Veterinary Medicine Research & Development
Pfizer Inc
Kalamazoo, Michigan 49001-0199
United States

Pfizer Animal Health

Environmental Assessment for the Concurrent Use of EAZI-BREED™ CIDR® Cattle Insert and Lutalyse® Sterile Solution in Lactating Dairy Cows

Active Ingredients: Progesterone, Dinoprost tromethamine

APPROVAL

Dated: 16 JUNE 2008 Signed: Walter J. Smolenski

Walter J. Smolenski, MS
Senior Scientist
Veterinary Medicine R & D
Pfizer Animal Health
Kalamazoo, MI 49001 USA

Dated: 16 June 2008 Signed: Richard C. Greening

Richard C. Greening, BS
Senior Scientist
Veterinary Medicine R & D
Pfizer Animal Health
Kalamazoo, MI 49001 USA

Dated: 16 June 2008 Signed: John R. Chenault

John R. Chenault, PhD
Associate Director
Veterinary Medicine R & D
Pfizer Animal Health
Kalamazoo, MI 49001 USA

Dated: 16 June 2008 Signed: Joseph A. Robinson

Joseph A. Robinson, PhD
Research Fellow
Veterinary Medicine R & D
Pfizer Animal Health
Kalamazoo, MI 49001 USA

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OVERVIEW

In accordance with the Code of Federal Regulations (21CFR 25.15(a)), "All applications or petitions requesting agency action require the submission of an EA [environmental assessment] or a claim of Categorical Exclusion." A Finding Of No Significant Impact (FONSI) for the concurrent use of EAZI-Breed™ CIDR® Cattle Insert (CIDR Insert) and Lutalyse® Sterile Solution (Lutalyse SS) for synchronization of estrus in dairy cows is sought for this submission. The concurrent use of the CIDR Insert and Lutalyse SS in dairy cattle complies with CFR 25.33(c) and in question two of the FDA/CVM Guidance #89 for Industry Phase I Decision Tree, ("2. *Is the VMP a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment? Yes*, then *STOP*") i.e., the active ingredients are natural substances, progesterone and prostaglandin $F_{2\alpha}$, with no significant environmental exposure expected. In this EA, it will be demonstrated that the amount of progesterone, the active ingredient in the CIDR Insert potentially excreted by the cow, is insignificant relative to natural production from cows not treated with the CIDR Insert. Therefore, CIDR Insert and Lutalyse SS are Phase I products and a full Phase II assessment is not required. Nevertheless, since progesterone is a hormone and that given the concerns for the impact of hormones in the environment, the potential toxicity of progesterone in the environment is assessed. It is concluded that the concurrent use of CIDR Insert and Lutalyse SS for the indications described in this EA will not cause harm to the environment when used according to label instructions. Also, the information in this EA provides justification for a FONSI under the aforementioned regulation.

1. PHASE I ASSESSMENT

1.1. Introduction

With this submission, Pharmacia and Upjohn, a division of Pfizer Inc. (P&U) applies for the registration of the concurrent use of CIDR Insert brand of progesterone releasing intravaginal Insert and Lutalyse SS brand of dinoprost tromethamine. Approval of this application will allow for concurrent use of these two products. P&U is not requesting approval for a new product or a combination of two previously approved products. It is only for approval of concurrent use of the two marketed products.

The approach taken in evaluating the potential of this concurrent use to impact the environment was based on the FDA/CVM Guidance for Industry #89, "VICH GL6 (Ecotoxicity Phase I) guideline on Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) - Phase I (June 2000)". Additional information is provided in this EA related to the potential impact of progesterone and its related metabolites on non-target aquatic species in the environment.

1.2. Indication

CIDR Insert and Lutalyse SS will be used concurrently for synchronization of estrus in dairy cows.

1.3. Composition, Physico-chemical and Pharmacological Properties

1.3.1. Composition

1.3.1.1. CIDR Insert

CIDR Insert is an intravaginal progesterone releasing insert consisting of a "T" shaped nylon spine, the body of which is approximately 13.5 cm long and the "wings" are each approximately 7.5 cm in length. The device is coated by injection molding with a 1 mm thick layer of silicone rubber containing 1.38 g progesterone [1]. The wings are closed during insertion by utilization of an insertion applicator. The CIDR Insert is equipped with a polyester "tail" to facilitate removal from the vagina at the end of the seven-day administration period. In the animal, as progesterone is absorbed by the vaginal mucosa from the surface of the CIDR Insert, progesterone deeper in the silicone rubber continuously diffuses toward the reduced concentrations nearer the surface of the CIDR Insert.

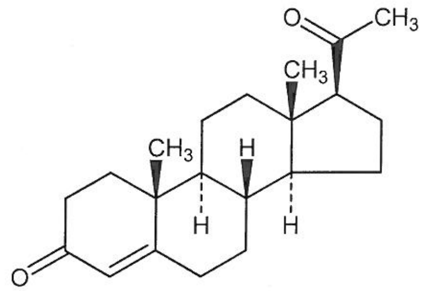
1.3.1.2. Lutalyse SS

Lutalyse SS is a clear, sterile, aqueous solution for injection. Each mL of Lutalyse SS contains 5 mg dinoprost (as the tromethamine salt), 1.65% benzyl alcohol as a preservative and water.

1.3.2. Physico-chemical properties

1.3.2.1. Progesterone

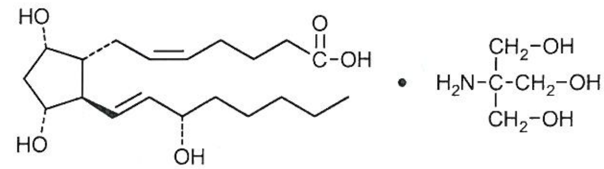
International non-proprietary name:	Progesterone
Chemical Abstracts Service (CAS) name and number:	Pregn-4-ene-3,20-dione; 57-83-0 [2]
Compound classification:	Progestogen
Synonyms and company identification number:	Progesterone (8CI); Δ4-Pregnene-3,20-dione; Agolutin; Bio-luton; CIDR Insert; Corlutin; Corlutina; Corluvite; Corporin; Corpus luteum hormone; Crinone; Cyclogest; Duraprogen; Estima; Flavolutan; Fologenon; Gesterol; Gestiron; Gestone; Gestormone; Gestron; Glanducorpin; Gynlutin; Gynolutone; Hormoflaveine; Hormoluton; Lipo-Lutin; Lucorteum Sol; Lugesteron; Luteal Hormone; Luteinique; Luteocrin normale; Luteodyn; Luteogan; Luteohormone; Luteol; Luteopur; Luteosan; Luteostab; Luteovis; Luteum; Lutex; Lutidon; Lutin; Lutociclina; Lutocuclin M; Lutocyclin; Lutocyclin M; Lutocylin; Lutoform; Lutogyl; Lutren; Lutromone; NSC 64377; NSC 9704; Nalutron; Percutacrine Luteinique; Piaponon; Primolut; Progeffik; Progekan; Progestan; Progestasert; Progesterol; Progestin; Progestogel; Progestol; Progeston; Progestone; Progestron; Prolets; Prolidon; Proluton; Prometrium; Prontogest; Protormone; Syngesterone; Syngestrets; Syntolutan; Utrogest; Utrogestan; Vitarrine [3];U-3672

Molecular formula:	$C_{21}H_{30}O_2$
Molecular weight:	314.46
Structure:	
Water solubility:	The aqueous solubility of progesterone is 7.3 mg/L [4].
Octanol/water partition coefficient (K_{OW}):	The log K_{OW} for progesterone is 3.87 [5].
Soil Binding (K_{OC})	The mean K_{OC} for progesterone measured in five soils is 8,248 [6].
Vapor pressure:	The estimated vapor pressure of progesterone is 2.69E-6 mm Hg at 25°C [5]. Thus, progesterone is not likely to partition into the atmosphere.

1.3.2.2. Dinoprost tromethamine

International non-proprietary name:	Dinoprost tromethamine
Chemical Abstracts Service (CAS) name and number:	(5Z,9.alpha.,11.alpha.,13E,15S)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid, tromethamine salt; #38562-01-5 [7]
Compound classification:	Oxytocic; abortifacient
Synonyms and company identification number:	7-[3,5 dihydroxy-2-(3-hydroxy-1-octenyl)cyclopentyl]-5-heptenoic acid, tromethamine salt; 583e; 5-Heptenoic Acid, 7-(3,5-Dihydroxy-2-(3-Hydroxy-1-Octenyl)Cyclopentyl)-, Tham; Pgf2-Alpha Tham; Pgf2-Alpha Tris salt; Pgf2-Alpha Tromethamine; Pgf2-Alpha Tromethamine salt; Prostaglandin F2-Alpha Tham; Prostaglandin F2a Tromethamine; Prostaglandin F2-Alpha Tham salt; Prostaglandin F2-Alpha Tromethamine; Prostaglandin F2-Alpha- Tham salt; Tham Pgf2-Alpha; Tromethamine Prostaglandin F2-Alpha; Prostin F_2 Alpha; dinoprost; PGF2.alpha.; Enzaprost F; Glandin; Lutalyse; Prostarmon F; Pronalgon F; U-14583; U-14,583E; PNU-0014583E [7, 8]
Molecular formula:	$C_{20}H_{34}O_5 \cdot C_4H_{11}NO_3$
Molecular weight:	475.62

Structure:



Water solubility: Dinoprost tromethamine is readily soluble in water to at least 200 mg/mL [7].

Melting point: The melting point for dinoprost tromethamine is 100 – 101°C [8].

Vapor pressure: The estimated vapor pressure of dinoprost is 4.26E-12 mm Hg at 25°C [9]. Thus, dinoprost is not likely to partition into the atmosphere.

1.3.3. Pharmacological properties

1.3.3.1. Progesterone

The CIDR Insert delivers progesterone at a controlled rate across the vaginal mucosa into the blood stream. This suppresses the release of gonadotrophin releasing hormone (GnRH) and consequently luteinizing hormone (LH) from the anterior pituitary, inhibiting follicle maturation and thus, controlling the estrous cycle. After removal of the device, circulating plasma levels of progesterone fall precipitously within 6 h, allowing follicle maturation, behavioral estrus, and ovulation [10].

1.3.3.2. Dinoprost

The therapeutic use of dinoprost (natural prostaglandin $F_{2\alpha}$, ($PGF_{2\alpha}$)) in food producing animals is to induce regression of corpora lutea. This response is observed in many mammals. The intracellular mechanism by which dinoprost induces luteolysis remains unknown. Various other physiological responses to dinoprost are known. These include stimulation of smooth muscle including vascular, bronchial, uterine and gastrointestinal muscle. Following administration, dinoprost tromethamine is rapidly dissociated to dinoprost ($PGF_{2\alpha}$). This compound has an extremely short half-life in plasma of only a few minutes in the bovine [11] and is extensively metabolized in mammals by lung and liver [12]. Near complete clearance occurs on one to two passages through the lungs or liver. No accumulation of dinoprost or residues has been observed in plasma following repeated daily injections in cattle [13]. Highest tissue concentrations of dinoprost are observed at the injection site which deplete to background concentrations by 24 - 48 h post-injection. Residue concentrations in milk of cows peak at 2 h post-injection (0.47 ppb) and decrease rapidly thereafter, with all other post-treatment values equivalent to the pre-treatment value (0.10 ppb) [14]. All (96%) of the ^{3}H-radiolabeled dinoprost was excreted in the urine, feces and milk of cows within 48 h of administration and metabolism of biologically active material by 72 h was complete as the radioactivity was found to be in the form of tritiated water [15].

1.4. Posology and Method of Administration

Concurrent use of CIDR Insert and Lutalyse SS will be used primarily by commercial dairy cattle breeders who use artificial insemination as a means of improving the genetics of their herds. A single CIDR Insert is administered into the vagina by means of an insertion applicator. The CIDR Inserts are allowed to remain in the vagina for seven days. In a study using CIDR Inserts containing 1.34 g progesterone, *ca.* 0.62 g (46.3%) of the progesterone was absorbed by the cow during a seven-day administration period [10]. Each intramuscular dose of 5 mL Lutalyse SS contains 25 mg dinoprost. A single dose is administered at the time of CIDR Insert removal.

1.5. Proper Disposal of CIDR Insert and Limited Potential for Environmental Exposure

Upon removal from the cow, the used CIDR Insert, will still contain some unabsorbed progesterone. To instruct the user in proper disposal of the CIDR Insert, the product label provides additional instructions for environmental safety and disposal. The truncated label language applicable to the environment is as follows:

> "Human Warning: Avoid contact with skin by wearing protective gloves when handling the EAZI-BREED CIDR Cattle Inserts. Keep this and all medications out of reach of children."
>
> "Environmental Warning: Store used (removed) EAZI-BREED CIDR Cattle Inserts in a plastic bag or other sealable container until they can be properly disposed in accordance with applicable local, state and Federal regulations."
>
> "DIRECTIONS: Used (removed) EAZI-BREED CIDR Cattle Inserts still contain some progesterone. Used EAZI-BREED CIDR Cattle Inserts must be stored in a sealable container until disposed. Sealed bag/container with used EAZI-BREED CIDR Cattle Inserts must be properly disposed in accordance with applicable local, state and Federal regulations."

There is a potential for progesterone to migrate from the CIDR Insert into the environment if it is improperly disposed. However, this potential is minimized by the physical properties of progesterone. For progesterone to be exposed to the environment, it would be dependent on water coming in contact with the surface of the CIDR Insert. Because the solubility of progesterone in water is limited (7.3 mg/L [4]), this reduces the potential for progesterone to diffuse out of the CIDR Insert. Progesterone has a high K_{oc} value that makes it relatively immobile in soil. The mean K_{oc} for progesterone in five soils was 8,248 [6]. Therefore, it is unlikely that the progesterone in the CIDR Inserts left on the ground would migrate into surface waters.

In addition, when inserts are stored in a plastic bag or other storage container prior to disposal, as required by the product label, the water barrier provided by the plastic bag or container will eliminate the potential for progesterone to reach the environment. Also, when disposed in its proper disposal packaging, the inserts will pose no risk to the environment.

1.6. Estimation of Progesterone Absorption from CIDR Insert in Dairy Cattle

The intravaginal progesterone CIDR Insert is administered for 7 days, an interval shorter than the normal luteal phase of the estrous cycle when plasma concentrations of progesterone are high, naturally.

In the modern lactating US dairy cows administered a CIDR Insert in the absence of a corpus luteum, the concentration of progesterone detected in plasma (≤1 ng/mL) [16] does not exceed that observed during the luteal phase of the estrous cycle (5-10 ng/mL [17, 18] or during pregnancy (10 to 12 ng/mL) [18, 19]. Therefore, the amount of progesterone absorbed from the CIDR Insert and potentially excreted into the environment is less than that normally excreted by cattle on a daily basis. A total of 0.62 g of progesterone was absorbed from the intravaginal CIDR Insert during a 7-day insertion period [10], based on a CIDR Insert which contained 1.34 g progesterone; the equivalent amount absorbed for a 1.38 g CIDR Insert would be 0.64 g. This equates to 91.4 mg/day, which is considerably lower than the estimated 375 mg/day (Section 2.2) produced by dairy cows with a functional corpus luteum. Moreover, the amount of progesterone absorbed from a CIDR Insert (0.64 g) represents only 0.5% [= (0.64 g/119 g) x 100%] of the annual endogenous progesterone production by a dairy cow (Section 2.2).

Progesterone from the CIDR Insert is metabolized by the animal using the same metabolic pathways as progesterone from endogenous sources prior to excretion into the environment. Therefore, parent progesterone absorbed from the CIDR Insert is excreted by the animal into the environment as metabolites that have modified biological activity compared with that of progesterone.

For initial estimates of predicted environmental concentrations (PEC) of progesterone (Section 1.8), it is assumed that the CIDR Inserts will release 46.3% of 1.38 g (= 0.64 g) progesterone which is absorbed during a seven-day administration period, equivalent to 91.4 mg/day. The initial worst-case PEC estimates (Section 1.8) are later refined in the assessment based on data for progesterone metabolism and estimates of excretion.

1.7. PEC Definitions

Throughout this document predicted environmental concentrations (PEC) will be determined for manure (PEC_{manure}), soil (PEC_{soil}) and surface water (PEC_{water}). Worst-case PEC estimates that have not had any adjustment factors applied will have an additional subscript added ($PEC_{manure\text{-}initial}$, $PEC_{soil\text{-}initial}$ and $PEC_{water\text{-}initial}$). Later in this document an adjustment factor will be applied to these initial PECs to adjust for the amount excreted due to metabolism ($PEC_{manure\text{-}metab}$, $PEC_{soil\text{-}metab}$ and $PEC_{water\text{-}metab}$). Additionally, for surface water PEC values, an additional adjustment factor that uses the compound's K_{oc} is applied to adjust the surface water concentration for equilibrium with the underlying sediment ($PEC_{water\text{-}metab\text{-}Koc}$).

1.8. Worst-Case Predicted Environmental Concentrations in Soil ($PEC_{soil\text{-}initial}$)

Calculations of the worst-case PEC in soil ($PEC_{soil\text{-}initial}$) of the active ingredients of CIDR Insert and Lutalyse SS, *viz.*, progesterone and dinoprost, respectively, are presented below. The calculations are based on the following worst-case assumptions:

- 12-month breeding cycle (typical calving interval in the US is 13-14 months [20]
- 100% of the cows within the herd will be treated
- one CIDR Insert will be used per cow per year for this concurrent use
- one injection of Lutalyse SS per cow per year for this concurrent use
- dairy cows are bred uniformly throughout the year
- 100% of excreted dose of the active ingredients is parent drug
- 27200 kg manure/acre
- no biodegradation of progesterone or dinoprost in manures or soils
- manures applied to soil; average plow depth is 15 cm

The PEC_{soil} of progesterone and dinoprost are calculated based on the methods described in Draft 10 AHI/FDA Forum Guidance [21].

A: Amount of active ingredient/kg excreta ($PEC_{manure\text{-}initial}$)

- Animal: dairy cows; mean body weight (BW) = 500 kg
- Manure production period: 90 d
- Concentration of active ingredient in excreta: a = (b x c x d)/e, where;
  a = $PEC_{manure\text{-}initial}$ = wet weight concentration of active ingredient in manure in µg/kg
  b = total dose administered to each animal/day = 91.4 mg progesterone and 25 mg dinoprost
  c = fraction of animals treated: (assuming all cows treated = 1.0)
  d = number of days animals are treated (= 7 days for progesterone and 1 day for dinoprost)
  e = total amount of manure produced per animal during manure production period = (31.8 kg/day x 90 days = 2862 kg)
  a = Progesterone $PEC_{manure\text{-}initial}$ = (91.4 x 1.0 x 7)/2862 = 0.224 mg/kg excreta
  = Dinoprost $PEC_{manure\text{-}initial}$ = (25 x 1.0 x 1)/2862 = 0.00874 mg/kg excreta

B: Concentration of active ingredient in soil ($PEC_{soil\text{-}initial}$)

- Amount of cow manure applied to 1 acre of land: 27200 kg
- Weight of soil in an acre which is 15 cm (6") deep: 910500 kg
- $PEC_{soil\text{-}initial}$ = ($PEC_{manure\text{-}initial}$ x kg of manure/acre)/910500 kg of soil
- Progesterone $PEC_{soil\text{-}initial}$ = (0.224 x 27200)/910500 = 0.00669 mg/kg = 6.69 µg/kg
  Dinoprost $PEC_{soil\text{-}initial}$ = (0.00874 x 27200)/910500 = 0.000261 mg/kg = 0.26 µg/kg

The worst-case $PEC_{soil\text{-}initial}$ values of 6.69 µg/kg and 0.26 µg/kg are approximately 15-fold and 380-fold lower than the VICH Phase I trigger limit of 100 µg/kg for progesterone and dinoprost, respectively.

1.9. Worst-Case Predicted Environmental Concentrations in Water ($PEC_{water\text{-}initial}$)

A conservative estimation of the amount of a compound reaching surface water from runoff can be calculated assuming that 1% of the total drug per acre applied to 10 acres of soil moves into a 1 acre pond which is 2 m deep (AHI/CVM ERA Forum guidance Draft 10 [21]). The equation used to calculate these values is as follows:

1.9.1. Calculation of progesterone $PEC_{water\text{-}initial}$

$$\text{Progesterone } PEC_{water\text{-}initial} = \frac{PEC_{soil}\ \mu g/kg \times 9.1 \times 10^{5}\ kg/acre \times 0.01 \times 10\ acre}{8.1 \times 10^{6}\ L}$$

$$= \frac{6.69\ \mu g/kg \times 9.1 \times 10^{5}\ kg/acre \times 0.01 \times 10\ acre}{8.1 \times 10^{6}\ L}$$

$$= 0.075\ \mu g/L \text{ or } 75\ ng/L$$

1.9.2. Calculation of dinoprost $PEC_{water\text{-}initial}$

$$\text{Dinoprost } PEC_{water\text{-}initial} = \frac{PEC_{soil}\ \mu g/kg \times 9.1 \times 10^{5}\ kg/acre \times 0.01 \times 10\ acre}{8.1 \times 10^{6}\ L}$$

$$= \frac{0.26\ \mu g/kg \times 9.1 \times 10^{5}\ kg/acre \times 0.01 \times 10\ acre}{8.1 \times 10^{6}\ L}$$

$$= 0.0029\ \mu g/L \text{ or } 2.9\ ng/L$$

1.9.3. PECwater-initial summary

The worst-case $PEC_{water\text{-}initial}$ values for progesterone and dinoprost are calculated to be 75 ng/L and 2.9 ng/L, respectively.

1.10. Phase I Conclusions

The information provided in this EA is submitted in support of the registration of the concurrent use of CIDR Insert and Lutalyse SS in dairy cattle. The approach taken in evaluating the potential for the active ingredients of the products to impact the environment was based on the FDA/CVM Guidance for Industry #89, VICH GL6 (Ecotoxicity Phase I) guideline on Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) - Phase I (June 2000). Worst-case $PEC_{soil\text{-}initial}$ calculations were done for progesterone and dinoprost; the $PEC_{soil\text{-}initial}$ values were 6.69 and 0.26 µg/kg, respectively, which are well below the VICH Phase I trigger limit of 100 µg/kg. The worst-case $PEC_{water\text{-}initial}$ values for progesterone and dinoprost are calculated to be 75 ng/L and

2.9 ng/L, respectively. The concurrent use of CIDR Insert and Lutalyse SS in dairy cattle is aligned with question 1 of the Phase I Decision Tree, ("*1. Is the VMP exempt from the need for an EIA by legislation and/or regulation? Yes,* then *STOP*"). Additionally, the progesterone in the CIDR Insert and dinoprost in Lutalyse SS should be classified as among those VMPs in compliance with CFR 25.33(c) "… substances that occur naturally in the environment", and in question two of the Phase I Decision Tree, ("*2. Is the VMP a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment? Yes,* then *STOP*") i.e., they are natural substances with no significant environmental exposure expected. The amount of CIDR Insert progesterone absorbed and then potentially excreted by the cow is insignificant (0.5%) relative to an untreated cow's yearly endogenous progesterone production. Therefore, CIDR Insert and Lutalyse SS are Phase I products and have a low potential to cause environmental effects.

Although the CIDR Insert conforms with the criteria for a Phase I product and the assessment would normally stop at this point, there is, nevertheless an elevated awareness of the potential impact of steroidal hormones in the environment. Therefore, this assessment is expanded to include additional information on progesterone in the environment from the use of the CIDR Insert.

2. METABOLISM AND EXCRETION OF PROGESTERONE

2.1. Overview of Progesterone

The progesterone in the CIDR Insert is indistinguishable from naturally produced progesterone and the release of progesterone from the CIDR Insert does not significantly change physiological concentrations (<1 ng/mL) of progesterone in plasma [16]. Thus, progesterone from the CIDR Insert is metabolized by the cow via the same metabolic pathways as is used for endogenously produced progesterone.

Studies have demonstrated that during administration of CIDR Inserts, the concentration of progesterone in the plasma from modern US lactating dairy cows does not exceed physiological concentrations of <1 to about 12 ng/mL [16, 17, 18, 19]. In fact, in the modern US dairy cow, the CIDR Insert provided about an additional 1 ng/mL in plasma [16]. In lactating New Zealand dairy cows in which endogenous production of progesterone was blocked, the mean concentration of progesterone in plasma during the last 7 days of a 10 day CIDR Insert administration period was 1.3 ng/mL and the peak daily concentration occurred on day 1 after administration, and averaged <2 ng/mL [22]. The concentration of progesterone in plasma during the luteal phase of the estrous cycle varies from about 5 to 10 ng/mL (see Section 2.2). When CIDR Inserts were administered to estrous cycling lactating cows during the luteal phase of the estrous cycle, when the concentration of progesterone is highest, the progesterone concentration in fat free milk never exceeded the concentration observed in pregnant cows and increased concentrations of progesterone ≤1 ng/mL during the 7 day administration period [23, 24].

Prior to excretion, progesterone is extensively metabolized by the cow to many metabolites. [25, 26, 27, 28]. Several metabolites of progesterone, including 5α and 5β reduced pregnanediones, pregnanolones, and pregnanediols, were detected in feces from cattle [28, 29], however, according to Schwarzenberger, "unmetabolized progesterone was barely present, if at all" [28]. Over several decades, researchers have been unable to detect progesterone in any significant amounts in fecal samples of mammals. Attempts to measure progesterone in feces as a means to determine reproductive status of various mammals have largely been unsuccessful. This led to the development of a group of non-specific antibodies to metabolites of progesterone to be used in assays for this purpose. The estimated yearly excretion of gestagens by cycling and pregnant cattle in the US is 4 g/cow, a value derived from the data of Lange, et al. [Table 3 of reference 30]. The "progesterone/progestins" found in feces as reported in some literature citations [22, 29] actually consists of various metabolites of progesterone including 20α-OH-pregnanes, 20β-OH-pregnanes, 20-oxo-pregnanes, pregnanediol, and five reduced progesterone metabolites, all progestins that cross reacted with the group-specific antibodies used in the assays [22]. Additionally, no progesterone activity above the detection limit of the assay (<0.17 ng/mL) was seen in manures from dairy or beef cattle operations when a sensitive and specific progesterone receptor gene transcription activity assay was used [31].

Recently, Isobe et al. [32] reported a double extraction preparation process for fecal samples that removes progesterone metabolites and allows measurement of progesterone in feces. This paper reports the mean of the highest concentration of progesterone in feces from beef cows during the luteal phase of the estrous cycle was less than 100 ng/g wet feces (Fig. 4 of Isobe et al. paper [32]) with the highest concentration determined in an individual cow to be 140 ng/g (Fig. 5 in Isobe et al. paper).

2.2. Estimation of Endogenously Produced Progesterone in Dairy Cattle

An estimate of the amount of progesterone endogenously produced by a dairy cow annually in one year is as follows. The duration of the estrous cycle in cattle averages 21 days and gestation averages 282 days. During the estrous cycle, plasma progesterone increases to above 5 ng/mL on about day 8, and is about 10 ng/mL on days 10 to 18 declining thereafter during regression of the corpus luteum [19, 33, 34]. Plasma progesterone concentrations remain at about 10 ng/mL throughout gestation [18, 19, 35]. Plasma concentrations of progesterone in lactating dairy cows may be somewhat lower than that observed in beef cattle [34], at least in part due to increased rate of progesterone metabolism. The high feed intake to maintain high milk production in modern dairy cattle results in increased hepatic blood flow with attendant increased progesterone metabolism [36, 37].

A direct estimate of progesterone production by the corpus luteum of cattle could not be found in the scientific literature. However, it can be calculated from existing data utilizing a pharmacological approach based on progesterone metabolic clearance rate. At steady state, the input of a given substance will equal the output, or in this case, metabolism of the substance. This is one definition of steady state. Progesterone is at or very nearly at steady state during the mid-luteal phase of the estrous cycle of cattle. Progesterone metabolic clearance rate (MCR) was measured as 3485 L/h in fed lactating Holstein dairy cows, based on 1 – 4 h after-feeding measurements for cows fed 1.5X and 2.2X maintenance diets; an MCR of 2767 L/h was determined in non-fed cows during the same period [37]. For this exercise, a value of 3126 L/h (mean of fed and non-fed) will be used. Using plasma progesterone concentration of 5 ng/mL during the mid-luteal phase of the estrous cycle and during pregnancy in dairy cows, the total clearance rate of progesterone is 5 ng/mL X 3126 l/h = 15.63 mg/h or 375 mg/day. Therefore, the steady state production of progesterone is 375 mg/day for dairy cows. These are low estimates because the plasma concentrations of progesterone during both the luteal phase of the estrous cycle and during gestation are above 5 ng/mL in dairy cows.

During a production cycle, dairy cows would be expected to be in anestrum for about 20 days, cycle three times before becoming pregnant and then are pregnant for 282 days. Assuming no progesterone production during anestrum, 12 days of high progesterone concentration during the estrous cycle (days 6 through 17), three estrous cycles before becoming pregnant (12 x 3 = 36 days), 282 days of high progesterone during gestation, and a daily production of 375 mg progesterone during the luteal phase of the estrous cycle and during pregnancy, the annual production of progesterone is estimated to be 119 g per animal [(282 + 36) days x 375 mg/day = 119 g per animal].

2.3. Determination of CIDR Insert-Derived Progesterone Excretion in Manure after Metabolism in the Dairy Cow

An estimate of the percent of progesterone released from the dairy cow as parent compound derived from the CIDR Insert can be made by using data from Isobe et al. [32] where plasma concentrations of progesterone were correlated to fecal concentrations. Then, knowing the daily clearance rate of plasma progesterone, an estimate of the percentage progesterone excreted can be estimated as follows. The mean of the highest concentration of progesterone in feces from beef cows during the luteal phase of the estrous cycle was less than 100 ng/g wet feces (Fig. 4 of Isobe et al. paper [32]) with the highest concentration determined in an individual cow to be 140 ng/g (Fig. 5 in Isobe et al. paper). For this exercise, it is assumed that the fecal concentration of progesterone in beef cows approximates that of dairy cows. As described in Section 1.8, the daily fecal output for dairy cattle used in this risk assessment is 31.8 kg/day. Therefore, 4.5 mg/day (140 ng/g x 31.8 kg/d) is the maximum mass of progesterone excreted by cows during the luteal phase of the estrous cycle. The estimate of progesterone clearance from cattle is 375 mg/day (Section 2.2). Therefore, a conservative estimate of the amount of the progesterone synthesized per day and ultimately excreted as parent compound is estimated as (4.5 mg/375 mg) x 100% = 1.20%. Progesterone excreted by pregnant cows would be expected to be higher than the amount excreted by cows in the luteal phase of the estrous cycle.

Another way to confirm an estimate of the amount of progesterone that is excreted as parent is to use data from a separate study in which a CIDR Insert was administered to dairy cattle and the endogenous production of progesterone was blocked so that endogenous production would not interfere with the progesterone absorbed and metabolites produced from the CIDR Insert [22]. In that study, the authors measured fecal progestins because the assay was not specific for progesterone in feces. Their plasma progesterone data indicated that the plasma levels of progesterone resulting from CIDR Insert use had a mean of 1.25 ng/mL for a 10 day CIDR Insert administration period (Table 1 of Reference 22), and approximately 1.5 ng/mL for the first 7 days (Figure 2a of Reference 22). Again, in the paper by Isobe et al. [32], the plasma concentrations of progesterone were correlated with the fecal concentrations of progesterone. Also, Isobe et al. used an assay for progesterone that was not as sensitive to interference by metabolites or to environmental cross reaction. From Figure 3 of Isobe et al. [32] a plasma concentration of 1.2 - 1.5 ng/mL would produce a fecal progesterone concentration of approximately 30 ng/g. With 31.8 kg of manure produced per day, this plasma level corresponds with approximately 0.95 mg/day of progesterone excreted in the feces. Since 91.4 mg/day of progesterone is absorbed from the CIDR Insert (Section 1.6) and approximately 0.95 mg/day of progesterone from an Insert is excreted in the feces, only 1.0 % [(0.95 mg/91.4 mg) x 100%], of the progesterone absorbed from the CIDR Insert is excreted as parent.

In summary, if a prediction of the amount of progesterone absorbed from the CIDR Insert and then excreted intact is made based on the estimate of daily endogenous production, about 1.2% is excreted as parent progesterone. In a completely separate calculation, based on actual measured fecal progesterone levels and its correlation to blood plasma levels, along with actual measurements of progesterone in the plasma of CIDR Insert treated cattle with endogenous production blocked, it is estimated that 1.0% of the CIDR Insert progesterone absorbed being excreted as parent progesterone. The mean of these two values is 1.1% parent progesterone or in other words, 98.9% of absorbed CIDR Insert progesterone is transformed into metabolites.

3. PROGESTERONE PEC REFINEMENTS FOR METABOLISM PRIOR TO EXCRETION IN MANURE

The initial progesterone PEC calculations from Section 1.8 (0.224 mg/kg excreta and 6.69 μg/kg soil) are large overestimates of potential concentrations of progesterone since these calculations assume 100% of absorbed drug is excreted as intact progesterone and that 100% of cows are treated in a 90 day period. Also, progesterone degradation in manure, soil and water would further reduce these estimates.

In Section 2.3 the percent of progesterone absorbed from a CIDR Insert and excreted as progesterone in feces was calculated to be 1.1%. This metabolism value will be used to refine the worst-case ($PEC_{initial}$) estimates for manure, soil and water that were derived in Section 1.8.

3.1. Calculation of Progesterone PEC_{manure} and PEC_{soil} Refined for Metabolism

$PEC_{manure\text{-}metab}$ = $PEC_{manure\text{-}initial}$ x 1.1% excreted / 100%

= 0.224 mg/kg excreta x 0.011 = 0.0025 mg progesterone/kg excreta

$PEC_{soil\text{-}metab}$ = $PEC_{soil\text{-}initial}$ x 1.1% excreted / 100%

= 6.69 μg/kg soil x 0.011 = 0.074 μg progesterone/kg soil (74 ng/kg)

3.2. Calculation of Progesterone PEC_{water} Refined for Metabolism and Sediment Adsorption

A conservative estimation of the amount of a compound reaching surface water from runoff can be calculated assuming that 1% of the total drug per acre applied to 10 acres of soil moves into a 1 acre pond which is 2 m deep (AHI/CVM ERA Forum guidance Draft 10 [21]). The equation used to calculate these values is as follows:

$$\text{Progesterone } (PEC_{water\text{-}metab}) = \frac{PEC_{soil\text{-}metab}\ \mu g/kg \times 9.1 \times 10^5\ kg/acre \times 0.01 \times 10\ acre}{8.1 \times 10^6\ L}$$

$$= \frac{0.074\ \mu g/kg \times 9.1 \times 10^5\ kg/acre \times 0.01 \times 10\ acre}{8.1 \times 10^6\ L}$$

$$= 0.000831\ \mu g/L \text{ or } 0.831\ ng/L$$

Consistent with the AHI/CVM ERA Forum guidance Draft 10 [21], PEC_{water} can be further refined due to adsorption to sediment since the K_{oc} (average of five soils K_{oc} = 8248) is known for progesterone [6].

$$\text{Progesterone } PEC_{water\text{-}metab\text{-}Koc} = \frac{8.3 \times 10^6 \times PEC_{water\text{-}metab}}{8.1 \times 10^6 + (3.0 \times 10^5 \times K_d)}$$

$$= \frac{8.3 \times 10^6 \times 0.831 \text{ ng/L}}{8.1 \times 10^6 + (3.0 \times 10^5 \times 239.19)}$$

$$= 0.086 \text{ ng/L}$$

where: Kd is the partition coefficient (units of mL of soil water per g of soil) for this chemical = 0.029 x average Koc [21] = 0.029 x 8,248 = 239.19, assuming equilibration of the compound in water within the top 5 cm of sediment

3.3. Dairy Management Practices Spread Use Throughout the Year Providing Dilution from Untreated Cattle and Endogenous Progesterone Production

3.3.1. Dilution of manure from CIDR Insert treated cattle with manure from untreated cattle

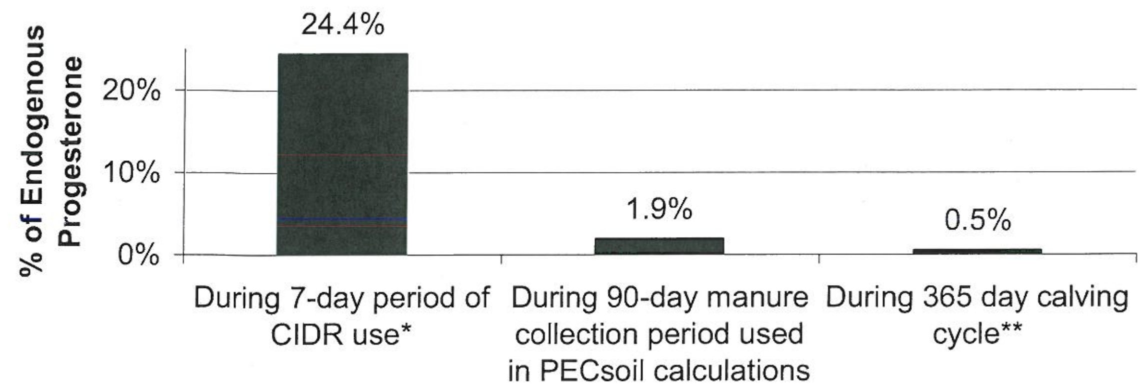
Dairy cattle calving management practice differs from beef cattle calving. In beef cattle, the majority of births are in the spring. However, with dairy, these births are evenly spread out over the 14 month birthing cycle so that there is usually a similar amount of animals producing milk at one time. Therefore, the scenario of 100% animal herd treatment (Section 1.8) at any given manure collection interval is not realistic. Since there are about 426 days per 14 month calving interval, even with 100% herd treatment, on average, only [(7 days/426 days) x 100%] = 1.64% of the herd would be treated within any 7-day CIDR Insert use window. For the typical 90-day manure collection period that is used in the Phase I EA PEC_{soil} calculations (Section 1.8), only [(90 d/426 days) x 100%] = 21% of the herd, on average, would be treated in a 90-day window.

In this EA, a value of 100% herd treatment was used. No refinement was applied to the PEC calculations for 21% herd treatment; hence the PEC estimates are conservative.

3.3.2. Dilution of CIDR Insert derived progesterone with progesterone endogenously produced by dairy cattle

The amount of progesterone released from a CIDR Insert during the 7-day administration (91.4 mg/day x 7 days = 640 mg) is inconsequential [(0.640 g/119 g) x 100% = 0.5%] relative to the amount of progesterone that an individual dairy cow makes each year, estimated to be 119 g (Section 2.2). The calculated daily absorption, 91.4 mg, is 24.4% of the estimated daily endogenous production (375 mg/day) of progesterone (Figure 1). Furthermore, the amount absorbed over the 7-day period (640 mg) is only 1.9% of the endogenous progesterone production that would occur during the typical 90 day manure collection period (375 mg x 90 days) used for PEC_{soil} calculations in this EA document (see Figure 1 below).

Figure 1. The Amount of CIDR Progesterone Absorbed as a Percentage of Endogenous Production in an Individual Dairy Cow



* During the 7-day period of CIDR Insert use, only 1.64% of the whole herd (on average) is treated at any one time. Therefore, CIDR Insert progesterone would be diluted by endogenous progesterone production of untreated cows. During the 90 and 365 day periods, the CIDR Insert progesterone is further diluted by endogenously produced progesterone beyond what is absorbed during the 7-day use period.

** Although a yearly production cycle is presented herein, actual management practice is for a 14 month cycle which would further reduce this estimate.

3.4. Summary of Refined Progesterone PEC Values

After refinement for metabolism the $PEC_{soil\text{-}metab}$ for progesterone is 74 ng progesterone/kg soil. After refinement for both metabolism and adjustment for sediment binding, the progesterone $PEC_{water\text{-}metab\text{-}Koc}$ is 0.086 ng/L. These estimates do not take into account any degradation that may take place in manure storage systems or biodegradation in soil or water. Also, we would expect only 21% of cows in a herd to be treated during a 90-day period, therefore, it is reasonable to assume that the manures from cows which receive the CIDR Insert concurrently with Lutalyse SS would be further diluted with manures from other categories of cattle that would not receive either of these products. Therefore, the actual environmental concentrations in soil and water are likely lower than those shown above and the calculated PEC values are very conservative. In addition, the average amount of progesterone absorbed by a cow given a CIDR Insert will represent only 1.9% of that produced endogenously by a dairy cow in the 90-day manure collection period. Consequently, the amount of progesterone from the CIDR Insert excreted by the cow would be negligible in contrast to the natural endogenous production rate of progesterone.

4. SURFACE WATER ASSESSMENT

In Phase I of this assessment it was shown that the CIDR Insert and Lutalyse SS for the indications described in this EA were Phase I products and will likely not cause harm to the environment when used according to label instructions.

The scientific literature has demonstrated that some hormones can cause adverse effects on fish in aquatic environments (see Section 4.4). In areas around paper mills it has been shown that progesterone derived from the degradation of pulp-derived phytosteroids in sediments can potentially be transformed to androstenedione (AED). AED has been implicated in potentially causing effects on fish. Therefore, the potential for CIDR Insert progesterone to be transformed to AED and potential subsequent effects on fish will be included in this portion of the assessment.

4.1. Endogenous Sources of Progesterone and Androstenedione (AED) that Impact Surface Water Species

Because progesterone and metabolites of progesterone are naturally occurring compounds that enter the environment in large quantities from numerous natural sources (e.g. wild and domesticated mammals, plant degradation, male fish pheromones, etc.), it is difficult to assess potential toxic effects on fish because of the negligible amount of progesterone and progesterone metabolites reaching the environment arising from CIDR Insert administration relative to other endogenous sources of these compounds.

4.1.1. Endogenous progesterone production from cattle

Following the 7-day treatment with the CIDR Insert, the animal will become pregnant and start continuous endogenous production of progesterone. Since the absorbed progesterone from the CIDR Insert represents only 0.5% of the total progesterone produced in a year by a dairy cow, and only 1.9% of the progesterone produced over a 90-day manure collection interval (see Section 3.3.2), we would anticipate that endogenous environmental concentrations would be 1/1.9% = 53x to 1/0.5% = 200x higher than the CIDR Insert derived PEC_{water} concentrations. Therefore, the potential for toxicity effects from CIDR Insert derived progesterone is 53 to 200 times lower than that of endogenously produced progesterone from the same dairy cow.

Also, it has been hypothesized that microbial transformations of phytosterols produce progesterone and AED in the intestinal tracts of livestock [38]. Thus, the total endogenous production rate of progesterone could be a combination of animal and plant derived steroidal compounds.

4.1.2. Endogenous production of AED by fish

Since AED is a fish pheromone, it is produced by fish in surface water. It has been documented that male goldfish can release AED at a rate of 50 ng/hour [39]. Furthermore, the males released up to 1000 ng/h of AED when sexually aroused by females or their pheromones [39]. Water concentrations of AED around spawning salmon and concentrations discharged from fish hatcheries into rivers have been measured to be near 1 ng/L [40].

4.1.3. Phytosterols and AED produced from plant degradation via microbes

It has been documented that progesterone and AED are detected in the water column downstream from paper mill effluents at concentrations of 6.55 nM (2060 ng/L) and 0.14 nM (40 ng/L), respectively [41]. It is hypothesized that these steroidal compounds are produced from the microbial transformation of pulp-derived phytosterols in the paper mill effluent [42].

If the degradation of paper mill effluent can produce these compounds, then they are likely also produced in other surface water areas from plant material decomposition [43].

4.2. Progesterone and AED Transformation to Androstadienedione (ADD)

In the microbiologically mediated transformation of progesterone to AED from phytosterols in paper mill effluent, AED can be further transformed to ADD [42]. In these environments the concentration of progesterone is typically greater than AED, and the AED concentration is typically greater than ADD. For example, in the Fenholloway river sediment at the outflow from the mill settling ponds, the concentrations of progesterone, AED and ADD were measured at 150 nM (47,169 ng/L), 4 nM (1146 ng/L) and 2.6 nM (739 ng/L), respectively [42]. These measured concentrations were for sediment not overlying surface water. AED and ADD have been shown to have an equal potential to act as an agonist in mammalian androgen receptor assays [42], but both were still 100 times less potent than the positive control, dihydrotestosterone (DHT), which is an active metabolite of the hormone testosterone. It is therefore conservatively assumed that ADD has a similar potential as AED to cause androgen related effects. Potential aquatic effects tests have principally focused on AED, with very little information found in the literature on ADD following a literature search.

4.3. AED PEC_{water} Calculations Assuming All of the Progesterone Equivalents that Reach Surface Water are Converted to AED.

To calculate the amount of AED in surface water, if progesterone was to be 100% transformed to AED, the same series of equations used to calculate progesterone PEC_{water} are used. However, because the molecular weight of progesterone (314.46) differs from the molecular weight of AED (286.42), the PEC_{soil} value must first be transformed. The progesterone $PEC_{soil\text{-}metab}$ was determined to be 0.074 µg/kg (Section 3.1). Therefore, the initial $PEC_{soil\text{-}metab}$ for AED is 0.074 µg /kg X (286.42/314.46) = 0.067 µg/kg.

$$\text{AED } PEC_{water\text{-}metab} = \frac{PEC_{soil\text{-}metab}\ \mu g/kg \times 9.1 \times 10^5\ kg/acre \times 0.01 \times 10\ acre}{8.1 \times 10^6\ L}$$

$$= \frac{0.067\ \mu g/kg \times 9.1 \times 10^5\ kg/acre \times 0.01 \times 10\ acre}{8.1 \times 10^6\ L}$$

$$= 0.000753\ \mu g/L \text{ or } 0.753\ ng/L$$

Consistent with the AHI/CVM ERA Forum guidance Draft 10 [21], $PEC_{water\text{-}metab}$ can be further refined due to adsorption to sediment;

$$\text{AED } PEC_{water\text{-}metab\text{-}Koc} = \frac{8.3 \times 10^6 \times PEC_{water\text{-}metab}}{8.1 \times 10^6 + (3.0 \times 10^5 \times Kd)}$$

$$= \frac{8.3 \times 10^6 \times 0.753 \text{ ng/L}}{8.1 \times 10^6 + (3.0 \times 10^5 \times 152.19)}$$

$$= 0.12 \text{ ng/L}$$

where: Kd is the partition coefficient (units of mL of soil water per g of soil. Lee et al. [44] determined the mean Log Koc of AED in three soils to be 3.72 (Koc = 5248).
The Kd = 0.029 x average Koc [21] = 0.029 x 5248 = 152.19, assuming equilibration of the compound in water within the top 5 cm of sediment

4.4. Potential for Fish Masculinization Effects from Progesterone, AED or ADD

The data indicate that progesterone derived from dairy cattle is principally metabolized in the animal prior to elimination (Section 2), and is relatively immobile in soil (Section 1.5) so there is a very low potential for progesterone from dairy cattle to directly cause aquatic effects on fish. Current research is primarily focused on testosterone analogs for these effects. In a river receiving paper mill effluent, progesterone derived from phytosterols was found to be converted by environmental microorganisms into androgenic compounds that may masculinize female mosquitofish (*Gambusia holbrooki*) [41, 42]. Downstream from these these paper mills, progesterone was measured at approximately 6.55 nM (2060) ng/L [41]. However the experts in the field of fish masculinization have not focused on progesterone as a possible cause, but have reported on other hormones (e.g. AED).

From the calculations in Section 4.3, we estimate a $PEC_{water\text{-}metab\text{-}koc}$ of 0.12 ng/L for AED if all of the progesterone excreted from CIDR Insert-treated cows was to be transformed to AED after reaching surface water. Therefore, there would be no masculinization effects on fish since the lowest value reported to cause this effect is 1.4 nM (400 ng/L) of AED with the NOEC concentration tested at 40 ng/L [45]. The 400 ng/L concentration produced an effect on anal fin ray elongation, but did not affect gonadosomatic index, vitellogenin expression or ovarian area. These effect values are lower than values for masculinization effects concentrations reported by Bandelj et al. [46] where 10,000 and 100,000 ng/L for AED and ADD respectively, were required to see the effect. Therefore, the potential NOEC differs among authors but the $PEC_{water\text{-}metab\text{-}Koc}$ value of AED (0.12 ng/L) potentially derived from the CIDR Insert is still well below any observed effects concentration. Although AED has been implicated in the masculinization of mosquitofish adjacent to paper mills, it has been shown by Durhan et al. that AED was not the active component which caused the observed androgenic activity of this species [47].

It is difficult to separate out effects of CIDR Insert use from the environmental background production of progesterone or AED (see Section 4.1). The environmental and endogenous sources of progesterone and AED are so large in contrast to the amount potentially derived from a CIDR Insert, that if effects from environmental and endogenous production occurred, they would greatly overshadow any possible effects resulting from the use of the CIDR Insert.

In summary, the potential concentrations of progesterone, AED or ADD in surface water derived from the progesterone in the CIDR Insert and excreted into the environment from dairy cattle are not large enough to cause masculinization effects on fish.

4.5. Comparison of PEC_{water} Calculations to Observed Environmental Concentrations

Since the absorbed progesterone from the CIDR Insert represents only 0.5% of the total progesterone produced in a year and only 1.9% of the progesterone produced over a 90-day manure collection interval (see Section 3.3.2), we would anticipate that endogenous environmental concentrations of progesterone or AED would be 53x to 200x higher than the CIDR Insert derived PEC_{water} concentrations. Therefore, we would expect to see high surface water concentrations of progesterone from natural endogenous sources and potentially AED if endogenously produced progesterone from dairy cattle was transformed to AED. For example, with a progesterone $PEC_{water\text{-}metab\text{-}Koc}$ at 0.085 ng/L and endogenous progesterone production at 53x to 200x times higher than CIDR Insert derived progesterone, a predicted surface water concentrations from endogenous progesterone production by cattle would be 4.5 to 17 ng/L (0.085 x 53 to 0.085 x 200). This surface water prediction is not supported by field data from water sources around dairy farms. In a study where 32 surface water samples from around dairy farms were analyzed for seven different steroids, no progesterone or AED was detected above the limits of detection of the assays, which were 0.4 and 0.3 ng/L, respectively (summarized in Table 1 of reference 38 and the footnote in Table 2 of reference 40). In another study where steroid concentrations in a dairy waste lagoon were as high as 650 ng/L [40], samples from groundwater monitoring wells, surface water and tile drainage fields that were expected to be impacted by the dairy operations, there was no evidence that progesterone or AED were reaching these water sources. Therefore, there is no evidence that for the dairy cattle manure management scenario, the above calculations of PEC_{water} are appropriate since surface water concentrations of progesterone (or AED derived from progesterone) produced endogenously from dairy cattle do not reach levels of 4.5 to 17 ng/L adjacent to dairy operations. A possible reason for this discrepancy is that the above equations do not allow for the inclusion of physical properties (K_{oc} and solubility) of progesterone or AED that prevent it from moving though soil and reaching surface water, or allow for degradation in environmental matrices.

In a large survey on the occurrence of pharmaceuticals, hormones and other organic species in watercourses susceptible to urbanization and livestock production, progesterone was detected in only 3 of the 70 watercourses analyzed for progesterone [48]. In those samples in which progesterone was detected, the median concentration was 110 ng/L [48]. The sources of progesterone in these infrequent positive samples were not reported but are consistent with potential paper mill effluent concentrations of progesterone (Section 4.1.3).

5. ADDITIONAL INFORMATION ON FATE AND EFFECTS OF PROGESTERONE AND DINOPROST ON NON-TARGET ENVIRONMENTAL ORGANISMS

Following a comprehensive search of the scientific literature, no articles were found concerning the effects of dinoprost (prostaglandin $F_{2\alpha}$) on non-target environmental species. However, dinoprost is a natural substance and it is rapidly metabolized by the dairy cow. Should trace levels of dinoprost be found in excreta from dairy cows, they are not expected to have negative effects on non-target environmental species.

Following an additional comprehensive search of the scientific literature, only a few pertinent articles were found concerning the effects of progesterone on non-target environmental species. No articles were found on the effects of progesterone on terrestrial species. In an article concerning the effects of progesterone on aquatic invertebrates, it was concluded that at most, a minimal, transient effect was found on the male to female ratio of *Daphnia magna* offspring in a long-term (26 d) test at a concentration of 100,000 ng/L [49]. No effects of exogenous progesterone at 1 mg/L on adults or on the development of juvenile larvae of the estuarine copepod, *Acartia tonsa*, were seen [50]. Progesterone has a protective effect on young fish against heavy metals when tested at up to 250 mg/kg BW [51].

There is scant information on rates of progesterone mineralization in soil and water systems. Since progesterone is a natural compound produced by mammals, it is very likely that it is readily metabolized in the environment. The rates of environmental transformation will likely differ from environment to environment. A soil metabolism study was conducted with a synthetic progesterone analog, melengestrol acetate (MGA), a potent progesterone agonist (data previously submitted to the agency NADA 34-254, MGA® 100/200 Premixes, NADA 39-402, MGA® 500 Liquid Premix (Type A Medicated Articles), Melengestrol Acetate (MGA) for Suppression of Estrus for Heifers Intended for Breeding). The metabolism in soil study demonstrated the mineralization of MGA in soil, with half-life estimates in three soils of 4.3, 5.3 and 27.8 days. Also, progesterone is quickly degraded in sewage treatment plants [52] and by the microbiota of surface waters adjacent to sewage treatment plants and in rivers [53]. Although the potential for surface waters to metabolize progesterone are relevant to this assessment, the degradation rates in sewage treatment plants only indicate that anaerobic environments (i.e. sediment) have the potential metabolic capacity to degrade progesterone, albeit, at a reduced rate.

6. ESTIMATE OF NUMBER OF IMPLANTS TO BE USED IN THE U.S. COMPARED TO THE RATE OF ENDOGENOUS PRODUCTION OF PROGESTERONE FROM DAIRY COWS

This application is for concurrent use of two separate products that are approved for use individually in lactating dairy cows. It is not a combination product or a new product. Therefore, this application is not expected to result in a substantial increase in CIDR Insert use over current use.

6.1. Potential Market and Usage of CIDR Insert Compared to Annual Endogenous Progesterone Production from Cattle

As of 1 July 2007 the US cattle inventory for female cattle totaled 59 million [54]. This represents the total number of cattle potentially producing endogenous progesterone that could release progesterone metabolites into the environment.

The number of calves born from beef and dairy cattle in the US in 2005 and 2006 were 37.6 million per year for both of these years [54]. In Section 2.2, it was estimated that annual production of progesterone from a dairy cow was 119 g per animal, predominantly during pregnancy. For the purposes of this assessment, it is assumed that the number of calves born equals the number of successful pregnancies in a year.

6.2. Yearly Endogenous Production of Progesterone (Dairy and Beef)

Endogenous progesterone production (all cattle births, dairy + beef)	=	Total number of births x yearly endogenous progesterone production rate
	=	37,600,000 animals x 0.119 kg progesterone/animal =
	=	4,474,400 kg progesterone/year

6.3. Yearly Endogenous Progesterone Production (Dairy Cattle)

There are approximately 9,150,000 milking dairy cattle in the US [54]. The current calving interval in the US is about 14 months. Therefore, of the 9,150,000 cattle (12 months per year/14 months per cycle) = 7,840,000 will become pregnant or calve each year.

Endogenous progesterone production (diary cattle)	=	Total number of births x yearly endogenous progesterone production rate
	=	7,840,000 x 0.119 kg progesterone/animal
	=	933,000 kg progesterone/year

6.4. CIDR Insert Usage in Dairy Cattle

Of the 9,150,000 milking dairy cattle, there are approximately 7,840,000 dairy cattle as a potential market in the US (see Section 6.3). If P&U was to achieve 100% market penetration for this new use of CIDR Inserts and treat every available dairy cow with the CIDR Insert (very unrealistic) then approximately 7,840,000 cattle could be treated per year. The CIDR Insert contains 1.38 g progesterone, 46% of which is absorbed by the cow.

Total maximum CIDR Insert progesterone for all diary cattle	=	Total number cattle x kg progesterone in CIDR Insert x % absorbed
	=	7,840,000 x 0.00138 kg progesterone per CIDR Insert x 46% absorbed
	=	5,000 kg CIDR Insert progesterone absorbed/year

6.5. Maximum Possible CIDR Insert Progesterone as a Percentage of Endogenous Production in Dairy Cattle

Maximum CIDR Insert progesterone for all diary cattle as a percent of endogenous progesterone production	=	kg CIDR Insert progesterone absorbed /endogenous progesterone production (diary cattle)
	=	(5,000/933,000) x 100%
	=	0.54%

This value represents the maximum potential and is a significant overestimate since it is unlikely that the CIDR Insert would be used for this new indication in every available dairy cow. Even with the gross overestimate of potential use, it still only represents an increase in progesterone in dairy cattle of 0.54% and only [(5,000/4,474,400) x 100%] = 0.11% of total progesterone produced endogenously from all female cattle. This negligible increase over endogenous production would be undetectable in the environment, since it represents a minor change in potential concentrations.

6.6. Adjustment from 100% Treatment of All Dairy Cattle to Maximum Potential Market

If P&U market share for this product was to increase from its present value to ⅓ of the potential dairy cattle market (a very optimistic value) then the total potential increase in progesterone production compared to endogenous production from all dairy cattle through the use of CIDR Insert would be 0.54% x ⅓ market share = 0.18% of endogenous progesterone production from dairy cattle, and is only 0.04% of total progesterone produced endogenously from all female cattle [(0.0018 x 933,000)/4,474,400 = 0.04%]. A ⅓ market share for all potential dairy cattle would represent 2.6 million CIDR Inserts (⅓ x 7,840,000 dairy cows), and is an overly optimistic projection for dairy cattle since this value is far above current sales.

6.7. Estimate of Increased Progesterone Use from Approval of this Application

The CIDR Insert is currently approved for use in lactating dairy cattle; this application is for a change in the label of CIDR Insert to allow concurrent use with Lutalyse SS. With the approval of this application it is unlikely that sales of CIDR Insert will increase by a substantial extent. However, the change in the product label would allow concurrent use and provide supporting human food safety data for the producer and consumer.

7. TABLE OF ALL PEC VALUES

Summary Table of PEC Values

PEC Variable	Dinoprost	Progesterone	AED
$PEC_{manure\text{-}initial}$	0.00874 mg/kg	0.224 mg/kg	*
$PEC_{soil\text{-}initial}$	0.26 µg/kg	6.69 µg/kg	*
$PEC_{water\text{-}initial}$	2.9 ng/L	75 ng/L	ND†
$PEC_{manure\text{-}metab}$	ND	0.0025 mg/kg	*
$PEC_{soil\text{-}metab}$	ND	74 ng/kg	*
$PEC_{water\text{-}metab}$	ND	0.831 ng/L	0.75 ng/L
$PEC_{water\text{-}metab\text{-}Koc}$	ND	0.086 ng/L	0.12 ng/L

* If AED was produced from progesterone, it would occur in sediment. Therefore, theoretical manure and soil concentrations are not presented.
† ND – Not Determined

8. SUMMARY

The CIDR Insert and Lutalyse SS are currently being used by dairy farmers to manage the estrous cycles of their livestock. Approval of this application will allow for concurrent use of these two products for synchronization of estrus. The active ingredient in CIDR Insert (progesterone) and Lutalyse SS (dinoprost, a prostaglandin) are naturally occurring compounds in mammals. Both of these active ingredients are extensively metabolized in the dairy cow prior to excretion. Even using very conservative assumptions, without consideration for metabolism in the animal and pattern of use, both progesterone and dinoprost initial PEC_{soil} estimates were well below the Phase I trigger limit of concern of 100 µg/kg soil. Therefore, these actives should be classified as Phase I products. For progesterone, specifically, there are many potential endogenous environmental sources of this hormone from mammals and plants. It is impossible to separate out potential environmental effects from CIDR Insert use from effects of endogenously produced progesterone. Progesterone occurs naturally in the environment and the administration of the CIDR Insert to dairy cows is not expected to alter significantly the concentration or distribution of progesterone, its metabolites, or degradation products in the environment. The amount of progesterone excreted into the environment from use of the CIDR Insert in an individual dairy cow is only 0.5% of that individual dairy cow's annual endogenous rate of progesterone production. It has been demonstrated that in sediment around paper mills that progesterone can be microbiologically transformed to AED and ADD. Both AED and ADD have been implicated in masculinization of female mosquitofish (*Gambusia holbrooki*) in these waters adjacent to paper mills. If all of the progesterone from the CIDR Insert that could potentially reach surface water was transformed to AED or ADD, their concentrations would not be high enough to elicit a masculinization effect on mosquitofish.

In summary, it is not anticipated that progesterone and dinoprost levels potentially excreted from cattle that are treated concurrently with the Lutalyse SS and CIDR Insert products would have any impacts on non-target environmental organisms.

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