Date of Approval: September 14, 2013

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-406

NEXGARD

Afoxolaner

Chewable Tablet

Dogs

NEXGARD kills adult fleas and is indicated for the treatment and prevention of flea infestations (*Ctenocephalides felis*), and the treatment and control of American Dog tick (*Dermacentor variabilis*) infestations in dogs and puppies 8 weeks of age and older, weighing 4 pounds of body weight or greater, for one month.

Sponsored by:

Merial Ltd.

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I. GENERAL INFORMATION

A. File Number

NADA 141-406

B. Sponsor

Merial Ltd.
3239 Satellite Blvd., Bldg. 500
Duluth, GA 30096-4640

Drug Labeler Code: 050604

C. Proprietary Name

NEXGARD

D. Established Name

Afoxolaner

E. Pharmacological Category

Antiparasitic

F. Dosage Form:

Chewable tablet

G. Amount of Active Ingredient:

Each chewable contains 11.3 mg, 28.3 mg, 68 mg, or 136 mg afoxolaner.

H. How Supplied:

NEXGARD is available in four sizes of beef-flavored, soft chewables: 11.3, 28.3, 68, or 136 mg afoxolaner. Each chewable size is available in color-coded packages of 3 or 6 beef-flavored chewables.

I. Dispensing Status

Rx

J. Dosage Regimen

NEXGARD is given orally once a month, at the minimum dosage of 1.14 mg/lb (2.5 mg/kg).

Dosing schedule:

Body Weight	Afoxolaner Per Chewable (mg)	Chewables Administered
4.0 to 10.0 lbs.	11.3	One
10.1 to 24.0 lbs.	28.3	One
24.1 to 60.0 lbs.	68	One
60.1 to 121.0 lbs.	136	One
Over 121.0 lbs.	Administer the appropriate combination of chewables	Administer the appropriate combination of chewables

K. Route of Administration

Oral

L. Species/Class

Dogs

M. Indications

NEXGARD kills adult fleas and is indicated for the treatment and prevention of flea infestations (*Ctenocephalides felis*), and the treatment and control of American Dog tick (*Dermacentor variabilis*) infestations in dogs and puppies 8 weeks of age and older, weighing 4 pounds of body weight or greater, for one month.

II. EFFECTIVENESS

A. Dosage Characterization

The minimum dose (2.5 mg/kg) was selected based on three exploratory laboratory effectiveness studies. The studies were conducted to evaluate the pharmacokinetics and determine the lowest effective dose of NEXGARD using afoxolaner in an experimental oral solution formulation over a dosage range of 0.75 to 3.5 mg/kg. Doses were administered under fed conditions (the 2.5 mg/kg dose included an additional group of dogs administered the drug in the fasted state). Effectiveness was demonstrated by challenging animals with adult fleas (*Ctenocephalides felis*) and ticks (*Dermacentor variabilis* or *Rhipicephalus sanguineus*) following treatment with NEXGARD. Arithmetic means were used to evaluate effectiveness results of all three studies.

The first dose-ranging study evaluated the effectiveness of 1.5, 2.5, and 3.5 mg/kg. Single oral dosages of NEXGARD at 2.5 mg/kg demonstrated 100% effectiveness against fleas for 28 days and ≥ 96% effectiveness against *D. variabilis* ticks for 30 days post-treatment. Plasma concentrations averaged 0.18 µg/mL 33 days post-treatment at the 2.5 mg/kg dose level, and were similar under fed or fasted conditions.

The second dose-ranging study also evaluated the effectiveness of single oral doses of 1.5, 2.5, and 3.5 mg/kg afoxolaner. A dose of 2.5 mg/kg showed similar effectiveness and pharmacokinetic properties as the first study with 100% effectiveness against fleas for 32 days and ≥ 95% effectiveness against ticks (*R. sanguineus*) for 30 days post-treatment. Plasma concentrations averaged 0.18 µg/mL 33 days post-treatment at the 2.5 mg/kg dose level.

In the third study, Beagles administered a single oral dose of 0.75, 1.5, 2.5, or 3.5 mg/kg afoxolaner confirmed that plasma concentrations greater than 0.08 µg/ml were 100% effective against fleas. The 0.75 mg/kg dosage was shown to be too low to maintain the desired flea effectiveness for a month after a single dose. The 2.5 mg/kg dosage maintained a 0.1 µg/mL mean plasma concentration 28 days post-treatment and an average of 99.8% effectiveness against fleas on day 31 post-treatment.

Based on the results of these studies, a minimum dose of 2.5 mg/kg was selected for further development.

B. Substantial Evidence

1. For the Treatment and Prevention of Flea Infestations:

a. Laboratory Dose Confirmation Study PR&D 0232901

Title: Efficacy and Prevention of Infestation of ML-3,663,925 Against Induced Infestations of Adult Fleas (*Ctenocephalides felis)* on Dogs After a Single Oral Dose Administered to Achieve at Least 2.5 mg/kg

(1) Investigator:

David R. Young, DVM, PhD
Turlock, CA

(2) Study Design:

(a) Objective:

Confirm the effectiveness of a single oral dose (at least 2.5 mg/kg) of afoxolaner for the treatment and prevention of induced adult *C. felis* infestations on dogs.

(b) Study Animals:

32 Beagle dogs (22 males, 10 females), 12-168 months of age, weighing between 9.1-19.1 kg.

(c) Treatment Groups:

Table 1: Treatment groups for Study PR&D 0232901.

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (untreated)	Once on Day 0	16 (13 M, 3 F)
2	2.5 mg/kg	NEXGARD	Once on Day 0	16 (9 M, 7 F)

(d) Drug Administration:

All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Day 0, dogs were fed 4 hours after treatment administration.

(e) Measurements and Observations:

Physical examinations were conducted on Day -7. On Days -1, 7, 14, 21, 28, and 35 each dog was infested with 100 ±5 unfed adult fleas *(C. felis)*. Dogs were treated on Day 0, followed by flea and flea egg collections 12 and 24 hours post-treatment. Twelve and 24 hours after each infestation date, flea comb counts and flea egg collections were conducted. General health observations were conducted at least once daily for all dogs. On Day 0, post-dosing clinical observations were conducted hourly for the first four hours post-dose for evidence of vomiting or other adverse events.

(f) Statistical Methods:

For flea and egg counts, percent effectiveness of the treated group with respect to the control group was calculated using the formula $[(C - T)/C]x100$, where C = geometric mean for the control group and T = geometric mean for the treated group for each time point. The comparisons were tested using the (two-sided) 5% significance level. The mixed model analysis was used to analyze log-counts, with treatment group as a fixed effect and the allocation blocks as random effects.

(3) Results:

NEXGARD was ≥ 93% effective at 12-hours post-infestation through Day 21, and on Day 35. On Day 28, NEXGARD was 81.1% effective 12 hours post-infestation (Table 2). NEXGARD was 100.0% effective at 24 hours post-infestation up through Day 35 (Table 3). Live flea counts were significantly reduced ($P \leq 0.003$) for each of the 12- and 24-hour post-infestation points in comparison with the control group. Flea egg counts were also significantly reduced ($P \leq 0.028$) at each of the 12- and 24-hour post-infestation points in comparison with the control group (Tables 4 and 5).

Table 2: Geometric mean of adult flea counts and percent effectiveness of NEXGARD for the treatment of induced *C. felis* infestations of dogs, 12 hours after infestation.

Days After Treatment[1]	Control Group Geometric Mean	NEXGARD Group Geometric Mean	Percent Effectiveness at 12 Hours Post-infestation
0	78.1	0.6	99.2
7	85.2	4.1	95.2
14	78.8	1.9	97.5
21	86.2	2.0	97.7
28	82.6	15.6	81.1
35	93.8	6.5	93.0

[1]For Day 0, fleas were applied to the dogs on Day -1.

Table 3: Geometric mean of adult flea counts and percent effectiveness of NEXGARD for the treatment of induced *C. felis* infestations of dogs, 24 hours after infestation.

Days After Treatment[1]	Control Group Geometric Mean	NEXGARD Group Geometric Mean	Percent Effectiveness at 24 Hours Post-infestation
0	60.9	0.0	100.0
7	72.2	0.0	100.0
14	81.8	0.0	100.0
21	72.4	0.0	100.0
28	76.2	0.0	100.0
35	80.9	0.0	100.0

[1]For Day 0, fleas were applied to the dogs on Day -1.

Table 4: Geometric mean of *C. felis* flea egg counts and range on dogs, 12 hours after infestation.

Days After Treatment[1]	Control Group Geometric Mean (Range)	NEXGARD Group Geometric Mean (Range)
0	15.3 (4-90)	1.7 (0-11)
7	13.4 (4-47)	0.0 (0)
14	21.7 (9-62)	0.2 (0-1)
21	18.8 (3-81)	0.1 (0-1)
28	20.1 (6-56)	0.0 (0)
35	10.7 (3-32)	0.0 (0)

[1]For Day 0, fleas were applied to the dogs on Day -1.

Table 5: Geometric mean of *C. felis* flea egg counts and range on dogs, 24 hours after infestation.

Days After Treatment[1]	Control Group Geometric Mean (Range)	NEXGARD Group Geometric Mean (Range)
0	27.2 (0-118)	3.9 (1-17)
7	25.5 (11-141)	0.0 (0)
14	39.7 (8-133)	0.1 (0-1)
21	38.5 (11-108)	0.0 (0)
28	22.8 (7-69)	0.0 (0)
35	16.7 (1-124)	0.0 (0)

[1]For Day 0, fleas were applied to the dogs on Day -1.

(4) Adverse Reactions:

One treated dog had diarrhea within the first hour post-treatment.

(5) Conclusions:

This study confirmed the 30-day effectiveness of a single monthly dose of NEXGARD for the treatment of adult *C. felis* infestations on dogs 24 hours post-infestation. Because the study demonstrated 81.1% effectiveness 12 hours post-infestation on Day 28, the study did not confirm 30-day effectiveness at 12 hours post-infestation. The study also confirmed that for new flea infestations, NEXGARD killed adult fleas before they can lay eggs, thus contributing to the prevention of flea infestations.

b. Field Study PR&D 02341

Title: Safety and Effectiveness of Monthly Oral Treatments With a Novel Ectoparasiticide Against Flea Infestations (*Ctenocephalides felis)* on Dogs Under Field Conditions with Additional Consideration of Tick Infestations

(1) Investigators:

Robert Zielinski, DVM, San Bernadino, CA
Carl Eric Fulton, DVM, Greenbrier, AR
Larry Hendricks, DVM, Germantown, MD
W. Joey Gross, DVM, Bogart, GA
Becky E. Marks, DVM, Portland, OR
Daniel Renfro, DVM, Richmond, MO
Edward Jezbera, DVM, Riverside, CA
Kinsey Phillips, DVM, Commerce, GA
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Kathy Taylor, DVM, Shelbyville, KY
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Edward J. Migneco, DVM, St. Louis, MO
Kevin R. Shimanek, DVM, Enid, OK

Of the 17 sites, one site was invalidated due to lack of protocol compliance and one site did not enroll any animals. A third site did not enroll enough evaluable households for evaluation of effectiveness but was used for evaluation of safety. Thus, 15 sites were used for safety evaluation, while 14 sites were used for assessing effectiveness.

(2) Study Design:

This was a masked, multi-center, field safety and effectiveness study using a randomized block design based on order of enrollment of households comparing NEXGARD (afoxolaner) to an active control, spinosad. Households were recruited from 17 different sites in 4 distinct geographical regions across the United States to enroll dogs for this study. Using a randomized block design for each study site, blocks of three households were formed based on order of enrollment. Within blocks, and because of the minimum age that can be treated with the active control, each household in which all dogs were ≥14 weeks of age was randomly allocated to one of two treatment groups in a 2:1 overall ratio of NEXGARD (afoxolaner; Treatment Group 1) to spinosad (active control; Treatment Group 2). In households that had a dog between 8 and 14 weeks of age, the household was automatically assigned to the afoxolaner group. Treatments were administered on, or within 4 days of, Days 0, 30, and 60, with flea counts performed prior to treatment on Visit 1 (Day 0), and on Visit 2 (Day 30), Visit 3 (Day 60), and Visit 4 (Day 90). Ticks, if present, were counted and removed.

(a) Objective:

The primary study objectives were to assess the safety and effectiveness of afoxolaner against natural infestations of the cat flea (*Ctenocephalides felis*) on dogs when used monthly for three consecutive months, under field conditions. The secondary objective was to evaluate tick counts collected as secondary indicators of effectiveness.

(b) Study Animals:

415 NEXGARD-treated dogs and 200 spinosad-treated dogs were evaluated for safety. The effectiveness analysis for Day 30 was performed on 186 NEXGARD-treated dogs and 96 spinosad-treated dogs.

Healthy dogs from households with up to five dogs and cats were included. For a household to be included, at least one dog (sentinel dog) had to harbor ≥10 fleas. In a household where more than one dog had at least 10 fleas, the dog to be designated the sentinel dog was randomly selected. In multiple dog households, the sentinel dog was assessed for effectiveness and safety and the non-sentinel dogs were only assessed for safety. All cats in the household were treated with a EPA-registered flea control product. All dogs (sentinel and non-sentinel) and cats in a household had to participate in the study, and all dogs in the household were treated with the same investigational or control product.

(c) Treatment Groups:

Table 6: Treatment groups for Study PR&D 02341.

Treatment Group	Dose, Route, and Frequency
NEXGARD	≥ 2.5 mg/kg afoxolaner orally, per label dose bands, once monthly for 3 consecutive months
Spinosad	≥ 30 mg/kg spinosad orally, per label dose bands, once monthly for 3 consecutive months

(d) Drug Administration:

Owners dosed their dogs (sentinel and non-sentinel) with NEXGARD once monthly with or without food for three consecutive months. Owners were instructed to administer spinosad with food.

(e) Measurements and Observations:

Physical examinations, body weights, and flea counts were performed on all dogs in the household at the first visit. Physical examinations and flea counts on the sentinel dog were then performed on visit 2 (~Day 30), visit 3 (~Day 60), and visit 4 (~Day 90). Monthly flea counts from the sentinel dog in each household were used to evaluate effectiveness. Tick counts were evaluated as secondary indicators of effectiveness.

(f) Statistical Methods:

For flea counts, the effectiveness evaluation of the treated group with respect to the control group was based on the transformed variable log(count + 1). The percent effectiveness was calculated using the formula 100x[(C-T)/C], where C = geometric mean of the baseline count (anti-logarithm of the least square mean minus 1 from the analysis below) and T = geometric mean of the visit day count (antilogarithm of the least square mean minus 1).

(3) Results:

Both afoxolaner-treated dogs and spinosad-treated dogs showed a statistically-significant ($p<0.001$) reduction in fleas from baseline (pre-treatment; Visit 1) to the end of the study, and both showed $\geq$90% effectiveness, as shown in Table 7 below.

Table 7: Geometric mean live flea counts and percent reduction compared to baseline (Visit 1).

Treatment Group	Visit 1 (Pre-treatment)	Visit 2 (Day 30)	Visit 3 (Day 60)	Visit 4 (Day 90)
Afoxolaner	(26.5)	98.0% (0.5)	99.7% (0.1)	99.9% (0)
Spinosad	(27.3)	95.7% (1.2)	99.4% (0.2)	99.7% (0.1)

For all comparisons of the post-treatment visit days with baseline, the difference was statistically significant ($p<0.001$) for all days for both treatment groups.

At the end of the study (Day 90), there were no ticks on dogs in either group; however, there were insufficient dogs with pre-existing tick infestations, and low numbers of ticks, to derive any conclusions.

(4) Adverse Reactions:

Evaluation of safety was completed over the 90-day period (Visits 2, 3, and 4) through in-clinic physical examinations or through reporting of abnormalities by the owner for both sentinel and non-sentinel dogs. The safety database included 415 dogs administered afoxolaner and 200 dogs administered spinosad. No serious adverse reactions were attributed to afoxolaner or spinosad. Adverse reactions that were reported at an incidence of greater than 1% are provided in Table 8. The most frequently reported finding in dogs in both groups was vomiting. The occurrence of vomiting was generally self-limiting and of short duration and tended to decrease with subsequent doses in both groups. The incidence of vomiting in both groups tended to decrease with subsequent doses. Five treated dogs experienced anorexia during the study, and two of those dogs experienced anorexia with the first dose but not subsequent doses.

Table 8: Dogs with adverse reactions.

Adverse Reactions	Afoxolaner N[1]	Afoxolaner % (n=415)	Spinosad N[2]	Spinosad % (n=200)
Vomiting (with and without blood)	17	4.1	25	12.5
Dry/ Flaky Skin	13	3.1	2	1.0
Diarrhea (with and without blood)	13	3.1	7	3.5
Lethargy	7	1.7	4	2.0
Anorexia	5	1.2	9	4.5

[1]Number of dogs in the afoxolaner treatment group with the identified abnormality.
[2]Number of dogs in the control group.

One dog with a history of seizures experienced a seizure on the same day after receiving the first dose and on the same day after receiving the second dose of NEXGARD. This dog experienced a third seizure one week after receiving the third dose. The dog remained enrolled and completed the study. Another dog with a history of seizures had a seizure nineteen days after the third dose of NEXGARD. The dog remained enrolled and completed the study. A third dog with a history of seizures received NEXGARD and experienced no seizures throughout the study.

(5) Conclusions:

The results of this study demonstrate that NEXGARD, when used monthly at the minimum labeled dose of 2.5 mg/kg, is safe and effective for the treatment and prevention of flea infestations in dogs under field conditions. Vomiting was the most commonly reported adverse reaction. Two dogs with a history of seizures experienced seizure activity during this study.

2. Prevention of Flea Infestations:

a. Overall conclusions on the prevention of flea infestations indication

In the laboratory dose confirmation study (PR&D Study 0232901), all dogs were infested with fleas on Day 0, 24 hours prior to treatment with NEXGARD. During this time, the fleas had the ability to initiate feeding, begin mating and start laying eggs, which was confirmed as dogs in both the treated and control groups generated eggs (0-11 eggs and 1-17 eggs at 12- and 24-hours, and 4-90 eggs and 0-118 eggs at 12- and 24-hours post-treatment, respectively). At subsequent evaluations, fleas from dogs in the treated group were essentially unable to produce any eggs (0-1 eggs), while fleas from dogs in the control group continued to produce eggs (1-141 eggs). Therefore, any adult fleas that hatched from the eggs generated immediately post-treatment and infested a treated dog would be killed, thus inhibiting the perpetuation of the flea infestation. Because female fleas initiate egg laying 24 to 36 hours after their initial blood meal (Dryden, 1989[1]), any subsequent generations of adult fleas that infested a treated dog would be killed before they are able to lay eggs, thus inhibiting the perpetuation of the flea infestation.

Additionally, the field study supporting substantial evidence of effectiveness for the flea indication (PR&D Study 02341) was conducted in households with existing flea infestations of varying severity. After NEXGARD was administered, and the initial flea kill (treatment indication) occurred, dogs were likely re-infested with adult fleas generated from the pre-adult stages already present in the environment. At that point, the study evaluated the perpetuation of flea infestations (prevention indication). Because the field study demonstrated continued effectiveness of NEXGARD against fleas, this study confirmed that NEXGARD inhibited flea infestations on dogs.

Collectively, data from the laboratory dose confirmation (Study PR&D 0232901) and field effectiveness study (PR&D Study PR&D 02341) support a 30-day effectiveness of a single monthly dose of NEXGARD for the prevention of flea (*C. felis*) infestations on dogs.

[1] Dryden, M.W. 1989. Biology of the cat flea, *Ctenocephalides felis felis*. Companion Anim. Prac. 19:23-27

3. For the Treatment and Control of Tick Infestations:

a. Laboratory Dose Confirmation Study PR&D 0233201

Title: Efficacy of ML-3,663,925 Against Induced Infestations of Adult *Dermacentor variabilis* on Dogs After a Single Oral Dose Administered to Achieve at Least 2.5 mg/kg

(1) Investigator:

William R. Everett, PhD
Greenbrier, AR

(2) Study Design:

(a) Objective:

Confirm the effectiveness of a single oral dose (at least 2.5 mg/kg) of afoxolaner for the treatment and control of induced infestations of adult stages of *D. variabilis* on dogs.

(b) Study Animals:

16 Beagle dogs (6 males, 10 females), 6.1-7.9 months of age, weighing between 5.7-9.3 kg.

(c) Treatment Groups:

Table 11: Treatment groups for Study PR&D 0233201.

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (untreated)	Once on Study Day 0	8 (3 M, 5 F)
2	2.5 mg/kg	NEXGARD	Once on Study Day 0	8 (3 M, 5 F)

(d) Drug Administration:

All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Day 0, dogs were fed 4 hours after treatment administration.

(e) Measurements and Observations:

Physical examinations were conducted on Day -12. On Days -1, 7, 14, 21, 28, and 35 each dog was infested with 50 ±5 unfed adult ticks *(D. variabilis).* Twenty-four hours after infestation, dead ticks were counted from collection pans beneath each animal cage. At 48-hours post-infestation live and dead ticks were removed and counted from individual animals, and dead ticks were counted from collection pans. For the Day -1 infestation, ticks found dead in the collection pans were counted at 48 and 72 hours post-infestation. General health observations were conducted at least once daily for all dogs. On Day 0, post-dosing clinical observations were conducted hourly for the first four hours post-dose for evidence of vomiting or other adverse events.

(f) Statistical Methods:

For live tick counts, percent effectiveness of the treated group with respect to the control group was calculated using the formula [(C − T)/C]x100, where C = geometric mean for the control group and T = geometric mean for the treated group for each time point. For dead tick counts, the formula was reversed as [(T - C)/T]x100. The comparisons were tested using the (two-sided) 5% significance level. The mixed model analysis was used to analyze log-counts, with treatment group as a fixed effect and the allocation blocks as random effects.

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

(3) Results:

NEXGARD was ≥97.3% effective against live ticks at 48 hours post-infestation through Day 30 (Table 12). Live tick counts were significantly reduced ($P \leq 0.002$) following each of the infestation time points in comparison with the control group. Total dead tick counts were significantly increased ($P \leq 0.002$, Table 13) in comparison with the control group following each tick infestation.

A minimum of 25% of the original ticks used to infest the animal at each time point evaluated was considered to be an adequate infestation, and a minimum of six adequately infested control dogs was required for the study to be considered valid. An adequate infestation was achieved for all time points evaluated.

Table 12: Geometric mean live tick counts and percent effectiveness of NEXGARD for the control of induced *D. variabilis* infestations of dogs, 48 hours after infestation.

Days After Treatment	Control Group Geometric Mean	NEXGARD Group Geometric Mean	Percent Effectiveness at 48 Hours Post-infestation
2	40.1	0.0	100.0
9	40.3	0.0	100.0
16	33.3	0.0	100.0
23	33.9	0.1	99.7
30	29.3	0.8	97.3
37	22.7	2.5	88.9

Table 13: Geometric mean dead tick counts of NEXGARD for the treatment of induced *D. variabilis* infestations of dogs, 48 hours after infestation.

Days After Treatment	Control Group Geometric Mean	NEXGARD Group Geometric Mean
2	0.3	40.4
9	2.4	35.0
16	2.5	35.6
23	2.5	29.9
30	3.4	24.2
37	3.9	14.8

(4) Adverse Reactions:

There were no adverse reactions during this study.

(5) Conclusions:

NEXGARD was >97% effective against adult *D. variabilis*, when measured 48 hours after infestation, for 30 days. The increased number of dead ticks and the reduction of live ticks support the treatment and control indication for *D. variabilis*, respectively.

b. Laboratory Dose Confirmation Study PR&D 0233202

Title: Efficacy of ML-3,663,925 Against Induced Infestations of Adult *Dermacentor variabilis* on Dogs After a Single Oral Dose Administered to Achieve at Least 2.5 mg/kg

(1) Investigator:

William R. Everett, PhD
Greenbrier, AR

(2) Study Design:

(a) Objective:

Confirm the effectiveness of a single oral dose (at least 2.5 mg/kg) of afoxolaner for the treatment and control of induced infestations of adult stages of *D. variabilis* on dogs.

(b) Study Animals:

16 Beagle dogs (6 males, 10 females), 7.6-9.8 months of age, weighing 5.9-9.6 kg.

(c) Treatment Groups:

Table 14: Treatment groups for Study PR&D 0233202.

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (untreated)	Once on Study Day 0	8 (5 M,3 F)
2	2.5 mg/kg	NEXGARD	Once on Study Day 0	8 (5 M, 3 F)

(d) Drug Administration:

All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Day 0, dogs were fed 4 hours after treatment administration.

(e) Measurements and Observations:

Physical examinations were conducted on Day -8. On Days -1, 7, 14, 21, 28, and 35 each dog was infested with 50 ±5 unfed adult ticks *(D. variabilis).* Twenty-four hours after infestation, dead ticks were counted from collection pans beneath each animal cage. At 48-hour post-infestation live and dead ticks were removed and counted from individual animals, and dead ticks were counted from collection pans. For the Day -1 infestation, ticks found dead in the collection pans were counted at 48 and 72 hours post-infestation. General health observations were conducted at least once daily for all dogs. On Day 0, post-dosing clinical observations were conducted hourly for the first four hours post-dose for evidence of vomiting or other adverse events.

(f) Statistical Methods:

For live tick counts, percent effectiveness of the treated group with respect to the control group was calculated using the formula [(C − T)/C]x100, where C = geometric mean for the control group and T = geometric mean for the treated group for each time point. For dead tick counts, the formula was reversed as [(T - C)/T]x100. The comparisons were tested using the (two-sided) 5% significance level. The mixed model analysis was used to analyze log-counts, with treatment group as a fixed effect and the allocation blocks as random effects.

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

(3) Results:

NEXGARD was ≥98.5% effective against live ticks at 48 hours post-infestation through Day 30 (Table 15). Live tick counts were significantly reduced ($P \leq 0.006$) following each of the infestation time points in comparison with the control group. Total dead tick counts were significantly increased ($P \leq 0.001$, Table 16) in comparison with the control group following each tick infestation.

A minimum of 25% of the original ticks used to infest the animal at each time point evaluated was considered to be an adequate infestation, and a minimum of six adequately infested control dogs was required for the study to be considered valid. An adequate infestation was achieved for all time points evaluated.

Table 15: Geometric mean live tick counts and percent effectiveness of NEXGARD for the control of induced *D. variabilis* infestations of dogs, 48 hours after infestation.

Days After Treatment	Control Group Geometric Mean	NEXGARD Group Geometric Mean	Percent Effectiveness at 48 Hours Post-infestation
2	40.0	0.0	100.0
9	29.1	0.0	100.0
16	26.2	0.0	100.0
23	27.0	0.1	99.7
30	32.7	0.5	98.5
37	24.2	2.7	88.8

Table 16: Geometric mean dead tick counts of NEXGARD for the treatment of induced *D. variabilis* infestations of dogs, 48 hours after infestation.

Days After Treatment	Control Group Geometric Mean	NEXGARD Group Geometric Mean
2	0.6	46.4
9	7.1	40.8
16	5.1	36.1
23	4.5	29.2
30	2.0	27.0
37	2.1	19.4

(4) Adverse Reactions:

There were no adverse reactions during this study.

(5) Conclusions:

NEXGARD was >98% effective against adult *D. variabilis*, when measured 48 hours after infestation, for 30 days. The increased number of dead ticks and the reduction of live ticks support the treatment and control indication for *D. variabilis*, respectively.

III. TARGET ANIMAL SAFETY:

A. Margin of Safety Study PR&D 0234001

Title: Safety of ML-3,663,925 When Administered Orally at 1, 3 and 5X the Maximum Exposure Dose (i.e. 6.3 mg/kg) in Eight-Week-Old Puppies at Three, One Month Dose Intervals and Three, Two Week Dose Intervals.

1. Investigator:

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2. Study Design:

a. Objective:

Evaluate the margin of safety of afoxolaner when administered to eight-week-old puppies at 1, 3, or 5X the maximum exposure dose of 6.3 mg/kg at three, one month-dose intervals and three, two-week dose intervals.

b. Study Animals:

32 Beagle dogs (16 males, 16 females), 8 to 9 weeks of age, weighing between 2.3 and 4 kg.

c. Treatment Groups:

Table 17: Treatment groups for Study PR&D 0234001.

Treatment Group	Dose
1	0 mg/kg
2	6.3 mg/kg (1X)
3	18.9 mg/kg (3X)
4	31.5 mg/kg (5X)

d. Drug Administration:

All treatments were administered orally with food for three monthly treatments on Days 0, 28, and 56, followed by three bi-weekly treatments on Days 84, 98, and 112, for a total of six treatments per dog. Dogs in the control group were sham-dosed. Treatments were administered to dogs in Groups 3 and 4 as two approximately equal doses, 3 hours apart. The dogs were fed portions of their daily ration immediately before, during, and after each dosing.

e. Measurements and Observations:

All animals were observed twice daily, except on treatment days. On treatment days, because of the divided dosing schedule, dogs were observed for abnormal clinical signs continuously for the first 15 minutes after the first dosing, and at least hourly thereafter for approximately three hours. Following the second dosing, dogs were similarly observed for abnormal clinical signs for at least four hours post-treatment. Physical examinations, body weights, and food consumption were recorded during the study.

Clinical pathology (hematology, clinical chemistry, and coagulation parameters) were evaluated twice pre-treatment and on Days 14, 27, 42, 55, 70, 83, 97, 111, and 125. Pre-dose blood samples were collected for plasma afoxolaner concentration measurements on Days 14, 27, 55, 83, 97, 111, 112 (3 hours after second treatment), and 125. Plasma samples were assayed using a validated LC/MS/MS method. Urinalyses were performed pre-treatment and on Days 27 and 126. On Day 126, two weeks following the last treatment, all dogs were euthanized and necropsied. Organ weights, and any gross pathology were recorded, and tissues were collected for histopathology.

f. Statistical Methods:

The physical exam variables (respiration rate, temperature, heart rate, and body weight), continuous clinical pathology values (with the exception of percentage values for white blood cell differentials), and urinalysis (specific gravity from refractometer only) were analyzed by repeated measures analysis of covariance (RMANCOVA) including treatment, sampling day, sex, and the interaction terms "treatment by sex," "treatment by sampling day," "sex by sampling day," and "treatment by sex by sampling day" as fixed effects. The "cohort" and "block within cohort by sex" terms were the random effects. The covariate was the most recent baseline measurement.

3. Results:

There were no deaths during the course of the study. There were no clinically relevant treatment-related changes in daily food consumption, body weight, physical examination variables, clinical pathology (hematology, clinical chemistries, or coagulation tests), gross pathology, histopathology, or organ weights. Vomiting occurred throughout the study, with a similar incidence across all groups, including one dog in the 5X group that vomited four hours after treatment. Plasma ML-3,663,925 concentrations increased throughout the study. Following the three monthly doses, plasma afoxolaner concentrations were less than proportional with dose administered. Following the three doses administered every 2 weeks, plasma afoxolaner concentrations on Day 125 were dose proportional up to 3X and more than dose proportional at 5X.

4. Conclusions:

Afoxolaner is well tolerated when administered to 8-week old Beagle dogs at 1, 3, or 5X the maximum potential exposure dose of 6.3 mg/kg at three, 28-day dose intervals followed by three, 2-week dose intervals for a total of 6 doses.

IV. HUMAN FOOD SAFETY:

This drug is intended for use in dogs, which are non-food animals. 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:

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

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 and 21 CFR part 514. The data demonstrate that NEXGARD, when used according to the label, is safe and effective for killing adult fleas and for the treatment and prevention of flea infestations (*Ctenocephalides felis*), and treatment and control of American Dog tick (*Dermacentor variabilis*) infestations in dogs and puppies 8 weeks of age and older, weighing 4 pounds of body weight or greater, for one month.

A. Marketing Status:

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise is needed to monitor for and respond to adverse reactions.

B. Exclusivity:

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of the approval because no active ingredient of the new animal drug has previously been approved.

C. Patent Information:

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