Date of Approval: March 8, 2018

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-492

Centragard™

eprinomectin and praziquantel transdermal solution

Transdermal Solution

Cats

CENTRAGARD is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*, and for the treatment and control of roundworms (adult and fourth stage larval *Toxocara cati*), hookworms (adult and fourth stage larval *Ancylostoma tubaeforme;* adult *Ancylostoma braziliense*), and tapeworms (adult *Dipylidium caninum* and *Echinococcus multilocularis*), in cats and kittens 7 weeks of age and older and 1.8 lbs or greater.

Sponsored by:

Merial, Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-492

B. Sponsor

Merial, Inc.
3239 Satellite Blvd., bldg. 500
Duluth, GA 30096-4640

Drug Labeler Code: 050604

C. Proprietary Name

Centragard™

D. Product Established Name

Eprinomectin and praziquantel transdermal solution

E. Pharmacological Category

Antiparasitic

F. Dosage Form

Transdermal solution

G. Amount of Active Ingredient

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

H. How Supplied

0.3 mL and 0.9 mL unit applicator

I. Dispensing Status

Rx

J. Dosage Regimen

Centragard is dosed at a minimum of 0.055 mL/lb (0.12 mL/kg), which delivers a minimum dose of 0.23 mg/lb eprinomectin and 4.55 mg/lb praziquantel. Administer the entire contents of a Centragard unit applicator topically once a month as specified in the following table:

Cat weight (lb)	Volume (mL)	Eprinomectin (mg)	Praziquantel (mg)
1.8-5.5	0.3	1.2	24.9
5.6-16.5	0.9	3.6	74.7
16.6-22.0	0.3 + 0.9	4.8	99.6
22.1-33.0	0.9 + 0.9	7.2	149.4

K. Route of Administration

Transdermal

L. Species/Class

Cats

M. Indication

Centragard is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*, and for the treatment and control of roundworms (adult and fourth stage larval *Toxocara cati*), hookworms (adult and fourth stage larval *Ancylostoma tubaeforme;* adult *Ancylostoma braziliense*), and tapeworms (adult *Dipylidium caninum* and *Echinococcus multilocularis*), in cats and kittens 7 weeks of age and older and 1.8 lbs or greater.

II. EFFECTIVENESS

The effectiveness of Centragard™ was demonstrated in 18 well-controlled laboratory studies described below. No adverse reactions were reported in any of the 226 cats administered the labeled dose. These studies demonstrate that Centragard™ is effective against induced heartworm infections after a single application. The effectiveness studies also demonstrate that Centragard™ is effective against roundworms (adult and fourth stage larval *T. cati*), hookworms (adult and fourth stage larval *A. tubaeformae*, adult *A. braziliense*), and tapeworms (adult *D. caninum* and *E. multilocularis*) after a single administration or when given monthly as part of a heartworm prevention program.

A. Dosage Characterization

The minimum dose of eprinomectin (0.5 mg/kg) and praziquantel (10 mg/kg) was selected based on exploratory laboratory effectiveness studies. The studies were conducted to evaluate the lowest effective dose of each active. Five studies evaluated doses of eprinomectin over a dose range of 0.1 to 1.0 mg/kg against experimental or natural infections of *Toxocara 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 *Dipylidium 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

Effectiveness studies were conducted with formulation ML-635, containing 8.3% fipronil, 0.4% eprinomectin, 8.3% praziquantel, and 10% (S)-methoprene. The doses of eprinomectin and praziquantel in ML-635 are equivalent to the approved formulation of Centragard™ (eprinomectin and praziquantel transdermal solution).

1. For the prevention of heartworm disease *(Dirofilaria immitis)*

   Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 to Prevent Heartworm Disease in Cats, When administered 30 Days after Infection with *Dirofilaria immitis* Larvae. (PR&D 0221001)

   Study Location and Dates: Athens, GA; September 15, 2010-March 14, 2011

   Study Design
   Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg to prevent heartworm disease in cats.

   Study Animals: Twenty-eight cats (14 males and 14 females), 3 to 4 months of age and weighing 1.5 to 2.8 kg, were randomized into 14 cats per treatment group.

   Treatment Groups:
   Group 1: Untreated control
   Group 2: ML-635

   Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -2 bodyweights.

   Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

   Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were inoculated with third-stage (L_3) *D. immitis* larvae on Day -30. On Day 0, the cats in Group 2 were treated with ML-635.

   Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. Necropsy was performed on each animal on Day 150 for heartworm recovery and enumeration.

   Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as a fixed effect and Replicate (block by body weight) within sex as a random effect.

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

Percent Effectiveness = 100 x (c_c-c_t) / c_c
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 1: *D. immitis*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	4.8 (2-17)	N/A
ML-635	0.0 (0)[a]	100.0%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

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 study to be considered valid. Twelve of the 14 control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: ML-635 was effective for the prevention of *D. immitis* infections in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 to Prevent Heartworm Disease in Cats, when administered 30 Days after Infection with *Dirofilaria immitis* Larvae. (PR&D 0221002)

Study Location and Dates: Athens, GA; September 29, 2010-March 28, 2011

Study Design
Objective: To confirm the effectiveness of ML-635 to prevent heartworm disease in cats at the recommended minimum dose of 0.12 mL/kg.

Study Animals: Twenty-eight cats (14 males and 14 females), 3 to 5 months of age and weighing 1.7 to 3.4 kg, were randomized into 14 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -2 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were inoculated with third-stage (L_3) *D. immitis* larvae on Day -30. On Day 0 the cats in Group 2 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. Necropsy was performed on each animal on Day 150 for heartworm recovery and enumeration.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as a fixed effect and Replicate (block by body weight) within sex as a random effect.

Effectiveness was determined on the basis of the percent reduction in heartworm 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 worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 2: *D. immitis*

Treatment	**Geometric Mean worm count (range)**	**% Effectiveness**
Untreated control	3.4 (2-25)	N/A
ML-635	0.0 (0)[a]	100%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

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 study to be considered valid. Ten of the 14 control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: ML-635 was effective for the prevention of *D. immitis* infections in cats.

2. For the treatment and control of gastrointestinal nematodes and cestodes

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 against Natural *Toxocara cati* Infection in Cats. (PR&D 0218801)

Study Location and Dates: Tirana, Albania; July 19, 2010 to July 26, 2010

Study Design
Objective: To confirm the effectiveness of ML-635 against *T. cati* at the recommended minimum dose of 0.12 mL/kg in naturally infected cats.

Study Animals: Twenty cats (6 males and 14 females), 3 to 50 months of age and weighing 0.7 to 3.6 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -3 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Fecal samples were examined microscopically for helminth eggs and macroscopically for cestode proglottids. All cats included in the study were shedding *T. cati* eggs. In addition, six out of ten cats in each group were shedding *D. caninum* eggs/proglottids. On Day 0, the cats in Group 2 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites recovered were identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results: The effectiveness of ML-635 against the natural *T. cati* infection was 98.6%; the mean parasite counts of the two groups were significantly different ($p<0.001$). The effectiveness of treatment with ML-635 against *D. caninum* was 98.9%; the mean parasite count of the treatment group being significantly different from the untreated control group ($p=0.002$).

Table 3: *T. cati*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	5.1 (1-16)	N/A
ML-635	0.1 (0-1)[a]	98.6%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

Table 4: *D. caninum*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	10.4 (0-140)	N/A
ML-635	0.1 (0-2)[a]	98.9%

[a] There was a significant difference between the geometric means of the treated and control groups ($p=0.002$).

A minimum of five (*T. cati*) and two (*D. caninum*) worms 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 10 control cats satisfied the adequacy of infection requirement for *T. cati* and 8 of 10 control cats satisfied the adequacy of infection requirement for *D. caninum*.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against *T. cati* infection and *D. caninum* infection in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 against Natural *Ancylostoma tubaeforme* Infection in Cats. (PR&D 0218901)

Study Location and Dates: Tirana, Albania; October 11, 2010 to October 18, 2010

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against *A. tubaeforme* in naturally infected cats.

Study Animals: Twenty cats (10 males and 10 females), 4 to 96 months of age and weighing 0.9 to 3.3 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -3 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Fecal samples were examined microscopically for nematode and cestode eggs and macroscopically for cestode proglottids. All cats included in the study were shedding hookworm eggs. On Day 0, the cats in Group 2 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites recovered were identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 5: *A. tubaeforme*

Treatment	**Geometric Mean worm count (range)**	**% Effectiveness**
Untreated control	19.0 (2-71)	N/A
ML-635	0.2 (0-2)[a]	99.0%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

A minimum of twenty worms 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 10 control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against *A. tubaeforme* infection in cats.

Title: Efficacy of Single Treatments with ML-635 and Eprinomectin Administered Topically against Naturally Acquired Infections of *Dipylidium caninum* in Cats. (PR&D 0213901)

Study Location and Dates: Tirana, Albania; June 28, 2010 to July 5, 2010

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against *D. caninum* in naturally infected cats, and that eprinomectin alone is not effective against *D. caninum.*

Study Animals: Thirty cats (13 males and 17 females), 7 months to 5 years of age and weighing 1.2 to 3.6 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635
Group 3: Eprinomectin

Treatment Dose: Cats in Group 2 were dosed topically with 0.12 mL/kg based on Day -3 bodyweights, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene.

Cats in Group 3 were dosed topically, based on Day -3 bodyweights, with 0.12 mL/kg of eprinomectin, delivering 0.5 mg/kg.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Fecal samples were examined microscopically for helminth eggs and macroscopically for *D. caninum* proglottids. All cats included in the study were naturally infected with *D. caninum*. On Day 0, the cats in Groups 2 and 3 were treated with ML-635 and eprinomectin, respectively.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites recovered were identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 x (c_c-c_t) / c_c
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 6: *D. caninum*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	13.7 (0-181)	N/A
ML-635	0.1(0-2)[a]	99.2%
Eprinomectin	11.4 (0-371)	17.0%

[a] There was a significant difference between the geometric means of the ML-635 treated and control groups (p=0.001). There was no difference between the geometric means between the eprinomectin and control groups (p=0.792).

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 study to be considered valid. Eight of the 10 control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against *D. caninum* infection in cats, the addition of eprinomectin did not interfere with the effectiveness of praziquantel, and the lack of effectiveness of eprinomectin against *D. caninum* infection in cats justifies the inclusion of praziquantel in the formulation.

Title: A Study to Determine the Efficacy of a Single Treatment with ML-635 Administered Topically against Natural *Ancylostoma braziliense* Infections in Cats. (PR&D 0224801)

Study Location and Dates: Bloemfontein, Republic of South Africa; March 15, 2011 to March 22, 2011

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against *A. braziliense* in naturally infected cats.

Study Animals: Twenty cats (13 males and 7 females), 4 months of age to adults (≥ 12 months) and weighing 1.4 to 3.3 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -1 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Fecal samples were examined microscopically for nematode and cestode eggs and macroscopically for cestode proglottids. All cats included in the study were shedding hookworm eggs. On Day 0, the cats in Group 2 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites recovered were identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 7: *A. braziliense*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	31.3 (10-114)	N/A
ML-635	2.8 (0-25)[a]	91.0%

[a] There was a significant difference between the geometric means of the treated and control groups (p=0.002).

A minimum of five worms 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. All 10 control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against *A. braziliense* infection in cats.

Title: A Study to Determine the Efficacy of Three Different Doses of ML-635 when Administered once Topically in a Spot-on Formulation against Induced Infections of *Toxocara cati* in Cats. (PR&D 0220101)

Study Location and Dates: Rohrdorf, Germany; July 12, 2010 to July 19, 2010.

Study Design
Objective: To determine the effectiveness of three different doses of ML-635 against induced infections of *T. cati* in cats.

Study Animals: Forty cats (23 males and 17 females), 18 to 21 weeks of age and weighing 1.2 to 2.4 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635 0.5X
Group 3: ML-635 1X
Group 4: ML-635 2X

Treatment Dose: Cats were dosed topically at 0.5X, 1X, and 2X the minimum effective dose of 0.12 mL/kg based on Day -3 bodyweights, with the 1X dose delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with approximately 100 *Toxocara cati* larvated eggs on Days -63, -62, and -61 and the presence of eggs was confirmed in their feces on Day -5. On Day 0, the cats in Groups 2-4 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites were recovered and identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 8: *T. cati*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	70.9 (37-150)	N/A
ML-635 0.5X	0.6 (0-9) [a]	99.2%
ML-635 1X	0.1 (0)[a]	100%
ML-635 2X	0.1 (0-2)[a]	99.8%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

A minimum of five worms 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. Ten of the 10 control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 at 0.12 mL/kg was effective against adult *T. cati* infection in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 or with Praziquantel Alone, Administered against Induced Infections of *Toxocara cati* and *Ancylostoma tubaeforme* in Cats. (PR&D 0214901)

Study Location and Dates: Rockwood, TN; October 25, 2010 to November 1, 2010

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against induced infections of *T. cati* and *A. tubaeforme,* and the lack of effectiveness of praziquantel at the recommended minimum dose in cats with induced infections of both parasites.

Study Animals: Thirty-two cats (28 males and 4 females), 12 to 13 weeks of age and weighing 1.9 to 3 kg, were randomized into 10/11 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635
Group 3: Praziquantel

Treatment Dose: Cats in Group 2 were dosed topically with 0.12 mL/kg based on Day -3 bodyweights, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene.

Cats in Group 3 were dosed topically, based on Day -3 bodyweights, with 0.12 mL/kg of praziquantel, delivering 10 mg/kg.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with approximately 100 *T. cati* larvated eggs on Days -63, -62, and -61, and 50 *A. tubaeforme* larvae on Days -28, -27, and -26. The presence of eggs was confirmed in their feces on Days -5, -4, -3, or -1. On Day 0 the cats in Groups 2 and 3 were treated with ML-635 and praziquantel, respectively.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites were recovered and identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 9: *T. cati*

Treatment	**Geometric Mean worm count (range)**	**% Effectiveness**
Untreated control	17.4 (1-131)	N/A
ML-635	0.1 (0-2)[a]	99.4%
Praziquantel	40.0 (17-91)	0%

[a] There was a significant difference between the geometric means of the ML-635 treated and control groups ($p<0.001$). There was no difference between the geometric means between the praziquantel and control groups ($p=0.0576$).

A minimum of five (*T. cati*) and twenty (*A. tubaeforme*) worms 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. Eight of the 10 control cats satisfied the adequacy of infection requirement for *T. cati,* but the control group failed to meet the criteria for adequacy of infection for *A. tubaeforme*

Adverse Reactions: No adverse reactions were reported.

Conclusions: A single topical treatment with ML-635 was effective against *T. cati* infection in cats, the addition of praziquantel did not interfere with the effectiveness of eprinomectin, and the lack of effectiveness of praziquantel against *T. cati* infection in cats justifies the inclusion of eprinomectin in the formulation.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 against Larval Stages of *Toxocara cati* in Cats following Induced Infections. (PR&D 0256201)

Study Location and Dates: Rohrdorf, Germany; June 20, 2011 to July 28, 2011

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against larval stages of *T. cati* in cats with induced infections.

Study Animals: Twenty cats (10 males and 10 females), 3 to 4 months of age and weighing 1.3 to 2.3 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635 – Treatment on Day 14

The timing of treatment for Group 2 was designed to target the *T. cati* L_4 stage.

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -3 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with 250 *T. cati* eggs on Day -5. On Day 14, Group 2 cats were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites were recovered and identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 x (c_c-c_t) / c_c
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 10: *T. cati*

Treatment	**Geometric Mean worm count (range)**	**% Effectiveness**
Untreated control	3.3 (0-10)	N/A
ML-635	0.0 (0)[a]	100%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

A minimum of five worms 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 10 control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment of ML-635 was effective against fourth stage larvae infection of *T. cati* in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 against Larval Stages and/or Adults of *Toxocara cati* and/or *Ancylostoma tubaeforme* in Cats following Induced Infections. (PR&D 0219001)

Study Location and Dates: Rohrdorf, Germany; June 20, 2011 to July 28, 2011

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against larval stages and/or adults of *T. cati* and/or *A. tubaeforme* in cats with induced infections.

Study Animals: Thirty cats (15 males and 15 females), 13 to 14 weeks of age and weighing 0.9 to 1.7 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635 – Treatment on Day 0
Group 3: ML-635 – Treatment on Day 14

The timing of treatment for Group 2 was designed to target the *A. tubaeforme* L_4 stage. The timing of treatment for Group 3 was designed to target the *T. cati* L_4 stage.

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -1 (Group 2) or Day 13 (Group 3) bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with approximately 300 *T. cati* eggs on Day -5 and 200 *A. tubaeforme* larvae on Day -7. On Day 0, Group 2 cats were treated and on Day 14, Group 3 cats were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites were recovered and identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 11: *T. cati*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	4.2 (0-21)	N/A
ML-635	0.0 (0)[a]	100%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

Table 12: *A. tubaeforme*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	45.1 (2-129)	N/A
ML-635	0.0 (0)[a]	100%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

A minimum of five (*T. cati*) and twenty (*A. tubaeforme*) worms 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 10 control cats satisfied the adequacy of infection requirement for *T. cati* and 8 of 10 control cats satisfied the adequacy of infection requirement for *A. tubaeforme*.

Adverse Reactions: No adverse reactions were reported.

Conclusions: A single topical treatment with ML-635 was effective against fourth stage larvae infection of *T. cati* and fourth stage larvae infection of *A. tubaeforme* in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 against Larval Stages and/or Adults of *Toxocara cati* and/or *Ancylostoma tubaeforme* in Cats following Induced Infections. (PR&D 0219002)

Study Location and Dates: Rohrdorf, Germany; March 21, 2011 to April 28, 2011

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against larval stages and/or adults of *T. cati* and *A. tubaeforme* in cats with induced infections.

Study Animals: Thirty cats (15 males and 15 females), 13 to 16 weeks of age and weighing 1.2 to 2.1 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635 – Treatment on Day 0
Group 3: ML-635 – Treatment on Day 14

The timing of treatment for Group 2 was designed to target the *A. tubaeforme* L_4 stage. The timing of treatment for Group 3 was designed to target the *T. cati* L_4 stage.

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -3 (Group 2) or Day 13 (Group 3) bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with approximately 400 *T. cati* eggs on Day -5 and 300 *A. tubaeforme*

larvae on Day -7. On Day 0, Group 2 cats were treated with ML-635 and on Day 14, Group 3 cats were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites were recovered and identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 13: *A. tubaeforme*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	69.1 (3-152)	N/A
ML-635	0.0 (0)[a]	100%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

A minimum of five (*T. cati*) and twenty (*A. tubaeforme*) worms 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. Nine of 10 control cats satisfied the adequacy of infection requirement for *A. tubaeforme*. The study failed to satisfy the adequacy of infection requirement for *T. cati*.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against fourth stage larvae infection of *A. tubaeforme* in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 against Larval Stages and/or Adults of *Toxocara cati* and/or *Ancylostoma tubaeforme* in Cats following Induced Infections. (PR&D 0220701)

Study Location and Dates: Stanwood, MI; July 20, 2010 to August 13, 2010

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against larval stages and/or adults of *T. cati* and *A. tubaeforme* in cats with induced infections.

Study Animals: Thirty cats (15 males and 15 females), 10 to 11 weeks of age and weighing 0.9 to 1.4 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635 – Treatment on Day 0
Group 3: ML-635 – Treatment on Day 14

The timing of treatment for Group 2 was designed to target the *A. tubaeforme* L_4 stage. The timing of treatment for Group 3 was designed to target the *T. cati* L_4 stage.

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -1 (Group 2) or Day 13 (Group 3) bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with approximately 200 *T. cati* eggs on Day -5 and 200 *A. tubaeforme* larvae on Day -7. On Day 0, Group 2 cats were treated with ML-635 and on Day 14, Group 3 cats were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites were recovered and identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 14: *T. cati*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	7.2 (0-38)	N/A
ML-635	1.2 (0-9)[a]	83.2%

[a] There was a significant difference between the geometric means of the treated and control groups (p=0.021).

Table 15: *A. tubaeforme*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	105.1 (11-338)	N/A
ML-635	0.2 (0-1)[a]	99.8%

[a] There was a significant difference between the geometric means of the treated and control groups (p<0.001).

A minimum of five (*T. cati*) and twenty (*A. tubaeforme*) worms 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. Eight of the 10 control cats satisfied the adequacy of infection requirement for *T. cati* and 9 of 10 control cats satisfied the adequacy of infection requirement for *A. tubaeforme*.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against fourth stage larvae infection of *A. tubaeforme* in cats.

Title: Efficacy of a Single Topical Treatment of ML-635 against Induced Infection of *Ancylostoma braziliense* in Cats. (PR&D 0228601)

Study Location and Dates: Stanwood, MI; May 16, 2011 to June 25, 2011

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against induced infections of *A. braziliense* in cats.

Study Animals: Twenty cats (12 males and 8 females), approximately 5 months of age and weighing 2 to 2.7 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -2 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with 300 *A. braziliense* infective larvae on Day -28 and the presence of eggs was confirmed in their feces on Day -2. On Day 0 the cats were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites were recovered and identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 16: *A. braziliense*

Treatment	**Geometric Mean worm count (range)**	**% Effectiveness**
Untreated control	137.0 (49-339)	N/A
ML-635	0.6 (0-2)[a]	99.5%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

A minimum of five worms 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. Ten of the 10 control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against adult *A. braziliense* infection in cats.

Title: Efficacy of a Single Treatment with ML-635 Administered Topically in a Spot-on Formulation against Naturally Acquired Infections of *Dipylidium caninum* in Cats (PR&D 0258701).

Study Location and Dates: Coyoacan, Mexico D.F.; December 1, 2011-December 8, 2011

Study Design
Objective: To confirm the effectiveness of ML-635 against *Dipylidium caninum* at the recommended minimum dose of 0.12 mL/kg in naturally infected cats.

Study Animals: Twelve cats (3 males and 9 females), 8 months to 4 years of age and weighing 2.2 and 3.9 kg, were randomized into 6 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 10 mg/kg of praziquantel, 0.5 mg/kg of eprinomectin, 10 mg/kg of fipronil, and 12 mg/kg of (S)-methoprene, based on Day 0 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Fecal samples were examined macroscopically for proglottids and microscopically for eggs. All cats included in the study were naturally infected with *Dipylidium caninum*. On Day 0, the cats in Group 2 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine and large intestine (including cecum) were removed. The parasites recovered were identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment as a fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 17: *D. caninum*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	54.9 (10-279)	N/A
ML-635	1.3 (0-142)[a]	97.7%

[a] There was a significant difference between the geometric means of the treated and control groups (P=0.008)

A minimum of two *D. caninum* worms 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. All six of the control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against *D. caninum* infections in cats.

Title: Efficacy of a Single Topical Treatment of ML-635 against Induced Infections with *Echinococcus multilocularis* in Cats (PR&D 0270701).

Study Location and Dates: Saint-Vulbas, France; January 24, 2012-April 20, 2012

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against induced infections of *E. multilocularis* in cats.

Study Animals: Twenty cats (10 males, 10 females), aged 31 to 49 weeks and weighing 2.3 to 6.1 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats were dosed topically with 0.12 mL/kg, delivering 10 mg/kg of praziquantel, 0.5 mg/kg of eprinomectin, 10 mg/kg of fipronil, and 12 mg/kg of (S)-methoprene, based on Day -2 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. On Day -22, all animals were orally infected with approximately 38,000 *E. multilocularis* protoscolices. On Day 0, the cats in Group 2 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine and large intestine (including cecum) were removed. The parasites recovered were identified.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Troup as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 18: *E. multilocularis*

Treatment	Geometric Mean worm count (range)	% Effectiveness
Untreated control	100.8 (0-5445)	N/A
ML-635	0.0 (0) [a]	100.0%

[a] There was a significant difference between the geometric means of the treated and control groups ($P<0.001$)

A minimum of 10 worms 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. A minimum of eighteen *Echinococcus multilocularis* scolices were recovered from 8 out of 10 control animals satisfying the requirements for adequate infection.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against *E. multilocularis* infections in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 against Induced Infections of *Ancylostoma tubaeforme* in Cats (PR&D 0276901)

Study Location and Dates: Stanwood, Michigan; June 23, 2012 to June 30, 2012

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against induced infections of adult *A. tubaeforme* in cats.

Study Animals: Twenty-four cats (12 males and 12 females), 16 weeks of age and weighing 1.4 to 2.6 kg, were randomized into 12 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats in Group 2 were dosed topically at 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -3 bodyweights.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with approximately 100 *A. tubaeforme* larvae on Days -28, -27, and -26, and the presence of eggs was confirmed in their feces on Days -5 or -4. On Day 0, the cats in Group 2 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment and daily thereafter. At necropsy, the stomach, small intestine, and large intestine (including cecum) were removed. The parasites were recovered, identified, and counted.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 19: Adult *A. tubaeforme*

Treatment	**Geometric Mean Worm Count (Range)**	**% Effectiveness**
Untreated Control	54.8 (21 – 125)	N/A
ML-635	0 (0 - 0) [a]	100%

[a] There was a significant difference between the geometric means of the treated and control groups ($p<0.001$).

A minimum of twenty worms 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. All twelve control cats satisfied the adequacy of infection requirement.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against infections of adult *A. tubaeforme* in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 Against Larval Stages of *Toxocara cati* in Cats Following Induced Infection (PR&D 0286302)

Study Location and Dates: Rockwood, TN; April 2, 2013- April 23, 2013

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against fourth stage larval *Toxocara cati* in cats with induced infections.

Study Animals: 30 cats (14 males and 16 females), 12 weeks of age and weighing 1.0 to 1.5 kg, were randomized into 10 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635 administered on Day 0
Group 3: ML-635 administered on Day 14

The timing of treatment for Group 3 was designed to target the L4 stage of *T. cati.*

Treatment Dose: Cats in Group 2 and 3 were dosed topically at 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -1 (Group 2) and Day 12 (Group 3) bodyweight.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with approximately 250 *T. cati* eggs on Day -5. The cats in Group 2 were treated with ML-635 on Day 0 and the cats in Group 3 were treated with ML-635 on Day 14.

Variables Measured: In addition to once daily observations, cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment. At necropsy, the stomach, small intestine and large intestine (including cecum) were removed. The parasites were recovered, identified, and counted.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 x (c_c-c_t) / c_c
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 20: Fourth stage *T. cati* larvae

Treatment	Geometric Mean Worm Count (Range)	% Effectiveness
Untreated Control	56.4[a] (29 – 89)	N/A
ML-635 Day 14	0.5[b] (0 – 3)	99.2%

[ab] There was a significant difference between the geometric means of the treated and control groups ($P < 0.001$).

A minimum of five worms 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. All 10 control cats satisfied the adequacy of infection requirement for *T. cati*.

Adverse Reactions: No adverse reactions due to treatment were reported.

Conclusion: A single topical treatment with ML-635 was effective against fourth stage larvae *Toxocara cati* infection in cats.

Title: A Study to Determine the Efficacy of a Single Topical Treatment with ML-635 against Induced Infections of *Echinococcus multilocularis* in Cats (PR&D 0228701)

Study Location and Dates: Stanwood, MI; May 18, 2013- May 25, 2014

Study Design
Objective: To confirm the effectiveness of ML-635 at the recommended minimum dose of 0.12 mL/kg against induced infections of *Echinococcus multilocularis* in cats.

Study Animals: 18 cats (9 males and 9 females), 4 months of age and weighing 2.4 to 3.9 kg, were randomized into 9 cats per treatment group.

Treatment Groups:
Group 1: Untreated control
Group 2: ML-635

Treatment Dose: Cats in Group 2 were dosed topically at 0.12 mL/kg, delivering 0.5 mg/kg of eprinomectin, 10 mg/kg of praziquantel, 10 mg/kg of fipronil, and 12 mg/kg of (*S*)-methoprene, based on Day -2 bodyweight.

Drug Administration: A single, topical treatment, applied directly on the skin in the midline of the neck, between the base of the skull and the shoulder blades.

Experimental Design: This study was conducted using principles of Good Clinical Practice (GCP). This study was a randomized block design. Cats were infected with approximately 30,000 *E. multilocularis* protoscolices on Day -21. On Day 0, the cats in Group 2 were treated with ML-635.

Variables Measured: Cats were observed for adverse reactions at 1, 2, 3, and 4 hours post-treatment, and daily thereafter. At necropsy, the stomach, small intestine and large intestine (including cecum) were removed. The parasites were recovered, identified, and counted.

Statistical methods: The logarithm of the (counts+1) was analyzed using the mixed procedure with Treatment Group as fixed effect and Replicate (block by body weight) as a 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 = $100 \times (c_c - c_t) / c_c$
Where c_c = Geometric mean number of worms in the control group
c_t = Geometric mean number of worms in the treatment group

Results:

Table 21: *E. multilocularis*

Treatment	Geometric Mean Worm Count (Range)	% Effectiveness
Untreated Control	23.4[a] (0 – 350)	N/A
ML-635	0[b] (0 – 0)	100%

[ab] There was a significant difference between the geometric means of the treated and control groups ($P = 0.01$).

A minimum of 10 worms 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. A minimum of ten *Echinococcus multilocularis* scolices were recovered from 7 out of 9 control animals satisfying the requirements for adequate infection.

Adverse Reactions: No adverse reactions were reported.

Conclusion: A single topical treatment with ML-635 was effective against infection of *Echinococcus multilocularis* in cats.

III. TARGET ANIMAL SAFETY

The safety of Centragard™ was demonstrated in 3 well-controlled laboratory studies and in one well-controlled field study described below. The purpose of these studies was to provide information on the safety of Centragard™ when used according to the label in cats. In the margin of safety study, which includes groups of cats administered elevated doses of the drug, adverse reactions in cats administered 3X and 5X the maximum exposure dose included signs consistent with avermectin toxicity, such as hypersalivation, pupillary changes, ataxia, and depression. These reactions were not observed in cats administered the 1X dose. Oral tolerance was evaluated to assess the effects of accidental oral ingestion in cats, including from licking or grooming the application site. Oral administration resulted in excessive salivation in all cats immediately following administration, as well as lethargy and vomiting in some cats for 1-2 hours post-oral administration. Correct application of Centragard™ will minimize the occurrence of such events. A safety study in cats with experimentally induced heartworm infections was conducted to assess the safety of Centragard™ in cats infected with adult heartworms. One cat in that study administered a 1X dose exhibited abnormal respiratory signs lasting 24 hours that were not seen upon repeated dosing. Finally, a well-controlled field study was conducted in client-owned cats. The study included 314 cats administered the labeled dose. Adverse reactions included vomiting, anorexia, lethargy, alopecia, and application site hair change. These safety studies, in combination with the safety information collected in the effectiveness studies, demonstrate the safety of Centragard™ when used according to the label.

Safety studies were conducted with formulation ML-635, containing 8.3% fipronil, 0.4% eprinomectin, 8.3% praziquantel, and 10% (S)-methoprene. The doses of eprinomectin and praziquantel in ML-635 are equivalent to the approved formulation of Centragard™ (eprinomectin and praziquantel transdermal solution).

A. Margin of Safety Study (PR&D 0214501)

Title: Safety and Local Tolerance of a Combination of ML-635 when Administered Topically at 1, 3, and 5X the Maximum Exposure Dose in Cats.

Study Location and Dates: Saint-Vulbas, France; June 1, 2010 to October 18, 2010.

Study Design
Objective: The study objective was to evaluate the safety and local tolerance of a combination of fipronil, eprinomectin, praziquantel, and (S)-methoprene when administered to cats topically at 1X, 3X, and 5X the maximum exposure dose (of 0.375 mL/kg) six times at 28-day intervals.

Study Animals: Thirty-two (16 male and 16 female) kittens ranging in age from 7 - 9 weeks on Day 0 and weighing 0.5 - 1.2 kg (1.1 - 2.6 lbs) were included in this negative controlled, masked safety study.

Treatment Groups:

Table 22. Treatment Groups for Study PR&D 0214501

Treatment Group	Dose (mL/kg)	Number & Gender of Animals
1	0X, mineral oil at 1 mL/kg	8 (4 male, 4 female)
2	1X, 0.375 mL/kg	8 (4 male, 4 female)
3	3X, 1.125 mL/kg	8 (4 male, 4 female)
4	5X, 1.875 mL/kg	8 (4 male, 4 female)

The maximum intended dose (1X) for the product is 0.375 mL/kg. The lower end weight cut-off for this product is 1.8 lbs (0.8 kg).

Treatment Dose: Cats were dosed according to Table 22 on Days 0, 28, 56, 84, 112, and 140. The formulation contained eprinomectin (0.4% w/v), fipronil (8.3% w/v), S-methoprene (10% w/v), and praziquantel (8.3% w/v).

Drug Administration: Treatments were administered topically to the inter-scapular area by parting the hair on the midline of the neck between the base of the skull and the shoulder blades and applying the formulation directly onto the skin in one spot. Treatments were administered using 1 mL tuberculin-type disposable syringes. Combinations of such syringes were used when the volume exceeded 1 mL. To avoid run off, the 0X (mineral oil), 3X, and 5X doses were administered in several fractions in the interscapular area when necessary.

Variables Measured: Physical exams were conducted pre-study, and at 3 and 6 hours post-treatment on Days 0, 28, 56, 84, 112, and 140. Physical exams were also conducted twice daily on Days 1-3, 29-31, 57-59, 85-87, 113-115, and 141-143. Urine from each cat was collected once during each of these timeframes. Blood samples were collected on Days 1, 29, 57, 85, 113, and 141. General health observations were performed continuously for 1 hour post-treatment; at 2, 3, 4, 5, 6, 7, 8, 12, and 18 hours post-treatment; and twice daily on non-dosing days. Necropsy, blood, and urine collection were completed on Days 167, 168, 169, or 170.

Statistical Methods: Analyses of clinical pathology variables (plasma chemistry, hematology, coagulation, and urinalysis) were performed using analysis of covariance for a repeated measures design, including the factors sex, treatment, sampling day and all interactions involving those factors as fixed effects, and randomization block nested within sex-by-treatment as a random effect. For each variable, the Day -4 measurement was included as a covariate. Follow-up pairwise mean comparisons between the zero dose group and each non-zero dose group were performed, at two-sided significance level of 0.10.

Results: One 5X kitten exhibited ataxia, disorientation, and lethargy for 12 hours and exhibited pupil dilation for 24 hours following the 3rd treatment (Days 56-57). This 5X kitten exhibited ataxia, disorientation, and lethargy for 6 hours, and moderate pupil dilation for 24 hours following the 4th treatment (Days 84-85), and pupil dilation following the 5th treatment (Days 112-113). The cat recovered after each treatment. One 5X and one 3X kitten had hypersalivation at one time point post-treatment on Day 0 and on Day 84, respectively. One 5X kitten had slow pupillary light responses for one day after treatment. One 3X kitten had slow pupillary light responses for 3 hours after a treatment and one control cat

had marked pupil dilation and slow pupillary light responses for 2 hours after a treatment. Observations in the 1X group were similar to observations in the control group. Immediately post-treatment, cats in all groups commonly scratched or groomed at the application site.

Kittens in the 3X group had higher mean blood urea nitrogen (BUN) values at Day 167-170 compared to the controls. The 5X kittens had lower testes to body weight ratios compared to controls. These findings were not associated with any clinical signs.

Table 23. Adverse Reactions Chart

Adverse Reactions[1]	Control Group	1X Group	3X Group	5X Group
Ataxia, disoriented, depressed	0	0	0	1*
Mild pupillary dilation	3	5	8	7
Moderate pupillary dilation	0	0	5	5
Marked pupillary dilation, slow PLRs	1	0	1	1
Hypersalivation	0	0	1	1
Vomiting	0	1	0	0
Loose stool, diarrhea	6	4	5	4

[1] Individual cats may have exhibited more than one sign and these signs may have occurred after one or more treatments.
* One 5X cat exhibited these signs after treatment on Days 56 and 84 (third and fourth treatments).

Conclusions: The study supports the safe use of Centragard™ (eprinomectin and praziquantel transdermal solution) in cats when used at the labeled dose. Administration of a combination of fipronil, eprinomectin, praziquantel, and (S)-methoprene to kittens at 3X and 5X the maximum exposure dose resulted in signs consistent with avermectin toxicity, such as hypersalivation, pupillary changes, ataxia, and depression. Pupillary changes seen in the 1X group were similar to those seen in the control group.

B. Oral Tolerance Study (PR&D 0215501)

Title: Study to Determine the Safety in Cats of the topical formulation of ML-635 Administered Orally at the Potential Maximum Dose (i.e. 0.375 mL/kg).

Study Location and Dates: Fulton, MO; November 2, 2010 to November 17, 2010.

Study Design
Objective: To determine the safety of a combination of fipronil, eprinomectin, praziquantel, and (S)-methoprene orally administered at the maximum label dose to cats (0.375 mL/kg), delivering 1.5 mg/kg of eprinomectin, 31 mg/kg of praziquantel, 31 mg/kg of fipronil, and 37.5 mg/kg of (S)-methoprene, based on Day -4 bodyweights.

Study Animals: Sixteen cats (8 male and 8 female) ranging in age from 9.2 - 10.8 months on Day 0 and weighing 2.90 - 5.00 kg on Day -4 were included in the study.

Treatment Groups:

Table 24. Treatment Groups for Study PR&D 0215501

Treatment Group	Dose (mL/kg)	Number & Gender of Animals
1	Sham	8 (4 male, 4 female)
2	0.375 mL/kg orally	8 (4 male, 4 female)*

*One cat in oral tolerance study did not receive the oral dose of ML-635 due to an immediate regurgitation reaction.

Treatment Dose: Cats were dosed once orally on Day 0. Cats from Treatment Group 1 were sham dosed (i.e. handled in the same manner as the treated cats, using an empty syringe placed into the back of the mouth). Cats from Treatment Group 2 were orally administered a combination of fipronil, eprinomectin, praziquantel, and (S)-methoprene at 0.375 mL/kg. The formulation contained eprinomectin (0.4% w/v), fipronil (8.3% w/v), S-methoprene (10% w/v), and praziquantel (8.3% w/v).

Variables Measured: Physical exams were conducted twice daily on Days 0-3 and once on Day 14. General health observations were performed continuously for 1 hour post-administration; at 1, 2, 3, 4, 5, 6, 7, 8, 12, and 18 hours post-administration; and twice daily on Days 1-14. Body weight, and food and water consumption were monitored. Baseline and Day 14 hematology and clinical chemistry profiles were evaluated.

Results: All 8 cats in Group 2 exhibited excessive salivation immediately following administration of ML-635. For one hour following oral administration, all Group 2 cats licked their mouths and excessively salivated. One treated cat regurgitated a small amount of the solution immediately after administration. Within one hour post-treatment, two treated cats vomited and three treated cats were lethargic. Three treated cats had excessive salivation at the 2 hour observation point. One treated cat that was lethargic, vomited, and excessively salivated for 2 hours following administration had an increased ALT and AST on Day 14. One treated cat vomited on Day 12.

Conclusions: The oral administration of a combination of fipronil, eprinomectin, praziquantel, and (S)-methoprene to 9-10 month old cats induced clinical signs of excessive salivation immediately following administration. Lethargy and vomiting were observed in 3 cats for 1-2 hours post-oral administration.

C. Heartworm Positive Study (PR&D 0222501)

Title: A Study to Determine the Safety of ML-635 in Cats Experimentally Infected with Adult *Dirofilaria immitis*, when Administered Topically Three Times at 28 Day Intervals at One and Three Times the Expected Maximum Exposure Dose

Study Location and Dates: Athens, GA; December 17, 2010 to March 2, 2011.

Study Design
Objective: To evaluate the safety of a combination of fipronil, eprinomectin, praziquantel, and (S)-methoprene in cats infected with adult *Dirofilaria immitis* via jugular transplantation, when administered three times, 28 days apart, at 1X and 3X the expected maximum exposure dose (0.375 mL/kg and 1.125 mL/kg, respectively).

Study Animals: Thirty-six heartworm-infected Domestic Shorthair cats (18 male and 18 female), weighing 1.88 - 4.54 kg and approximately 4.7 - 6.6 months of age at study initiation, were enrolled in the study.

Treatment Groups:

Table 25. Treatment Groups for Study PR&D 0222501

Treatment Group	Dose (mL/kg)	Number & Gender of Animals
1	0X; mineral oil, 1.125 mL/kg	12 (6M/6F)
2	1X; ML-635, 0.375 mL/kg	12 (6M/6F)
3	3X; ML-635, 1.125 mL/kg	12 (6M/6F)

Heartworm Infection: *D. immitis* infections were induced by transplantation of adult *D. immitis*. Each cat was placed under anesthesia and received 3 male and 3 female live adult *D. immitis* heartworms by surgical transplantation into a jugular vein per the implantation technique described by Rawlings and McCall.[1] The *D. immitis* strain was a recent (<3 years old) USA field isolate.

All cats were negative for heartworm antibody, antigen, and microfilariae prior to transplantation. Two weeks after transplantation (Day -6), immunoserology verified positive heartworm antigen and the presence of microfilaria in all enrolled cats. All cats were negative for microfilaria at Day 84, including the controls. All cats, except one cat in the 1X group, were positive via heartworm antibody tests on Day -6 and all cats were positive on Day 84.

Treatment Dose: All animals were dosed once on Day 0, Day 28, and Day 56, except two cats in Group 1 (controls), which were dosed on Days 0 and 28 but died before Day 56.

Drug Administration: All treatments were applied topically on the midline of the neck between the base of the skull and the shoulder blades, using one or more 1 mL disposable syringes without the needle.

Variables Measured: Physical examinations were performed at baseline, 24 and 48 hours after each treatment, and on Day 83, at the end of the study. Daily health observations were performed twice daily on Days 0 – 83. On Days 0, 28,

[1] Rawlings CA, McCall JW. Surgical transplantation of adult *Dirofilaria immitis* to study heartworm infection and disease in dogs. *Am J Vet Res*. 1985; 46(1): 221-224.

and 56 post-treatment observations were performed at 1, 2, 4, 6, 8, and 24 hours after treatment. Cats were weighed prior to each treatment and on Day 83.

Results: Two control cats died during the study due to hypertrophic cardiomyopathy complicated by severe heartworm-associated systemic vasculitis. On Day 1, a 1X cat had cyanotic mucous membranes and had an increased respiratory rate lasting 24 hours. The cat recovered and exhibited no abnormal signs following two subsequent treatments.

There was no significant difference in the number of heartworms recovered from each group. See Table 26, below.

Table 26. Adult Heartworms Recovered at Necropsy

Treatment Group	# of cats per group	# of cats with worms	Worm range (Min, Max)	# of dead worms per group	Average# of worms
0X	12*	11	0, 6	1	4.25
1X	12	12	1, 6	9	4.08
3X	12	12	2, 6	2	3.92

* Two control cats died prior to study conclusion.

Conclusions: Topical administration of a combination of fipronil, eprinomectin, praziquantel, and (S)-methoprene was well tolerated in cats experimentally-infected with adult heartworms. One cat administered 1X the expected maximum dose exhibited abnormal respiratory signs lasting 24 hours.

D. Field Safety

In a well-controlled field study, 314 cats were topically administered a combination of fipronil, eprinomectin, praziquantel, and (S)-methoprene (ML-635). Adverse reactions (and percentage of cats that exhibited the adverse reaction during the study) included vomiting (5.7%), anorexia (3.8%), lethargy (2.9%), alopecia (3.5%), and application site hair change (4.1%).

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 Centragard™:

Not for human use. Keep out of reach of children. Avoid contact with the application site for 5 hours following treatment. Wash hands after administering the product. If the product accidentally gets into the eyes, wash 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 and 21 CFR part 514. The data demonstrate that Centragard™, 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 roundworms (adult and fourth stage larval *Toxocara cati*), hookworms (adult and fourth stage larval *Ancylostoma tubaeforme;* adult *Ancylostoma braziliense*), and tapeworms (adult *Dipylidium caninum* and *Echinococcus multilocularis*), in cats and kittens 7 weeks of age and older and 1.8 lbs or greater.

A. Marketing Status

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status) because professional expertise is needed to diagnose and monitor the parasites treated.

B. Exclusivity

Centragard™, 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 Centragard™.

C. Patent Information:

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