

Date of Approval: December 20, 2006

FREEDOM OF INFORMATION SUMMARY

NADA 141-251

ADVANTAGE MULTI for Dogs

Imidacloprid + Moxidectin

ADVANTAGE MULTI for Dogs is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*. ADVANTAGE MULTI for Dogs kills adult fleas and is indicated for the treatment of flea infestations (*Ctenocephalides felis*). ADVANTAGE MULTI for Dogs is also indicated for the treatment and control of the following intestinal parasites:

| Intestinal Parasite | | Intestinal Stage | | |
|---------------------|-------------------------------|------------------|----------------|---------------------|
| | | Adult | Immature Adult | Fourth Stage Larvae |
| Hookworm Species | <i>Ancylostoma caninum</i> | X | X | X |
| | <i>Uncinaria stenocephala</i> | X | X | X |
| Roundworm Species | <i>Toxocara canis</i> | X | | X |
| | <i>Toxascaris leonina</i> | X | | |
| Whipworm | <i>Trichuris vulpis</i> | X | | |

Sponsored by:
Bayer HealthCare LLC
Animal Health Division
P.O. Box 390
Shawnee Mission, KS 66201

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I. GENERAL INFORMATION:

- a. File Number: NADA 141-251
- b. Sponsor: Bayer HealthCare LLC
Animal Health Division
P.O. Box 390
Shawnee Mission, KS 66201

Drug Labeler Code: 000859
- c. Established Name: imidacloprid + moxidectin
- d. Proprietary Name: ADVANTAGE MULTI for Dogs
- e. Dosage Form: Solution
- f. How Supplied: Unit applicator tube
Applicator tube size and applications per package:
6 x 0.4 mL tubes
6 x 1.0 mL tubes
6 x 2.5 mL tubes
6 x 4.0 mL tubes
- g. How Dispensed: Rx
- h. Amount of Active Ingredients: 10% imidacloprid + 2.5% moxidectin
- i. Route of Administration: Topical
- j. Species/Class: Dogs
- k. Recommended Dosage:

The recommended minimum dose is 4.5 mg/lb (10 mg/kg) imidacloprid and 1.1 mg/lb (2.5 mg/kg) moxidectin, once a month by topical administration, as specified by the following table.

| Dog Weight (lb) | ADVANTAGE MULTI for Dogs | Volume (mL) | Imidacloprid (mg) | Moxidectin (mg) |
|------------------------|---------------------------------|--------------------|--------------------------|------------------------|
| 3 – 9 | ADVANTAGE MULTI 9 | 0.4 | 40 | 10 |
| 9.1 – 20 | ADVANTAGE MULTI 20 | 1.0 | 100 | 25 |
| 20.1 – 55 | ADVANTAGE MULTI 55 | 2.5 | 250 | 62.5 |
| 55.1 – 88* | ADVANTAGE MULTI 88 | 4.0 | 400 | 100 |

*Dogs over 88 lbs should be treated with the appropriate combination of ADVANTAGE MULTI for Dogs tubes.

l. Pharmacological Category: Antiparasitic

m. Indications:

ADVANTAGE MULTI for Dogs is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*. ADVANTAGE MULTI for Dogs kills adult fleas and is indicated for the treatment of flea infestations (*Ctenocephalides felis*). ADVANTAGE MULTI for Dogs is also indicated for the treatment and control of the following intestinal parasites:

| Intestinal Parasite | | Intestinal Stage | | |
|---------------------|-------------------------------|------------------|----------------|---------------------|
| | | Adult | Immature Adult | Fourth Stage Larvae |
| Hookworm Species | <i>Ancylostoma caninum</i> | X | X | X |
| | <i>Uncinaria stenocephala</i> | X | X | X |
| Roundworm Species | <i>Toxocara canis</i> | X | | X |
| | <i>Toxascaris leonina</i> | X | | |
| Whipworm | <i>Trichuris vulpis</i> | X | | |

2. EFFECTIVENESS:

a. Dosage Characterization for the Treatment of Flea Infestations:

- 1) Effectiveness Evaluation of Imidacloprid 10% Solution Applied Dermally for Control of Fleas on Dogs (Report #74572)

Purpose: The objective of this study was to evaluate the initial and residual flea effectiveness of three imidacloprid dose levels on dogs.

Investigator: Jerry Cunningham, M.S.

Location: Ag Research Consultants, Inc., Greenbrier, AR

Animals: 32 dogs (18 females and 14 males), 2 to 9 years of age, 8 dogs per treatment group (Groups 1 and 2 had 4 females and 4 males; Groups 3 and 4 had 5 females and 3 males.)

Treatment Groups: Group 1: 3.75 mg/kg of 10% Imidacloprid
Group 2: 7.50 mg/kg of 10% Imidacloprid
Group 3: 10.0 mg/kg of 10% Imidacloprid
Group 4: Control (vehicle without active ingredient)

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Each dog was infested with 100 unfed adult fleas on Days -1, 6, 13, 20, 27, and 33. Dogs were treated once on Day 0. Dogs were observed for adverse reactions at 0.5, 1, 3, and 5 hours post-treatment and daily thereafter. Dogs were visually examined for fleas on Days 1, 7, 14, 21, and 28. On Day 34, the dogs were combed and fleas removed and counted.

Results: Effectiveness of imidacloprid against adult fleas on dogs compared to the control is shown in the following table:

Table 1: Effectiveness of Three mg/kg Doses of Imidacloprid Against Fleas

| Day | Control | Group 1 (3.75 mg/kg) | | Group 2 (7.5 mg/kg) | | Group 3 (10 mg/kg) | |
|-----|-------------|-------------------------|-----------------------|------------------------|-----------------------|-----------------------|-----------------------|
| | Total Fleas | Total Fleas | Percent Effectiveness | Total Fleas | Percent Effectiveness | Total Fleas | Percent Effectiveness |
| 1 | 553 | 17 | 96.9 | 12 | 97.8 | 4 | 99.3 |
| 7 | 751 | 7 | 99.1 | 0 | 100 | 0 | 100 |
| 14 | 911 | 51 | 94.4 | 9 | 99.0 | 1 | 99.9 |
| 21 | 953 | 30 | 96.8 | 12 | 98.7 | 0 | 100 |
| 28 | 873 | 40 | 95.4 | 14 | 98.4 | 6 | 99.3 |
| 34 | 1171 | 98 | 91.6 | 28 | 97.6 | 36 | 96.9 |

Adverse Reactions: No adverse reactions were reported.

Conclusions: Imidacloprid applied topically as a single dose of 3.75 mg/kg, 7.5 mg/kg or 10 mg/kg was effective against adult fleas on dogs. Effectiveness at Day 34 was 91.6%, 97.6% and 96.9% after receiving a single topical dose of imidacloprid for 3.75, 7.5, or 10 mg/kg, respectively. The dose of 10 mg/kg was selected for the treatment of flea infestations.

b. Substantial Evidence of Effectiveness for the Treatment of Flea Infestations:

1) Effectiveness of Topically Applied Imidacloprid + Moxidectin Against Flea (*Ctenocephalides felis*) Infestations on Dogs (Study #150.902, Report #75238)

Purpose: The objectives of this study were to 1) determine the effectiveness of topically applied imidacloprid + moxidectin against flea infestations on dogs and 2) to demonstrate that moxidectin does not interfere with the insecticidal activity of imidacloprid against fleas.

Investigator: David R. Young, DVM, Ph.D.

Location: Young Veterinary Research Services, Turlock, CA

Animals: 32 dogs (16 females and 16 males), 6 to 10 years of age, various hair coat lengths, between 21.2 and 81.5 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: 10% Imidacloprid + 2.5% Moxidectin
Group 2: 2.5% Moxidectin
Group 3: 10% Imidacloprid
Group 4: Control (vehicle without active ingredients)

Treatment Dosages:

| | |
|----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤20 lbs | 1.0 mL |
| ≥ 20.1 ≤55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Each dog was infested with 100 unfed adult fleas (*Ctenocephalides felis*) on Day -5. A comb count was performed by Day -4 to establish pretreatment parasite burdens. On Days -1, 6, 13, 20, 27, and 34, each dog was infested with approximately 100 unfed adult fleas. Dogs were treated once on Day 0. Dogs were observed for adverse reactions at 1, 2, 3, 4, 6, 8, 12, 18, and 24 hours post-treatment and twice daily thereafter. Fleas were combed, counted, and removed on Days 1, 7, 14, 21, 28, and 35.

Variables Measured: Flea counts

Results: Effectiveness against flea infestations was calculated by comparing the geometric mean number of fleas on the control group with that of the treated groups. See Table 2:

Table 2: Percent Flea Effectiveness and Geometric Mean Number of Fleas

| Day | Group 1 Imidacloprid/Moxidectin | | Group 2 Moxidectin | | Group 3 Imidacloprid | | Group 4 Control |
|-----|------------------------------------|-------------|-----------------------|------------|-------------------------|------------|--------------------|
| | Percent Effectiveness | Flea Count* | Percent Effectiveness | Flea Count | Percent Effectiveness | Flea Count | Flea Count |
| 1 | 99.5 | 0.4 | 14.0 | 62.4 | 99.5 | 0.4 | 72.5 |
| 7 | 99.9 | 0.1 | 69.4 | 25.9 | 100 | 0.0 | 84.7 |
| 14 | 100 | 0.0 | 62.8 | 26.8 | 100 | 0.0 | 72.0 |
| 21 | 99.7 | 0.2 | 63.8 | 28.0 | 100 | 0.0 | 77.5 |
| 28 | 99.9 | 0.1 | 64.3 | 28.5 | 99.6 | 0.3 | 79.9 |
| 35 | 99.7 | 0.3 | 58.5 | 36.3 | 99.0 | 0.9 | 87.5 |

*All flea counts are geometric means

Adverse Reactions: Most of the dogs in all treatment groups had an oily or “spiked” appearance of the hair at the treatment site on Day 0 and Day 1.

Conclusion: Effectiveness of imidacloprid either alone or in combination with moxidectin against flea infestations was 99.0% to 100% up to Day 35. Moxidectin alone provided limited flea effectiveness. The combination product imidacloprid + moxidectin was as effective as imidacloprid alone against flea infestations. Moxidectin did not interfere with the activity of imidacloprid against fleas.

2) Effectiveness of Topically Applied Imidacloprid + Moxidectin Against Flea (*Ctenocephalides felis*) Infestations on Dogs (Study #151.038, Report #75258)

Purpose: The objective of this study was to confirm the effectiveness of topically applied imidacloprid + moxidectin against flea infestations on dogs.

Investigator: Robyn Slone, B.S.

Location: Professional Laboratory and Research Services (PLRS), Corapeake, NC

Animals: 16 dogs (9 females and 7 males), various breeds and hair coat lengths, between 18.8 and 54 lbs, 8 dogs per treatment group

Treatment Groups: Group 1: 10% Imidacloprid + 2.5% Moxidectin
Group 2: Control (vehicle without active ingredients)

Treatment Dosages:

| | |
|---------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥10.1 ≤20 lbs | 1.0 mL |
| ≥20.1 ≤55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Each dog was infested with 100 unfed adult fleas (*Ctenocephalides felis*) on Day -6. A comb count was performed by Day -5 to establish pretreatment parasite burdens. On Days -1, 6, 13, 20, 27, and 34 each dog was infested with approximately 100 unfed adult fleas. Dogs were treated once on Day 0. Dogs were observed for adverse reactions at 1, 2, 3, 4, 6, 8, 12, 18, and 24 hours post-treatment and twice daily thereafter. Fleas were combed, counted, and removed on Days 1, 7, 14, 21, 28, and 35.

Variables Measured: Flea counts

Results: Effectiveness of imidacloprid + moxidectin against flea infestations was calculated by comparing the geometric mean number of fleas on the control group with that of the treated groups. See Table 3:

Table 3: Percent Flea Effectiveness and Geometric Mean Number of Fleas

| Day | Group 1 Imidacloprid + Moxidectin | | Group 2 Control |
|-----|--------------------------------------|---------------------------|---------------------------|
| | Percent Effectiveness | Geometric Mean # of Fleas | Geometric Mean # of Fleas |
| 1 | 99.8 | 0.1 | 71.4 |
| 7 | 100 | 0.0 | 57.8 |
| 14 | 99.5 | 0.3 | 60.7 |
| 21 | 99.1 | 0.9 | 96.6 |
| 28 | 97.7 | 2.0 | 85.6 |
| 35 | 95.1 | 4.3 | 86.4 |

Adverse Reactions: One control group dog vomited on Day 2 and had diarrhea on Day 14.

Conclusion: Effectiveness of imidacloprid + moxidectin against flea infestations was 95.1% to 100% up to Day 35.

3) Evaluation of the Effects of Shampooing or Water Immersion on the Initial and Residual Effectiveness of Imidacloprid for Flea Control on Dogs (Report #74792)

Purpose: The objective of this study was to evaluate the initial and residual effectiveness of imidacloprid for flea control on dogs following shampooing or water immersion.

Investigator: Ronald Everett, Ph.D.

Location: AgResearch Consultants, Inc., Greenbriar, AR

Animals: 24 dogs (13 females and 11 males), 2 to 11 years of age, 8 dogs per treatment group (Group 1 had 5 females and 3 males, Groups 2 and 3 had 4 females and 4 males)

Treatment Groups:

Group 1: 10% Imidacloprid 10 mg/kg: Shampooed 4 days post-treatment

Group 2: 10% Imidacloprid 10 mg/kg: Water immersion weekly post-treatment

Group 3: Control (vehicle without active ingredient):

4 dogs shampooed 4 days post-treatment

4 dogs immersed in water weekly post-treatment

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Each dog was infested with 100 adult fleas (*Ctenocephalides felis*) on Days -1, 6, 13, 20, 27, and 34. Dogs were treated once on Day 0. Dogs were observed for adverse reactions at 0.5, 1, 3, and 5 hours post-treatment and daily thereafter. Flea comb counts were performed on Days 1, 7, 14, 21, 28, and 35. Dogs in Group 1 were shampooed with non-medicated shampoo on Day 4. Dogs in Group 2 were immersed in a tank of tap water for 1 minute and allowed to air dry on Days 4, 11, 18, 25, and 32. Each dog's head was thoroughly wetted three times during the immersion procedure. Dogs in Group 3 were divided into two groups. Half were shampooed on Day 4 and the other half were immersed in water weekly.

Variables Measured: Flea counts

Results: Effectiveness of imidacloprid against flea infestations after bathing or water immersion was calculated by comparing the geometric mean number of fleas on the control group with that of the treated groups. See Table 4:

Table 4: Effectiveness of Imidacloprid after Bathing or Water Immersion

| Day | Imidacloprid Shampoo | | Imidacloprid Water Immersion | | Control Shampoo | Control Water Immersion |
|-----|-----------------------|-------------|------------------------------|------------|-----------------|-------------------------|
| | Percent Effectiveness | Flea Count* | Percent Effectiveness | Flea Count | Flea Count | Flea Count |
| 1 | 100 | 0 | 100 | 0 | 94.3 | 66.6 |
| 7 | 98.9 | 0.9 | 99.8 | 0.1 | 90.0 | 74.3 |
| 14 | 97.7 | 2.2 | 99.4 | 0.5 | 94.5 | 85.8 |
| 21 | 94.4 | 5.4 | 95.7 | 3.8 | 95.6 | 87.5 |
| 28 | 96.3 | 3.7 | 96.7 | 2.7 | 100.4 | 83.9 |
| 35 | 71.4 | 25.5 | 86.8 | 12.2 | 89.1 | 92.5 |

*All flea counts are geometric means

Adverse Reactions: No adverse reactions were reported.

Conclusion: Imidacloprid applied topically as a single dose of 10 mg/kg was from 94.4% to 100% effective against adult flea infestations for 28 days after shampooing 4 days post-treatment or immersion in water weekly post-treatment. This study supports the labeling statement: Shampooing or water immersion 4 days after treatment will not reduce the effectiveness of ADVANTAGE MULTI for Dogs against flea infestations.

c. Dosage Characterization for the Prevention of Heartworm Disease:

Refer to section e., Dosage Characterization for the Treatment and Control of Intestinal Nematodes, which establishes a minimum effective dose for moxidectin.

d. Substantial Evidence for the Prevention of Heartworm Disease:

- 1) Evaluation of Post-Treatment Bathing/Shampooing on the Effectiveness of Topically Applied Imidacloprid + Moxidectin for the Prevention of Heartworm Disease in Dogs (Report #75459)

Purpose: The objective of this study was to establish the safety and effectiveness of topically applied imidacloprid + moxidectin against experimentally induced larval heartworm (*Dirofilaria immitis*) infections of dogs. The study also determined the effects of post-treatment bathing/shampooing on product effectiveness.

Investigator: Larry E. Travis, B.S.

Location: Inhausen Research Institute, Inc., Ft. Collins, CO

Animals: 24 purpose bred Beagles (12 females and 12 males), 11 to 12 months of age, between 20.7 and 27.7 pounds, 6 dogs per treatment group

Treatment Groups: Group 1: 10% Imidacloprid + 2.5% Moxidectin
 Group 2: 10% Imidacloprid + 2.5% Moxidectin,
 Bathed/shampooed 90 minutes post-treatment
 Group 3: Control (vehicle without active ingredients)
 Group 4: Control (vehicle without active ingredients),
 Bathed/shampooed 90 minutes post-treatment

Treatment Dosages: ≤10 lbs 0.4 mL
 ≥ 10.1 < 20 lbs 1.0 mL
 ≥ 20.1 < 55 lbs 2.5 mL
 >55 lbs 4.0 mL

Route of Administration: Topical on the dorsal midline

Frequency of Treatment: Single treatment

Study Design: Heartworm diagnostic tests were performed on Day -34. On Day -33 each dog was experimentally inoculated with 50 *D. immitis* larvae harvested from infected mosquitoes (*Aedes aegypti*). Dogs were treated once on Day 0. Dogs were observed for adverse reactions at 1, 2, 3, 4, 6, 8, 12, and 24 hours post-treatment and then once or twice daily thereafter. Ninety minutes after treatment, dogs in Groups 2 and 4 were bathed. The shampoo was allowed to remain in contact with the skin and hair coat for 2-3 minutes. The dogs were rinsed and the bathing procedure was repeated. Heartworm diagnostic tests were repeated on Day 87 or 91. On Days 113 or 114, dogs were euthanized and adult heartworms were recovered and enumerated.

Variables Measured: Adult heartworm counts

Results: Effectiveness of imidacloprid + moxidectin against the development of adult heartworms was calculated by comparing the geometric mean number of adult heartworms in the control groups with that of the treated groups. See Table 5:

Table 5: Heartworm Effectiveness

| Group | Treatment | Geometric Mean # of Heartworms | Percent Effectiveness |
|-------|--|--------------------------------|-----------------------|
| 1 | Imidacloprid + Moxidectin | 0 | 100 |
| 2 | Imidacloprid + Moxidectin (bathed/shampooed 90 minutes post-treatment) | 0 | 100 |
| 3 | Control | 34.5 | NA* |
| 4 | Control (bathed/shampooed 90 minutes post-treatment) | 35.1 | NA |

*Not Applicable

Adverse Reactions: Post-treatment, 7 dogs in Group 1, and 1 dog in Group 2 had diarrhea. Dogs in all groups had reduced food intake post-treatment.

Conclusions: Imidacloprid + moxidectin applied topically was 100% effective against larval heartworm infections in dogs when treatment was applied 33 days following experimental infection with *D. immitis* larvae. Bathing/shampooing dogs 90 minutes post-treatment did not reduce the effectiveness of imidacloprid + moxidectin for prevention of heartworm disease.

2) Evaluation of Post-Treatment Bathing/Shampooing on the Effectiveness of Topically Applied Imidacloprid + Moxidectin for Prevention of Heartworm Disease in Dogs (Report #75484)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin against experimentally induced larval heartworm (*Dirofilaria immitis*) infections of dogs. The study also evaluated the effects of post-treatment bathing/shampooing and simulated swim/rain exposure on product effectiveness.

Investigator: Robyn L. Slone, B.S.

Location: Professional Laboratory & Research Services, Inc., Corapeake, NC

Animals: 40 purpose bred Beagles (20 females and 20 males), 6.5 to 8 months of age, between 16.2 and 29.7 pounds, 8 dogs per treatment group

Treatment Groups:

- Group 1: 10% Imidacloprid + 2.5% Moxidectin
Simulated swim/rain exposure 60 minutes post-treatment
- Group 2: 10% Imidacloprid + 2.5% Moxidectin,
Simulated swim/rain exposure 24 hours post-treatment, and
repeated 7, 14, 21, and 28 days post-treatment
- Group 3: 10% Imidacloprid + 2.5% Moxidectin
Bathed/shampooed 4 hours post-treatment
- Group 4: 10% Imidacloprid + 2.5% Moxidectin,
Bathed/shampooed 1 day post-treatment
- Group 5: Control (10% Imidacloprid)
Simulated swim/rain exposure 24 hours post-treatment, and
repeated 7, 14, 21, and 28 days post-treatment

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline (one spot between the shoulder blades for dogs ≤ 20 lbs, and 3 spots from the shoulder to the base of the tail in dogs > 20 lbs)

Frequency of Treatment: Single treatment

Study Design: Heartworm diagnostic tests were performed on Day -44. On Day -34 each dog was experimentally inoculated with 50 *D. immitis* larvae harvested from infected mosquitoes (*Aedes aegypti*). Dogs were treated once on Day 0. Dogs were observed for adverse reactions at 1, 6.5, 8, and 12 hours post-treatment and once or twice daily thereafter. Dogs in Groups 1, 2, and 5 were exposed to water to simulate swimming or heavy rainfall. These simulations were enacted by immersing the dog's entire body (up to but not including the face) in a tank of warm water for approximately 2 minutes. Dogs in Groups 3 and 4 were bathed/shampooed at 4 and 24 hours post-treatment, respectively. The shampoo was allowed to remain in contact with the skin and hair-coat for 2 to 3 minutes. The dogs were rinsed and the bathing procedure was repeated. Heartworm diagnostic tests were repeated on Day 75. On Day 119 (153 days post-infection) the dogs were euthanized and adult heartworms were recovered and enumerated.

Variables Measured: Adult heartworm counts

Results: Effectiveness of imidacloprid + moxidectin against the development of adult heartworms was calculated by comparing the geometric mean number of adult heartworms in the control group with that of the treated groups. See Table 6:

Table 6: Heartworm Effectiveness

| Group | Treatment | Geometric Mean # of Heartworms | Percent Effectiveness |
|--------------|--|---------------------------------------|------------------------------|
| 1 | Imidacloprid + Moxidectin (simulated rain/swim 60 minutes post-treatment) | 0 | 100 |
| 2 | Imidacloprid + Moxidectin (simulated rain/swim 24 hours, 7, 14, 21 and 28 days post-treatment) | 0 | 100 |
| 3 | Imidacloprid + Moxidectin (bathed/shampooed 4 hours post-treatment) | 0 | 100 |
| 4 | Imidacloprid + Moxidectin (bathed/shampooed 1 day post-treatment) | 0 | 100 |
| 5 | Control – Imidacloprid (rain/swim schedule as in Group 2) | 21.7 | NA |

Adverse Reactions: Diarrhea, with or without blood, occurred in 3 dogs in Group 5.

Conclusions: Imidacloprid + moxidectin applied topically was 100% effective for the prevention of heartworm disease when treatment was applied 34 days following experimental infection with *D. immitis* larvae. Imidacloprid treatment alone was not effective for the prevention of heartworm disease in dogs. Imidacloprid did not interfere with the activity of moxidectin for prevention of heartworm disease in dogs. Single or multiple exposure to water, as may occur with swimming or rainfall, beginning as early as 60 minutes post-treatment, and shampooing dogs 4 hours or 1 day post-treatment did not reduce the effectiveness of imidacloprid + moxidectin for prevention of heartworm disease.

3) Effectiveness of Topically Applied Imidacloprid + Moxidectin for Prevention of Heartworm Disease in Dogs (Report #75490)

Purpose: The objective of this study was to determine the safety and effectiveness of topically applied imidacloprid + moxidectin against experimentally induced larval heartworm (*Dirofilaria immitis*) infections of dogs. The study was designed to confirm that imidacloprid alone has no activity against larval stages of *D. immitis* and imidacloprid does not interfere with the activity of moxidectin against *D. immitis* in the combined formulation.

Investigator: Dwight Bowman, Ph.D.

Location: Cheri-Hill Kennel, Stanwood, MI

Animals: 24 purpose bred Beagles (12 females and 12 males) 5 to 7 months of age, between 19.7 and 28.9 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: 10% Imidacloprid + 2.5% Moxidectin
Group 2: 2.5% Moxidectin
Group 3: Control (10% Imidacloprid)

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline (one spot between the shoulder blades for dogs ≤ 20 lbs, and 3 spots from the shoulder to the base of the tail in dogs > 20 lbs)

Frequency of Treatment: Single treatment

Study Design: Heartworm diagnostic tests were performed on Day -46. On Day -45 each dog was experimentally inoculated with 50 *D. immitis* larvae harvested from infected mosquitoes (*Aedes aegypti*). Dogs were treated once on Day 0. Dogs were observed for adverse reactions at 1, 2, 3, 4, 6, 8, 12, and 18 hours post-treatment and once or twice daily thereafter. Heartworm diagnostic tests were repeated on Day 75. On Day 119 (164 days post-infection) the dogs were euthanized and adult heartworms were recovered and enumerated.

Variables Measured: Adult heartworm counts

Results: Effectiveness of imidacloprid + moxidectin against the development of adult heartworms was calculated by comparing the geometric mean number of adult heartworms in the control group with that of the treated groups. See Table 7:

Table 7: Heartworm Effectiveness

| Group | Treatment | Geometric Mean # of Heartworms | Percent Effectiveness |
|-------|---------------------------|--------------------------------|-----------------------|
| 1 | Imidacloprid + Moxidectin | 0 | 100 |
| 2 | Moxidectin | 0 | 100 |
| 3 | Control – Imidacloprid | 37.6 | NA |

Adverse Reactions: Twenty-two dogs had damp or stiff fur at the application site. Thirteen dogs had a white residue at the application site. Twenty-two dogs had diarrhea during the study and ten dogs (3 dogs each in Groups 1 and 2, and 4 dogs in Group 3) vomited at least once post-treatment.

Conclusions: Imidacloprid + moxidectin and moxidectin solution alone applied topically were 100% effective against larval stages of *D. immitis* in dogs when treatment was administered 45 days post-infection. Imidacloprid solution alone was not effective against larval stages of *D. immitis* and imidacloprid did not interfere with the activity of moxidectin against larval stages of *D. immitis* in the combined formulation.

e. Dosage Characterization for the Treatment and Control of Intestinal Nematodes:

Nine dosage characterization studies were conducted by Bayer in Germany and Australia using different concentrations of moxidectin combined with 10% imidacloprid. The dosage volume was fixed at 0.1 mL/kg to maintain the same dosage schedule established for ADVANTAGE. For studies conducted in Germany, DRONTAL Plus Tablets (praziquantel/febantel/pyrantel) were given to the dogs after treatment with imidacloprid + moxidectin as a reference standard to remove any remaining intestinal nematodes rather than necropsy the dogs and collect the remaining worms.

The results of the dosage characterization studies are summarized in Table 8. The data indicates that moxidectin at a dose of 2.5 mg/kg was consistently greater than 90% effective against *Toxocara canis* and *Ancylostoma caninum*. The bolded doses were less than 90% effective.

Table 8: Summary of Dosage Characterization Studies

| Study # | Location | Parasite | Moxidectin dose (mg/kg) | Percent Effectiveness | # of dogs |
|---------|-----------|--------------------------------|-------------------------|-----------------------|-----------|
| 75414 | Germany | Adult <i>A. caninum</i> | 2.5 | 100 | 5 |
| | | | 1.75 | 100 | 5 |
| | | | 1.0 | 100 | 5 |
| | | Adult <i>T. canis</i> | 2.5 | 91.8 | 5 |
| | | | 1.75 | 96.5 | 5 |
| | | | 1.0 | 79.1 | 5 |
| 75415 | Germany | Adult <i>T. canis</i> | 7.5 | 100 | 4 |
| 75416 | Germany | Adult <i>T. canis</i> | 2.5 | 91.0 | 5 |
| | | | 1.75 | 96.4 | 5 |
| | | | 1.0 | 76.5 | 5 |
| 75417 | Germany | Adult <i>A. caninum</i> | 5.0 | 100 | 2 |
| 75418 | Germany | Adult <i>A. caninum</i> | 2.5 | 100 | 5 |
| | | | 1.75 | 100 | 5 |
| | | | 1.0 | 100 | 5 |
| 75419 | Germany | 14-day-old <i>T. canis</i> | 5.0 | 100 | 5 |
| 75420 | Australia | 7-day-old <i>A. caninum</i> | 5.0 | 100 | 4 |
| | | | 2.5 | 100 | 5 |
| 75421 | Australia | Adult <i>A. caninum</i> | 7.5 | 100 | 5 |
| | | | 5.0 | 100 | 2 |
| | | | 2.5 | 100 | 11 |
| | | | 1.0 | 100 | 4 |
| 75422 | Australia | Adult <i>T. canis</i> | 2.5 | 98.2 | 6 |
| | | | 1.75 | 71.4 | 5 |
| | | | 1.0 | 83.3 | 5 |

f. Substantial Evidence for the Treatment and Control of Adult Intestinal Nematodes:

- 1) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Natural Infections with *Toxocara canis* in Dogs (Study #150.970, Report #75312)

Purpose: The objectives of this study were 1) to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with adult *Toxocara canis* in dogs and 2) to demonstrate a lack of interference between imidacloprid and moxidectin when combined in the same formulation.

Investigator: William Barton, PhD

Location: Central Arizona Veterinary Laboratory (CAVL), Amarillo, TX

Animals: 32 mongrel dogs (16 females and 16 males), 2 to 24 months of age, between 1.8 and 47.4 pounds, 8 dogs enrolled per treatment group

Treatment Groups:

- Group 1: Control (vehicle without active ingredients)
- Group 2: 2.5% Moxidectin
- Group 3: 10% Imidacloprid + 2.5% Moxidectin
- Group 4: 10% Imidacloprid

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Toxocara canis* were treated once on Day 0. Following a 9 to 10 day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *T. canis* were recovered, identified, and counted.

Variables Measured: Adult *Toxocara canis* worm counts

Results: Effectiveness against adult *Toxocara canis* was calculated by comparing the geometric mean number of adult *Toxocara canis* in the control group with that of the treated groups. See Table 9:

Table 9: Effectiveness of Imidacloprid + Moxidectin against Adult *Toxocara canis*

| Group | Treatment | Geometric Mean # of <i>T. canis</i> | Percent Effectiveness |
|--------------|---------------------------|--|----------------------------------|
| 1 | Control | 7.7 | NA |
| 2 | Moxidectin | 0.5 | 94.0 |
| 3 | Imidacloprid + Moxidectin | 0.3 | 96.2 |
| 4 | Imidacloprid | 7.5 | 2.7 |

Adverse Reactions: During both pre- and post-treatment, diarrhea was observed in dogs from all 4 treatment groups. Four dogs died during the study. Post-mortem examinations determined that one dog from Group 1 died due to anemia and emaciation associated with enteric helminthiasis and one dog each from Groups 1, 2 and 3 died from complications associated with parvoviral gastroenteritis.

Conclusions: A single topical dose of imidacloprid + moxidectin was 96.2% effective against natural infections with adult *Toxocara canis* in dogs. Imidacloprid alone demonstrated little or no activity against *T. canis*. The anthelmintic activity of moxidectin was not diminished when combined with imidacloprid.

2) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Natural Infections with *Toxocara canis* in Dogs (Study #151.257, Report #75326)

Purpose: The objectives of this study were 1) to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with adult *Toxocara canis* in dogs and 2) to demonstrate a lack of interference between imidacloprid and moxidectin when combined into the same formulation.

Investigator: Merle Olson, DVM, MSc

Location: DVM Services, Calgary, Alberta

Animals: 32 mongrel dogs (16 females and 16 males), 2.5 to 4 months of age, between 6.4 and 20.6 pounds, 8 dogs enrolled per treatment group

Treatment Groups: Group 1: Control (vehicle without active ingredients)
Group 2: 10% Imidacloprid + 2.5% Moxidectin
Group 3: 2.5% Moxidectin
Group 4: 10% Imidacloprid

| | | |
|---------------------------|-----------------|--------|
| <u>Treatment Dosages:</u> | ≤10 lbs | 0.4 mL |
| | ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| | ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| | >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Toxocara canis* were treated once on Day 0. Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *T. canis* were recovered, identified, and counted.

Variables Measured: Adult *Toxocara canis* worm counts

Results: Effectiveness against adult *Toxocara canis* was calculated by comparing the geometric mean number of adult *Toxocara canis* in the control group with that of the treated groups. See Table 10:

Table 10: Effectiveness of Imidacloprid + Moxidectin against Adult *Toxocara canis*

| Group | Treatment | Geometric Mean # of <i>T. canis</i> | Percent Effectiveness |
|--------------|---------------------------|--|----------------------------------|
| 1 | Control | 5.2 | NA |
| 2 | Imidacloprid + Moxidectin | 0.0 | 100 |
| 3 | Moxidectin | 0.0 | 100 |
| 4 | Imidacloprid | 6.3 | NA |

Adverse Reactions: During both pre- and post-treatment, diarrhea was occasionally observed in dogs from all 4 treatment groups. One dog in Group 4 was euthanized on Day 2 and an intestinal intussusception was diagnosed during post-mortem examination. Mild, self-limiting depression was observed post-treatment in another dog from Group 4.

Conclusions: A single topical dose of imidacloprid + moxidectin was 100% effective against natural infections with adult *Toxocara canis* in dogs. Imidacloprid alone demonstrated no activity against *T. canis*. The anthelmintic activity of moxidectin was not diminished by its combination with imidacloprid.

3) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Natural Infections with *Toxascaris leonina* in Dogs (Report #75327)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with adult *Toxascaris leonina* in dogs.

Investigator: Merle Olson, DVM, MSc

Location: DVM Services, Calgary, Alberta

Animals: 16 mongrel dogs (7 females and 9 males), approximately 9 months of age, between 36.7 and 50.5 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: Control (vehicle without active ingredients)
Group 2: 10% Imidacloprid + 2.5% Moxidectin

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 < 20 lbs | 1.0 mL |
| ≥ 20.1 < 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Toxascaris leonina* were treated once on Day 0. Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *Toxascaris leonina* were recovered, identified, and counted.

Variables Measured: Adult *Toxascaris leonina* worm counts

Results: Effectiveness against adult *Toxascaris leonina* was calculated by comparing the geometric mean number of adult *Toxascaris leonina* in the control group with that of the treated group. See Table 11:

Table 11: Effectiveness against Adult *Toxascaris leonina*

| Group | Treatment | Geometric Mean # of <i>T. leonina</i> | Percent Effectiveness |
|-------|---------------------------|---------------------------------------|-----------------------|
| 1 | Control | 20.4 | NA |
| 2 | Imidacloprid + Moxidectin | 0.0 | 100 |

Adverse Reactions: Post-treatment, diarrhea was occasionally observed in dogs from both treatment groups. One control group dog had a poor appetite on Days 1, 4, and 5. One imidacloprid + moxidectin group dog vomited once on Day 5.

Conclusions: A single topical dose of imidacloprid + moxidectin was 100% effective against natural infections with adult *Toxascaris leonina* in dogs.

4) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Artificial Infections with *Toxascaris leonina* in Dogs (Report #75351).

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for experimentally induced infections with *Toxascaris leonina* in dogs.

Investigator: John W. McCall, PhD

Location: TRS Labs, Inc., Athens, GA

Animals: 16 purebred Beagles (7 females and 9 males), 9 to 10 months of age, between 15.8 and 35.8 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: Control (vehicle without active ingredients)
Group 2: 10% Imidacloprid + 2.5% Moxidectin

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: On Day -76, dogs were experimentally infected with *Toxascaris leonina*. Dogs were treated once on Day 0 (replicate 1) or on Day 14 (replicate 2). Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *Toxascaris leonina* were recovered, identified, and counted.

Variables Measured: Adult *Toxascaris leonina* worm counts

Results: Effectiveness against adult *Toxascaris leonina* was calculated by comparing the geometric mean number of adult *Toxascaris leonina* in the control group with that of the treated group. See Table 12:

Table 12: Effectiveness against Adult *Toxascaris leonina*

| Group | Treatment | Geometric Mean # of <i>T. leonina</i> | Percent Effectiveness |
|-------|---------------------------|---------------------------------------|-----------------------|
| 1 | Control | 19.1 | NA |
| 2 | Imidacloprid + Moxidectin | 0.6 | 97.0 |

Adverse Reactions: One dog in the imidacloprid + moxidectin group had diarrhea 30 minutes, 1, 2, and 4 hours post-treatment. Two dogs in the imidacloprid + moxidectin group started vomiting 1 and 2 days post-treatment, respectively.

Conclusions: A single topical dose of imidacloprid + moxidectin was 97.0% effective against experimentally induced infections with *Toxascaris leonina* in dogs.

5) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Natural Infections with *Ancylostoma caninum* in Dogs (Report #75308).

Purpose The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with adult *Ancylostoma caninum* in dogs.

Investigator: William Barton, PhD

Location: Central Arizona Veterinary Laboratory (CAVL), Amarillo, TX

Animals: 18 mongrel dogs (6 females and 12 males), 3 to 96 months of age, between 17 and 60.1 pounds, 9 dogs per treatment group

Treatment Groups: Group 1: 10% Imidacloprid + 2.5% Moxidectin
Group 2: Control (vehicle without active ingredients)

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Ancylostoma caninum* were treated once on Day 0. Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *Ancylostoma caninum* were recovered, identified, and counted.

Variables Measured: Adult *Ancylostoma caninum* worm counts

Results: Effectiveness against adult *Ancylostoma caninum* was calculated by comparing the geometric mean number of adult *Ancylostoma caninum* in the control group with that of the treated group. See Table 13:

Table 13: Effectiveness against Adult *Ancylostoma caninum*

| Group | Treatment | Geometric Mean # of <i>A. caninum</i> | Percent Effectiveness |
|-------|---------------------------|---------------------------------------|-----------------------|
| 1 | Imidacloprid + Moxidectin | 0.0 | 100 |
| 2 | Control | 43.4 | NA |

Adverse Reactions: One dog in the control group was depressed on Days 1 through 7. In the imidacloprid + moxidectin group, one dog had diarrhea on Day 7, another dog had diarrhea on Days 5 and 9, and another dog had a dry stool on Day 3 and diarrhea on Day 9. The remaining dogs had at least one incidence of diarrhea or bloody diarrhea, which started during the pre-treatment period.

Conclusions: A single topical dose of imidacloprid + moxidectin was 100% effective against natural infections with adult *Ancylostoma caninum* in dogs.

6) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Natural Infections with *Ancylostoma caninum* in Dogs (Report #75310)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for adult *Ancylostoma caninum* in dogs.

Investigator: Larry Cruthers, PhD

Location: Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 16 mongrel dogs (7 females and 9 males), 9 weeks to 6 years of age, between 6.2 and 73 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: Control (vehicle without active ingredients)
 Group 2: 10% Imidacloprid + 2.5% Moxidectin

Treatment Dosages:

| | |
|----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤20 lbs | 1.0 mL |
| ≥ 20.1 ≤55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Ancylostoma caninum* were treated once on Day 0. Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *Ancylostoma caninum* were recovered, identified, and counted.

Variables Measured: Adult *Ancylostoma caninum* worm counts

Results: Effectiveness against adult *Ancylostoma caninum* was calculated by comparing the geometric mean number of adult *Ancylostoma caninum* in the control group with that of the treated group. See Table 14:

Table 14: Effectiveness against Adult *Ancylostoma caninum*

| Group | Treatment | Geometric Mean # of <i>A. caninum</i> | Percent Effectiveness |
|--------------|---------------------------|--|----------------------------------|
| 1 | Control | 37.1 | NA |
| 2 | Imidacloprid + Moxidectin | 0.0 | 100 |

Adverse Reactions: During both pre- and post-treatment, diarrhea was occasionally observed in dogs from each treatment group. One dog in the imidacloprid + moxidectin group had diarrhea on Day 2 and vomited once on Day 4. Four dogs in the control group developed diarrhea post-treatment. One dog in the control group developed a non-productive cough on Days 9 and 10.

Conclusions: A single topical dose of imidacloprid + moxidectin was 100% effective against natural infections with adult *Ancylostoma caninum* in dogs.

7) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Natural Infections with *Uncinaria stenocephala* in Dogs (Report #75309).

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with adult *Uncinaria stenocephala* in dogs.

Investigator: Larry Cruthers, PhD

Location: Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 16 mongrel dogs (11 females and 5 males), 3 to 36 months of age, between 10.2 and 53.4 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: Control (vehicle without active ingredients)
Group 2: 10% Imidacloprid + 2.5% Moxidectin

Treatment Dosages: ≤10 lbs 0.4 mL
≥ 10.1 ≤ 20 lbs 1.0 mL
≥ 20.1 ≤ 55 lbs 2.5 mL
>55 lbs 4.0 mL

Route of Administration: Topical

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Uncinaria stenocephala* were treated once on Day 0. Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *Uncinaria stenocephala* were recovered, identified, and counted.

Variables Measured: Adult *Uncinaria stenocephala* worm counts

Results: Effectiveness against adult *Uncinaria stenocephala* was calculated by comparing the geometric mean number of adult *Uncinaria stenocephala* in the control group with that of the treated group. See Table 15:

Table 15: Effectiveness against Adult *Uncinaria stenocephala*

| Group | Treatment | Geometric Mean # of <i>U. stenocephala</i> | Percent Effectiveness |
|--------------|---------------------------|---|------------------------------|
| 1 | Control | 32.7 | NA |
| 2 | Imidacloprid + Moxidectin | 0.0 | 100 |

Adverse Reactions: During both pre- and post-treatment, ocular discharge was occasionally observed in several dogs from the imidacloprid + moxidectin group. One dog in the imidacloprid + moxidectin group developed nasal and ocular discharge at 4 hours post-treatment which did not resolve until Day 10. One dog in the control group vomited on Day 4.

Conclusions: A single topical dose of imidacloprid + moxidectin was 100% effective against natural infections with adult *Uncinaria stenocephala* in dogs.

8) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Natural Infections with *Uncinaria stenocephala* in Dogs (Report #75304)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with adult *Uncinaria stenocephala* in dogs.

Investigator: William Barton, PhD

Location: Central Arizona Veterinary Laboratory (CAVL), Amarillo, TX

Animals: 14 mongrel dogs (6 females and 8 males), 1 to 4 years of age, between 30.7 and 67.2 pounds, 7 dogs per treatment group

Treatment Groups: Group 1: 10% Imidacloprid + 2.5% Moxidectin
Group 2: Control (vehicle without active ingredients)

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Uncinaria stenocephala* were treated once on Day 0. Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *Uncinaria stenocephala* were recovered, identified, and counted.

Variables Measured: Adult *Uncinaria stenocephala* worm counts

Results: Effectiveness against adult *Uncinaria stenocephala* was calculated by comparing the geometric mean number of adult *Uncinaria stenocephala* in the control group with that of the treated group. See Table 16:

Table 16: Effectiveness against Adult *Uncinaria stenocephala*

| Group | Treatment | Geometric Mean # of <i>U. stenocephala</i> | Percent Effectiveness |
|-------|---------------------------|--|-----------------------|
| 1 | Imidacloprid + Moxidectin | 0.0 | 100 |
| 2 | Control | 221.3 | NA |

Adverse Reactions: One dog in the imidacloprid + moxidectin group exhibited depression and unsteadiness on Days 6 through 9. The signs in this dog may have been related to peak serum levels of moxidectin, which vary between dogs, and occur between 1 and 21 days after topical application of imidacloprid + moxidectin. Diarrhea was observed in dogs from each treatment group before and after treatment. Three dogs in the control group and one dog in the imidacloprid + moxidectin group developed bloody stools post-treatment. Two dogs in the control group became anorexic post-treatment.

Conclusions: A single topical dose of imidacloprid + moxidectin was 100% effective against natural infections with adult *Uncinaria stenocephala* in dogs.

9) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Natural Infections with *Trichuris vulpis* in Dogs (Study #151.430, Report #75467)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with adult *Trichuris vulpis* in dogs.

Investigator: Tony Janes

Location: CAVL, Amarillo, TX

Animals: 22 mixed-breed dogs (11 females and 11 males), 1 to 5 years of age, between 19.4 and 72.9 pounds, 11 dogs per treatment group

Treatment Groups: Group 1: 10% Imidacloprid + 2.5% Moxidectin
Group 2: Control (vehicle without active ingredients)

Treatment Dosages:

| | |
|----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤20 lbs | 1.0 mL |
| ≥ 20.1 ≤55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline (one spot at the base of the skull for dogs < 20 lbs, and 3-4 spots from the shoulder to the tail in dogs > 20 lbs)

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Trichuris vulpis* were treated once on Day 0. Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *Trichuris vulpis* were recovered, identified, and counted.

Variables Measured: Adult *Trichuris vulpis* worm counts

Results: Effectiveness against adult *Trichuris vulpis* was calculated by comparing the geometric mean number of adult *Trichuris vulpis* in the control group with that of the treated group. See Table 17:

Table 17: Effectiveness against Adult *Trichuris vulpis*

| Group | Treatment | Geometric Mean # of <i>T. vulpis</i> | Percent Effectiveness |
|--------------|---------------------------|---|----------------------------------|
| 1 | Imidacloprid + Moxidectin | 0.4 | 97.2 |
| 2 | Control | 15.5 | NA |

Adverse Reactions: No adverse reactions were reported. Compared to the control group, the number of observations of loose stools decreased post-treatment in the imidacloprid/moxidectin group.

Conclusions: A single topical dose of imidacloprid + moxidectin was 97.2% effective against natural infections with adult *Trichuris vulpis* in dogs.

10) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Adult *Trichuris vulpis* in Dogs (Study #151.468, Report #75507)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with adult *Trichuris vulpis* in dogs.

Investigator: Larry Cruthers, PhD

Location: Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 16 mixed-breed dogs (13 females and 3 males), approximately 6 months to 10 years of age, between 17.6 and 64.2 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: Control (vehicle without active ingredients)
Group 2: 10% Imidacloprid + 2.5% Moxidectin

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dog's neck at the base of the skull

≥ 20.1 ≤ 55 lbs 2.5 mL
>55 lbs 4.0 mL

Route of Administration: Topical on the dog's neck at the base of the skull

Frequency of Treatment: Single treatment

Study Design: Dogs naturally infected with adult *Trichuris vulpis* were treated once on Day 0. Following a 10-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, the gastrointestinal tract was opened and surviving adult *Trichuris vulpis* were recovered, identified, and counted.

Variables Measured: Adult *Trichuris vulpis* worm counts

Results: Effectiveness against adult *Trichuris vulpis* was calculated by comparing the geometric mean number of adult *Trichuris vulpis* in the control group with that of the treated group. See Table 19:

Table 19: Effectiveness against Adult *Trichuris vulpis*

| Group | Treatment | Geometric Mean # of <i>T. vulpis</i> | Percent Effectiveness |
|-------|---------------------------|--------------------------------------|-----------------------|
| 1 | Imidacloprid + Moxidectin | 2.5 | 90.1 |
| 2 | Control | 24.9 | NA |

Adverse Reactions: Compared to the control group, the number of observations of loose stools increased in the imidacloprid + moxidectin group after treatment. Two dogs in the imidacloprid + moxidectin group vomited: one at 4 hours post-treatment and the other at 24 hours post-treatment. Slight erythema was observed at the application site in 3 of the imidacloprid + moxidectin group dogs between 2 and 8 hours post-treatment.

Conclusions: A single topical dose of imidacloprid + moxidectin was 90.1% effective against natural infections with adult *Trichuris vulpis* in dogs.

g. Substantial Evidence for the Treatment and Control of Immature Intestinal Nematodes:

- 1) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Immature (Fourth Stage Larvae and Immature Adults) *Toxocara canis* in Dogs (Report #75366)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with fourth stage larvae and immature adult *T. canis* in dogs.

Investigator: Larry Cruthers, PhD

Location: Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 32 purebred Beagles (16 females and 16 males), approximately 2 months of age, between 4.6 and 7.6 pounds, 8 dogs per treatment group

Treatment Groups:

- Group 1: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 14, Necropsy on Day 20
- Group 2: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 14, Necropsy on Day 20
- Group 3: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 24, Necropsy on Day 29
- Group 4: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 24, Necropsy on Day 29

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 < 20 lbs | 1.0 mL |
| ≥ 20.1 < 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: On Day 0, dogs were experimentally infected with embryonated *Toxocara canis* eggs. Dogs were treated once, on Day 14 or Day 24, to coincide with specific immature stages (fourth stage larvae or immature adults, respectively). Following a 5 to 6 day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, *Toxocara canis* recovered from the gastrointestinal tract were identified to stage and counted.

Variable Measured: Adult, immature adult, and fourth stage larvae *Toxocara canis* worm counts

Results: Effectiveness of imidacloprid + moxidectin against fourth stage larvae and immature adult *T. canis* in dogs is shown in Table 20:

Table 20: Effectiveness against Fourth Stage Larvae and Immature Adult *T. canis*

| Treatment Group | # of <i>Toxocara canis</i> Recovered | | | Geometric Mean # of <i>T. canis</i> | Percent Effectiveness |
|--|--------------------------------------|-----------------|--------|-------------------------------------|-----------------------|
| | 4 th Stage Larvae | Immature Adults | Adults | | |
| Group 1: Control (Day 14) | 67 | 80 | 1 | 9.2 | NA |
| Group 2: Imidacloprid + Moxidectin (Day 14) | 2 | 2 | 0 | 0.2 | 97.6 |
| Group 3: Control (Day 24) | 30 | 205 | 3 | 15.4 | NA |
| Group 4: Imidacloprid + Moxidectin (Day 24) | 0 | 2 | 0 | 0.2 | 98.7 |

Adverse Reactions: No adverse reactions were reported

Conclusions: The effectiveness of a single topical dose of imidacloprid + moxidectin against fourth stage larvae and immature adult *T. canis* in dogs was 97.6% and 98.7%, respectively.

2) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Immature (Fourth Stage Larvae and Immature Adults) *Toxocara canis* in Dogs (Report #75412)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with fourth stage larvae and immature adult *T. canis* in dogs.

Investigators: Dara J. Cooke, BSc and Martin Murphy, MVB, MS, PhD, MRCVS

Location: Biological Laboratories Europe Ltd., Glenamoy, Ireland

Animals: 32 purebred Beagles (16 females and 16 males), 3.5 months of age, between 10.4 and 15.3 pounds, 8 dogs per treatment group

Treatment Groups:

- Group 1: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 14, Necropsy on Day 19
- Group 2: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 14, Necropsy on Day 19
- Group 3: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 24, Necropsy on Day 29
- Group 4: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 24, Necropsy on Day 29

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 < 20 lbs | 1.0 mL |
| ≥ 20.1 < 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: On Day 0, dogs were experimentally infected with embryonated *Toxocara canis* eggs. Dogs were treated once, on Day 14 or Day 24, to coincide with specific immature stages (fourth stage larvae or immature adults, respectively). Following a 5-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, *Toxocara canis* recovered from the gastrointestinal tract were identified to stage and counted.

Variable Measured: Adult, immature adult, and fourth stage larvae *Toxocara canis* worm counts

Results: Effectiveness of imidacloprid + moxidectin against the fourth stage larvae of *T. canis* in dogs is shown in Table 21. Control Group 1 did not have adequate infections of *T. canis*. However, control Group 4 had adequate infections of fourth stage larvae, which provides evidence of effectiveness of Group 3 against fourth stage larvae. However, because control Group 4 did not have an adequate infection of immature adult or adult *T. canis*, the study does not provide evidence of effectiveness against immature adult *Toxocara canis*.

Table 21: Effectiveness against Fourth Stage Larvae of *T. canis*

| Treatment Group | # of <i>Toxocara canis</i> Recovered | | | Geometric Mean # of <i>T. canis</i> | Percent Effectiveness |
|---|--------------------------------------|-----------------|--------|-------------------------------------|-----------------------|
| | 4 th Stage Larvae | Immature Adults | Adults | | |
| Group 3: Imidacloprid + Moxidectin (Day 24) | 3 | 0 | 0 | 0.3 | 98.2 |
| Group 4: Control (Day 24) | 119 | 0 | 0 | 13.8 | NA |

Adverse Reactions: One dog developed lameness immediately after treatment with imidacloprid + moxidectin.

Conclusions: The effectiveness of topical imidacloprid + moxidectin against fourth stage *T. canis* larvae in dogs was 98.2% (treatment at 24 days post-infection).

3) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Immature (Fourth Stage Larvae and Immature Adults) *Ancylostoma caninum* in Dogs (Report #75317)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with fourth stage larvae and immature adult *A. caninum* in dogs.

Investigator: Craig R. Reinemeyer, DVM, PhD

Location: East Tennessee Clinical Research (ETCR), Inc., Knoxville, TN

Animals: 32 purebred Beagles (16 females and 16 males), approximately 2.5 months of age, between 5.4 and 10 pounds, 8 dogs per treatment group

Treatment Groups:

- Group 1: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 7, Necropsy on Day 12
- Group 2: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 7, Necropsy on Day 12
- Group 3: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 11, Necropsy on Day 16
- Group 4: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 11, Necropsy on Day 16

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: On Day 0, dogs were experimentally infected with *A. caninum* larvae. Dogs were treated once, on Day 7 or Day 11, to coincide with specific immature stages (fourth stage larvae or immature adults, respectively). Following a 5-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, *Ancylostoma caninum* recovered from the gastrointestinal tract were identified to stage and counted.

Variable Measured: Adult, immature adult, and fourth stage larvae *Ancylostoma caninum* worm counts

Results: Effectiveness of imidacloprid + moxidectin against fourth stage larvae of *Ancylostoma caninum* in dogs is shown in Table 22. Treatment 7 days after infection targets the fourth stage larvae. Because control Group 1 had an adequate infection after treatment at Day 7, the study provides evidence of effectiveness against the fourth stage larvae of *A. caninum*. However, because control Group 3 did not have adequate infections of *A. caninum*, the study does not provide evidence of effectiveness against immature adult *Ancylostoma caninum*.

Table 22: Effectiveness against Fourth Stage Larvae of *A. caninum*

| Treatment Group | # of <i>A. caninum</i> Recovered | | | Geometric Mean # of <i>A. caninum</i> | Percent Effectiveness |
|--|----------------------------------|-----------------|--------|---------------------------------------|-----------------------|
| | 4 th Stage Larvae | Immature Adults | Adults | | |
| Group 1: Control (Day 7) | 0 | 0 | 982 | 90.4 | NA |
| Group 2: Imidacloprid + Moxidectin (Day 7) | 0 | 0 | 0 | 0.0 | 100 |

Adverse Reactions: During both pre- and post-treatment, diarrhea and ocular discharge were occasionally observed in dogs from both treatment groups. Post-treatment, mild erythema was observed at the treatment site of 4 dogs (2 dogs each from Groups 3 and 4). One imidacloprid + moxidectin group dog experienced diarrhea and flaky, peeling skin on the abdomen 2 and 8 hours post-treatment.

Conclusions: The effectiveness of topical imidacloprid + moxidectin against fourth stage *A. caninum* larvae in dogs was 100% (treatment at 7 days post-infection).

4) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Immature (Fourth Stage Larvae and Immature Adults) *Ancylostoma caninum* and *Uncinaria stenocephala* in Dogs (Report #75316)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with fourth stage larvae and immature adult *A. caninum* and *U. stenocephala* in dogs.

Investigator: Larry Cruthers, PhD

Location: Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 32 mongrel puppies (15 females and 17 males), approximately 2 months of age, between 3.6 and 7.8 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 7, Necropsy on Day 12
Group 2: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 7, Necropsy on Day 12
Group 3: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 11, Necropsy on Day 17
Group 4: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 11, Necropsy on Day 17

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: On Day 0, dogs were experimentally infected with *A. caninum* and *U. stenocephala* larvae. Dogs were treated once, on Day 7 or Day 11, to coincide with specific immature stages (fourth stage larvae or immature adults, respectively). Following a 5 or 6 day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, *Ancylostoma caninum* and *U. stenocephala* recovered from the gastrointestinal tract were identified to species and stage and counted.

Variable Measured: Adult, immature adult, and fourth stage larvae *Ancylostoma caninum* and *Uncinaria stenocephala* worm counts

Results: Effectiveness of imidacloprid + moxidectin against fourth stage larvae and immature adult *Ancylostoma caninum* in dogs is shown in Table 23. Due to inadequate infections, effectiveness against *U. stenocephala* could not be determined.

Table 23: Effectiveness against Fourth Stage Larvae and Immature Adult *A. caninum*

| Treatment Group | # of <i>A. caninum</i> Recovered | | | Geometric Mean # of <i>A. caninum</i> | Percent Effectiveness |
|---|----------------------------------|-----------------|--------|---------------------------------------|-----------------------|
| | 4 th Stage Larvae | Immature Adults | Adults | | |
| Group 1: Control (Day 7) | 2 | 124 | 27 | 17.9 | NA |
| Group 2: Imidacloprid + Moxidectin (Day 7) | 0 | 0 | 0 | 0.0 | 100 |
| Group 3: Control (Day 11) | 0 | 0 | 170 | 20.8 | NA |
| Group 4: Imidacloprid + Moxidectin (Day 11) | 0 | 0 | 0 | 0.0 | 100 |

Adverse Reactions: During both pre- and post-treatment, diarrhea was occasionally observed in pups from all 4 treatment groups. One imidacloprid + moxidectin group dog had diarrhea starting ½ hour post-treatment and started vomiting 1 hour post-treatment. Four dogs had white deposits on the tips of their fur immediately post-treatment that completely resolved by eight hours post-treatment.

Conclusions: Effectiveness of a single topical dose of imidacloprid + moxidectin against fourth stage larvae and immature adult *A. caninum* in dogs was 100%.

5) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Immature (Fourth Stage Larvae and Immature Adults) *Ancylostoma caninum* and *Uncinaria stenocephala* in Dogs (Report #75307)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with fourth stage larvae and immature adult *A. caninum* and *U. stenocephala* in dogs.

Investigator: Larry Cruthers, PhD

Location: Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 32 Beagle or hound-type puppies (11 males and 21 males), 2 to 3 months of age, between 2.4 and 28 pounds, 8 dogs per treatment group

Treatment Groups: Group 1: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 7, Necropsy on Day 12
Group 2: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 7, Necropsy on Day 12
Group 3: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 11, Necropsy on Day 17
Group 4: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 11, Necropsy on Day 17

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: On Day 0, dogs were experimentally infected with *A. caninum* and *U. stenocephala* larvae. Dogs were treated once, on Day 7 or Day 11, to coincide with specific immature stages (fourth stage larvae or immature adults, respectively). Following a 5 or 6 day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, *Ancylostoma caninum* and *U. stenocephala* recovered from the gastrointestinal tract were identified to species and stage and counted.

Variable Measured: Adult, immature adult, and fourth stage larvae *Ancylostoma caninum* and *Uncinaria stenocephala* worm counts

Results: Effectiveness of imidacloprid + moxidectin against fourth stage larvae and immature adult *Ancylostoma caninum* and *U. stenocephala* in dogs are shown in Table 24 and Table 25, respectively.

Table 24: Effectiveness against Fourth Stage Larvae and Immature Adult *A. caninum*

| Treatment Group | # of <i>A. caninum</i> Recovered | | | Geometric Mean # of <i>A. caninum</i> | Percent Effectiveness |
|---|----------------------------------|-----------------|--------|---------------------------------------|-----------------------|
| | 4 th Stage Larvae | Immature Adults | Adults | | |
| Group 1: Control (Day 7) | 18 | 41 | 50 | 9.3 | NA |
| Group 2: Imidacloprid + Moxidectin (Day 7) | 0 | 0 | 0 | 0.0 | 100 |
| Group 3: Control (Day 11) | 0 | 0 | 122 | 9.4 | NA |
| Group 4: Imidacloprid + Moxidectin (Day 11) | 0 | 0 | 0 | 0.0 | 100 |

Table 25: Effectiveness against L4 Larvae and Immature Adult *U. stenocephala*

| Treatment Group | # of <i>U. stenocephala</i> Recovered | | | Geometric Mean # of <i>U. stenocephala</i> | Percent Effectiveness |
|---|---------------------------------------|-----------------|--------|--|-----------------------|
| | 4 th Stage Larvae | Immature Adults | Adults | | |
| Group 1: Control (Day 7) | 182 | 248 | 51 | 36.7 | NA |
| Group 2: Imidacloprid + Moxidectin (Day 7) | 0 | 0 | 0 | 0.0 | 100 |
| Group 3: Control (Day 11) | 0 | 0 | 557 | 44.3 | NA |
| Group 4: Imidacloprid + Moxidectin (Day 11) | 0 | 0 | 0 | 0.0 | 100 |

Adverse Reactions: Four dogs in the imidacloprid + moxidectin group developed diarrhea post-treatment. Three out of those four dogs had blood in the diarrhea. One dog in the imidacloprid + moxidectin group and one dog in the control group developed a cough post-treatment. Fifteen dogs had deposits on the hair immediately post-treatment that resolved within 2 to 48 hours.

Conclusions: A single topical dose of imidacloprid + moxidectin was 100% effective against the developmental stages (fourth stage larvae and immature adults) of both *A. caninum* and *U. stenocephala* in dogs.

6) Evaluation of the Effectiveness of a Combination of Imidacloprid and Moxidectin against Immature (Fourth Stage Larvae and Immature Adults) *Uncinaria stenocephala* in Dogs (Study #141.330, Report #75411)

Purpose: The objective of this study was to confirm the safety and effectiveness of topically applied imidacloprid + moxidectin as a treatment for infections with fourth stage larvae and immature adult *U. stenocephala* in dogs.

Investigator: Dara J. Cooke, BSc

Location: Biological Laboratories Europe Ltd., Glenamoy, Ireland

Animals: 32 purebred Beagles (16 females and 16 males), 2.7 to 3.4 months of age, between 7 and 13 pounds, 8 dogs per treatment group

Treatment Groups:

- Group 1: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 7, Necropsy on Day 12
- Group 2: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 7, Necropsy on Day 12
- Group 3: 10% Imidacloprid + 2.5% Moxidectin,
Infected on Day 0, Treated on Day 11, Necropsy on Day 16
- Group 4: Control (vehicle without active ingredients)
Infected on Day 0, Treated on Day 11, Necropsy on Day 16

Treatment Dosages:

| | |
|-----------------|--------|
| ≤10 lbs | 0.4 mL |
| ≥ 10.1 ≤ 20 lbs | 1.0 mL |
| ≥ 20.1 ≤ 55 lbs | 2.5 mL |
| >55 lbs | 4.0 mL |

Route of Administration: Topical on the dorsal midline between the shoulder blades

Frequency of Treatment: Single treatment

Study Design: On Day 0, dogs were experimentally infected with *U. stenocephala* larvae. Dogs were treated once, on Day 7 or Day 11, to coincide with specific immature stages (fourth stage larvae or immature adults, respectively). Following a 5-day post-treatment observation period, dogs were euthanized and necropsied. At necropsy, *U. stenocephala* recovered from the gastrointestinal tract were identified to stage and counted.

Variable Measured: Adult, immature adult, and fourth stage larvae *Uncinaria stenocephala* worm counts

Results: Effectiveness of imidacloprid + moxidectin against fourth stage (L4) larvae and immature adult *U. stenocephala* in dogs is shown in Table 26.

Table 26: Effectiveness against L4 Larvae and Immature Adult *U. stenocephala*

| Treatment Group | # of <i>U. stenocephala</i> Recovered | | | Geometric Mean # of <i>U. stenocephala</i> | Percent Effectiveness |
|---|---------------------------------------|-----------------|--------|--|-----------------------|
| | 4 th Stage Larvae | Immature Adults | Adults | | |
| Group 1: Control (Day 7) | 83 | - | - | 10.2 | NA |
| Group 1: Control (Day 7) | - | 13 | - | 1.1 | NA |
| Group 2: Imidacloprid + Moxidectin (Day 7) | 0 | 0 | 0 | 0.0 | 100 |
| Group 3: Imidacloprid + Moxidectin (Day 11) | 0 | 0 | 0 | 0.0 | 100 |
| Group 4: Control (Day 11) | 19 | - | - | 1.3 | NA |
| Group 4: Control (Day 11) | - | 66 | - | 7.2 | NA |
| Group 4: Control (Day 11) | - | - | 28 | 1.6 | NA |

Adverse Reactions: Both pre- and post-treatment, diarrhea was observed in 23 dogs in all treatment groups. Post-treatment, diarrhea was occasionally observed in 1 dog from Group 1 and 2 dogs from Group 2.

Conclusions: The effectiveness of a single topical dose of imidacloprid + moxidectin against fourth stage larvae and immature adult *U. stenocephala* in dogs was 100%.

3. TARGET ANIMAL SAFETY:

- 1) Evaluation of the Safety of Imidacloprid/Moxidectin Topical Solution in Puppies (Study #150.872, Report #75158) Good Laboratory Practice (GLP) study

Purpose: The objective of this study was to demonstrate the safety of 10.0% imidacloprid + 2.5% moxidectin topical solution when administered topically to puppies at 14-day intervals for 6 treatments.

Study Director: Albert Abraham, BVSc, PhD

Location: Desoto Research Facility, DeSoto, KS

Animals: 48 Beagle puppies (6 females and 6 males per treatment group), 7 weeks of age and weighing between 2.6 and 5.7 pounds at initial treatment

Treatment Dosages: Dogs in the control group (Group 1) were treated with mineral oil at 5 times (5X) the recommended unit dose volume for their weight range. Dogs in Groups 2, 3, and 4 were treated topically with 10% imidacloprid + 2.5% moxidectin at 1X, 3X, and 5X multiples of the recommended unit dose volumes, respectively. [For example, since the recommended (label) unit dose volume for dogs weighing 3 to 9 lbs is 0.4 mL, dogs in the 3X group that weighed 3 to 9 lbs were treated with 1.2 mL of imidacloprid + moxidectin.] See Table 27 for the range of mg/kg doses applied in each group.

Table 27: Minimum and Maximum Imidacloprid and Moxidectin Doses Applied

| Treatment Group | Imidacloprid | | Moxidectin | |
|-----------------|--------------|-----------|------------|-------------|
| | Minimum | Maximum | Minimum | Maximum |
| 1X | 9 mg/kg | 30 mg/kg | 2.25 mg/kg | 7.5 mg/kg |
| 3X | 27 mg/kg | 85 mg/kg | 6.75 mg/kg | 21.25 mg/kg |
| 5X | 44 mg/kg | 142 mg/kg | 11 mg/kg | 35.5 mg/kg |

Route of Administration: Topical, applied to different sites on back of head, neck and shoulders

Frequency of Treatment: Once every 14 days for a total of 6 applications

Study Design: Physical examinations were performed on Days -12 and 83. Body weights were taken on Days -12, -1, 13, 27, 41, 55, 69, 77, and 83. The puppies were treated on Days 0, 14, 28, 42, 56, and 70. On treatment days, clinical observations were conducted pre-dosing, immediately after dosing, and at 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dosing. On other study days, clinical observations were performed twice daily. Food consumption was monitored twice daily. Blood samples were collected for clinical pathology on Days -7, -1, 1, 15, 29, 43, 57, 71, and 83. The puppies were necropsied on Days 84 and 85.

Variables Measured: Clinical observations – systemic and local (application sites), physical examinations, body weight, food consumption, hematology, clinical chemistry, gross pathology, and histopathology

Statistical Method: A repeated measures analysis of covariance was used to model each of the endpoints. The fixed effects included in the model were treatment, sex, day, treatment-by-sex, and treatment-by-day. The random effect included in the model was animal. Statistical analysis was assessed at the 10% level of significance.

Results:

Clinical Observations: Vomiting was reported in one puppy from the 1X group (Day 57), in two puppies from the 3X group (Days 1 and 79), and in one puppy from the 5X group (Day 1). Loose stools and diarrhea were observed in all groups, including the controls, throughout the study. One puppy from the control group died of a jejunal intussusception on Day 23.

One puppy in the 1X group had pruritus for one hour following the fifth treatment. Puppies treated with imidacloprid + moxidectin had a rough hair coat at the application site post-treatment.

A puppy from the 5X group was observed to have rapid, difficult respiration with moist rales 4 to 8 hours post-dosing on Day 14. Treatment was not necessary. A puppy from the control group had heavy respiration post-dosing on Day 0 and on Day 27.

Body Weights and Food Consumption: Male puppies in the 1X and 3X groups gained less weight during the study compared to the control group puppies. Two puppies in each group treated with imidacloprid + moxidectin had decreased appetite within 24 hours post-dosing. Puppies in the control group did not exhibit a decreased appetite.

Hematology, Chemistry, and Necropsy: Although all individual bilirubin values were within the normal range, the number of puppies with increases in direct bilirubin was higher in the treated groups: 1/11 puppies in the control group, 8/12 puppies in the 1X group, 6/12 puppies in the 3X group, and 6/12 puppies in the 5X group. No differences were noted between groups on hematology, necropsy, or histopathology.

Conclusions: Imidacloprid + moxidectin is safe following topical administration to seven-week-old Beagle puppies. Clinical observations included occasional vomiting, decreased appetite, and a rough coat at the application site after treatment. One 1X group puppy had pruritus post-treatment. Transient, rapid and difficult respiration occurred in one 5X group puppy.

- 2) Dermal Dose Tolerance Study with Imidacloprid/Moxidectin Topical Solution in the Dog (Study #150.874, Report #75160) Good Laboratory Practices (GLP) laboratory study

Purpose: The objective of this study was to demonstrate the safety of 10.0% imidacloprid/ 2.5% moxidectin topical solution when administered topically to dogs at ten times (10X) the recommended unit dose volume for their weight range.

Study Director: R. D. Jones, DVM, PhD, Dipl. ABVT & ABT

Location: Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS

Animals: 16 Beagle dogs (4 females and 4 males per treatment group), 7 to 8 months of age, and between 20.9 and 31.0 pounds

Treatment Dosages: Dogs in the control group (Group 1) were treated topically with mineral oil at 10X the recommended unit dose volume for their weight range. Dogs of Group 2 were treated topically with 10% imidacloprid + 2.5% moxidectin at 10X the recommended (label) unit dose volume for their weight range. The label provides for a minimum of 10 mg/kg imidacloprid and 2.5 mg/kg moxidectin. See Table 28 for the range of mg/kg doses applied.

Table 28: Minimum and Maximum Imidacloprid and Moxidectin Doses Applied

| Imidacloprid | | Moxidectin | |
|--------------|-------------|------------|------------|
| Minimum | Maximum | Minimum | Maximum |
| 183.7 mg/kg | 262.4 mg/kg | 45.9 mg/kg | 65.6 mg/kg |

Route of Administration: Topical, applied along the dorsal midline from the cervical to sacral region

Frequency of Treatment: Single Treatment

Study Design: Physical examinations were performed on Days -14 and 17. Body weights were taken on Days -14, -1, and 17. The dogs were treated once on Day 0. Clinical observations were conducted pre-dosing, immediately after dosing, and at 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dosing on Day 0, and twice daily on other study days. Food consumption was monitored daily. Blood samples were collected for clinical pathology on Days -4, 1, and 17.

Variables Measured: Clinical observations – systemic and local (application sites), physical examinations, body weight, food consumption, hematology and clinical chemistry

Results:

Clinical Observations: One female in the imidacloprid + moxidectin group was observed rubbing and rolling its back on the cage immediately post-dosing.

In the imidacloprid + moxidectin group, one female vomited 6 hours post-dosing and one male vomited food on Day 6.

Body Weights and Food Consumption: Dogs in the imidacloprid + moxidectin group did not gain as much weight as dogs in the control group.

Hematology and Chemistry: Compared to the control group on Day 17, the imidacloprid + moxidectin group had increased direct bilirubin and activated partial thromboplastin, and the males had increased red blood cell (RBC) counts and

hemoglobin.

Conclusions: A single topical administration of imidacloprid + moxidectin topical solution at 10X the recommended dose was associated with behavioral signs of application site discomfort, vomiting, decreased weight gain, and increased direct bilirubin, activated partial thromboplastin, RBC counts, and hemoglobin.

3) Dermal Safety Study with Imidacloprid/Moxidectin Topical Solution in the Ivermectin-Sensitive Collie (Study #150.876, Report #75162)
 GLP laboratory study

Purpose: The objective of this study was to demonstrate the safety of 10.0% imidacloprid + 2.5% moxidectin topical solution when administered topically to prescreened ivermectin-sensitive Collies.

Study Director: Allan J. Paul, DVM, MS

Location: University of Illinois, College of Veterinary Medicine, Urbana, IL

Animals: 21 Collies (10 females and 11 males), prescreened for ivermectin sensitivity, 1 to 8 years of age, between 38.9 and 89.8 pounds, 9 Collies in the 5X group, 3 Collies in the 3X group, and 9 Collies in the control group

Treatment Dosages: To maximize drug exposure, dogs were treated topically with multiples of the maximum mg/kg dose for their weight range, based on the recommended (label) unit doses of 10% imidacloprid + 2.5% moxidectin. [For example, the recommended unit dose volume for dogs within the 55.1-88 lb weight range is 4.0 mL, which provides a maximum of 4.0 mg/kg moxidectin. Collies in the 3X group that weighed 55.1 to 88 lb were treated with doses of imidacloprid + moxidectin that provided 12 mg/kg moxidectin.] The actual 3X and 5X moxidectin exposures are provided in Table 29. The control group dogs received mineral oil.

Table 29: Minimum and Maximum Moxidectin Exposures

| Recommended (Label) Dose & Collie Safety Study Groups | Range of Actual Moxidectin Exposures with Applications of Imidacloprid + Moxidectin | |
|---|---|------------|
| | Minimum | Maximum |
| The Label Dose for Dogs 20.1 – 55 lb is 2.5 mL | 2.5 mg/kg | 6.9 mg/kg |
| The Label Dose for Dogs 55.1 – 88.0 lb is 4.0 mL | 2.5 mg/kg | 4.0 mg/kg |
| 3X Group in Collie Study | 12.0 mg/kg | 19.5 mg/kg |
| 5X Group in Collie Study | 20 mg/kg | 32.5 mg/kg |

Route of Administration: Topical, first applied to the skin between the shoulder blades and then to the back area if needed to accommodate the volume

Frequency of Treatment: Once every 28 days, for a total of three applications

Study Design: The Collies were previously screened for ivermectin sensitivity. Collies were treated on Days 0, 28, 56, and 84. See Table 30 for the treatment schedule. Mineral oil was used for the control. On treatment days, clinical observations were conducted pre-dosing, and at 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dosing. On other study days, clinical observations were performed twice daily until the end of the study on Day 98.

Table 30: Treatment Schedule

| Study Day | Topical Treatment Administered | | |
|-----------|--------------------------------|--------------|-------------|
| | Control Group | 3X Group | 5X Group |
| 0 | Mineral Oil | 3X dose | Mineral Oil |
| 28 | Mineral Oil | 3X dose | 5X dose |
| 56 | Mineral Oil | 3X dose | 5X dose |
| 84 | Mineral Oil | No Treatment | 5X dose |

Variables Measured: Clinical observations – systemic and local (application sites)

Results: No clinical abnormalities were recorded for any Collie during the study.

Conclusions: Topical application of imidacloprid + moxidectin was not associated with clinical abnormalities in ivermectin-sensitive Collies at 3 or 5 times the recommended dose.

4) Evaluation of the Safety of Imidacloprid/Moxidectin Topical Solution in Heartworm-Positive Dogs (Study #150.873, Report #75159)
GLP laboratory study

Purpose: The objective of this study was to demonstrate the safety of 10 % imidacloprid + 2.5 % moxidectin topical solution when administered topically to heartworm infected dogs at 14-day intervals for 3 treatments.

Study Director: John W. McCall, PhD

Location: TRS Labs, Inc., Athens, GA

Animals: 24 Beagle dogs (4 females and 4 males per treatment group), 43 weeks to 48 weeks of age at the beginning of the study

Treatment Dosages: Dogs of Groups 1 and 2 were treated topically with 10%

imidacloprid + 2.5% moxidectin at 1X and 5X multiples of the recommended (label) unit dose volumes, respectively. The label provides a minimum of 10 mg/kg imidacloprid and 2.5 mg/kg moxidectin. See Table 31 for the range of mg/kg doses applied in each group. Dogs of the control group (Group 3) were treated with mineral oil at the 5X volume.

Table 31: Minimum and Maximum Imidacloprid and Moxidectin Doses Applied

| Group | Imidacloprid | | Moxidectin | |
|-------|--------------|------------|-------------|-------------|
| | Minimum | Maximum | Minimum | Maximum |
| 1X | 13.3 mg/kg | 25.8 mg/kg | 3.3 mg/kg | 6.4 mg/kg |
| 5X | 57 mg/kg | 135 mg/kg | 14.25 mg/kg | 33.75 mg/kg |

Route of Administration: Topical, applied to the skin on the back of the head, neck, and between the shoulder blades

Frequency of Treatment: Once every 14 days, for 3 applications

Study Design: On Day -63, active live adult heartworms, 8 male and 8 female, were inserted into the jugular vein of each dog. Physical examinations were performed on Days -64, -3, and 34. Body weights were taken on Days -64, -3, 0, 14, 28, and 34. The dogs were treated on Days 0, 14, and 28. On treatment days, clinical observations were conducted pre-dosing, immediately after dosing, and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dosing. On other study days, clinical observations were performed twice daily. Food consumption was monitored daily. Blood samples were collected for clinical pathology and heartworm antigen testing on Days -7 and 33. Blood was collected for modified Knott test and microfilaria counts on Days, -3, 0, 1, 14, 15, 28, and 29. The dogs were necropsied 6 days after the third treatment (on Day 34) and all heartworms were recovered, enumerated, sexed and counted as live or dead adult heartworms per dog.

Variables Measured: Clinical observations – systemic and local (application sites), physical examinations, body weight, food consumption, hematology, clinical chemistry, heartworm antigen testing, microfilaria counts, and adult heartworm counts

Results:

Clinical Observations: One dog in the 5X group vomited 3 hours after treatment on Day 14.

Modified Knott Tests: Microfilaria counts decreased following treatment with imidacloprid + moxidectin.

Heartworm Counts: Heartworm recovery at necropsy was similar for all treatment groups. See Table 32.

Table 32: Heartworm Counts at Necropsy

| Group | Dead Heartworms per Dog | | Live Heartworms per Dog | |
|-------|-------------------------|-----------|-------------------------|-------------|
| | Mean | Range | Mean | Range |
| 0X | 0.375 worms | 0-2 worms | 13 worms | 8-15 worms |
| 1X | 0.25 worms | 0-1 worms | 12.1 worms | 9-15 worms |
| 5X | 0.375 worms | 0-2 worms | 13.3 worms | 11-15 worms |

Conclusions: Imidacloprid + moxidectin topical solution, administered topically at 1 and 5 times the recommended dose to dogs with adult heartworm infections and circulating microfilaria, did not cause adverse reactions associated with heartworm or microfilaria status. Although microfilaria counts decreased following treatment with imidacloprid + moxidectin, hypersensitivity reactions were not observed. One dog in the 5X group vomited 3 hours after the second treatment.

5) Oral Safety Study with Imidacloprid/Moxidectin Topical Solution in the Beagle Dog (Study #150.875, Report #75161). GLP laboratory study

Purpose: The objective of this study was to demonstrate the safety of 10.0% imidacloprid + 2.5% moxidectin topical solution when administered as a single oral dose to Beagle dogs.

Study Director: Albert Abraham, BVSc, PhD

Location: Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, KS

Animals: 24 Beagle dogs (6 females and 6 males per treatment group), 19 to 76 months of age, and between 15 and 39 pounds

Treatment Dosages: The recommended unit dose volume was administered orally to the dogs in Group 1. Dogs 9.1 to 20.0 lb were dosed with 1.0 mL and dogs 20.1 to 55 lb were dosed with 2.5 mL of 10% imidacloprid + 2.5% moxidectin. The label provides a minimum of 10 mg/kg imidacloprid and 2.5 mg/kg moxidectin. See Table 33 for the range of mg/kg doses administered orally. Tap water was administered to the Group 2 (control) dogs.

Table 33: Minimum and Maximum Doses Administered Orally

| Imidacloprid | | Moxidectin | |
|--------------|------------|------------|-----------|
| Minimum | Maximum | Minimum | Maximum |
| 11.1 mg/kg | 25.4 mg/kg | 2.8 mg/kg | 6.4 mg/kg |

Route of Administration: Oral by gavage, followed by 5 mL tap water

Frequency of Treatment: Single treatment

Relationship to feeding: Fasted

Study Design: Physical examinations were performed on Days -1, and 14. Body weights were taken on Days -1 and 14. The dogs were fasted and dosed once on Day 0. On Day 0, clinical observations were conducted pre-dosing, and at 1, 2, 3, 4, 6, 8, 12, 18, and 24 hours post-dosing. On other study days, clinical observations were performed twice daily. Food consumption was monitored twice daily. Blood samples were collected for clinical pathology on Days -7 and 13.

Variables Measured: Clinical observations, physical examinations, body weight, food consumption, hematology, and clinical chemistry

Results:

Clinical Observations: Six dogs in the imidacloprid + moxidectin group vomited within 1 hour of dosing, 2 of these dogs vomited again at 2 hours post-dosing, and 1 vomited again at 18 hours post-dosing.

One dog in the imidacloprid + moxidectin group exhibited shaking (nervousness) 1 hour post-dosing.

Another dog in the imidacloprid + moxidectin group exhibited abnormal neurological signs (circling, ataxia, generalized muscle tremors, and dilated pupils with a slow pupillary light response) starting at 4 hours post-dosing through 18 hours post-dosing. Without treatment, this dog was neurologically normal at 24 hours post-dosing. Food consumption, although depressed during the initial 30 hours post-dosing, was normal by 48 hours post-dosing. This dog received an oral dose of 4.65 mg/kg moxidectin.

Conclusions: Oral administration of imidacloprid + moxidectin to adult Beagles caused vomiting and signs of avermectin toxicity (ataxia, muscle tremors, and mydriasis). The results of this study support the bolded Contraindications label statement “Do not administer this product orally” and a Warnings statement to ensure that dogs do not lick the product after topical application.

- 6) Pilot Oral Safety Study with Imidacloprid/Moxidectin Topical solution in the Ivermectin-Sensitive Collie (Study #150.877, Report #75163).
Pilot GLP laboratory study

Purpose: The objective of this study was to provide information regarding the safety of 10.0% imidacloprid + 2.5% moxidectin topical solution when ingested by the ivermectin-sensitive Collie.

Investigator: Allan J. Paul, DVM, MS

Location: University of Illinois, College of Veterinary Medicine, Urbana, IL

Animals: Five ivermectin-sensitive Collies (2 females and 3 males), 47 to 78 pounds, all 5 Collies were in the same treatment group

Treatment Dosages: All 5 Collies were dosed orally with the same escalating mg/kg doses of the 10% imidacloprid + 2.5% moxidectin topical solution. The label provides a minimum of 10 mg/kg imidacloprid and 2.5 mg/kg moxidectin. At 2-week intervals, the Collies were dosed orally with 4%, then 10%, and then 40% of the minimum label dose. See Table 34.

Table 34: Treatment Dosages Administered Orally

| Treatment Day | Volume mL/kg | Dosage | Comment |
|----------------------|---------------------|---|--|
| Day 0 | 0.004 | 0.1 mg/kg moxidectin 0.4 mg/kg imidacloprid | This mg/kg dose is 4% of the minimum label dose |
| Day 14 | 0.01 | 0.25 mg/kg moxidectin 1.0 mg/kg imidacloprid | This mg/kg dose is 10% of the minimum label dose |
| Day 28 | 0.04 | 1.0 mg/kg moxidectin 4.0 mg/kg imidacloprid | This mg/kg dose is 40% of the minimum label dose |

Route of Administration: Orally, by gavage

Frequency of Treatment: Once at each dose level, on Days 0, 14, and 28

Study Design: The Collies were previously screened for ivermectin sensitivity. On Day 0, all 5 Collies were dosed orally with 0.004 mL/kg of the 10% imidacloprid + 2.5% moxidectin topical solution. On Day 14 they were dosed orally with 0.01 mL/kg of the product. On Day 28 they were dosed orally with 0.04 mL/kg of the product. On treatment days, clinical observations and scores for moxidectin toxicosis were conducted immediately after dosing and at 1, 2, 3, 4, 6, 8, 12, 14, and 24 hours post-dosing. On other study days, clinical observations were performed twice daily until the end of the study on Day 42. Dogs were weighed prior to dosing on Days 0, 14, and 28.

Variables Measured: Clinical observations (systemic and oral cavity), scores for neurological signs typical of moxidectin toxicosis (depression, ataxia, mydriasis, salivation, muscle fasciculation, and coma), and body weights

Results:

Clinical Observations: The prescreened ivermectin-sensitive Collies remained asymptomatic following oral administration of 0.004 mL/kg and 0.01 mL/kg of the 10% imidacloprid + 2.5% moxidectin topical product on Days 0 and 14, respectively.

However, following oral administration of 0.04 mL/kg on Day 28, 4 of the 5 Collies developed signs of severe moxidectin toxicity. Two hours post-dosing, one dog had ataxia. Clinical signs progressed from 3 to 8 hours post-dosing. Adverse reactions included depression, ataxia, mydriasis, salivation, muscle fasciculation, and coma. The four Collies that became comatose were euthanized at 8 hours post-dosing without attempting treatment. One Collie did not exhibit any signs of moxidectin toxicity throughout the study. See Table 35.

Table 35: Number of Collies Affected with Clinical Signs Post-Dosing

| Clinical Signs | Hours Post Dosing | | | | | | |
|----------------------|-------------------|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 6 | 8 |
| Depression | 0 | 0 | 0 | 3 | 3 | 4 | 4 |
| Ataxia | 0 | 0 | 1 | 3 | 3 | 4 | 4 |
| Mydriasis | 0 | 0 | 0 | 3 | 2 | 4 | 3 |
| Salivation | 0 | 0 | 0 | 2 | 2 | 3 | 0 |
| Muscle Fasciculation | 0 | 0 | 0 | 3 | 3 | 4 | 0 |
| Coma | 0 | 0 | 0 | 0 | 0 | 0 | 4 |

Conclusions: Oral administration of 0.04 mL/kg (40% of the minimum label dose) of 10% imidacloprid + 2.5% moxidectin caused ataxia, depression, mydriasis, salivation, muscle fasciculation, and coma in ivermectin-sensitive Collies. The results of this study support the bolded Contraindications label statement “Do not administer this product orally” and a Warnings statement to ensure that dogs do not lick the product after topical application.

7) Clinical Safety Evaluation of Imidacloprid + Moxidectin Applied Dermally to Dogs (Study #151.252, Report #75280)

Purpose: The objective of this study was to assess the clinical safety of topically-applied imidacloprid + moxidectin in dogs when administered by dog owners under the conditions of actual field-use.

Study Monitor: Daniel K. Ciszewski, DVM, MS

Investigators and Locations:

Lisa Arthur, DVM, Harris Blvd Veterinary Clinic, Charlotte, NC
 Roger Becker, DVM, Independence Animal Hospital, Independence, MO
 Richard Mauldin, DVM, Hillcrest Animal Hospital, Oklahoma City, OK
 Craig Staehle, DVM, The Gentle Doctor, O’Fallon, MO

Animals: A total of 207 client-owned dogs from 92 different households were enrolled in the study. A total of 196 dogs, from 83 households, completed the study. Of the 196 dogs, 128 (59 males and 69 females) were in the imidacloprid + moxidectin group, and 68 dogs (28 males and 40 females) were in the active control

group. Of the 196 dogs, 45 breeds were represented with the predominant breeds being mixed (33/ 16.8%), Yorkshire Terrier (13/ 6.6%), Labrador Retriever (9/ 4.6%), Shetland Sheepdog (8/ 4.1%), Dachshund (8/ 4.1%), and Australian Shepherd (7/ 3.6%). Also included were Collie crosses (4/ 2.0%), Shetland Sheepdog crosses (3), Border Collie crosses (2), Australian Shepherd cross (1), Shetland Sheepdog/Australian Shepherd cross (1), and Australian Cattle dog (1). Dogs treated with imidacloprid + moxidectin had an age range of 3 months to 15 years old, and a weight range of 4 to 157 pounds.

Treatment Dosages: Each dog was treated with the recommended (label) unit dose volume for its weight range.

ADVANTAGE MULTI for Dogs (10% imidacloprid + 2.5% moxidectin)

The label provides for a minimum of 10 mg/kg imidacloprid and 2.5 mg/kg moxidectin

Active Control: REVOLUTION (selamectin)

The label provides for a minimum of 6 mg/kg selamectin

Route of Administration: Topical. For dogs \leq 20 lbs, imidacloprid + moxidectin was applied directly to the skin in one spot between the shoulder blades. For dogs $>$ 20 lbs, imidacloprid + moxidectin was applied evenly in 3-4 spots on the top of the back from the shoulder to the base of the tail.

Frequency of Treatment: Dog owners administered one treatment every 30 days for a total of 3 applications.

Study Design: The study was randomized and controlled, and attending veterinarians and dog owners were masked to treatment. Attending veterinarians performed baseline physical examinations and heartworm tests on all dogs. Treatments were administered by the dog owners according to written and oral instructions provided by the veterinarian. Owners treated their dogs on Days 0, 30, and 60. On treatment days, owners observed their dogs for untoward reactions at 30-60 minutes, 4-6 hours, and 22-26 hours after treatment. Within 7 days following the final treatment, owners brought their dogs back to the veterinary clinic for a post-study physical examination and assessment.

Variables Measured: Dog owners recorded their observations on study forms.

Results: See Table 36 for owner-recorded post-treatment observations.

Table 36: Owner-Recorded Post-treatment Observations

| Observation | Imidacloprid/Moxidectin (128 dogs) | Active Control (68 dogs) |
|--------------------|---|-------------------------------------|
| Pruritus | 19 dogs (14.8%) | 7 dogs (10.3%) |
| Residue | 9 dogs (7.0%) | 5 dogs (7.4%) |
| Medicinal Odor | 5 dogs (3.9%) | None observed |
| Lethargy | 1 dog (0.8%) | 1 dog (1.5%) |
| Inappetence | 1 dog (0.8%) | 1 dog (1.5%) |
| Hyperactivity | 1 dog (0.8%) | None observed |

Concurrent Medications: Other products used concurrently with imidacloprid/moxidectin during this field study included ACE inhibitors, anticonvulsants, antihistamines, antimicrobials, chondroprotectants, corticosteroids, immunotherapeutics, MAO inhibitors, NSAIDs, ophthalmic medications, sympathomimetics, synthetic estrogens, thyroid hormones, and urinary acidifiers.

Conclusions: Adverse reactions associated with the administration of imidacloprid + moxidectin topical solution once monthly for 3 months included: pruritus, flaky/greasy residue at the application site, medicinal odor, lethargy, inappetence, and hyperactivity.

- 8) Serum Pharmacokinetics of Imidacloprid 10% w/v / Moxidectin 2.5% w/v Spot-on in Dogs Applied Once per Month for Three Consecutive Intervals (Study #141.329, V00-006, Report #24953) GLP laboratory study
 [In a non-GLP follow-up study to Study #141.329, V00-006, serum concentrations of moxidectin were assessed at 8, 12, and 16 weeks after the third (last) imidacloprid + moxidectin application. Report #24965]

Purpose: The objective of this study was to evaluate the pharmacokinetics of topical 10% imidacloprid + 2.5% moxidectin solution at the minimum label dose of 0.1 mL/kg.

Investigator & Location: Dr. G. Beddies, Bayer AG, Animal Health – R&D, Leverkusen, Germany

Animals: 10 adult Beagle dogs, 1.5 to 2.75 years of age, between 21.6 and 34.8 pounds, 4 males and 6 females per treatment group

Treatment Dosages: 0.1 mL/kg of 10% imidacloprid + 2.5% moxidectin topical solution, providing 10 mg/kg imidacloprid and 2.5 mg/kg moxidectin

Route of Administration: Topically in one spot onto the skin of each dog's back between the shoulder blades

Study Design: The dogs were treated once monthly on Days 0, 30, and 60. PK blood samples were collected pretreatment and at 1, 2, 4, 6, 8, and 12 hours and 1, 2, 3, 7, 14, 21, and 28 days after the first treatment; and at 2, 4, and 8 hours and 1, 2, 3, 7, 14, 21, and 28 days after the second and third treatments. [In the follow-up study, PK samples were collected from 8 of the 10 dogs, 8, 12, and 16 weeks after the third treatment.] Serum moxidectin concentrations were measured using High Pressure Liquid Chromatography (HPLC) with fluorescent detection and imidacloprid concentrations were analyzed with turbulent flow chromatography/tandem mass spectrometry. Clinical examinations were conducted on Days 1, 14, 28, 30, 44, 58, 60 and 88.

Results: See Tables 37, 38, and 39 for pharmacokinetic (PK) results.

Table 37: Serum Imidacloprid PK Variables for 28 Days after Each Dose

| Application Interval | C_{max}¹ mcg/L mean; range | T_{max}², time post-dosing, mean; range | Range, last day of quantifiable concentrations of imidacloprid in serum³ |
|---------------------------------|--|---|--|
| Day 0 – 28, Dosed on Day 0 | 69.5 mean; 27.6 – 207.4 | 31 hrs mean; 8 hrs – 3 days | Days 3 to 7 (3 to 7 days post dosing) |
| Day 30 – 58, Dosed on Day 30 | 49.1 mean; 17.5 – 98.9 | 31 hrs mean; 1 – 2 days | Days 33 to 44 (3 to 14 days post dosing) |
| Day 60 – 88, Dosed on Day 60 | 44.8 mean; 19.3 – 130.6 | 25 hrs mean; 4 hrs – 2 days | Days 61 to 67 (1 to 7 days post dosing) ⁴ |

¹ C_{max} is the maximum serum concentration

² T_{max} is the time of C_{max} post-dosing

³ Limit of quantification 10 mcg/L

⁴ One dog did not have quantifiable concentrations at any time during this dosing interval

Table 38: Serum Moxidectin PK Variables for 28 Days after Each Dose

| Application Interval | C_{max} mcg/L mean; SD¹ | AUC² for 28 day interval mcg•day/L | T_{max}, time post-dosing, mean; range | Moxidectin (mcg/L) 28 days post-dosing |
|-----------------------------|---|--|---|---|
| Day 0 -- 28 | 18.1 mean; 5.1 SD | 324.6 mean; 68.5 SD | 9.3 days mean; 1 – 21 days | On Day 28: 10.5 mean; 2.9 SD |
| Day 30 -- 58 | 22.6 mean; 3.6 SD | 471.5 mean; 79.0 SD | 4.6 days mean; 2 hrs – 7 days | On Day 58: 15.9 mean; 3.3 SD |
| Day 60 -- 88 | 33.5 mean; 6.1 SD | 596.4 mean; 91.8 SD | 4.6 days mean; 8 hrs – 28 days | On Day 88: 18.8 mean; 4.1 SD |

¹ SD is the standard deviation

² AUC is area under the curve

Table 39: Serum Moxidectin Levels at 8, 12 and 18 Weeks after the Day 60 Dose

| # of Weeks after Day 60 Dose | Serum Moxidectin Concentration (mcg/L) |
|------------------------------|--|
| 8 weeks | 6.3 mean; 2.3 SD |
| 12 weeks | 2.7 mean; 1.4 SD |
| 16 weeks | 1.3 mean; 0.7 SD |

Conclusions: When imidacloprid + moxidectin topical solution was administered topically to 10 adult Beagle dogs at the minimum recommended dose of 10 mg/kg imidacloprid and 2.5 mg/kg moxidectin, serum concentrations tended to fluctuate over one dosing interval within any given subject and the time to peak concentrations occurred from about 1 day to 21 days after topical administration. At 28 days after treatment with imidacloprid + moxidectin topical solution, serum levels of moxidectin were still high. Steady state conditions are to be expected within four to five consecutive 30-day application intervals. With regard to imidacloprid, up to a 10-fold variation in peak concentrations was observed, but the duration of systemic exposure was consistently short among all study subjects.

4. *HUMAN FOOD SAFETY:*

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

5. *USER SAFETY:*

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to ADVANTAGE MULTI for Dogs:

“Not for human use. Keep out of the reach of children.

Children should not come in contact with application sites for two (2) hours after application.

Causes eye irritation. Harmful if swallowed. Do not get in eyes or on clothing. Avoid contact with skin. **Wash hands thoroughly with soap and warm water after handling.** If contact with eyes occurs hold eyelids open and flush with copious amounts of water for 15 minutes. If eye irritation develops or persists, contact a physician. If swallowed, call poison control center or physician immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by the poison control center or physician. People with known hypersensitivity to benzyl alcohol, moxidectin, or imidacloprid should administer the product with caution. In case of allergic reaction, contact a physician. If contact with skin or clothing occurs, take off contaminated clothing. Wash skin immediately with plenty of soap and water. Call a poison control center or physician

for treatment advice. The Material Safety Data Sheet (MSDS) provides additional occupational safety information.

For a copy of the Material Safety Data Sheet (MSDS) or to report adverse reactions call Bayer Veterinary Services at 1-800-422-9874. For consumer questions call 1-800-255-6826.”

The bolded human warnings “**Children should not come in contact with application sites for two (2) hours after application**” and “**Wash hands thoroughly with soap and warm water after handling**” were based on Human Risk Assessment determinations. The risk assessment estimated the potential human (adult and toddler) acute and chronic dermal, toddler hand-to-mouth oral exposure levels, and levels of concern from contact with a treated dog. The risk assessment factors, pet surface-to-human transfer dose, dermal absorption, and No Observable Adverse Effect Levels (NOAEL), were derived from data for imidacloprid, moxidectin, or related compounds in toxicity or pharmacokinetic studies in laboratory animals, a pharmacokinetic study in adult human volunteers, or cotton glove stroking (drug recovery) studies in dogs.

6. 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 ADVANTAGE MULTI for Dogs, when used according to the label, is safe and effective for the prevention of heartworm disease caused by *Dirofilaria immitis*. ADVANTAGE MULTI for Dogs kills adult fleas and is indicated for the treatment of flea infestations (*Ctenocephalides felis*). ADVANTAGE MULTI for Dogs is also indicated for the treatment and control of the following intestinal parasites:

| Intestinal Parasite | | Intestinal Stage | | |
|---------------------|-------------------------------|------------------|----------------|---------------------|
| | | Adult | Immature Adult | Fourth Stage Larvae |
| Hookworm Species | <i>Ancylostoma caninum</i> | X | X | X |
| | <i>Uncinaria stenocephala</i> | X | X | X |
| Roundworm Species | <i>Toxocara canis</i> | X | | X |
| | <i>Toxascaris leonina</i> | X | | |
| Whipworm | <i>Trichuris vulpis</i> | X | | |

a. Marketing Status:

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise and proper diagnosis are required to determine the existence of heartworm infections and to monitor the safe use of the product.

b. Exclusivity:

Under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of approval. Exclusivity is based on the conduct of new studies for substantial evidence of effectiveness and new target animal safety data.

c. Patent Information:

ADVANTAGE MULTI for Dogs is protected under the following U.S. patent numbers:

| <u>U.S. Patent Number</u> | <u>Date of Expiration</u> |
|---------------------------|---------------------------|
| 6,232,328 | May 12, 2015 |
| 6,001,858 | November 27, 2015 |

7. ATTACHMENTS:

Facsimile labeling is attached as indicated below:

- Package Insert
- Calendar Reminder Stickers
- Tube Labels
- Foil Backing for Blister Pack
- Dispensing Container Carton (Multi Carton)
- Display Carton
- Shipping Carton