Date of Approval: April 20, 2023

CORRECTED FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-570

NexGard® COMBO

(esafoxolaner, eprinomectin, and praziquantel topical solution)

Cats

NexGard® COMBO is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (fourth stage larval and adult *Toxocara cati*), hookworm (fourth stage larval and adult *Ancylostoma tubaeforme;* adult *Ancylostoma braziliense*), and tapeworm (*Dipylidium caninum*) infections. NexGard® COMBO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations and the treatment and control of *Ixodes scapularis* (black-legged tick) and *Amblyomma americanum* (lone star tick) infestations for one month in cats and kittens 8 weeks of age and older, and weighing 1.8 lbs or greater.

Sponsored by:

Boehringer Ingelheim Animal Health USA, Inc.

Executive Summary

NexGard® COMBO (esafoxolaner, eprinomectin, and praziquantel topical solution) is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (fourth stage larval and adult *Toxocara cati*), hookworm (fourth stage larval and adult *Ancylostoma tubaeforme*; adult *Ancylostoma braziliense*), and tapeworm (*Dipylidium caninum*) infections. NexGard® COMBO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations and the treatment and control of *Ixodes scapularis* (black-legged tick) and *Amblyomma americanum* (lone star tick) infestations for one month in cats and kittens 8 weeks of age and older, and weighing 1.8 lbs or greater.

NexGard® COMBO is an antiparasitic drug with three active ingredients and is applied to cats topically once a month.

Safety and Effectiveness

The sponsor conducted 16 laboratory studies and one clinical field study in a total of 186 laboratory and 244 client-owned cats to demonstrate the effectiveness of NexGard® COMBO against a variety of internal and external parasites. The 17 studies are as follows:

- Five laboratory studies showing that NexGard® COMBO is effective at preventing heartworm disease caused by *D. immitis*.
- One clinical field study in client-owned cats and one laboratory study showing that the drug is effective at killing adult fleas (*C. felis*) and treating and preventing flea infestations.
- Five laboratory studies showing that the drug is effective at treating and controlling *I. scapularis* and *A. americanum* tick infestations.
- Two laboratory studies showing that the drug is effective at treating and controlling adult roundworms (*T. cati*).
- Three laboratory studies showing that the drug is effective at treating and controlling tapeworms (*D. caninum*).

The most common adverse reactions from the clinical field study were emesis, hair changes to the application site, anorexia, lethargy, and hypersalivation.

The sponsor conducted a margin of safety study and an oral tolerance study to demonstrate the safety of NexGard® COMBO in cats. In the margin of safety study, one kitten receiving NexGard® COMBO at five times the labeled dose exhibited ataxia, dorsal recumbency, hypothermia (95.1°F), disorientation, tremors, lethargy, hypersalivation, and pupil dilation with slight response to light. This kitten received supportive care and appeared to be fully recovered within 48 hours. No other treatment-related effects were seen in any of the cats in the study. There were no treatment-related changes in body weight, food consumption, water consumption, clinical pathology parameters (hematology, serum chemistry, coagulation, and urinalysis), or organ weights at any dose level.

The oral tolerance study evaluated the effects of accidental oral ingestion in cats, such as from licking or grooming the application site. Orally administering NexGard® COMBO to cats caused them to salivate profusely immediately after dosing. The hypersalivation resolved by one-hour post-dosing. Correct application of NexGard® COMBO topically and in the proper location on the cat will minimize the chance of accidental oral ingestion of the drug and subsequent hypersalivation.

The sponsor established the safety of NexGard® COMBO in cats infected with adult heartworms using the safety study described in the FOI Summary for the original approval of Centragard® (eprinomectin and praziquantel transdermal solution). Eprinomectin is the active ingredient that is effective against *D. immitis*, and the concentration of eprinomectin (0.4% w/v) in NexGard® COMBO is identical to the concentration of eprinomectin in Centragard®.

User Safety
Users should avoid direct contact with a cat's application site for 4 hours or until the site is visibly dry. NexGard® COMBO may be a mild to moderate eye irritant.

Conclusions
Based on the data submitted by the sponsor for the approval of NexGard® COMBO, the Food and Drug Administration (FDA) determined that the drug is safe and effective when used according to the labeling.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-570

B. Sponsor

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

Drug Labeler Code: 000010

C. Proprietary Name

NexGard® COMBO

D. Drug Product Established Name

esafoxolaner, eprinomectin, and praziquantel topical solution

E. Pharmacological Category

Antiparasitic

F. Dosage Form

Topical solution

G. Amount of Active Ingredient

Esafoxolaner – 12 mg/mL
Eprinomectin – 4 mg/mL
Praziquantel – 83 mg/mL

H. How Supplied

0.3 mL and 0.9 mL unit applicator

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

NexGard® COMBO is dosed at a minimum of 0.055 mL/lb (0.12 mL/kg), which delivers 0.65 mg/lb (1.44 mg/kg) esafoxolaner, 0.22 mg/lb (0.48 mg/kg) eprinomectin and 4.53 mg/lb (9.98 mg/kg) praziquantel. Administer the entire contents of NexGard® COMBO unit applicator topically once a month as specified in the following table:

Cat Weight (lb)	Volume (mL)	Esafoxolaner (mg)	Eprinomectin (mg)	Praziquantel (mg)
1.8 – 5.5	0.3	3.6	1.2	24.9
5.6 – 16.5	0.9	10.8	3.6	74.7
16.6 – 22.0	0.3 + 0.9	14.4	4.8	99.6
22.1 – 33.0	0.9 + 0.9	21.6	7.2	149.4

K. Route of Administration

Topical

L. Species

Cats

M. Indication

NexGard® COMBO is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (fourth stage larval and adult *Toxocara cati*), hookworm (fourth stage larval and adult *Ancylostoma tubaeforme*; adult *Ancylostoma braziliense*), and tapeworm (*Dipylidium caninum*) infections. NexGard® COMBO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations and the treatment and control of *Ixodes scapularis* (black-legged tick) and *Amblyomma americanum* (lone star tick) infestations for one month in cats and kittens 8 weeks of age and older, and weighing 1.8 lbs or greater.

II. EFFECTIVENESS

The effectiveness of NexGard® COMBO was demonstrated in 16 well-controlled laboratory studies and one clinical field safety and effectiveness study described below. These studies demonstrate that NexGard® COMBO is effective against a variety of both internal and external parasites. NexGard® COMBO was administered to 186 laboratory and 244 client-owned cats as part of the demonstration of effectiveness. The most common adverse reactions from the clinical field safety and effectiveness study were emesis, application site hair change, anorexia, lethargy, and hypersalivation.

A. Dosage Characterization

The minimum dose of esafoxolaner (1.4 mg/kg) was selected based on laboratory effectiveness studies. The effectiveness of early formulations containing 0.8% w/v esafoxolaner, 0.4% w/v eprinomectin, and 8.3% w/v praziquantel was tested on experimental infestations of *I. scapularis* and showed sub-optimal duration of

effectiveness at a dose of 1.0 mg/kg. The effectiveness of 1.4 mg/kg and 0.7 mg/kg was then tested with the final formulation, containing 1.2% w/v esafoxolaner, 0.4% w/v eprinomectin, and 8.3% w/v praziquantel in two dose determination studies, on experimental infestations of *Ixodes ricinus* and *Otodectes cynotis* respectively. The studies showed that the 1.4 mg/kg dose was effective against both parasites, while the lower dose of 0.7 mg/kg was not effective against both parasites.

The minimum dose of eprinomectin (0.51 mg/kg) and praziquantel (10.0 mg/kg) was selected based on laboratory effectiveness studies. The studies were conducted to evaluate the lowest effective dose of each active ingredient. Five studies evaluated doses of eprinomectin over a dose range of 0.1 to 1.0 mg/kg against experimental or natural infections of *T. cati* and/or *Ancylostoma tubaeforme* in cats. Two studies evaluated doses of praziquantel over a dose range of 6.0 to 10.0 mg/kg against natural infections of *D. caninum* in cats. These studies demonstrated that 0.5 mg/kg eprinomectin and 10 mg/kg praziquantel were the lowest dose that was consistently effective against gastrointestinal nematodes and tapeworms, respectively.

B. Substantial Evidence

1. For the prevention of heartworm disease caused by *D. immitis*

   Five studies were performed to demonstrate substantial evidence of effectiveness of NexGard® COMBO against two heartworm isolates for the prevention of heartworm disease in cats. For these studies, a minimum of two adult heartworms was considered to be an adequate infection and a minimum of six adequately infected control cats was required for the study to be considered valid.

   Study PR&D 0420601 demonstrated adequacy of infection and 100% effectiveness against one recent heartworm isolate. Four studies (PR&D 0373801, 0373803, 0373804, 0420401) were conducted using a second heartworm isolate. However, none of the individual studies using the second isolate met the requirement for adequacy of infection. When the four studies were evaluated together, the analysis demonstrated that NexGard® COMBO is effective in the prevention of heartworm disease when NexGard® COMBO is administered once a month for three consecutive months.

   a. **Title:** A Study to Determine the Efficacy of a Single Topical Treatment with NexGard® COMBO to Prevent Heartworm Disease in Cats. (Study No. PR&D 0420601)

      Study Dates: November 9, 2018 to September 28, 2019.

      Study Location: Waverly, New York

      Study Design:

      Objective: To confirm the effectiveness of NexGard® COMBO to prevent heartworm disease in cats when applied once topically at the recommended minimum dose, 30 days after infection with *D. immitis* L_3

stage larvae.

Study Animals: Twenty cats (10 males and 10 females), 6.1 to 6.4 months of age and weighing 2.4 to 5.5 kg, were randomized into 10 cats per treatment group stratified by sex.

Experimental Design: This study was conducted in accordance with Good Clinical Practice (GCP) guidelines. Cats were inoculated with third-stage (L_3) *D. immitis* larvae on Day -30. Cats were administered mineral oil or NexGard® COMBO once on Day 0.

Treatment Groups:

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg mineral oil. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. On Day 150, the animals were humanely euthanized and a necropsy was performed for heartworm recovery and enumeration.

Statistical Methods:

The logarithm of the (counts + 1) was analyzed using the Mixed procedure in SAS 9.4 with treatment group, sex, and sex-by-treatment as fixed effects.

Effectiveness was determined based on the percent reduction in worm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
Where c_c = Geometric mean number of worm counts in the control group
c_t = Geometric mean number of worm counts in the treated group

Results: There was 100% prevention of development of *D. immitis* in the group of cats administered NexGard® COMBO. Eight of the 10 control cats were infected with at least two worms; therefore, the study was considered to satisfy the adequacy of infection requirement for validation purpose.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with NexGard® COMBO was 100% effective in the prevention of *D. immitis* infections in cats.

b. **Title:** Study to Determine the Efficacy of a Single or Multiple Topical Treatments with NexGard® COMBO to Prevent Heartworm Disease in Cats. (A Combined Analysis of Study No. PR&D 0373801, 0373803, 0373804, and 0420401)

Study Dates and Locations:

Study 1 (PR&D 0373801): April 28, 2017 to November 29, 2018, Athens, GA
Study 2 (PR&D 0373803): December 12, 2017 to January 29, 2019, Athens, GA
Study 3 (PR&D 0373804): July 20, 2018 to September 26, 2019, Athens, GA
Study 4 (PR&D 0420401): October 10, 2018 to September 30, 2019, Stanwood, MI

Study Designs:

Objective: A combined analysis of four studies to confirm the effectiveness of NexGard® COMBO to prevent heartworm disease in cats when applied once (all studies) or three times at monthly intervals (Studies 1, 2, and 3) topically at the recommended minimum dose, starting 30 days after infection with *D. immitis* L_3 stage larvae. All studies used the same laboratory isolate of *D. immitis.* The studies were conducted in accordance with GCP guidelines.

Study Animals:

Study 1: Thirty cats (15 males and 15 females), 3.8 to 4.5 months of age and weighing 1.9 to 3.4 kg.
Study 2: Thirty cats (15 males and 15 females), 3.5 to 3.7 months of age and weighing 2.0 to 3.0 kg.
Study 3: Thirty cats (15 males and 15 females), 3.3 to 3.8 months of age and weighing 1.8 to 3.0 kg.
Study 4: Twenty cats (9 males and 11 females), 8.0 to 8.7 months of age and weighing 2.6 to 5.4 kg.

Experimental Designs: The studies were conducted in accordance with GCP guidelines. Cats were inoculated with third-stage (L_3) *D. immitis* larvae on Day -30. Control cats were topically administered mineral oil on Day 0 or on Days 0, 30, and 60. Treated cats were topically administered NexGard® COMBO once on Day 0 or on Days 0, 30, and 60. Cats were randomized to the following treatment groups, 10 cats per treatment group:

Study 1, 2, and 3

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO; Mineral oil (control)
- Group 3: NexGard® COMBO

Study 4
- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

Drug Administration:

Studies 1, 2, and 3:
- Cats in Group 1 were topically administered 0.12 mL/kg mineral oil on Days 0, 30, and 60.
- Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel, once on Day 0 and were topically administered 0.12 mL/kg mineral oil on Days 30 and 60.
- Cats in Group 3 were topically administered 0.12 mL/kg NexGard® COMBO, delivering 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel, on Days 0, 30, and 60.

Study 4:
- Cats in Group 1 were topically administered 0.12 mL/kg mineral oil on Day 0.
- Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel, on Day 0.

A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. On Day 147 (Study 1), Day 150 (Study 2 and 3), or Day 149 (Study 4) the animals were humanely euthanized and a necropsy was performed for heartworm recovery and enumeration.

Results: There was 100% prevention of development of *D. immitis* in all groups of cats administered NexGard® COMBO. A minimum of two worms was considered to be an adequate infection and a minimum of six adequately infected control cats was required for the individual study to be considered valid. None of the studies met the adequacy of infection requirement (at least 6 cats in the control group infected with at least 2 worms), and therefore these studies were not evaluated individually for effectiveness.

Additional Effectiveness Evaluation:

An additional effectiveness assessment was performed analyzing the results from Studies 1–4. Table II.1 presents the observed heartworm counts. No heartworms were observed in the 70 animals administered NexGard® COMBO (from all 7 treated groups from the 4 studies included).

Table II.1. Heartworm counts from Studies 1–4.

Study	Number of Animals	Control Group Number of Heartworms Observed in Each Individual Animal (from low to high)	NexGard® COMBO Number of Heartworms Observed in Each Individual Animal (from low to high)
1	Control = 10 NexGard® COMBO = 20	0, 0, 0, 0, 0, 0, 1, 2, 4, 7	All 0
2	Control = 10 NexGard® COMBO = 20	0, 0, 0, 1, 1, 1, 3, 6, 6, 7	All 0
3	Control = 10 NexGard® COMBO = 20	0, 0, 0, 0, 0, 0, 0, 0, 1, 1	All 0
4	Control = 10 NexGard® COMBO = 20	0, 0, 0, 0, 0, 0, 1, 2, 4, 7	All 0

The results from these 4 studies were retrospectively assessed to examine what inference the studies, collectively, can provide towards the effectiveness of NexGard® COMBO. Because the studies did not individually demonstrate substantial evidence of effectiveness, the probabilities of observed outcomes under different levels of presumed effectiveness were examined.

The analysis was conducted using data from these 4 studies for all animals administered NexGard® COMBO, including those treated once and those treated monthly for three consecutive months. Therefore, this collective analysis only supports conclusions on the effectiveness of NexGard® COMBO when it is administered monthly for a minimum of three consecutive months.

Two probability simulation assessments were performed based on the combined data to address two concerns. First, the distribution of animals adequately exposed to heartworm disease among the 70 treated animals was calculated based on the assumption that the level of infection observed over the 4 control groups is indicative of the level of infection that would have been observed in the treated groups in these studies, had these animals been untreated after inoculation. Second, the probability of observing 0 heartworms in the treated animals given the level of presumed infectious challenge in the 70 treated animals was calculated under various hypothetical levels of true effectiveness: 90%, 95%, or 99%.

A Monte Carlo procedure, replicated 2 million times, was used to perform these evaluations. The procedure included a computational algorithm that is used to generate random samples from a specified probability

distribution[1], where the distributional parameters were derived from the empirical distribution of heartworm counts observed in the 40 control animals from Studies 1-4.

Results:

The results of the first probability simulation assessment shows the relevant percentiles for describing the distribution of the number of adequately infected animals among 70 treated animals (see Table II.2). This means that if the 70 animals in the treated group were not administered NexGard® COMBO (untreated), and assuming that the distribution of heartworm counts in this untreated group of 70 would be the same as in the 40 control animals (from Table II.1), then the number of adequately infected animals would likely follow a distribution summarized in Table II.3. Under these assumptions there will be 12 or fewer adequately infected cats 10% of the time and fewer than 17 adequately infected cats approximately half the time. In summary, about 95% of the time, there will be between 10 and 26 adequately infected animals.

Table II.2. Calculated distribution of number of adequately infected animals out of 70, assuming the distribution of heartworm counts follows the same distribution as that observed from the control group.

Minimum	2.5%	5%	10%	25%	Median	75%	90%	95%	97.5%	Maximum
1	10	11	12	15	17	20	23	24	26	40

The second simulation assesses the probability of observing no heartworms in all 70 treated animals given an assumed rate of the true effectiveness of NexGard® COMBO. The simulation assumes heartworm infection distribution is consistent with that observed in the 40 control cats. The results are as follows: if the true underlying effectiveness is 90%, the probability of observing no heartworm in all 70 animals is 0.04%. If effectiveness is 95%, the probability of observing no heartworm in all 70 animals is slightly higher at 1.36%. If the effectiveness is 99%, observing no heartworm in all 70 animals is more likely, at 39.17%.

Adverse Reactions: No adverse reactions were reported in Studies 1, 2, or 4. In Study 3 one Group 3 cat exhibited skin abnormalities (irritation/erythema and alopecia) in the area of the application site from Days 33 to 59 following the second administration of NexGard® COMBO. These abnormalities were assessed as possibly related to treatment with NexGard® COMBO. Abnormalities of the skin (irritation/erythema and alopecia) in the region of the application site were also assessed as possibly related to administration of mineral oil in 2 cats in Group 1 and in 2 cats in Group 2 following administration of mineral oil on Day 30.

[1] Rubinstein, R. Y., & Kroese, D. P. (2016). *Simulation and the Monte Carlo method* (Vol. 10). John Wiley & Sons.

Conclusion: Topical treatment with NexGard® COMBO was 100% effective in prevention of *D. immitis* infection in cats when administered once a month for three consecutive months after infection.

2. For the Treatment and Prevention of Flea Infestations (*C. felis)*

   Two studies, one laboratory dose confirmation study and one multi-site field safety and effectiveness study in client-owned animals, were conducted to demonstrate the effectiveness of NexGard® COMBO to kill adult fleas for the treatment and control of flea infestations.

   a. **Title:** Dose Confirmation of a Single Topical Treatment with NexGard® COMBO against Induced Infestations of *C. felis* on Cats. (Study No. PR&D 0376201)

      Study Dates: July 26, 2017 to April 4, 2019

      Study Location: Turlock, CA

      Study Design:

      Objective: To confirm the effectiveness of NexGard® COMBO when applied once topically at the recommended minimum dose for the treatment and control of induced infestations of adult *C. felis* on cats, for inhibition of flea egg production, and for inhibition of flea egg development.

      Study Animals: Twenty cats (9 males and 11 females), 16 to 101 months of age and weighing 2.8 to 6.8 kg on the day before treatment.

      Experimental Design: This study was conducted in accordance with GCP guidelines. This study followed a randomized block design based on pre-treatment flea count. Cats were treated once on Day 0. Cats were randomized to the following treatment groups of 10 cats each:

      - Group 1: Mineral oil (control)
      - Group 2: NexGard® COMBO

      Drug administration was on Day 0. Cats were infested with approximately 100 adult *C. felis* on Days -1, 7, 14, 21 and 30.

      Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

      Measurements and Observations: Live fleas were counted on Day 1, at 24 hours after treatment; and on Days 8, 15, 22, and 31, at 24 hours after infestation. Flea eggs were collected from the area beneath each cage on Days 1, 8, 15, 22, and 31 and counted. All eggs were incubated for 3 days, and hatched flea larvae were counted on Days 4, 11, 18, 25, and

34. Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter.

Statistical Methods:

The live flea counts or the egg count were analyzed using the Mixed procedure in SAS version 9.4 with treatment group as the fixed effect and allocation blocks as the random effect. The arithmetic mean of the live flea counts and larval hatch at each timepoint was calculated using the least squares mean (LSMeans) obtained from the Mixed procedure. For determination of the viability of flea eggs the larval counts for the treated group were compared to the counts of the control group using the Mixed procedure in SAS version 9.4 if at least two animals in each group had positive egg counts.

Effectiveness was determined on the basis of the percent reduction in flea and egg counts in the NexGard® COMBO group compared to the control group. To demonstrate effectiveness, comparison analysis at all timepoints should be statistically different and in favor of the NexGard® COMBO group.

Percent Effectiveness (Adult Fleas) = 100 x $(c_c - c_t)/c_c$
Where c_c = Arithmetic mean (LSMeans) of flea counts in the control group
c_t = Arithmetic mean (LSMeans) of flea counts in the NexGard® COMBO group

Percent Effectiveness (egg counts) = 100 x $(c_c - c_t)/c_c$
Where c_c = Arithmetic mean (LSMeans) mean of egg counts in the control group
c_t = Arithmetic mean (LSMeans) mean of egg counts in the NexGard® COMBO group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$

Results:

Adult Fleas: At least 9 of 10 cats in the control group had an adequate infestation, defined as at least 50 fleas present, at each count.

Table II.3: Summary of Analyses of adult *C. felis* counts

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	Percent Effectiveness	P-value
1	68.2	5.4	92.1	< 0.0001
8	74.7	0.1	99.9	< 0.0001
15	68.8	0.0	100.0	< 0.0001
22	77.2	0.4	99.5	< 0.0001
31	73.0	3.3	95.5	< 0.0001

Flea counts on all counting days demonstrated > 90% effectiveness and

the results were significantly different when comparing the NexGard® COMBO group to the control group.

Egg production:

Table II.4: Summary of Analyses of *C. felis* egg counts

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	Percent Effectiveness	P-value
1	74.5	29.8	60	0.0073
8	58.3	0.1	99.8	0.0009
15	47.6	0.0	100.0	0.0045
22	58.3	0.0	100.0	0.0003
31	49.1	0.0	100.0	0.0017

Flea egg collection on Day 1 showed a 60% reduction of flea egg production when comparing the NexGard® COMBO group to the control group. The cats had been infested with fleas on Day -1 allowing the fleas to mate and start producing eggs prior to being exposed to the NexGard® COMBO. After Day 1, inhibition of flea egg production was consistently above 99% when comparing the NexGard® COMBO group to the control group.

Egg development:

Table II.5: Summary of Analyses of *C. felis* larval hatch counts

Study Day	N*	Control Group Arithmetic Mean of Larval Hatch	N*	NexGard® COMBO Group Arithmetic Mean of Larval Hatch	P-Value
4	10	47.3	9	17.1	0.0109
11	9	49.6	1	1.0	-
18	10	41.4	0	-	-
25	9	54.7	0	-	-
34	10	34.1	0	-	-

*N = number of animals from which eggs were produced

On Day 4, the NexGard® COMBO group had a significantly different ($p = 0.0058$) and numerically lower larval hatch count than the control group. Statistical analysis was only meaningful on Day 4.

Adverse Reactions: No adverse reactions were reported.

Conclusion: NexGard® COMBO is safe and effective in cats to kill adult fleas (*C. felis*) and for the treatment and prevention of flea infestations over a period of one month.

b. **Title:** Safety and Effectiveness of NexGard® COMBO Against Flea Infestations and Flea Allergy Dermatitis on Cats Under Field Conditions. (Study No. PR&D 03736)

Study Dates: April 20, 2017 to June 11, 2019

Study Locations:

West Palm Beach, FL
Springfield, MO
Washington, MO
Portland, OR
Knoxville, TN
Commerce, GA
Zachary, LA
Lake Worth, FL
Jackson, MS
Largo, FL
Plaquemine, LA

Study Design:

Objective: The objective of this study was to determine the safety and effectiveness of NexGard® COMBO, when administered as a topical solution to cats three times at one-month intervals, against fleas and to evaluate the effect of treatment on signs of flea allergy dermatitis (FAD) in a multi-site field study.

Study Animals: Two-hundred-one households were enrolled in the study. The households contained 380 cats. The cats were 8 weeks to 17 years old and weighed 1.8 to 23.9 lbs at Visit 1. Cats were predominantly mixed domestic short, medium, and long-haired breeds; some pure-bred cats were also included. The enrolled cats consisted of 144 neutered males, 28 intact males, 169 spayed females, and 39 intact females. One-hundred-seventeen cats treated with NexGard® COMBO were evaluated for effectiveness at all visits. Fifty cats treated with an active control were evaluated for effectiveness at all visits.

Experimental Design: This study was conducted in accordance with GCP guidelines. This was a positive-controlled, blinded, multicenter, clinical safety and effectiveness study using a randomized block design based on order of enrollment of households. The experimental unit for the study was the household, each of which was represented by a sentinel cat. All enrolled households had at least one cat naturally infested with at least five fleas. In multi-cat households, one sentinel cat was selected at random if it had five or more fleas and signs of FAD (if no cat in the household had signs of FAD, then a cat with five or more fleas was selected). At visit 1, all cats in a household had a physical examination and were evaluated for fleas and signs of FAD. At Visits 2, 3, and 4 (Days 30, 60, and 90), flea counts, evaluation of signs of FAD (when applicable), and physical examinations were performed on the sentinel cat. A physical

examination was performed on all cats in the household at Visit 4.

Cats were randomized to the following treatment groups:

- Group 1: NexGard® COMBO
- Group 2: Topical selamectin (active control)

Drug Administration: Cats in Group 1 were topically administered NexGard® COMBO at the intended label dose (1.4 to 4.2 mg/kg of esafoxolaner, 0.5 to 1.5 mg/kg of eprinomectin, 10.0 to 30.0 mg/kg of praziquantel) on Days 0, 30, and 60. Cats in Group 2 were treated with the active control according to the approved label on Days 0, 30, and 60. NexGard® COMBO was applied as a single spot directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades. The active control was applied according to the approved label directions.

Measurements and Observations: Live fleas were counted from the sentinel cats on Visits 2, 3, and 4 for effectiveness evaluation. Clinical signs of FAD (alopecia, miliary dermatitis, excoriation, erythema, and/or scaling) were evaluated on the sentinel cats (with signs of FAD at Visit 1) on Visits 2, 3, and 4. Physical examinations and abnormalities reported throughout the study were used for evaluation of safety. Personnel performing flea counts and health observations were masked to treatment.

Statistical Methods:

The log-counts from each treatment group were analyzed by post-treatment clinic visit using a repeated measures general linear mixed model. Visit time (Day) was the fixed effect for comparing the post-treatment counts and the baseline counts, and site, block (site), and site-by-day were the random effects. Visit time was the repeated measure with the subject defined as cat-within-site. The pivotal analysis compared the counts of Day 30, Day 60, and Day 90 visit with baseline for each treated group.

Effectiveness was determined on the basis of the percent reduction in flea counts on Day 30, Day 60, and Day 90 visit compared to the Day 0 visit for the treated groups.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
Where c_c = Geometric mean of flea counts on Day 0
c_t = Geometric mean of flea counts on post treatment visits

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$

Changes in the severity of clinical signs related to FAD were summarized and the percent of cats showing improvement for each sign was calculated.

Adverse reactions that occurred during the study were summarized and the frequency of occurrence was tabulated.

Results:

For each Visit 2, 3, and 4, the effectiveness of NexGard® COMBO, based on geometric means, was ≥ 97.8%.

Table II.6: PR&D 03736 Percent Effectiveness against live adult *C. felis*

Treatment Group	Visit	Baseline Geometric Mean Flea Count	Visit Geometric Mean Flea Count	Percent Effectiveness	P-value
NexGard® COMBO	2; Day 30	15.31	0.34	97.8	< .0001
NexGard® COMBO	3; Day 60	15.35	0.07	99.6	< .0001
NexGard® COMBO	4; Day 90	15.41	0.01	99.9	< .0001
Active control	2; Day 30	13.30	2.38	82.1	< .0001
Active control	3; Day 60	13.63	0.99	92.8	< .0001
Active control	4; Day 90	13.56	0.72	94.7	< .0001

Fifty-two NexGard® COMBO treated cats and 20 active control-treated cats were included in the analysis of FAD at Visit 4. Percent of cats showing improvement in the observed clinical signs of FAD are summarized in table II.7.

Table II.7: Number and Percent Improvement in Clinical Signs of FAD at Day 90

FAD Sign	NexGard® COMBO Number Improved	NexGard® COMBO Percent Improved	Active Control Number Improved	Active Control Percent Improved
Alopecia	28 of 31	90.3	11 of 12	91.7
Miliary Dermatitis	28 of 28	100	8 of 8	100
Excoriation	18 of 19	94.7	7 of 9	77.8
Erythema	23 of 25	92.0	12 of 12	100
Scaling	15 of 15	100	4 of 5	80.0

Adverse Reactions:

Health abnormalities that may have been related to treatment included emesis, application site hair change, anorexia, and lethargy. Hypersalivation was also seen but was likely due to licking the application site.

There were no serious health abnormalities that were attributed to treatment. Concurrent administration of various medications and vaccines did not have an impact on the safety of NexGard® COMBO. There were three deaths in the NexGard® COMBO group and 3 in the active control. None of the deaths were related to treatment with NexGard® COMBO or active control.

Table II.8. Adverse Reactions in Study PR&D 03736

Event	n[1]	Treatment Group NexGard® COMBO % (n = 244)	n[2]	Treatment Group Active Control % (N = 136)
Emesis	16	6.56	8	5.88
Application Site Hair Change	9	3.69	0	0.00
Anorexia	7	2.87	4	2.94
Lethargy	6	2.46	5	3.68
Bacterial skin infection	4	1.64	1	0.74
Pruritus	4	1.64	0	0.00
Sneezing	4	1.64	5	3.68
Desquamation	3	1.23	2	1.47
Diarrhea	3	1.23	3	2.21
Epiphora	3	1.23	1	0.74
Hypersalivation	3	1.23	0	0.00
Hyperthermia	3	1.23	0	0.00

[1]Number of cats treated with NexGard® COMBO with the identified abnormality.

[2]Number of cats treated with active control with the identified abnormality.

Conclusion: The results of this study demonstrate that NexGard® COMBO is safe and effective for the treatment and prevention of flea infestations under field conditions. The cats treated with NexGard® COMBO showed an improvement in clinical signs related to FAD over the course of the study as a direct result of eliminating fleas.

3. For the Treatment and Control of Tick Infestations

Five studies were conducted to support the effectiveness of NexGard® COMBO for the treatment and control of tick infestations. Two dose confirmation studies, Study 0378801 and 0378802, demonstrated effectiveness for the treatment and control of *I. scapularis* infestations on cats.

Three studies were conducted to demonstrate effectiveness against *Amblyomma Americanum* (Studies 0396501, 0404201, and 2021016). Although Study PR&D 0396501 failed to demonstrate > 90% effectiveness on Days 17 and 33, and Study 2021016 failed to demonstrate > 90% effectiveness on Day 3, the average effectiveness for all studies combined is > 90% at all time points tested (Days 3, 10, 17, 24, and 33). In addition, > 90% effectiveness was demonstrated at these time points in at least two of the three studies. Therefore, the combined data demonstrates that NexGard®

COMBO is effective for the treatment and control of *A. americanum* infestations on cats when assessed at 72 hours following treatment.

Ixodes scapularis

a. **Title:** Dose Confirmation of a Single Topical Treatment with NexGard® COMBO against Induced Infestations of *Ixodes scapularis* on Cats. (Study No. PR&D 0378801)

Study Dates: February 5, 2018 to May 8, 2019

Study Location: Fulton, MO

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO when applied once topically at the recommended minimum dose for the treatment and control of induced infestations of adult stages of *I. scapularis* on cats.

Study Animals: Twenty cats (8 males and 12 females), 20.3 to 59.0 months of age and weighing 3.0 to 6.2 kg.

Experimental Design: This study was conducted in accordance with GCP guidelines. This study followed a randomized block design based on pre-treatment live tick count. Cats were randomized into the following treatment groups of 10 animals each:

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

Cats were treated once on Day 0. Cats were infested with approximately 50 adult *I. scapularis* on Days -2, 7, 14, 21, and 30.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg Control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for health abnormalities at least once daily throughout the study. Post-dosing clinical observations were conducted at 1, 2, 3, and 4 hours after treatment and once daily thereafter. Live ticks were counted on Days 2, 9, 16, 23, and 32 at 48 hours after infestations or treatment. Dead ticks were counted in the collection pan under each cat on Days 1, 2, 8, 9, 15, 16, 22, 23, 31, and 32. Personnel performing tick counts and health observations were masked to treatment group.

Statistical Methods:

Live tick counts were analyzed by timepoint using the Mixed procedure in SAS version 9.4 with treatment group as the fixed effect and allocation blocks as the random effect. The arithmetic mean of the live tick counts at each timepoint was calculated using the least squares mean (LSMeans) obtained from the Mixed procedure.

Effectiveness was determined on the basis of the percent reduction in live tick counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
Where c_c = Arithmetic mean (LSMeans) of live tick counts in the control group
c_t = Arithmetic mean (LSMeans) of live tick counts in the NexGard® COMBO group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$

Results:

At least 8 out of 10 cats in the control group had an adequate infestation, defined as at least 13 live *I. scapularis* ticks (25% of the infestation dose of 50 ticks per cat), at each tick count day.

The percent effectiveness using arithmetic means of the NexGard® COMBO group (Treatment Group 2) versus the control group (Treatment Group 1) was 95.1, 99.3, 100, 98.8, and 100% on Days 2, 9, 16, 23, and 32. The NexGard® COMBO group (Treatment Group 2) had significantly different ($p < 0.0001$) and numerically lower live tick counts than the control group (Treatment Group 1).

The NexGard® COMBO group had higher dead tick counts than the control group (Treatment Group 1) for all study days.

Table II.9. Study PR&D 0378801 Summary of Analysis of Live Tick Counts

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	Percent Effectiveness	P-value
2	26.5	1.3	95.1	< 0.0001
9	27.9	0.2	99.3	< 0.0001
16	30.4	0.0	100	< 0.0001
23	32.1	0.4	98.8	< 0.0001
32	32.5	0.0	100	< 0.0001

Table II.10. Study 0378801: *I. scapularis* Dead Tick Count Results

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	P-value
2	3.6	22.2	< .0001
9	2.1	32.5	< .0001
16	5.0	25.5	< .0001
23	4.3	24.8	< .0001
32	1.6	27.2	< .0001

Adverse Reactions: No adverse reactions were reported.

Conclusion: NexGard® COMBO was effective against *I. scapularis* ticks when assessed 48 hours after treatment of an existing infestation and 48 hours after approximately weekly re-infestation for one month.

b. **Title:** Dose Confirmation of a Single Topical Treatment with NexGard® COMBO against Induced Infestations of *Ixodes scapularis* on Cats. (Study No. PR&D 0378802)

Study Dates: December 18, 2017 to January 31, 2019

Study Location: Athens, GA

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO when applied once topically at the recommended minimum dose for the treatment and control of induced infestations of adult *stages of I. scapularis* on cats.

Study Animals: Twenty cats (11 males and 9 females), 8.1 to 12.2 months of age and weighing 2.2 to 5.3 kg.

Experimental Design: This study was conducted in accordance with GCP guidelines. This study followed a randomized block design based on pre-treatment live tick count. Cats were randomized into the following treatment groups of 10 animals each:

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

Cats were treated once on Day 0. Cats were infested with approximately 50 adult *I. scapularis* on Days -2, 7, 14, 21, and 30.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg Control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for health abnormalities at least once daily throughout the study. Post-dosing clinical observations were conducted at 1, 2, 3, and 4 hours after treatment and once daily thereafter. Live ticks were counted on Days 2, 9, 16, 23, and 32 at 48 hours after infestations or treatment. Dead ticks were counted in the collection pan under each cat on Days 1, 2, 8, 9, 15, 16, 22, 23, 31, and 32. Personnel performing tick counts and health observations were masked to treatment group.

Statistical Methods:

Live tick counts were analyzed by timepoint using the Mixed procedure in SAS 9.4 with treatment group as the fixed effect and allocation blocks as the random effect. The arithmetic mean of the live tick counts at each timepoint was calculated using the least squares mean (LSMeans) obtained from the Mixed procedure.

Effectiveness was determined on the basis of the percent reduction in live tick counts in the NexGard® COMBO group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
Where c_c = Arithmetic mean (LSMeans) of tick counts in the control group
c_t = Arithmetic mean (LSMeans) of tick counts in the NexGard® COMBO group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$

Results:

At least 8 out of 10 cats in the control group had an adequate infestation, defined as at least 13 live *I. scapularis* ticks (25% of the infestation dose of 50 ticks per cat), at each tick count day.

The percent effectiveness using arithmetic means of the NexGard® COMBO (Treatment Group 2) versus the control group (Treatment Group 1) was 98.8, 100, 100, 99.6, and 98.2% on Days 2, 9, 16, 23, and 32, respectively. The NexGard® COMBO (Treatment Group 2) had significantly different ($p<0.0001$) and numerically lower live tick counts than the control group (Treatment Group 1).

NexGard® COMBO (Treatment Group 2) had higher dead tick counts than the control group (Treatment Group 1) at all time points.

Table II.11. Study PR&D 0378802 Summary of Analysis of Live Tick Counts

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	Percent Effectiveness	P-value
2	24.5	0.3	98.8	< .0001
9	26.5	0.0	100.0	< .0001
16	27.2	0.0	100.0	< .0001
23	24.4	0.1	99.6	< .0001
32	22.2	0.4	98.2	< .0001

Table II.12. Study 0378802: *I. scapularis* Dead Tick Count Results

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	P-value
2	1.3	11.8	< .0001
9	11.6	27.5	< .0001
16	10.6	31.3	< .0001
23	10.1	24.4	0.0068
32	11.4	20.4	0.0234

Adverse Reaction: No treatment-related adverse reactions were reported.

Conclusion: NexGard® COMBO was effective against *I. scapularis* when assessed 48 hours after treatment of an existing infestation and 48 hours after weekly re-infestation for one month.

Amblyomma americanum

c. **Title:** Dose Confirmation of a Single Topical Treatment with NexGard® COMBO against Induced Infestations of *Amblyomma americanum* on Cats. (Study No. PR&D 0404201)

Study Dates: May 18, 2018 to March 28, 2019

Study Location: Athens, GA

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO for the treatment and control of induced infestations of adult stages of *Amblyomma americanum* on cats when applied once topically at the recommended minimum dose.

Study Animals: Twenty cats (10 male, 10 female), 6.8 to 7.8 months of age and weighing 2.52 to 5.64 kg.

Experimental Design: The study was conducted according to the principles of GCP. This study followed a randomized block design based on pre-

treatment live tick count. Cats were randomized into the following treatment groups of 10 animals each:

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

Cats were treated once on Day 0. Cats were infested with approximately 50 adult *A. americanum* on Days -2, 14, and 30.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for health abnormalities at least once daily throughout the study. Post-dosing clinical observations were conducted at 1, 2, 3, and 4 hours after treatment. Live ticks were counted on Days 3, 17, and 33 at 72 hours after infestations or treatment. Dead ticks were counted in the collection pan under each cat on Days 1, 2, 3, 15, 16, 17, 31, 32, and 33. Personnel performing tick counts and health observations were masked to treatment group.

Statistical Methods:

Live tick counts were analyzed by timepoint using the Mixed procedure in SAS version 9.4 with treatment group as the fixed effect and block as the random effect. The arithmetic mean of the live tick counts at each timepoint was calculated using the least squares mean (LSMeans) obtained from the Mixed procedure.

Effectiveness was determined based on the percentage reduction in live tick counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
Where c_c = Arithmetic mean (LSMeans) of live tick counts in the control group
c_t = Arithmetic mean (LSMeans) of live tick counts in the NexGard® COMBO group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$

Results:

At least 9 out of 10 cats in the control group had an adequate infestation, defined as at least 12 live *A. americanum* ticks (25% of the infestation dose of 50 ticks per cat), at each tick count day.

The percent effectiveness using arithmetic means of the NexGard® COMBO (Treatment Group 2) versus the control group (Treatment Group

1) was 99.3, 100, and 100% on Days 3, 17, and 33, respectively. The NexGard® COMBO (Treatment Group 2) had significantly different ($p < 0.0001$) and numerically lower live tick counts than the control group (Treatment Group 1) for all time points.

NexGard® COMBO (Treatment Group 2) had higher dead tick counts than the control group (Treatment Group 1) at all time points.

Table II.13. Study 0404201: *A. americanum* Live Tick Count Results

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	Percent Effectiveness	P-value
3	27	0.2	99.3	< 0.0001
17	32.1	0	100.0	< 0.0001
33	26.3	0	100.0	< 0.0001

Table II.14. Study 0404201: *A. americanum* Dead Tick Count Results

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	P-value
3	1.4	16.9	< .0001
17	1.9	25.4	< .0001
33	1.8	18.3	< .0001

Adverse Reactions: No treatment-related adverse reactions were reported. On Day 15, one control cat had a brief and acute fatal illness diagnosed as cytauxzoonosis based on clinical and post mortem findings. Cytauxzoonosis is known to be endemic in the area where the ticks had been collected.

Conclusion: A single dose of NexGard® COMBO was effective against *A. americanum* within 72 hours after treatment and following two re-infestations for one month.

d. **Title:** Dose Confirmation of a Single Topical Treatment with NexGard® COMBO against Induced Infestations of *Amblyomma americanum* on Cats. (Study No. PR&D 0396501)

Study Dates: September 14, 2017 to May 14, 2018

Study Location: Turlock, CA

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO for the treatment and control of induced infestations of adult stages of *Amblyomma americanum* on cats when applied once topically at the recommended minimum dose.

Study Animals: Twenty cats (10 male, 10 female), 33 to 97 months of age and weighing 2.7 to 6.2 kg, were randomized into two groups of 10 cats each.

Experimental Design: The study was conducted according to the principles of GCP. This study followed a randomized block design based on pre-treatment live tick count. Cats were randomized into the following treatment groups of 10 animals each:

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

Cats were treated once on Day 0. Cats were infested with approximately 50 adult *A. americanum* on Days -2, 7, 14, 21, and 30.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for health abnormalities at least once daily throughout the study. Post-dosing clinical observations were conducted at 1, 2, 3, and 4 hours after treatment. Live ticks were counted on Days 3, 10, 17, 24, and 33, at 72 hours after infestations or treatment. Dead ticks were counted in the collection pan under each cat on Days 1, 2, 3, 8, 9, 10, 15, 16, 17, 22, 23, 24, 31, 32, and 33. Personnel performing tick counts and health observations were masked to treatment group.

Statistical Methods:

Live tick counts were analyzed by timepoint using the Mixed procedure in SAS version 9.4 with treatment group as fixed effect and block as the random effect. The arithmetic mean of the live tick counts at each timepoint was calculated using the least squares mean (LSMeans) obtained from the Mixed procedure.

Effectiveness was determined based on the percentage reduction in live tick counts in the treated group compared to the control group.

Percent Effectiveness = 100 x $(c_c - c_t)/c_c$
Where c_c = Arithmetic mean (LSMeans) of live tick counts in the control group
c_t = Arithmetic mean (LSMeans) of live tick counts in the NexGard® COMBO group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with α = 0.05

Results:

At least 8 out of 10 cats in the control group had an adequate infestation, defined as at least 12 live *A. americanum* ticks (25% of the infestation dose of 50 ticks per cat), at each tick count day.

The percent effectiveness using arithmetic means of the NexGard® COMBO (Treatment Group 2) versus the control group (Treatment Group 1) was 99.5, 98.1, 87.6, 94,2, and 88.5% on Days 3, 10, 17, 24, and 33, respectively. The NexGard® COMBO (Treatment Group 2) had significantly different ($p < 0.0001$) and numerically lower live tick counts than the control group (Treatment Group 1) for all time points.

NexGard® COMBO (Treatment Group 2) had higher dead tick counts than the control group (Treatment Group 1) at all time points.

Table II.15. Study 0396501: *A. americanum* Live Tick Count Results

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	Percent Effectiveness	P-value
3	18.8	0.1	99.5	< 0.0001
10	24.0	0.5	98.1	< 0.0001
17	23.7	2.9	87.6	< 0.0001
24	22.7	1.3	94.2	< 0.0001
33	25.0	2.9	88.5	< 0.0001

Table II.16. Study 0396501: *A. americanum* Dead Tick Count Results

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	P-value
3	0.1	14.0	< .0001
10	2.0	19.0	0.0003
17	2.2	17.3	< .0001
24	1.1	16.2	0.0001
33	0.6	14.9	< .0001

Adverse Reactions: No adverse reactions were reported.

Conclusion: NexGard® COMBO was greater than 90% effective against *A. americanum* on Days 3, 10, and 24, but less than 90% effective on Days 17 and 33, when assessed within 72 hours after treatment and following weekly re-infestations for a month.

e. **Title:** Dose Confirmation of a Single Topical Treatment of NexGard® COMBO against Induced Infestations of Adult *Amblyomma americanum* on Cats. (Study No. 2021016)

Study Dates: May 6 to December 8, 2021.

Study Location: Fulton, MO

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO for the treatment and control of induced infestations of adult stages of *Amblyomma americanum* on cats when applied once topically at the recommended minimum dose.

Study Animals: Twenty cats (9 male, 11 female), 14.1 to 78.0 months of age and weighing 2.79 to 6.04 kg, were randomized into two groups of 10 cats each.

Experimental Design: The study was conducted according to the principles of GCP. This study followed a randomized block design based on pre-treatment live tick count. Cats were randomized into the following treatment groups of 10 animals each:

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

Cats were treated once on Day 0. Cats were infested with approximately 50 adult *A. americanum* on Days -2, 7, 14, 21, and 30.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for health abnormalities at least once daily throughout the study. Post-dosing clinical observations were conducted at 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after treatment. Live ticks were counted on Days 3, 10, 17, 24, and 33 at 72 hours after infestations or treatment. Dead ticks were counted in the collection pan under each cat on Days 1, 2, 3, 8, 9, 10, 15, 16, 17, 22, 23, 24, 31, 32, and 33. Personnel performing tick counts and health observations were masked to treatment group.

Statistical Methods:

Live tick counts were analyzed by timepoint using the Mixed procedure in SAS version 9.4 with treatment group as the fixed effect. The arithmetic mean of the live tick counts at each timepoint was calculated using the least squares mean (LSMeans) obtained from the Mixed procedure.

Effectiveness was determined based on the percentage reduction in live tick counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
Where c_c = Arithmetic mean (LSMeans) of live tick counts in the control group
c_t = Arithmetic mean (LSMeans) of live tick counts in the NexGard® COMBO group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$.

Results:

At least 6 out of 10 cats in the control group had an adequate infestation, defined as at least 12 live *A. americanum* ticks (25% of the infestation dose of 50 ticks per cat), at each tick count days.

The percent effectiveness using arithmetic means of the NexGard® COMBO (Treatment Group 2) versus the control group (Treatment Group 1) was 89.9, 100, 99.2, 100, and 99.4% on Days 3, 10, 17, 24, and 33, respectively. The NexGard® COMBO (Treatment Group 2) had significantly different ($p < 0.0001$) and numerically lower live tick counts than the control group (Treatment Group 1) for all time points.

NexGard® COMBO (Treatment Group 2) had higher dead tick counts than the control group (Treatment Group 1) at all time points.

Table II.17. Study 2021016: *A. americanum* Live Tick Count Results

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	Percent Effectiveness	P-value
3	20.7	2.1	89.9	< .0001
10	16.5	0.0	100.0	< .0001
17	12.1	0.1	99.2	< .0001
24	20.4	0.0	100.0	< .0001
33	15.8	0.1	99.4	< .0001

Table II.18. Study 2021016: *A. americanum* Dead Tick Count Results

Study Day	Control Group Arithmetic Mean	NexGard® COMBO Group Arithmetic Mean	P-value
3	0.9	17.2	< .0001
10	1.6	18.7	< .0001
17	0.7	13.0	< .0001
24	1.1	14.4	< .0001
33	0.4	15.3	< .0001

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

Conclusion: NexGard® COMBO was less than 90% effective against *A. americanum* on Day 3, but greater than 90% effective on Days 10 through 33 when assessed within 72 hours after treatment and following re-infestations for a month.

4. For the Treatment and control of roundworms and hookworms

   Studies to support substantial evidence of effectiveness of a topical eprinomectin dose of 0.48 mg/kg for the treatment and control of roundworms (fourth stage larval and adult *T. cati*) and hookworms (fourth stage larval and adult *Ancylostoma tubaeforme*; adult *Ancylostoma braziliense*) were conducted for the approval of Centragard® (eprinomectin and praziquantel transdermal solution); refer to the Freedom of Information Summary for the original approval of NADA 141-492, dated March 8, 2018. The concentration of eprinomectin in NexGard® COMBO is identical to the concentration of eprinomectin in Centragard® transdermal solution for cats. To demonstrate that NexGard COMBO® is effective against the same roundworm and hookworm indications as Centragard®, the sponsor conducted two dose confirmation studies to demonstrate effectiveness against the least susceptible nematode species, *T. cati*, as established in dose confirmation studies for Centragard®.

   Toxocara cati (adult)

   a. **Title:** Effectiveness of a Single Topical Treatment with NexGard® COMBO, or of a Single Topical Treatment with Esafoxolaner Administered Alone, against Induced Adult *Toxocara cati* Infection in Cats. (Study No. PR&D 0375701)

      Study Dates: January 31, 2018 to January 16, 2019

      Study Location: Rockwood, TN

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO against adult *T. cati*-induced infections in cats, and the ineffectiveness of esafoxolaner alone, when applied once topically at the recommended minimum dose.

Study Animals: Thirty cats (16 male and 14 female), 10 to 12 weeks of age at the time of *T. cati* inoculation and weighing 2.0 to 2.9 kg two days before treatment.

Experimental Design: This study was conducted in accordance with GCP guidelines. All cats were confirmed negative for *Toxocara* eggs prior to randomization. Cats were randomized into the following treatment groups (10 cats per treatment group) using a completely randomized design.

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO
- Group 3: Esafoxolaner

Cats were inoculated orally on three consecutive days (Days -63, -62 and -61), each time with approximately 34 infective larvated *T. cati* eggs. All cats were diagnosed positive for *T. cati* ova prior to treatment. Cats were treated once on Day 0. The timing of treatment was designed to target the *T. cati* adult stage. Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. Cats in Group 3 were topically administered esafoxolaner at 0.12 mL/kg, delivering 1.4 mg/kg of esafoxolaner. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: On Day 7, the animals were humanely euthanized and a necropsy was performed for parasite recovery and enumeration.

Statistical Methods:

The logarithm of the (counts + 1) was analyzed using the GLM procedure in SAS 9.4 with treatment group as the fixed effect in the model.

Effectiveness was determined on the basis of the percent reduction in worm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
Where c_c = Geometric mean of worm counts in the control group
c_t = Geometric mean of worm counts in the treated group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with α = 0.05

Results:

Adequate infection was defined as at least five *Toxocara cati* worms found in at least 6 of the control animals. All 10 control animals had 14 or more worms on necropsy.

The percent effectiveness using geometric means was 100% for the NexGard® COMBO treated group versus the control group, and the counts of adult *T. cati* were significantly different ($p < 0.0001$) and numerically lower in NexGard® COMBO treated group compared to the control group. The percent effectiveness using geometric means was less than 0% for the esafoxolaner alone treated group versus the control group and the counts of adult *T. cati* in these two groups were not significantly different ($p = 0.8858$).

Table II.19. Study PR&D 0375701 Summary of Analyses of Adult *T. cati* Counts

Treatment Group	Geometric Mean	Percent Effectiveness	P-Value
Control	29.5	-	-
NexGard® COMBO	0.00	100.0%	< 0.0001
Esafoxolaner	30.5	-3.2%	0.8858

Adverse Reactions: No adverse reactions were reported.

Conclusion: NexGard® COMBO was effective for the treatment and control of adult *T. cati* following induced infection in cats. Esafoxolaner was not effective against adult *T. cati* infection.

b. **Title:** Effectiveness of a Single Topical Treatments with NexGard® COMBO Against Natural *Toxocara cati* Infections in Cats. (Study No. PR&D 0375802)

Study Dates: August 11, 2017 to April 6, 2018

Study Location: Tirana, Albania

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO against natural *T. cati* infections in cats, when applied once topically at the recommended minimum dose.

Study Animals: Twenty cats (7 males and 13 females), 5 to 8 months of age and weighing 1.0 to 2.1 kg at the time of treatment.

Experimental Design: This study was conducted in accordance with GCP guidelines. All cats in the study were naturally infected with *T. cati*. All

cats were diagnosed positive to *Toxocara* eggs prior to treatment, confirming that they were infected with adult *T. cati*. Cats were randomized into the following treatment groups (10 cats per treatment group) using a block design, with block based on pre-treatment body weight.

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

The animals were enrolled in two phases comprising 8 and 2 blocks, respectively. Cats were treated once on Day 0 Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: On Day 7, the animals were humanely euthanized and a necropsy was performed for parasite recovery and enumeration.

Statistical Methods:

The logarithm of the (counts + 1) was analyzed using the Mixed procedure in SAS 9.4 with treatment group as the fixed effect, and the phase and block within phase as random effects.

Effectiveness was determined on the basis of the percent reduction in worm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
Where c_c = Geometric mean of worm counts in the control group
c_t = Geometric mean of worm counts in the treated group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$

Results:

Adequate infection was defined as at least five *T. cati* worms found in at least 6 of the control animals. Eight of 10 control animals had 7 or more worms.

The percent effectiveness using geometric means was 98.8% for the NexGard® COMBO treated group versus the control group, and the counts of adult *T. cati* were significantly lower in NexGard® COMBO treated group compared to the control group ($p < 0.0001$).

Table II.20. Study 0375802 Summary of Analyses of Adult *T. cati* Counts

Treatment Group	Geometric Mean	Percent Effectiveness	P-Value
Control	12.14	-	-
NexGard® COMBO	0.14	98.8%	< 0.0001

Adverse Reactions: No adverse reactions were reported.

Conclusion: NexGard® COMBO was effective for the treatment and control of adult *T. cati* in cats with natural *T. cati* infection.

5. For the Treatment and Control of Tapeworms *(D. caninum)*

Three laboratory dose confirmation studies were conducted to support the effectiveness of NexGard® COMBO for the treatment and control of *Dipylidium caninum.* Two studies (2021-1375 and 0375501) fulfilled the following requirements: an adequate level of infection in 6 control animals, a statistically significant difference between treated and control groups at $P < 0.05$, and a calculated effectiveness of 90% or greater using geometric means for the recovered *D. caninum* counts in the control and treated groups. However, although study 2020476 had an adequate level of infection in 6 control animals and a statistically significant difference between treated and control groups at $P < 0.05$, the calculated effectiveness was below 90%. The average percent effectiveness for the three studies (92.8%) is greater than 90%, supporting the effectiveness of NexGard® COMBO for the treatment and control of *D. caninum.*

a. **Title:** A Study to Confirm the Efficacy of a Single Topical Treatment with NexGard® COMBO and the Inefficacy of Esafoxolaner against *Dipylidium caninum* Induced Infection in Cats. (Study No. PR&D 0375501)

Study Dates: July 11, 2017 to August 6, 2018

Study Location: Bloemfontein, Republic of South Africa

Study Design:

Objectives: To confirm the effectiveness of NexGard® COMBO against *D. caninum*-induced infections in cats, and the ineffectiveness of esafoxolaner alone, when applied once topically at the recommended minimum dose.

Study Animals: Twenty-four cats (13 males and 11 females), 1 to 8 years of age and weighing 2.5 to 4.6 kg two days before treatment.

Experimental Design: This study was conducted in accordance with GCP guidelines. Cats were experimentally infected using the intermediate host, fleas known to be harboring *D. caninum*. All cats were diagnosed positive for *D. caninum* proglottids prior to treatment. Cats were randomized to

the following treatment groups (eight cats per treatment group) using a block design with block based on pre-treatment bodyweight.

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO
- Group 3: Esafoxolaner

On Day 0, cats in Groups 1, 2 and 3 were administered control, NexGard® COMBO, or esafoxolaner, respectively.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. Cats in Group 3 were topically administered esafoxolaner at 0.12 mL/kg, delivering 1.4 mg/kg of esafoxolaner. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: On Day 7, the animals were humanely euthanized and a necropsy was performed for parasite recovery and enumeration. Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter.

Statistical Methods:

The logarithm of the (counts + 1) was analyzed using the Mixed procedure in SAS 9.4 with treatment group as the fixed effect and allocation blocks as the random effect.

Effectiveness was determined on the basis of the percent reduction in worm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/cc$
Where c_c = Geometric mean of worm counts in the control group
c_t = Geometric mean of worm counts in the treated group

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$.

Results:

A minimum of two *D. caninum* scolicies was considered to be an adequate infection and a minimum of six adequately infected control cats was required for the study to be considered valid. Seven of the eight control cats satisfied the adequacy of infection requirement.

The percent effectiveness using geometric means was 98.0% for the NexGard® COMBO treated group versus the control group, and the counts of *D. caninum* were significantly lower in NexGard® COMBO treated group compared to the control group ($p = 0.0018$).

Table II.21. Study PR&D 0375501 Summary of Analyses of *D. caninum* Counts

Treatment Group	Geometric Mean	Percent Effectiveness	P-Value
Control	28.9	-	-
NexGard® COMBO	0.6	98.0	0.0018
Esafoxolaner	56.5	-95.8	-

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with NexGard® COMBO was 98% effective against *D. caninum*-induced infection in cats. Esafoxolaner alone was ineffective against *D. caninum*-induced infection.

b. **Title:** A Study to Confirm the Efficacy of a Single Topical Treatment with NexGard® COMBO Against Natural *Dipylidium caninum* Infection in Cats. (Study No. 2021-1375)

Study Dates:; October 28, 2021 to July 14, 2022

Study Location: Mexico City, Mexico

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO against *D. caninum* natural infections in cats, when applied once topically at the recommended minimum dose.

Study Animals: Twenty cats (5 males and 15 females), approximately 4 months to 12 years of age and weighing 1.6 to 5.5 kg one day before treatment. The study was conducted in two subsequent phases including cohorts of 14 cats and 6 cats, respectively.

Experimental Design: This study was conducted in accordance with GCP guidelines. All cats included in the study were naturally infected with *D. caninum*. All cats were diagnosed positive for dipylidiid proglottids prior to treatment, confirming that they were infected with dipylidiid cestodes. Cats were randomized into the following treatment groups (10 cats per group), using a block design with block based on pre-treatment body weight:

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

On Day 0, cats in Groups 1 and 2 were administered control or NexGard® COMBO, respectively.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied

directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for adverse reactions hourly for six hours and then approximately 8, 12, and 24 hours post-treatment and daily thereafter. On Day 11 and 12, the animals were humanely euthanized and a necropsy was performed on the same day for parasite recovery and enumeration.

Statistical Methods:

The logarithm of the (counts + 1) was analyzed using the Mixed procedure in SAS 9.4 with treatment group as the fixed effect and the phase and block within phase as random effects.

Effectiveness was determined based on the percent reduction in worm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$
where c_c = Geometric mean of worm counts in the control group calculated from the back-transformed least squares mean from the MIXED procedure
c_t = Geometric mean of worm counts in the treated group calculated from the back-transformed least squares mean from the MIXED procedure

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$.

Results:

A minimum of two *D. caninum* scolicies was considered to be an adequate infection and a minimum of six adequately infected control cats was required for the study to be considered valid. Six of the eight control cats satisfied the adequacy of infection requirement.

The percent effectiveness using geometric means was 99.1% for the NexGard® COMBO treated group versus the control group, and the counts of *D. caninum* were significantly lower in NexGard® COMBO treated group compared to the control group ($p < 0.001$).

Table II.21. Study 2021-1375 Summary of Analyses of *D. caninum* Counts

Treatment Group	Geometric Mean	Percent Effectiveness	P-Value
Control	68.1	-	-
NexGard® COMBO	0.6	99.1	< 0.001

Adverse Reactions: No adverse reactions related to treatment with NexGard® COMBO were observed.

Conclusion: A single topical treatment with NexGard® COMBO at the minimum recommended dose was 99.1% effective against natural infections with *D. caninum* in cats.

c. **Title:** A Study to Confirm the Efficacy of a Single Topical Treatment with NexGard® COMBO Against *Dipylidium caninum* Induced Infection in Cats. (Study No. 2020476)

Study Dates: December 15, 2020 to August 22, 2021

Study Location: Bloemfontein, Republic of South Africa

Study Design:

Objective: To confirm the effectiveness of NexGard® COMBO against *D. caninum*-induced infections in cats when applied once topically at the recommended minimum dose.

Study Animals: Sixteen cats (9 males and 7 females), 14 months to 7 years of age and weighing 2.1 to 4.7 kg one day before treatment.

Experimental Design: This study was conducted in accordance with GCP guidelines. Cats were experimentally infected using the intermediate host, fleas, known to be harboring *D. caninum*. All cats were diagnosed positive for dipylidiid proglottids prior to treatment, confirming that they were infected with dipylidiid cestodes. Cats were randomized into the following treatment groups of 8 cats each using a completely randomized design within each phase:

- Group 1: Mineral oil (control)
- Group 2: NexGard® COMBO

The study was conducted in five subsequent phases including cohorts of 4, 4, 4, 2, 2 cats, respectively.

Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Measurements and Observations: Cats were observed for adverse reactions hourly for approximately six hours and at 8, 12 and 24 hours post-treatment and daily thereafter. On Day 7, the animals were humanely euthanized and a necropsy was performed on the same day for parasite recovery and enumeration.

Statistical Methods:

The logarithm of the (counts + 1) was analyzed using the Mixed procedure in SAS 9.4 with treatment group as the fixed effect and the phase listed as a random effect.

Effectiveness was determined based on the percent reduction in worm counts in the NexGard® COMBO -treated group compared to the control group.

Percent Effectiveness = 100 x $(c_c - c_t)/c_c$
where c_c = Geometric mean of worm counts in the control group calculated from the back-transformed least squares mean from the MIXED procedure
c_t = Geometric mean of worm counts in the treated group calculated from the back-transformed least squares mean from the MIXED procedure

Statistical significance was determined based on a two-sided test for comparison of the treatment groups with $\alpha = 0.05$.

Results:

A minimum of two *D. caninum* scolicies was considered to be an adequate infection and a minimum of six adequately infected control cats was required for the study to be considered valid. Six of the eight control cats satisfied the adequacy of infection requirement.

The percent effectiveness using geometric means was 81.4% for the NexGard® COMBO treated group versus the control group, and the counts of *D. caninum* were not significantly lower in NexGard® COMBO treated group compared to the control group ($p = 0.0242$).

Table II.22. Study 2020476 Summary of Analyses of *D. caninum* Counts

Treatment Group	Geometric Mean	Percent Effectiveness	P-Value
Control	3.3	-	-
NexGard® COMBO	0.6	81.4	0.0242

Adverse Reactions: No adverse reactions related to treatment with NexGard® COMBO were observed.

Conclusion: A single topical treatment with NexGard® COMBO at the minimum recommended dose was 81.4% effective against induced infections with *D. caninum* in cats.

III. TARGET ANIMAL SAFETY

The safety of NexGard® COMBO was demonstrated in two well-controlled laboratory studies described below. The purpose of these studies was to provide information on the safety of NexGard® COMBO when used according to the label in cats. In the margin of safety study, one kitten receiving NexGard® COMBO at five times the labeled dose exhibited ataxia, dorsal recumbency, hypothermia (95.1°F), disorientation, tremors, lethargy, hypersalivation, and pupil dilation with slight response to light. This kitten received supportive care and appeared to be fully recovered within 48 hours. No other treatment-related effects were detected in any of the cats in this study. There were no treatment-related alterations in body weight, food consumption, water consumption, clinical pathology parameters (hematology, serum chemistry, coagulation, and urinalysis), or organ weights at any dose level.

In a separate study evaluating the pharmacokinetics of repeated monthly doses of the combined topical formulation at the target dose of 1.45 mg/kg esafoxolaner, 0.51 mg/kg eprinomectin, and 10.0 mg/kg praziquantel, steady state was reached by the fourth dose for esafoxolaner and after the second dose for eprinomectin and praziquantel, suggesting that in the margin of safety study, cats were exposed to steady state levels of esafoxolaner, eprinomectin, and praziquantel.

Oral tolerance was evaluated to assess the effects of accidental oral ingestion in cats, including from licking or grooming the application site. All cats orally administered NexGard® COMBO salivated profusely immediately after dosing. Hypersalivation was no longer present at the first hourly post-dose observation and afterwards. Correct application of NexGard® COMBO will minimize the occurrence of such events.
The safety of NexGard® COMBO in cats infected with adult *D. immitis* is established based on the study supporting the safety of Centragard® transdermal solution for cats; see Freedom of Information Summary for NADA 141-492. The concentration of eprinomectin in NexGard® COMBO is identical to the concentration of eprinomectin in Centragard® transdermal solution for cats (0.4% w/v eprinomectin).

These safety studies, in combination with the safety information collected in the effectiveness studies, demonstrate the safety of NexGard® COMBO when used according to the label.

A. Margin of Safety Study (PR&D 0363901)

Title: A Target Animal Safety and Local Tolerance Study of a Combination of ML-3,314,163, ML-3,179,374, and ML-1,653,648 when Administered Topically at 1, 3, or 5X the Maximum Exposure Dose Six Times at Four-Week Intervals in Kittens. (Study No. PR&D 0363901)

Study Dates: April 11, 2017 to May 1, 2019

Study Location: Ashland, Ohio

Study Design:

Objective: To evaluate the potential toxicity of the combination of esafoxolaner, eprinomectin, and praziquantel when administered to domestic kittens topically

six times at 4-week intervals, at one time (1X), 3 times (3X), and five times (5X) the maximum exposure dose.

Study Animals: Thirty-two (16 male and 16 female) kittens ranging in age from 8-9 weeks on Day -1 and weighing 698 g to 1156 g (1.54 to 2.55 lbs) were included in this study.

Experimental Design: The study was a placebo-controlled, randomized, masked laboratory study conducted in accordance with Good Laboratory Practice regulations. Animals were randomized to 4 study groups following a block design (based on pre-treatment bodyweight) stratified by sex.

Treatment Groups: Cats were dosed according to Table III.1 on Days 0, 28, 56, 84, 112, and 140. The final NexGard® COMBO formulation was used, containing 1.2% w/v esafoxolaner, 0.4% w/v eprinomectin, and 8.3% w/v praziquantel.

Table III.1. Treatment Groups for Study PR&D 0363901

Treatment Group Number	Control and Test Article	Test Article Dosage Level	Dose Volume (mL/kg)	Number of Animals Males	Number of Animals Female
1	Control Article (mineral oil)	0	1	4	4
2	NexGard® Combo	1x[a]	0.375	4	4
3	NexGard® Combo	3x[b]	1.125	4	4
4	NexGard® Combo	5x[c]	1.875	4	4

[a]the 1X dose provided: 4.5 mg/kg esafoxolaner, 1.5 mg/kg eprinomectin, 31.1 mg/kg praziquantel
[b]the 3X dose provided: 13.5 mg/kg esafoxolaner, 4.5 mg/kg eprinomectin, 93.4 mg/kg praziquantel
[c]the 5X dose provided: 22.5 mg/kg esafoxolaner, 7.5 mg/kg eprinomectin, 155.6 mg/kg praziquantel

Drug Administration: Doses were administered topically, in one spot directly on the skin on the midline of the neck between the base of the skull and the shoulder blades.

Measurements and Observations: Clinical observations were performed twice daily on non-dosing days, and on dosing days prior to dosing, within 10 minutes after dosing of the last animal, and at 1, 2, 3, 4, 6, and 8 hours (± 15 minutes) after dosing of the last animal. Following dosing on Days 84, 112, and 140, additional clinical observations were conducted on all cats at 9 and 10 hours (± 15 minutes) after dosing of the last animal. At each clinical observation, the eyes were examined and scored for pupillary dilation light reflex. More detailed physical observations were performed prior to treatment, weekly during the study, and at necropsy. The condition of the dosing application site was also documented. Detailed veterinary physical examinations were conducted prior to

treatment, on the day after each treatment, on the day of necropsy, and as needed to assess adverse events.

Body Weights: Animals were weighed prior to dosing and weekly during the study period, including the day before each dose administration, and prior to necropsies.

Food and Water Consumption: Food and water consumption were quantitatively measured daily starting on Day -1.

Clinical Pathology: Samples were obtained for hematology, serum chemistry profiles, coagulation parameters, and urinalysis testing prior to dosing, on the day after each dosing, and prior to necropsy.

A complete necropsy with organ weights and microscopic examinations was completed at the end of the study.

Statistical Methods:

Numerical variables were summarized descriptively for each group by study time point, where appropriate. Categorical variables were summarized using counts and percentages. All pair wise comparisons were two-sided and performed at a 0.10 significance level, unless specified. No adjustment was made for multiple comparisons. The experimental unit was the individual cat.

Incidence rates of adverse events were analyzed over the full study using Fisher's Exact test, where appropriate. In addition, pupil dilation scores from the first 48 hours post-treatment were analyzed using the highest pupil dilation score (most severe) as the analysis endpoint for each cat. If there were sufficient data for analysis, pupil dilation scores were statistically analyzed with a two-sided Mantel-Haenszel test. These analyses were also conducted for each treatment interval window as well as across the entire study.

The continuous variables measured repeatedly, such as respiration rate, body temperature, heart rate, and body weight, as well as serum chemistry, hematology values, and urine specific gravity, were analyzed using repeated measure analysis of covariance (RMANCOVA). The model included treatment, sampling day, sex, and all interactions among these effects as fixed effects, the weight block-within-sex group as the random effect, and the most recent measurement prior to the start of treatment as a covariate. The same model without the baseline covariate was used for the analysis of food and water intake. The covariance structure of the model was chosen based on the Akaike Information Criterion.

The continuous variables measured only once such as absolute organ weights and relative organ weight to body weight and relative organ weight to brain weight, were analyzed using analysis of variance (ANOVA) including treatment, sex, and the interaction term "treatment by sex" as fixed effects, and the weight block-within-sex as the random effect.

Results:

At approximately 9 hours after completion of the third treatment on Day 56, a 5X kitten exhibited ataxia, dorsal recumbency, hypothermia (95.1°F), disorientation, tremors, lethargy, hypersalivation, and pupil dilation with slight response to light. The application site was wiped off with water and later cleaned with chlorhexidine. Diazepam (0.4 mg/kg) and subcutaneous fluids were administered once and a heat lamp was provided. At a veterinary recheck on the following morning (Day 57), the kitten was mildly ataxic and otherwise normal. At the scheduled veterinary examination (approximately 24 hours after treatment), this kitten had decreased activity and pupil dilation. Clinical pathology results from the scheduled blood sampling did not reveal any abnormality. Mild pupil dilation with a decreased response to light and injected sclera were the only abnormalities observed in the afternoon of Day 57, and pupillary responses were back to normal on the morning of Day 58. This kitten did not exhibit any adverse reaction after its two previous and three subsequent 5X treatments.

No other treatment-related effects were detected in any of the cats in this study. There were no treatment-related alterations in body weight, food consumption, water consumption, clinical pathology parameters (hematology, serum chemistry, coagulation, and urinalysis), or organ weights at any dose level. Upon visual inspection of the treatment sites at necropsy, a dark red subcutaneous area (≤5mm diameter) was observed in three cats (2 males and 1 female) in the 5X group. Microscopic examination of these sites, however, revealed no histologic abnormalities.

Conclusion: This study supports the safe use of NexGard® COMBO transdermal solution in cats when dosed according to the label. Administration of 5 times the maximum exposure dose to kittens may result in adverse signs such as ataxia, disorientation, recumbency, tremors, lethargy, hypersalivation, and pupil dilation. With the lack of adverse clinical signs in the 1X and 3X dose groups, and only one cat being affected in the 5X group, the study demonstrated an adequate margin of safety.

B. Oral Tolerance Study (PR&D 0375101)

Title: A Study to Determine the Safety in Cats of the Topical Formulation NexGard® COMBO Administered Orally at the Maximum Dose. (Study No. PR&D 0375101)

Study Dates: October 5, 2017 to February 1, 2019

Study Location: Saint-Vulbas, France

Study Design:

Objective: To determine the safety in cats of the topical formulation NexGard® COMBO when administered orally once at the maximum label dose of 0.375 mL/kg.

Study Animals: Sixteen (8 male and 8 female) kittens, 7.4 to 8.9 weeks of age and weighing 0.6 to 1.0 kg (1.34 to 2.29 lbs) on Day -1, were included in this study.

Experimental Design: The study was a placebo-controlled, randomized, masked laboratory study conducted in accordance with Good Laboratory Practice regulations. Animals were randomized to the following two study groups following a block design (based on pre-treatment bodyweight) stratified by sex.

- Group 1: Sterile water (control)
- Group 2: NexGard® COMBO

Drug Administration: Treatment occurred on Day 0. Cats in Group 1 were orally administered 0.375 mL sterile water/kg body weight. Cats in Group 2 were orally administered NexGard® COMBO at 0.375 mL/kg, delivering, 4.5 mg/kg of esafoxolaner, 1.5 mg/kg of eprinomectin, and 31.1 mg/kg of praziquantel. The calculated amount of control or test article (0.375 mL/kg) was rounded up to the next 0.01 mL and drawn up into a syringe. Doses were administered by inserting the syringe into the mouth and expelling the contents aiming at the back of the tongue.

Measurements and Observations: Physical examinations were performed in an examination room by a veterinarian on Day -4; on Day 0, 6 hours (± 90 minutes) after dose administration; and on Days 1, 7, and 14. The examinations, included measurement of heart rate, rectal temperature, observation of the head (eyes, ears, mouth), and evaluation of the circulatory, respiratory, digestive, musculoskeletal, neurological, genital and skin/hair systems.

Cats were also observed by qualified technicians in their pens twice daily, from acclimation start (Day -7) to end of in-life, for general health and pupil dilation status. The floor of the pens were also observed for the presence of vomit or diarrhea. Additional observations were conducted 1, 2, 3, 4, 8, and 12 hours post-dosing on Day 0.

Body weights were measured on Days -4, 0, 1, 7, and 14.

Food consumption for each individual animal was measured daily from Day -7 to Day 14.

Blood and urine samples were collected on Days -1 and Day 14 for the determination of hematology, plasma chemistry profiles, and urinalysis.

Statistical Analysis: Blood biochemistry, hematology values, urinalysis parameter specific gravity, and daily average feed consumption were analyzed using analysis of covariance (ANCOVA) including treatment, sex, and treatment-by-sex interaction as fixed effects, and the pre-treatment measurement as a covariate. All pairwise comparisons used an unadjusted 0.10 significance level.

Results: All cats orally administered NexGard® COMBO salivated profusely immediately after dosing. Hypersalivation was no longer present at the first hourly post-dose observation and afterwards. These signs were attributed to oral administration of NexGard® COMBO. There were no clinically relevant differences

between the two groups in food consumption, body weight, and clinical pathology (hematology, serum chemistry, and urinalysis).

Conclusion: NexGard® COMBO administered orally at 0.375 mL/kg caused excessive salivation immediately after administration but was otherwise well-tolerated.

C. Safety in Cats Infected with Heartworms

The concentration of eprinomectin in NexGard® COMBO is identical to the concentration of eprinomectin in Centragard® transdermal solution for cats (0.4% w/v eprinomectin). Therefore, the safety of NexGard® COMBO when used in cats infected with adult *D. immitis* is established based on the study supporting the safety of Centragard® in this animal population. Refer to the Freedom of Information (FOI) Summary for Centragard® transdermal solution for cats (NADA 141-492) for detailed information on this study.

IV. HUMAN FOOD SAFETY

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

V. USER SAFETY

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

Not for human use. Keep this and all drugs out of the reach of children.

Avoid direct contact with application site for 4 hours or until visibly dry.

This product may act as a mild to moderate eye irritant.

Keep product in the original packaging until use. Wash hands after product administration. If the product accidentally gets into the eyes, rinse thoroughly with water. If wearing contact lenses, flush the eyes first with water and then remove the lenses and continue to flush thoroughly with water. In case of accidental ingestion, or if skin or eye irritation occurs, contact a poison control center or 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® COMBO, 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 (fourth stage larval and adult *Toxocara cati*), hookworm (fourth stage larval and adult *Ancylostoma tubaeforme*; adult *Ancylostoma braziliense*), and tapeworm (*Dipylidium caninum*) infections. NexGard® COMBO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations and the treatment and control of *Ixodes*

scapularis (black-legged tick) and *Amblyomma americanum* (lone star tick) infestations for one month in cats and kittens 8 weeks of age and older, and weighing 1.8 lbs or greater.

A. Marketing Status

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

B. Exclusivity

NexGard® COMBO 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 FD&C Act because the sponsor submitted an original NADA that contains new studies that demonstrate the safety and effectiveness of NexGard® COMBO.

C. Patent Information

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

VII. Appendix: Details of Correction

The indications on the title sheet of this summary, in the Executive Summary, under General Information, Section N, and in the Agency Conclusions was corrected to clarify the duration of the treatment and control indications.

Original Indications
NexGard® COMBO is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis.* NexGard® COMBO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations, the treatment and control of *Ixodes scapularis* (black-legged tick) and *Amblyomma americanum* (lone star tick) infestations, and the treatment and control of roundworms (fourth stage larval and adult *Toxocara cati*), hookworms (fourth stage larval and adult *Ancylostoma tubaeforme*; adult *Ancylostoma braziliense*), and tapeworms (*Dipylidium caninum*) in cats and kittens 8 weeks of age and older, and weighing 1.8 lbs or greater.

Corrected Indications, July 14, 2023
NexGard® COMBO (esafoxolaner, eprinomectin, and praziquantel topical solution) is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (fourth stage larval and adult *Toxocara cati*), hookworm (fourth stage larval and adult *Ancylostoma tubaeforme*; adult *Ancylostoma braziliense*), and tapeworm (*Dipylidium caninum*) infections. NexGard® COMBO kills adult fleas (*Ctenocephalides felis*) and is indicated for the treatment and prevention of flea infestations and the treatment and control of *Ixodes scapularis* (black-legged tick) and *Amblyomma americanum* (lone star tick) infestations for one month in cats and kittens 8 weeks of age and older, and weighing 1.8 lbs or greater.

The Dosage Regimen listed under the General Information was corrected to rectify rounding errors in the minimum dose of each active ingredient delivered as calculated from the dosing table.

Original Dosage Regimen
NexGard® COMBO is dosed at a minimum of 0.055 mL/lb (0.12 mL/kg), which delivers 0.66 mg/lb (1.45 mg/kg) esafoxolaner, 0.23 mg/lb (0.51 mg/kg) eprinomectin and 4.55 mg/lb (10.0 mg/kg) praziquantel.

Corrected Dosage Regimen, July, 14, 2023
NexGard® COMBO is dosed at a minimum of 0.055 mL/lb (0.12 mL/kg), which delivers 0.65 mg/lb (1.44 mg/kg) esafoxolaner, 0.22 mg/lb (0.48 mg/kg) eprinomectin and 4.53 mg/lb (9.98 mg/kg) praziquantel.

The Drug Administration section of the effectiveness study summaries was corrected to rectify rounding errors in the minimum dose of each active ingredient delivered as calculated from the dosing table.

Original Drug Administration (effectiveness study summaries)
Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.45 mg/kg of esafoxolaner, 0.51 mg/kg of eprinomectin, and 10.0 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Corrected Drug Administration (effectiveness study summaries), July, 14, 2023
Drug Administration: Cats in Group 1 were topically administered 0.12 mL/kg control. Cats in Group 2 were topically administered 0.12 mL/kg NexGard® COMBO, delivering, 1.44 mg/kg of esafoxolaner, 0.48 mg/kg of eprinomectin, and 9.98 mg/kg of praziquantel. A single spot was applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.