Date of Approval: July 2, 2019

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-519

ProHeart® 12

(moxidectin)

Dogs

For use in dogs 12 months of age and older for the prevention of heartworm disease caused by *Dirofilaria immitis* for 12 months. For the treatment of existing larval and adult hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) infections.

Sponsored by:

Zoetis Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-519

B. Sponsor

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

Drug Labeler Code: 054771

C. Proprietary Name

ProHeart® 12

D. Drug Product Established Name

Moxidectin

E. Pharmacological Category

Antiparasitic

F. Dosage Form

Sterile Suspension

G. Amount of Active Ingredient

Each mL of constituted suspension contains 10 mg moxidectin

H. How Supplied

ProHeart® 12 consists of two separate vials that require mixing prior to administration or use. One vial contains 10% moxidectin sterile microspheres and the second vial contains sterile vehicle for constitution; only this sterile diluent should be used for the constitution. The constituted suspension is ready for administration 30 minutes after mixing.

ProHeart® 12 is available in two sizes:

10 mL size (889 mg/vial moxidectin plus 8 mL/vial sterile vehicle)
50 mL size (4444 mg/vial moxidectin plus 40 mL/vial sterile vehicle)

I. Dispensing Status

Rx

J. Dosage Regimen

The recommended subcutaneous dose is 0.05 mL of the constituted suspension/kg body weight (0.023 mL/lb). This amount of suspension will provide 0.5 mg moxidectin/kg body weight (0.23 mg/lb). To ensure accurate dosing, calculate each dose based on the dog's weight at the time of treatment.

K. Route of Administration

Subcutaneous injection

L. Species/Class

Dogs 1 year of age and older

M. Indication

ProHeart® 12 is indicated for use in dogs 12 months of age and older for the prevention of heartworm disease caused by *Dirofilaria immitis* for 12 months.

ProHeart® 12 is indicated for the treatment of existing larval and adult hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) infections.

II. EFFECTIVENESS

The effectiveness of ProHeart® 12 administered at 0.23 mg/lb (0.5 mg/kg) body weight for the prevention of heartworm disease caused by *Dirofilaria immitis* was demonstrated in two laboratory dose confirmation studies and a field study which enrolled 297 client owned dogs that were administered the product. All studies demonstrated the product to be 100% effective in the prevention of heartworm disease caused by *Dirofilaria immitis* for twelve months. Adverse events observed in the field study included vomiting, diarrhea, anorexia, and seizures. Adverse events occurred at a similar rate to that observed in the control group. The effectiveness of ProHeart® 12 for the treatment of existing larval and adult hookworms was established by referencing the data presented in the Freedom of Information Summaries for the original approval of ProHeart® 6 (moxidectin) dated June 6, 2001 (NADA 141-189), and the supplemental approval dated June 13, 2002. ProHeart® 12 and ProHeart® 6 are the same formulation, but ProHeart® 12 is three times the concentration of ProHeart® 6.

A. Dosage Characterization

A laboratory effectiveness study demonstrated that a single subcutaneous dose of 0.5 mg/kg moxidectin in an extended release injectable formulation was 100% effective against heartworm for 12 months. In another laboratory effectiveness study, a single subcutaneous dose of 0.5 mg/kg moxidectin in an extended release formulation was 98.6% effective for reducing naturally acquired nematode infections. Therefore, a single subcutaneous dose of 0.5 mg/kg moxidectin was selected as the dosage for the product for the prevention of heartworm disease caused by *Dirofilaria immitis* for 12 months and for the treatment of larval and adult hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) infections in dogs that exist at the time of administration.

B. Substantial Evidence

Substantial Evidence for the Prevention of Heartworm Disease:

1. Dose Confirmation Study

Title: Dose Confirmation of ProHeart® 12 (Sustained-Release Injectable Moxidectin Formulation) Given at 0.5 mg/kg for One-Year Prevention of Heartworm Disease Caused by *Dirofilaria immitis* in Dogs (Study number A166C-US-14-362)

Study Dates: January 2015 to August 2017.

Study Location: Spring Lake, MI

Study Design:

Objective: To confirm the effectiveness of a moxidectin extended release formulation (ProHeart® 12) when administered by subcutaneous injection at a dose of 0.5 mg/kg for protection against *Dirofilaria immitis* infections for one year in dogs.

Study Animals: 20 Beagle dogs (11 male, 9 female) approximately 12 months of age.

Table II.1: Treatment Groups for Study Number A166C-US-14-362

Treatment Group	Moxidectin Dose	Route	Treatment Day	Days of Heartworm Testing	Day of L3 Inoculation	Necropsy Day	Number of Animals
Saline	0 mg/kg	SC	Day 0	-19 (±2), 240 (±2), 485 (±2), and 515 (±5 days)	365	515 (±5)	10
ProHeart® 12	0.5 mg/kg	SC	Day 0	-19 (±2), 240 (±2), 485 (±2), and 515 (±5 days)	365	515 (±5)	10

L3 = *D. immitis* third-stage larvae; SC = Subcutaneous injection

Measurements and Observations: All dogs were determined to be negative for *D. immitis* by antigen test and modified Knott's test prior to initiation of the study and on Day 240. All dogs were treated on Day 0. At Day 365 (12 months) following treatment, all dogs were inoculated with 50 *Dirofilaria immitis* L3 larvae. Infections were allowed to develop for 150 days (approximately 5 months) at which time each animal was euthanized and necropsied to determine the presence of heartworms. Following treatment, dogs were observed at hours 1, 3, 6, and 24 for adverse reactions to treatment. General health observations were made twice daily throughout the study. On Days 485 and 515, blood samples were collected for adult heartworm antigen and modified Knott's testing to confirm heartworm infection status. The heart and lungs of each test dog were removed at necropsy for heartworm recovery and quantification.

Results: Adult *D. immitis* were recovered from all dogs administered saline (placebo). Worm counts ranged from 22 to 37 worms per dog (geometric mean, 30.2). No adult *D. immitis* were recovered from the ProHeart® 12-treated dogs. Adult *D. immitis* counts in the ProHeart® 12 treatment group were significantly different and numerically lower than adult *D. immitis* counts in the saline treatment group ($P<0.0001$).

Adverse Reactions: There were no treatment-related adverse reactions during the study.

Conclusion: A single subcutaneous injection of ProHeart® 12, at a dose of 0.5 mg moxidectin/kg body weight, was 100% effective in preventing the development of *D. immitis* infections in dogs inoculated with third-stage larvae (L3) 365 days after treatment.

2. Dose Confirmation Study

Title: Dose Confirmation of ProHeart® 12 (Sustained-Release Injectable Moxidectin Formulation) Given at 0.5 mg/kg for One-Year Prevention of Heartworm Disease Caused by *Dirofilaria immitis* in Dogs (Study number A166C-US-14-376)

Study Dates: January 2015 to August 2017.

Study Location: Greenfield, IN

Study Design:

Objective: To confirm the effectiveness of a moxidectin extended release formulation (ProHeart® 12) when administered by subcutaneous injection at a dose of 0.5 mg/kg for protection against *Dirofilaria immitis* infections for one year in dogs.

Study Animals: 20 Beagle dogs (11 male, 9 female) approximately 15 months of age.

Table II.2: Treatment Groups for Study Number A166C-US-14-376

Treatment Group	Moxidectin Dose	Route	Treatment Day	Days of Heartworm Testing	Day of L3 Inoculation	Necropsy Day	Number of Animals
Saline	0 mg/kg	SC	Day 0	-12 (±2), 240 (±2), 485 (±2), and 515 (±5 days)	365	515 (±5)	10
ProHeart® 12	0.5 mg/kg	SC	Day 0	-12 (±2), 240 (±2), 485 (±2), and 515 (±5 days)	365	515 (±5)	10[a]

L3 = *D. immitis* third-stage larvae; SC = Subcutaneous injection
[a] One dog in the ProHeart® 12-treated group was excluded from the effectiveness analysis per protocol due to incomplete dosing.

Measurements and Observations: All dogs were determined to be negative for *D. immitis* by antigen test and modified Knott's test prior to initiation of the study and on Day 240. All dogs were treated on Day 0. At Day 365 (12 months) following treatment, all dogs were inoculated with 50 *Dirofilaria immitis* L3 larvae. Infections were allowed to develop for 150 days (approximately 5 months) at which time each animal was euthanized and necropsied to determine the presence of heartworms. Following treatment, dogs were observed at hours 1, 3, 6, and 24 for adverse reactions to treatment. General health observations were made twice daily throughout the study. On Days 485 and 515, blood samples were collected for adult heartworm antigen and modified Knott's testing to confirm heartworm infection status. The heart and lungs of each test dog were removed at necropsy for heartworm recovery and quantification.

Results: Adult *D. immitis* were recovered from all dogs administered saline (placebo). Worm counts ranged from 22 to 44 worms per dog (geometric mean, 32.6). No adult *D. immitis* were recovered from the ProHeart® 12-treated dogs. Adult *D. immitis* counts in the ProHeart® 12 treatment group was significantly different and numerically lower than adult *D. immitis* counts in the saline treatment group (P=0.0112)

Adverse Reactions: There were 3 treatment-related adverse reactions during the study. Three dogs experienced injection site reactions. One dog administered saline exhibited mild subcutaneous swelling at the 3 and 6 hour post dosing observations which dissipated by the 24 hour post dosing observation. Two dogs, one each in the saline and ProHeart® 12 treatment groups, were observed to have a round area of mild redness in the injection site area at the 24-hour post dosing observation. The injection site reaction resolved after 2 days for the dog in the saline treatment group and after 29 days for the dog in the ProHeart® 12 treatment group.

Conclusion: A single subcutaneous injection of ProHeart® 12, at a dose of 0.5 mg moxidectin/kg body weight, was 100% effective in preventing the development of *D. immitis* in dogs inoculated with third-stage larvae (L3) 365 days after treatment.

3. Clinical Field Study

Title: Efficacy and Safety of ProHeart® 12 (Sustained-Release Injectable Moxidectin Formulation) in the Prevention of Heartworm Disease Caused by *Dirofilaria immitis* in Dogs Presented as Veterinary Patients

Study Dates: December 17, 2014 to September 29, 2017

Study Locations: This was a multi-center study conducted at 19 veterinary practices located in geographically diverse areas of the United States including the Southeast.

New Braunfels, TX
Dallas, TX
Quakertown, PA
Decatur, IL
Cropwell, AL
Leawood, KS
Germantown, TN
Seguin, TX
Zachary, LA
Augusta, ME
Fort Collins, CO
Gainesville, FL
Ocala, FL
Starke, FL
Savannah, GA
Memphis, TN
Lake Charles, LA
Pensacola, FL
Bradenton, FL

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

Objective: To confirm the effectiveness and safety of ProHeart® 12 (moxidectin) extended release injectable suspension at a dose of 0.23 mg/lb (0.5 mg/kg) body weight for the prevention of heartworm disease caused by *Dirofilaria immitis* when given as two single subcutaneous injections, twelve months apart, under field conditions.

Study Animals: A total of 593 client-owned dogs (297 in the ProHeart® 12 group, 296 in the active control group) of various breeds from 1 year to 14 years of age.

Experimental Design:

Table II.3: Treatment Groups for Study Number A161C-US-13-330

Treatment	Dosage	Regimen (Route of administration)	Dogs per Group	Days of Treatment*	Days of Heartworm Testing*
ProHeart® 12	0.2273 mg/lb (0.5 mg/kg)	2 doses, 12 months apart (Subcutaneous injection)	297	0, 365	-3 to 0, 120, 240, 365, 480, 605
Ivermectin and pyrantel pamoate tablets	Ivermectin per label 2.72 µg/lb (6 µg/kg)	Monthly for 20 months (Oral)	296	0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 365, 390, 420, 450, 480, 510, 540, 570	-3 to 0, 120, 240, 365, 480, 605

*±7 days for all activities after screening between Day -3 to Day 0

Drug Administration: ProHeart® 12 was administered at a dose of 0.5 mg/kg by subcutaneous injection every 12 months. The active control oral tablets (ivermectin and pyrantel pamoate) were administered every 30 days.

Study Duration: Approximately 605 days

Measurements and Observations: The primary variable for the determination of effectiveness was heartworm testing (adult antigen and microfilariae) at Days 365 and 605. Scheduled visits occurred on study Days 120, 240, 365, 480, and 605. Clinical observations and administration site evaluations were conducted prior to dosing and at 1 and 2 hours after treatment. Owner contact was conducted on Days 1, 7, 14, 21, 30, 42, 60, 90, 150, 180, 210, 270, 300, 330, 366, 372, 379, 386, 390, 407, 420, 450, 510, 540, and 570 to confirm dosing and check for adverse events, including assessment of the injection site. Heartworm testing occurred on Days -3 to 0, 120, 240, 365, 480, and 605; and clinical pathology (hematology, serum biochemistry, and urinalysis) was conducted on Days -3 to 0 and 605.

Results: A total of 235, 226, and 222 ProHeart® 12-treated dogs completed the heartworm testing on Days 365, 480, and 605, respectively. None of these animals tested positive for heartworm on any of the test days.

Adverse Reactions: A total of 593 dogs, ranging in age from 1 year to 14 years, were included in a field study safety summary. Adverse reactions reported in dogs treated with ProHeart® 12 and the active control are summarized in Table II.4.

Table II.4: Number of Dogs* with Adverse Reactions Reported During the Field Study with ProHeart® 12

Adverse Reaction	ProHeart® 12 n=297 (%)	Ivermectin and Pyrantel Pamoate; n=296 (%)
Vomiting	75 (25.3)	78 (26.4)
Lethargy	46 (15.5)	34 (11.5)
Diarrhea (with and without blood)	43 (14.5)	46 (15.5)
Anorexia	41 (13.8)	31 (10.5)
Seizures	10 (3.4)	7 (2.4)
Hepatopathy	8 (2.7)	3 (1.0)
Hypersalivation	7 (2.4)	3 (1.0)
Hypersensitivity Reactions	6 (2.0)	4 (1.4)

* Some dogs may have experienced more than one adverse reaction or more than one occurrence of the same adverse reaction during the study.

Two ProHeart® 12-treated dogs had mild to moderate anaphylactoid/ hypersensitivity-related drug reactions within the first 24 hours following the initial treatment. In one dog, hives and facial swelling were treated symptomatically with full recovery in 24 hours. In the second dog, redness and swelling about the face and paws, followed by vomiting, polydipsia, and elevated heart rate was treated symptomatically and signs resolved completely within 4 days. Both dogs received a second dose 12 months later. The second dog was pre-treated with diphenhydramine, and neither dog had a reaction to the second dose. One active control-treated dog experienced anaphylactoid/hypersensitivity-related clinical signs within the first 24 hours. The dog was withdrawn from the study prior to the second monthly dose.

The cases of anorexia observed in the study were generally mild and short in duration. Some cases occurred in association with ProHeart® 12 dosing, however the majority were not associated with the day of dosing. All cases resolved by the conclusion of the study.

Ten ProHeart® 12-treated dogs reported single incidents of seizures, most were mild and did not appear to occur in association with the day of dosing.

Mild injection site reactions occurred in six ProHeart® 12-treated dogs and were observed from one to seven days post dosing and included warmth, swelling, and pruritus. One of these cases included mild pruritus at the injection site, that resolved spontaneously within 24 hours of administration.

Conclusions: ProHeart® 12 was demonstrated to be safe and effective when administered to dogs by subcutaneous injection every 12 months at a dosage of 0.2273 mg/lb (0.5 mg/kg) of body weight for prevention of heartworm disease caused by *Dirofilaria immitis*.

Substantial Evidence for treatment of existing larval and adult hookworm infections (*Ancylostoma caninum* and *Uncinaria stenocephala*)

The effectiveness of ProHeart® 12 administered at 0.2273 mg/lb (0.5 mg/kg) body weight for the treatment of existing larval and adult hookworms was established by referencing the data presented in the Freedom of Information Summaries for the original approval of ProHeart® 6, dated June 6, 2001 (NADA 141-189), and the supplemental approval, dated June 13, 2002. These studies confirmed a single subcutaneous injection of ProHeart® 6 given at a dose of 0.17 mg moxidectin/kg bodyweight was ≥90% effective against larval and adult stages of *Ancylostoma caninum* and *Uncinaria stenocephala*. ProHeart® 12 and ProHeart® 6 are the same formulation, but ProHeart® 12 is three times the concentration of ProHeart® 6.

III. TARGET ANIMAL SAFETY

The safety of ProHeart® 12 was evaluated in five laboratory studies including a margin of safety study, a study to evaluate safety in dogs infected with heartworms, a study to evaluate safety in ivermectin-sensitive Collies, and reproductive safety studies in both female and male dogs. In total, safety was evaluated in 107 laboratory animals. The safety of ProHeart® 12 was also evaluated in client-owned dogs (n=297) in the field study summarized above. The margin of safety study included dose groups receiving 1, 3, and 5 times the label dose every six months for one year (3 doses), and the only treatment-related finding was injection site inflammation. The heartworm positive safety study showed mild injection site reactions and a slight decrease in the number of adult heartworms compared to control dogs. No adverse events were observed in the ivermectin-sensitive Collie study. No adverse reproductive outcomes were observed in either reproductive safety study. In addition to the studies presented here, the safety of ProHeart® 12 was evaluated through the review of pharmacovigilance data, including post-approval data for ProHeart® 6 (moxidectin microspheres at a dose of 0.17 mg/kg), and data voluntarily reported from foreign market use of ProHeart® 12.

A risk minimization action plan (RiskMAP) was developed for this product and ProHeart® 6 because of the post-approval adverse drug experiences reported for ProHeart® 6. ProHeart® 12 and ProHeart® 6 are the same formulation, but ProHeart® 12 is three times the concentration of ProHeart® 6. The ProHeart® 12 and ProHeart® 6 RiskMAP provides educational materials to the veterinarian, veterinary staff, and the dog owner explaining the risks and proper use of ProHeart® 12 and ProHeart® 6. ProHeart® 12 and ProHeart® 6 are for use in dogs only and are available through a restricted distribution program to veterinarians that have completed the RiskMAP training and certification module. Technicians/assistants that have completed the training and are certified can administer ProHeart® 12 and ProHeart® 6.

Veterinarians are expected to report all adverse events that occur in animals or humans to the manufacturer.

A. Margin of Safety Study – Study Number A362N-US-15-524

Title: ProHeart® 12: Margin of Safety of 3 Doses Administered Every 6 Months to Beagle Dogs.

Study Dates: August 26, 2015 to September 7, 2016

Study Location: Madison, Wisconsin

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

Objective: The purpose of this study was to demonstrate the margin of safety of ProHeart® 12 administered by subcutaneous injection, once every 6 months, in dogs at 1, 3, and 5 times the label dose of 0.5 mg/kg body weight. Related secondary objectives include characterizing the pharmacokinetics and evaluating residual moxidectin concentration at the 1X dose injection sites after 1 year.

Study Animals: Thirty-two (32) purpose-bred Beagle dogs (16 males and 16 females) approximately 6 to 7 months of age at initiation of the study and weighing between 8.5 to 11.2 kg for males and 7.1 to 8.9 kg for females were enrolled.

Experimental Design:

Table III.1: Treatment Groups (Study Number 362N-US-15-524)

Treatment Group	Number of Dogs	Dose Level	Dose Volume
Control (saline)	4M and 4F	0	0.25 mL/kg
ProHeart® 12 (1X)	4M and 4F	0.5 mg/kg	0.05 mL/kg
ProHeart® 12 (3X)	4M and 4F	1.5 mg/kg	0.15 mL/kg
ProHeart® 12 (5X)	4M and 4F	3.5 mg/kg	0.25 mL/kg

Drug Administration: Dogs were administered ProHeart® 12 moxidectin microspheres reconstituted with ProHeart® 12 vehicle or control (0.9% sodium chloride for injection) subcutaneously at 6-month intervals on Days 1, 183, and 365 into marked locations just to the left (Day 1) or right (Days 183 and 365) of the dorsal midline, anterior to scapulae. Reconstituted ProHeart® 12 was stored refrigerated and protected from light for 8 weeks prior to each administration. Dose volumes were calculated based on the most recently recorded body weight.

Measurements and Observations: Clinical observations, physical examinations, body weights, food consumption, hematology, serum chemistry, coagulation, urinalysis, injection site observations, gross necropsy (Day 379), organ weights, and histopathology. Blood samples were collected on Days 1, 2, 4, 11, 31, 46, 60, 91, 121, 183, 184, 186, 193, 213, 228, 242, 273, 303, 365, 368, and 375 to measure moxidectin plasma concentration. Plasma and tissue concentrations were measured using validated UPLC-MS/MS.

Statistical Methods: In all analyses the experimental unit was the individual animal. Variables measured once (organ weights) were analyzed for treatment effects by using a mixed linear model. For continuous variables measured more than once (body weight, feed consumption, hematology, serum chemistry,

coagulation, and urine), data were examined by using a general linear mixed model for repeated measures, with a covariate when appropriate. A non-compartmental analysis was used to estimate pharmacokinetic parameters.

Results: There were no effects of ProHeart® 12 on physical examinations, body weight, or food consumption. The only ProHeart® 12-related clinical finding was edema and thickening of skin at injection sites. There were no treatment related effects in hematology, coagulation, or clinical chemistry. There were no treatment related differences in organ weights. Gross lesions associated with administration of the test article include dose related subcutaneous thickening of injection sites. Microscopically, there was granulomatous inflammation noted at the injection site of treated dogs consistent with a gradually resolving, steady state tissue response to the presence of microspheres.

Pharmacokinetics: Following the first dose, the maximum concentration ("C_{max}") ranged from 8.5 – 15.9 ng/mL and the time of C_{max} (T_{max}) ranged from 10 – 30 days in 8 dogs receiving the therapeutic dose. Area under the concentration-time curve to the last measured time point ("AUC0-t") and C_{max} increased in a less than dose-proportional manner from 1X to 5X. The general trend of less than dose-related proportionality in AUC0-t and C_{max} from 1x to 5x continued for the subsequent doses. The observed C_{max} values following the three administrations 6 months apart showed little or no accumulation. The minimum drug concentrations measured 6 months after the first dose – which is halfway through the prescribed dosing regimen – ranged from 0.326 ng/mL to 2.26 ng/mL in 8 dogs receiving the 1X dose.

Moxidectin residue at 1X (0.5 mg/kg) injection sites: The amount of moxidectin remaining in the microspheres at the injection site one year after administration of the 1X (0.5 mg/kg) dose had relatively large between-subject variability, with amounts ranging from 1.8 to 1506 µg, corresponding to 0.045% to 37.6% of the administered dose.

Conclusions: Subcutaneous administration of ProHeart® 12 to male and female Beagle dogs at 0.5, 1.5, or 2.5 mg/kg on Days 1, 183, and 365 was well tolerated and did not result in any systemic adverse effects. ProHeart® 12-related findings included edema and thickening of the injection sites that was not associated with adverse clinical signs. These results support the safety of ProHeart® 12 when administered to dogs at a dosage of 0.5 mg/kg once every 12 months.

B. Safety in Dogs with Heartworm Infections – Study Number 0899-C-US-39-02

Title: Clinical Observations Following the Administration of [ProHeart® 12] (moxidectin for extended release injectable suspension) Given at 3X to Dogs with Implanted Adult Heartworm (*Dirofilaria immitis*) Infections in Georgia.

Study Dates: May 16, 2003-February 10, 2004

Study Location: Athens, Georgia

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

Objective: The objective of this study was to evaluate the effect of treatment of heartworm-positive dogs with ProHeart® 12.

Study Animals: Sixteen (16) purpose-bred Beagle dogs (8 males and 8 females) approximately 7 to 17 months of age at the time of surgical implantation with adult heartworms (9 to 19 months at time of treatment) were used in this study. The males weighed 7.4 to 12.75 kg and the females weighed 8.55 to 11.55 kg the day before treatment.

Experimental Design:

Table III.2: Treatment Groups (Study Number 0899-C-US-39-02)

Treatment Group	Number of Dogs	Dose Level
Control (saline)	4M and 4F	0
ProHeart® 12 (3X)	4M and 4F	1.5 mg/kg

Drug Administration: Dogs were administered a single dose of ProHeart® 12 moxidectin microspheres reconstituted with ProHeart® 12 vehicle or control (0.9% sodium chloride for injection) between the shoulder blades 63 days after heartworm implantation. Dose volumes were calculated based on the most recently recorded body weight.

Measurements and Observations: Dogs were confirmed to be negative for heartworm infection prior to the study by both antigen testing and by microscopic analysis for microfilariae in blood smears. Dogs were implanted with 20 adult heartworms (10 male and 10 female) on Day 0, and treated on Day 63. Health observations were generally made at least twice a day throughout the study. On treatment day, observations were made prior to treatment, then 2, 4, 6, 8, and 12 hours post-treatment. There were three observations per day during the time of expected peak blood levels (Days 70-77). Occasionally animals were observed once during weekends and holidays. Physical examinations were carried out prior to treatment, and on 2, 14, 28, and 56 days post-treatment. Microfilaria counts and heartworm antigen assessments were made beginning on Day 14 and repeated every 2 weeks through Day 56. Prior to treatment with test or control items on Day 63, the schedule was adjusted relative to treatment such that tests occurred 2 days prior, then 1, 3, 7, 14, 21, 28, 42, and 56 days post-treatment (Days 61, 64, 66, 70, 77, 84, 91, 105, and 119, respectively). At necropsy, remaining adult worms in each dog were counted and sexed.

Statistical Methods: Microfilaria counts and adult male, female, and total heartworm counts at necropsy were analyzed using a two-way analysis of variance. The value for the count was log-transformed upon analysis, and the significance was tested at the 5% level.

Results: One dog in the ProHeart® 12 treatment group had a 5 cm X 3 cm injection site swelling 7 days post-treatment but not at other times. At necropsy, 5 dogs in the ProHeart® 12 treatment group had small off-white deposit of suspected test article, but no associated tissue reaction, at the subcutaneous

injection sites. No other ProHeart® 12-related reactions or effects were noted in any of the dogs.

The number of heartworms recovered at necropsy for the ProHeart® 12 treatment group was less than the number of heartworms recovered for the control group. A statistically significant difference was observed ($p = 0.016$) between the ProHeart® 12 treatment group and the control group. One dog with the lowest counts of adult male and female worms ($n = 7$ and 6, respectively) was microfilaria-negative throughout the study. One dog in the ProHeart® 12 treatment group with 8 male and 9 female worms at necropsy had 2 worm fragments.

All dogs were positive for heartworm antigen from the first post-treatment assessment until the end of the study. Fifteen of the sixteen dogs were microfilaria-positive at the first post-infection blood collection (14 days after implantation). All eight control dogs remained microfilaria-positive until the end of the study. Seven of the eight dogs in the ProHeart® 12 treatment group were microfilariae-positive 14 days after the induced infection and prior to treatment. Microfilarial counts reached a peak in the seven positive dogs in the ProHeart® 12 group the day after treatment, then decreased over the following two weeks to a low level for the remainder of the study.

Conclusions: A single subcutaneous injection of ProHeart® 12, equivalent to 3X the label dose level, administered to dogs with implanted adult heartworm infections did not result in any adverse clinical or gross pathological effects.

C. Safety in Ivermectin-Sensitive Dogs - Study Number 0899-C-US-38-02

Title: Clinical Observations from the Administration of ProHeart® 12 (moxidectin for extended release injectable suspension) in Ivermectin-Sensitive Dogs.

Study Dates: May 13, 2003-June 23, 2003

Study Location: Urbana, Illinois

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

Objective: The objective of this study was to determine the safety of a single dose of ProHeart® 12 at 1, 3, or 5 times the proposed label dose in ivermectin-sensitive Collies.

Study Animals: Fifteen (15) Collies (6 males and 9 females) approximately 7 months to 6.5 years of age at the time of treatment were used in this study. Each dog had previously demonstrated toxicity to ivermectin at 120 µg ivermectin/kg orally and confirmed to be negative for heartworm (modified Knott's test; adult heartworm antigen-negative).

Experimental Design:

Table III.3: Treatment Groups (Study Number 0899-C-US-38-02)

Treatment Group	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Dogs (Day 0)		Number of Dogs (Day 21)	
			Moxidectin	Saline	Moxidectin	Saline
ProHeart® 12 (1X)	0.5	0.05	3 (2M, 1F)	2 (1M, 1F)	2 (1M, 1F)	3 (2M, 1F)
ProHeart® 12 (3X)	1.5	0.15	3 (1M, 2F)	2 (2F)	2 (2F)	3 (1M, 2F)
ProHeart® 12 (5X)	3.5	0.25	1 (1M)	4 (1M, 3F)	4 (1M, 3F)	1 (1M)

Drug Administration: Each dog received both ProHeart® 12 and saline. On Day 0 and 21, dogs were administered a dose of either ProHeart® 12 moxidectin microspheres reconstituted with ProHeart® 12 vehicle or 0.9% sodium chloride subcutaneously in the dorsolateral neck cranial to the scapula. No more than 3 mL was injected in a single site. Dogs administered moxidectin on Day 0 were administered the same dose volume of saline on Day 21. Similarly, dogs administered moxidectin on Day 21 were administered the same dose volume of saline on Day 0.

Measurements and Observations: Health observations, including observations for depression, ataxia, mydriasis, and excessive salivation were made 1, 2, 3, 4, 5, 6, 7, 8, 12, and 18 hours after treatment, then twice daily for 20 days after each injection.

Results: No adverse reactions to ProHeart® 12 were observed in any dogs.

Conclusions: A single subcutaneous injection of ProHeart® 12, equivalent to either 1X, 3X, or 5X the recommended dose level, administered to ivermectin-sensitive Collie dogs did not result in any observed adverse reactions.

D. Female Reproductive Safety – Study Number 0899-C-US-33-02

Title: A reproduction Study of ProHeart® 12 (moxidectin for extended release injectable suspension) in Female Beagle Dogs.

Study Dates: May 20, 2002-November 9, 2002

Study Location: Mattawan, Michigan

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

Objective: The objective of this study was to assess the effect of ProHeart® 12 at a 3X dose level in female laboratory Beagle dogs, administered either one month prior to mating, at mating, during gestation, or shortly after whelping.

Study Animals: Forty (40) adult female purpose-bred laboratory Beagle dogs with a history of successful breeding performance and free of infectious disease were included in this study. Twenty-five untreated males were used in the breeding phase of the trial.

Experimental Design:

Table III.4: Treatment Groups (Study Number 0899-C-US-33-02)

Treatment Group	ProHeart® 12 Dose Level (mg moxidectin/ kg body weight)	Number of Females Preselected	Number of Females Mated
1	0X (Control)	12	8
2	3X (1.5): Premating	12	9[a]
3	3X (1.5): Mating	12	9[b]
4	3X (1.5): Mid-Gestation	12	8
5	3X (1.5): Lactation	12	8

[a] One dog was paired for mating only once and was excluded from the summary.
[b] One dog was dosed two days late and was excluded from the summary.

Drug Administration: All dogs in treatment groups 2 to 5 were administered a single subcutaneous injection containing 3X the recommended dose level of ProHeart® 12 (1.5 mg moxidectin/kg body weight) moxidectin microspheres reconstituted with ProHeart® 12 vehicle. The timing of treatments covered the following critical periods of the reproductive cycle. Dogs in treatment group 1 received no test article.

"Control": Untreated control.
"Pre-mating": Dosed approximately one month prior to anticipated mating.
"Mating": Dosed the day after the first mating.
"Mid-Gestation": Dosed on Day 28 of gestation period.
"Lactation": Dosed the fifth day after whelping.

Duration of the Study: Each female dog remained under observation until her puppies were weaned at six weeks of age.

Measurements and Observations: Adult dogs were given a complete physical examination prior to study initiation, and following the completion of parturition. Semen from males was evaluated prior to mating. Body weight, food consumption, and general health of females were monitored weekly beginning with acclimatization and continuing until the end of the study. Breeding procedures/observations, including estrus detection and monitoring, were designed to ensure identification and treatment of 8 females prior to breeding and to ensure that each female mated twice during estrus. Whelping was monitored and assistance made available if necessary. Duration of gestation, dystocia, vaginal discharge, and milk production were documented as applicable. The number, sex, weight, and viability of pups were recorded. A necropsy was performed on stillborn puppies and a cause of death determined, if possible. Puppies were observed twice daily (cage side) for survival and obvious changes in appearance and behavior. Individual body weight measurements and detailed clinical examinations were performed on Days 1 (or Day 0 for some litters), 4, 7,

14, 21, 28, 35, and 42 of lactation. A complete post mortem examination was performed on each puppy that died during the observation period.

Statistical Methods: Binary data for the female dog such as conception, female mating, pregnancy, and gestation were analyzed by Fisher's exact test. Whelping index and puppy viability were analyzed by pairwise comparison of each active group with the control group using arcsin square root transformation. Other continuous variables such as female dog body weight, female dog body weight change, gestation length, and litter size were analyzed by pairwise t-tests on non-transformed values. Mean pup weight was analyzed by analysis of covariance. In addition, sex distribution of puppies within treatment groups were analyzed by Chi-Square test for homogeneity.

Results: All mated dogs, except one in the mid-gestation group, became pregnant and delivered at least one puppy. Table III.5 shows the major reproductive parameters measured

Table III.5: Reproductive Parameters (Study Number 0899-C-US-33-02)

Treatment Group	Number with Litters	Mean Gestation Period (days)	Mean Pups per litter at birth	Mean Pups per litter at Day 4	Mean Pups per litter at Day 42
Control	8/8	62.8	4.3	4.1	3.9
Pre-mating	9/9	63.2	5.8	5.4	5.2
Mating	9/9	62.7	5.2	4.7	4.7
Mid-gestation	7/8	63.7	6.1	5.7	5.3
Lactation	8/8	63.9	6.3	5.6	5.6

Conclusions: A single subcutaneous injection of ProHeart® 12, equivalent to 3X the recommended dose level, administered to reproducing female dogs at critical times throughout their reproductive cycles (pre-mating through post-whelping) had no adverse effects on conception, pregnancy maintenance, and the development, growth, and health of the puppies.

E. Male Reproductive Safety – Study Number 0899-C-CN-01-02

Title: Effect of ProHeart® 12 (moxidectin for extended release injectable suspension) on the Seminal Quality in Male Beagles.

Study Dates: January 31, 2003-June 12, 2003

Study Location: Fergus, Ontario, Canada

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

Objective: The purpose of this study was to assess the effects of the treatment of adult breeding male dogs with 3X levels of ProHeart® 12 on semen quality.

Study Animals: Sixteen (16) healthy, sexually mature, male purpose-bred laboratory Beagle dogs between 15 to 31 months of age at the start of the experimental period, and 10.2 to 18.3 kg body weight on the day before treatment, were used in this study. Dogs were confirmed heartworm-negative by Knott's test and by assay of serum for adult heartworm antigen. Each enrolled dog had shown acceptable semen quality prior to enrollment.

Experimental Design:

Table III.6: Treatment Groups (Study Number 0899-CN-01-02)

Treatment Group	Number of Dogs	Dose Level
Control (saline)	8	0
ProHeart® 12 (3X)	8	1.5 mg/kg

Drug Administration: All dogs were treated with a single subcutaneous injection of either 3X the recommended dose level of ProHeart® 12 (1.5 mg moxidectin/kg body weight) moxidectin microspheres reconstituted with ProHeart® 12 vehicle or control (0.9% sodium chloride for injection) on Day 0.

Measurements and Observations: The semen quality (sperm motility, sperm morphology and count, cytology (other cell types), debris, crystals, etc.), of each dog was determined on ejaculates collected two times pretreatment (Days -21 and -7) and seven times post-treatment on Days 7, 21, 35, 42, 63, 77, and 91. Animals were observed for general health at least twice daily during the study. The dogs received complete physical exams by a veterinarian during acclimation (Day -35), the day prior to treatment (Day -1), after treatment on Day 0, and on Days 35 and 91 post-treatment.

Statistical Methods: Sperm data were analyzed by a mixed model analysis of variance using SAS PROC MIXED procedure.

Results: No clinically significant changes or abnormalities were noted in semen quality (motility, morphology, volume, and concentration). Minor injection site thickening was noted by palpation in four dogs in the ProHeart® 12 treatment group. Two dogs had thickening noted at the 1-week observation time that persisted throughout the 13-week post-treatment period. Two other dogs had minor thickening that was first noted at the 3-week observation time and were resolved by 9 or 11-weeks post-treatment.

Conclusions: A single subcutaneous injection of ProHeart® 12, equivalent to 3X the recommended dose level, did not have any adverse effect on the semen quality of dogs. Minor thickening at the injection site was observed in four of the eight dogs administered ProHeart® 12.

IV. HUMAN FOOD SAFETY

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

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to ProHeart® 12:

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

If contact with your skin occurs, wash thoroughly with water. May be irritating to the eyes. If product accidentally gets into your eyes, flush eyes 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 and show the package insert to the physician. Take care to avoid accidental self-injection. In case of accidental self-injection, seek medical advice and show the package insert or the label to the physician. The Safety Data Sheet (SDS) contains more detailed occupational safety information.

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 ProHeart® 12, when used according to the label, is safe and effective for use in dogs 12 months of age and older for the prevention of heartworm disease caused by *Dirofilaria immitis* for 12 months and for the treatment of existing larval and adult hookworm *(Ancylostoma caninum* and *Uncinaria stenocephala)* infections.

A. Marketing Status

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

B. Exclusivity

ProHeart® 12, 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 ProHeart® 12.

C. Patent Information

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