

FREEDOM OF INFORMATION SUMMARY

Original New Animal Drug Application

NADA 141-188

Marquis™
(15% w/w ponazuril)
Antiprotozoal Oral Paste

“...for the treatment of equine protozoal myeloencephalitis (EPM) caused by
Sarcocystis neurona.”

For Oral Use in Horses

Sponsored by:

Bayer Corporation
Agriculture Division
Animal Health

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I. GENERAL INFORMATION

NADA Number: 141-188

Sponsor: Bayer Corporation
Agriculture Division
Animal Health
P. O. Box 390
Shawnee Mission, Kansas 66201

Generic Name: ponazuril

Trade Name: Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste

Marketing Status: This is a prescription (Rx) product which carries the following caution statement: “Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.”

II. INDICATIONS FOR USE

Marquis (ponazuril) is indicated for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*.

III. DOSAGE FORM, ROUTE OF ADMINISTRATION, AND DOSAGE

Dosage Form: Marquis (ponazuril) is available in cartons containing four (4) syringe applicators and one (1) syringe plunger. Each syringe barrel of Marquis (ponazuril) will deliver 127 grams of paste. Each gram of paste contains 150 mg of ponazuril (15% w/w).

Route of Administration: Marquis (ponazuril) is administered orally.

Recommended Dosage: Each syringe barrel of Marquis (ponazuril) contains enough paste to treat one (1) 1,200 lb. (545 kg) horse for seven (7) days, at a dose rate of 5 mg/kg (2.27 mg/lb.) body weight. The plunger contains a dosage ring calibrated for a dose rate of 5 mg/kg (2.27 mg/lb.) body weight and marked for horse weights from 600 to 1,200 lb. (273 to 545 kg). The syringe barrels are packaged in units of four with one reusable plunger. This package provides sufficient paste to treat one 1,200 lb. (545 kg) horse for 28 days at a dose rate of 5 mg/kg (2.27 mg/lb.) body weight.

IV. EFFECTIVENESS

A. Dosage Characterization

To characterize the dose of ponazuril necessary to treat infections of *Sarcocystis neurona*, several lines of evidence were considered. Studies have demonstrated that ponazuril is an effective anticoccidial drug for the treatment of gastrointestinal and somatic coccidiosis in several species of mammals and birds, including cattle, sheep, swine, rabbits and chickens at doses of 7-20 mg/kg. Therefore, a series of studies was conducted to determine a dose of ponazuril that would be effective in horses against *Sarcocystis neurona*. First, an *in vitro* test was conducted to determine the dose of drug necessary in the cerebrospinal fluid of the horse. Second, a pharmacokinetic study defined the level of drug that could be obtained in both serum and CSF. Finally, two doses were tested in naturally infected horses in a clinical field study.

1. *In vitro* Susceptibility Study

a. Type of Study/Purpose: To evaluate the activity of ponazuril against the intracellular stages of *Sarcocystis neurona* in cell culture.

b. Investigators:

J. P. Dubey, Ph.D., USDA, Agricultural Research Service, Livestock and Poultry Sciences Institute, Parasite Biology and Epidemiology Laboratory, Beltsville, MD 20705

D. S. Lindsay, Ph.D., Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 1410 Prices Fork Road, Blacksburg, VA 24061-0342

T. J. Kennedy, Ph.D., Bayer Corporation, Agriculture Division Animal Health, P.O. Box 390, Shawnee Mission, KS 66201

c. General Design: *In vitro* evaluation of effectiveness to inhibit merozoite development in cell cultures.

1) Test Media: Bovine turbinate or African green monkey kidney cells grown to confluence in supplemented RPMI media.

2) Treatments: 0.001, 0.01, 0.1, 1.0, 5.0 µg/mL of ponazuril in the culture media

3) Procedure: All cultures were grown in 25 cm² plastic cell culture flasks, incubated at 37°C in a reduced atmosphere and stored under the same conditions. The assay involved an assessment of merozoite production. Cell monolayers were infected with *S. neurona* merozoites, SN6 strain, isolated from a horse with EPM. Two hours after inoculation of the cells with merozoites, various concentrations of ponazuril were added to the media one time and the cultures allowed to incubate for 10 days. After 10 days of incubation, visual damage to the cell monolayer was assessed, media removed and quantified and the number of merozoites per mL determined by counting using a hemocytometer.

d. Results: Using a time of 10 days post-treatment, ponazuril inhibited merozoite production by more than 94% in bovine turbinate cultures of *S. neurona* treated with 0.1-1.0 µg/mL ponazuril and greater than 97% inhibition of merozoite production was observed when infected cultures were treated with 5 µg/mL. Results are shown in Table IV.1

Table IV.1 Percent reduction in merozoite production in cell cultures infected with *S. neurona* and treated with various concentrations of ponazuril

African green monkey cells		
Ponazuril Concentration (µg/mL)	Merozoite count ±1sd	% Reduction
None	1,406,438 ±276,817	NA
0.1	412,062 ±102,227	70.7
1.0	139,125 ±200,878	90.1
5.0	40,125 ±43,758	97.1
Bovine turbinate cells		
Ponazuril Concentration (µg/mL)	Merozoite count ±1sd	% Reduction
None	4,175,125 ±464,794	NA
0.01	3,040,688 ±410,257	27.2
0.1	231,813 ±93,988	94.4
1.0	63,313 ±46,594	98.5
5.0	86,375 ±58,853	97.9

e. **Conclusion:** Ponazuril is effective against the merozoite stage of *Sarcocystis neurona* in cell culture.

2. Pharmacokinetic Study

a. **Type of Study/Purpose:** Single phase multiple dose study with blood and cerebrospinal fluid samplings to determine the pharmacokinetics of ponazuril when given at 5 mg/kg as a 15% w/w oral paste formulation in normal horses. To determine the level of ponazuril in the serum and cerebrospinal fluid of horses prior to, during and after the administration of 5 mg/kg daily for 28 days.

b. **Investigator:**

Dr. Martin Furr, Marion duPont Scott Equine Center, Virginia Tech University, Old Waterford Rd. at Morven Park, P.O. Box 1938, Leesburg, VA 20177

c. **General Design:**

- 1) Animals: Ten healthy horses, all male castrates, ranging in age from 3-15 years
- 2) Treatment: Daily dose of 5 mg/kg (2.27 mg/lb) for 28 days with a 15% oral paste formulation of ponazuril
- 3) Treatment group assignment: Animals all received the same treatment.
- 4) Treatment Dose, Route, Frequency and Duration: Animals received ponazuril at a rate of 5 mg/kg body weight daily for 28 days.

- 5) **Clinical Examination/Clinical Scoring:** Animals were evaluated by physical examination, hematology and serum chemistry and cerebrospinal fluid indices.
- 6) **Samples:** Serum was collected for ponazuril assay at Days 0, 7, 14, 21, 28, 35, 36, 37, 38, 39, 40, 41, 42 and 49. Cerebrospinal fluid was collected for ponazuril assay at Days 0, 7, 14, 21, 28, 35, 42 and 49.
- d. **Results:** Maximum calculated serum and CSF concentrations were 5.59 ug/mL and 0.21 ug/mL, respectively. These occurred on days 18.2 and 15 for serum and CSF, respectively. The terminal elimination half-life for serum (calculated using Day 28 to 42 data) was 4.50 ± 0.57 days. Steady state was achieved by approximately 20 days post dose, which is consistent with the terminal elimination half-life of approximately 4.5 days. The ratio of CSF to serum was approximately 4%.

The mean concentration vs. time profiles in serum and CSF are provided in Tables 1 and 2 below.

Table 1. Serum Concentrations (ug/mL) of Ponazuril, By Days

	0	7	14	21	28	35	36	37	38	39	40	41	42	49
Mean	0	4.33	5.26	5.18	4.66	0.394	0.289	0.236	0.2	0.167	0.152	0.124	0.106	0.101
SD	0	1.098	0.877	1.187	0.6	0.186	0.131	0.103	0.105	0.085	0.102	0.088	0.073	0.086

Table 2. CSF Concentrations (ug/mL) of Ponazuril, By Days

	0	7	14	21	28	35	42	49
Mean	0	0.182	0.156	0.169	0.156	0.02	0.001	0.001
SD	0	0.076	0.033	0.055	0.048	0.011	0.003	0.003

- e. **Conclusions:** Ponazuril, when dosed at 5 mg/kg for 28 days, provides measurable drug concentrations in both serum and cerebrospinal fluid.
3. **Summary of Dose Characterization:** First, studies have demonstrated that ponazuril is an effective anticoccidial drug in several species of mammals and birds. Second, an *in vitro* evaluation has shown that ponazuril can significantly reduce *Sarcocystis neurona* merozoite production in cell cultures. Levels of drug in the range of 0.1-1.0 ug/mL were 94% effective when tested in bovine turbinate cell cultures and 70.7% effective when tested in African Green monkey cells. Third, ponazuril, when dosed to healthy horses at 5 mg/kg once daily for 28 days, provided mean daily steady state CSF concentrations ranging between 0.156 ug/mL to 0.182 ug/mL. In bovine cell culture this level was adequate to effect a 94% parasite kill rate.

B. Clinical Field Study 1

1. Type of Study: The study was conducted as a field evaluation of two doses of ponazuril (5 mg/kg and 10 mg/kg). The study was conducted by seven investigators involving a total of 102 acceptable cases.

2. Investigators:

Dr. Frank Andrews, Large Animal Clinical Medicine, College of Veterinary Medicine, University Of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1701

Dr. Fairfield Bain, Hagyard-Davidson-Mcgee, 4250 Ironworks Road, Lexington, KY 40511-8412

Dr. Bill Bernard, Rood & Riddle Equine Hospital, 2150 Georgetown Road, Lexington, KY 40580

Dr. Doug Byars, Hagyard-Davidson-Mcgee, 4250 Ironworks Road, Lexington, KY 40511-8412

Dr. Martin Furr, Marion duPont Scott Equine Center, Virginia Tech University, Old Waterford Rd. at Morven Park, P.O. Box 1938, Leesburg, VA 20177

Dr. Robert Mackay, Dept. Large Animal Clinical Sciences, College of Veterinary Medicine, Box 100-136, University of Florida, Gainesville, FL 32610

Dr. Steven Reed, Dept. Large Animal Medicine, College of Veterinary Medicine, The Ohio State University, 611 Vernon L. Tharp Street, Columbus, OH 43210-6610

3. General Design: The use of historical controls in the evaluation of compounds for effectiveness is described in 21 CFR 514.117 (b)(4)(iv). Equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*, is a neurologic disease of horses that most often results in asymmetric incoordination (ataxia), weakness, and spasticity. EPM can occur as a peracute, acute, or chronic condition. The clinical signs are caused by direct neuronal damage by the parasite, as well as damage secondary to inflammation and the pressure exerted by inflammation within neural spaces. The evaluation of ponazuril as a potential anti-protozoal agent for the treatment of EPM was conducted using historical controls. Historical controls were used for this study due to the progressive nature of the disease if left untreated and the lack of approved therapeutics for the treatment of this disease. Effectiveness was based on a standardized neurologic grade scoring method with neurological examination corroborated with videotape reviews by an expert panel.

a. Purpose: This study was designed to evaluate the safety and effectiveness of 15% w/w ponazuril oral paste formulation when used according to label directions under field conditions in adult horses infected with *Sarcocystis neurona* and exhibiting neurological signs of equine protozoal myeloencephalitis (EPM).

b. Animals: There were one hundred two adult horses, 32 females, 9 males and 61 geldings, ranging in age from 2-30 years. Approximately 70% were Thoroughbreds

and Quarter Horses, with the remainder represented by Saddlebreds, Tennessee Walkers, Hanoverians, Appaloosas, Arabians and mixed breeds.

- c. **Enrollment Criteria:** 2 years of age or older, weight appropriate for age and breed, acceptable overall physical condition score on physical examination, hematology, and serum chemistry values clinically acceptable, and a diagnosis of EPM supported by clinical examination and a positive Western Blot for *S. neurona* on CSF.

Primary Diagnostic Criterion - Standardized clinical examination consistent with EPM (presence of an asymmetric neurological deficit)

Neurologic Grading Scale:

- 0 – Normal, no deficit detected
- 1 – Deficit just detected at normal gait
- 2 – Deficit easily detected and is exaggerated by backing, turning, swaying, loin pressure or neck extension
- 3 – Deficit very prominent on walking, turning, loin pressure or neck extension
- 4 – Stumbling, tripping and falling down spontaneously
- 5 - Recumbent, unable to rise

Other enrollment criteria: cervical radiographs with no suggestion of spinal cord compression or vertebral canal stenosis (determined by calculation of adjusted minimal sagittal vertebral canal diameters and subjective assessment of radiographs), cerebrospinal fluid (CSF) positive Western blots for *Sarcocystis neurona* IgG, CSF cytology (less than 500 red blood cells/mL), CSF indices-(Total Protein <90, IgG index >0.3, albumin quotient <2.2), negative CSF for equine herpes virus-1 (EHV-1 titer below 1:4), and normal serum values for Vitamin E (> 2.0 µg/mL).

- d. **Exclusion Criteria:** Animals that received therapy for EPM within the last 3 months, animals outside of the specifications above, animals with a questionable diagnosis (seizure disorders, behavioral disorders), any other clinical signs that may indicate diseases other than EPM.
- e. **Treatment Groups and Controls:** Animals were randomly assigned to one of two treatment groups and dosed orally with either 5 mg/kg or 10 mg/kg of ponazuril in a 15% paste body weight daily for 28 days. The client did not know the dose. By study conclusion, 47 had been assigned to the 5 mg/kg dose and 55 to the 10 mg/kg dose.
- f. **Challenge:** Natural infection.
- g. **Dosage form:** The formulation used during this study was identical to the product intended for market, a 15% w/w ponazuril paste.
- h. **Route of Administration:** Oral
- i. **Dose, Frequency and Duration:** Animals were dosed at either 5 or 10 mg/kg body weight once per day for 28 days.
- j. **Treatment Success or Failure:** Success was based on the clinical response and a masked scoring of videotapes made during the neurological examinations. Animals had to improve at least one grade on the neurological examination at 90 days after the

end of treatment, and had to be corroborated by video review to be considered a success.

4. **Results:** Of 113 horses evaluated, 11 were eliminated for non-compliance with the protocol, six at 5 mg/kg and five at 10 mg/kg. At a dose rate of 5 mg/kg (2.27 mg/lb.) for 28 days, 28 of 47 horses (60%) improved at least one grade by Day 118 based upon investigator's evaluation. At 10 mg/kg (4.54 mg/lb.) for 28 days, 32 of 55 animals (58%) improved at least one grade by Day 118 based upon investigator's evaluation.

In order to corroborate the cases deemed successes by the clinical investigators, independent experts reviewed the videotapes of the success cases in a masked fashion. Of the 28 success cases at the 5 mg/kg dose, four horses did not have sufficient videotapes for review. Thus, of the remaining 24 success cases with videotapes, 18 were corroborated as successes (75% corroboration). Likewise, in the 10 mg/kg group, there were 32 success cases, 7 of which lacked videotapes. Thus of the remaining 25 cases with videotapes, 14 were corroborated as successes (56% corroboration).

Because there was no difference between the 5 and 10 mg/kg doses with respect to the clinical investigators' assessments of success, the 5 mg/kg dose was selected.

**EPM EFFECTIVENESS – USING CLINICAL INVESTIGATOR’S
NEUROLOGICAL SCORES ONLY
DAY 0 EPM SCORES FROM CLINICAL INVESTIGATOR’S ASSESSMENT**

Treatment	EPM SCORE D0 = 2	IMPROVED D118*	EPM SCORE D0 = 3	IMPROVED D118*	EPM SCORE D0 = 4	IMPROVED D118*
5 mg/kg **	28	15	15	10	4	3
% Improved		54%		67%		75%
10 mg/kg **	26	15	25	16	4	1
% Improved		58%		64%		25%

* based on clinical investigator’s neurological assessment

** included all horses, whether videotaped or not

**EPM EFFECTIVENESS – USING CORROBORATED VIDEOTAPE OUTCOMES
DAY 0 EPM SCORES FROM CLINICAL INVESTIGATOR’S ASSESSMENT**

Treatment	EPM SCORE D0 = 2	IMPROVED D118*	EPM SCORE D0 = 3	IMPROVED D118*	EPM SCORE D0 = 4	IMPROVED D118*
5 mg/kg **	13	10	9	7	2	1
% Improved		77%		78%		50%
10 mg/kg **	12	5	12	8	1	1
% Improved		42%		67%		100%

* based on corroborated video outcomes

** did not include horses that were classified as successes by the clinical investigator but were missing a videotaped examination (N = 4 for 5 mg/kg group and N = 7 for 10 mg/kg group)

5. **Statistical Analysis:** Twenty-eight out of 47 (60%) and 32 out of 55 (58%) animals received a lower maximum score by the clinical investigator at three month’s post-treatment when compared with pretreatment in the 5 and 10 mg/kg dose groups, respectively. There was no significant difference in the proportion of animals that showed improvement when comparing the two dose groups (Pearson’s Chi-square, $p=0.8867$).

No statistical analyses were performed on the CSF Western Blot data. All animals were found Western Blot CSF positive by at least one of two laboratories at the beginning of the study. The Western Blot of the CSF did not appear to be a major factor in determining treatment success nor a reliable measure of treatment success. The neurological examination (corroborated with masked videotape reviews) appeared to be the most reliable source for determining treatment success.

Descriptive statistics were calculated for serum and cerebrospinal fluid variables. For serum IgG, 27.5% and 31.9% of the animals experienced an increase in the serum IgG Index in the 5 mg/kg and 10 mg/kg dose groups, respectively. The CSF IgG levels

were decreased in the two treatment groups by 77.5% and 61.7%, respectively. Changes in Relative Quantity (RQ), an experimental test purported to measure the relative quantity of protein produced in response to parasite infection, showed no correlation with the corroborated EPM success/failure outcomes. No statistical differences were found between successes and failures with respect to RQ differences (Chi-square, $p > 0.05$).

Descriptive statistics were generated for hematology, serum chemistry and CSF indices. The primary purpose of these evaluations was to determine if significant changes were seen in these variables and to determine the medical significance of such changes.

The masked videotape reviews were also assessed using descriptive statistics. The corroboration of each clinical investigator's neurological examinations (improved horses) with those of independent videotape reviews indicated over 65% corroboration for both treatment groups combined.

6. **Conclusions:** At 5 mg/kg for 28 days, 28 of 47 horses (60%) improved at least one grade by Day 118. Seventy-five percent (75%) of those improved, that had also been videotaped, were corroborated by videotape assessment. At 10 mg/kg, 32 of 55 animals (58%) improved at least one grade by Day 118 and 56% of those improved, that had also been videotaped, were corroborated using videotape assessment.
7. **Adverse Reactions:** Eight animals were noted to have unusual daily observations. Two horses exhibited blisters on the nose and mouth at some point in the study, three animals showed a skin rash or hives for up to 18 days, one animal had loose stools throughout the treatment period, one had a mild colic on one day and one animal had a seizure while on medication.

C. Clinical Field Study 2

1. **Type of Study:** The study was conducted as an additional field evaluation using historical controls.

2. **Investigators**

Dr. Frank Andrews, Large Animal Clinical Medicine, College of Veterinary Medicine, University of Tennessee P.O. Box 1071, Knoxville, TN 37901-1701

Dr. Bill Bernard, Rood & Riddle Equine Hospital, 2150 Georgetown Road Lexington, KY 40580

Dr. Martin Furr, Marion duPont Scott Equine Center, Virginia Tech University, Old Waterford Rd. at Morven Park, P.O. Box 1938, Leesburg, VA 20177.

Dr. Robert MacKay, Dept. Large Animal Clinical Sciences, College of Veterinary Medicine, Box 100-136, University of Florida, Gainesville, FL 32610

Dr. Steven Reed, Dept. Large Animal Medicine, College of Veterinary Medicine, The Ohio State University, 611 Vernon L. Tharp Street, Columbus, OH 43210-6610

3. **General Design:** The use of historical controls in the evaluation of compounds for effectiveness is described in 21 CFR 514.117 (b)(4)(iv). Equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*, is a neurologic disease of

horses that most often results in asymmetric incoordination (ataxia), weakness, and spasticity. EPM can occur as a peracute, acute, or chronic condition. The clinical signs are caused by direct neuronal damage by the parasite as well as damage secondary to inflammation and the pressure exerted by inflammation within neural spaces. The evaluation of ponazuril as a potential anti-protozoal agent for the treatment of EPM was conducted using historical controls. Historical controls were used for this study due to the progressive nature of the disease if left untreated and the lack of approved therapeutics for the treatment of this disease. Effectiveness was based on improvement in the neurologic grade from day 0 to day 28.

- a. **Purpose:** This study was designed to evaluate the safety and effectiveness of 15% w/w ponazuril oral paste formulation when used according to label directions under field conditions in adult horses infected with *Sarcocystis neurona* and exhibiting neurological signs of equine protozoal myeloencephalitis (EPM).
- b. **Animals:** There were twelve adult horses, 6 females, 2 males and 4 geldings, ranging in age from 2-19 years. Seventy-five percent were Thoroughbreds and Quarter Horses, with the remainder represented by 1 Saddlebred, 1 Tennessee Walker and 1 Hanoverian.
- c. **Enrollment Criteria:** 2 years of age or older, weight - appropriate for age and breed, acceptable overall physical condition score on physical examination, hematology and serum chemistry values clinically acceptable and a diagnosis of EPM supported by clinical examination.

Primary Diagnostic Criterion-standardized clinical examination consistent with EPM, (presence of an asymmetric neurological deficit)

- 0 – Normal, no deficit detected
- 1 – Deficit just detected at normal gait
- 2 – Deficit easily detected and is exaggerated by backing, turning, swaying, loin pressure or neck extension
- 3 – Deficit very prominent on walking, turning, loin pressure or neck extension
- 4 – Stumbling, tripping and falling down spontaneously
- 5 - Recumbent, unable to rise

Other enrollment criteria: cervical radiographs with no suggestion of spinal cord compression or vertebral canal stenosis (determined by calculation of adjusted minimal sagittal vertebral canal diameters and subjective assessment of radiographs)

Optional enrollment criteria: cerebrospinal fluid (CSF) positive Western blots for *Sarcocystis neurona* IgG, CSF cytology (less than 500 red blood cells/mL), CSF indices-(Total Protein <90, IgG index >0.3, albumin quotient <2.2), Negative CSF for equine herpes virus-1 (EHV-1 titer below 1:4), normal serum values for Vitamin E (> 2.0 µg/mL).

Animals in this study were allowed to have received therapy for EPM within the last 3 months prior to enrollment in this study.

- d. **Exclusion criteria:** Animals outside of the specifications above, animals with a questionable diagnosis (seizure disorders, behavioral disorders), any other clinical signs that may indicate diseases other than EPM.
 - e. **Treatment Groups and Controls:** Animals were randomly assigned to one of two treatment groups and dosed orally with either 5 mg/kg or 10 mg/kg of ponazuril in a 15% w/w paste daily for 28 days. The client did not know the dose. By study conclusion, 7 horses had been assigned to the 5 mg/kg dose and 5 horses to the 10 mg/kg dose.
 - f. **Challenge:** Natural infection.
 - g. **Dosage form:** The formulation used during this study was identical to the product intended for market, a 15% w/w ponazuril paste.
 - h. **Route of Administration:** Oral
 - i. **Dose, Frequency and Duration:** Animals were dosed at either 5 or 10 mg/kg body weight once per day for 28 days.
 - j. **Treatment Success or Failure:** Success was based on the clinical response. Animals had to improve at least one grade on the neurological examination at the end of the 28 day treatment period to be considered a success.
4. **Results:** At a dose rate of 5 mg/kg (2.27 mg/lb.) for 28 days, 7 of 7 horses (100%) improved by Day 28. At 10 mg/kg (4.54 mg/lb.) for 28 days, 5 of 5 animals (100%) improved by Day 28.

TREATMENT	EPM SCORE D0 = 2	IMPROVED D28	EPM SCORE D0 = 3	IMPROVED D28	EPM SCORE D0 = 4	IMPROVED D28
5 mg/kg	2	2	4	4	1	1
% Improved		100		100		100
10 mg/kg	4	4	0		1	1
% Improved		100				100

- 5. **Statistics:** Due to the small number of animals in this study, no statistical analyses were completed.
- 6. **Conclusions:** Animals exhibiting signalment of equine protozoal myeloencephalitis that were treated with ponazuril at a dose of 5 or 10 mg/kg for 28 days showed improvement in clinical score of at least one grade in 100% of the cases in this study.
- 7. **Adverse Reactions:** There were no adverse reactions recorded during the study.

V. ANIMAL SAFETY

A. General Safety Study

- 1. **Type of Study:** Target Animal Safety- Toxicity Study

2. Investigator

John W. Campbell, Ph.D.
Southwest Bio-Labs, Inc.
Las Cruces, NM

3. General Design

- a. **Purpose:** This study was designed to evaluate the safety of 15% w/w ponazuril oral paste formulation when given in multiple doses in the adult horse.
- b. **Test Animals:** Sixteen adult mixed breed horses, 8 males and 8 females, ranging in age from 3-15 years.
- c. **Control Animals:** Eight adult mixed breed horses, 4 males and 4 females, ranging in age from 3-15 years.
- d. **Physical condition:** All animals were evaluated and found to be physically sound prior to the start of the study.
- e. **Dosage form:** The formulation used during this study was identical to the product intended for market, a 15% w/w ponazuril paste.
- f. **Route of Administration:** Oral
- g. **Dosage Used:** Animals were dosed with ponazuril paste at 0 mg/kg, 10 mg/kg or 30 mg/kg body weight daily for 28 or 56 days.
- h. **Variables Measured:** During the treatment phase, body weights were taken on Study Days 0, 7, 14, 21, 27, 42, 49, and 55, whole blood was collected for hematology, coagulation, and serum chemistry profiles on Study Days 7, 14, 21, 27 (all animals) and Days 35, 42, 49 and 55 for the horses treated for 56 days. Complete physical examinations were conducted on Study Days 27 (for all animals) and 55 for those horses treated for 56 days. Horses were observed once per week by a licensed veterinarian, and daily observations of the horses' general well being were made by the animal caretaker. Those making observations of the horses' physical condition were masked to treatment group. On Study Day 28, two pre-selected animals of each sex and from each group (12 total) were euthanized by an injection of euthanasia solution followed by exsanguination. A complete post-mortem examination was performed and all organ systems were examined and abnormalities were noted. Tissues were collected and prepared for histopathological examination by placing them into 10% buffered formalin. The eyes were injected with, then immersed in Davidson's fixative. A bone marrow smear was also prepared. The fixed tissues were sectioned and mounted, then routinely stained with hematoxylin and eosin and examined. The remaining 12 animals were euthanized and examined as above on Study Day 56.

4. Statistical Analysis:

Two analyses were conducted for each analysis endpoint. The first analysis included data from all 24 animals (8 animals/group) from day 0 through day 28 (Day 28 Analysis). The second analysis included data from day 0 through day 56 for the 12 animals (4 animals/group) that were treated for 56 days (Day 56 Analysis).

Each clinical pathology parameter was analyzed with a repeated measures analysis of covariance including terms for fixed effects, random effects and pre-treatment measurements as the covariate. The covariance structure was modeled as autoregressive order 1. Body weights were analyzed using the same model as described for clinical pathology with the day –1 measurement included as the covariate and the covariance structure modeled as compound symmetric. All tests were conducted at the 0.10 significance level.

During the study time period of 28 days, the analysis indicated that direct bilirubin was statistically significantly higher in both the 10 mg/kg and 30 mg/kg doses ($p=0.094$ and $p=0.0542$, respectively); white blood cell counts were statistically significantly higher in the 30 mg/kg dose ($p=0.0016$) and basophils were statistically significantly lower in the 30 mg/kg dose ($p=0.0690$).

In addition, at day 7, sodium was statistically significantly lower in the 30 mg/kg dose ($p=0.0935$); alanine aminotransferase was statistically significantly lower in both the 10 mg/kg and 30 mg/kg doses ($p=0.0521$ and $p=0.0143$, respectively); mean corpuscular volume was statistically significantly lower in both the 10 mg/kg and 30 mg/kg doses ($p=0.0008$ and $p<0.0001$, respectively); and mean corpuscular hemoglobin concentration was statistically significantly higher in the 10 mg/kg and 30 mg/kg doses ($p=0.0195$ and $p=0.0015$, respectively). At day 14, gamma glutamyltransferase was statistically significantly higher in the 30 mg/kg dose ($p=0.0306$); mean corpuscular volume was statistically significantly lower in both the 10 mg/kg and 30 mg/kg doses ($p=0.084$ and $p=0.0053$, respectively); and mean corpuscular hemoglobin concentration was statistically significantly higher in the 30 mg/kg dose ($p=0.0016$). At days 21 and 28, blood urea nitrogen was statistically significantly higher in the 30 mg/kg dose ($p=0.0404$ and $p=0.0385$, respectively).

During the study time period of 56 days, the analysis showed that chloride was statistically significantly higher in both the 10 mg/kg and 30 mg/kg doses ($p=0.0431$ and $p=0.0837$, respectively); total bilirubin was statistically significantly higher in the 30 mg/kg dose ($p=0.0664$); alanine aminotransferase was statistically significantly lower in the 30 mg/kg dose ($p=0.0505$); phosphate and gamma glutamyltransferase were statistically significantly higher in the 10 mg/kg dose ($p=0.0017$ and $p=0.0649$, respectively).

In addition, at day 7, mean corpuscular volume was statistically significantly lower in both the 10 mg/kg and 30 mg/kg doses ($p=0.0838$ and $p=0.0263$, respectively). At day 14, mean corpuscular hemoglobin was statistically significantly lower in the 10 mg/kg dose ($p=0.0677$); and mean corpuscular hemoglobin concentration was statistically significantly higher in the 30 mg/kg dose ($p=0.0572$). At day 28, mean corpuscular hemoglobin was statistically significantly lower in the 30 mg/kg dose ($p=0.0044$). At day 35, potassium was statistically significantly higher in the 10 mg/kg dose ($p=0.0805$).

For inappetence data, since only a few animals (4 animals) showed inappetence, there was no need to conduct statistical analysis.

The analysis of fecal scores using a generalized linear mixed model showed that both the 10 mg/kg and 30 mg/kg dose groups had statistically significantly higher abnormal

feces rates than the control group ($p=0.0049$ and $p=0.0613$, respectively) over the study time period of days 0-28; and only the 30 mg/kg dose group had statistically significantly higher abnormal feces rate than the control ($p=0.0799$) over the study time period of days 0-56.

The analysis of contingency tables summarizing fecal scores at each observation day using Fisher's Exact test indicated that a treatment effect was found for study days 5,6,15,19,20, and 39 ($p\leq 0.1$). For the pair-wise comparison between the control group and the 10 mg/kg group, statistically significant differences were shown for study days 5,6, and 20 ($p\leq 0.1$). Similarly, study days 24 and 25 exhibited statistically significant differences between the control group and the 30 mg/kg group ($p\leq 0.1$).

Body weight analyses resulted in no statistically significant effects for either study time periods of 28 or 56 days.

5. Results

a. **Clinical Results-Hematology/Serum Chemistry:** Sporadic findings outside of normal ranges were found in some animals. Any values noted as significantly different between groups were then subjected to further scrutiny to determine if a pattern or syndrome was evident. When values were noted as significantly different among groups and the values were noted consistently, the values were then compared to a table of standard values from the testing laboratory. The results of this evaluation showed the following:

- 1) **Sodium (NA):** This value is transient on day 7 and is not significant on any day after that. This transient hyponatremia may be due to increased water intake or increased sodium loss through the kidneys or manure. Values were not out of the normal range.
- 2) **Direct Bilirubin (DBIL):** The increased direct bilirubin concentration was not out of the normal range. Increased DBIL may be associated with mild biliary stasis and is related to increased GGT concentration on day 14, which is when the DBIL is the highest.
- 3) **Blood Urea Nitrogen (BUN):** There was a mild increase in BUN but not outside the normal range. Increased BUN would suggest increased metabolism of proteins or increased non-protein nitrogen absorption from the GI tract. The change in BUN was not associated with renal disease or decreased renal function. Serum creatinine, a more sensitive indicator of reduction in renal function, was not abnormal.
- 4) **Potassium (K):** There was a transient increase in potassium by day 35 but still within normal range. This value is actually normal but the control horses showed a decreased potassium level on that day. It would seem that the controls had a spuriously low concentration of potassium and potassium stayed low in controls throughout the study.
- 5) **Chloride (Cl):** There was an increased chloride concentration over time in treated horses, although within normal ranges for chloride. The change was not related to

dehydration, as NA does not increase with time, thus most likely it is related to decreased loss from kidneys.

- 6) Total Bilirubin (TBIL): Mild increase in total bilirubin may be related to mild biliary stasis as noted above.
- 7) White Blood Cells (WBC): There was a transient increase on Day 14. These values are still within the normal range. Increased WBC may be due to alteration in GI flora.
- 8) Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH): There was a transient decrease in RBC size, but still within the normal range which is related to the increase in MCHC. There is no known mechanism that increases MCHC over the normal range. The smaller RBCs are probably the result of older RBCs entering the circulation. This change is not associated with anemia, otherwise cells would be getting bigger or show an increased MCV.
- 9) Basophils (BASO): Basophils decreased over time but were within the normal range. These cells are a small number of the normal WBCs, can be quite variable, and usually increase with allergic reactions. The decrease would not be associated with allergic reaction in this case.
- 10) Gamma Glutamyltransferase (GGT): Increase in GGT over time suggests biliary stasis when noted with bilirubin changes. However, the change noted here was transient and of no clinical significance.
- 11) Phosphate (PO4): The increased phosphate noted in the 10 mg/kg group was within normal limits.

b. Clinical Results- Gastrointestinal-

- 1) Loose Feces: The incidence of loose feces, while noted in all groups including the controls, was greater in the 30 mg/kg group compared to controls.
- 2) Inappetence: Only four animals showed inappetence for some days during the study period. One animal was in the control group, two were in the 10 mg/kg dose group and one was in the 30 mg/kg dose group. Inappetence does not appear to be treatment related.

c. Body Weights: No statistical differences were noted in body weights among groups. However, one animal in the 10 mg/kg group lost weight over the course of 56 days.

d. Histopathology: Necropsy revealed moderate edema in uterine tissues of three of the four females in the 30 mg/kg group (two after 28 days of treatment and one after 56 days of treatment). The edema appeared to be treatment related.

6. Conclusions: Administration of ponazuril at a dose of 10 or 30 mg/kg body weight for 28 or 56 days produced transient episodes of loose feces as well as changes in uterine tissues at 30 mg/kg. Overall, ponazuril administered as a 15% oral paste, at 2X or 6X the recommended dosage, was demonstrated to be generally safe for adult horses.

VI. HUMAN SAFETY

Data on human safety, pertaining to consumption of drug residues in food, were not required for approval of this NADA. The drug is to be labeled for use in horses, which are non-food animals.

Human warnings are provided on the product label as follows:

WARNING: For use in animals only. Not for use in horses intended for food. Not for human use. Keep out of the reach of children.

VII. AGENCY CONCLUSIONS:

The data in support of this NADA comply with the requirements of Section 512 of the Act and Section 514 of the implementing regulations. The data demonstrate that Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste, when used under labeled conditions of use, is safe and effective.

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise is required to diagnose EPM and to monitor the safe use of this product.

Under Section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of approval because no active ingredient (including any ester or salt of the active ingredient) has been approved in any other application.

Bayer Animal Health, Inc. has the following patent: U.S. Patent No. 5,833,095 which expires on August 7, 2017.

VIII. APPROVED PRODUCT LABELING

1. Package Insert: Side 1 & 2
2. 127g Syringe Label
3. Carton for 4 x 127g Syringe Applicators plus Reusable Plunger: Front Panel, Side Panel, Back Panel, Top Flap, Inside Top