Date of Approval: July 22, 2010

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-200

EAZI-BREED CIDR Cattle Insert

Progesterone
Solid Matrix
Lactating Dairy Cows

This supplement provides for the concurrent administration of progesterone solid matrix and dinoprost tromethamine for synchronization of estrus in lactating dairy cows

Sponsored by:

Pharmacia & Upjohn Co., a Division of Pfizer, Inc.

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I. GENERAL INFORMATION:

A. File Number:	NADA 141-200
B. Sponsor:	Pharmacia & Upjohn Co., a Division of Pfizer, Inc. 235 East 42d St. New York, NY 10017 Drug Labeler Code: 000009
C. Proprietary Name:	EAZI-BREED CIDR Cattle Insert
D. Established Name:	Progesterone
E. Pharmacological Category:	Steroid Hormone
F. Dosage Form:	Solid Matrix
G. Amount of Active Ingredient:	Each insert contains 1.38 grams progesterone in molded silastic over a nylon spine.
H. How Supplied:	10 inserts per polyethylene bag
I. How Dispensed:	OTC
J. Dosage:	Administer one EAZI-BREED CIDR Cattle Insert for 7 days
K. Route of Administration:	Intravaginal for 7 days
L. Species/Class:	Cattle: lactating dairy cows
M. Indication:	For synchronization of estrus in lactating dairy cows
N. Effect of Supplement:	This supplement provides for the concurrent administration of progesterone solid matrix and dinoprost tromethamine for synchronization of estrus in lactating dairy cows.

II. EFFECTIVENESS:

A. Dosage Characterization:

This supplemental approval does not change the previously approved dosages. The Freedom of Information (FOI) Summaries for the original and supplemental approvals of NADA 141-200, dated May 2, 2002, and July 29, 2003, respectively, contain dosage characterization information for the EAZI-BREED CIDR Cattle Insert (CIDR) for beef and dairy heifers, and beef and dairy cows. The FOI Summaries for supplemental approvals of NADA 108-901, dated February 20, 1981, and February 11, 1983, contain dosage characterization information for LUTALYSE Sterile Solution (LUTALYSE, dinoprost tromethamine) in dairy and beef cows, and dairy and beef heifers.

B. Substantial Evidence:

Clinical Field Study

a. Title:

Pivotal Field Efficacy Study For Use of EAZI-BREED CIDR Cattle Insert (Progesterone Releasing Intravaginal Insert) with Concurrent Injection of LUTALYSE Sterile Solution (Dinoprost Tromethamine) for Synchronization of Estrus in Lactating Dairy Cows, Study Number 1930C-06-60-519

b. Investigators and Study Locations:

This study was conducted at 5 commercial dairies (locations, sites) in the Southern, Western, Midwestern and Northeastern regions of the US. The Investigators and locations of study sites are listed in Table 1.

Table 1. Summary of investigators and study locations.

Investigator	Study Site Location
Michael Capel, DVM	Mount Morris, NY
Paul Busman, DVM	Ravenna, MI
Michael Bossom, DVM	Charlotte, IA
Chad Wright, DVM	Bakersfield, CA
Carlos Risco, DVM	Bell, FL

c. Study Design:

1) *Objective:* The objective of this study was to determine the effectiveness and field safety of the treatment regimen consisting of a 7-day administration of the CIDR with an injection of 5 mL LUTALYSE (25 mg dinoprost) at the day of insert removal for synchronization of estrus in lactating dairy cows.

2) *Test Animals:* Five hundred lactating dairy cows (Holstein, Jersey, or crossbred), 100 at each site, 50 in each of two treatment groups, were enrolled in the study. Enrollment criteria were that cows must have completed the farm specified post partum voluntary wait period (> 40 days post partum), be clinically healthy, be ≤ 110 days postpartum, be inseminated no more than 3 times during current lactation, have a body condition score ≥ 2 and ≤ 4 using a 5 point scale, and be eligible for breeding.

3) *Experimental Design:* The study design was a randomized block with cows blocked on order-of-presentation for enrollment within site, without regard to parity, to each of two treatment groups. Cows were enrolled by weekly (or longer) breeding cohorts. All cows in a block and all cows within a breeding cohort were housed in the same pen. More than one breeding cohort could be enrolled on a given week. There was a minimum of 2 enrollment days at each site.

4) *Treatment Groups:*

Table 2. Summary of treatment groups.

Treatment group	Dose	Route of Administration	Number of animals	
			Per Site	Total
T01; LUTALYSE	5 mL*; administered on Study Day 7	Intramuscular	50	250
T02; EAZI-BREED CIDR Cattle Insert and LUTALYSE	One CIDR administered on Study Day 0 and Removed on Study Day 7 5 mL; administered at time of EAZI-BREED CIDR Cattle Insert removal on Study Day 7	Intra-vaginal Intramuscular	50	250

* 5 mg dinoprost/ mL

5) *Measurements and Observations:* Enrolled cows were observed at least once daily for estrus and clinical signs of health abnormalities on Study Days 1-14. Cows observed in estrus on Study Days 3 to 14 were artificially inseminated following site specific procedures. Cows that were not inseminated by Study Day 14 completed the study on Study Day 14. Cows that were inseminated from Study Day 3 to 14 were observed for general health weekly from Study Day 15 until pregnancy evaluation 45-55 days post-insemination.

   At the time of CIDR removal (Study Day 7), a vaginal mucus score was assigned based on the following criteria: 1 = No Mucus Observed, 2 = Clear Mucus Observed, 3 = Cloudy Mucus Observed, 4 = Yellow Mucus Observed, 5 = Dark Red or Brown Mucus Observed, 6 = Mucus with Bright Red Fresh Blood.

d. Statistical Analysis:

Effectiveness: Effectiveness was determined in individual cows by presence or lack of a synchronized estrus, defined as observed in estrus during the synchronization period, Study Day 9 through 12 (days 2 to 5 post CIDR removal and/or LUTALYSE injection). Synchronization rate [(number of cows in estrus during the synchronization period divided by number of cows enrolled) X 100] was analyzed as a binary variable (1=showed estrus, 0=no estrus) using a generalized linear mixed model (Glimmix procedure with a binomial error distribution and a logit link function). The statistical model included the fixed effect of treatment and the random effects of site, site by treatment, cohort within site, block within site and cohort, and residual. The model included parity (first parity vs. second and greater parity) as a covariate. Least squares means are presented as estimates of treatment means. Standard errors of least squares means were estimated and 95% confidence intervals were constructed. Back-transformed least squares means are presented. The null hypothesis was that the treatment effect of CIDR plus LUTALYSE (T02) is less than or equal to the control effect LUTALYSE alone (T01) and the alternative hypothesis was that the treatment effect was greater than the control. A one-sided test was conducted using α=0.05.

Reproductive safety: Reproductive safety was addressed by evaluation of pregnancy rate and conception rate. These variables were analyzed in a similar approach as described for effectiveness:

Pregnancy Rate = number of cows pregnant at 50 ± 5 days after insemination ÷ number of cows enrolled X 100

Conception Rate = number of cows pregnant at 50 ± 5 days after insemination ÷ number of cows inseminated X 100

The null hypothesis was that the treatment effect of CIDR plus LUTALYSE (T02) was greater than or equal to the control effect LUTALYSE alone (T01) and the alternative hypothesis was that the treatment effect was less than the control. These variables were evaluated at an alpha of 0.10.

There were two analyses each for Pregnancy Rate and Conception Rate based on different inclusion criteria:

A) All cows inseminated during Study Days 3 through 14.

B) The same analysis as in A but the cows in T02 that were in estrus during Study Day 3 to 7 were coded as not pregnant.

Field Safety: Conditions of use safety were evaluated by mucus scores and adverse events, which were summarized but not analyzed.

e. Results:

Effectiveness: Synchronization rate (Table 3) was greater in cows administered the CIDR and LUTALYSE than cows administered LUTALYSE alone. In the CIDR group, 67.5% of cows were observed in estrus during the synchronization period compared to 46.7% of cows in LUTALYSE only group.

Table 3. Statistical analysis for synchronization rate and percent of animals with a synchronized estrus.

Treatment	**T01[1]**	**T02[2]**
Number of Animals	248	247
Synchronization Rate		
Least Squares Means	-0.1333	0.7303
95% Confidence Interval (Lower, Upper)	(-0.7425, 0.4758)	(0.1045, 1.3560)
Back-Transformed % of Animals		
Least Squares Means	46.7	67.5
95% Confidence Interval (Lower, Upper)	(32.2, 61.7)	(52.6, 79.5)
One-Sided P-Value	0.0227	

[1] T01 = 5 mL LUTALYSE only

[2] T02 = 7-day administration period of the CIDR with an injection of 5 mL LUTALYSE at the time of insert removal

Reproductive safety:

Pregnancy rate, whether determined by including all cows inseminated during Study Days 3-14 (Table 4) or excluding cows inseminated Study Days 3-7 (Table 5) were not statistically different between cows administered the CIDR and LUTALYSE and cows administered LUTALYSE alone. When considering all cows inseminated during Study Days 3-14, pregnancy rate was 34.5% in cows administered LUTALYSE alone, compared to 26.4% in cows administered the CIDR and LUTALYSE. Similarly when cows inseminated on Study Days 3-7 were excluded from the analysis, pregnancy rate was 34.4% in cows administered LUTALYSE alone, compared to 25.9% in cows administered the CIDR and LUTALYSE.

Table 4. Statistical analyses for pregnancy rate[1] for all cows inseminated study days 3-14.

Treatment	**T01[2]**	**T02[3]**
Number of Animals	244	245
Least Square Means	-0.6419	-1.0242
95% Confidence Interval (Lower, Upper)	(-1.5449, 0.2611)	(-1.9367, -0.1117)
Back-transformed Mean (%)		
Least Squares Means	34.5%	26.4%
95% Confidence Interval (Lower, Upper)	(17.6, 56.5)	(12.6, 47.2)
One-sided P-value	0.1283	

[1] Pregnancy Rate = number of animals pregnant at 45-55 days post insemination/number of animals enrolled

[2] T01 = 5 mL LUTALYSE only

[3] T02 = 7-day administration period of the CIDR with an injection of 5 mL LUTALYSE at the time of insert removal

Table 5. Statistical analyses for pregnancy rate[1] with all cows in treatment group T02 in estrus study days 3-7 coded not pregnant.

Treatment	**T01[2]**	**T02[3]**
Number of Animals	244	245
Least Square Means	-0.6468	-1.0487
95% Confidence Interval (Lower, Upper)	(-1.5476, 0.2540)	(-1.9597, -0.1377)
Back-transformed Mean (%)		
Least Squares Means	34.4%	25.9%
95% Confidence Interval (Lower, Upper)	(17.5, 56.3)	(12.4, 46.6)
One-sided P-value	0.1233	

[1] Pregnancy Rate = number of animals pregnant at 45-55 days post insemination/number of animals enrolled

[2] T01 = 5 mL LUTALYSE only

[3] T02 = 7-day administration period of the CIDR with an injection of 5 mL LUTALYSE at the time of insert removal

Conception rate, whether determined by including all cows inseminated during Study Days 3-14 (Table 6) or excluding cows inseminated Study Days 3-7 (Table 7) were not statistically different between cows administered the CIDR and LUTALYSE and cows administered LUTALYSE alone. When considering all cows inseminated during Study Days 3-14, conception rate was 46.9% in cows administered LUTALYSE alone, compared to 36.3% in cows administered the CIDR and LUTALYSE. Similarly when cows inseminated on Study Days 3-7 were excluded from the analysis, conception rate was 46.7% in cows administered LUTALYSE alone, compared to 35.6% in cows administered the CIDR and LUTALYSE.

Table 6. Statistical analyses for conception rate[1] for all cows inseminated study days 3-14.

Treatment	T01[2]	T02[3]
Number of Animals	244	245
Least Square Means	-0.1242	-0.5633
95% Confidence Interval (Lower, Upper)	(-0.9159, 0.6676)	(-1.3536, 0.2269)
Back-transformed Mean (%)		
Least Squares Means	46.9%	36.3%
95% Confidence Interval (Lower, Upper)	(28.6, 66.1)	(20.5, 55.6)
One-sided P-value	0.1431	

[1] Conception Rate = number of animals pregnant at 45-55 days post insemination/number of animals inseminated
[2] T01 = 5 mL LUTALYSE only
[3] T02 = 7-day administration period of the CIDR with an injection of 5 mL LUTALYSE at the time of insert removal

Table 7. Statistical analyses for conception rate[1] with all cows in treatment group T02 in estrus study days 3-7 coded not pregnant.

Treatment	T01[2]	T02[3]
Number of Animals	244	245
Least Square Means	-0.1311	-0.5921
95% Confidence Interval (Lower, Upper)	(-0.9221, 0.6599)	(-1.3825, 0.1983)
Back-transformed Mean (%)		
Least Squares Means	46.7%	35.6%
95% Confidence Interval (Lower, Upper)	(28.5, 65.9)	(20.1, 54.9)
One-sided P-value	0.1358	

[1] Conception Rate = number of animals pregnant at 45-55 days post insemination/number of animals inseminated
[2] T01 = 5 mL LUTALYSE only
[3] T02 = 7-day administration period of the CIDR with an injection of 5 mL LUTALYSE at the time of insert removal

Field Safety: Across all locations, 26.8% of inserts had either no mucus observed (score 1) or clear mucus (score 2). A total of 72.4% inserts had scores of 3 or 4, indicating localized irritation. No cows had a score of 5 and 0.9% inserts had a score of 6, indicating potential vaginitis or damage during insert removal.

The overall incidence of clinical observations was low, with 21 adverse events observed in 19 of 248 cows administered LUTALYSE (T01), and 37 adverse events were observed in 31 of 247 cows administered CIDR plus LUTALYSE

(T02). These events were summarized into general categories and a majority of these observations were related to feet and legs, mastitis, and injuries. The incidence of these observations was comparable between treatments. This summary indicated that there were no general health observations documented that would indicate a detrimental effect attributable to the concurrent use of CIDR and LUTALYSE for synchronization of estrus in lactating dairy cows.

f. Conclusion: The results of this study support the conclusion that the CIDR when used for a 7-day administration period with an injection of 5 mL LUTALYSE (25 mg dinoprost) at the time of insert removal, is safe for the dairy cow and is effective for synchronization of estrus in lactating dairy cows, and is more effective in synchronizing estrus than administration of LUTALYSE alone.

III. TARGET ANIMAL SAFETY:

CVM did not require target animal safety studies for this supplemental approval. The FOI Summary for the original approval of NADA 141-200 dated May 2, 2002, contains a summary of the target animal safety study for the use of the CIDR in dairy heifers. The FOI Summary for the supplemental approval of NADA 108-901 dated November 2, 1979, contains a summary of the target animal safety study for use of LUTALYSE in beef and dairy cattle. Field safety and reproductive safety observations for the concurrent use of the CIDR plus LUTALYSE were evaluated in the clinical effectiveness study and the data were reviewed for this supplemental application (see Section II above).

IV. HUMAN FOOD SAFETY:

A. Toxicology

Progesterone is regulated based on allowable incremental increase limits for residues. Using the revised daily consumption values, FDA has updated the allowable incremental increase limits for residues of progesterone in edible tissues to 5 ppb for muscle, 15 ppb for liver, 30 ppb for kidney and 30 ppb for fat.

Adequate studies have been submitted previously to NADA 108-901 to determine the toxicity of dinoprost tromethamine, a naturally occurring prostaglandin $F_{2\alpha}$.

B. Residue Chemistry

1. Summary of Residue Chemistry Studies

FOI Summaries regarding previous approvals for the CIDR and LUTALYSE for cattle were described under NADA 141-200 and NADA 108-901, respectively. There is no tissue withdrawal period or milk discard time requirement associated with these approvals. This supplement approval is for the concurrent use of the CIDR with LUTALYSE in lactating dairy cows. The CIDR is administered for 7 days, and 5 mL LUTALYSE is administered at the time of the CIDR removal.

Regarding progesterone residue safety in edible tissues, FDA has determined that human food safety for progesterone residues in edible tissues can be demonstrated by showing that concentrations of progesterone residues in muscle and fat tissues of lactating dairy cows treated with the CIDR and LUTALYSE do not exceed the revised allowable incremental increase limits above those of untreated control cows.

There are no codified allowable incremental increase limits for progesterone in milk. FDA has determined that human food safety for progesterone residues in milk can be demonstrated by showing that concentrations of progesterone residues in milk as the result of the concurrent treatment with the CIDR and LUTALYSE do not exceed the concentrations in the untreated pregnant cows.

There are neither codified tolerances nor allowable incremental increase limits for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) residues in tissues or milk. FDA has determined that human food safety for $PGF_{2\alpha}$ residues in edible tissues can be demonstrated by showing that the $PGF_{2\alpha}$ residue concentrations at the injection site as the result of the concurrent treatment with the CIDR and LUTALYSE do not exceed the concentrations resulting from the treatment with LUTALYSE alone.

FDA has determined that human food safety for $PGF_{2\alpha}$ residues in milk can be demonstrated by showing that the $PGF_{2\alpha}$ residue concentrations in milk as the result of the concurrent treatment with the CIDR and LUTALYSE do not exceed the concentrations resulting from the treatment with LUTALYSE alone.

a. Residue Depletion Studies

Residue depletion information for the use of the CIDR (NADA 141-200) and LUTALYSE (NADA 108-901) in cattle were previously described in the FOI Summaries for the approval of respective products. Additional data generated regarding the concurrent use of the CIDR with LUTALYSE in lactating dairy cows are described below.

<u>Progesterone and $PGF_{2\alpha}$ Residues in Muscle and Fat</u>

Study 1531N-60-07-583 --"Concentration of progesterone and concentration of $PGF_{2\alpha}$ in the edible tissues of lactating dairy cows treated concurrently with EAZI-BREED CIDR Cattle Insert and LUTALYSE Sterile Solution"

1. Objectives

   The study was conducted to determine the concentrations of progesterone and $PGF_{2\alpha}$ in the tissues of lactating dairy cows that were treated with a CIDR + LUTALYSE and to compare the results with those of the control groups. The study was designed such that all cows were targeted for enrollment on days 1-7 of the estrous cycle with sacrifice during days 8 to 14. At this time in the cycle, at least one large corpus luteum (CL) was expected to be on the ovaries and progesterone from endogenous sources (*i.e,.* the CL) was being produced in the greatest concentrations.

2. Study Director, Study Location, and Dates

   Mr. Ryan Roof, Pfizer Animal Health, Kalamazoo, Michigan

   Animal Phase--Halbert Dairy Farm, LLC, 23675 Banfield Rd, Battle Creek, MI

   Analytical Phase--Bldg 300, 333 Portage Street, Kalamazoo, MI

   April 30, 2009, to November 23, 2009

3. General Design of the Study

   a) Test Substance: A CIDR containing 1.38 g of progesterone and LUTALYSE in a 5 mg/mL formulation

   b) Test Animals: Lactating, non-pregnant, estrous cycling Holstein dairy cows. Age and weight were not selection criteria.

   c) Number of Animals and Treatment Groups: Thirty-six cows in the early phase of the estrous cycle (days 1-7) on Study Day 0 were randomly assigned to three treatment groups (Groups T01, T02 and T03) with twelve animals in each group. Enrollment days were staggered over three weeks due to the limitation of animals at the correct stage of estrous cycle.

   d) Dose: Cows in Treatment Group T01 received a CIDR on Study Day 0. On Study Day 7 and 8-12 hours (10 ± 1 h target) prior to euthanasia, the CIDR was removed and the cows were immediately administered 5 mL LUTALYSE via intramuscular (IM) injection. The animals were euthanized at 8-12 hours, with a target of 10 ± 1 hours,

after removal of the CIDR and injection of LUTALYSE. Treatment Group T02 received a single 5 mL IM injection of LUTALYSE on Study Day 7 at 8-12 hours (10 ± 1 h target) prior to euthanasia. Group T03 received no treatment and was sacrificed on Study Day 7.

e) Sample Collection: Approximately 500 g muscle (composite from thigh and loin), 500 g fat (perirenal and omental) and 500 g injection site tissues were collected after euthanasia.

f) Assay: Concentrations of progesterone and $PGF_{2\alpha}$ were determined using validated Liquid Chromatography-Mass Spectrometry (LC-MS) methods for analyzing progesterone in bovine muscle and fat and for analyzing $PGF_{2\alpha}$ at the injection site. The limit of quantitation for progesterone in muscle is 2 ppb. The limit of quantitation for progesterone in fat is 3 ppb. The limit of quantitation for $PGF_{2\alpha}$ in muscle is 0.6 ppb.

4. Results--Progesterone Residues in Muscle and Fat (Table 8)

Table 8. Upper range and mean of progesterone concentrations in the muscle and fat tissues from untreated cows (T03) and CIDR + LUTALYSE treated cows (T01).

Group	Muscle (ng/g)	Fat (ng/g)
T03 (Untreated Control Cows)		
Upper Range[#]	19.6	340
Mean ± Std[#]	10.9 ±5.3	171.2±71.7
T01 (CIDR and LUTALYSE Treated)		
Upper Range[#]	12.4	344
Mean ± Std[#]	6.3±4.0	186.3±97.0

[#] includes only the animals with corpus lutea (11 animals for T03 group and 9 animals for T01 group)

5. Conclusions on Progesterone Concentrations in Muscle and Fat

Muscle--The mean (6.3) and upper range (12.4) of progesterone concentrations in muscle of the treated animals (T01) were below the mean (10.9) and upper range (19.6) of the untreated control cows (T03), respectively.

Fat--The mean (186.3) and upper range (344) of progesterone concentrations in fat of the treated animals (T01) did not exceed more than 30 ppb (the allowable incremental increase limit for fat) above the mean (171.2) and upper range (340) of untreated cows (T03), respectively.

The progesterone concentrations in muscle and fat tissues of cows treated with the CIDR and LUTALYSE do not exceed the allowable incremental increase limits for progesterone above the concentrations naturally present in untreated cows. Therefore, the use of the CIDR and LUTALYSE in lactating dairy cows does not require a withdrawal period as far as progesterone residue safety in edible tissues is concerned.

6. Results – $PGF_{2\alpha}$ Residues at Injection Site (Table 9)

Table 9. The range and mean $PGF_{2\alpha}$ residue concentrations at the injection site of cows treated with CIDR + LUTALYSE (T01, n=12) and cows treated with LUTALYSE Alone (T02, n=12).

	Treatment Group	
	T01	**T02**
Range	2.91 to 497	2.04 to 2620
Mean ± Std	108.0 ± 178.6	311.4 ± 751.8

7. Conclusions on $PGF_{2\alpha}$ concentrations in injection site tissue

The mean (108) and upper range (497) of the $PGF_{2\alpha}$ concentrations in the injection site tissue of cows treated with the CIDR and LUTALYSE (T01) did not exceed the mean (311.4) and upper range (2620) of the $PGF_{2\alpha}$ concentrations in the injection site tissue of cows treated with LUTALYSE alone (T02).

LUTALYSE has been approved for use in lactating dairy cows with no withdrawal period requirement. Therefore, the concurrent use of the CIDR and LUTALYSE in lactating dairy cows does not require a withdrawal period as far as $PGF_{2\alpha}$ residue safety in edible tissues is concerned.

Progesterone and $PGF_{2\alpha}$ Residues in Milk

Study Title: "Concentration of progesterone and $PGF_{2\alpha}$ in the milk of lactating dairy cows treated concurrently with EAZI-BREED CIDR Cattle Insert and LUTALYSE Sterile Solution" -- GLP Study 1531N-60-08-702.

1. Objectives

   In this study, the concentrations of progesterone in the milk of the animals treated with the CIDR and LUTALYSE (Group T01) were determined at specific times before and after removal of the CIDR and injection of LUTALYSE. The concentrations were compared with those in the untreated pregnant cows (Group T03). The concentrations of $PGF_{2\alpha}$ in the milk of animals treated with the CIDR and LUTALYSE (Group T01) were compared with those from the cows that received a single injection of LUTALYSE alone (Group T02)

2. Study Director, Study Location and Dates

   Mr. Ryan Roof, Pfizer Animal Health, Kalamazoo, MI

   Animal Phase--Halbert Dairy Farm, LLC, 23675 Banfield Rd., Battle Creek, MI

   Analytical Phase--Pfizer Research Farm, 5300 N. 28th Street, Richland, MI and Bldg 300, 333 Portage Street, Kalamazoo, MI

   January 9, 2009, to December 3, 2009

3. General Design of the Study

   a) Test Substance: A CIDR containing 1.38 g of progesterone and LUTALYSE in a 5 mg/mL formulation

   b) Test Animals: Lactating, estrous cycling Holstein dairy cows and lactating, pregnant Holstein dairy cows.

   c) Treatment Groups, Dose and Milk Sample Collection:

      Estrous cycling cows (n=12) in treatment group T01 each received a CIDR after the AM milking on Study Day 0. On Study Day 7, after the AM milking, the CIDR was removed and a single intramuscular 5 mL injection of 5 mg/mL LUTALYSE was administered. Milk samples were taken from the group twice a day approximately 12 hours apart, from Day 0 until Day 10.

Estrous cycling cows (n=12) in treatment group T02 received a single intramuscular 5 mL injection of 5 mg/mL LUTALYSE on Study Day 7 after the AM milking. Milk samples were taken from group T02 twice daily starting at the AM milking on Study Day 7 and continued until Study Day 10.

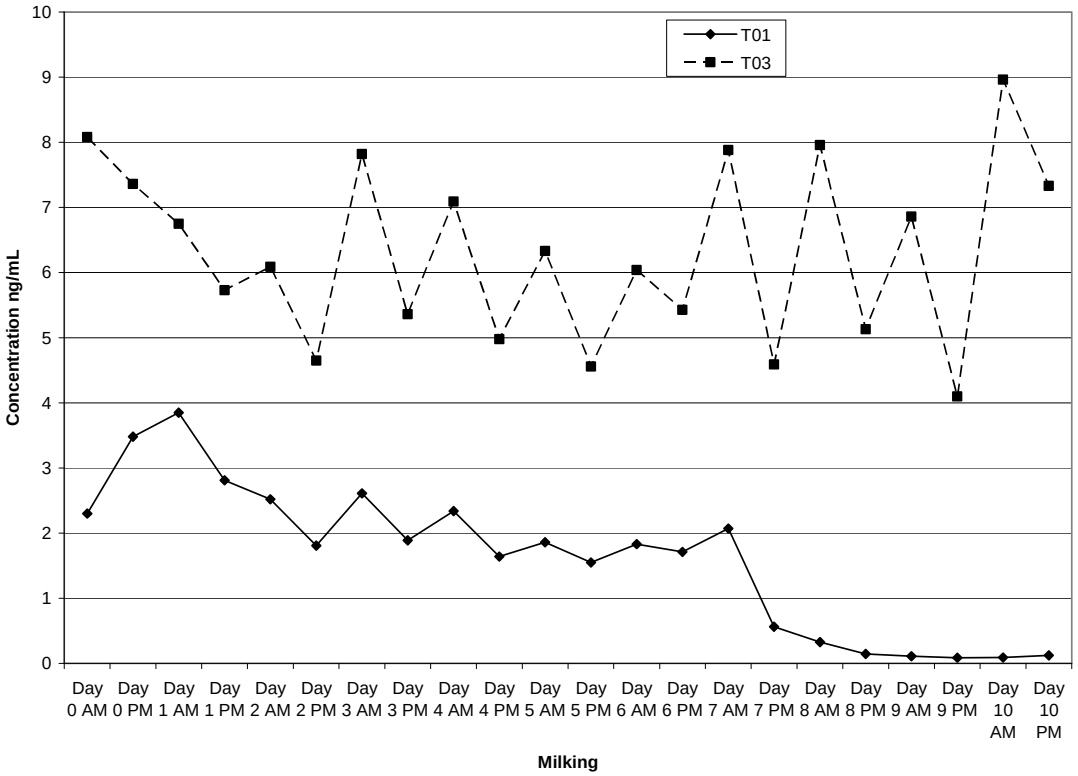
Animals (n=12) in treatment group T03 were pregnant dairy cows that received no treatment with either the CIDR or LUTALYSE. Six cows were between days 60 and 149 of gestation and 6 cows were between days 150 and 220 of gestation on Study Day 0. Milk samples were taken from this group twice a day beginning on Study Day 0 and continuing to Study Day 10.

d) Assay: Concentrations of progesterone (Day 0 to Day 10) and $PGF_{2\alpha}$ (Day 7 to Day 10) in milk were determined using validated Liquid Chromatography-Mass Spectrometry (LC-MS) methods.

4. Results – Progesterone Concentrations in Milk

The means of the progesterone residue concentrations in milk of animals in the T01 (CIDR + LUTALYSE treated) and T03 (untreated pregnant lactating cows) are shown in Figure 1 below.

Figure 1. Mean progesterone concentrations in the skim milk from the CIDR + LUTALYSE treated cows (T01, n = 12) and the pregnant, untreated Cows (T03, n = 12).



5. Conclusions on Progesterone Residue Concentrations in Milk

   a) Within each sampling time point, the mean progesterone concentration in milk of the CIDR and LUTALYSE (T01) treated cows was numerically lower than that observed in the pregnant cows (T03). Many of the individual sample concentrations of the T01 group animals were below the limit of quantitation (LOQ) of 0.6 ng/mL, starting at Day 7 PM milking and onward.

   b) The progesterone AUC values in milk from the CIDR and LUTALYSE treated cows were compared in a non-superiority statistical test relative to those in milk from untreated pregnant cows. The progesterone milk residue concentrations from the CIDR and LUTALYSE treated cows were non-superior to those from the milk of the pregnant cows.

   c) Within the average milking herd, many cows are pregnant. Therefore, it is concluded that the use of the CIDR and LUTALYSE in lactating

dairy cows does not cause human food safety concerns for progesterone residues in milk. As far as progesterone residue is concerned, such use does not require a milk discard time, either during or after the CIDR administration period.

6. Results – PGF $_{2\alpha}$ Residues in Milk

The results of the $PGF_{2\alpha}$ residue assays for the CIDR + LUTALYSE treated cows (T01) and the LUTALYSE alone treated cows (T02) are summarized in Table 10. The limit of detection (LOD) and limit of quantitation (LOQ) of the assay were 0.030 ng/mL and 0.075 ng/mL, respectively.

Table 10. $PGF_{2\alpha}$ Concentrations in skim milk (ng/mL) from the CIDR + LUTALYSE treated Cows (T01) and the LUTALYSE alone treated cows (T02***) from study Day 7 through 10.**

Animal ID	Treatment	Day 7 AM	Day 7 PM	Day 8 AM	Day 8 PM	Day 9 AM	Day 9 PM	Day 10 AM	Day 10 PM
3341	T01	0.0596 †	0.0807	0.0380 †	0.0453 †	0.0438 †	0.0586 †	0.0370 †	0.0643 †
3382[#]	T01	0.122	0.152	0.0762	0.110	0.0997	0.118	0.134	0.113
3517	T01	0.0626 †	0.0517 †	<LOD	0.0530 †	0.0584 †	0.054 †	0.0418 †	0.0591 †
3624	T01	0.0647 †	0.136	<LOD	0.0410 †	0.0464 †	0.0442 †	0.0372 †	0.0557 †
4182	T01	0.0562 †	0.109	<LOD	0.0856	0.0600 †	0.0462 †	0.0528 †	0.0663 †
4754	T01	0.0524 †	0.0595 †	<LOD	0.0450 †	0.0364 †	0.0345 †	0.0374 †	0.0585 †
4755	T01	0.0645 †	0.0834 ∗	<LOD	0.0549 †	0.0437 †	0.0385 †	0.0454 †	0.0539 †
4786	T01	0.0454 †	0.0479 †	ND	0.0335 †	0.0391 †	0.0303 †	<LOD	0.0525 †
4809	T01	0.0803	0.0982	<LOD	0.0576 †	0.0501 †	0.0570 †	0.0569 †	0.0668 †
4853	T01	0.0625 †	0.0479 †	<LOD	0.0539 †	0.0612 †	0.0451 †	0.0646 †	0.0667 †
4933	T01	0.0695 †	0.114	<LOD	0.0643 †	0.0638 †	0.0591 †	0.0778	0.0771
7815	T01	0.0656 †	0.158	<LOD	0.0442 †	0.0522 †	0.0545 †	0.0424 †	0.0675 †
Mean	T01	0.065 †	0.087	<LOD	0.054 †	0.053 †	0.050 †	0.050 †	0.065 †
573	T02	0.0810	0.151	0.0461 †	0.0565 †	0.0654 †	0.0636 †	0.0719 †	0.0633 †
2128	T02	0.0549 †	0.0414 †	<LOD	0.0411 †	0.0426 †	0.0515 †	0.0441 †	0.0382 †
3259	T02	0.0575 †	0.0781	<LOD	0.0471 †	0.0405 †	0.0333 †	0.0301 †	0.0312 †
3455	T02	0.0518 †	0.0990	<LOD	0.0549 †	0.0536 †	0.0565 †	0.0553 †	0.0507 †
3790	T02	0.0465 †	0.153	<LOD	0.029 ‡	0.0417 †	0.0398 †	0.0304 †	0.0300 †
4164	T02	0.0547 †	0.0755	<LOD	0.0448 †	0.0511 †	0.0439 †	0.0444 †	0.0333 †
4178	T02	0.106	0.149	0.0767	0.135	0.107	0.103	0.0926	0.0903
4818	T02	0.0772	0.334	0.0642 †	0.0640 †	0.0638 †	0.0742 †	0.0628 †	0.0599 †
4869	T02	0.0601 †	0.0432 †	<LOD	0.0463 †	0.0447 †	0.0495 †	0.0471 †	0.0435 †
4892	T02	0.0633 †	0.152	<LOD	0.0691 †	0.0557 †	0.0583 †	0.0482 †	0.0403 †
4974	T02	0.0853	0.126	<LOD	0.0878	0.0758	0.0566 †	0.0645 †	0.0487 †
4988	T02	0.0420 †	<LOD	<LOD	0.0483 †	0.0488 †	<LOD	0.0317 †	0.0335 †
Mean	T02	0.063 †	0.093	<LOD	0.056 †	0.055 †	0.052 †	0.049 †	0.044 †

† -- > LOD (0.030 ng/mL), but < LOQ (0.075 ng/ml)

**-- CIDR administered on Study Day 0 after the AM milking and removed on Study Day 7, followed by LUTALYSE injection 8-12 hr prior to the PM milking on Study Day 7.

*** -- Cows administered LUTALYSE 8-12 h prior to the PM milking on Study Day 7.

[#] -- Animal 3282 did not have CIDR.

7. Conclusions on $PGF_{2\alpha}$ Residue Concentrations in Milk

   The milk residue concentrations in the T01 and T02 treatment groups before the LUTALYSE injection and at 24 hrs after the injection were comparable. The majority of the samples were below the LOQ. An increase in $PGF_{2\alpha}$ residues relative to the rest of the samples was observed only at 8-12 hrs post LUTALYSE injection (Day 7 PM milking) in both the T01 and T02 treatment groups, when most of the residue data were above the LOQ. The mean and upper range of the $PGF_{2\alpha}$ residues at the Day 7 PM milking were comparable between the two groups. The data support the conclusion that the concurrent use of the CIDR and LUTALYSE does not result in milk $PGF_{2\alpha}$ residues above those resulting from the treatment with LUTALYSE alone. LUTALYSE has been approved for use in lactating dairy cows with no milk discard time. Therefore, the concurrent use of the CIDR and LUTALYSE does not require milk discard time as far as $PGF_{2\alpha}$ residue safety in milk is concerned.

2. Target Tissue and Marker Residue Assignment

It is not necessary to assign a target tissue or marker residue for progesterone and $PGF_{2\alpha}$.

3. Tolerance Assignments

Progesterone is regulated on the basis of allowable incremental increases (21 CFR 556.540). No residues of progesterone are permitted in excess of the following revised increments above the concentrations of progesterone naturally present in uncooked edible tissues of untreated steers and calves: (1) 5 ppb for muscle; (2) 15 ppb for liver; (3) 30 ppb for kidney; and (4) 30 ppb for fat. It is not necessary to establish allowable incremental increase limits for progesterone in milk.

It is not necessary to establish allowable incremental increase limits for $PGF_{2\alpha}$ in tissue or milk.

4. Withdrawal and Milk Discard Times

The use of the CIDR and LUTALYSE in lactating dairy cows does not compromise human food safety; therefore, such use does not require a withdrawal period or milk discard time (*i.e.*, zero withdrawal and zero milk discard time).

C. Microbial Food Safety

The Agency carefully considered the impact of progesterone in combination with dinoprost tromethamine for cattle on antimicrobial resistance among bacteria of public health concern. Progesterone and dinoprost tromethamine are not normally known to, nor been reported to affect antimicrobial resistance among bacteria; therefore, information or data on microbial food safety for these drugs is not necessary at this time.

D. Analytical Methods for Residues

Regulatory methods for progesterone and $PGF_{2\alpha}$ are not required.

V. USER SAFETY:

The labeling for the EAZI-BREED CIDR Cattle Insert contains the following human warning regarding safety to humans handling, administering or exposed to the EAZI-BREED CIDR Cattle Insert:

"Avoid contact with skin by wearing protective gloves when handling the inserts. Keep this and all medications out of the reach of children."

The labeling for LUTALYSE contains the following human warning regarding safety to humans handling, administering, or exposed to LUTALYSE:

*"Not for human use. Women of childbearing age, asthmatics, and persons with bronchial and other respiratory problems should exercise **extreme caution** when handling this product. In the early stages, women may be unaware of their pregnancies. Dinoprost tromethamine is readily absorbed through the skin and can cause abortion. Accidental spillage on the skin should be washed off **immediately** with soap and water."*

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that the concurrent use of the EAZI-BREED CIDR Cattle Insert and LUTALYSE, when used according to the label, is safe and effective for synchronization of estrus in lactating dairy cows. Additionally, data demonstrate that residues in food products derived from cattle treated concurrently with the EAZI-BREED CIDR Cattle Insert and LUTALYSE will not represent a public health concern when used according to the label.

A. Marketing Status:

The EAZI-BREED CIDR Cattle Insert can be marketed over-the-counter because the approved labeling contains adequate directions for the use by laypersons and the conditions of use prescribed on the label are reasonably certain to be followed in practice.

B. Exclusivity:

Under section 512(c)(2)(F)(iii) 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 concurrent administration of progesterone solid matrix and dinoprost tromethamine for synchronization of estrus in lactating dairy cows for which this supplement is approved.

C. Supplemental Applications:

This supplemental NADA required a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2).

D. Patent Information:

The EAZI-BREED CIDR Cattle Insert is under the following U.S. patent numbers:

U.S. Patent Number	Date of Expiration
6,423,039	June 22, 2017
6,663,608	June 22, 2017

For current information on patents, see the Animal Drugs @ FDA database (formerly the Green Book) on the FDA CVM internet website.

VII. ATTACHMENTS:

Facsimile Labeling:

Product Bag Label
Inner Case Label
Outer Case Label