Approval Date: June 25, 2003

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-041

Estradiol Benzoate

Celerin ä C
Microencapsulated Estradiol Benzoate
Suspension Implant

For increased rate of weight gain in suckling beef calves.

Sponsored by:

PR Pharmaceuticals, Inc.
1716 Heath Parkway
Fort Collins, Colorado 80524

FREEDOM OF INFORMATION SUMMARY

Celerin ä C (estradiol benzoate)
Microencapsulated Estradiol Benzoate
Suspension Implant

1. GENERAL INFORMATION:

a. File Number:	NADA 141-041
b. Sponsor:	PR Pharmaceuticals, Inc. 1716 Heath Parkway Fort Collins, Colorado 80524 Drug Labeler Code: 067210
c. Established Name:	Estradiol benzoate
d. Proprietary Name:	Celerin™ C
e. Dosage Form:	Suspension Implant
f. How Supplied:	Each package contains one vial of microencapsulated estradiol benzoate powder and one vial of sterile diluent for suspension. The entire package constitutes 50 X 10 mg doses of estradiol benzoate.
g. How Dispensed:	OTC
h. Amount of Active Ingredients:	One mL dose contains 10 mg estradiol benzoate.
i. Route of Administration:	Subcutaneous injection in the ear only. A 16 to 20 gauge needle is recommended.
j. Species/Class:	Suckling beef calves. Do not use in veal calves, calves intended for reproduction, or calves less than 30 days old.

k. Recommended Dosage:	One mL dose containing 10 mg of estradiol benzoate
l. Pharmacological Category:	Steroid hormone
m. Indications:	For increased rate of weight gain in suckling beef calves.

2. EFFECTIVENESS:

A. Dosage Characterization:

Dose titration was performed as part of the effectiveness study (see section 2.B below).

B. Substantial Evidence:

The new animal drug application for Celerin™ C (estradiol benzoate) contains data from an adequate and well-controlled investigation demonstrating the effectiveness of the new animal drug for the indication for use and dosage as given in Section 1 above.

Effectiveness of microencapsulated estradiol benzoate was established in a three-location, clinical effectiveness/dose titration study. A uniform protocol was used by each site to allow pooled summary and analysis of study data. Research was conducted in major beef calf producing areas of the United States.

Investigators/Study Locations:

Dr. Bill C. Clymer
Kuhlman Ranch
Canyon, TX

Dr. John Vetterling
J Ranch
Granby, CO

Dr. Bill C. Clymer
Rocking C Ranch
Molalla, OR

The objective was to determine the dose response for microencapsulated estradiol benzoate (MEB) with lactide/glycolide polymer on the rate of weight gain in suckling beef calves. The test animals were suckling steer and heifer cross-bred calves of English beef breeds. For each site, calves from 1-3 months of age and weighing an average of approximately 145 lbs. were randomly assigned by weight to one of five treatment groups, and administered either 0, 2.5, 5.0, 10, or 15 mg MEB. The number of calves started at each site was 250 (23-27 calves per group), 116 (8-10 calves per group), and 113 (9-12 calves per group) for the Colorado, Texas, and Oregon sites, respectively. The calves were weaned on day 168 after drug administration.

Each animal was administered MEB (in 1 mL sterile diluent) via subcutaneous injection on the backside of the mid-ear. The control cattle were not injected. The animals were administered MEB once at the initiation of each study.

Average daily gain data for the steers and heifers are summarized in Table 1, respectively, presented by individual study site, and pooled across sites.

TABLE 1. EFFECTIVENESS AND DOSE RESPONSIVENESS TO MICROENCAPSULATED ESTRADIOL BENZOATE (MEB) OF SUCKLING STEERS AND HEIFERS.

Locations				
MEB (mg)	Oregon	Texas	Colorado	Pooled LS Means
Average Daily Gain (lbs)				
0	1.98	1.62	1.82	1.82
2.5	2.04	1.78	1.86	1.87
5.0	2.15	1.81	1.90	1.93
10.0	2.13	1.73	1.91	1.94
15.0	2.36	1.82	1.95	1.99

The data were pooled and subjected to analysis of variance and to linear plateau modeling to determine the significance of the effect of MEB on average daily gain. There was a significant ($P<.05$) dose effect on average daily gain with a linear response through 15 mg of MEB. Doses of 5 mg and above were shown to be significantly different ($P<.05$) from controls.

No treatment-related adverse conditions were reported at any of the sites.

Based on the data cited above, it is concluded that MEB is effective for the indications for use at the dosage as given in Section 1.

3. TARGET ANIMAL SAFETY:

A. Target Animal Safety Study:

A target animal safety study was conducted by Dr. Bill C. Clymer, Clymer Research & Consulting, Inc., Amarillo, TX. The purpose of the study was to assess the safety to beef cattle of MEB when injected subcutaneously in the ear.

Forty-eight (24 bulls/24 heifers) crossbred calves of predominantly Angus and Brahman breeds, averaging 35 days of age (age range of 5-57 days) and 112 lbs in weight (weight range of 66-156 lbs), were assigned to treatment in a restricted randomized design by weight (restricted by birth date and balanced by gender within treatment).

Treatment consisted of administering either 0 (no injection), 2X (1 mL in the left ear), 6X (2 mL in left ear and 1 mL in right ear), or 10X (same as the 6X treatment plus 2 mL subcutaneously in the right side on the neck) of MEB (1 mL = 20 mg/mL MEB). Six bulls and 6 heifers were used per treatment group. The calves were maintained with their dams in a common pasture for the 140-day experiment period. All animals were observed at least twice weekly for general signs of health, including appetite, movement, and respiration. The calves were weighed, subjected to physical examination and blood collection, and the ears were clinically evaluated by palpation at 28-day intervals during the test period. At Day 140, two bulls and two heifers (previously selected at random at start of study) from each test group were subjected to necropsy. All tissues were examined histologically from the highest dose and control group.

The treatment of beef calves with MEB by subcutaneous injection at 2, 6, and 10 times the use level did not result in any adverse effects on health. Treatment-related abnormalities noted on Day 28 consisted of slight udder swelling (occurred in 3 of 12 animals from the highest dose group), and injection site reactions (abscesses, open lesions, and scars occurring in 15 of 36 treated calves). These conditions were not observed on Day 56. Animals in all treated groups showed increases in overall weight gain compared to control animals. No adverse treatment-related changes were observed in hematology and serum chemistry variables. At termination of the study, no significant macroscopic or microscopic abnormalities were observed. The ears from all necropsied calves were examined microscopically, and were determined to have no injection site reactions.

B. Corroborative Research:

Research was conducted at six locations to evaluate the effects of administering MEB on the injection site in suckling beef calves.

Name of Investigators and Location of Studies:

Dr. Roger McCraw Rose Hill Farm Nashville, NC	Dr. Thomas Kennedy Armstrong Ranches Emmett, AR
Mr. W. R. Thomson Horned M Ranch White Oak, SC	Dr. Johnnie Copeland Russ Putnal Ranch Myakka City, FL
Dr. Thomas Kennedy Alvord Ranch Princeton, OR	Dr. George Reese Julius Tompkins Farm Fitzpatrick, AL

A total of 500 suckling steer and heifer beef calves were administered 20 mg MEB via subcutaneous injection in the ear, and were compared to 503 control animals (administered vehicle). The injection sites on all animals were examined at 24-28 days for ear infections. Seventeen of the calves administered MEB were observed to have either a minor scab or swelling on their ears, compared to only one control animal. No major abscesses were observed in any of the MEB treated animals.

Research was conducted at one location to evaluate the degree of movement of microspheres after subcutaneous implantation in the ear of light weight cattle and to assess the effects of direct venous implantation of BLIP microspheres in cattle.

Name of Investigators and Location of Study:

T.J. Kennedy and P.W. Geeding
Cosby, MO

Study Number: 635-0007-91B-001

Title: Assessment of the Migration of BLIP Microspheres after Subcutaneous Implantation in Cattle.

Objective: To define, by qualitative means, the degree of movement of microspheres after subcutaneous implantation in the ear of light weight cattle.

Methods:

Six Holstein cattle, approximately 6-10 months of age, were assigned to this study. Animals were treated with 1 mL microspheres (as per use conditions of this product) containing 100 mg/mL carbon black for subsequent gross and histological observation. Animals were necropsied in pairs at days 14, 35, and 63 after treatment. Animals in the day 14 slaughter group were given treatment in each ear, while animals in the other two groups were given treatment in the left ear only. At necropsy, for animals in the day 14 group, both ears and a skin flap 15 cm wide by 30 cm long starting at the dorsal aspect of the ear and continuing along the margin of the mandible to the ventral fold of the neck groove was removed. Also, the lateral and medial retropharyngeal lymph nodes under the flap were removed. These tissues were preserved in glutaraldehyde/formaldehyde for transmission electron microscopy. Animals necropsied on days 35 and 63, were processed in the same manner as those necropsied on day 14, except that only tissue from the left ear was processed.

Results:

Upon gross evaluation of the injection site at all three necropsy times post-treatment, black pigmented areas with discrete margins, denoting placement and location of the "implant" were noted. For all three necropsy times, no migration tracks were seen from the injection site toward the base of the ear. Histologically, no evidence for microsphere migration to the drainage lymph nodes was evident.

Based on these observations through day 63 after treatment, it was concluded that the microspheres stayed at the site of placement, and would likely remain there for the duration of the active drug life.

Name of Investigator and Location of Study:

T.J. Kennedy
Cosby, MO

Study Number: 635-0243-91B-017

Title: Consequences of Venous Implantation of BLIP Microspheres in Cattle

Objective: To assess the effects of direct venous implantation of BLIP microspheres in cattle.

Methods:

Twelve steers weighing approximately 900 lb. and one 80 lb calf were given either control microspheres (n = 2), or microspheres containing 15 mg/mL estradiol benzoate. The two

control and nine treated steers were given treatments by intravenous administration in an ear vein, while one steer and the light weight calf were given intravenous treatments in the jugular vein. For intravenous treatment in the ear vein, steers were given 1, 3, or 5 mL of the estradiol benzoate microsphere suspension (15, 45, or 75 mg estradiol benzoate). The steer given treatment via the jugular vein was given 20 mL of suspension (300 mg estradiol benzoate), while the calf was given 5 mL of suspension (75 mg). Injections were made using a 16 gauge needle. The light weight calf was necropsied 3 hours after treatment to determine if the microspheres might localize in the lungs and heart. The remaining 12 steers on the study were observed at least daily for three weeks for adverse events and general health issues.

Results:

Necropsy of the light weight calf with subsequent histological evaluation of the lungs and heart was an attempt to locate microspheres before any possible degradation or encapsulation. No microspheres were found in any of the sections of lung or heart tissue. There were also no gross pathological findings.

With respect to administration of control and test articles via the ear veins of steers, all injections were made with great difficulty. The size of the needle (16 gauge) made injection possible at only one of the two mid-rib ear veins, and animals had to be completely restrained to cannulate the vein with this size needle. For two of the animals, backflow of test article occurred from the injection site. The investigator concluded, that based upon the extreme measures taken to administer control and test articles into the ear vein, it would be rare that this route of administration would occur under field conditions.

With respect to animal health in control and treated steers during the 3-week, post-treatment period, no pathological signs were noted. There were no physical changes in any animals compared to controls immediately after nor up to 21 days post-treatment.

It was concluded that intravenous administration of microencapsulated estradiol benzoate via an ear vein, caused no adverse events through three weeks after treatment.

Based on the data from the studies cited above, in conjunction with the data from the efficacy studies (in which no treatment-related adverse conditions were reported), it is concluded that MEB is safe for the indications for use and at the dosage as given in Section 1.

4. HUMAN SAFETY:

- **Allowable Incremental Increases:**

Allowable incremental increases of estradiol ($E_2\beta$) have been established by the agency under 21 CFR 556.240. Residues for estradiol and related esters may not exceed the following increments above the concentrations of estradiol naturally present in the untreated animals; in the uncooked edible tissues of heifers, steers, and calves, 120 parts per trillion (ppt) for muscle, 480 ppt in fat, 360 ppt for kidney, and 240 ppt for liver.

- **Residue Depletion Studies:**

1. Type of Study

Sixty calves were randomly allotted to two treatment groups of 30 calves each (15 heifers and 15 steers). After administration of the test article (or sham dosing) the calves in each treatment group were further randomly allotted to one of three slaughter groups (Days 15, 30, or 90 post-treatment).

2. Investigator:

Bill C. Clymer, Ph.D.
C.R.C., Inc.
Amarillo, TX

Statistician:

Thomas J. Keefe, Ph.D.
Envirostat Associates
Fort Collins, CO

3. General Design of Study

a. Purpose of Study: To establish that the exogenous administration of a naturally-occurring hormone does not augment the level of hormone in the edible tissue of treated animals above that of the background level for non-treated calves.

b. Test Animals: Sixty commercial crossbred calves (30 heifers and 30 steers), 100-250 pounds body weight, estimated at 2-4 months of age.

c. Dosage Form: Injectable microspheres containing estradiol benzoate suspended with sterile injection vehicle.

d. Route of Administration: All treated calves received 1 mL (15 mg estradiol benzoate) in each ear subcutaneously for a total of 30 mg estradiol benzoate (1.5X).

e. Time and Duration of Dosing: Each treated calf received a single subcutaneous injection in each ear on Day 0 of the test. Control calves received a sham injection.

4. Results

Tissue samples to be assayed for estradiol residues were collected from treated and control animals at 15, 30, and 90 days post-injection. Tissue samples included kidney, intra-abdominal fat, liver and longissimus muscle. Table 2 lists the results of the analysis.

TABLE 2. ESTRADIOL RESIDUES IN ANIMAL TISSUE FOLLOWING INJECTION WITH 30 mg MICROENCAPSULATED ESTRADIOL BENZOATE

Estradiol Residues in Edible Tissue (pg/g)						
	Assay Period	N [d]	Muscle [a]	Kidney [b]	Liver [b]	Fat [a]
TREATED	Day 15 mean	10	8.3	26.99	25.00	8.45
	(S.D.[c])		2.52	3.67	0.00	2.25
	Day 30 mean	9	6.07	25.32	25.00	10.91
	(S.D.)		0.14	0.97	0.00	4.01
	Day 90 mean	8	6.53	26.23	25.00	19.03
	(S.D.)		0.87	2.68	0.00	19.97
CONTROL	Day 15 mean	10	6.48	25.00	25.00	9.70
	(S.D.)		1.10	0.01	0.00	6.16
	Day 30 mean	10	6.29	25.00	25.00	6.87
	(S.D.)		0.64	0.00	0.00	1.01
	Day 90 mean	10	6.89	25.23	25.12	6.41
	(S.D.)		1.47	0.71	0.36	0.55

[a] LOQ = 6 pg/g
[b] LOQ = 25pg/g
[c] S.D. = Standard Deviation
[d] N = Number of animals

5. Statistical Analysis

The data were statistically analyzed by a three-way analysis of variance to determine the significance of the variation of tissue residue over the withdrawal times. This variation was further evaluated by orthogonal polynomial contrasts within analysis of variance and further, the relationships among tissue were examined via linear correlation analysis for all calves and for those sampled on Day 90.

Mean assayed levels of estradiol in kidney and muscle from estradiol benzoate-treated calves were not different from the corresponding samples in control calves at any of the sampled time points. Additionally, when the kidney and muscle residues in the treated calves were compared to the kidney and muscle residues in the control calves, the difference in residue concentrations was found to be significantly less than the allowable incremental increases permitted under 21 CFR 556.240. While mean assay levels of estradiol in fat samples were higher in the estradiol benzoate-treated calves than the corresponding levels in control calves for the 30- and 90-day samples, they were less than the control calves at the 15-day sampling time. When the fat residues in the estradiol benzoate-treated calves were compared to the fat residues in the control calves, the difference in residue concentrations was found to be significantly less than the allowable incremental increases permitted under 21 CFR 556.240. Residues of estradiol in the liver samples of treated and control calves were essentially equal to the Limit of Quantification (LOQ) for the method. When the liver residues in the estradiol benzoate-treated calves were compared to the liver residues in the control calves, the difference in residue concentrations was found to be significantly less than the allowable incremental increases permitted under 21 CFR 556.240.

- **Withdrawal Time:**

Residues of estradiol in calves treated with Celerin™ C were several times less than the allowable incremental increases permitted under 21 CFR 556.240. No withdrawal period is required.

- **Regulatory Method:**

Celerin™ C qualifies for a zero withdrawal and, as such, a regulatory analytical method for residues is not required.

- **User Safety Concerns:**

  The product labeling contains the following statement:

  1. "Keep out of reach of children."

5. 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 estradiol benzoate when administered at 10 mg/mL is safe and effective for the claim indicated in section 1 of this FOI Summary.

Allowable incremental increases of estradiol ($E_2\beta$) have been established by the agency under 21 CFR 556.240. Residues for estradiol and related esters may not exceed the following increments above the concentrations of estradiol naturally present in the untreated animals; in the uncooked edible tissues of heifers, steers, and calves, 120 parts per trillion (ppt) for muscle, 480 ppt in fat, 360 ppt for kidney, and 240 ppt for liver. Residues of estradiol in calves treated with Celerin™ C were several times less than the allowable incremental increases permitted under 21 CFR 556.240. Celerin™ C qualifies for a zero withdrawal and, as such, a regulatory analytical method for residues is not required.

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 the approval. The three years of marketing exclusivity applies only to the use of the product (Celerin™ C) containing 10 mg per mL estradiol benzoate for which this original new animal drug application is approved.

The Center for Veterinary Medicine has concluded that, for this product, adequate directions of use by the layperson have been provided and the product will have over-the-counter (OTC) status. Label directions provide detailed instruction in plain language. The drug product is not a controlled substance. Thus, the drug product is assigned OTC status, and the labeling is adequate for the intended use.

Estradiol benzoate is under the following U.S. patent numbers:

U.S. Patent Number	Date of Expiration
5,288,496	February 22, 2011
5,401,507	March 28, 2012
5,427,796	February 22, 2011

6. ATTACHMENTS:

Facsimile Labeling is attached as indicated below:

Inner Carton Label (50 – 10 mg doses)
Vial Label (Celerin™ C – Microencapsulated estradiol benzoate powder – 50 – 10 mg doses)
Vial Label (50 mL sterile diluent)
Case Label (20 units of 50 – 10 mg doses)
Package Insert