

Date of Approval:

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FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-268

PROTAZIL Antiprotozoal Pellets

1.56% diclazuril
Oral Pellets
Horses

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

Sponsored by:

Schering-Plough Animal Health Corp.

2007.141.268

FOIS 1

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I. GENERAL INFORMATION:

- A. File Number:** NADA 141-268
- B. Sponsor:** Schering-Plough Animal Health Corp.
556 Morris Avenue
Summit, NJ 07901
- Drug Labeler Code: 000061
- C. Proprietary Name(s):** PROTAZIL Antiprotozoal Pellets
- D. Established Name(s):** Diclazuril
- E. Pharmacological Category:** Antiprotozoal
- F. Dosage Form(s):** Oral pellets
- G. Amount of Active Ingredient(s):** 1.56% (w/w) diclazuril
- H. How Supplied:** PROTAZIL Antiprotozoal Pellets are supplied in 2 lb (0.9 kg) and 10 lb (4.5 kg) containers.
- I. How Dispensed:** Rx
- J. Dosage(s):** PROTAZIL Antiprotozoal Pellets are administered as a top-dress in the horse's daily grain ration at a rate of 1 mg diclazuril per kilogram (0.45 mg/lb) of body weight for 28 days.
- K. Route(s) of Administration:** Oral
- L. Species/Class(es):** Horses
- M. Indication(s):** PROTAZIL Antiprotozoal Pellets are indicated for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona* in horses.

II. EFFECTIVENESS:

A. Dosage Characterization:

Published literature reports *Sarcocystis neurona* is sensitive to the inhibitory effects of diclazuril. Diclazuril at a concentration of 0.1 ng/mL inhibited merozoite production of *Sarcocystis neurona* in cell culture by more than 80%. At *in vitro* concentrations of 1 ng/mL, inhibition was greater than 95%. However, the clinical relevance of the *in vitro* cell culture data has not been determined.

Predictions of the systemic drug concentrations of diclazuril for daily 1 mg/kg doses of the pelleted formulation were based upon published equine pharmacokinetic data¹ generated with a non-market formulation and Schering-Plough pilot equine data at higher doses (5-5.6X clinical dose). The study by Dirikolu et al. involved four horses that received a single oral gavage dose of a poultry premix (CLINACOX) and two EPM horses that received 21 daily doses of CLINACOX. The Schering-Plough pilot study employed a two-period and two-treatment crossover design where six horses received single doses of diclazuril as an intravenous injection and as the oral formulation. A 21-day washout period separated administration of the two treatments. Based upon the information, the oral bioavailability of diclazuril from the PROTAZIL Antiprotozoal Pellets is estimated to be approximately 5%. Diclazuril concentrations in the cerebral spinal fluid (CSF) of the two EPM horses used in the study by Dirikolu et al. were found to be approximately 1% - 5% of the concentrations observed in the plasma.

Two target animal safety studies were conducted toward approval of this drug and provided further dose characterization. SPAH Study #04008 demonstrated that the 1 mg/kg dose of PROTAZIL Antiprotozoal Pellets (containing diclazuril: 1.56% w/w) results in steady-state mean plasma diclazuril concentrations in the range 2000 – 2500 ng/mL, while SPAH Study #99306 demonstrated a linear relationship between plasma diclazuril concentrations and CSF diclazuril concentrations.

Considering the intended labeled dose (1 mg/kg daily for 28 days) and the ratio of concentrations in plasma versus the CSF, one can expect the steady state CSF diclazuril concentrations with daily oral administration of 1 mg/kg PROTAZIL pellets to range from 20-70 ng/mL. These concentrations exceed the targeted concentration in the CSF (1 ng/mL, *in vitro* IC₉₅), justifying the dose selected in the field studies.

¹ Dirikolu L, Lehner F, Natrass C, et al. Diclazuril in the horse: its identification and detection and preliminary pharmacokinetics. *J. Vet. Pharmacol. Therap.* 1999;22:374-379.

B. Substantial Evidence:

1. Type of Study: Clinical Field Study

a. Title: Diclazuril (SCH 209203) safety and effectiveness in the treatment of equine protozoal myeloencephalitis (EPM) in the horse (Study Number C99-558).

b. Investigator(s):

Andrews Knoxville, TN	Dr. John Jagar Millbrook, NY
Dr. Greg Staller Califon, NJ	Dr. Tom Divers Ithaca, NY
Dr. Corinne Sweeney Kennett Square, PA	Dr. Rob MacKay Gainesville, FL
Dr. Steve Reed Columbus, OH	Dr. Martin Furr Leesburg, VA
Dr. Jerry Johnson Lexington, KY	Dr. Charles MacAllister Stillwater, OK

c. Study Design:

1) Objectives: The purpose of the study was to demonstrate the safety and effectiveness of diclazuril for the treatment of equine protozoal myeloencephalitis (EPM) in horses naturally infected with EPM under field conditions. PROTAZIL (1.56% diclazuril) Antiprotozoal Pellets were administered once a day at a dose of 1 mg diclazuril per kilogram of body weight for 28 consecutive days.

2) Study Animals: Two hundred and fourteen mares, stallions, and geldings of various breeds, ranging in age from 9.6 months to 30 years, were enrolled in a multi-center field study. All horses were confirmed EPM-positive based on the results of clinical examinations and laboratory testing, including CSF Western Blot analyses. The three most represented breeds enrolled were Quarter Horses, Thoroughbreds, and Tennessee Walking Horses.

3) Treatment Groups:

- a. Control:

EPM is typically a progressive neurological disease. It has been estimated that up to 55-65%² of horses respond favorably to treatment. However, it is further estimated that only a small percentage (no more than 10%) of treated horses recovers completely. Studies evaluating drugs to treat EPM necessitated using each horse as its own control because of the poor prognosis in untreated horses. Furthermore, at the time the field study was conducted, there was no FDA-approved treatment for EPM.
 - b. Diclazuril:

PROTAZIL (1.56% diclazuril) Antiprotozoal Pellets were administered to horses as a top-dress on their daily grain ration at 1 mg/kg, 5 mg/kg, or 10 mg diclazuril/kg body weight for 28 days.
- 4) Drug Administration: PROTAZIL (1.56% diclazuril) Antiprotozoal Pellets was supplied in buckets, each of which contained approximately 6000 -7000 mL of pellets. A graduated cylinder was supplied with each bucket to measure the dose. Clinical investigators used a dosage calculation chart based on the horse's weight to determine the dose. Animals were dosed once daily for 28 days by applying the pellets on top of the normal grain ration. Owners documented the amount of pellets consumed on a daily basis.
- 5) Measurements and Observations:
- a. Ataxia Score: In order to be enrolled in the study, a horse had to exhibit a grade 2 or greater ataxia score in at least one limb and have a positive Western Blot for *S. neurona* performed on CSF. Clinical investigators performed a standardized neurological examination on Day 0, Day 27 and Day 48. The Day 0 and Day 48 ataxia scores were compared to determine if at least a one grade improvement was seen. Clinical investigators assigned ataxia scores based on the modified Mayhew scale as follows:

0 = Normal, Neurologic deficits not detected.
1 = Neurologic deficits may be detectable at normal gaits; Exacerbated with manipulative procedures (e.g., backing, turning in tight circles, walking with head elevation, etc.).
2 = Neurological deficit obvious at normal gaits or posture; Signs exacerbated with manipulative procedures.
3 = Neurologic deficit very prominent at normal gaits; Horses give the impression that they may fall (but do not) and buckle or fall with manipulative procedures.

² Granstrom DE. Understanding Equine Protozoal Myeloencephalitis: Your Guide to Horse Health Care and Management. Lexington: The Blood-Horse Inc, 1997:10.

4 = Neurologic deficit is profound at normal gait; Horse frequently stumbles or trips and may fall at normal gaits or when manipulative procedures are utilized.

5= Horse is recumbent, unable to rise.

b. Western Blot (WB): Serum and cerebrospinal fluid (CSF) were collected and tested on Day 0, Day 27, and Day 48 for antibodies to *Sarcocystis neurona*. Horses had to have a positive Day 0 CSF Western Blot in order to be enrolled in the study. Cytology to detect blood contamination, albumin quotient, IgG index, and total protein were also measured on all CSF samples obtained from horses enrolled in the study.

c. Videotape Evaluation: The neurological examinations were videotaped for each horse on Days 0, 27, and 48. In order to provide objectivity and corroborate the clinical investigator's evaluation, three independent equine experts reviewed and graded each videotape. The experts were masked to the date (chronological order) and the identification of the horse while viewing the tape. If the majority of the experts indicated that the horse improved at least one grade, then the case was considered a corroborated success. Horses that were WB CSF negative on Day 48 were considered successes and were not further reviewed.

d. Exclusion Criteria: A horse was excluded from the study if it received therapy for EPM within the last 6 months, was in poor health for causes not attributable to EPM, was pregnant or was a breeding stallion, or was showing signs of other conditions or diseases that also result in neurological signs. In order to rule out other conditions or diseases that also result in neurological signs, additional diagnostics were performed. Cervical radiographs were taken to rule out spinal cord compression or vertebral canal stenosis. Serum samples were tested for antibodies to Equine Herpes Virus-1 and West Nile Virus. Serum samples were also tested for Vitamin E levels to rule out equine degenerative myelopathy (EDM). CSF cytology was performed to determine nucleated cell count and eosinophils. An eosinophil value of >20% of the nucleated cell count or an elevated nucleated cell count (>100 cells/uL) was suggestive of migrating parasite larvae. Horses with abnormal findings were removed from the study.

- 6) Statistical Methods: Success was based on the clinical investigator's neurological evaluations on Day 0 and 48, the CSF Western Blot results taken on Days 0 and 48, and the masked evaluations of the Day 0 and Day 48 neurological examination videotapes.

A Clinical Investigator Success was defined as a horse that improved at least one grade on the neurological examination at Day 48 as compared to Day 0. Western Blot Success was defined as a horse with a negative CSF Western Blot by Day 48, regardless of the horse's neurological status. A

Corroborated Clinical Success was a case that was judged to be a success by the clinical investigator which was also corroborated by the masked evaluation of the videotapes. The panel did not review videotapes of horses that seroconverted to Western Blot CSF negative status, as they were considered true treatment successes.

- d. **Results:** Of the 214 horses enrolled in the study, 68 were dosed at 1 mg diclazuril/kg body weight for 28 days. After excluding cases meeting the exclusion criteria, a total of 42 horses at the 1 mg/kg dose were evaluated for effectiveness. All 214 horses were evaluated for safety. Table 1 summarizes the cases evaluated for effectiveness at Day 48.

Table 1. Breakdown of Success Cases.

Treatment Group	Day 48 Protocol Compliant Cases	Western Blot Successes (seroconversion from positive to negative)	Day 48 Clinical Investigator Successes	Expert Successes (corroborating the clinical investigator successes)	Overall Successes (WB conversions and expert-corroborated successes)
1 mg/kg	42	3	25*	10	13

*One case at 1 mg/kg was rated as a success by the clinical investigator but did not have videotapes available for expert review. This case was not included in the calculation of percentage of corroborated clinical successes.

Based on the numbers of horses that seroconverted to negative Western Blot status and the numbers of horses classified as successes by the clinical investigators, 28 of 42 horses (67%) were considered successes at 1 mg/kg. With regard to independent expert masked videotape assessments, 10 of 24 horses (42%) at 1 mg/kg were considered successes. There was no clinical difference in success rates among the horses treated with 1, 5, or 10 mg/kg.

- e. **Adverse Reactions:** Adverse events were reported for two cases. In one case, a horse was enrolled showing severe neurologic signs. Within 24 hours of dosing, the horse was recumbent, biting, and exhibiting signs of dementia. The horse died, and no cause of death was determined. In the second case, the horse was reported to be walking stiffly approximately 13 days after the start of dosing. The referring veterinarian reported that the horse had been fed grass clippings and possibly had laminitis.
- f. **Conclusions:** PROTAZIL Antiprotozoal Pellets dosed at 1 mg diclazuril/kg body weight for 28 consecutive days are an effective treatment for EPM.

III. TARGET ANIMAL SAFETY:

Two studies were conducted to evaluate the safety of diclazuril when administered orally to horses for 42 days as a top-dress on the grain ration.

A. Type of Study: Toxicity Study

1. Title: Diclazuril (SCH 209203) Target Animal Safety Study in Horses. SPAH Study # 99306/SBL Study # 99389H
2. Study Director: John W. Byrd, PhD
Southwest Bio-Labs, Inc.
Las Cruces, NM
3. Study Design:
 - a) Objectives: To evaluate the safety of diclazuril in horses, the target animal, for the Equine Protozoal Myeloencephalitis therapeutic program at 0, 5, 15, 25, and 50 times the clinical dose, administered for at least 42 days as a top-dress on the grain ration.
 - b) Study Animals: Thirty horses (15 males, intact and 15 females) ranging in weight from 163 to 268 kg, and 5 to 9 months of age at start of dose administration. Breeds were Quarter Horses, Thoroughbreds, and Quarter Horse crosses.
 - c) Treatment Groups:

Table 2. Treatment Groups

Tx Group	Dose mg/kg	Number and Sex of Animals
1	0	3 Males, 3 Females
2	5 mg/kg (5X)	3 Males, 3 Females
3	15 mg/kg (15X)	3 Males, 3 Females
4	25 mg/kg (25X)	3 Males, 3 Females
5	50 mg/kg (50X)	3 Males, 3 Females

- d) Drug Administration: The test article was diclazuril top-dress pellets, containing 1.56% diclazuril. The control was top-dress pellets without diclazuril.
- e) Measurements and Observations: Physical examination, daily clinical observations, body weight, feed and water consumption, blood collection (hematology, coagulation and serum chemistry), urinalysis, fecal

examination, and necropsy including gross pathology, histopathology, and organ weights. Plasma diclazuril drug concentration was evaluated during the study and cerebrospinal fluid (CSF) diclazuril drug concentration was evaluated at necropsy.

- f) Statistical Methods: Body weight, clinical pathology, and other variables observed at several time points during the study were each modeled using a repeated measures analysis of variance/covariance. The fixed effects included in the model were treatment, day and gender, and their 2- and 3-way interactions. The mean baseline values of the dependent variables were included in the model as covariates. Follow-up mean comparisons between each treated group and control using linear contrasts were performed as necessary.

4. Results:

- a) Clinical Observations and Physical Examinations: There were no test article-related findings observed in this study during clinical observations or physical examinations. Signs relating to upper respiratory disease (fever, coughing, nasal discharge, enlarged or draining lymph nodes), lice, ringworm, parasitism, rough hair coat, alopecia, warts, as well as some wounds both old and new from infighting, were all commonly seen throughout the study in many horses. Some cervical swellings that formed during the pretest period indicated that some of these young horses were exposed to *Streptococcus equi* (strangles).
- b) Body Weights: The young, growing horses in all dose groups in this study did not gain their expected amount of weight (1.2 to 1.7 pounds per day) during the study. This was especially noticeable in the 50 mg/kg group. This may be related to the fact that horses were limit-fed to 2% of their body weight per day and that horses in the 50 mg/kg group had the lowest body weights at the start of the study.
- c) Feed and Water Consumption: Test article-related effects were limited to a decrease in the grain/top-dress consumption in two horses (1 male and 1 female) in the 50 mg/kg group. The other horses ate all of their grain/top-dress and hay ration during the dosing period.

There were no test article-related differences from the control group in water consumption.

- d) Hematology and Coagulation: It could not be determined if there were any test article-related hematology/coagulation changes. Evaluation of some parameters including white blood cell (WBC) and fibrinogen in some horses early in the study was complicated by an apparent strangles infection. Fifty percent of the horses had WBC counts above some of the normal published

ranges at least once during the study. Activated partial thromboplastin time APTT increased in 3-5 out of 6 horses in each group, including the control group (who were also exposed to diclazuril) by at least a factor of two as compared to their baseline values by the end of the study.

- e) Serum Chemistry: It could not be determined if there were any test article-related serum chemistry changes. Possible dose-related trends were noted for increasing blood urea nitrogen (BUN), creatinine, and sorbitol dehydrogenase (SDH) with increasing dosage, although values remained within normal range. Elevated levels of serum calcium (Ca⁺⁺) and chloride (Cl⁻) were seen at days 21 and 28 and elevated levels of serum sodium (Na⁺) were seen on day 28 in every dose group.
- f) Urinalysis: There were no test article-related changes in the urine.
- g) Fecal Analysis: There were no test article-related changes in the feces. Evidence of fecal parasites was seen in 30% of the horses pre-study and 73% of the horses at termination.
- h) Necropsy, Histopathology and Organ Weights: There were no test article-related findings on necropsy or histopathology. Necropsy findings included numerous bots in the horses' stomachs and ascarid nematodes in the intestinal lumen.

Increased liver size in relation to body weight was seen in the 50 mg/kg horses, although this group of horses may have been underweight. A trend for decreasing kidney weights with increasing dose was seen with lower kidney weights in the 25 and 50 mg/kg groups compared to the 0 mg/kg group. Decreasing heart weight in relation to body weight was seen in 25 mg/kg males without any dose-related trends. The clinical relevance of these findings is not known.

- i) Drug Concentration Analysis in the Plasma and CSF: Diclazuril concentrations in plasma (weekly) and CSF (at necropsy) tended to show a dose-dependent, but less than dose-proportional increase in all treatment groups. Three of the six control horses also had detectable diclazuril concentrations in the CSF and plasma.
5. Adverse Reactions: There were no test article-related findings observed in this study during clinical observations or physical examinations.
6. Conclusions: The safety of diclazuril top dress administered to horses at 1 mg/kg once daily cannot be determined based solely on this study because of the lack of an adequate control group (control horses tested positive for the test drug). However, possible findings associated with the drug were limited to

elevations in BUN, creatinine, and SDH and less than anticipated weight gain. Definitive test article-related effects were decreased grain/top-dress consumption in horses in the 50 mg/kg group (equal to 50X).

B. Type of Study: Toxicity Study

1. Title: SCH 209203 (Diclazuril) - PROTAZIL Top Dress: 42-day Oral Target Animal Safety Study in Horses. SPAH Study #04008.
2. Investigator: Terry N. Terhune, DVM, PhD
HMS Veterinary Development, Inc.
Tulare, CA
3. Study Design:
 - a) Objectives: To assess the safety of PROTAZIL Top Dress (containing diclazuril: 1.56% w/w) when administered to horses orally at 0, 1, and 5 times the proposed clinical diclazuril dose of 1 mg/kg/day, administered daily for a total of 42 consecutive days.
 - b) Study Animals: Twenty-four horses (12 castrated males and 12 females) between 2 and 8 years old, and weighing between 357-584 kg at the start of treatment. Breeds were Thoroughbreds, Arabians, Quarter Horses, and a Mustang.
 - c) Treatment Groups:

Table 3. Treatment Groups

Tx Group	Dose (mg/kg)	Number and Sex of Animals
1	0, control article alfalfa pellets at 5X volume	4 males, 4 females
2	1 mg/kg or 4.2 mL top dress/ 100 lbs bw (1X)	4 males, 4 females
3	5 mg/kg or 21 mL top dress/ 100 lbs bw (5X)	4 males, 4 females

- d) **Drug Administration:** The test article was a pelleted formulation containing 1.56% (w/w) diclazuril as the final market formulation. The control article was commercially available alfalfa pellets.
- e) **Measurements and Observations:** Daily clinical observations, physical examinations, body weight, food and water consumption, clinical pathology (serum chemistry, hematology, diclazuril plasma concentrations), and fecal analysis.
- f) **Statistical Methods:** Body weight, clinical pathology, and other variables observed at several time points during the study were each modeled using a repeated measures analysis of variance/covariance. The fixed effects included in the model were treatment, day and gender, and their 2- and 3-way interactions. The mean baseline values of the dependent variables were included in the model as covariates. Follow-up mean comparisons between each treated group and control using linear contrasts were performed as necessary.

4. Results:

- a) **Clinical Observations and Physical Examinations:** There were no test article-related findings observed during clinical observations or physical examinations during the study.
- b) **Body Weight:** There were no test article-related effects on body weight.
- c) **Feed Consumption, Water Consumption, and Average Daily mg/kg Consumption of Test Article:** There were no test article-related effects on feed consumption. The horses were fed a percentage of their body weight daily. All of the horses ate the amount that was fed each day. There were no test article-related effects on water consumption.
- d) **Hematology:** There were no test article-related effects on hematology.
- e) **Serum Chemistry:** There was a dose dependent trend toward increasing gamma-glutamyl transferase (GGT) levels, especially in the 5 mg/kg dose group.
- f) **Fecal Examination:** There were no test article-related fecal examination findings.
- g) **Diclazuril Plasma Concentration Analysis:** Group average diclazuril concentrations in equine plasma were approximately two fold higher in the 5 mg/kg dose group as compared to the 1 mg/kg dose group. There were no measurable diclazuril levels in the placebo group. In general, the females tended to have lower diclazuril plasma concentrations than the males.

5. Adverse Reactions: There were no test article-related findings observed during clinical observations or physical examinations during the study.
6. Conclusions: This target animal safety study has shown that PROTAZIL (1.56% diclazuril) Antiprotozoal Pellets, when administered to horses orally at the proposed clinical diclazuril dose of 1 mg/kg/day for forty-two consecutive days, are safe.

IV. HUMAN FOOD SAFETY:

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

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to PROTAZIL Antiprotozoal Pellets:

“WARNING: For use in horses only. Do not use in horses intended for human consumption. Not for human use. Keep out of reach of children.”

The following items were examined to ensure human user safety: the MSDS for diclazuril, the FOI Summary for CLINACOX (NADA 140-951), and the data submitted in support of this NADA. According to the MSDS for the active ingredient (dated 1995, Janssen Pharmaceutica), the active ingredient is a powder which can be absorbed through inhalation and ingestion. Absorption of the active ingredient from PROTAZIL Antiprotozoal Pellets by inhalation or ingestion is unlikely, as the active ingredient is incorporated into extruded pellets, which are not overly friable based on information reviewed for approval of this NADA.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514. The data demonstrate that PROTAZIL Antiprotozoal Pellets, when used according to the label, are safe and effective for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*.

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is judged to be critical for the diagnosis of equine protozoal myeloencephalitis in horses and for 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 because the active ingredient has been approved in another application. With regard to this application, the sponsor has conducted new studies providing substantial evidence of effectiveness and demonstrating safety of the active ingredient in horses.

C. Patent Information:

PROTAZIL Antiprotozoal Pellets are under the following U.S. patent numbers:

<u>U.S. Patent Number</u>	<u>Date of Expiration</u>
4,631,278	August 1, 2007
5,830,893	April 23, 2017
5,883,095	August 7, 2017

VII. ATTACHMENTS:

Facsimile Labeling:

- a. Expandable content label for 2 lb container
- b. Expandable content label for 10 lb container

