Date of Approval: July 19, 2023

# FREEDOM OF INFORMATION SUMMARY

## ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-554

NexGard® PLUS

(afoxolaner, moxidectin, and pyrantel chewable tablets)

Dogs

NexGard® PLUS is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*. NexGard® PLUS is indicated for the treatment and control of adult hookworm *(Ancylostoma caninum, Ancylostoma braziliense*, and *Uncinaria stenocephala)* and roundworm *(Toxocara canis* and *Toxascaris leonina)* infections. NexGard® PLUS kills adult fleas and is indicated for the treatment and prevention of flea infestations *(Ctenocephalides felis)* and the treatment and control of *Ixodes scapularis* (black-legged tick), *Rhipicephalus sanguineus* (brown dog tick), *Dermacentor variabilis* (American dog tick), and *Amblyomma americanum* (lone star tick) infestations for one month in dogs and puppies eight weeks of age and older, weighing four pounds of body weight or greater.

Sponsored by:

Boehringer Ingelheim Animal Health USA, Inc.

**Executive Summary**
NexGard® PLUS (afoxolaner, moxidectin, and pyrantel chewable tablets) is approved for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of adult hookworm (*Ancylostoma caninum, Ancylostoma braziliense*, and *Uncinaria stenocephala*) and roundworm (*Toxocara canis* and *Toxascaris leonina*) infections. NexGard® PLUS kills adult fleas and is indicated for the treatment and prevention of flea infestations (*Ctenocephalides felis*) and the treatment and control of *Ixodes scapularis* (black-legged tick), *Rhipicephalus sanguineus* (brown dog tick), *Dermacentor variabilis* (American dog tick), and *Amblyomma americanum* (lone star tick) infestations for one month in dogs and puppies eight weeks of age and older, weighing four pounds of body weight or greater.

NexGard® PLUS is an antiparasitic drug with three active ingredients and is available in five strengths of soft chewable tablets that are given orally once a month.

**Safety and Effectiveness**
The sponsor conducted seven laboratory studies and one clinical field study, including a total of 70 laboratory and 134 client-owned dogs, to demonstrate the effectiveness of NexGard® PLUS against a variety of internal and external parasites. The eight studies are as follows:

- One clinical field study in client-owned dogs showing that NexGard® PLUS is safe and effective at preventing heartworm disease caused by *D. immitis* under field conditions.

- Two laboratory studies showing that the drug is effective at preventing heartworm disease caused by *D. immitis*.

- One laboratory study showing that the drug is effective at killing adult fleas (*C. felis*) and treating and preventing flea infestations.

- Two laboratory studies showing that the drug is effective at treating and controlling tick infestations. The same minimum dose of afoxolaner (2.5 mg/kg) has already been shown to be effective at treating and controlling *I. scapularis*, *R. sanguineus*, *D. variabilis*, and *A. americanum* infestations in dogs under NADA 141-406. Therefore, the sponsor only conducted the two laboratory studies using the dose-limiting parasite for afoxolaner (*A. americanum*) to show that NexGard® PLUS is effective against all listed tick species. A dose-limiting parasite is defined as the parasite that requires the highest dose of an antiparasitic drug to achieve the established minimum effectiveness.

- Two laboratory studies showing that the drug is effective at treating and controlling gastrointestinal nematode infections. The same minimum dose of pyrantel pamoate (5 mg/kg) has already been shown to be effective at treating and controlling adult roundworm (*T. canis* and *T. leonina*) and adult hookworm (*A. caninum*, *A. braziliense*, and *U. stenocephala*) infections in dogs under NADA 140-971 for Heartgard® PLUS (ivermectin/pyrantel). Therefore, the sponsor only conducted the two laboratory studies using the dose-limiting parasite for pyrantel

pamoate (*T. canis*) to show that NexGard® PLUS is effective against all listed species of adult roundworms and hookworms.

The most common adverse reactions from the clinical field study were diarrhea, vomiting, lethargy, itching, dermatitis, anorexia, and muscle tremors.

The sponsor conducted a margin of safety study in young, healthy, male and female beagles. The dogs were given NexGard® PLUS orally at 0X, 1X, 3X, and 5X the maximum exposure dose for each of the three active ingredients every 28 days for six treatments. No dogs showed signs of avermectin sensitivity or had serious health abnormalities related to the drug.

The sponsor conducted a safety study in young, healthy male and female beagles that were heartworm positive. The dogs were given NexGard® PLUS orally at 0X, 1X, and 3X the maximum exposure dose for each of the three active ingredients every 28 days for three treatments. The drug was well-tolerated in dogs with pre-existing adult heartworm infections and circulating microfilariae. No dogs showed signs of avermectin toxicity or had serious health abnormalities related to the drug.

The sponsor also conducted a safety study in avermectin-sensitive collies, which have a mutation at the multidrug resistance (MDR1) gene that makes them more sensitive to adverse effects of ivermectin. Healthy male and female collies confirmed to have the MDR1 mutation (they are MDR1 deficient) were given NexGard® PLUS orally at 0X, 1X, 3X, and 5X the maximum exposure dose for each of the three active ingredients for one treatment. A second treatment was given 28 days later to the 0X and 1X groups. The drug was well-tolerated in MDR1-deficient, avermectin-sensitive collies. No dogs showed any clinical signs of avermectin toxicity during the study.

**Conclusions**
Based on the data submitted by the sponsor for the approval of NexGard® PLUS, FDA determined that the drug is safe and effective when used according to the labeling.

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# I. GENERAL INFORMATION

## A. File Number

NADA 141-554

## B. Sponsor

Boehringer Ingelheim Animal Health USA, Inc.
3239 Satellite Blvd.
Duluth, GA 30096

Drug Labeler Code: 000010

## C. Proprietary Name

NexGard® PLUS

## D. Drug Product Established Name

afoxolaner, moxidectin, and pyrantel chewable tablets

## E. Pharmacological Category

Antiparasitic

## F. Dosage Form

Chewable Tablet

## G. Amount of Active Ingredient

Each chewable contains:

9.375 mg afoxolaner, 45 mcg moxidectin, and 18.75 mg pyrantel*
18.75 mg afoxolaner, 90 mcg moxidectin, and 37.5 mg pyrantel*
37.5 mg afoxolaner, 180 mcg moxidectin, and 75 mg pyrantel*
75 mg afoxolaner, 360 mcg moxidectin, and 150 mg pyrantel*
150 mg afoxolaner, 720 mcg moxidectin, and 300 mg pyrantel*
*As pamoate salt

## H. How Supplied

NexGard® PLUS is available in five strengths of beef-flavored soft chewables. Each chewable size is available in color-coded packages of one, three, or six chewables.

## I. Dispensing Status

Prescription (Rx)

## J. Dosage Regimen

NexGard® PLUS is given orally once a month at the minimum dosage of 1.14 mg/lb (2.5 mg/kg) afoxolaner, 5.45 mcg/lb (12 mcg/kg) moxidectin, and 2.27 mg/lb (5.0 mg/kg) pyrantel (as pamoate salt). For heartworm disease prevention, give once monthly for at least six months after last exposure to mosquitoes.

Dosing Schedule:

| Body Weight (lbs.) | Afoxolaner Per Chewable (mg) | Moxidectin Per Chewable (mcg) | Pyrantel* Per Chewable (mg) | Chewables Administered |
|---|---|---|---|---|
| 4.0-8.0 | 9.375 | 45 | 18.75 | One |
| 8.1-17.0 | 18.75 | 90 | 37.5 | One |
| 17.1-33.0 | 37.5 | 180 | 75 | One |
| 33.1-66.0 | 75 | 360 | 150 | One |
| 66.1-132.0 | 150 | 720 | 300 | One |
| Over 132.0 | Not Applicable | Not Applicable | Not Applicable | Administer the appropriate combination of chewables |

*As pamoate salt.

## K. Route of Administration

Oral

## L. Species/Class

Dogs

## M. Indication

NexGard® PLUS is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis.* NexGard® PLUS is indicated for the treatment and control of adult hookworm (*Ancylostoma caninum, Ancylostoma braziliense*, and *Uncinaria stenocephala*) and roundworm (*Toxocara canis* and *Toxascaris leonina*) infections. NexGard® PLUS kills adult fleas and is indicated for the treatment and prevention of flea infestations (*Ctenocephalides felis*) and the treatment and control of *Ixodes scapularis* (black-legged tick), *Rhipicephalus sanguineus* (brown dog tick), *Dermacentor variabilis* (American dog tick), and *Amblyomma americanum* (lone star tick) infestations for one month in dogs and puppies eight weeks of age and older, weighing four pounds of body weight or greater.

## II. EFFECTIVENESS

### A. Dosage Characterization

**Afoxolaner: treatment and prevention/control of flea and tick infestations**

Dosage characterization information is contained in the Freedom of Information (FOI) Summary for the original approval of an afoxolaner chewable tablet for dogs (NADA 141-406 dated September 14, 2013). Those data support a dose of 1.14 mg/lb (2.5 mg/kg), administered orally once per month, to kill adult fleas and for the treatment and prevention of flea infestations and the treatment and control of tick infestations.

**Pyrantel (as pamoate salt): treatment and control of gastrointestinal nematodes**

Dosage characterization information is contained in the FOI Summary for the original approval of an ivermectin and pyrantel pamoate chewable tablet for dogs (NADA 140-971 dated January 15, 1993). Those data support a dose of 2.27 mg/lb (5.0 mg/kg), administered orally once per month, for the treatment and control of hookworm and roundworm infections.

**Moxidectin: prevention of heartworm disease**

Multiple exploratory studies were conducted evaluating moxidectin at doses from 3 mcg/kg to 100 mcg/kg and for varying durations. The exploratory studies demonstrated that increasing the dose and the number of monthly administrations improved the effectiveness of moxidectin against certain laboratory *Dirofilaria immitis* isolates. Therefore, a dosage of 12 mcg/kg, administered six times at monthly intervals, was selected for the prevention of heartworm disease.

### B. Substantial Evidence

The effectiveness of NexGard® PLUS was demonstrated in seven well-controlled laboratory studies and one clinical field study described below. These studies demonstrate that NexGard® PLUS is effective against a wide variety of both internal and external parasites. NexGard® PLUS was administered to a total of 70 laboratory and 134 client-owned dogs in these studies. The most common adverse reactions reported in the clinical field study were diarrhea, vomiting, lethargy, itching, dermatitis, anorexia, and muscle tremor. In addition, a field study and multiple well-controlled laboratory studies were conducted to demonstrate the effectiveness and clinical safety of afoxolaner in support of the approval of NexGard® (afoxolaner) Chewable Tablets for Dogs [Refer to the Freedom of Information Summary for NADA 141-406 dated September 14, 2013].

***Prevention of Heartworm Disease***

1. Field Safety and Effectiveness Study

   **Title:** Safety and Effectiveness Against *Dirofilaria immitis* of Monthly Oral Treatments Containing a Combination of Afoxolaner + Moxidectin + Pyrantel Pamoate in Client-Owned Dogs. (Study No. PR&D 04197)

   **Study Dates:** March 2019 to June 2021

   **Study Locations:**

   Bradenton, FL
   Brownstown, IN
   Commerce, GA
   Enid, OK
   Harleysville, PA
   Lake Worth, FL

   **Study Design:**

   Objective: To determine the safety and effectiveness of NexGard® PLUS administered at monthly intervals for the prevention of heartworm disease caused by *Dirofilaria immitis* in dogs in a field setting in the USA.

   Study Animals: Dogs were included in the evaluation of safety if they received at least one dose of test article. One hundred thirty-four (134) dogs administered NexGard® PLUS and 138 dogs administered the active control were evaluated for safety. One hundred twenty (120) dogs administered NexGard® PLUS and 124 dogs administered the active control were included in the effectiveness evaluation. Dogs intended for breeding, and pregnant and lactating dogs, were not eligible for enrollment.

   Experimental Design: A masked, multicenter, field safety and effectiveness study was conducted using a randomized block design based on the order of enrollment of the individual dogs on a per-site basis. The study was conducted in accordance with Good Clinical Practice (GCP) guidelines. The study compared NexGard® PLUS to an active control containing both imidacloprid and moxidectin. Dogs were confirmed healthy and negative for heartworm infection (by *Dirofilaria immitis* antigen and blood microfilariae testing) prior to enrollment. Dogs were enrolled from six veterinary clinics located in heartworm-endemic regions of the USA. Owners administered NexGard® PLUS or the active control to the dog in the dog's home environment on approximately Days 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 330. Treatments could be administered with or without food.

**Table II.1. Study PR&D 04197 Treatment Groups**

| Treatment | Minimum Dosage | Approximate Days of Treatment | Number of Dogs Evaluable for Safety | Number of Dogs Evaluable for Effectiveness | Approximate Days of Heartworm Testing |
|---|---|---|---|---|---|
| NexGard® PLUS | 2.5 mg/kg afoxolaner + 12 mcg/kg moxidectin + 5.0 mg/kg pyrantel | 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 330 | 134 | 120 | -30 to 0, 60, 120, 180, 240, and 330 |
| Active Control | 10 mg/kg imidacloprid + 2.5 mg/kg moxidectin | 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 330 | 138 | 124 | -30 to 0, 60, 120, 180, 240, and 330 |

Drug Administration: Owners administered NexGard® PLUS or the active control to their dog monthly in the dog's home environment with or without food. The active control contained imidacloprid and moxidectin.

Measurements and Observations: Physical examinations, including body weight measurements, were performed on all dogs prior to treatment on Day 0 and on Days 60, 120, and 180. In addition, body weights were measured on growing puppies on Days 30, 90, and 150 such that dosing adjustments could be made as needed. Blood was collected for hematology and clinical chemistry prior to treatment on Day 0 and on Day 330. Blood was collected on Days 60, 120, 180, 240, and 330 for *Dirofilaria immitis* antigen and blood microfilariae testing.

**Statistical Methods:** The statistical analysis plan indicated that if 100% effectiveness was demonstrated for NexGard® PLUS, the study would be considered to have shown effectiveness, and no statistical analysis would be performed. Because all heartworm antigen and microfilariae tests performed on Days 180, 240, and 330 were negative, no statistical analysis was required.

**Results:** Percent effectiveness for NexGard® PLUS was 100%, as none of the 120 dogs administered NexGard® PLUS tested positive for adult heartworms at any time point.

**Adverse Reactions:**

Evaluation of safety was completed over the 330-day period through in-clinic physical examinations, clinical pathology, and reporting of abnormalities by the owner. The safety database included 134 dogs administered NexGard® PLUS and 138 dogs administered the active control.

There were no serious treatment-related adverse events noted during the study. The most commonly reported adverse reactions are summarized in Table II.2 below.

**Table II.2. Study PR&D 04197 Adverse Reactions**

| Clinical Sign | NexGard® PLUS n = 134 Number (Percentage) | Active Control n = 138 Number (Percentage) |
|---|---|---|
| Diarrhea | 9 (6.7%) | 7 (5.1%) |
| Vomiting | 6 (4.5%) | 7 (5.1%) |
| Lethargy | 3 (2.2%) | 5 (3.6%) |
| Itching | 3 (2.2%) | 3 (2.2%) |
| Dermatitis | 2 (1.5%) | 1 (0.7%) |
| Anorexia | 1 (0.7%) | 4 (2.9%) |
| Muscle tremor | 1 (0.7%) | 1 (0.7%) |

One dog in the NexGard® PLUS group was reported to exhibit muscle tremors along with nausea and depression for one day after the Day 0 treatment. The dog remained in the study and muscle tremors were not reported after any subsequent treatments.

**Conclusions:** This study demonstrated that NexGard® PLUS is safe and effective for the prevention of heartworm disease in dogs under field conditions.

Two well-controlled laboratory studies were conducted to assess the effectiveness of NexGard® PLUS against *Dirofilaria immitis* in dogs.

2. Laboratory Dose Confirmation Study PR&D 0409401: Prevention of Heartworm Disease

   **Title:** Efficacy of Afoxolaner + Moxidectin + Pyrantel Pamoate Administered Orally in a Chewable Formulation Against *Dirofilaria immitis* in Dogs. (Study No. Study PR&D 0409401)

   **Study Dates:** November 5, 2018 to February 25, 2020

   **Study Location:** Athens, GA

   **Study Design:**

   Objective: To confirm the effectiveness of NexGard® PLUS when administered six times at monthly intervals to dogs starting 30 days after infection with *Dirofilaria immitis* third-stage larvae (L3).

Study Animals: Twenty dogs (10 males and 10 females), 4.3 to 4.9 months of age and weighing 6.3 to 9.4 kg, were included in the study.

Experimental Design: Within sex, dogs were randomly allocated to one of the two treatment groups. The study was conducted in accordance with GCP guidelines.

**Table II.3. Study PR&D 0409401 Treatment Groups**

| Treatment | Treatment Days | Afoxolaner (mg/kg) | Moxidectin (mcg/kg) | Pyrantel Pamoate (mg/kg) | Number of Dogs |
|---|---|---|---|---|---|
| NexGard® PLUS | 0, 30, 60, 90, 120, 150 | 2.5 | 12 | 5 | 10 |
| Control (not treated) | Not Applicable | 0 | 0 | 0 | 10 |

Drug Administration: Dogs were fed prior to treatment. All treatments were administered orally. Control dogs did not receive any treatment.

Measurements and Observations: Physical examinations were conducted, and blood was collected for antigen and microfilaria tests, on Day -36. Each dog was inoculated with 50 third-stage infective *Dirofilaria immitis* larvae once on Day -30. Dogs were treated on Days 0, 30, 60, 90, 120, and 150 based on body weights collected on Days -1, 28, 59, 89, 119, and 147, respectively. General health observations were performed at least once daily. Post-dosing clinical observations were conducted 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after the first treatment and 1, 2, 3, and 4 hours after subsequent treatments for all dogs. Blood was collected from each dog on Day 89 for heartworm antigen testing. On Day 181, dogs were humanely euthanized and *Dirofilaria immitis* were collected and counted by sex.

**Statistical Methods:** Parasite counts were transformed to the natural logarithm of (count + 1). The MIXED procedure was used for the analysis of the log-counts at a two-sided $\alpha = 0.05$. The percent effectiveness was calculated as 100[(C - T)/C], where C and T were the geometric means obtained by the back-transformed least squares means, respectively, of the control group and treated group as obtained from the model.

**Results:** All 10 control dogs harbored at least five live adult *Dirofilaria immitis* at necropsy. NexGard® PLUS was 100% effective against *Dirofilaria immitis* in this study; there were no worms present in any dog administered NexGard® PLUS. The mean worm count of *Dirofilaria immitis* for the treated dogs was less than the mean worm count of *Dirofilaria immitis* for the control dogs and a statistically significant difference ($P < 0.0001$) was observed between the mean worm count for the treated dogs and the mean worm count of the control dogs.

**Table II.4. Study PR&D 0409401 Percent Effectiveness**

| Treatment | *Dirofilaria immitis* Count (Geometric Mean) | Percent Effectiveness |
|---|---|---|
| NexGard® PLUS | 0.0 | 100% |
| Control | 29.7 | Not Applicable |

**Adverse Reactions**: During the post-treatment health observations, vomiting was reported in two dogs administered NexGard® PLUS and diarrhea with or without blood was reported in one dog administered NexGard® PLUS.

**Conclusions**: NexGard® PLUS was 100% effective against induced infection with *Dirofilaria immitis* when administered once a month for six consecutive months following infection.

3. Laboratory Dose Confirmation Study PR&D 0427201: Prevention of Heartworm Disease

**Title:** Efficacy of Afoxolaner + Moxidectin + Pyrantel Pamoate Administered Orally in a Chewable Formulation Against *Dirofilaria immitis* in Dogs. (Study No. PR&D 0427201)

**Study Dates:** March 12, 2019 to February 21, 2020

**Study Location:** Colbert, GA

**Study Design:**

Objective: To confirm the effectiveness of NexGard® PLUS when administered six times at monthly intervals to dogs starting 30 days after infection with *Dirofilaria immitis* third-stage larvae (L3).

Study Animals: Twenty dogs (10 males and 10 females), 5.2 to 5.8 months of age and weighing 7.1 to 9.9 kg, were included in the study.

Experimental Design: Within sex, dogs were randomly allocated to one of the two treatment groups. The study was conducted in accordance with GCP guidelines.

**Table II.5. Study PR&D 0427201 Treatment Groups**

| Treatment | Treatment Days | Afoxolaner (mg/kg) | Moxidectin (mcg/kg) | Pyrantel Pamoate (mg/kg) | Number of Dog |
|---|---|---|---|---|---|
| NexGard® PLUS | 0, 30, 60, 90, 120, 150 | 2.5 | 12 | 5 | 10 |
| Control (not treated) | Not applicable | 0 | 0 | 0 | 10 |

Drug Administration: Dogs were fed prior to treatment. All treatments were administered orally. Control dogs did not receive any treatment.

Measurements and Observations: Physical examinations were conducted, and blood was collected for antigen and microfilaria tests, on Day -36. Each dog was inoculated with 50 third-stage infective *Dirofilaria immitis* larvae once on Day -30. Dogs were treated on Days 0, 30, 60, 90, 120, and 150 based on body weights collected on Days -3, 28, 59, 89, 119, and 147, respectively. General health observations were performed at least once daily. Post-dosing clinical observations were conducted 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after the first treatment and 1, 2, 3, and 4 hours after subsequent treatments for all dogs. Blood was collected from each dog on Day 89 for heartworm antigen testing. On Day 181, dogs were humanely euthanized and *Dirofilaria immitis* were collected and counted by sex.

**Statistical Methods:** Parasite counts were transformed to the natural logarithm of (count + 1). The MIXED procedure was used for the analysis of the log-counts at a two-sided $\alpha = 0.05$. The percent effectiveness was calculated as 100[(C - T)/C], where C and T were the geometric means obtained by the back-transformed least squares means, respectively, of the control group and treated group as obtained from the model.

**Results:** All 10 control dogs harbored at least five live adult *Dirofilaria immitis* at necropsy. NexGard® PLUS was 100% effective against *Dirofilaria immitis* in this study; there were no worms present in any dog administered NexGard® PLUS. The mean worm count of *Dirofilaria immitis* for the treated dogs was less than the mean worm count of *Dirofilaria immitis* for the control dogs and a statistically significant difference ($P < 0.0001$) was observed between the mean worm count for the treated dogs and the mean worm count of the control dogs.

**Table II.6. Study PR&D 0427201 Percent Effectiveness**

| Treatment | *Dirofilaria immitis* Count (Geometric Mean) | Percent Effectiveness |
|---|---|---|
| NexGard® PLUS | 0.0 | 100% |
| Control | 35.5 | Not Applicable |

**Adverse Reactions:** There were no treatment-related adverse events during this study.

**Conclusions:** NexGard® PLUS was 100% effective against induced infection with *Dirofilaria immitis* when administered once a month for six consecutive months following infection.

***Kills Adult Fleas and For the Treatment and Prevention of Flea Infestations***

A well-controlled laboratory study was conducted to assess the effectiveness of NexGard® PLUS against adult *Ctenocephalides felis*. In addition, a multi-site field study and two well-controlled laboratory studies, including one flea speed of kill study, were conducted to demonstrate the effectiveness of afoxolaner, at a dose of

2.5 mg/kg, against fleas in support of the approval of NexGard® (afoxolaner) Chewable Tablets for Dogs [Refer to the Freedom of Information Summary for NADA 141-406 dated September 14, 2013].

4. Laboratory Dose Confirmation Study PR&D 0431501: Killing of Adult Fleas and Treatment and Prevention of Flea Infestations

   **Title:** Efficacy of a Single Treatment with Afoxolaner, Moxidectin, and Pyrantel Pamoate Administered Orally in a Chewable Formulation Against Induced Infestations of Adult Fleas *(Ctenocephalides felis)* on Dogs. (Study No. PR&D 0431501)

   **Study Dates:** July 22, 2019 to August 5, 2020

   **Study Location:** Colbert, GA

   **Study Design:**

   Objective: To confirm the effectiveness of NexGard® PLUS when administered once for the treatment and control of induced infestations of adult *Ctenocephalides felis* on dogs.

   Study Animals: Twenty (20) dogs (10 males and 10 females), 8.2 to 14.8 months of age and weighing 7.7 to 13.2 kg, were included in the study.

   Experimental Design: The study followed a randomized block design based on pre-treatment flea counts. Pre-treatment flea counts were used to form 10 blocks (two dogs per block). Within blocks, each dog was randomly allocated to one of the two treatment groups. The blocks were randomly assigned to three different rooms for housing. The study was conducted in accordance with GCP guidelines.

   **Table II.7. Study PR&D 0431501 Treatment Groups**

| Treatment | Dosage | Day of Treatment | Number of Dogs | Days of Flea Infestation | Days of Flea Count |
|---|---|---|---|---|---|
| Control (sham-dosed) | Not Applicable | Day 0 | 10 | -1, 7, 14, 21, and 28 | 1, 8, 15, 22, and 29 |
| NexGard® PLUS | 2.5 mg/kg afoxolaner + 12 mcg/kg moxidectin + 5 mg/kg pyrantel | Day 0 | 10 | -1, 7, 14, 21, and 28 | 1, 8, 15, 22, and 29 |

   Drug Administration: Dogs were fed prior to treatment. All treatments were administered orally. Control dogs did not receive any test article.

Measurements and Observations: Each dog was infested with approximately 100 unfed adult *Ctenocephalides 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, 2, 3, 4, 5, 6, 8, 12, and 24 hours after treatment. General health observations were conducted at least once daily. Flea counts and health observations were conducted masked to treatment.

**Statistical Methods:**

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 calculated from the least squares mean of live flea counts for the control group and T = arithmetic mean calculated from the least squares mean of live flea counts for the treated group.

Flea counts were analyzed separately at each time point using an F-test. The MIXED procedure in SAS was used for the analysis, with treatment group as a fixed effect and room and block within room as random effects. Testing was two-sided at the significance level $\alpha = 0.05$.

**Results:**

Control dogs maintained adequate flea infestations on Days 1, 8, 15, 22, and 29, 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 NexGard® PLUS-treated dogs. The NexGard® PLUS-treated group had a 100% reduction in live flea counts 24 hours after treatment of the existing infestation, and ≥ 99.8% reduction in live flea counts 24 hours after weekly re-infestations for 29 days (Table II.8).

Live flea counts for the NexGard® PLUS-treated group were significantly different ($P < 0.0001$) and numerically lower than the control group on all post-treatment count days.

**Table II.8. Adult *Ctenocephalides felis* Effectiveness; Arithmetic Mean Live Flea Count and Percent Effectiveness**

| Days of Flea Count | Control Group Adult Flea Count (Arithmetic Mean) | NexGard® PLUS Group Adult Flea Count (Arithmetic Mean) | Percent Effectiveness |
|---|---|---|---|
| 1 | 88.9 | 0.0 | 100% |
| 8 | 91.5 | 0.2 | 99.8% |
| 15 | 93.2 | 0.0 | 100% |
| 22 | 87.3 | 0.0 | 100% |
| 29 | 81.9 | 0.0 | 100% |

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

**Conclusions:**

This study demonstrated the effectiveness of NexGard® PLUS for the treatment and control of adult *Ctenocephalides felis* when assessed 24 hours after treatment of an existing infestation, and 24 hours after weekly re-infestation for one month.

Collectively, the data from the well-controlled laboratory effectiveness study of NexGard® PLUS summarized above, and the three effectiveness studies of afoxolaner alone (two laboratory and one field) summarized under NADA 141-406, support the effectiveness of a single monthly dose of NexGard® PLUS for the treatment and prevention of flea *(Ctenocephalides felis)* infestations on dogs.

***Treatment and Control of Tick Infestations***

Multiple studies were conducted to demonstrate the effectiveness of afoxolaner, at a dose of 2.5 mg/kg, for the treatment and control of *Ixodes scapularis*, *Rhipicephalus sanguineus*, *Dermacentor variabilis*, and *Amblyomma americanum* in support of the approval of NexGard® (afoxolaner) Chewable Tablets for Dogs [Refer to the Freedom of Information Summary for NADA 141-406 dated September 14, 2013]. Therefore, only two laboratory studies were conducted against the dose-limiting tick species for afoxolaner *(Amblyomma americanum)* to demonstrate effectiveness of NexGard® PLUS against ticks.

5. Laboratory Dose Confirmation Study PR&D 0424001: Treatment and Control of Induced Infestations of *Amblyomma americanum.*

   **Title:** Efficacy of a Single Treatment with Afoxolaner, Moxidectin, and Pyrantel Pamoate Administered Orally in a Chewable Formulation Against Induced Infestations of Adult *Amblyomma americanum* on Dogs. (Study No. PR&D 0424001)

   **Study Dates:** March 12, 2019 to July 27, 2020

   **Study Location:** Athens, GA

   **Study Design**:

   Objective: To confirm the effectiveness of NexGard® PLUS when administered once for the treatment and control of induced infestations of adult *Amblyomma americanum* on dogs.

   Study Animals: Twenty (20) dogs (10 males and 10 females), 7.1 to 8.6 months of age and weighing 5.84 to 8.10 kg, were included in the study.

Experimental Design: The study followed a randomized block design based on pre-treatment tick counts. Pre-treatment tick counts were used to form 10 blocks of two dogs. Within blocks, each dog was randomly allocated to one of the treatment groups. Dogs were randomly allocated to cages within a single room. The study was conducted in accordance with GCP guidelines.

**Table II.9. Study PR&D 0424001 Treatment Groups**

| Treatment | Dosage | Day of Treatment | Number of Dogs | Days of Tick Infestation | Days of Tick Count |
|---|---|---|---|---|---|
| Control (sham-dosed) | Not Applicable | Day 0 | 10 | -1, 7, 14, 21, and 28 | 3, 10, 17, 24, and 31 |
| NexGard® PLUS | 2.5 mg/kg afoxolaner + 12 mcg/kg moxidectin + 5 mg/kg pyrantel | Day 0 | 10 | -1, 7, 14, 21, and 28 | 3, 10, 17, 24, and 31 |

Drug Administration: Dogs were fed prior to treatment. All treatments were administered orally. Control dogs did not receive any test article.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *Amblyomma 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, 2, 3, 4, 5, 6, 8, 12, and 24 hours after treatment. General health observations were conducted at least once daily. Tick counts and health observations were conducted masked to treatment.

**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 calculated from the least squares mean of live tick counts for the control group and T = arithmetic mean calculated from the least squares 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 calculated from the least squares mean of dead tick counts for the control group and T = arithmetic mean calculated from the least squares mean of dead tick counts for the treated group.

Tick counts for treated and control dogs at each time point were compared using an F-test. The MIXED procedure in SAS was used for the analysis, with treatment group as a fixed effect, and allocation block as a random effect. 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 NexGard® PLUS-treated group had a 97% 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 31 days (Table II.10).

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

**Table II.10. *Amblyomma americanum* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness**

| Days of Tick Count | Control Group Live Tick Count (Arithmetic Mean) | NexGard® PLUS Group Live Tick Count (Arithmetic Mean) | Percent Effectiveness |
|---|---|---|---|
| 3 | 29.9 | 0.9 | 97.0% |
| 10 | 25.7 | 0.4 | 98.4% |
| 17 | 24.9 | 0.1 | 99.6% |
| 24 | 24.1 | 0.2 | 99.2% |
| 31 | 19.2 | 0.1 | 99.5% |

Dead tick counts for the NexGard® PLUS-treated group were significantly different ($P < 0.0001$) and numerically higher than the control group on all post-treatment count days.

**Table II.11. *Amblyomma americanum* Arithmetic Mean Dead Tick Count**

| Days of Tick Count | Control Group Dead Tick Count (Arithmetic Mean) | NexGard® PLUS Group Dead Tick Count (Arithmetic Mean) |
|---|---|---|
| 3 | 0.4 | 29.7 |
| 10 | 1.1 | 22.8 |
| 17 | 1.8 | 25.0 |
| 24 | 1.0 | 26.3 |
| 31 | 3.4 | 29.1 |

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

**Conclusions:** This study demonstrated the effectiveness of NexGard® PLUS for the control (reduced live ticks) and treatment (increased dead ticks) of *Amblyomma americanum* when assessed 72 hours after treatment of an existing infestation and 72 hours after weekly re-infestation for one month.

6. Laboratory Dose Confirmation and Non-Interference Study PR&D 0425701: Treatment and Control of Induced Infestations of *Amblyomma americanum*

**Title**: Efficacy of Moxidectin and Pyrantel Pamoate, With and Without Afoxolaner, Administered Once Orally in a Chewable Formulation Against Induced Infestations of Adult *Amblyomma americanum* on Dogs. (Study No. PR&D 0425701)

**Study Dates:** August 6, 2019 to September 11, 2020

**Study Location:** Greenbrier, AR

**Study Design:**

Objective: To confirm the effectiveness of NexGard® PLUS when administered once for the treatment and control of induced infestations of adult *Amblyomma americanum* on dogs. The effectiveness of a chewable tablet formulation of moxidectin and pyrantel pamoate, when administered once at a dose of at least 12 mcg/kg and 5 mg/kg, respectively, was also evaluated.

Study Animals: Thirty (30) dogs (11 males and 19 females), 20.6 to 94 months of age and weighing 7.2 to 12.7 kg, were included in the study.

Experimental Design: The study followed a randomized block design based on pre-treatment tick counts. Pre-treatment tick counts were used to form 10 blocks of three dogs. Within blocks, each dog was randomly allocated to one of the treatment groups. The blocks were randomly assigned to two different rooms for housing. The study was conducted in accordance with GCP guidelines.

**Table II.12. Study PR&D 0425701 Treatment Groups**

| Treatment | Dosage | Day of Treatment | Number of Dogs | Days of Tick Infestation | Days of Tick Count |
|---|---|---|---|---|---|
| Control (sham-dosed) | Not Applicable | Day 0 | 10 | -1, 7, 14, 21, and 28 | 3, 10, 17, 24, and 31 |

| Treatment | Dosage | Day of Treatment | Number of Dogs | Days of Tick Infestation | Days of Tick Count |
|---|---|---|---|---|---|
| NexGard® PLUS | 2.5 mg/kg afoxolaner + 12 mcg/kg moxidectin + 5 mg/kg pyrantel | Day 0 | 10 | -1, 7, 14, 21, and 28 | 3, 10, 17, 24, and 31 |
| Moxidectin and Pyrantel Pamoate | 12 mcg/kg moxidectin + 5 mg/kg pyrantel | Day 0 | 10 | -1, 7, 14, 21, and 28 | 3, 10, 17, 24, and 31 |

Drug Administration: Dogs were fed prior to treatment. All treatments were administered orally. Control dogs did not receive any test article.

Measurements and Observations: Each dog was infested with approximately 50 unfed adult *Amblyomma 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, 2, 3, 4, 5, 6, 8, 12, and 24 hours after treatment. General health observations were conducted at least once daily. Tick counts and health observations were conducted masked to treatment.

**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 calculated from the least squares mean of live tick counts for the control group and T = arithmetic mean calculated from the least squares 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 calculated from the least squares mean of dead tick counts for the control group and T = arithmetic mean calculated from the least squares mean of dead tick counts for the treated group.

Group comparison between the treated group and the control group (Group 2 vs. Group 1) only included data from Group 1 and Group 2. Group comparison between the treated group and the control group (Group 3 vs. Group 1) only included data from Group 1 and Group 3. Tick counts for treated and control dogs were compared using an F-test. The MIXED procedure in SAS was used for the analyses, with treatment group as a fixed effect and room and block-within-room as random effects, at each time point for live tick counts and dead

tick counts separately. The counts of *Amblyomma americanum* in the moxidectin + pyrantel pamoate group vs. the control group were not significantly different (P = 0.1789). Testing was two-sided at the 5% significance level for the comparison of Group 2 vs. Group1. For the comparison of Group 3 vs. Group 1, the MIXED model was performed to obtain the least squares means, but inferential testing was not performed.

**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 3, there were no live ticks on any NexGard® PLUS-treated dogs. The NexGard® PLUS-treated group had a 100% reduction in live tick counts 72 hours after treatment of the existing infestation, and ≥ 98.9% reduction in live tick counts 72 hours after weekly re-infestations for 31 days (Table II.13).

The moxidectin and pyrantel pamoate group did not demonstrate effectiveness, with 0 percent effectiveness at all time points.

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

**Table II.13. *Amblyomma americanum* Live Tick Effectiveness; Arithmetic Mean Live Tick Count and Percent Effectiveness**

| Days of Tick Count | Control Group Live Tick Count (Arithmetic Mean) | NexGard® PLUS Group Live Tick Count (Arithmetic Mean) | Percent Effectiveness | Moxidectin, Pyrantel Pamoate Group Live Tick Count (Arithmetic Mean) | Percent Effectiveness |
|---|---|---|---|---|---|
| 3 | 40.5 | 0.0 | 100% | 42.2 | 0% |
| 10 | 36.6 | 0.0 | 100% | 40.8 | 0% |
| 17 | 28.2 | 0.0 | 100% | 35.2 | 0% |
| 24 | 24.6 | 0.0 | 100% | 32.4 | 0% |
| 31 | 27.5 | 0.3 | 98.9% | 32.3 | 0% |

Dead tick counts for the NexGard® PLUS-treated group were significantly different (P < 0.0001) and numerically higher than the control group on all post-treatment count days.

**Table II.14. *Amblyomma americanum* Arithmetic Mean Dead Tick Count**

| Days of Tick Count | Control Group Dead Tick Count (Arithmetic Mean) | NexGard® PLUS Group Dead Tick Count (Arithmetic Mean) | Moxidectin, Pyrantel Pamoate Group Dead Tick Count (Arithmetic Mean) |
|---|---|---|---|
| 3 | 0.4 | 31.9 | 0.2 |
| 10 | 0.8 | 34.2 | 0.3 |
| 17 | 1.0 | 28.9 | 1.3 |
| 24 | 0.5 | 27.1 | 0.6 |
| 31 | 1.2 | 30.8 | 1.2 |

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

**Conclusions:**

This study demonstrated the effectiveness of NexGard® PLUS for the control (reduced live ticks) and treatment (increased dead ticks) of *Amblyomma americanum* when assessed 72 hours after treatment of an existing infestation and 72 hours after weekly re-infestation for one month.

The test article containing moxidectin and pyrantel pamoate was not effective against induced *Amblyomma americanum* infestations in dogs, justifying the need for afoxolaner in the combination.

***For the Treatment and Control of Roundworm and Hookworm Infections***

Studies to confirm the effective dose of pyrantel pamoate for the treatment and control of adult roundworm (*Toxocara canis* and *Toxascaris leonina*) and adult hookworm (*Ancylostoma caninum*, *Ancylostoma braziliense*, and *Uncinaria stenocephala*) were conducted with Heartgard® PLUS (ivermectin/pyrantel) [Refer to the Freedom of Information Summary for NADA 140-971 dated October 3, 1996]. Therefore, only two laboratory studies were conducted against the dose-limiting parasite for pyrantel pamoate *(Toxocara canis)* to demonstrate effectiveness of NexGard® PLUS against both adult roundworms and hookworms.

7. Laboratory Dose Confirmation and Non-Interference Study PR&D 0410401: Treatment and Control of Induced Infections of *Toxocara canis*

   **Title:** Efficacy of Afoxolaner + Moxidectin, With and Without Pyrantel Pamoate, Administered Orally in a Chewable Formulation to Dogs Against Induced Infections of *Toxocara canis.* (Study No. PR&D 0410401)

   **Study Dates:** February 28, 2019 to March 23, 2020

   **Study Location:** Stanwood, MI

**Study Design:**

Objective: To confirm the effectiveness NexGard® PLUS when administered once against induced infections of adult *Toxocara canis* in dogs. The effectiveness of a chewable tablet formulation of afoxolaner + moxidectin, when administered once at a dose of at least 2.5 mg/kg + 12 mcg/kg, respectively, was also evaluated.

Study Animals: Thirty dogs (16 males and 14 females), approximately 8.6 to 9.3 weeks of age and weighing 3.1 to 7.3 kg, were included in the study.

Experimental Design: Dogs were ranked based on decreasing Day -6 body weights and allocated to 10 blocks of 3 animals each. Within blocks, dogs were randomly allocated to one of the three treatment groups. The study was conducted in accordance with GCP guidelines.

**Table II.15. Study PR&D 0410401 Treatment Groups**

| Treatment | Dosage | Treatment Day | Number of Dogs |
|---|---|---|---|
| NexGard® PLUS | 2.5 mg/kg afoxolaner + 12 mcg/kg moxidectin + 5 mg/kg pyrantel | 0 | 10 |
| Afoxolaner + Moxidectin | 2.5 mg/kg afoxolaner + 12 mcg/kg moxidectin | 0 | 10 |
| Control (not treated) | Not Applicable | Not Applicable | 10 |

Drug Administration: Dogs were fed prior to treatment. All treatments were administered orally. Control dogs did not receive any treatment.

Measurements and Observations: Fecal samples collected on Day -61 were examined microscopically and the absence of patent nematode infection was confirmed in all dogs. Physical examinations were conducted on Day -59 and Day -6. Each dog was inoculated orally with approximately 300 larvated *Toxocara canis* eggs divided over three days on Days -58, -57, and -56. On Day -5, fecal samples were examined microscopically to confirm the presence of *Toxocara canis* eggs in all dogs. Dogs were treated on Day 0 based on body weights collected on Day -6. General health observations were conducted at least once daily for all dogs. Post-dosing clinical observations were conducted 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after treatment for all dogs. On Day 7, all dogs were humanely euthanized and necropsied for parasite recovery, speciation, and enumeration.

**Statistical Methods:** Parasite counts were transformed to the natural logarithm of (count + 1). The MIXED procedure was used for the analysis of the log-counts with the treatment groups listed as a fixed effect, and the allocation blocks listed as a random effect. Comparisons were tested at a two-sided $\alpha = 0.05$. The percent effectiveness was calculated as 100[(C - T)/C], where C and T were the geometric means obtained by the back-transformed least squares means, respectively, of the control group and each treated group as obtained from the model.

**Results:** Eight of 10 control dogs harbored adult *Toxocara canis* at necropsy, with each of those eight dogs harboring five or more adult worms. NexGard® PLUS was 95.2% effective against *Toxocara canis* in this study. The mean worm count of *Toxocara canis* for dogs treated with NexGard® PLUS was less than the mean worm count of *Toxocara canis* for the control dogs and a statistically significant difference (P = 0.0015) was observed between the mean worm count for dogs treated with NexGard® PLUS and the mean worm count of the control dogs. The afoxolaner + moxidectin group did not demonstrate effectiveness, with 53.2 percent effectiveness against *Toxocara canis* in this study. The counts of adult *Toxocara canis* in the afoxolaner + moxidectin group versus the control group were not significantly different (P = 0.1789).

**Table II.16. Study PR&D 0410401 Percent Effectiveness**

| Treatment | *Toxocara canis* Count (Geometric Mean) | Percent Effectiveness |
|---|---|---|
| NexGard® PLUS | 0.3 | 95.2% |
| Afoxolaner + Moxidectin | 3.1 | 53.2% |
| Control | 6.7 | Not Applicable |

**Adverse Reactions:** There were no treatment-related adverse events during this study.

**Conclusions:** NexGard® PLUS was effective in the treatment and control of induced *Toxocara canis* infections in dogs. A chewable containing afoxolaner and moxidectin was not effective against induced *Toxocara canis* infections in dogs, justifying the need for pyrantel pamoate in the combination.

8. Laboratory Dose Confirmation Study PR&D 0426601: Treatment and Control of Natural Infections of *Toxocara canis*

**Title:** Efficacy of Afoxolaner + Moxidectin + Pyrantel Pamoate, Administered Orally in a Chewable Formulation to Dogs Against Naturally Acquired Infection with *Toxocara canis*. (Study No. PR&D 0426601)

**Study Dates:** June 28, 2019 to March 12, 2020

**Study Location:** Tirana, Albania

**Study Design:**

Objective: To confirm the effectiveness of NexGard® PLUS when administered once in dogs naturally infected with adult *Toxocara canis*.

Study Animals: Twenty dogs (10 males and 10 females), approximately 3 months to 2 years of age and weighing 5.0 to 11.4 kg, were included in the study.

Experimental Design: Dogs were ranked based on decreasing Day -3 body weights and allocated to 10 blocks of 2 animals each. Within blocks, dogs were randomly allocated to one of the two treatment groups. The study was conducted in accordance with GCP guidelines.

**Table II.17. Study PR&D 0426601 Treatment Groups**

| Treatment | Dosage | Treatment Day | Number of Dogs |
|---|---|---|---|
| NexGard® PLUS | 2.5 mg/kg afoxolaner + 12 mcg/kg moxidectin + 5 mg/kg pyrantel | 0 | 10 |
| Control (sham-dosed) | Not Applicable | Not Applicable | 10 |

Drug Administration: Dogs were fed prior to treatment. All treatments were administered orally. Control dogs did not receive any test article.

Measurements and Observations: Physical examinations were conducted on Day -8. On Day -6, fecal samples were examined microscopically to confirm the presence of *Toxocara canis* eggs in all dogs. Dogs were treated on Day 0 based on body weights collected on Day -3. General health observations were conducted at least once daily for all dogs. Post-dosing clinical observations were conducted 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after treatment for all dogs. On Day 8, all dogs were humanely euthanized and necropsied for parasite recovery, speciation, and enumeration.

**Statistical Methods:** Parasite counts were transformed to the natural logarithm of (count + 1). The MIXED procedure was used for the analysis of the log-counts at a two-sided $\alpha = 0.05$. The percent effectiveness was calculated as 100[(C - T)/C], where C and T were the geometric means obtained by the back-transformed least squares means, respectively, of the control group and treated group as obtained from the model.

**Results:** All 10 control dogs harbored adult *Toxocara canis* at necropsy, with 6 of the 10 dogs harboring 5 or more adult worms. NexGard® PLUS was 98.4% effective against *Toxocara canis* in this study. The mean worm count of *Toxocara*

*canis* for the treated dogs was less than the mean worm count of *Toxocara canis* for the control dogs and a statistically significant difference ($P < 0.0001$) was observed between the mean worm count for the treated dogs and the mean worm count of the control dogs.

**Table II.18. Study PR&D 0426601 Percent Effectiveness**

| Treatment | *Toxocara canis* Count (Geometric Mean) | Percent Effectiveness |
|---|---|---|
| NexGard® PLUS | 0.07 | 98.4% |
| Control | 4.48 | Not Applicable |

**Adverse Reactions:** There were no treatment-related adverse events during this study.

**Conclusions:** NexGard® PLUS was effective in the treatment and control of naturally acquired *Toxocara canis* infections in dogs.

## III. TARGET ANIMAL SAFETY

### A. Margin of Safety Study in Eight-Week-Old Dogs Study PR&D 0416301

**Title:** Safety of a Combination of Afoxolaner + Moxidectin + Pyrantel Pamoate When Administered Orally in a Chewable Formulation at 1, 3, and 5X the Maximum Exposure Dose in Eight-Week-Old Puppies Treated Every 28 Days for Six Treatments. (Study No. PR&D 0416301)

**Study Dates:** June 24, 2019 to April 16, 2021

**Study Location:** Fulton, MO

**Study Design:**

Objective: To determine the safety profile of NexGard® PLUS when administered orally six times, 28 days apart, at 1, 3, and 5X the maximum exposure doses to eight-week-old puppies.

Study Animals: Thirty-two (32) Beagle dogs (16 males, 16 females), aged 8 to 9 weeks and weighing 1.95 to 3.00 kg, were included in the study.

Experimental Design: This 168-day margin of safety study was conducted in accordance with Good Laboratory Practice (GLP) Regulations (21 CFR Part 58).

**Table III.1. Study PR&D 0416301 Treatment Groups**

| Dose Multiple | Treatment | Treatment Days | Afoxolaner (mg/kg) | Moxidectin (mcg/kg) | Pyrantel Pamoate (mg/kg) | Number of Dogs |
|---|---|---|---|---|---|---|
| 0X | Control (sham-dosed) | 0, 28, 56, 84, 112, 140 | Not Applicable | Not Applicable | Not Applicable | 8 |
| 1X | NexGard® PLUS | 0, 28, 56, 84, 112, 140 | 5 | 24 | 10 | 8 |
| 3X | NexGard® PLUS | 0, 28, 56, 84, 112, 140 | 15 | 72 | 30 | 8 |
| 5X | NexGard® PLUS | 0, 28, 56, 84, 112, 140 | 25 | 120 | 50 | 8 |

Drug Administration: All treatments were administered to dogs in the fasted state. On each treatment day, control dogs were sham-dosed and dogs in the 1X, 3X, and 5X groups were administered their calculated doses orally.

Measurements and Observations: General health observations were conducted twice daily throughout the study. Food consumption was recorded twice daily and reported weekly. Puppies were observed at 1, 2, 3, 4, 5, 6, and 9 hours after each treatment. Observations for avermectin toxicity were also performed 4 hours after each treatment. Physical examinations, including body weights and observations for avermectin toxicity, were performed on Days -14 (physical exams only), -4, 1, 27, 29, 55, 57, 83, 85, 111, 113, 139, 141, and 167. Ophthalmic examinations were conducted on Days -8 and 163. Blood was collected for clinical pathology evaluation (hematology, coagulation, and plasma chemistry) on Days -4, 27, 55, 83, 111, 139, and 167. Urine was collected on Days -5 and 168. A complete necropsy with organ weights and microscopic examination was completed on Day 168.

**Statistical Methods:** Organ weights were analyzed using analysis of variance (ANOVA); the statistical model included treatment, sex, and the interaction term “treatment by sex” as fixed effects and cohort and block within cohort by sex as random effects. Food consumption was analyzed using repeated measures analysis of variance (RMANOVA). Continuous clinical pathology values, respiration rate, temperature, heart rate, and body weight were analyzed using repeated measures analysis of covariance (RMANCOVA). Both models for RMANOVA and RMANCOVA included treatment, sampling day, sex, the interaction terms “treatment by sex,” “treatment by sampling day,” “sex by sampling day,” and “treatment by sex by sampling day” as fixed effects and cohort and “block within cohort by sex” as random effects. The covariate was the most recent baseline measurement. All fixed model effects were tested at a significance level of $\alpha = 0.10$ except the three-way treatment-by-sex-by-time interaction, which was tested at $\alpha = 0.05$. Pairwise mean comparisons between each treatment group and the control group were performed using a significance level of $\alpha = 0.10$. Incidence of specific abnormalities was compared between treatment groups and the control group using the Fisher’s exact test at a significance level of $\alpha = 0.10$.

**Results:**

There was no evidence of test article-related alterations in food consumption, body weight, physical examination variables (heart rate, respiratory rate, body temperature), ophthalmic examination variables, and anatomical or clinical pathology findings. No signs of avermectin sensitivity were observed. No serious health abnormalities related to the administration of NexGard® PLUS were observed.

Vomiting was observed sporadically across all groups, including the control group, and did not appear to be related to treatment. Mild, self-limiting diarrhea (with and without blood) was observed sporadically and was possibly related to treatment, as there were more incidences in the groups administered NexGard® PLUS than the control group throughout the study, including within 48 hours after treatment.

**Conclusion:** The study supports the safe use of NexGard® PLUS in dogs when dosed monthly at the labeled dose.

**B. Safety Study in Heartworm Positive Dogs Study PR&D 0430201**

**Title:** A Study to Evaluate the Safety of a Combination of Afoxolaner + Moxidectin + Pyrantel Pamoate When Administered Orally in a Chewable Formulation at 1X and 3X the Maximum Exposure Dose of 5 mg/kg, 24 mcg/kg, and 10 mg/kg, Respectively, to Heartworm Positive Dogs. (Study No. PR&D 0430201)

**Study Dates:** September 5, 2019 to March 23, 2021

**Study Location:** Athens, GA

**Study Design:**

Objective: To evaluate the safety of NexGard® PLUS when administered orally three times, approximately 28 days apart, at 1X and 3X the maximum exposure doses to dogs infected with *Dirofilaria immitis*.

Study Animals: Twenty-four (24) healthy Beagle dogs (14 males, 10 females), aged 6.4 to 7.4 months and weighing 6.44 to 12.68 kg, were included in the study.

Experimental Design: Twenty-four dogs, surgically implanted with *Dirofilaria immitis* were used in this study. Blood collected for testing on Day -4 was confirmed positive for heartworm antigen, and all dogs had at least 1000 microfilariae/mL. Microfilariae and heartworm antigen testing were repeated on samples collected on Days 7, 28, and 56. This study was conducted in accordance with GLP regulations (21 CFR Part 58).

**Table III.2. Study PR&D 0430201 Treatment Groups**

| Dose Multiple | Treatment | Treatment Days | Afoxolaner (mg/kg) | Moxidectin (mcg/kg) | Pyrantel Pamoate (mg/kg) | Number of Dogs |
|---|---|---|---|---|---|---|
| 0X | Control (sham-dosed) | 0, 29, 57 | Not Applicable | Not Applicable | Not Applicable | 8 |
| 1X | NexGard® PLUS | 0, 29, 57 | 5 | 24 | 10 | 8 |
| 3X | NexGard® PLUS | 0, 29, 57 | 15 | 72 | 30 | 8 |

Drug Administration: All treatments were administered to dogs in the fasted state. On Days 0, 29, and 57, control dogs were sham-dosed and dogs in the 1X and 3X groups were administered their calculated doses orally.

Measurements and Observations: All dogs were observed for general health at least once daily following heartworm transplantation and at least twice daily beginning on Day 0. Dogs were observed at approximately 1, 2, 3, 4, 6, 8, 12, and 24 hours after each treatment. Clinical observations for signs of avermectin toxicity were conducted prior to each treatment and at approximately 4 hours post-treatment. Complete physical examinations were conducted prior to each treatment, at approximately 8 hours post-treatment, and on Day 84. Blood was collected for hematology and serum biochemistry profiles at baseline (Day -7) and on Day 84. A necropsy with examination for adult *Dirofilaria immitis* recovery and enumeration was performed on Day 85.

**Statistical Methods:** Heartworm counts were transformed to the natural logarithm of (count + 1) for the comparison of the 1X or 3X treatment group against the control at $\alpha = 0.10$ using ANOVA with treatment as a fixed effect. Microfilariae counts on Days 7, 28, and 56 were transformed to the natural logarithm of (count + 1) for the comparison of the 1X or 3X treatment group against the control at each time point separately at $\alpha = 0.10$ using analysis of covariance (ANCOVA) with treatment as a fixed effect and pre-treatment microfilariae counts as a covariate. Continuous clinical pathology variables were analyzed using ANCOVA with treatment as a fixed effect and baseline measurements of each variable and pre-treatment microfilariae counts as covariates. Physical exam variables were analyzed using RMANCOVA, including treatment, sampling day, and the interaction term “treatment by sampling day” as fixed effects. For each variable, pre-treatment microfilariae counts were included as a covariate and, where applicable, the most recent baseline physical exam measurement was included as covariate. All fixed model effects were tested at a significance level of $\alpha = 0.10$ except the three-way treatment-by-sex-by-time interaction, which was tested at $\alpha = 0.05$.

**Results:**

Mild, self-limiting diarrhea was observed in one dog in the 1X group and in three dogs in the 3X group within 8 hours of treatment. Vomiting was observed in two dogs in the 3X group within 4 hours of treatment and resolved without the need for

medication. No signs of avermectin toxicity were observed at any time during the study, and there were no other treatment-related health abnormalities.

There were no clinically relevant, treatment-related effects on clinical pathology parameters (hematology and serum chemistry).

At all time points (Days 7, 28, and 56) evaluated, microfilariae counts in the 1X and 3X groups were significantly different ($p \leq 0.0028$) and numerically lower than the control group. There were no hypersensitivity reactions observed and no abnormalities noted on the physical examinations performed 8 hours after each treatment. All dogs had adult heartworms at necropsy. Additionally, fragments of worms were found in one control dog, five 1X dogs, and three 3X dogs, and dead worms were recovered in one 1X dog. Live worm recoveries were 90.6%, 80.0%, and 83.3% in the control, 1X, and 3X groups, respectively.

**Conclusion:** NexGard® PLUS, administered orally three times at approximately 28-day intervals, at 1X and 3X the maximum exposure doses, was well tolerated in dogs with pre-existing adult heartworm infections and circulating microfilariae.

**C. Safety Study in Avermectin-Sensitive Collie Dogs Study PR&D 0423901**

**Title**: Safety Evaluation of a Combination of Afoxolaner + Moxidectin + Pyrantel Pamoate When Administered Orally in a Chewable Formulation at 1, 3, and 5X the Maximum Exposure Dose of 5 mg/kg, 24 mcg/kg, and 10 mg/kg, Respectively, to Collie Dogs Known to Be MDR1 Deficient. (Study No. PR&D 0423901)

**Study Dates:** February 11, 2020 to February 23, 2021

**Study Location:** Fulton, MO

**Study Design:**

Objective: To evaluate the safety of NexGard® PLUS when administered orally at 1, 3, and 5X the maximum exposure doses to Collie dogs known to be MDR1 deficient. The study included a second administration to the sham-dosed control and 1X groups on Day 28.

Study Animals: Twenty-four (24) healthy MDR1-deficient Collie dogs (19 males, 5 females), aged 3.13 to 10.17 years and weighing 20.45 to 47.30 kg, were included in the study. Dogs were confirmed to be MDR1 deficient (mutant/mutant) and sensitive to ivermectin at ≤ 120 mcg/kg.

Experimental Design: This 43-day safety study was conducted in accordance with GLP regulations (21 CFR Part 58).

**Table III.3. Study PR&D 0423901 Treatment Groups**

| Dose Multiple | Treatment | Treatment Day(s) | Afoxolaner (mg/kg) | Moxidectin (mcg/kg) | Pyrantel Pamoate (mg/kg) | Number of Dogs |
|---|---|---|---|---|---|---|
| 0X | Control (sham-dosed) | 0, 28 | Not Applicable | Not Applicable | Not Applicable | 6 |
| 1X | NexGard® PLUS | 0, 28 | 5 | 24 | 10 | 6 |
| 3X | NexGard® PLUS | 0 | 15 | 72 | 30 | 6 |
| 5X | NexGard® PLUS | 0 | 25 | 120 | 50 | 6 |

Drug Administration: All treatments were administered to dogs in the fasted state. On Day 0, control dogs were sham-dosed and dogs in the 1X, 3X, and 5X groups were administered their calculated doses orally. On Day 28, control dogs were sham-dosed and dogs in the 1X group were administered their calculated doses orally.

Measurements and Observations: All dogs were observed for general health at least twice daily throughout the study. On Days 0 and 28, health observations were conducted at approximately 1 and 3 hours post-treatment. Health observations and clinical observations for signs of avermectin toxicity were conducted at approximately 2, 4, 6, 8, 12, 18, and 24 hours post-treatment, as well as on Days 1, 2, 3, 4, 29, 30, 31, and 32. The study ended on Day 43.

**Statistical Methods:** No statistical analysis was performed.

**Results:** No clinical signs of avermectin toxicity were noted in any dog at any time during the study. Vomiting was observed in the 3X and 5X groups within 8 hours of treatment and resolved without intervention. Diarrhea, with or without blood, was observed in some dogs in all of the NexGard® PLUS groups within 24 hours of treatment and was self-limiting.

**Conclusion:** NexGard® PLUS, administered orally at 1X, 3X, and 5X the maximum exposure doses, was well tolerated in MDR1-deficient, avermectin-sensitive Collie dogs.

## IV. HUMAN FOOD SAFETY

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

## V. USER SAFETY

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

Not for use in humans. Keep this and all drugs out of the reach of children. In case of accidental ingestion, contact a physician for treatment advice.

## VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that NexGard® PLUS, when used according to the label, is safe and effective for the prevention of heartworm disease caused by *Dirofilaria immitis.* NexGard® PLUS is indicated for the treatment and control of adult hookworm (*Ancylostoma caninum, Ancylostoma braziliense*, and *Uncinaria stenocephala*) and roundworm (*Toxocara canis* and *Toxascaris leonina*) infections. NexGard® PLUS kills adult fleas and is indicated for the treatment and prevention of flea infestations (*Ctenocephalides felis*) and the treatment and control of *Ixodes scapularis* (black-legged tick), *Rhipicephalus sanguineus* (brown dog tick), *Dermacentor variabilis* (American dog tick) and *Amblyomma americanum* (lone star tick) infestations for one month in dogs and puppies eight weeks of age and older, weighing four pounds of body weight 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

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

### C. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.