

I. GENERAL INFORMATION

A. File Number

NADA 141-036

B. Sponsor

The Upjohn Company
7000 Portage Road
Kalamazoo, Michigan 49001

C. Proprietary Name

Pirsue™ Aqueous Gel

D. Established Name

pirlimycin hydrochloride aqueous gel

E. Dosage Form, Route of Administration and Recommended Dosage

Pirlimycin hydrochloride is available in 10 mL plastic syringes (plastets) with cannula for intramammary infusion. Each plastet contains pirlimycin hydrochloride equivalent to 50 mg pirlimycin (5 mg/mL). Infuse the contents of one (1) syringe into each affected quarter. Repeat this treatment once, after a 24-hour interval.

F. Dispensing Status

This is a prescription product and will carry the following caution statement:
"Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian."

G. Species/Class

Dairy cattle

H. Indication

Pirlimycin is indicated for the treatment of clinical and subclinical mastitis in lactating dairy cattle. Pirlimycin 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.

II. EFFECTIVENESS

A. Pivotal Studies (2)

Two separate clinical studies were conducted which lead to the conclusion that pirlimycin is effective for the treatment of mastitis in lactating dairy cattle when administered via intramammary infusion at 50 mg per 10 mL twice, with the second infusion 24 hours after the first.

A field trial involving 39 herds, 486 cows, and 509 qualifying quarters was conducted to determine an effective dosage of intramammary infusion of pirlimycin for treatment of clinical mastitis in lactating dairy cattle. A regime of two intramammary infusions (at a 24-hour interval) of 10 mL formulations, each containing 50, 100, or 200 mg pirlimycin, was compared to no treatment in a randomized complete block design. All three drug containing treatments were effective as therapy for clinical mastitis, and there were no significant differences among the three drug concentrations.

A clinical trial including 25 lactating dairy cattle with induced *Staphylococcus aureus* infections in 70 quarters was completed to further evaluate dosages of pirlimycin for treatment of clinical and subclinical mastitis. A regime of two intramammary infusions of 10 mL formulations of pirlimycin at 25, 50, or 100 mg pirlimycin was evaluated relative to a contemporary non-medicated control group. The 50 and 100 mg dose groups were similar and each significantly better than the non-medicated control group, while the 25 mg group was not significantly better than the control group.

Data from the studies are summarized in Table 4.1.

Table 4.1. Percent Cure Rates from Clinical Trials

Study	Pirlimycin Dose Groups (mg per 10 mL.)				
	0	25	50	100	200
Clinical Mastitis Field Trial					
No. Cows	118	--	123	122	123
No. Quarters*	118	--	134	127	130
Overall Cure Rate	28(a)	--	48(b)	46(b)	43(b)
Induced Staph. aureus - Clinical Trial					
No. Cows	4	7	7	7	--
No. Quarters*	11	21	22	16	--
Overall Cure Rate	9(a)	38(a,b)	64(b,c)	69(c)	--

*These clinical cases included staphylococcal and streptococcal isolates which include *Staph. aureus*, *Strep. agalactiae*, *Strep. dysgalactiae*, and *Strep. uberis*, each bacterial pathogens associates with bovine mastitis.

a,b,c Treatment groups within a study not having a common superscript are significantly different at $P < 0.05$.

Based on these data it was concluded that 50 mg pirlimycin. in a 10 mL formulation administered twice. at a 24-hour interval. via intramammary infusion. was effective for therapy of mastitis in lactating dairy cattle.

1. Clinical Mastitis Dose Finding Field Trial

- a. Type of Study: This was a dose determination clinical trial conducted in herds and cows representative of the target population.
- b. Investigators:

Robert Bushnell, D.V.M., M.S.
University of California

Davis, CA 95616 John McDonald, D.V.M., Ph.D.
Washington State University
College of Veterinary Medicine
Pullman, WA 99164-6610

Roger Natzke, Ph.D.
University of Florida
Dairy Science Department
Gainesville, FL 32611

c. General Design:

- (i) Purpose of Study: The purpose of this study was to determine, within limits of this study, an optimal dosage of pirlimycin when administered as two intramammary infusions at a 24-hour interval for the treatment of clinical mastitis in lactating dairy cows.
- (ii) Test Animals: Test animals were representative samples from the population for which this product is intended; that is, lactating dairy cattle of various ages and in varying stages of lactation and each with clinical mastitis. There were 486 cows from 39 herds distributed among treatment groups as described in Table 4.2.

Table 4.2. Number of Cows in each Treatment Group

	Dose of Pirlimycin (mg per 10 mL)			
	0	50	100	200
Number of Cows	118	123	122	123

- (iii) Controls: A non-medicated contemporary control group was included in this randomized complete block design with a control animal assigned at random within a block of four animals with mastitis.
- (iv) Diagnosis: A trained dairy employee monitored each herd for cows with clinical mastitis by the routine use of the strip cup or equivalent methods to detect abnormal milk from the cow's udder quarter(a). A quarter was considered to have signs of clinical mastitis if the quarter milk was visually abnormal. Quarter swelling or heat, pain, and tenderness on palpation may, but did not have to, accompany these changes in quarter milk. A pre-therapy single quarter milk sample was obtained from each of the affected quarter(s) and the milk cultured for the presence of organisms associated with mastitis. A quantitative somatic cell count was also obtained from milk of affected quarters. These data were neither available for nor used for treatment assignments.
- (v) Dosage Form: All drug dosages were aqueous gel formulations provided as 10 mL intramammary infusions.
- (vi) Route of Administration: Drug was administered via intramammary infusion.

- (vii) Dosages Used: The 10 mL formulations contained 50, 100, or 200 mg pirlimycin.
- (viii) Test Duration: Animals were monitored for 11 days after enrollment day and evaluations based on the milking at the end of this interval.
- (ix) Pertinent Parameters Measured:
 - milk status (normal or abnormal)
 - clinical signs
 - bacteriology on last milking of test interval
 - quantitative somatic cell count on last milking of test interval
- (x) Decisions Variables:
 - A qualifying quarter was defined as a cure if, at the end of the study, it had:
 - normal milk, and
 - no bacteria present in last milking,
 - no clinical signs of mastitis.
 - A qualifying cow was deemed a cure only if all qualifying quarters were deemed cures. Any other outcome deemed the cow a failure.

- d. Results: The overall cure rates (percent, Freeman-Tukey transformed) 11 days post-enrollment for lactating cows with clinical mastitis administered pirlimycin by intramammary infusions are described in Table 4.3.

Table 4.3. Percent Cure Rate by Treatment Group

	Pirlimycin Dose Groups (mg per 10 mL.)			
	0	50	100	200
% Cure Rates Transformed	28(a)	48(b)	46(b)	43(b)
Number of Cows	118	123	122	123
Number of Quarters	118	134	127	130

a,b Treatment means without a common superscript are significantly different when tested at $P < 0.05$.

- e. Statistical Analysis:
- (i) Statistical methods used: Analysis of variance procedures were used to analyze these data. Within each herd, a cow cure rate was determined then transformed via the Freeman-Tukey procedure, and an analysis completed using a model as described in Table 4.4.

Table 4.4. Analysis of Variance

Source	DF	Mean Squares	P-Value
Investigator	2	0.4801	--
Herds (Investigator)	36	0.1544	--
Treat	3	0.3489	0.0055
Error	114	0.0788	

- (ii) Comparisons with Control: Each drug treatment mean was significantly different from control, and there were no significant differences among the three drug treatment groups.
- (iii) Level of Significance: A $P < 0.05$ level of significance was used for comparisons among treatment groups.

- f. Conclusions: Based on these results, and consistent with conditions of this study. It was concluded that all three pirlimycin dosage regimens were effective as therapy for clinical mastitis in lactating dairy cattle.
- g. Adverse Reactions: There were no drug related adverse reactions reported from this study.
- h. Special Issues: None.

2. Induced *Staphylococcus aureus* (SA) Infections in Lactating Dairy Cattle

- a. Type of Study: This clinical study was designed to further evaluate pirlimycin doses for the treatment of clinical and subclinical mastitis in lactating dairy cows.
- b. Investigator:

Robert J. Yancey, Jr., Ph.D.
Microbiology and Nutrition R&D
The Upjohn Company
Kalamazoo, MI 49001
- c. General Design: Purpose of Study: The purpose of this study was to further evaluate dosages of pirlimycin for the treatment of clinical and subclinical mastitis in lactating dairy cows when the drug was administered as two 10 mL intramammary infusions at a 24-hour interval.
 - (i) Test Animals: Test animals were representative samples from the target population; that is, lactating dairy cattle of various ages and varying stages of lactation. All were of the Holstein breed.
 - (ii) Allocation to treatment group: There were 25 lactating dairy cows with 70 quarters with induced *Staph. aureus* mastitis infections distributed among treatment groups as described in Table 4.5.

Table 4.5. Number of Cows and Quarters per Treatment Group

	Pirlimycin Dose Groups (mg per 10 mL.)			
	0	25	50	100
Number of Cows	4	7	7	7
Number of Quarters	11	21	22	16

- (iii) Controls: A non-medicated contemporary control group was included with this study. A complete randomized design was used with 4, 7, 7, and 7 cows randomly assigned to control, 25, 50, or 100 mg drug treatments, respectively. Fewer animals (4) were assigned to the control group than to each of the drug treatment groups because of the known low percent of spontaneous recoveries and because there was an emphasis to differentiate among drug treatment groups, rather than to differentiate between a drug group and the control group.
- (iv) Diagnosis: Subsequent to *Staph. aureus* (SA) injections into each quarter of a cow, animals were monitored for 14 days and quarters were qualified as "infected" anytime during this interval based on recovery of SA. Degree (severity) of infection was further classified based on 1) strip cup scores, 2) somatic cell count, and 3) physical condition. All quarters of a cow were assigned to the same treatment group.
- (v) Dosage Form: All drug dosages were aqueous gel formulations, provided as 10 mL intramammary infusions.
- (vi) Route of Administration: Drug was administered via intramammary infusion.
- (vii) Dosages Used: The 10 mL formulations contained 25, 50, or 100 mg pirlimycin.
- (viii) Test Duration: Following injection of *Staph. aureus* into each quarter there was a maximum of 14 days for quarters to qualify as infected. There was a 28-day observation period following the last of the two treatments.
- (ix) Pertinent Parameters: Each of the following was measured at Days 7, 11, 14, 21, and 28 after last treatment.
- presence of *Staph. aureus*
 - milk quality (normal or abnormal)
 - clinical health
- (x) Decision Variables: Two different procedures were used with the most stringent targeted, *a priori*, for dose selection purposes. - One (Evaluation I) provided for cures to be defined as:
- absence of *Staph. aureus* on Day 11 post treatment,

normal milk (via strip cup) at Day 11, and

- The other (Evaluation II) provided for: absence of *Staph. aureus* at each sample time post treatment through 28 days, plus all other criteria listed in I.
- d. Results: The overall cure rates (percent) were lower when the second of the above two decision variable methods was applied as summarized in Table 4.6.

Table 4.6. Percent Cure Rates by Dose Groups

	Pirlimycin Dose Groups (mg per 10 mL.)			
Dose	0	25	50	100
Total Cows	4	7	7	7
Total Quarters	11	21	22	16
Evaluation I	11	18	22	16
Evaluation II	11	21	22	16
Overall Cure Rate				
Evaluation I	18.2(a)	38.9 (a)	72.7(b)	75.0(b)
Evaluation II	9.1(a)	38.1(a,b)	63.6(b,c)	68.7(c)

a,b,c Treatment means within a method not having a common superscript are significantly different at $P < 0.05$.

- e. Statistical Analysis:

A log-linear model was fit for each of the two evaluation methods. Factors accounted for were parity and treatment. Probabilities of a larger Chi-Square for these effects are shown below:

Table 4.7. Log-linear Model (P-Value)

Source	DF	Evaluation I	Evaluation II
Parity	1	0.22	0.43
Treatment	3	<.01	0.01

- (i) Comparisons: In addition, pairwise comparisons were made for each evaluation method with results shown in the summary table above.
- (ii) Level of Significance: $P < 0.05$ was used for first determining whether or not significant treatment effects existed and, if so, $P < 0.05$ was also used for the pairwise contrasts.

- f. Conclusions: Consistent with conditions of this study, it was concluded that 50mg pirlimycin administered twice at a 24-hour interval, via intramammary infusion, was an optimum dosage for the treatment of mastitis in lactating dairy cattle. The 25 mg dosage provided a sub-optimal response, while the 100 mg dosage provided no enhancement, relative to the 50 mg dosage.
- g. Adverse Reactions: None observed during the course of this study.
- h. Special Issues: None.

B. Corroborative Studies (none)

III. TARGET ANIMAL SAFETY

Summary: Two pivotal studies address target animal safety. These studies were nonclinical GLP studies. Individually and collectively these studies indicate that the intramammary formulation of pirlimycin is safe and non-irritating to the lactating dairy cow. The two non-pivotal studies included both efficacy studies previously mentioned. During both studies, no udder irritation was noted due to udder infusion with the product.

A. Pivotal Studies

1. Safety/Toxicity Study

- a. Type of Study: The study was designed as a GLP study to assess mammary irritation in 12 normal lactating dairy cows following two intramammary infusions of pirlimycin (at a 24-hour interval) in each of their 4 quarters. The formulation contained 200 mg (4X) of pirlimycin in an aqueous vehicle. Milk production, strip cup examination of milk, udder palpation, electronic somatic cell counts (ESCC), and body temperatures were the parameters measured before and after treatment.
- b. Investigator: Study Director:

R.J. Yancey, Jr. Ph.D.
The Upjohn Company
Kalamazoo, MI 49001
- c. General Design:
 - (i) Purpose of Study: The purpose of this study was to determine mammary tissue irritation in lactating dairy cows following infusion of an experimental aqueous gel formulation containing pirlimycin hydrochloride (HCI) administered twice at a 24-hour interval.
 - (ii) Test Animals: Twelve (12) Holstein and Guernsey lactating dairy cattle divided equally as 6 mature cows and 6 first calf heifers. The goal was to have half (3) from each age group with daily milk production above 22.7 kg (50 lb; early lactation) and half (3) below 11.4 kg (25 lb; late lactation).

- (iii) Controls: Pretreatment samples served as a within-animal control.
- (iv) Dosage Form: 200 mg of pirlimycin in an aqueous gel vehicle in a 10 mL plastic syringe (Expreset™ plastet).
- (v) Route of Administration: Intramammary (IMM)
- (vi) Dosages Used: IMM infusion of one plastet (200 mg pirlimycin/10 mL) per quarter in all four quarters of each cow twice, at a 24-hour interval.
- (vii) Test Duration: The duration of the study was from 36 hours prior to drug administration to 144 hours after the second dose for a total of 9 days of observation.
- (viii) Pertinent Parameters: Milk weights, strip cup readings, palpation of the udder, rectal temperatures, and electronic somatic cell counts were recorded.

d. Results:

- (i) Milk Production: Total daily milk production was averaged for the 4 pre-treatment milkings and compared to the average total milk produced for the 14 milkings following the first treatment. Posttreatment average milk production ranged from a 3.6% increase to a decrease of 12.9% with an average decrease of 3.8%. Some decrease in production was anticipated due to the late stage of lactation for some of the cows. The pre-treatment values were within the standard deviation of the post-treatment values for 10 of 12 cows.
- (ii) Strip Cup Evaluation/Udder Palpation: Strip cup evaluation of quarter milk showed some transient abnormalities in 5 of 48 quarters pretreatment and in 13 of the 48 total quarters post-treatment. Abnormalities in one of the affected quarters post-treatment were due to mastitis, and slight to severe swelling was also observed in this quarter. Swelling was not observed in the remaining 47 quarters during the trial.
- (iii) Rectal Temperatures: Body temperatures remained normal for all cows during the entire study.
- (iv) Milk Somatic Cell Counts: Two of the 48 quarters treated with pirlimycin had ESCC of $>3 \times 10^6$. One quarter of one cow exhibited elevated ESCC at the time of second treatment, while the other elevated quarter was observed from 60 to 132 hours post-treatment and was mastitis-related.

- e. Statistical Analysis: Statistical methods are not applicable to this study.
- f. Conclusions: Based on the data collected, the experimental aqueous gel formulation containing 200 mg pirlimycin per 10 mL appeared to be nonirritating to the udders of normal lactating cows.

2. Safety/Toxicology Study

- a. Type of Study: The study was designed as a GLP study to assess mammary tissue irritation in normal lactating dairy cows following the intramammary (IMM) infusions of 50 mg of pirlimycin in an aqueous gel formulation per quarter in all 4 quarters twice, at a 24-hour interval.

- b. Investigator: Study Director:

R.A. Rzepkowski
The Upjohn Company
Kalamazoo, MI 49001

- c. General Design:

- (i) Purpose of study: The purpose of this study was to determine mammary tissue irritation in lactating cows following infusion of the final aqueous gel formulation with 50 mg of pirlimycin per 10 mL plastet.
- (ii) Test Animals: Eight first and eight second or greater lactation Holstein cows in varying stages of lactation were used for the study. An attempt was made to split the groups into high and low groups by milk production. The high group were cows producing greater than 22.7 kg. (50 lb) and the low group produced less than 11.4 kg (25 lb).
- (iii) Controls: Pretreatment samples served as a within animal control.
- (iv) Dosage Form: 50 mg of pirlimycin in the final aqueous gel formulation in a 10 mL plastet.
- (v) Route of Administration: intramammary (IMM)
- (vi) Dosages Used: IMM infusion of one plastet (50 mg pirlimycin per 10 mL) per quarter in all four quarters of each cow twice, at a 24-hour interval.
- (vii) Test Duration: Seven days pretreatment, 3 days treatment, 6 days post-treatment.
- (viii) Pertinent Parameters: ESCC, milk production, percent milk fat, body temperature, milk quality of foremilk, physical palpation of the mammary gland, bacteriology, and general health were measured and assessed.

d. Results:

- (i) ESCC: There were no significant group X period interactions. There was a marginal increase in ESCC between pre-treatment (35,100) and treatment (43,600). There was no difference between pre-treatment (35,100) and post-treatment (38,100) periods.
- (ii) Milk Production: There was neither a significant group X period interaction nor a significant difference among the three periods for milk production.
- (iii) Percent Milk Fat: There was no significant group X period interaction. A marginal decrease in percent milk fat was noted between pretreatment and treatment periods. A significant reduction in percent milk fat between treatment and post-treatment periods was also noted. There was no significant difference between production groups for milk fat. All milk fat percentage values were within an acceptable range for Holstein dairy cattle.
- (iv) Body Temperature: There was neither a significant group X period interaction nor significant differences among the three periods.
- (v) Milk Quality of Foremilk: All enrolled quarters were examined during the study period for evidence of abnormal milk. One cow (#46) with clinical mastitis showed abnormal foremilk from the last treatment milking and the next five milkings. One additional cow (#50) had flakes in one quarter but maintained a low ESCC (124 x 103). All other samples were considered normal.
- (vi) Physical Palpation of the Mammary Gland: One cow (#46) had slight to moderate swelling as a result of clinical mastitis. Another cow (#52) had a broken down median suspensory ligament which resulted in some scar tissues. All other cows maintained soft, pliable udders.
- (vii) Bacteriology: Two quarters of two cows were eliminated from the study due to the isolation of a *Staphylococcus aureus* and a *Staphylococcus hyicus*. Three additional quarters yielded bacteria during the study.

- e. Statistical Analysis: All animals received treatment and served as their own controls. A 2 X 2 factorial design was used to remove lactation and production effects. Weighted analysis of variance testing was performed on each period for the four variables.
- f. Conclusions: The variables measured in this study (ESCC, milk production, milk fat percentage, body temperature, milk quality of the foremilk, physical palpation of the mammary gland, bacteriology, and general health) are supportive of the claim that this aqueous gel formulation containing 50 mg pirlimycin per 10 mL plastet syringe is non-irritating to the mammary gland of the normal lactating dairy cow when administered twice at a 24-hour period.

B. Non-Pivotal Studies: During both efficacy studies previously mentioned under IV. A., no udder irritation was noted due to udder infusion with the product.

IV. HUMAN FOOD SAFETY

A. Toxicity Studies 1.

- 1. 13-Week Oral Toxicity Study in Sprague Dawley Rats with U57930E
 - a. Technical Report N°. : 7220-88-043, Study No. J-338 (86-338)
 - b. Starting Date: December 1-3, 1986
 - c. Termination Date: March 3-6. 1987
 - d. Study Investigator: T.A. Jackson
 - e. Study Location:

Hazleton-Wisconsin Laboratories
Madison, WI
 - f. Identification of Test Substance: pirlimycin hydrochloride (U-57,930E)
Dosage Form Tested: solution in purified water
 - g. Species and Strain of Test Animal: rat - Sprague-Dawley Cd:CD® (SD) BR
 - h. Number of Animals/Sex: 5 groups of 20 males and 20 females
 - i. Dose Levels: 0, 10, 30, 100, 300 mg/kg/day
Dose Volume: 10 ml/kg/day
Dose Duration: 13 weeks (91 days)
 - j. Route of Administration: oral
 - k. Parameters Studied: mortality, body weight, clinical observations, hematology, clinical chemistry, urinalysis, organ weight, ophthalmic observations, gross necropsy observations, and microscopic observations.

- i. Significant Toxicities Observed: Pirlimycin has a low overall overt toxicity. Reductions in serum protein and serum globulin levels in male and female rats in the 30 mg/kg group and higher were noted.
 - m. NOEL: 10 mg/kg
 - n. Statistical Analysis: Standard one-way analysis of variance was used to analyze body weight, body weight gain; food consumption; clinical chemistry and hematology; urine pH, volume and specific gravity; organ weight; organ to body weight %; and organ to brain weight ratio.
 - o. Conclusion: Five test groups consisting of 20 Sprague Dawley rats per sex were administered 0, 10, 30, 100, or 300 mg/kg pirlimycin per day by gavage. The NOEL for this study was determined to be 10 mg/kg and was based on reductions in serum protein and serum globulin levels in male and female rats in the 30 mg/kg group and higher.
- 2. U-57,930E: 91-Day Oral Toxicity and Drug Safety Study in the Beagle Dog
 - a. Technical Report N°. : 7220-89-006, Study No., 87-037
 - b. Starting Date: September 14, 1987
 - c. Termination Date: December 15-16, 1987
 - d. Study Director: T.A. Jackson
 - e. Study Location:

The Upjohn Co.
Drug Safety Research
Kalamazoo, MI 49001
 - f. Identification of Test Substance: pirlimycin hydrochloride (U-57,930E)
 - g. Dosage Form Tested: drug in gelatin capsules
 - h. Species and Strain of Test Animal: Beagle dog
 - i. Number Animals/Sex: 5 groups of 5 males and 5 females
 - j. Dose Levels: 0 (empty capsule), 4, 16, 44), and 160 mg/kg
Dose: 1 capsule/dog/daily
Dose Duration: 92 days males, 93 days females
 - k. Route of Administration: oral
 - l. Parameters Studied: mortality, body weight change, clinical observations, hematology, clinical chemistry, urinalysis, organ weight, ophthalmic observation, gross necropsy observation, and microscopic observations.
 - m. Significant Toxicities Observed: Toxicity was observed in the 40 and 160mg/kg groups and included salivation, vomiting, gastric irritation and

elevations in aspartate aminotransferase and alanine aminotransferase levels.

- n. NOEL: 16 mg/kg
- o. Statistical Analysis: Treatment group differences were analyzed using analysis of variance on raw data in conjunction with analysis of variance on ranks of data.
- p. Conclusion: Five test groups consisting of five Beagle dogs per sex were administered 0, 4, 16, 40 and 160 mg pirlimycin/kg/ day via capsule. Toxicity was observed in the 40 and 160 mg/kg groups and included salivation, vomiting, gastric irritation and elevations in aspartate aminotransferase and alanine aminotransferase levels. The NOEL was determined to be 16 mg/kg.

3. A Segment II Teratology Study (Oral) of U-57,930E in Rats

- a. Technical Report N°. : 7227-88-129, Study No. 87-426
- b. Starting Date: November 23, 1987
- c. Termination Date: December 10, 1987
- d. Study Director: T.A. Marks
- e. Study Location:

The Upjohn Co.
Drug Safety Research
Kalamazoo, MI 49001
- f. Identification of Test Substance: pirlimycin hydrochloride (U-57,930E)
- g. Species/Strain of Test Animal: rat - Upj:TUC (SD) spl.
- h. Number Animals/Sex/group: 24 females/group
- i. Dose Level: 0 (vehicle), 200, 400, 800 mg/kg/day
Dose Vehicle: solution in purified water
Dose Duration: daily treatment; Days 6 to 15 of gestation
- j. Route of Administration: oral
- k. Parameters Studies: Maternal effects, mortality, weight gain, clinical observation, % conception, mean corpora lutea/litter, mean resorption/litter, dams with resorption, mean implantation rate, mean live/litter, sex ratio (M:F), mean dead/litter, and fetal development/malformation.
- l. Significant Toxicities Observed: There was no indication of teratogenic effects even at the highest level of 800 mg/kg but both 400 and 800 mg levels were toxic to the dam (soft stool. urogenital staining. post dosing salivation).

- m. NOEL for Teratogenic Effects: 800 mg/kg
 - n. Statistical Analysis: Exact One Tailed Test, Modified Jonckheere Ordered Alternatives Test and Jack Knife methodology
 - o. Conclusion: Four test groups of mated Sprague Dawley females (Day 1 post insemination) were administered 0, 200, 400 or 800 mg/kg pirlimycin per day by gavage on days 6 through 15 of gestation. Cesarean section was performed on day 20. There was no indication of teratology even at the highest level of 800 mg/kg but both the 400 and 800 mg/kg levels were toxic to the dam (soft stools, urogenital staining, post dosing salivation). The NOEL for terata is 800 mg/kg. The NOEL for maternal toxicity is 200 mg/kg.
4. A Two-Generation Reproduction Study (Oral) in Rats given U-57,930E
- a. Technical Report No.: 7227-88-010, Study No. J-285 (86-285)
 - b. Starting Date: August 25, 1986
 - c. Termination Date: April 20, 1987
 - d. Study Director: T.A. Marks
 - e. Study Location:

The Upjohn Co.
Drug Safety Research
Kalamazoo, MI 49001
 - f. Identification of Test Substance: pirlimycin hydrochloride (U-57,930E)
 - g. Species/Strain of Test Animal: rat- TUC (SD)apr
 - h. Number Animals/Sex/Group: 30 males. 30 females/group
 - i. Dose Levels: 0 (vehicle). 100, 200. and 400 mg/kg/day
Dose Vehicle: purified water
Dose Duration:

F0 male- 60 days pre-breeding and during mating
F0 female- 14 days pre-breeding; through mating, gestation to weaning (21 days post-gestation)
F1 male- day last litter weaned until end of 14-day breeding period (sacrifice)
F1 female - day last litter weaned until Day 21 postpartum
 - j. Route of Administration: oral
 - k. Parameters Studied: Maternal effects: mortality, mean study weight gain, clinical observation, fertility index, mean gestation length; paternal effects: mortality. mean weight gain. clinical observations; reproduction data: mean post-implant loss/pregnant dam, mean implants/pregnancy, mean

live/litter, mean dead/litter, mean pup weight Day 0, sex ratio (male/female), gross pup observations; offspring data: skeletal observation, mean live/litter, mean pup weight; F1 offspring data Days 1, 4, 14, and 21; F1 maternal effects; F1 paternal effects; F1 reproductive data; F2 offspring data Day 1, 4, 14, and 21.

- l. Significant Toxicity Observed: Toxic effects in the F0 parenteral animals included decreased body weight gains for males given 400 mg/kg, salivation in all dose groups and staining of the rear quarters in females given 200 mg/kg and in both males and females given 400 mg/kg. Nasal discharge, struggling during dosing and diarrhea also occurred in the 400 mg/kg group. In the F1 parenteral animals given 400 mg/kg, both sexes exhibited nasal discharge and salivation; reduced body weight gain occurred only in males and staining of the rear quarters was present only in females. The fertility index was reduced for both F1 males and females in the 200 and 400 mg/kg dose groups.
 - m. NOEL: 100 mg/kg
 - n. Statistical Analysis: Analyzed variance, modified Jonckheere Ordered Alternative Test and Jack-knife methodology
 - o. Conclusion: Four test groups consisting of 30 Upj:TUC (SD) spf rats per sex were exposed to 0, 100, 200, or 400 mg/kg/day pirlimycin in purified water by gavage. Only one litter per generation was produced. Toxic effects in the F0 parenteral animals included salivation in all groups, staining of the rear quarters in females of the mid-dose group, and salivation, staining, nasal discharge, struggling during dosing and diarrhea in the high dose group. Males in the high dose group also had reduced body weight gain. Decreased body weight gain, nasal discharge, salivation, and staining occurred only in the 400 mg/kg group of the F1 generation. The fertility index was reduced for both F1 males and females in the mid and high-dose groups. The NOEL for this study was determined to be 100 mg/kg.
- 5. *U-57,930E: Evaluation of the in vitro* Unscheduled DNA Synthesis (UDS) Assay in Rat Primary Hepatocytes
 - a. Technical Report N°. : 7227-89-024, Study No. 88-395
 - b. Starting Date: November 3, 1988
 - c. Termination Date: November 11, 1988
 - d. Study Director: C.S. Aaron
 - e. Study Location:

The Upjohn Co.
Drug Safety Research
Kalamazoo, MI 49001

- f. Identification of Test Substance: pirlimycin hydrochloride (U-57,930E)
 - g. Species/Strain: Rat Primary Hepatocytes--Fischer 344
 - h. Number Animals/Sex: n/a; cell culture *in vitro* system
 - i. Dose Levels: (1st) 1, 3, 10, 30, 100, 300 mcg/mL
(2nd) 3, 10, 30, 100, and 300 mcg/mL
Dose Form: pirlimycin was placed in cell culture medium
Dose Duration: 18 to 20-hour exposure
 - j. Route of Administration: *In vitro* test system
 - k. Parameters Tested: The *in vitro* hepatocyte DNA repair assay is used to measure the ability of pirlimycin to induce unscheduled DNA synthesis as an indicator of genotoxicity after the cell culture is exposed to a single dose of the test article. Primary hepatic cell cultures are obtained and incubated with labeled thymidine, cultured and then fixed and stained for evaluation. A test article is considered positive if the mean net grain count is greater than 0 for the group, and if the percentage of cells in repair are greater than 20%. A minimum of three slides were scored per animal.
 - l. Significant Results: The positive and negative controls acted as expected. Doses of 1000 and 3000 mcg/mL were toxic and the slides were unscorable. All net grains/nucleus (NG) counts for cultures treated with pirlimycin were less than zero, and, therefore, met our criteria for a negative response.
 - m. NOEL: Not applicable
 - n. Statistical Analysis: Effect: Net grain count; Dose Response: Regression Analysis and Modified Jonckheere Test; Mean Analysis: Dunnett's t-test.
 - o. Conclusion: Pirlimycin was negative under the conditions of the *in vitro* rat hepatocyte DNA repair assay.
6. *Evaluation of U-57,930E in the Salmonella/microsome Test (Ames Assay)*
- a. Technical Report N°. : 7227-89-030, Study No. 88-402
 - b. Starting Date: November 3, 1988
 - c. Termination Date: November 16, 1988
 - d. Study Director: C.S. Aaron
 - e. Study Location:

The Upjohn Co.
Drug Safety Research
Kalamazoo, MI 49001

- f. Identification of Test Substance: pirlimycin hydrochloride (U-57,930E)
 - g. Test System: *Salmonella typhimurium*/microsome test (Ames Assay)
 - h. Strains: TA97, TA98, TAI00, TAI02, TA1535
 - i. Dose Levels Tested: 625, 1250, 2500, 5000 mcg/plate
Negative Control: dimethylsulfoxide (DMSO)
Positive Controls with Activation (S-9): 2-aminoanthracene (all strains)
Positive Controls without Activation: Dexon (TA97), 2-nitrofluorene TA09, TA100, Cumene hydroperoxide (TA102), sodium azide (TA1535)
 - j. Route of Administration: *In vitro* test system
 - k. Parameters Tested: The procedures for the Ames assay are the standard ones developed in the laboratory of Dr. B.N. Ames. Briefly, histidine auxotrophs of *Salmonella typhimurium* are mixed with the test compound in 0.1 mL dimethylsulfoxide, the 9,000 X g supernatant of liver homogenates (or saline) in molten (45 °C) agar. The molten agar mix is poured onto a Petri plate containing a histidine-deficient base agar. Revertants to histidine prototrophy are scored as colonies, after incubation at 37°C for 2 days. Triplicate plates are used for each dose level and the experiment is repeated. Vehicle controls are run in triplicate for each strain in each experiment and reported as an average of the three values.
 - l. Significant Results: No evidence of toxicity/solubility limitations were at 5000 mcg/plate. The data show no evidence of bacterial mutagenicity at any dose, whether or not exogenous *in vitro* liver homogenates (S-9) was used to supply metabolic activation.
 - m. NOEL: Not applicable
 - n. Statistical Analysis: Not applicable
 - o. Conclusion: There was no evidence of mutagenicity under the conditions of this test.
7. Evaluation of U-57,930E in the AS52/Xprt and CHO/HPRT Mammalian Cell Forward Mutation Assay
- a. Technical Report No.: 7228-89-023, Study No. 88-393
 - b. Starting Date: November 14, 1988
 - c. Completion Date: April 26, 1989
 - d. Study Investigator: C.S. Aaron
 - e. Study Location:

Pharmacon Research International
Waverly, PA

- f. ID of Test Substance: pirlimycin hydrochloride (U-57,930E)
 - g. Test System: CHO/HPRT Mammalian cell line
AS52/XPRT Genetically engineered cell line from Chinese hamster carrier
 - h. Strains: CHO, AS52
 - i. Dose Levels Tested: With S-9: 50, 100, 250, 500, 1000, 1500, 2000, and 2500 mcg/mL; doses $\geq$ 1500 mcg/mL (AS52) and $\geq$ 2000 mcg/mL (CHO) were too toxic to evaluate.

Positive Control: dimethylnitrosamine (100 mcg/mL)
Negative Control: untreated cultures and solvent [dimethylsulfoxide (DMSO)] controls

Without S-9: 50, 100, 250, 500, 750, 1000, 1250, and 1500 mcg/mL; doses $\geq$ 1000 mcg/mL (AS52) and $\geq$ 1250 mcg/mL (CHO) were too toxic to evaluate.

Positive Control: ethyl methanesulfonate (200 mcg/mL) Negative Control: untreated cultures and solvent (DMSO) controls
 - j. Route of Administration: *In vitro* test system
 - k. Parameters Tested: Pirlimycin was evaluated in the AS52/XPRT and CHO-HPRT Mammalian Cell Forward Gene Mutation Assays to determine its ability to induce mutations at the xanthine-guanine phosphoribosyl transferase (XPRT) locus in cultured AS52 Chinese Hamster Ovary (CHO) cells and at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in CHO-KI-BH4 cells.
 - l. Significant Results: Doses $\geq$ 1500 mcg/mL (AS52) and $\geq$ 2000 mcg/mL (CHO) were too toxic to evaluate with S-9. Doses $\geq$ 1000 mcg/mL (AS52) and $\geq$ 1250 mcg/mL (CHO) were too toxic to evaluate without S-9. Pirlimycin failed to induce mutation at the XPRT locus of the AS52 cells or at the HPRT locus of the CHO cells both in the presence and absence of S-9 metabolic activation.
 - m. NOEL: Not applicable.
 - n. Statistical Analysis: Least-squares methods of linear regression
 - o. Conclusion: Pirlimycin did not show evidence of statistically significant or dose dependent induction of mutations in either the AS52 or standard CHO cell line.
8. Evaluation of Pirlimycin (U-57,930E) in the Drosophila Sex-Linked Recessive Lethal Assay
- a. Technical Report N°.: 7228-89-054; Study No. 88-394
 - b. Starting Date: January 25, 1989

- c. Termination Date: September 15, 1989
 - d. Study Investigator: C.S. Aaron
 - e. Study Location:

Department of Zoology
University of Wisconsin
Madison, WI
 - f. Identification of Test Substance: pirlimycin hydrochloride (U-57,930E)
 - g. Species/Strain: *Drosophila melanogaster* (Canton-S. wild type)
 - h. Dose Level Tested: Negative control (vehicle), positive control Dimethylnitrosamine (DMN; 25 ppm), and 1000 ppm and 7500 ppm pirlimycin.
 - i. Dosage Vehicle: Aqueous solution containing 5% sucrose.
 - j. Dose Volume: 1.0 mL feeding solution/vial
 - k. Parameters Tested: This study evaluated the ability of pirlimycin to induce recessive lethal mutations in the post-meiotic germ cells of adult *Drosophila melanogaster* males treated by feeding. Mutation-induced mortality was measured.
 - l. Significant Results: The mutation frequency in the offspring of males treated with pirlimycin was not enhanced (26 lethals in 12,501 tested chromosomes at 7500 ppm; 22 lethals in 11,634 treated chromosomes at 1000 ppm; versus 23 lethals in 10,795 negative control chromosomes).
 - m. NOEL: Not applicable
 - n. Statistical Analysis: Margolin, Normal Test, and Poisson Distribution
 - o. Conclusion: Pirlimycin is not a germ line mutagen under the conditions of this assay.
9. Evaluation of U-57,930E in the Micronucleus Test in Mouse Bone Marrow
- a. Technical Report N°.: 7227/89/077
 - b. Starting Date: January 17. 1989
 - c. Completion Date: February 16, 1989
 - d. Study Investigator: C.S.Aron
 - e. Study Location:

Pharmacon Research International
Waverly, PA

- f. Identification of Test Substance: pirlimycin hydrochloride U-57,930E)
- g. Test System: micronucleus test in mouse bone marrow
- h. Species/Strain of Test Animal: CDI Mouse
- i. Number of Animals/Sex/Group: 5 males and 5 females/group
- j. Dose Levels: 0, 175, 250, and 375 mg/kg
- k. Dose Vehicle: distilled water
- l. Dose Duration: single dose
- m. Negative Control: distilled water
- n. Positive Control: triethylenemelamine (TEM. 0.5 mg/kg)
- o. Route of Administration: intraperitoneal
- p. Parameters Tested: Pirlimycin was evaluated in the *in vivo* micronucleus assay to determine its ability to induce the formation of micronucleated polychromatic erythrocytes in mouse bone marrow.
- q. Significant Toxicity Observed: There were no statistically significant increases in micronuclei in polychromatic erythrocytes (MNPCE) at any dose in either sex due to the treatment with pirlimycin.
- r. Treated
 - 175 mg/kg 0.90 ± 1.60 MNPCE/1000 PCE
 - 250 mg/kg 0.70 ± 0.68 MNPCE/1000 PCE
 - 375 mg/kg 1.00 ± 1.05 MNPCE/1000 PCE
- s. Negative Control - 0.90 ± 0.74 MNPCE/1000 PCE
- t. Positive Control - 33.50 ± 13.54 MNPCE/1000 PCE
- u. Statistical Analysis: One-tailed t-test
- v. Conclusion: Pirlimycin is judged to be non-mutagenetic under the conditions of this assay.

B. No Observable Effect Levels and the Safe Concentration Determination for Pirlimycin HCl

The Safe Concentration of total residue was determined from the lowest No Observable Effect Level (NOEL) in the most sensitive species from the various toxicology studies conducted. A summary of these studies follows:

Table 6.1. Summary of these studies for Pirlimycin HCl

Study Type	Species	Doses (mg/kg/day)	NOEL (mg/kg/day)
90-day oral	Rat	0,10, 30, 100, 300	10
90-day oral	Dog	0, 4, 16, 40, 160	16
Two-generation oral reproduction	Rat	0, 100, 200, 400	100
Segment II teratology Study (oral)	Rat	0, 200, 400, 800	200

Therefore, the lowest No Observed Effect Level (rat) = 10 mg/kg/day. The calculation for Safe Concentrations (SC) for tissues and milk is:

$$SC = \frac{\text{Allowable Daily Intake} \times \text{Human Weight}}{\text{Daily Consumption of Meat}}$$

where Human Weight = 60 kg and Daily Meat consumption is 500 grams,
ADI = lowest NOEL/Safety Factor

where SF = 1,000 because 90-day data are used for the NOEL determinations,
therefore, ADI = (10 mg/kg)/1,000 or 0.01 mg/kg/day and

$$SC = \frac{(0.01 \text{ mg/kg/day}) \times 60 \text{ kg}}{500 \text{ g/day}} = 1.2 \text{ mg/kg or } 1.2 \text{ ppm}$$

Table 6.2. Food Consumption Factors

Tissue	Consumption Factors	Calculated Safe Concentrations for Pirlimycin (ppm)
Muscle	1X	1.2
Fat	4X	4.8
Liver	2X	2.4
Kidney	3X	3.6
Milk	0.33X	0.4

C. This compound is a Category A compound derived from the Threshold Assessment considerations.

Based on structural activity assessment, it was assigned Category C (non-carcinogen). Subsequent to this, the 90-day feeding studies and genotox data allowed it to be classified as Category A. Because of the therapeutic use on specific animals, it is considered a low use drug. Chronic studies were not required, and based on 90-day studies, a Safety Factor of 1000 is used in the Safe Concentration (SC) calculations.

D. Total Residue Depletion and Metabolism Studies

Two total residue and metabolism studies were conducted in lactating dairy cattle, and one metabolism study was conducted in Sprague-Dawley rats. The studies and the conclusions drawn from them are summarized below.

1. Lactating Dairy Cow Total Residue and Metabolism Study 1

a. Principal Investigators:

R.E. Hornish
The Upjohn Co., Kalamazoo, MI T.S. Arnold
The Upjohn Co., Kalamazoo, MI

b. Animal Species: bovine

Breed/Sex: Holstein cows, lactating

Number of Animals: 12; 3 sacrificed at each of 4 time points post-treatment

Stage of Lactation: 2nd or 3rd lactation, in mid-cycle

Health Status: healthy, mastitis free

c. Route of Dose Administration: intramammary

d. Dose Rate: 200 mg/quarter x all 4 quarters (4X label)

e. Duration of dosing: 2 doses/quarter at 24 hour interval

f. Radioisotope: ^{14}C located as shown in the structure (Appendix I).

g. Total Pirlimycin Related Residue Depletion Data

Samples of liver, kidney, muscle and fat from each animal were radioassayed by combustion of the sample, trapping the resulting $^{14}\text{C}^{18}\text{O}_2$, addition of scintillant, and liquid scintillation counting (LSC). Milk samples were radioassayed by dilution with scintillant and LSC. The mean total radioactivity per sampling time, expressed as parts per million (ppm) pirlimycin, is shown in tables Table 6.3 and 6.4.

Table 6.3. Total Radioactivity in Tissues of Cows Receiving Two 200mg/quarter Doses [in ppm pirlimycin] ($\pm$ S.D.)(1)

Tissue	Withdrawal Time			
	4 days	6 days	14 days	28 days
Liver	9.18 $\pm$ 1.37	7.13 $\pm$ 1.28	3.57 $\pm$ 0.39	0.50 $\pm$ 0.37
Kidney	1.964 $\pm$ 0.71	0.78 $\pm$ 0.17	0.26 $\pm$ 0.05	0.01 $\pm$ 0.008
Muscle	0.10 $\pm$ 0.036	0.05 $\pm$ 0.01	0.02 $\pm$ 0.005	<0.01
Fat	0.22 $\pm$ 0.22	0.03 $\pm$ 0.008	0.01 $\pm$ 0.005	<0.01

(1) Average of 3 animals per time point. Animals were dosed at a rate 4 times label dose.

Table 6.4. Total Radioactivity in Milk of Cows Receiving Two 200mg/quarter Doses [in ppm pirlimycin] ($\pm$ S.D.)(1)

Milking Time	Total Residue
dose 1 + 12 hours	50.6 $\pm$ 15.3
dose 1 + 24 hours	5.28 $\pm$ 4.45
dose 2 + 12 hours	44.0 $\pm$ 17.6
" 24 hours	5.14 $\pm$ 4.39
" 36 hours	1.53 $\pm$ 1.72
" 48 hours	0.79 $\pm$ 1.09
" 60 hours	0.39 $\pm$ 0.35
" 72 hours	0.23 $\pm$ 0.13
" 84 hours	0.17 $\pm$ 0.08
" 96 hours	0.14 $\pm$ 0.06

(1) Average of 12 animals per time point. Animals were dosed at a rate 4 times label dose.

- h. Metabolite Profiles: Tissues, milk and excreta were extracted, the pirlimycin-related residues were fractionated via high performance liquid chromatography (HPLC). and the relative amounts of metabolites were quantified by liquid scintillation counting. Data collected from the 12 dairy cows used in Study 1 are shown in Table 6.5.

Table 6.5. Target Animal Metabolism Summary

		Residue/Metabolite Composition		
Sample	Dose Disposition	Pirlimycin	Sulfoxide(1)	Other(2)
MILK	50%	>95%	<5%	-
Urine	10%	80%	8%	12% (3)
Feces(2)	24%	45%	2%	53%
Liver: average of Days 4 to 28 of withdrawal		~22%	~76%	-

(1) Biological Activity is <1% of pirlimycin

(2) Fecal metabolites identified as adenosine adducts of both pirlimycin and pirlimycin sulfoxide likely produced by GI tract micro flora.

(3) Probably transferred from feces to urine since urine and feces were partially commingled before collection.

2. Lactating Dairy Cow Total Residue and Metabolism Study 2

a. Principal Investigators:

R.E. Hornish
The Upjohn Co., Kalamazoo, MI T.S. Arnold
The Upjohn Co., Kalamazoo, MI.

b. Animal Species: bovine

Breed/Sex: Holstein cows, lactating
Number of Animals: 23; 5 sacrificed at each of 3 time points
and 8 sacrificed at one time point post-treatment
Stage of lactation: 1st through 4th lactation, in mid-cycle
Health Status: healthy, mastitis free

c. Route of Dose Administration: intramammary

d. Dose Rate: 50 mg/quarter x all 4 quarters (1X label)

e. Duration of dosing: 2 doses/quarter at a 24-hour interval

f. Radioisotope: ^{14}C located as shown in the structure (Appendix I).

g. Total Pirlimycin Related Residue Depletion Data: Samples were assayed for total residues as described above. The results follow in Table 6.6 and Table 6.7.

Table 6.6. Total Radioactivity in Tissues of Cows Receiving Two 50mg/quarter Doses [in ppm pirlimycin] (+S.D.)(1)

Tissue	Withdrawal Time			
	4 days	6 days	14 days	28 days
No. Cows	(5)	(5)	(8)	(5)
Liver	2.18±1.08	1.89±1.10	0.99±0.51	0.89±0.65
Kidney	0.30±0.19	0.15±0.07	0.06±0.03	0.04±0.02
Muscle	0.02±0.01	0.01±0.004	<0.01	<0.01
Fat	0.01±0.01	0.01±0.004	<0.01	<0.01

1 Average of n animals per time point. Animals were dosed at the label rate of 50 mg/quarter, all 4 quarters treated.

Table 6.7. Total Radioactivity in Milk of Cows Receiving Two 50mg/quarter Doses [in ppm pirlimycin] ($\pm$ S.D.)

Milking Time	Total Residue
dose 1 + 12 hours	19.5 $\pm$ 17.3
dose 1 + 24 hours	2.67 $\pm$ 4.23
dose 2 + 12 hours	18.4 $\pm$ 18.3
" 24 hours	2.03 $\pm$ 1.99
" 36 hours	0.42 $\pm$ 0.40
" 48 hours	0.17 $\pm$ 0.15
" 60 hours	0.11 $\pm$ 0.08
" 72 hours	0.08 $\pm$ 0.05
" 84 hours	0.06 $\pm$ 0.03
" 96 hours	0.05 $\pm$ 0.02

- h. Metabolite Profiles: Liver and milk were extracted. the pirlimycinrelated residues were fractionated via high performance liquid chromatography (HPLC), and the relative mounts of metabolites were quantified by liquid scintillation counting. Data collected from the 23 dairy cows used in study 2 are shown below in Table 6.8.

Table 6.8. Target Animal Metabolism Summary

Sample	Dose Disposition	Residue/Metabolite Composition		
		Pirlimycin	Sulfoxide(1)	Other(2)
MILK	50.7%	>92%	ND	ND
Urine	12.7%	ND	ND	ND
Feces(2)	27.6%	ND	ND	ND
Liver: average of Days 6 to 18 of withdrawal		24.5%	61.8%	9.8% (2)

(1) Biological Activity is <1% of pirlimycin

(2) Tentatively identified as pirlimycin surfone.

ND = not determined

3. Test Animal Study Rat Comparative Metabolism Study

a. Principal Investigator:

R.E. Hornish
The Upjohn Co., Kalamazoo, MI

- b. Species: rat
Strain: Sprague-Dawley
Number: 12
Sex: 6 M and 6 F
Age: mature adults, 8 weeks
- c. Dose Route: Oral, by gavage
- d. Dose Level: 30 mg/kg/day (Lowest Observable Effect Level)
- e. Dose Duration and Interval: 5 days, 24-hour intervals
- f. Sacrifice interval: 2 to 4 hours post-5th-dose
- g. Radioisotope: ¹⁴C located as shown in the structure (Appendix I).
- h. Rat liver and excreta were extracted, the pirlimycin-related residues were fractionated via high performance liquid chromatography (HPLC), and the relative amounts of metabolites were quantified by liquid scintillation counting. The data showed that the rats formed the pirlimycin sulfoxide metabolite. Because parent pirlimycin and the sulfoxide account for at least 90% of the metabolites observed in cattle milk and tissues, the Sprague Dawley rat is a suitable test animal upon which to base the safe concentration for pirlimycin residues. These results satisfactorily addressed the comparative metabolism requirement.

4. Tolerance for the marker residue

- a. MILK As noted in section B, the safe concentration for total pirlimycin residues in milk is 0.4 ppm. The metabolism data showed that parent pirlimycin constitutes virtually all of the pirlimycin-related residue found in the milk of cows dosed by the intramammary route. Pirlimycin constituted 95% of the total residue in milk in study 1 and 92% of the total residue in study 2. Therefore, pirlimycin was established as the marker residue in milk, and the tolerance (R_m) for pirlimycin in milk was set at 0.4 ppm.
- b. TISSUE

The total residue data shown above established that liver is the tissue from which pirlimycin residues deplete most slowly to the safe concentration. The metabolism data confirmed that liver would be the target tissue because there were two pirlimycin-related substances present in liver at measurable levels at the time total residues deplete to the safe concentration. Those two substances are parent pirlimycin and pirlimycin sulfoxide. Either pirlimycin or the sulfoxide would be an acceptable marker residue, as each has a well-defined relationship to total residue over the time frame of interest. However, as noted above, parent pirlimycin is the marker residue in milk, so parent pirlimycin was also chosen as the marker residue in liver. Having the same marker residue in tissue and milk simplifies the process of monitoring for residues of the drug. The livers from cows used in the total residue studies were assayed for parent pirlimycin using HPLC/MS determinative method, which is described below. When the total residue in liver was in the range of the safe concentration

(2.4 ppm), the concentration of pirlimycin averaged approximately 21% of the total residue concentration. Therefore, a tolerance (Rm) of 0.5 ppm was established for the marker residue, parent pirlimycin, in cattle liver.

5. *Study establishing the Milk Discard Period in the Lactating Dairy Cow* The following study addressed the milk discard time in terms of when the marker residue fell below the milk Rm of 0.4 ppm, with considerations given to confidence intervals about the actual residue concentrations as a function of time.

a. Principal Investigators

S.T. Chester

The Upjohn Co, Kalamazoo, MI F.S. Yein

The Upjohn Co, Kalamazoo, MI

b. Animal Species: bovine

Strain/Breed: Holstein

Sex: female, lactating

No. of Animals: 26, from 8 herds

Stage of lactation: 1st or 2nd lactation, in mid-cycle

Weight: 440 to 730 Kg

Health Status: one or more quarters mastitic

c. Route of Dose Administration: intramammary

d. Dose Rate: 50 mg/quarter x all 4 quarters

e. Duration of dosing: 2 doses/quarter at a 24-hour interval

f. Pirlimycin Residue Depletion Data in MILK

Table 6.8. Pirlimycin Residue Depletion Data in MILK

Milk Collection (Hours(1))	Concentration PPM	
	Mean	Standard Deviation
12	8.41	9.04
24	0.88	0.80
36	0.23	0.18
48	0.11	0.06
60	0.07	0.04
72	0.05	0.03
84	0.03	0.02
96	0.02	0.02

(1) Time of collection post-last-treatment

g. Calculation of the milk discard period

The residue depletion data were analyzed by a statistical method which determines the statistical tolerance limit for the 99th percentile of the population with 95% confidence as outlined in the Agency's *Guideline for*

Establishing a Withdrawal Period. The milk discard period for pirlimycin in the dairy cow was determined to be 36 hours.

6. *Studies establishing the Pre-Slaughter Withdrawal Period in the Lactating Dairy Cow* The following studies addressed the pre-slaughter withholding time in terms of when the marker residue fell below the Rm of 0.50 ppm parent pirlimycin in liver, with considerations given to confidence intervals about the actual residue concentrations as a function of time.

a. Marker Residue Depletion Study I

(i) Principal Investigators

R.E. Hornish
The Upjohn Co., Kalamazoo, MI S.T. Chester
The Upjohn Co., Kalamazoo, MI

(ii) Animal Species: bovine (Dairy Cow)

Strain/Breed: Holstein

Sex: female, lactating

Number of Animals: 31

Stage of lactation: 1st to 4th lactation, in mid-cycle Weight: 438 to 865 Kg

Health Status: healthy, mastitis free

(iii) Route of Dose Administration: intramammary

(iv) Dose Rate: 50 mg/quarter in all 4 quarters

(v) Duration of dosing: 2 doses/quarter at a 24-hour interval

(vi) Pirlimycin Residue Depletion Data in LIVER: Residues of pirlimycin were measured using the determinative method described in Section 7.

Table 6.9. Mean Pirlimycin Residue in ppm in LIVER

Sacrifice Interval (days)	N(1)	Mean	Standard Deviation
2	5	2.33	1.00
6	5	1.07	0.36
10	8	0.60	0.31
14	8	0.53	0.28
21	5	0.43	0.13

(1) Total cows per group.

b. Marker Residue Depletion Study 2

(i) Principal Investigators:

R.E. Hornish
The Upjohn Co., Kalamazoo, MI S.T. Chester
The Upjohn Co, Kalamazoo, MI

- (ii) Animal Species: bovine (Dairy Cow)
Strain/Breed: Holstein
Sex: female, lactating
Number of Animals: 33, from 2 herds
Stage of lactation: 1st to-4th lactation, in mid-cycle
Weight: 420 to 765 Kg
Health Status: healthy, mastitis free
- (iii) Route of Dose Administration: intramammary
- (iv) Dose Rate: 50 mg/quarter in all 4 quarters
- (v) Duration of dosing: 2 doses/quarter at a 24-hour interval
- (vi) Pirlimycin Residue Depletion Data in LIVER: Residues of pirlimycin were measured using the determinative method described in Section 7.

Table 6.9. Mean Pirlimycin Residue in ppm in LIVER

Sacrifice Interval (days)	N(1)	Mean	Standard Deviation(2)	N(2)
7	5	0.49	0.15	5
14	5	0.07	0.03	5
21	11	0.04	0.01	5
30	12	0.06	0.03	8

(1) Total cows per group.

(2) Only values > or = 0.03 ppm were included in the mean and standard deviation calculations. All other values were <0.03 ppm.

- (vii) Calculation of the pre-slaughter withdrawal period.

The residue depletion profiles for these two marker residue depletion studies are different, with the second study showing a faster depletion rate. Possible causes for this difference were considered. The mean dose per unit body weight was essentially the same in both studies, so that was not likely a factor. Average milk production was higher in the second study (about 60 pounds/day vs. 42 pounds/day), and it is possible that contributed to the higher depletion rate seen in that study. In addition, the cows in the second study were treated on site in their home dairy environment, without the stress of transportation and acclimation to a new environment (such as the Upjohn experimental farm, which was the site of the first study).

However, based on the criteria stated in CVM guidelines, the data from both studies appear to be valid for use in calculating a withdrawal time. That is, the studies were done under field conditions (although different types of field conditions) in the target population, the proposed label dose was used, and residues were measured using the regulatory determinative method. It was concluded that the data from the two studies represent the range of residues that may result from post-approval use of the drug.

Therefore, the results of the studies were combined to calculate a withdrawal time. The data were analyzed using an algorithm for censored regression (PROC LIFEREG, available from SAS, Inc.), which allows consideration of data below the limit of quantitation of the method, and the CVM guideline parameters of 99th percentile and 95% confidence. Based on this analysis, the withdrawal time following use of pirlimycin was determined to be 28 days.

7. Regulatory Methods

- a. Milk Simultaneous Determinative and Confirmatory Method Type:
HPLC/Thermospray/Mass Spectrometer

Sample Work-Up: Organic Solvent Partition/Solid Phase

Extraction/Purification

Qualitative Diagnosis: Selective Ion Monitoring mass spectrometry detection of four ions--m/z 158, 375, 411, 413--at the appropriate retention time with a minimum intensity (S:N) of 3:1

LOC = 0.20 ppm

(LOC = Limit of Confirmation)

Quantitative Measurement: Based on m/z 411 (MH⁺) ion, using an internal standard

LOQ = 0.20 ppm

(LOQ = Limit of Quantitation)

The LOC and LOQ will vary depending on the specific instrumentation used.

- b. Liver Simultaneous Determinative and Confirmatory Method

Type: HPLC/Thermospray/Mass Spectrometer

Sample Work-Up: Organic Solvent Extraction/Solvent-Solvent
Extraction/Purification

Qualitative Diagnosis: Selective Ion Monitoring mass spectrometry detection of four ions---m/z 158, 375, 411, 413--at the appropriate retention time with a minimum intensity (S:N) of 3:1

LOC = 0.25 ppm

(LOC = Limit of Confirmation)

Quantitative Measurement: Based on m/z 411 (MH(+)) ion, using an internal standard

LOQ = 0.05 ppm

(LOQ = Limit of Quantitation)

The LOC and LOQ will vary depending on the specific instrumentation used.

- c. Results of Method Validation: The Method Trials of the simultaneous determinative and confirmatory methods for pirlimycin residues in bovine milk and liver were satisfactorily completed by FDA and USDA laboratories.
- d. Method Location: The validated regulatory analytical methods for pirlimycin residues are on display in Dockets Management Branch (HFV-305), Park Building (room 1-23), 12420 Parklawn Drive. Rockville, MD 20855. They are attached to the FOI summary.

V. 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 of the implementing regulations. The data demonstrate that pirlimycin hydrochloride (PIRSUE™ Aqueous Gel), a lincosamide antibiotic, when administered at 50 mg per infected quarter to lactating dairy cattle by intramammary infusion, twice, with the second infusion 24 hours after the first, is safe and effective for the treatment of clinical and subclinical mastitis caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*, each a major bacterial pathogen associated with bovine mastitis. Based on a battery of toxicology tests, the safe concentrations for total pirlimycin-related residues are 0.4 ppm in milk, 1.2 ppm in muscle, 2.4 ppm in liver, 3.6 ppm in kidney, and 4.8 ppm in fat. Based on metabolism studies, a tolerance (Rm) of 0.4 ppm has been established for parent pirlimycin in milk, and a tolerance (Rm) of 0.5 ppm has been established for parent pirlimycin in liver. These tolerances (Rm) refer to the residue measured by the regulatory methods described herein.

A pre-slaughter withdrawal period of 28 days and a milk discard period of 36 hours (3 milkings) have been established for this use of pirlimycin. These restrictions are based on statistical analyses of the depletion data, using an upper tolerance limits containing 99 percent of the population with a 95 percent confidence limit.

Labeling restricts this drug to use by or on order of a licensed veterinarian. This decision was based on the following factors: (a) the product contains a new antimicrobial entity intended only for therapeutic purposes, (b) adequate directions can not be written to enable lay persons to appropriately diagnose and subsequently use this product to treat clinical and subclinical mastitis caused by staphylococcal and streptococcal organisms, and (c) prevent indiscriminate use for violative tissue and milk residues, and reduce emergence of drug resistant organisms.

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human

environment and that an environmental impact statement is not required. The agency's finding of no significant impact (FONSI) and the evidence supporting that finding are contained in an environmental assessment, which may be seen in the Docket Management Branch (HFV-305), Park Building (Room 1-23), 12420 Parklawn Dr., Rockville, Maryland 20855.

Section 512(c)(2)(P)(i) of the act provides a five-year exclusivity period for a drug, no active ingredient (including any ester or salt of the active ingredient) of which has been approved in any other application under subsection (b)(1). This NADA qualifies for such an exclusivity period. Pirlimycin hydrochloride is under patent number, U.S. 4,278,789 expiring July 14, 1998.

VI. LABELING (Attached)

1. Labeling for PIRSUE™ Aqueous Gel

Copies of applicable labels may be obtained by writing to the:

Freedom of Information Office
Center for Veterinary Medicine, FDA
7500 Standish Place
Rockville, MD 20855

Appendix I

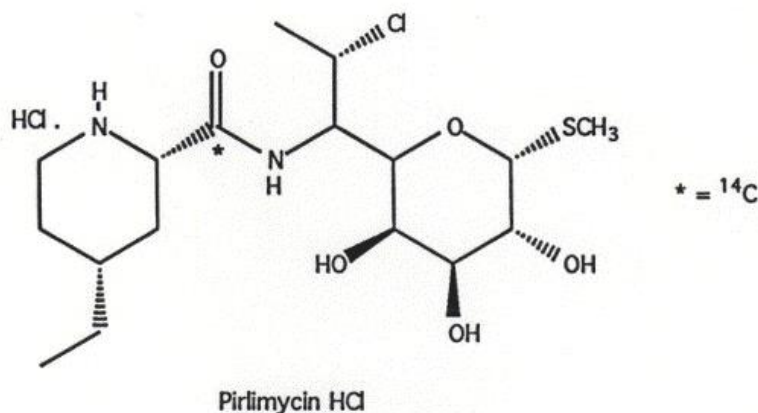


Figure 1. Structure of Pirlimycin HCl with location of ¹⁴C label noted.

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.