FREEDOM OF INFORMATION SUMMARY

Supplement to NADA 141-036

PIRSUE® Sterile Solution

(pirlimycin hydrochloride for lactating dairy cattle)

"...for intramammary treatment of clinical and subclinical staphylococcal and streptococcal mastitis in lactating dairy cattle."

Sponsored by:

PHARMACIA & UPJOHN COMPANY

Date of approval_____

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I. GENERAL INFORMATION

NADA Number: 141-036

Sponsor: Pharmacia & Upjohn Company
7000 Portage Road
Kalamazoo, Michigan 49001

Accepted Name: pirlimycin hydrochloride

Trade Name: PIRSUE®Sterile Solution

Marketing Status: This is a prescription product and bears the caution statement as follows: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

Supplemental Effect: Provides for a new sterile formulation with the same therapeutic claims. Also, provides for an Acceptable Daily Intake, a tolerance in cattle muscle, and a 9-day pre-slaughter withdrawal period.

II. INDICATIONS FOR USE IN CATTLE

PIRSUE®Sterile Solution is indicated (pirlimycin hydrochloride) for the treatment of clinical and subclinical mastitis in lactating dairy cattle. PIRSUE® Sterile Solution has been proven effective only against *Staphylococcus* species such as *Staphylococcus aureus* and *Streptococcus* species such as *Streptococcus agalactiae, Streptococcus dysgalactiae* and *Streptococcus uberis*. Cows with systemic clinical signs caused by mastitis should receive other appropriate therapy under the direction of a licensed veterinarian.

III. PRODUCT INFORMATION

A. Dosage Form: PIRSUE® Sterile Solution is available in 10 mL plastic syringes (plastets) with a cannula for intramammary infusion. Each plastet contains pirlimycin hydrochloride equivalent to 50 mg of pirlimycin (5 mg/mL).

B. Route of Administration: PIRSUE® Sterile Solution is to be administered to lactating dairy cattle by intramammary infusion.

C. Recommended Dosage: Infuse the contents of one (1) syringe (plastet) into each affected quarter. Repeat this treatment once, at a 24-hour interval.

IV. ANIMAL SAFETY AND EFFECTIVENESS

The original new animal drug application (NADA) for PIRSUE™ Aqueous Gel (NADA 141-036) for intramammary infusion in lactating dairy cattle for the treatment of clinical and subclinical mastitis was approved September 10, 1993. The effectiveness data provided in support of the original NADA is summarized in the original Freedom of Information (FOI) summary.

For the purpose of this supplemental approval, the following bioequivalence study was conducted to bridge the animal safety and effectiveness data from PIRSUE™ Aqueous Gel to PIRSUE® Sterile Solution.

Pivotal Bioequivalency Study # 782-9690-95-002

A. Type of Study: Milk and plasma pharmacokinetics of pirlimycin following intramammary infusion.

B. Principal Investigator: R. E. Hornish
Pharmacia & Upjohn Animal Health,
Kalamazoo, MI.

C.

Animal Species:	bovine
Strain/Breed:	Holstein
Sex:	female, lactating
No. of Animals:	20
Stage of Lactation:	2nd to 4th lactation, > 18 kg of milk/day
Weight:	449-772 kg
Health Status:	healthy cows
Enrollment Criteria:	lactating cows (2nd, 3rd or 4th lactation), weight (500-800 kg), previous monthly DHI SCC (<750,000), no antibiotic use within 30 days prior to study

D. Study Design: GLP, randomized two period, two sequence, two treatment crossover

E. Treatments: Test product: sterile formulation containing no carboxymethylcellulose (CMC)

Reference product: PIRSUE® Aqueous Gel containing 2% CMC

F. Route of administration: Intramammary

G. Dose rate: 100 mg pirlimycin into all four quarters

H. Duration of dosing: a single infusion.

I. Analytical methods: Milk - validated by microbiological cylinder plate method (LOQ = 0.02 μg/mL)

Plasma - validated by HPLC with fluorescence detection LOQ = 2 ng/mL)

J. Blood sampling schedule: predose and hrs 2, 4, 6, 8, 10, 12, 15, 24, 36, 48, 60,72, 84, and 96 post-dose

K. Milk sampling schedule: every 12 hrs up to hour 96 post-treatment. All four quarters milked-out and pooled by the milking machine to generate a composite 4-quarter milk sample per milking per cow.

L. Bioequivalence approval criteria: plasma AUC_{96} and C_{max} values are contained within the limits of +/- 20% relative to the corresponding reference means.

M. Results: The milk residue data, and milk and plasma bioequivalence data are presented in Tables 4.1 and 4.2, respectively.

Table 4.1. Milk residue data

Time of observation after treatment (hr.)	Mean concentration of pirlimycin in milk(μg/mL)				Mean amount of pirlimycin collected in milk at each milking time (mg)			
Formulation-order of treatment	0%-1st	0%-2nd	2%-1st	2%-2nd	0%-1st	0%-2nd	2%-1st	2%-2nd
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	19.7	20.6	18.6	28.7	133.82	123.17	154.18	200.94
24	2.04	1.63	1.32	2.73	14.45	10.75	12.32	11.40
36	0.51	0.34	0.36	0.66	3.30	2.36	3.20	2.63
48	0.17	0.19	0.14	0.23	1.34	1.53	1.45	0.92
60	0.11	0.10	0.09	0.13	0.81	0.74	0.81	0.72
72	0.07	0.07	0.06	0.09	0.60	0.61	0.64	0.42
84	0.06	0.06	0.05	0.12	0.43	0.39	0.45	0.43
96	0.04	0.03	0.04	0.05	0.35	0.23	0.35	0.21
Total Amount	NA	NA	NA	NA	155.1	139.77	173.4	217.68
Percent of dose	NA	NA	NA	NA	38.8	34.9	43.35	54.42

Table 4.2. Milk and plasma bioequivalence data

	0% CMC (Test Formulation)	2% CMC (Ref. Formulation)	Lower Confidence Interval (%)	Upper Confidence Interval (%)
Plasma AUC_{96} (ng* hr/mL)[a]	957.0 +/- 301.7	907.9 +/- 214.2	96*	112*
Plasma C_{max} (ng/mL)[a]	31.7 +/- 12.5	28.4 +/- 10.2	101*	120*
time (hrs) of last milk sample > 0.13μg/mL**	45.5	46.1	88	109
time (hrs) to milk drug concentrations of 0.4 μg/mL***	35.0	34.7	96	105

[a]arithmetic mean +/- SD

*90% confidence interval based on logarithmic means

**based upon observed data (where 0.13μg/mL represents a target pirlimycin concentration based upon pathogen MIC values)

***extrapolated value using linear regression methods (where 0.4 μg/mL represents the tolerance based upon human food safety).

N. Conclusion: The analysis of the plasma data indicated that the AUC_{96} for the two formulations were statistically equivalent and thus pirlimycin was equally bioavailable from the two formulations. The mean time to the tolerance concentration of 0.40 μg/mL were statistically equivalent. Based upon the concentrations of pirlimycin in milk and the equivalence of the blood levels, it is concluded that the new sterile formulation is comparable to the aqueous gel formulation.

V. HUMAN FOOD SAFETY

Complete summaries of all pivotal toxicology and metabolism studies are found in the Human Food Safety Section of the Freedom of Information Summary, dated September 10, 1993, for PIRSUE™Aqueous Gel under the original NADA 141-036. For PIRSUE®Sterile Solution, the NOEL (10 mg/kg from the 90 day oral rat study), the ADI (0.01 mg/kg/day), the residue marker (R_m, parent pirlimycin in milk and liver), the tolerance of parent pirlimycin in milk and liver (0.4 ppm in milk and 0.5 ppm in liver), and the regulatory methods for pirlimycin in milk and liver (HPLC/Thermospray/Mass Spec. method) remain identical for the pathogen free product.

A. Tolerances for Residues in Liver, Milk, and Muscle

As specified in 21 CFR 556.515, the tolerance for residues of parent pirlimycin (the marker residue) is established at 0.5 ppm in cattle liver (target tissue) and 0.4 ppm in milk.

As part of the approval of this supplement, the Agency has taken the opportunity to update the human food safety information on this product and codify an Acceptable Daily Intake (ADI) of 0.01 mg/kg body weight/day and a tolerance in cattle muscle.

Total and parent residue data from Study No. TR 782-7926-92-002 were compared with parent data from Study No. TR 782-7926-97-002 and were used as the basis for assigning a tolerance for pirlimycin in muscle. Data do not exist to adequately establish the ratio between parent pirlimycin and total pirlimycin residues in muscle. Therefore, the percent of parent pirlimycin in liver was used as a conservative estimate of the marker to total residue ratio in muscle. When the total residue in the liver was in the range of the muscle safe concentration (1.2 ppm), the concentration of parent pirlimycin was about 24% of the total. Using this conservative ratio estimate, parent pirlimycin levels in muscle should be less than 0.3 ppm when total residues of pirlimycin in muscle are below its safe concentration of 1.2 ppm. Accordingly, 0.3 ppm is assigned as the tolerance for pirlimycin in muscle.

B. Study to Confirm 36-Hour Milk Discard Period.

A milk residue depletion study was conducted at Pharmacia & Upjohn, Kalamazoo, MI, with pirlimycin in a sterile formulation. The purpose of the study was to confirm the 36-hour milk discard period that previously was established with the pathogen-free pirlimycin product under NADA 141-036.

Twenty-two lactating Holstein (20 test, 2 control) weighing between 494 and 771 kg body weight were used. The test cattle were treated by intramammary

infusion into all 4 udder quarters with two doses of a sterile aqueous formulation of pirlimycin HCl at a 24-hour interval at a dose rate of 50 mg of pirlimycin free base equivalents per quarter for a total of 400 mg per cow. Milk was collected from each cow as a composite 4-quarter milking every 12 ± 1 hours beginning just before treatment through 96 hours after the second treatment. The milk samples were analyzed for pirlimycin using the method described in Section D. The results are depicted in the following table.

Table 5.1. Mean pirlimycin residues (in ppm) in milk collected from cows treated by intramammary infusion with two doses of a sterile aqueous formulation of pirlimycin HCl at a 24-hour interval at a dose rate of 50 mg of pirlimycin free base equivalents per quarter

Milk collection time after treatment (hr.)	Mean pirlimycin residues	Standard deviation
12	10.4	4.99
24	0.82	0.76
36	0.21	0.31
48	0.11	0.07
60	0.07	0.02
72	0.05	0.02
84	0.03	0.01
96	0.02	0.01

LOQ = 0.02

The statistical method used to calculate the milk discard time was that described by FDA: VI. Guideline for Establishing a Withdrawal Period, September, 1994. Single data points were allowed for this study based on previously demonstrated sample repeatability and assay variability. The milk discard period was confirmed as 36 hours by using the 0.4 ppm tolerance and calculating the upper tolerance limit of the mean residue concentration at each timepoint with a 95% confidence interval on the 99th percentile.

C. Study to Establish Withdrawal Time

A tissue residue depletion study was conducted at Pharmacia & Upjohn, Kalamazoo, MI, with pirlimycin in a sterile formulation. The purpose of the study was to determine the depletion of parent pirlimycin (marker residue) in liver to below the 0.5 ppm tolerance for calculation of the withdrawal time. This study was a continuation of the milk discard study described in Section B.

Twenty-two lactating Holstein (20 test, 2 control) weighing between 494 and 771 kg body weight were used. The test cattle were treated by intramammary infusion into all 4 udder quarters with two doses of a sterile aqueous formulation of pirlimycin HCl at a 24-hour interval at a dose rate of 50 mg of pirlimycin free base equivalents per

quarter for a total of 400 mg per cow. Liver, kidneys, muscle, and adipose tissue were collected. The tissue samples were analyzed for pirlimycin using the method described in Section D. The results are depicted in the following table.

Table 5.2. Mean pirlimycin residues (in ppm) in liver collected from cows treated by intramammary infusion with two doses of a sterile aqueous formulation of pirlimycin HCl at a 24-hour interval at a dose rate of 50 mg of pirlimycin free base equivalents per quarter

Withdrawal time (days)	Mean pirlimycin residues (n=4)	Standard deviation
2	10.4	0.22
7	0.24	0.04
14	0.04	0.02
21	<0.025	---
28	<0.025	---

LOQ = 0.025

The statistical method used to calculate the withdrawal time was that described by FDA: VI. Guideline for Establishing a Withdrawal Period, September, 1994. Using a statistical tolerance limit for the 99th percentile of the population with a 95% confidence interval, the withdrawal time was determined to be 9 days to reach the tolerance of 0.5 ppm in liver.

D. Regulatory Methods

Pirlimycin in milk and in liver is analyzed by a simultaneous determinative and confirmatory method using HPLC/Thermospray/Mass Spectrometry. The limit of quantitation is 0.025 ppm for milk. The limit of quantitation for the liver assay was validated at 0.05 ppm. However, for this study using the sterile formulation, the limit of quantitation was 0.025 ppm. Both the milk and liver assays were submitted to method validation trials. The results of those trials are described in NADA 141-036. The two methods are on file at the Center for Veterinary Medicine, Food and Drug Administration, HFV-199, 7500 Standish Place, Rockville, MD 20855.
The section on Liver Sample Storage and Pre-sampling Preparation (page 11 in the September 2, 1993, determinative method for pirlimycin in liver) has been amended as follows: "The liver samples should be stored frozen (-15 to -20°C) and they should not be stored for more than 3 months prior to analysis. They should be thawed by partially submerging the container in an ambient temperature water bath for an hour or less prior to analysis. The samples may be allowed to experience two freeze thaw cycles prior to analysis."

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 514 of the implementing regulations. The data demonstrate that Pirsue® Sterile Solution is safe and effective for the treatment of clinical and subclinical mastitis in lactating dairy cattle by intramammary infusion of 50 mg pirlimycin hydrochloride sterile solution per quarter twice at a 24-hr interval.

An Acceptable Daily Intake (ADI) of 0.01 mg/kg body weight/day has been established for pirlimycin. A tolerance of 0.5 ppm in liver (the target tissue) has been established for residues of parent pirlimycin (the marker residue) in cattle. Concentrations of marker residue below the tolerance in the target tissue indicate that the total residues of the drug in all edible tissues are below their respective safe concentrations. A tolerance of 0.4 ppm in milk has been established for residues of parent pirlimycin (the marker residue) in lactating cattle. A milk discard time of 36 hours is required for the use of pirlimycin hydrochloride in lactating dairy cattle. A muscle tolerance of 0.3 ppm has been established. Residues of parent pirlimycin below 0.3 ppm in muscle indicate that muscle residues are below the muscle safe concentration. Residues of drug at or below the muscle tolerance are not indicative of the safety of other edible tissues in lactating cattle for human consumption.

A pre-slaughter withdrawal period of 9 days has been assigned for the use of pirlimycin hydrochloride in lactating dairy cattle. The withdrawal period was based on a statistical analysis of the depletion data, using an upper tolerance limit containing 99 percentile of the population with a 95 percent confidence interval.

The product remains a prescription drug for safe and effective use by a veterinarian in the treatment of staphylococcal and streptococcal mastitis in lactating dairy cattle.

In accordance with 21 CFR 514.106(b)(2)(i), this is a Category II change which did not require a reevaluation of the safety or effectiveness data in the parent application.

The agency has determined under 21 CFR 25.33(a)(1) that this action is of a type that does not individually or cumulatively have a significant impact on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

Under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act, this approval for food producing animals qualifies for THREE years of marketing exclusivity beginning on the date of approval because the application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or, in case of food producing animals, human food safety studies (other than bioequivalence or

residue studies) required for the approval of the application and conducted or sponsored by the applicant. The three years of marketing exclusivity applies only to the new sterile solution for which the supplemental application was approved.

Pirlimycin is under U.S. patent number 4,278,789 expiring November 23, 2002.

VII. APPROVED PRODUCT LABELING

See attachment.