 to 11 weeks of age, and 4.0 to 9.0 kg bodyweight.

Treatment Groups:

Table II.58. Treatment Groups for Study A166C-ZA-16-749

Group	Treatment	Dosage	Day of Treatment	Dogs per Group	Day of *Toxocara canis* Inoculation	Day of Necropsy and *Toxocara canis* Count
T01	Vehicle Control	NA	Day 0	8	-24	7
T02	Simparica Trio™ *	1.2 mg/kg Sarolaner + 24 µg/kg Moxidectin + 5 mg/kg Pyrantel	Day 0	8	-24	7

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally.

Measurements and Observations: Each dog was inoculated orally with 300 (±50) infective *Toxocara canis* L_3 larvated eggs 24 days prior to treatment administration. Clinical observations were conducted 1, 3, 6, and 24 hours after treatment. General health observations were conducted at least once daily. On Day 7 post treatment, all dogs were humanely euthanized and necropsied for recovery of *Toxocara canis*.

Statistical Methods: Effectiveness was determined on the basis of the percentage reduction in *Toxocara canis* worm counts in the treated group compared to the control group.

For the log-transformed *Toxocara canis* worm counts, percent effectiveness of the treated group with respect to the control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean (back-transformed mean) of worm counts for the control group and T = geometric mean (back-transformed mean) of worm counts for the treated group. 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. Treatment differences were assessed at the 5% level of significance.

Results: Control dogs had adequate *T. canis* infections.

Effectiveness of Simparica Trio™ against immature adult *Toxocara canis* is shown in Table II.59.

Table II.59. Effectiveness Against Immature Adult *T. canis* (Study A166C-ZA-16-749)

Treatment	*T. canis* Worm Counts: Range	*T. canis* Worm Counts: Geometric Mean	*T. canis* Worm Counts: Percentage Reduction
Control	1 to 128	15.2	NA
Simparica Trio™	0 to 2	0.3[1]	97.9%

[1] The geometric mean worm counts for the Simparica Trio™ -treated group was significantly lower than those from the control group (P=0.0008).

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusion: This study confirms that a single oral dose of Simparica Trio™ is effective in the treatment and control of immature adult *Toxocara canis.*

C. Pharmacology

During the development of Simparica Trio™, two non-final formulations were used in the clinical development program. Formulation 1 differed from the final formulation of Simparica Trio™ as it had a higher dose rate of sarolaner. Formulation 2 differed from the final formulation of Simparica Trio™ due to minor formulation changes in excipients. In order to bridge between these non-final formulations and the final Simparica Trio™ formulation, the following studies were conducted.

24. Bioequivalence study 1: Study A461N-US-17-861

Title: Two-Way Cross-Over Bioequivalence Study Comparing Moxidectin in a Final and Non-Final Formulation of Combination Tablet Containing Sarolaner, Moxidectin, and Pyrantel in Dogs

Study Dates: February 6, 2018 – December 7, 2018

Study Location: Concord, OH

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

Study Objective: The objective of this study was to determine if the moxidectin in a sarolaner, moxidectin, pyrantel pamoate tablet (non-final formulation #1) was bioequivalent to the moxidectin in the final Simparica Trio™ formulation in healthy dogs under fasted conditions.

Study Animals: Thirty-six male Beagle dogs, between 11.7 to 12.6 months and weighing 7.40 to 12.35 kg, were included in the study.

Treatment Groups: This study was a two treatment two period crossover design. A 105-day washout separated the period 1 and period 2 drug administrations.

Table II.60. Treatment Sequences for Study A461N-US-17-861

Sequence	Treatment Group Period 1	Treatment Group Period 2	Number of Animals
A	Non-final formulation #1 (T01)	Simparica Trio™ (T02)	18
B	Simparica Trio™ (T02)	Non-final formulation #1 (T01)	18

Drug Administration: All treatments were administered orally as outlined in Table 60 above after fasting overnight. Each dog was administered one chewable tablet containing 0.24 mg moxidectin in addition to sarolaner and pyrantel pamoate.

Measurements and Observations: Blood samples were collected from each dog pre-dose (0 hour) and at 0.5, 1, 2, 2.5, 3, 5, 8, 24, 32, 48, 72, and 96 hours and 7, 10, 15, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, and 105 days post-dose. The concentration of moxidectin was measured in canine plasma using a validated liquid chromatography mass spectrometry (LC-MS/MS) method.

Pharmacokinetic and Statistical Methods: Non-compartmental pharmacokinetic parameters were determined for each individual dog in each period using Phoenix WinNonlin version 6.4 (Pharsight Corp.). The area under the curve from time zero to the last sampling time associated with quantifiable drug concentration (AUClast) was estimated using the linear

trapezoidal method and the maximum observed concentration (Cmax) was the highest observed plasma concentration for each animal. These parameter estimates were transformed to the natural logarithm to determine bioequivalence between the two products using the linear mixed effects model in the bioequivalence module of Phoenix WinNonlin version 6.4. The fixed effects were subject, sequence, and period and the random effect was subject nested within sequence. For two products to be bioequivalent, the lower bound of the 90% confidence interval (CI) must be greater than 80% and the upper bound must be less than 125% for both Cmax and AUClast.

Results:

Table II.61. Assessment of bioequivalence for moxidectin between non-final formulation #1 (R) and Simparica Trio™ (T)

Variable	Formulation	Geometric Least Square Mean	Ratio T/R	90% CI lower	90% CI Upper
AUClast (ng*h/mL)	Non-final formulation #1 (R)	1027	92.97	89.61	96.48
AUClast (ng*h/mL)	Simparica Trio™ (T)	954.9	92.97	89.61	96.48
Cmax (ng/mL)	Non-final formulation #1 (R)	16.3	88.04	82.70	93.73
Cmax (ng/mL)	Simparica Trio™ (T)	14.3	88.04	82.70	93.73

AUClast = the area under the curve from time zero to the last sampling time associated with quantifiable drug concentration
Cmax = maximum observed concentration
CI = confidence interval

Conclusions: This study demonstrated that moxidectin in sarolaner, moxidectin, and pyrantel pamoate combination tablets (non-final formulation #1) and moxidectin in the final Simparica Trio™ chewable tablets formulation was bioequivalent following oral administration to dogs. The results of this study allow for a bridge to the results of the heartworm studies (*D. immitis*) conducted with non-final formulation #1.

25. Bioequivalence study 2: Study A461N-US-17-862

Title: Two-Way Cross-Over Bioequivalence Study Comparing Moxidectin and Sarolaner in a Final and Non-Final Formulation of Combination Tablet Containing Sarolaner, Moxidectin, and Pyrantel in Dogs

Study Dates: February 6, 2018 – December 14, 2018

Study Location: Concord, OH

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

Objective: The objective of this study was to determine if the moxidectin and sarolaner in a sarolaner, moxidectin, pyrantel pamoate tablet (non-final formulation #2) was bioequivalent to the moxidectin and sarolaner in the final Simparica Trio™ formulation in healthy dogs under fasted conditions.

Study Animals: Forty-eight male Beagle dogs, between 12.1 to 13.9 months and weighing 7.70 to 14.55 kg, were included in the study.

Treatment Groups: This study was a two treatment, two period crossover design. A 105-day washout separated the period 1 and period 2 drug administrations.

Table II.62. Treatment Sequence for Study A461N-US-17-862

Sequence	Treatment (Period 1)	Treatment (Period 2)	Number of Animals
A	Non-final formulation #2 (T01)	Simparica Trio™ (T02)	24
B	Simparica Trio™ (T02)	Non-final formulation #2 (T01)	24

Drug Administration: All treatments were administered orally as outlined in Table II.62 above after fasting overnight. Each dog was administered one chewable tablet containing 0.24 mg moxidectin and 12 mg sarolaner in addition to pyrantel pamoate.

Measurements and Observations: Blood samples were collected from each dog pre-dose (0 hour) and at 0.5, 1, 2, 2.5, 3, 5, 8, 24, 32, 48, 72, and 96 hours and 7, 10, 15, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, and 105 days post-dose. Concentrations of moxidectin and sarolaner were measured in canine plasma using a validated LC-MS/MS method.

Pharmacokinetic and Statistical Methods: Non-compartmental pharmacokinetic parameters were determined for each individual dog in each period using Phoenix WinNonlin version 6.4 (Pharsight Corp.). The area under the curve from time zero to the last sampling time associated with quantifiable drug concentration (AUClast) was estimated using the linear trapezoidal method and the maximum observed concentration (Cmax) was the highest observed plasma concentration for each animal. These parameter estimates were transformed to the natural logarithm to determine bioequivalence between the two products using the linear mixed effects model in the bioequivalence module of Phoenix WinNonlin version 6.4. The fixed effects were subject, sequence, and period and the random effect was subject nested within sequence. For two products to be bioequivalent, the lower bound of the 90% confidence interval (CI) must be greater than 80% and the upper bound must be less than 125% for both Cmax and AUClast.

Results:

Table 63: Assessment of bioequivalence for moxidectin between non-final formulation #2 (R) and Simparica Trio™ (T)

Variable	Formulation	Geometric Least Square Mean	Ratio T/R	90% CI Lower	90% CI Upper
AUClast (ng*h/mL)	Non-final formulation #2 (R)	917.69	106.81	102.31	111.51
AUClast (ng*h/mL)	Simparica Trio™ (T)	980.17	106.81	102.31	111.51
Cmax (ng/mL)	Non-final formulation #2 (R)	12.37	102.88	96.54	109.63
Cmax (ng/mL)	Simparica Trio™ (T)	12.73	102.88	96.54	109.63

AUClast = the area under the curve from time zero to the last sampling time associated with quantifiable drug concentration
Cmax = maximum observed concentration CI = confidence interval

Table 64: Assessment of bioequivalence for sarolaner between non-final formulation #2 (R) and Simparica Trio™ (T)

Variable	Formulation	Geometric Least Square Mean	Ratio T/R	90% CI Lower	90% CI Upper
AUClast (ng*h/mL)	Non-final formulation #2 (R)	172609.59	94.23	87.55	101.41
AUClast (ng*h/mL)	Simparica Trio™ (T)	162644.72	94.23	87.55	101.41
Cmax (ng/mL)	Non-final formulation #2 (R)	441.86	90.03	83.38	97.20
Cmax (ng/mL)	Simparica Trio™ (T)	397.79	90.03	83.38	97.20

AUClast = the area under the curve from time zero to the last sampling time associated with quantifiable drug concentration
Cmax = maximum observed concentration
CI = confidence interval

Conclusions: This study demonstrated that moxidectin and sarolaner in sarolaner, moxidectin, and pyrantel pamoate combination tablets (non-final formulation #2) and moxidectin and sarolaner in the final Simparica Trio™ chewable tablets formulation were bioequivalent following oral administration to dogs. The results of this study allow for a bridge to the results of the flea (*C. felis*) and tick (*A. americanum*, *A. maculatum*, *D. variabilis*, *I. scapularis*, and *R. sanguineus*) studies, the margin of safety study in 8-week old dogs, and the margin of safety study in heartworm positive dogs conducted with non-final formulation #2.

26.*In-vitro* bioequivalence of pyrantel pamoate: Study AQ62Z-IN-18-999

Type of Study: An *in vitro* Dissolution study was used *in lieu* of a pharmacokinetic study because pyrantel pamoate has minimal systemic absorption and acts locally in the gastrointestinal tract.

Study Location: Bengaluru, India.

General Design: Final Simparica Trio™ formulation and non-final formulation #2 sarolaner, moxidectin, and pyrantel pamoate combination tablet dissolution profiles were generated under multiple *in vitro* conditions to demonstrate that the formulations perform comparably across all segments of the canine gastrointestinal tract. Tablets containing 12 mg sarolaner, 0.24 mg moxidectin, and 50 mg pyrantel (as pamoate salt) were used.

Table 65: *In vitro* dissolution testing media and conditions

Media composition	USP Phosphate buffer with 1.5% w/v SLS	Canine FaSSGF	Canine FaSSIF
pH	6.8	1.5	7.5
USP Apparatus	Type II	Type II	Type II
Paddle Speed	75 rpm	75 rpm	75 rpm
Volume	1000 mL	1000 mL	1000 mL
Media Temperature	37°C ± 0.5°C	37°C ± 0.5°C	37°C ± 0.5°C

FaSSGF = Fasted State Simulated Gastric Fluid
FaSSIF = Fasted State Simulated Intestinal Fluid
SLS = Sodium Laurel Sulfate
USP= United States Pharmacopeia
rpm= revolution per minute

Sampling time points included 10, 15, 20, 30, 45, 60, 75, and 90 minutes. A high performance liquid chromatography with UV detection (HPLC-UV) method was used to quantify the concentrations of pyrantel in the dissolution vessels. Twelve tablets were tested independently for each formulation in each medium. The F2 metric was used to compare the profiles for similarity. An F2 value ≥50 was considered similar. If the variability of the vessel data was not acceptable (RSD ≤20% at first timepoint, RSD ≤10% at all subsequent timepoints) to apply the F2 testing criteria, the tolerance limit approach was applied to demonstrate comparability.

Results: The F2 criterion or tolerance limit, where appropriate, was met for pyrantel pamoate using each of medium.

Conclusion: This *in vitro* bioequivalence study demonstrated that pyrantel pamoate in sarolaner, moxidectin, and pyrantel pamoate combination tablets (non-final formulation #2) and pyrantel pamoate in the final Simparica Trio™ chewable tablet formulation was comparable following *in vitro* dissolution testing in multiple media and is therefore biologically equivalent.

27.*In vitro* dissolution comparison of additional tablet strengths

Type of Study: *In vitro* Dissolution

Study Location: Bengaluru, India.

General Design: *In vitro* dissolution was compared, within strengths, for the final Simparica Trio™ formulation with each of the non-final formulations (#1 and #2). The *in vitro* dissolution testing was conducted in 1000 mL of USP Phosphate buffer with 1.5% w/v sodium lauryl sulfate using paddle speed at 50 rpm (Tablet size 1), 75 rpm (Tablet size 2, 3, 4), and 100 rpm (Tablet size 5, 6). Temperature was maintained at 37° ± 0.5° C. Sampling time points included 10, 15, 20, 30, 45, 60, 75, and 90 minutes. A high performance liquid chromatography with ultraviolet (UV) detection (HPLC-UV) method was used to quantify the concentrations of moxidectin, sarolaner, and pyrantel pamoate in the dissolution vessels. The F2 metric was used to compare the profiles for similarity. F2 value ≥50 was considered similar. If the variability of the vessel data was not acceptable (Relative Standard Deviation (RSD) ≤20% at first timepoint, RSD ≤10% at all subsequent timepoints) to apply the F2 testing criteria, the tolerance limit approach was applied to demonstrate comparability.

Results: Simparica Trio™ is supplied in tablets containing the following concentration of drug:

3.0 mg sarolaner / 0.06 mg moxidectin / 12.5 mg pyrantel (as pamoate salt)
6.0 mg sarolaner / 0.12 mg moxidectin / 25.0 mg pyrantel (as pamoate salt)
12.0 mg sarolaner / 0.24 mg moxidectin / 50.0 mg pyrantel (as pamoate salt)
24.0 mg sarolaner / 0.48 mg moxidectin / 100 mg pyrantel (as pamoate salt)
48.0 mg sarolaner / 0.96 mg moxidectin / 200 mg pyrantel (as pamoate salt)
72.0 mg sarolaner / 1.44 mg moxidectin / 300 mg pyrantel (as pamoate salt)

For the first three tablet strengths listed above, Simparica Trio™ tablets had mean sarolaner and pyrantel pamoate release greater than 80% in 45 minutes and moxidectin release greater than 75% in 45 minutes. For the fourth through sixth tablet strengths listed above, Simparica Trio™ tablets had mean sarolaner and pyrantel pamoate release greater than 80% in 60 minutes and moxidectin release greater than 75% in 60 minutes. The F2 criterion or tolerance limit, where appropriate, was met for all strengths of the three pharmaceutical strengths.

Conclusion: The results of the dissolution study allow the bioequivalence results for the size evaluated in the *in vivo* bioequivalence studies (Study No. A461N-US-17-861 and A461N-US-17-862) and the *in vitro* dissolution study (Study No. AQ62Z-IN-18-999) to be inferred for the other Simparica Trio™ tablet sizes for moxidectin, sarolaner, and pyrantel pamoate.

III. TARGET ANIMAL SAFETY

A. Margin of Safety Study in 8-week old dogs

Title: A 25-Week Target Animal Safety Study (Once Every 4 Weeks Oral Tablet Administration for 7 Doses) of Sarolaner, Moxidectin, and Pyrantel (as Pamoate Salt) in 8-Week Old Beagle Dogs; Study A362N-US-16-670

Study Dates: July 21, 2016 – February 13, 2018

Study Location: Ashland, OH

Study Design:

Objective: To evaluate the safety margin of the sarolaner, moxidectin, and pyrantel combination (combination product) when administered orally at one (1X), three (3X), and five times (5X) the maximum labeled dose once monthly for 7 consecutive doses to Beagle dogs starting at 8 weeks of age. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR Part 58).

Study Animals: 32 Beagle dogs (16 male, 16 female), 8 weeks of age and 1.8 to 2.9 kg body weight.

Treatment Groups:

Table III.1. Treatment groups for Study A362N-US-16-670

Group Number	Treatment	Dose Level	Number of Animals
T01	Placebo control	NA	8
T02 (1X)	Simparica Trio™ *	2.4 mg/kg Sarolaner, 48 µg/kg Moxidectin, 10 mg/kg Pyrantel	8
T03 (3X)	Simparica Trio™ *	7.2 mg/kg Sarolaner, 144 µg/kg Moxidectin, 30 mg/kg Pyrantel	8
T04 (5X)	Simparica Trio™ *	12 mg/kg Sarolaner, 240 µg/kg Moxidectin, 50 mg/kg Pyrantel	8

*non-final formulation #2; see section II.C. Bioequivalence Studies

Drug Administration: All treatments were administered orally. Dogs were fed approximately 30 minutes prior to dosing. The control group received the number of placebo (empty) capsules corresponding to the 5X treatment group dogs.

Measurements and Observations: Clinical observations were performed on all animals prior to dosing; at 1, 2, 3, 4, 6, 8, 12, and 18 hours post-dosing on Study Days 0, 28, 56, 84, 112, 140, and 168; and daily for 5 days after dosing.

General health observations were conducted twice daily throughout the study. Immediately post-dosing, observations were made for emesis, choking, gagging, or drooling. Systems-based physical examinations were conducted weekly by veterinarians. Body weights were recorded twice weekly after the first dose and once weekly thereafter. Food consumption was recorded daily and reported weekly. Ophthalmic examination was conducted pre-randomization and near the end of treatment. Blood was collected for clinical pathology evaluation (hematology, coagulation, and serum chemistry) at pre-treatment, prior to each dose, and prior to necropsy. Urine samples were collected for urinalysis during acclimation; on Study Days 55, 111, and 167; and on the day of necropsy. A complete necropsy with organ weights and microscopic examination was completed at the end of the study.

Statistical Methods: Post-treatment body weight, post-treatment body temperature, post-treatment average weekly food consumption, and numerical clinical pathology data were analyzed using a mixed linear model for analysis of variance of repeated measures. Where appropriate, a baseline covariate (pre-treatment value) was included in the model. Organ weight variables (absolute weights, and relative weights to brain and body weight) were analyzed using a general linear mixed model for analysis of variance.

Results: During the end-of-study ophthalmic examination the following change was found: one 1X dog had retinal dysplasia (OS folds). All other results in the treated groups were similar to the control group.

Conclusion: The study supports the safe use of Simparica Trio™ (sarolaner, moxidectin, and pyrantel) in dogs 8 weeks of age and older when used at the labeled dose.

B. Safety Study in Heartworm Positive Dogs

Title: GLP Margin of Safety of Sarolaner, Pyrantel Pamoate, and Moxidectin When Administered Orally to Beagle Dogs Infected with Adult *Dirofilaria immitis*; Study A362N-US-15-533

Study Dates: January 24, 2017 – May 18, 2018

Study Location: Kalamazoo, MI

Study Design:

Objective: To evaluate the safety of the sarolaner, moxidectin, and pyrantel combination (combination product) following oral administration at one (1X) and three times (3X) the maximum labeled dose administered 3 times at 28-day intervals to dogs infected with adult *Dirofilaria immitis*. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR Part 58).

Study Animals: 24 beagle dogs (12 male, 12 female), approximately 10 months of age and 6.2 – 11.2 kg body weight at initiation

Treatment Groups:

Table III.2. Treatment groups for Study A362N-US-15-533

Group Number	Treatment	Dosage	Dogs per Group
T01 (0X)	Placebo control	NA	8
T02 (1X)	Simparica Trio™ *	2.4 mg/kg Sarolaner, 48 µg/kg Moxidectin, 10 mg/kg Pyrantel	8
T03 (3X)	Simparica Trio™ *	7.2 mg/kg Sarolaner, 144 µg/kg Moxidectin, 30 mg/kg Pyrantel	8

*non-final formulation #2; see section II.C. Bioequivalence Studies

Experimental Design: Twenty-four (24) dogs with pre-existing heartworm infections resulting from surgical transplantation of 10 male and 10 female adult *Dirofilaria immitis* were used in this study. Implantations were >6 months prior to study initiation and dogs were verified to be microfilaremic (>500 microfilaria/mL) and *D. immitis* antigen positive prior to study initiation.

Drug Administration: All treatments were administered orally. Dogs were fed approximately 30 minutes prior to dosing. The control group received the number of placebo (empty) capsules corresponding to the 3X treatment group dogs.

Measurements and Observations: Clinical observations were performed on all animals prior to dosing; 2-4 hours post-dosing on Study Days 0, 28, and 56; and daily for 3 days after dosing. Dogs were monitored for emesis for 30 minutes after dosing, and re-dosed if needed. The general health of each dog was evaluated twice daily. Clinical observations included evaluation for emesis, abnormalities in feces, urine, and appetite. Dogs were monitored for hypersensitivity reactions defined to include anaphylaxis, shock, collapse, respiratory distress, depression, or fever. Blood was collected for pharmacokinetic evaluation before each dose, and at 2, 8, 24, 72, 168, 336, 504, and 672 hours following each dose. Microfilaria counts were conducted at multiple timepoints through the study. *D. immitis* antigen testing was conducted pre-dose and on Days 28, 56, and 83. A necropsy with examination for adult *D. immitis* worms was completed at the end of the study.

Statistical Methods: Body weight and average daily food intake were statistically analyzed using a mixed linear model for analysis of variance of repeated measures. Log transformed microfilariae count were analyzed using a general linear mixed model for analysis of variance of repeated measures. The most recent (Day 0) pre-treatment microfilariae count was used as a covariate in the analysis. The number of adult heartworms from necropsy was summarized with descriptive statistics by treatment and worm sex.

Results: There were no treatment related effects on body weight although there were instances of decreased appetite. A single dog (3X) vomited at 30 minutes after the first dose. Diarrhea occurred more commonly in the dogs administered Simparica Trio™ and also more often in the 3X group compared to the 1X group.

Two dogs (1 each in 1X and 3X) had fever of less than 24 hours after the first dose, which may have been a transient reaction to a rapid microfilaria reduction. The dogs recovered without treatment or sequelae. Microfilaria counts decreased to near zero in both groups administered Simparica Trio™, but there were no other hypersensitivity reactions (defined as anaphylaxis, shock, collapse, respiratory distress, or depression). Adult heartworms were recovered at necropsy with a few dead worms in 1X and 3X groups, and live worm recovery of 89% (placebo control), 83% (1X), and 74% (3X).

Following oral dosing at 1X and 3X the maximum intended dose, sarolaner and moxidectin show dose related increases in AUC and C_{max} but pyrantel showed less than proportional increases in AUC and C_{max}. Sarolaner and moxidectin showed accumulation following the first dose with diminished accumulation following the second dose. Pyrantel showed variable pharmacokinetics but no significant accumulation. In this study, while the values of the exposure parameters (AUC, C_{max}, and T_{max}) were comparable to other PK studies conducted in healthy beagle dogs, the average terminal half-lives were longer and were 19 days for sarolaner and 22 days for moxidectin. It is not clear if this prolonged half-life is related to the disease state (infection with adult *Dirofilaria immitis*) of these animals.

Conclusions: The oral administration of Simparica Trio™ at 1X and 3X the maximum labeled dose to dogs with pre-existing adult heartworm infections and circulating microfilaria was well tolerated and did not cause severe adverse reactions in any dogs.

C. Tolerance Study in Avermectin-Sensitive Collie Dogs

Title: Tolerance of Sarolaner + Moxidectin + Pyrantel Pamoate Combination When Administered Orally to Avermectin-Sensitive (mdr-1 gene mutation) Collie Dogs; Study A366R-US-15-565

Study Dates: October 5, 2017 – May 3, 2018

Study Location: Stanwood, MI

Study Design:

Study Objective: To evaluate the safety of a combination product containing sarolaner, moxidectin, and pyrantel following a single oral administration at one (1X), three (3X), and five times (5X) the maximum labeled dose to avermectin-sensitive Collie dogs.

Study Animals: Thirty-two (32) avermectin-sensitive Collie dogs (16 male, 16 female), 1.5 to 10.8 years and 18.0 to 45.7 kg body weight. Dogs were pre-screened for sensitivity to 120 µg/kg ivermectin.

Treatment Groups:

Table III.3. Dose Levels

Group Number	Treatment	Dosage	Dogs per Group
T01 (0X)	Placebo control	NA	8
T02 (1X)	Simparica Trio™	2.4 mg/kg Sarolaner, 48 µg/kg Moxidectin, 10 mg/kg Pyrantel	8
T03 (3X)	Simparica Trio™	7.2 mg/kg Sarolaner, 144 µg/kg Moxidectin, 30 mg/kg Pyrantel	8
T04 (5X)	Simparica Trio™	12 mg/kg Sarolaner, 240 µg/kg Moxidectin, 50 mg/kg Pyrantel	7[a]

[a] One animal was withdrawn from the study on Day -7 following a seizure observed after the pre-study physical examination.

Drug Administration: All treatments were administered orally. Dogs were fed 30 minutes prior to dosing. The control group received the number of placebo (empty) capsules corresponding to the 5X treatment group dogs.

Measurements and Observations: Body weights were collected on Days -14, 2 and 7. General health observations were conducted twice daily on Days -7 through -1, and Days 4 through 7. Clinical observations were performed on all animals prior to dosing; at 1, 2, 3, 4, 6, 8, 12, and 18 hours post-dose; and twice daily on Days 1-3. Blood was collected for pharmacokinetic evaluation prior to dosing; at 2, 8, 24, 48, and 72 hours post dose; and on 30, 50, and 66 days after dosing.

Statistical Methods: Body weight was statistically analyzed using a mixed linear model for analysis of variance of repeated measures. General health and clinical observations were summarized. Plasma concentrations, C_{max}, and $AUC_{0\text{-}72h}$ were natural log transformed prior to summarization and back-transformed values were reported.

Results: Dogs from all groups were observed with mild to moderate signs associated with avermectin sensitivity including ataxia, depression, muscle fasciculation, mydriasis, and salivation. The 5X group had a greater number of observations overall. Most observations were noted on Day 0-1. Only a single dog in the 5X group had a test article related sign (ataxia) on Day 2.

Table III.4. Total Incidence of Clinical Observations by Treatment: Number of abnormal observations (number of affected animals)

Clinical Observation	T01 (0X)	T02 (1X)	T03 (3X)	T04 (5X)
Ataxia	2 (2)	3 (2)	4 (2)	12 (3)
Depression	6 (1)	0 (0)	0 (0)	1 (1)
Muscle Fasciculations	8 (1)	1 (1)	0 (0)	8 (3)
Mydriasis	1 (1)	0 (0)	3 (2)	4 (2)
Salivation	6 (4)	6 (3)	4 (4)	7 (3)

Following oral dosing at 1X, 3X, and 5X the maximum labeled dose in avermectin sensitive collies, sarolaner and moxidectin showed dose related increases in AUC and C_{max} but pyrantel showed less than proportional increases in AUC and C_{max}. Compared to beagle dogs used in other studies, MDR1 deficient collies had greater exposure (AUC and C_{max}) and prolonged terminal half-lives to moxidectin and sarolaner, but not to pyrantel.

Conclusions: Oral administration of Simparica Trio™ was well tolerated in phenotypically tested avermectin sensitive Collies (MDR1 negative) at up to 3X the maximum labeled dose. At 5X the maximum labeled dose, mild and self-limiting signs were observed that resolved within 3 days of treatment.

IV. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to Simparica Trio™:

Warnings: Not for use in humans. Keep this and all drugs out of reach of children.

V. 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 Simparica Trio™, when used according to the label, is safe and effective for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (immature adult and adult *Toxocara canis* and adult *Toxascaris leonina*) and adult hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) infections. Simparica TRIO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations, and the treatment and control of tick infestations with *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *Ixodes scapularis* (black-legged tick), and *Rhipicephalus sanguineus* (brown dog tick) for one month in dogs and puppies 8 weeks of age 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 the product is indicated for the prevention of heartworm infections (*Dirofilaria immitis*) in dogs, which requires veterinary examination and testing to ensure dogs are negative for adult heartworm disease prior to administration of the product to dogs.

B. Exclusivity

Simparica Trio™, as approved in our approval letter, qualifies for THREE years of marketing exclusivity beginning on 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 Simparica Trio™.

C. Patent Information

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

VI. APPENDIX: Details of Correction

The indications on the title sheet of this summary and under General Information, Section N, was corrected to clarify the treatment and control of roundworm and hookworm indications.

Original Indications
Simparica Trio™ prevents heartworm disease caused by *Dirofilaria immitis*, kills adult fleas *(Ctenocephalides felis)* and is indicated for the treatment and prevention of flea infestations, the treatment and control of tick infestations with *Amblyomma americanum* (lone star tick), *Amblyomma maculat*um (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *Ixodes scapularis* (black-legged tick), and *Rhipicephalus sanguineus* (brown dog tick), and the treatment and control of roundworm (immature adult and adult *Toxocara canis* and adult *Toxascaris leonina*) and adult hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) infections for one month in dogs and puppies 8 weeks of age and older, and weighing 2.8 pounds or greater.

Corrected Indications, April 13, 2020
SIMPARICA TRIO is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (immature adult and adult *Toxocara canis* and adult *Toxascaris leonina*) and adult hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) infections. Simparica TRIO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations, and the treatment and control of tick infestations with *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *Ixodes scapularis* (black-legged tick), and *Rhipicephalus sanguineus* (brown dog tick) for one month in dogs and puppies 8 weeks of age and older, and weighing 2.8 pounds or greater.