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

Environmental Assessment for Use of the EAZI-BREED™ CIDR® Cattle Insert for Induction of Estrous Cycles in Anestrous Lactating Dairy Cows

Active Ingredient: Progesterone

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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 use of EAZI-BREED™ CIDR® Cattle Insert (CIDR Insert) for induction of estrous cycles in anestrous lactating dairy cows in the USA is sought for this submission. The use of the CIDR Insert in dairy cattle complies with CFR 25.33(c) and in question two of the Guidance for Industry #89 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 ingredient is a natural substance, *viz.*, progesterone, 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. Nevertheless, since progesterone is a hormone and given the concerns for the impact of hormones in the environment, the potential toxicity of progesterone to non-target species in the environment is assessed. It is concluded that the use of the CIDR Insert 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.

CIDR Progesterone Intravaginal Inserts have been previously approved for multiple indications under NADA 141-200. Environmental Assessments have been prepared and FONSIs have been issued for the original approval in 2002, and the supplemental approval in 2010. A categorical exclusion from the requirement to prepare an EA was granted under 21 CFR 25.33(c) for the 2003 supplemental approval. The approved uses (and approval dates) are listed below:

- For Synchronization of Estrus in Suckled Beef Cows and Replacement Beef and Dairy Heifers, for Advancement of First Postpartum Estrus in Suckled Beef Cows, and for Advancement of First Pubertal Estrus in Replacement Beef Heifers. May 2, 2002.
- For Synchronization of the Return to Estrus in Lactating Dairy Cows Inseminated at the Immediately Preceding Estrus. July 29, 2003.
- For concurrent administration of progesterone solid matrix and dinoprost tromethamine (EAZI-BREED™ CIDR® Cattle Insert and LUTALYSE® Sterile Solution) for synchronization of estrus in lactating dairy cows. July 22, 2010.

The Center for Veterinary Medicine (CVM) had previously asked Pfizer to address the potential for masculinization of female fish from progesterone or the conversion of progesterone to androstenedione, and compare the release of progesterone use from CIDR to endogenous sources of progesterone in the environment in the 2008 EA prepared for approval of concurrent use of CIDR and LUTALYSE [1]. A FONSI was prepared by CVM in association with that EA. The current EA addresses an additional label claim to be added to the NADA, which represents a relatively small use in anestrous lactating dairy cows. The predicted environmental concentrations in this EA remain the same as those reported in the previous EA [1] because of the same conservative assumptions. In this EA, additional data are presented that have become available on environmental concentrations, endogenous environmental sources of these hormones, conversion of progesterone to androstenedione, and a recently published study on effects of progesterone on fish fecundity [48]. Also, additional information on the spatial and temporal characteristics of dairy farms, reproduction practices, current CIDR Insert use by dairy farms, farm management and

current marketing data on CIDR use in dairy and beef cattle are presented in Section 4. This additional information does not alter the conclusions reached in the previously accepted EA for which a FONSI was granted [1].

1. INTRODUCTION

With this submission, Pharmacia and Upjohn (P&U), a division of Pfizer Inc., is seeking a supplemental NADA approval for the use of CIDR Insert brand of progesterone releasing intravaginal insert for induction of estrous cycles in anestrous lactating dairy cows.

The approach taken in evaluating the potential of this 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 (EIAs) for Veterinary Medicinal Products (VMPs) - 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.

2. PRODUCT CHARACTERISTICS

2.1. Indications for use in dairy cattle

CIDR Progesterone Intravaginal Inserts are currently approved for multiple uses in beef cows and beef and dairy heifers, as discussed in the Overview of this EA. Specifically, for dairy cattle, CIDR Inserts are approved for (1) synchronization of estrus in dairy heifers, (2) synchronization of the return to estrus in lactating dairy cows inseminated at the immediately preceding estrus, and (3) concurrent use with LUTYLASE (dinoprost) for synchronization of estrus in lactating dairy cows. The new label indication sought with this application, is the use of CIDR Insert for induction of estrous cycles in anestrous lactating dairy cows.

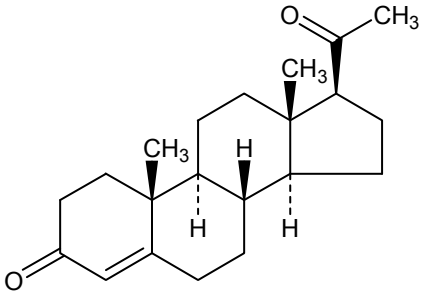
2.2. Composition, Physico-chemical and Pharmacological Properties

2.2.1. CIDR Insert composition

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 "wings" which 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. 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 7-d 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.

2.2.2. Progesterone physico-chemical properties

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); ∆4-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 [62].
Octanol/water partition coefficient (K_{ow}):	The log K_{ow} for progesterone is 3.87 [4].
Soil Binding (K_{oc})	The mean K_{oc} for progesterone measured in five soils is 8,248 [63].
Vapor pressure:	The estimated vapor pressure of progesterone is 2.69E-6 mm Hg at 25°C [4]. Thus, progesterone is not likely to partition into the atmosphere.

2.2.3. Progesterone pharmacological properties

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 [5].

2.3. 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 cow administered a CIDR Insert in the absence of a corpus luteum, the concentration of progesterone detected in plasma (≤1 ng/mL) [6] does not exceed that observed during the luteal phase of the estrous cycle (5 - 10 ng/mL [7, 8] or during pregnancy (10 to 12 ng/mL) [8, 9]. 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 administration period [5], 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 3.2) 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 (119 g) by a dairy cow (Section 3.2).

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, it is assumed that the CIDR Inserts will release 46.3% of 1.38 g (= 0.64 g) progesterone which is absorbed during a 7-day administration period. This is equivalent to 91.4 mg/day. The initial worst-case PEC estimates (Section 5.2) are later refined in the assessment based on data for progesterone metabolism and estimates of excretion.

2.4. Disposal of CIDR Insert

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 would require water coming into contact with the surface of the CIDR Insert. Because the solubility of progesterone in water is limited (7.3 mg/L [62]), 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 [63]. Therefore, it is unlikely that the progesterone in the CIDR Inserts left on the ground would migrate into surface waters.

In addition, when the CIDR Inserts are stored in a plastic bag or other storage container as recommended by the product label prior to disposal, the water barrier provided by the plastic will eliminate the potential for progesterone to reach the environment.

3. METABOLISM AND EXCRETION OF PROGESTERONE

3.1. Overview of Progesterone Metabolism

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 [6]. 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 [6, 7, 8, 9]. In fact, in the modern US dairy cow, the CIDR Insert provided about an additional 1 ng/mL in plasma [6]. 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 [10]. [Note: a 10-day CIDR Insert administration is not approved in the US]. The concentration of progesterone in plasma during the luteal phase of the estrous cycle varies from about 5 to 10 ng/mL (see Section 3.2). 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 $\leq$1 ng/mL during the 7-day administration period [11, 64].

Prior to excretion, progesterone is extensively metabolized by the cow to many metabolites. [12, 13, 14, 15]. Several metabolites of progesterone, including 5α and 5β reduced pregnanediones, pregnanolones, and pregnanediols, were detected in feces from cattle [15, 16], however, according to Schwarzenberger et al., “unmetabolized progesterone was barely present, if at all” [15]. 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 17]. The

"progesterone/progestins" found in feces as reported in some literature citations [10, 16] 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 [10]. 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 [18].

Isobe et al. [19] 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 [19]) with the highest concentration determined in an individual cow to be 140 ng/g (Fig. 5 in Isobe et al. paper).

3.2. 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 [9, 20, 21]. Plasma progesterone concentrations remain at about 10 ng/mL throughout gestation [8, 9, 22]. Plasma concentrations of progesterone in lactating dairy cows may be somewhat lower than that observed in beef cattle [21], 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 [23, 24].

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 [24]. 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 3 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].

The amount of progesterone released from a CIDR Insert during the 7-day administration (91.4 mg/day x 7 days = 640 mg) period is inconsequential (1.9%) compared to the endogenous progesterone production that would occur during the typical dairy cattle 90-day manure collection period (375 mg x 90 days). A comparison over a 90-day manure collection period is considered appropriate because this duration is used for calculating the predicted environmental concentration in soil (PEC_{soil}) in this EA (See Section 4.4).

3.3. 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 cow as parent compound derived from the CIDR Insert can be made by using data from Isobe et al. [19] where plasma concentrations of progesterone were positively 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 concentrations of progesterone in feces from cows during the luteal phase of the estrous cycle was less than 100 ng/g wet feces (Fig. 4 of Isobe et al. paper [19]) with the highest concentration determined in an individual cow to be 140 ng/g (Fig. 5 in Isobe et al. paper). As described in Section 5.2, 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 3.2). Therefore, an 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%. The amount of 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 [10]. 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 10), and approximately 1.5 ng/mL for the first 7 days (Figure 2a of Reference 10). Again, in the paper by Isobe et al. [19], the plasma concentrations of progesterone were positively 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. [19] 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 2.3) and approximately 0.95 mg/day of progesterone from a CIDR 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 is 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.

4. PRODUCT USE PATTERN

4.1. Intended Use-Pattern for All Approved and Proposed Indications for EAZI-BREED CIDR Inserts in Dairy Cattle

The product label for CIDR Inserts contains multiple indications for use in beef cows, and beef and dairy heifers. There are two indications on the label specifically for use in dairy cows. These are as follows:

a. For synchronization of the return to estrus in lactating dairy cows inseminated at the immediately preceding estrus.

b. For synchronization of estrus in lactating dairy cows with concurrent use of LUTALYSE.

The proposed indication addressed in this EA is for induction of estrous cycles in anestrous lactating dairy cows. The anestrous condition at the end of the voluntary wait period (VWP) is well recognized and can range from 10% to 60% of a herd with a mean of about 34% [25, 26, 27, 28]. For this new indication, treatment with a CIDR Insert is for individual treatment of cows determined to be anestrous, not whole herd based indications such as synchronization of estrus or fixed time AI (FTAI) treatment regimens.

4.2. Description of Reproduction Practices on US Dairy Operations

Common practice in commercial dairy herds in the US is to observe a period post-partum when cows are not inseminated even if observed in estrus, commonly called the herd voluntary wait period (VWP). During the VWP, the uterus undergoes involution in preparation for a new pregnancy and cows resume normal estrous cycles. The VWP may be as short as 42 days and as long as 80 days, with an average of approximately 60 days, depending on the reproductive program the manager of the herd is using for first inseminations following the VWP. However, not all cows resume normal estrous cycles by the end of the VWP and such cows are commonly called anestrous cows.

For dairies that utilize breeding programs for first inseminations following the VWP, relatively few dairies utilize synchronization of estrus programs requiring detection of estrus. Herds are more likely to utilize treatment regimens for FTAI. The core of FTAI programs is the Ovsynch protocol, which consists of a dose of gonadotropin releasing hormone (GnRH) followed 7 days later with administration of a prostaglandin product, a second dose of GnRH 48 to 56 h later with FTAI at either 24 or 18 h later, respectively [29]. Additional treatments may be applied before these FTAI programs (pre-synch programs) used to place cows in a physiological state more likely to respond to the Ovsynch protocol. In addition, programs culminating in a second FTAI have been developed to re-synchronize cows not conceiving to the first AI following the VWP [29]. Similarly, these additional programs utilize prostaglandin and/or GnRH products, and may include incorporation of the CIDR Insert.

The recent APHIS reports for dairy cattle health and management practiced in the United States provide a summary of current reproductive management practices [30, 31]. Artificial insemination to natural, spontaneous estrus was used for first AI following the VWP in a majority of cows in 54.7% of dairies. The second most common practice for first services in cows was natural service, at 21.7% of dairies. FTAI programs were only used for a majority of first services for cows in less than 7% of dairies, but were used more frequently for second or greater services for cows (39.6%). The extent of use of FTAI by dairies varies by region of the US with a higher percentage of herds in the East region 60.3% than in the West region (35.6%) using FTAI programs. Slightly over 60% of dairies had used FTAI programs for 5 years or more. The primary reasons FTAI was used were to catch up on nonpregnant cows (48.8%) and to control all first and subsequent services (27.7%).

There are various alternative programs for FTAI, including use of a CIDR Insert during the 7 days between the first dose of GnRH and the injection of the prostaglandin product. These programs have been well investigated by academicians. The addition of a CIDR Insert in these programs has demonstrated benefit in anestrous dairy cows [26, 27], but had limited benefit in estrous cycling dairy cows. Thus the role of CIDR Inserts has been marginalized for use in cows with high risk of being anestrous. These are cows that have not been observed in estrus by 80 to 100 days in milk and upon evaluation of ovaries using ultrasound a corpus luteum cannot be identified. Although the use of CIDR Inserts for inducing estrous cycles in anestrous cows is not yet approved, it has been reported that 65.7% of the dairy farms that have used CIDR inserts in the past 12 months (32.4% of all dairy farms in the U.S.) were already using CIDR Inserts for this purpose [30].

4.3. Calving Cycles

Dairy cows are bred and calve year round to maintain a constant supply of milk. A calving interval of 12-13 months is generally recommended to achieve maximum lifetime milk production. However, typical calving intervals in dairy cows in the US are 12 -14 months with a mean of about 13 months [30, 38]. The mean calving interval is longer than that recommended because many cows do not conceive from first or second inseminations. Therefore, the number of potential available target animals in a year is less than the full US dairy herd.

In this EA, for calculations, a calving interval of 13 months (395 d) will be used.

4.4. Dairy Management Practices and Expected Product Use

Because the demand for fresh milk and dairy products is relatively constant throughout the year, there is no temporal variability in the number of dairy cattle throughout the year, and they maintain constant milk production. Therefore, CIDR Insert use in dairy cows is also uniform through the 13 month birthing cycle. In addition, because CIDR Insert use in dairy cows will likely be uniform throughout the year, the scenario of 100% animal herd treatment (Section 5.2) at any given manure collection interval is not realistic. A 90-day manure collection period is typically used for dairy cattle when estimating the PEC_{soil} (Section 5.2) and this EA assumes that the number of cows treated in a single herd every 90-days would be equal. Therefore, with a 13 month calving cycle (395 d) only 23% of the herd, on average, would be treated in a 90-day manure collection window [i.e., (90 d/ 395 d) x 100% = 23%].

Based on these calculations, a realistic worst-case scenario assumes 23% of a dairy cow herd would be treated for induction of estrous cycles in anestrous dairy cows during 90-day period. However, it is likely that this percentage will be much lower because (1) CIDR Inserts are already being used use in dairy cows and (2) other programs are currently in place for artificial insemination and synchronization of estrus that do not involve the use of CIDR Inserts. Therefore, it is likely that adding this new indication for use will result in a minimal increase in CIDR Insert use over current use. As such, it is anticipated that there will be a negligible incremental increase of environmental progesterone through manure of dairy cows treated with CIDR Inserts for induction of estrous cycles in comparison to the amount of endogenous progesterone produced naturally in dairy cows.

4.5. Farm Management

Reviews of common farm management can be found in references [32 and 33]. Herd size varies from small 20-cow herds in WI to herds in excess of 6000 in CA, WA and ID. Therefore, herd management practices vary dramatically. Cow management for housing varies from total confinement in stanchions, to large free stall barns with head lock-ups with or without adjacent exercise pens, to dry lots with or without shade structures or wind breaks. Total grass feed operations are minimal and seasonal in some geographical regions, but are located throughout the country. Milking, in general, is conducted 2 or 3 times daily. In general, cows in large herds are fed a complete mixed ration; in smaller herds feeding may include direct feeding or top dressing of a supplement to dry matter. Feed types are regional in nature and include hay, haylage, corn silage, high moisture corn, soybean meal and various local commodities including by-products. Least-cost rationing may be utilized by larger dairies that purchase all or most of their feed. In contrast, some dairies produce most of their own feeds. Manure in free stall barns is cleaned several times daily, by water flush or scrapping. Manure is commonly stored in lagoons. Dry lots may be cleaned quarterly. In confinement facilities, the manure is cleaned once or twice daily and may be collected and spread onto fields as needed.

4.6. Dairy Cattle Farm Size

As shown in Table 1, which is taken from the 2007 USDA Agricultural Census [34], there were a total of 9,266,574 milk cows that had calved in that year. Although only 2.26% of dairies have a farm size of >1000 animal units (AU), nearly 40% of milk cows are on these larger farms. Almost 53% of milk cows are on farms with between 50 and 1000 AU.

Table 1. Farm Size, Number of Farms, and Number of Milk Cows from 2007 USDA Census

Farm Size # Animals	Number of Farms	Percent Farms	Number of Milk Cows	Percent of Milk Cows
1 to 9	14,426	20.64%	38,147	0.41%
10 to 19	3,568	5.11%	48,821	0.53%
20 to 49	16,344	23.39%	576,070	6.22%
50 to 99	18,986	27.17%	1,280,983	13.82%
100 to 199	8,975	12.84%	1,180,985	12.74%
200 to 499	4,307	6.16%	1,278,721	13.80%
500 to 999	1,702	2.44%	1,161,865	12.54%
1000 to 2499	1,104	1.58%	1,673,772	18.06%
>2499	478	0.68%	2,027,210	21.88%
Total	69,890	100.00%	9,266,574	100.00%

Data are from Table 17 of 2007 USDA Agricultural Census of Agriculture [34]
http://www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_US/index.asp

4.7. Dairy Cattle Top Production States

From the 2007 USDA Agricultural census, the states with the highest milk cow inventories are listed in Table 2.

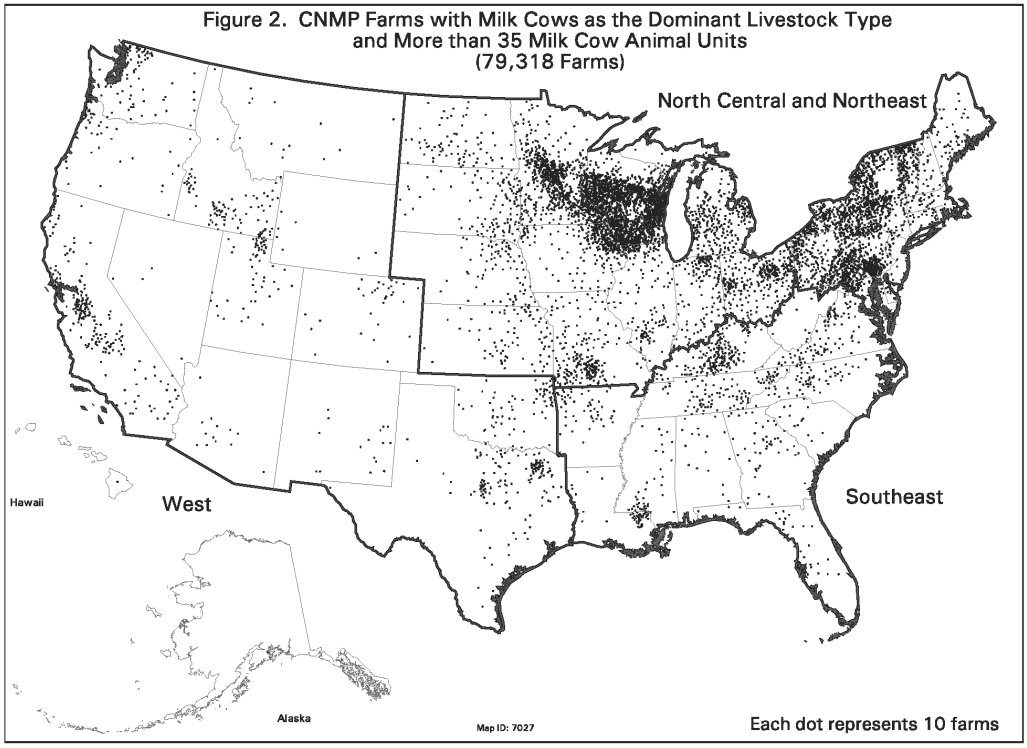
Table 2. Top Ten States in US for Milk Cow Inventory for 2007 USDA Census

Rank	State	# Milk Cows	% of Total
1	California	1,840,730*	19.9%
2	Wisconsin	1,249,309	13.5%
3	New York	626,455	6.8%
4	Pennsylvania	553,321	6.0%
5	Idaho	536,463	5.8%
6	Minnesota	459,752	5.0%
7	Texas	404,399	4.4%
8	Michigan	344,233	3.7%
9	New Mexico	326,400	3.5%
10	Ohio	271,938	2.9%

Source: USDA NASS Agricultural web site "Quick Stats" [35], http://quickstats.nass.usda.gov/
* In the 2011 survey, 85% of milk cows in California were located in the San Joaquin Valley agricultural district.

4.8. Dairy Cattle Spatial Distribution

Examination of USDA maps of the number of farms for beef and dairy cattle [36] indicates that these cattle groups are located principally in different regions of the US. Beef cattle farms are distributed within the corn belt of the Midwest and are not necessarily associated with human population centers. Beef production is higher in areas with less rainfall (western US) than where dairy is concentrated e.g., in the Northeast. Since milk is the primary product produced from dairy cattle and it is principally composed of water, making it heavy for shipping, being in close proximity to city centers that have a need for fresh milk and dairy products is an advantageous location. This is easily seen on the USDA map for dairy cattle where clusters exist around Austin and Dallas TX, New Orleans LA , Little Rock AR, Philadelphia PA, New York NY, Dayton and Cleveland OH , San Francisco CA, Salt Lake City UT, etc. See the following USDA dairy cattle map [36].



However, not all major cities have large numbers of dairy farms associated with them. Although the cities of Los Angeles, Miami, Houston and Denver support large human populations, there are a small number of dairy facilities in direct proximity. A second factor beside the human population influencing the distribution of dairy cattle is the resources necessary to produce milk at a low cost. A principle component of the dairy cattle's diet is hay and alfalfa which is a commodity that is more limited in dry areas such as Denver, Los Angeles and Miami, but much more plentiful (inexpensive) in the Northeast and the central valley of California. It is important to note that the dots on the USDA map represent the number of farms (each dot representing 10 farms), not the number of animals on a farm. Therefore, the lack of dots around cities like Los Angeles could also be influenced by farm size. California has a small number of dairy farms (2,165) in comparison to the US total (69,890), however it has a very large number of large dairies with >2499 head, with 36% (169/476) of these >2499 head dairies located in California [34]. Also, California outranks Wisconsin in the total number of milk cows, indicating that Wisconsin has, in general, smaller dairies than California (Table 2). California's dairies are principally in the central

valley with 85% of California milk cows in the San Joaquin Valley agricultural district [35]. In Wisconsin the dairies are more uniform than California. However, the populations are the lowest in the North and the highest in the central and southern locations with the south central agricultural district with the highest population representing 24% of the milk cows across the 9 agricultural districts [35].

The production of cheese, yogurt, ice cream and butter are other major uses for milk. These production facilities (i.e., cheese) do not need to be located close to human population centers and are located where milk can be produced and transported cheaply. From the USDA map for dairy cattle farms [36], it is consistent that Wisconsin and some areas of the northeastern US have a higher density of dairy cattle farms than anywhere in the nation.

The scientific and governmental literature has been searched for actual examples of watersheds that have more detailed information of the spatial orientation of dairy operations within a watershed, but these data are infrequent. A study was reported in the denser area of dairies in the Upper North Bosque River watershed of north central Texas. In Figure 1 of reference [37], individual dairies are denoted on the map of the watershed. From the mile scaling of the watershed, it can be seen that there are as many as 11 dairy facilities located within an approximate 25 square mile region in two areas of the watershed. In other areas the density is much lower.

5. TRADITIONAL PREVIOUSLY ACCEPTED METHODS FOR CALCULATION OF PEC_{soil} AND PEC_{water}

Calculations of predicted environmental concentrations (PEC) presented are based on traditional calculation methods that have been previously accepted for common agricultural practices and are the same as those used in the previously approved CIDR EA [1].

5.1. PEC Definitions

Throughout this document PECs 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 due to refinement of the calculations 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 ($PEC_{water\text{-}metab\text{-}Koc}$).

5.2. Worst-Case PEC in Soil

Calculation of the worst-case PEC in soil ($PEC_{soil\text{-}initial}$) of the active ingredient of the CIDR Insert, *viz*., progesterone, is presented below. The calculations are based on the following worst-case assumptions:

- 12-month breeding cycle (typical calving interval in the US is 12-14 months) [30, 38]
- 100% of the cows within the herd will be treated
- one CIDR Insert will be used per cow per year
- dairy cows are bred uniformly throughout the year
- 100% of excreted dose of the active ingredient is parent drug
- 27200 kg manure/acre
- no biodegradation of progesterone in manures or soils
- manures applied to soil; average plow depth is 15 cm

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
  c = fraction of animals treated: (assuming all cows treated = 1.0)
  d = number of days animals are treated (= 7 days for progesterone)
  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

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

The worst-case $PEC_{soil\text{-}initial}$ value of 6.69 µg/kg is approximately 15-fold lower than the VICH Phase I trigger limit of 100 µg/kg for progesterone.

5.3. Worst-Case PECs of Progesterone 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 [1]. The equation used to calculate these values is as follows:

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

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

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

The worst-case $PEC_{water\text{-}initial}$ value for progesterone is calculated to be 75 ng/L.

5.4. Progesterone PEC Refinements for Metabolism Prior to Excretion in Manure

The initial progesterone PEC calculations from Section 5.2 (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. Therefore, the calculations are overly conservative.

In Section 3.3 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 5.2.

5.5. 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)

5.6. 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 [1]. 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 previously accepted methods [1], 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 [63].

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

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

$$= 0.086\ ng/L$$

* *where*: Kd = is the partition coefficient (units of mL of soil water per g of soil) for this chemical = 0.029 x average Koc [1] = 0.029 x 8,248 = 239.19, assuming equilibration of the compound in water within the top 5 cm of sediment

5.7. Refinement for Temporal CIDR Insert Usage within Dairy Manure 90-d Collection Period and Use Pattern

As previously discussed (see Section 4.4), the initial assumption of 100% herd treatment at one time is an overestimate based on the temporal use CIDR Inserts in dairy cattle. Dairy cow pregnancies, and thus, the use of CIDR Inserts are evenly spread out over the year in order to have uniform amounts of animals producing milk at one time. Because the PEC_{water} is derived using a 90-d manure holding period for dairy cows, this value (90 d) is divided by the duration of the calving cycle (395 d) to estimate the percent of the herd treated with a CIDR Insert that will contribute to the total progesterone concentration in manure within a single manure holding period (23%). Therefore, the $PEC_{water\text{-}metab\text{-}Koc}$ is further refined as follows:

Progesterone $PEC_{water\text{-}metab\text{-}Koc}$	=	0.086 ng/L
Refinement for 23% herd treatment in 90-day manure collection window (Section 4.4)	=	0.086 x 23% = 0.020 ng/L

Also, the incidence of anestrous conditions in dairy cattle is 34% (Section 4.1), which would cause a further reduction in the PEC_{water} estimate if the PEC_{water} estimate was restricted to use for anestrous dairy cattle.

5.8. 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. It would be expected that only 23% of cows in a dairy herd have the potential to be treated during a 90-day manure collection period. Therefore, it is reasonable to assume that the manures from cows which receive the CIDR Insert would be further mixed with manures from other categories of cattle that would not receive the CIDR Insert. Therefore, the incremental increase in environmental concentration of progesterone originating from the CIDR Insert (0.020 ng/L) is very small in comparison to endogenously produced progesterone being released. These estimates also do not take into account any degradation that may take place in manure storage systems or biodegradation in soil or water, so actual environmental concentrations in soil and water are likely considerably lower than those shown above. Thus, the calculated PEC values are very conservative.

6. SURFACE WATER RISK ASSESSMENT

The scientific literature has demonstrated that some hormones can cause adverse effects on fish in aquatic environments (see Section 6.6). In areas around paper mills it has been shown that progesterone derived from the degradation of wood 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 its potential subsequent effects on fish will be included in this portion of the assessment.

6.1. 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 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.

6.1.1. Endogenous progesterone production from cattle

Following the 7-day treatment period with the CIDR Insert, the animal will become pregnant and start continuous endogenous production of progesterone. Because the absorbed progesterone from the CIDR Insert represents only 1.9% of the endogenous progesterone produced over a 90-day manure collection interval (see Section 3.2), we would anticipate that the total amount of progesterone originating from a CIDR Insert to be negligible in comparison to endogenously produced progesterone present in the environment. Therefore, the potential for toxic effects in aquatic organisms, as a result of CIDR Insert use, is unlikely.

Also, it has been hypothesized that microbial transformations of phytosterols produce progesterone and AED in the intestinal tracts of livestock [39]. Thus, the total endogenous production rate of progesterone could be a combination of animal and plant derived steroidal compounds.

In a study that examined the abundance of progesterone in dairy cattle solid waste, progesterone in fresh manure was less than the limit of quantitation (LOQ) for the assay [40]. However, when the manure was aged for 2 weeks, the progesterone concentration was 196 µg/kg manure. Progesterone was not detected in any of the three liquid manure lagoons. This study suggests that the progesterone may have been produced by metabolism in the manure and was not directly excreted by the cattle.

In a study that measured estrogens, testosterone, AED and progesterone in beef steers feedlots, fresh manure from steers did not contain AED or progesterone [41]. Although the testosterone was rapidly transformed in the feedlot soil it could only account for approximately 10% of the amount of AED produced if 100% of the testosterone detected was transformed into AED (see Figure 1 of reference 41). The production of progesterone and AED in the aged feedlot soil could have resulted from the transformation of natural sterols in the manure. These data indicate that endocrine active compounds are naturally produced from the manure components. The concentrations of progesterone, testosterone and AED declined approximately 85% after a simulated rainfall, indicating their potential for degradation. Because only a small mass of these steroids were recovered in the runoff of the simulated rainfall, the loss was likely due to degradation and not removal from runoff.

6.1.2. 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/h [42]. Furthermore, the males released up to 1000 ng/h of AED when sexually aroused by females or their pheromones [42]. Water concentrations of AED around spawning salmon and concentrations discharged from fish hatcheries into rivers have been measured to be near 1 ng/L [43].

6.1.3. 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 [44]. It is hypothesized that these steroidal compounds are produced from the microbial transformation of pulp-derived phytosterols in the paper mill effluent [45]. 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 [46].

6.2. 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 [45]. 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 (47169 ng/L), 4 nM (1146 ng/L) and 2.6 nM (739 ng/L), respectively [45]. 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 [45], 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 an exhaustive literature search.

6.3. 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 5.5). Therefore, the initial $PEC_{soil\text{-}metab}$ for AED is 0.074 µg/kg X (286.42/314.46) = 0.067 µg/kg.

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

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

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

Consistent with previously accepted methods [1], $PEC_{water\text{-}metab}$ can be further refined due to adsorption to sediment:

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

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

$$= 0.12\ ng/L$$

where: Kd = is the partition coefficient (units of mL of soil water per g of soil. Lee et al. [47] determined the mean Log Koc of AED in three soils to be 3.72 soil (Koc = 5248). The Kd = 0.029 x average Koc [1] = 0.029 x 5248 = 152.19, assuming equilibration of the compound in water within the top 5 cm of sediment.

6.4. Refinement for Temporal CIDR Insert Usage within Dairy Manure 90-d Collection Period and Use Pattern

As previously discussed, the initial assumption of 100% herd treatment is an overestimate based on the temporal use of CIDR Inserts in dairy cattle. Therefore, this value was reduced to 23% herd treatment, based on the assumptions that (1) the use of CIDR Inserts is evenly distributed over the 13-month calving cycle and (2) the amount of progesterone derived from CIDR Inserts that may be land applied in a 90-d manure holding period is also evenly distributed (see Section 4.4). Therefore, the AED $PEC_{water\text{-}metab\text{-}Koc}$ can be further refined as follows:

Androstenedione $PEC_{water\text{-}metab\text{-}Koc}$	=	0.12 ng/L
Refinement for 23% treatment in 90 day manure collection window (Section 4.4)	=	0.12 x 23% = 0.028 ng/L

6.5. Table of all PEC Values

Summary Table of PEC Values

PEC Variable	Progesterone	AED
$PEC_{manure\text{-}initial}$	0.224 mg/kg	†
$PEC_{soil\text{-}initial}$	6.69 µg/kg	†
$PEC_{water\text{-}initial}$	75 ng/L	ND*
$PEC_{manure\text{-}metab}$	0.0025 mg/kg	†
$PEC_{soil\text{-}metab}$	74 ng/kg	67 ng/kg
$PEC_{water\text{-}metab}$	0.831 ng/L	0.75 ng/L
$PEC_{water\text{-}metab\text{-}Koc}$	0.086 ng/L	0.12 ng/L
$PEC_{water\text{-}metab\text{-}Koc}$ Refinement for 23% herd treatment in 90 day manure collection window	0.020 ng/L	0.028 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

6.6. Potential for Fish Reproduction 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 3), and is relatively immobile in soil (Section 5.6) so there is a very low potential for progesterone from dairy cattle to directly cause 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*) [44, 45]. Downstream from these paper mills, progesterone was measured at approximately 6.55 nM (2060) ng/L [44].

A 21-d reproduction study was conducted with fathead minnows exposed to 10, 100 and 1000 ng/L progesterone in water under flow-through conditions [48]. This study was evaluated, but due to the study design and concerns with how it was conducted, a NOEC

could not be determined and it was found unsuitable to support a risk analyses in this EA. Deficiencies found with the study include: 1) progesterone concentrations in water were not measured because water samples were lost; 2) the exposure concentrations were spaced too far apart to accurately determine a NOEC or LOEC; 3) a dose-response relationship was not observed for cumulative fecundity; and 4) adverse effects on fecundity were observed in the lowest concentration tested (i.e., a NOEC could not be determined).

For AED, the lowest value reported to cause masculinization in female fish was 1.4 nM (400 ng/L) of AED with the NOEC concentration tested at 40 ng/L [49]. 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. [50] where 10,000 and 100,000 ng/L for AED and ADD respectively, were required to see the effect. If a safety factor of 10 is applied to the NOEC, the predicted no effect concentration (PNEC) for AED would be 4 ng/L.

From the calculations in Section 6.3, we estimate a AED ($PEC_{water\text{-}metab}$) = 0.753 ng/L and a $PEC_{water\text{-}metab\text{-}Koc}$ of 0.12 ng/L if all of the progesterone excreted from CIDR Insert-treated cows was to be transformed to AED after reaching surface water. This PEC was further refined to 0.028 ng/L (Section 6.4), based on the maximum percent of herd treatment in a 90-day period. These PECs are all below the PNEC for AED, and therefore the RQs are less than 1.0 indicating no risk to the environment.

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 [51].

It is difficult to separate out effects of CIDR Insert use from the environmental background production of progesterone or AED (see Section 6.1). 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. It should also be noted that these estimates do not consider any degradation in soil or water or manure storage systems, so the PEC values are overly conservative.

6.7. Summary of Potential Effects on Fish

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 expected to be considerably lower than those resulting from endogenously produced progesterone found in the environment from cattle and other sources. While AED and ADD are not anticipated to cause masculinization or reproduction effects in fish, adequate and reliable data are not currently available in the literature to confidently estimate a PNEC for fish exposed to progesterone. Thus, concentrations of progesterone that will not cause reproductive effects in fish are still unknown. However, it is estimated that progesterone released from use of CIDR Inserts in dairy cows will only contribute 0.02 ng/L to the total concentration of progesterone in the aquatic environment and based on what is known for other endocrine active compounds, this concentration is very unlikely to cause reproductive effects in fish.

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

Because the absorbed progesterone from the CIDR Insert represents only 1.9% of the endogenous progesterone produced over a 90-day manure collection interval (see Section 3.2), it is anticipated that endogenous environmental concentrations of progesterone or AED would be significantly greater than the CIDR Insert derived PEC_{water} concentrations. Therefore, we would expect to see higher 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, if the $PEC_{water\text{-}metab\text{-}Koc}$ for CIDR-derived progesterone is in the range from 0.02 - 0.085 ng/L, and this contribution is only 1.9% of the estimated endogenous progesterone production, then the predicted surface water concentrations from endogenous progesterone production by cattle would be approximately 53 times higher or 1.0 - 4.5 ng/L. Although surface water monitoring data are limited, this predicted concentration range is not supported by field data from water sources specifically 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 assay, which were 0.4 and 0.3 ng/L, respectively (summarized in Table 1 of reference 39 and the footnote in Table 2 of reference 43). In another study where steroid concentrations of 17α-estradiol, estrone, testosterone and AED were detected at concentrations as high as 650 ng/L in a dairy waste lagoon [43], samples from groundwater monitoring wells, surface water and tile drainage fields that were expected to be impacted by the dairy operations, and there was no evidence that progesterone or AED were reaching these water sources.

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 sampled [52]. In those samples analyzed in which progesterone was detected, the median and maximum concentrations were 110 ng/L and 199 ng/L, respectively [52]. The sources of progesterone in these infrequent positive samples were not reported, but they are consistent with potential paper mill effluent concentrations of progesterone (Section 6.1.3).

An extensive water monitoring project for hormones from agricultural sources within a farm (600 ha) was conducted at Purdue University Agricultural Experiment Station [53]. Natural and synthetic hormones were monitored from tile-drained fields that received waste from beef and dairy cattle, poultry and swine facilities. Both solids and liquids from collection ponds were applied to agricultural fields. The hormones 17α- and 17β-estradiol, estrone, estriol, testosterone, AED, 17α and 17β-trenbolone and trendione were monitored at 7 sampling stations over a 15-month period. Testosterone and AED were detected the most frequently among the androgens with the synthetic androgens detected in less than 15% of samples. At the 6 sampling sites the maximum concentration of AED ranged from 1.6 ng/L to 16 ng/L. Progesterone was not monitored in this study. The authors noted that due to sorption and potential microbial degradation of hormones between sampling and analysis, the actual environmental concentrations could potentially be higher.

A study was conducted looking at steroid hormones in surface water of agricultural, suburban and mixed use areas in Chester County PA [54]. Of the 21 locations sampled, progesterone was detected at a lower frequency than estradiol, estrone or estriol. When progesterone was detected, the concentration ranged from 7-12 ng/L.

The studies summarized above were presented to demonstrate that although there are multiple sources of progesterone into the environment, the frequency of detection is small and the concentration is relatively low. The monitoring data from these studies also suggest that dairy farms are likely not a major source of these steroids into surface waters.

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

Following a 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 [55]. 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 [56]. Progesterone had a protective effect on young fish exposed to copper when tested at up to 250 mg/kg BW [57].

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 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 related hormone, 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 this synthetic progesterone analog (MGA) in soil, with half-life estimates in three soils of 4.3, 5.3 and 27.8 d. Also, progesterone is quickly degraded in sewage treatment plants [58] and by the microbiota of surface waters adjacent to sewage treatment plants and in rivers [59]. Although the potential for organisms in 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.

8. NUMBER OF CIDR INSERTS TO BE USED IN THE U.S. COMPARED TO THE RATE OF ENDOGENOUS PRODUCTION OF PROGESTERONE FROM DAIRY COWS

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

As of 1 Jan 2012 the US cattle inventory for female cattle totaled 58.5 million [Table 3]. This represents the total number of cattle potentially producing endogenous progesterone that could release progesterone metabolites into the environment.

It is assumed that the number of calves born from beef and dairy cattle in the US is equal to the number of beef (29,882,900) and dairy cows (9,229,500) for a total of 39,112,400 calves born [Table 3]. In Section 3.2, 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.

Table 3. USDA Cattle Numbers by Category in the US on Jan 1 2012

Class	Value
Cattle and calves	90,768,500
Cows and heifers that have calved	39,112,400
Beef cows	29,882,900
Milk cows	9,229,500
Heifers ≥500 lbs	19,387,800
For beef cow replacement	5,211,600
Expected to calve†	3,201,300
For milk cow replacement	4,527,000
Expected to calve†	3,029,900
Other heifers	9,649,200
Steers ≥500 lbs	16,071,500
Bulls ≥500 lbs	2,052,000
Calves under 500 lbs	14,144,800
Cattle on feed	14,121,400

* Source: USDA NASS Cattle Inventory [60]
† Replacement heifers expected to calve during the year

8.2. 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

= 39,112,400 animals x 0.119 kg progesterone/animal

= 4,654,376 kg progesterone/year

8.3. Yearly Endogenous Progesterone Production (Dairy Cattle)

There are 9,229,500 milking dairy cattle in the U.S. [Table 3]. The current calving interval in the US is 13 months. Therefore, of the 9,229,500 milking dairy cattle (12 months per year/13 months per cycle), about 8,519,540 will become pregnant or calve each year.

Endogenous progesterone production (dairy cattle)	=	Total number of births x yearly endogenous progesterone production rate
	=	8,519,540 x 0.119 kg progesterone/animal
	=	1,013,825 kg progesterone/year

8.4. CIDR Insert Usage in Dairy Cattle in 2011

Based on total sales and the average selling price of a CIDR Insert, P&U estimates that in 2011 approximately 1.5 million CIDR Inserts were sold for use in cattle [61]. Approximately 43% of the inserts were used in dairy cattle and 57% in beef cattle. Therefore, approximately 645,000 dairy cattle were treated with a CIDR Insert in 2011. In the 2007 USDA agricultural census there were approximately 9,266,574 dairy cattle in the U.S. (Table 1), therefore about 7% of dairy cattle received a CIDR Insert [(645,000/9,266,574) X 100% = 7%].

CIDR Insert progesterone from use in dairy cattle in 2011	=	Total number inserts used in dairy cattle x kg progesterone in CIDR Insert x % absorbed
	=	645,000 inserts x 0.00138 kg progesterone per CIDR Insert x 46% absorbed
	=	409 kg CIDR Insert progesterone absorbed/year

Based on the 2011 sales of CIDR Inserts for use in dairy cattle, it is estimated that 409 kg of progesterone is absorbed, and potentially released into the environment. However, this contribution will increase slightly with the approval of the new indication for induction of estrous cycles in anestrous lactating dairy cattle.

8.5. CIDR Insert Progesterone as a Percentage of Endogenous Production in Dairy Cattle

Maximum CIDR Insert progesterone for dairy cattle as a percent of endogenous progesterone production	=	kg CIDR Insert progesterone absorbed /endogenous progesterone production (dairy cattle)
	=	(409 kg/1,013,825 kg) x 100%
	=	0.040%

This value (0.040%) represents the current sales of CIDR Inserts to dairy cattle as a percentage of total progesterone excreted by dairy cattle. Although it is anticipated that sales of CIDR Inserts will increase as a result of the new label indication, this is a negligible environmental contribution in comparison to endogenously produced progesterone. Even if

it is conservatively assumed that 100% of dairy cattle in the U.S. are treated with a CIDR Insert each year (i.e. 8,519,450 dairy cattle), rather than basing calculations on 2011 sales data, the overall percentage of progesterone in the environment that is derived from the CIDR Inserts (5,408 kg) is still negligible (0.53%).

8.6. Estimated Increase in Environmental Progesterone Resulting from Approval of this Indication

The CIDR Insert is currently approved for synchronization of estrus in lactating dairy cattle and beef and dairy heifers. The supplemental application seeks to add an indication for the use of CIDR Inserts for induction of estrous cycles in anestrous lactating dairy cows. This additional use may cause a minor increase in CIDR use in dairy cattle, but the magnitude of that increase is difficult to predict because dairy farmers have several alternatives to CIDR Inserts for inducing estrous cycles in anestrous cows. When refining the PEC_{water} to account for (1) metabolism in the body, (2) adsorption to soil, and (3) 23% of a herd potentially being treated in a 90-d window period, it is estimated that CIDR Inserts would add an additional 0.020 ng/L progesterone to the aquatic environment as a result of this new indication. Therefore, regardless of the potential for an increase in sales, the overall percentage of progesterone in the environment as a result of this approval will be negligible, and no significant environmental impacts are anticipated.

9. SOURCES OF UNCERTAINTY IN THE RISK ASSESSMENT AND METHODS TO CONTROL UNCERTAINTY

The models presented to predict environmental concentrations are worst-case models. This helps to control uncertainty associated with the magnitude of the predictions. Since progesterone is a natural hormone in the environment, and likely degraded in most ecosystems, the models are made even more conservative by not using degradation rates of progesterone in soil and water in the models.

10. CONCLUSIONS

The CIDR Insert is currently being used by dairy farmers to manage the estrous cycles of their livestock. Approval of this application will allow use of this product for induction of estrous cycles in anestrous lactating dairy cows. The active ingredient in CIDR Insert (progesterone) is naturally occurring compound in all animals. This active ingredient is 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, the progesterone initial PEC_{soil} estimate was very low. There are many potential endogenous environmental sources of progesterone from animals 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 (1.9%) compared to the endogenous progesterone production that would occur during the typical dairy cattle 90-day manure collection period. When marketing data are considered, CIDR Insert use in dairy cattle increases the progesterone production from dairy cattle by only 0.040%.

It has been demonstrated that in sediment near 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. It is estimated that progesterone released from use of CIDR Inserts in dairy cows will only contribute 0.02 ng/L to the total concentration of progesterone in the aquatic environment. Based on what is known for progesterone and other endocrine active compounds, this concentration is very unlikely to cause reproductive effects in fish.

In summary, it is anticipated that progesterone levels potentially excreted from cattle that are treated with the CIDR Insert for induction of estrous cycles in anestrous lactating dairy cows will not cause significant environmental impacts.

11. ALTERNATIVES TO THE PROPOSED USE

None.

12. SIGNATURE BLOCK

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Appendix 1. Table of Confidential Studies and Information

Table 4. Pfizer Confidential Studies and Marketing Data

Source Material	Reference
E-mail correspondence of CIDR Insert marketing data for dairy and beef cattle	[61] Appendix 2
Dobbin JB, Smolenski WJ. Determination of water solubility for progesterone. Pharmacia Study Report SR-0847-7926-2002-002, 17 July 2002.	[62] Appendix 3
Bradley B, Smolenski WJ, Robinson JA. Adsorption desorption of ^{14}C-progesterone in five soils. Pfizer Study Report SR-0847-7926-2003-002. 11 July 2003.	[63] Appendix 4
Hornish RE, Krabill LF, Boucher JF, Anderson YC, Chenault JR, Prough MJ. Determination of concentrations of progesterone in milk of untreated pregnant cows and estrous cycling cows with and without a CIDR™ 1380 progesterone-releasing intravaginal insert. Pharmacia Study Report Number 0847-7926-2002-001, 10 June 2002.	[64] Appendix 5

Appendix 2. Personal e-mail Correspondence with Pfizer US-Cattle Marketing

Based on total sales and the average selling price of a CIDR Insert we estimate that in 2011 approximately 1.5 million CIDR Inserts were sold for use in cattle [61]. Approximately 43% of the use was for dairy cattle and 57% in beef cattle.

Appendix 3. Determination of water solubility for progesterone

Dobbin JB, Smolenski WJ. 2002. Determination of water solubility for progesterone. Pharmacia Study Report SR-0847-7926-2002-002, 17 July.

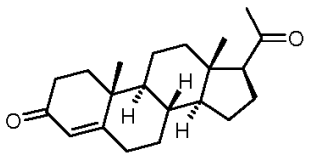
Abstract

Progesterone is a naturally occurring progestogen hormone used to suppress or synchronize estrus in cattle. Since progesterone is listed as insoluble in water in the Merck Index, this study was undertaken to determine the actual aqueous solubility. Knowledge of the aqueous solubility is of use for designing environmental fate studies such as soil adsorption/desorption and biodegradation in soil. The study was conducted following OECD Method 105 guidelines for solubility in water determination.

The column elution method was used for this determination. Progesterone was dissolved in methanol and applied to silica sand. The methanol was evaporated, leaving the silica sand coated with progesterone. The coated sand was placed in a glass column and reagent grade water was pumped through the column at 0.4 mL/min flow rate. The column effluent was assayed using HPLC with UV detection to determine the progesterone concentration. The mean progesterone concentration of ten consecutive fractions was determined to be 7.33 μg/mL at 20°C ± 0.5°C at a pH of 7. The experiment was repeated at a flow rate of 0.2 mL/min. Under these conditions the mean progesterone concentration of ten consecutive fractions was determined to be 7.30 μg/mL at 20°C ± 0.5°C at a pH of 7. Since the lower flow rate produced equivalent results as the higher flow rate, additional experiments with lower flow rates were not required. The mean of the two flow rates was used as the final summary.

Conclusion: The solubility of progesterone in water was determined to be 7.31 μg/mL at 20°C ± 0.5°C at a pH of 7. This value is the mean of the observations at flow rates of 0.4 mL/min and 0.2 mL/min.

Tabular Summary

Product name:	Generic name: Progesterone PNU-number: PNU-3672
SECTION: Environment	Reference: Investigating laboratory: ABC Laboratories Europe, 38 Castleroe Road, Coleraine, Northern Ireland BT51 3RL. Study period (years): 2002
Composition of test substance: Progesterone Sigma-Aldrich Co. Ltd, Lot# 100K0130, 99.3% Purity	Structure: 
Study objective: To determine the aqueous solubility of progesterone.	
Experimental conditions: The column elution method was used for this determination. Progesterone was dissolved in methanol and applied to silica sand. The methanol was evaporated, leaving the silica sand coated with progesterone. The coated sand was placed in a glass column and reagent grade water was pumped through the column at 0.4 mL/min. This study was repeated using a flow rate of 0.2 mL/min. The column effluent was assayed using HPLC with UV detection to determine the progesterone concentration.	
Principal of test: Test substance is applied to silica sand and water is pumped through the sand until the system reaches equilibrium. The concentration in ten consecutive fractions is determined. The flow rate is halved and the experiment repeated. Flow rate continues to be halved until the solubility does not change.	
Results: At a flow rate of 0.4 mL/min the mean progesterone concentration of ten consecutive fractions was determined to be 7.33 µg/mL at 20°C ± 0.5°C at a pH of 7. The experiment was repeated at a flow rate of 0.2 mL/min. Under these conditions the mean progesterone concentration of ten consecutive fractions was determined to be 7.30 µg/mL at 20°C ± 0.5°C at a pH of 7. Since the lower flow rate produced equalivant results as the high flow rate, additional experiments with even lower flow rates were not required. The mean of the two flow rates was therefore used as final summary.	
Conclusions: The solubility of progesterone in water was determined to be 7.31 µg/mL at 20°C ± 0.5°C at a pH of 7. This value is the mean of the observations at flow rates of 0.4 mL/min and 0.2 mL/min.	
Study in compliance with GLP: yes X no	

Appendix 4. Adsorption desorption of ^{14}C-progesterone in five soils

2. SUMMARY

2.1. Abstract

The objective of the study was to determine the soil adsorption/desorption properties of ^{14}C-progesterone by the batch equilibrium method in compliance with the the OECD Guideline for the Testing of Chemicals No. 106, and with consideration of the U.S. FDA Environmental Assessment Technical Assistance Handbook, Technical Assistance Document (TAD) 3.08. Five soil types were used and were selected based on characteristics to fit the guideline requirements.

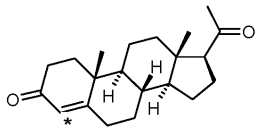
Soil I.D.	Soil type (USDA)	Collection Location	pH	% Organic Carbon	USDA Textural Class		
					% Sand	% Silt	% Clay
S122	Loamy Sand	Coleraine, N. Ireland	5.5	4.36	73	26	1
S123	Loam	Altuβheim, Germany	7.5	2.15	51	40	9
S124	Loam	Grandforks, ND, US	7.7	3.90	51	28	21
S125	Loamy Sand	Grandforks, ND, US	5.9	1.28	85	6	9
S126	Clay Loam	Glasgow, MT, US	8.1	0.99	35	26	39

Tier 1 and 2 tests indicated that a ratio of soil:solution of 1:25 w/v was appropriate for the test. The definitive tier 3 test was conducted with an adsorption/desorption equilibration time of 2 hours. These short equilibration times were chosen since tier 1 and 2 tests indicated that longer equilibration times resulted in degradation of progesterone in the soils, and may have influenced the results. The transformation by all soils at 2 hours was minimal (>90% recovery as progesterone). The definitive isotherm test was run in triplicate on all five soils. The progesterone concentrations were approximately 0.02, 0.04, 0.10, 0.50, and 2.00 µg/mL in 0.01 M $CaCl_2$, including blank samples. Incubations were conducted in the dark at 20°C ± 2°C.

Isotherm adsorption mean K_{oc} values were 7480, 1040, 6520, 15000, and 11200 mL/g for soils S122, S123, S124, S125, and S126, respectively. Progesterone was classified as having low mobility potential for soil S123, and being immobile for soils S122, S124, S125 and S126. The regression constant (n) varied only slightly from 1.00 indicating that there was no dependence on the progesterone concentration. Also the percent desorbed from the soil was lower than the percent not desorbed, which indicated that adsorption of the test item was not completely reversible and that the test item may not be freely desorbed from the test soil once bound. An obvious quantitative relationship between the adsorption K_d and a single soil variable (pH, percent organic matter, percent clay, cation exchange capacity, etc.), was not observed which suggests that the K_d is influenced by multiple factors.

Conclusions: The K_{oc} values ranged from 1040-15000 mL/g, indicating that progesterone mobility in soil can be classified from low mobility in one soil to immobile in the remaining 4 soils.

2.2. Tabular Summary

Product name:	Generic name: Progesterone PNU-3672
SECTION: Environment	Reference: Barney Bradley, Walter J. Smolenski and Joseph A. Robinson, Adsorption Desorption of ^{14}C-Progesterone in Five Soils, Study Report Number: SR-0847-7926-2003-002 Investigating laboratory: ABC Laboratories Europe, 38 Castleroe Road, Coleraine, Northern Ireland BT51 3RL. Study period (years): 2002
Composition of test substance: Progesterone Chemical Name: Pregn-4-ene-3, 20-dione Specific Activity: 50.8 mCi/mmoL Radiochemical purity: 99.5 % Molecular Weight 314.47 Chemical Formula: $C_{21}H_{30}O_2$	Structure: 
Study objective: To determine the soil adsorption/desorption properties of ^{14}C-Progesterone by the batch equilibrium method in five soils, in compliance with the OECD Guideline for the Testing of Chemicals No. 106.	
Experimental conditions: The definitive isotherm test was run in triplicate on all five soils. The concentrations were approximately 0.02, 0.04, 0.10, 0.50, and 2.00 µg/mL in 0.01 M $CaCl_2$, including blank samples. A ratio of soil:solution of 1:25 w/v was used with two- hour equilibration times. Incubations were conducted in the dark at 20°C ± 2°C.	
Principal of test: The equilibration of a chemical in soil between the sorbed and aqueous phases (0.01 M $CaCl_2$) depends on the physical-chemical properties of the compound and soil. The soil binding coefficient, normalized to organic carbon content of the soil (K_{oc}), is estimated using data obtained over a range of measured equilibration concentrations of the test substance.	
Results: Isotherm adsorption mean K_{oc} values were 7480, 1040, 6520, 15000, and 11200 mL/g for soils S122, S123, S124, S125, and S126, respectively. The test item was classified as having low mobility potential for soil S123, and being immobile for soils S122, S124, S125 and S126. Through the tier tests the data indicated that the ^{14}C-Progesterone degrades and shorter equilibrium times (2 h) were chosen so that the ^{14}C-progesterone did not transform in the soil. The regression constant (n) varied only slightly from 1.00 indicating that there was no dependence on the test item concentration. Also the percent desorbed from the soil was lower than the percent not desorbed, which indicated that adsorption of the test item was not completely reversible and that the progesterone may not freely desorbed from the test soil once bound. An obvious quantitative relationship between the adsorption K_d and a single soil variable (pH, percent organic matter, percent clay, cation exchange capacity, etc.), was not observed which suggests that the K_d is influenced by multiple factors.	
Conclusions: The K_{oc} values ranged from 1040-15000 mL/g, indicating that progesterone mobility in soil can be classified from low mobility in one soil to immobile in the remaining 4 soils.	
Study in compliance with GLP: yes X no	

Appendix 5. Concentrations of progesterone in milk

Study Report

Determination of Concentrations of Progesterone in Milk of Untreated Pregnant Cows and Estrous Cycling Cows with and without a CIDR™ 1380 Progesterone-Releasing Intravaginal Insert

Project Number:	AHRD-0847
Status of the Report:	FINAL
Study Number:	2001-0473
Protocol Number:	847-7926-I/O-YCA-02-001
GLP Status:	GLP
Previous Reports of the Study:	None
Study Director:	Rex E. Hornish, Ph.D. Preclinical Development Animal Health Pharmacia Corp. Kalamazoo, MI USA 49001

It is the policy of Pharmacia to conduct studies (including study conduct and the archiving of essential documents) in compliance with company SOPs and Standards, which incorporate the GLP requirements.

SUMMARY

Abstract

Introduction

The CIDR™ 1380 intravaginal progesterone insert, that contain 1.38 g of progesterone per insert, is being developed by Pharmacia Animal Health for synchronization of the return to estrus of previously inseminated lactating dairy cows. The proposed treatment regime for dairy cattle is to administer a CIDR insert 14±1 days after insemination with removal 7 days later. Most animals not pregnant as a result of the preceding insemination are expected to exhibit estrus within 1 to 3 days following insert removal. The purpose of this study was to determine the effect of CIDR inserts administered to dairy cows and retained *in situ* for 7 days on milk progesterone concentrations.

Study Objective

This study was designed to determine progesterone concentrations in milk of untreated pregnant dairy cows, untreated estrous cycling cows, and cycling cows treated with a CIDR 1380 insert containing 1.38 g of progesterone that were inserted intravaginally at 14±1 days after estrus for a 7-day period to establish whether or not the CIDR-treated cows produced an increase in levels of progesterone in their milk that was significantly less than the increase due to pregnancy.

Animal Phase

Sixty-four estrous cycling cows (>40 and <150 days after calving) were given a single IM injection of 5 mL of LUTALYSE® Sterile Solution (prostaglandin $F_2\alpha$; 6.7 mg dinoprost tromethamine/mL; 5 mg dinoprost equivalent/mL) on study day 0 to synchronize estrus. On study days 2-4, animals were observed for signs of estrous behavior. Twenty animals that were detected in estrus on study days 2-4 were enrolled in the study. These animals were not inseminated but were randomly assigned to one of two groups of 10 each: control cows or CIDR-treated cows. Control cows received no further treatment. CIDR cows received intravaginal administration of a CIDR 1380 insert on study day 17 (day 14±1 after estrus) with removal of the insert 7 days later (study day 24, day 21±1 after estrus). Composite milk samples from each animal in both groups were collected daily from study days 6 to 16 and then twice daily from study days 17 to 27. In addition, a group of 10 pregnant cows (≥60 and ≤220 days of gestation) were included in the study and milk samples were obtained for 11 consecutive days simultaneously with the control and CIDR treatment groups during study days 17 through 27.

Analytical Phase

The Coat-A-Count® Progesterone RIA kit manufactured by Diagnostic Products Corporation (DPC), Los Angeles, CA was used to assay the skim milk samples collected in this study for the concentration of progesterone. The kit was developed and validated by DPC to assay progesterone in human serum and plasma samples, but was modified and validated to assay bovine milk samples. The primary modification was that calibration standards were prepared in de-fatted, charcoal-stripped milk instead of human serum. Otherwise the kit was used as provided.

Data Analysis and Evaluation

The area under the curve (AUC) of the concentration-time plots for progesterone in milk were computed from days 17 through 27 of the study for each animal. These data were used as the decision variable to address differences in progesterone milk levels. Cows 8904 (a control cow) and 8917 (a CIDR cow) demonstrated aberrantly low levels of progesterone in their milk. Therefore, statistical analyses were performed with both the inclusion and exclusion of cows 8904 and 8917. A log transformation is typically applied to concentration type data to help stabilize the variance, thus analyses were conducted for both log-transformed data and untransformed data. The analysis of log transformed AUCs excluding cows 8904 and 8917 is the appropriate analysis to be used for decision-making.

The hypothesis under test was "is the increase in milk progesterone concentration for CIDR-treated cows, over that of estrous cycling control cows (increase due to CIDR insert administration), significantly less that the increase in milk progesterone in pregnant cows over that of estrous cycling cows (increase due to pregnancy)." To test this hypothesis, the difference between pregnant and control group mean AUCs were compared to the difference between CIDR and control group mean AUCs. The mean AUC for the pregnant cows was calculated as the arithmetic average. The means for the control and CIDR groups were determined using analysis of co-variance, since milk concentrations were higher pretreatment in cows of the control group than in the CIDR group. Therefore, milk progesterone concentration on study days 15, 16 and the AM milking on day 17 was used as a covariate as a method to adjust for differences in the pre-treatment period. If the 95% upper confidence limit on the difference in progesterone concentration between CIDR and control groups was less than the difference between pregnant and control groups then it could be concluded that the increase in progesterone concentration in milk due to CIDR insert administration is within an acceptable range.

For the log transformed AUC excluding cows 8904 and 8917, the pregnancy group mean was 3.81 day•ng/mL and the adjusted control group mean was 3.05 day•ng/mL. Thus, the increase due to pregnancy, Δ, was calculated as 0.76 day•ng/mL (3.81 – 3.05). This Δ represents an acceptable increase in milk progesterone. The hypothesis test is to determine if the increase due to use of the CIDR insert increases the milk progesterone significantly less than this acceptable increase. The CIDR group mean was 3.33 day•ng/mL thus the increase due to

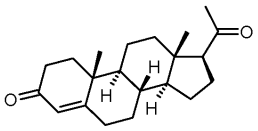
CIDR was 0.28 day•ng/mL (3.33 – 3.05) compared to the increase due to pregnancy of 0.76 day•ng/mL. Applying a 95% upper confidence interval to the increase due to CIDR gives a value of 0.70 day•ng/mL and because 0.70 day•ng/mL is less than 0.76 day•ng/mL the null hypothesis can be rejected. Therefore, it can be concluded that the use of the CIDR insert causes an increase in milk progesterone that is significantly less (α=0.05) than the increase resulting from pregnancy.

Conclusions

From the statistical analysis it can be concluded that the increase in milk progesterone due to the use of the CIDR intravaginal progesterone insert is significantly less than the increase in milk progesterone due to pregnancy. These results support the conclusion that use of a CIDR 1380 insert in lactating dairy cows should not require a milk discard period.

PNU-3672 SR-0847-7926-2002-001
Report of Study 2001-0473 10 June 2002

TABULAR SUMMARY

Product name: CIDR™ 1380	Generic name: CIDR – Progesterone
SECTION: Residues	Reference: Hornish RE, Krabill LF, Boucher JF, Anderson YC, Chenault JR. Prough MJ Determination of Concentrations of Progesterone in Milk of Untreated Pregnant Cows and Estrous Cycling Cows with and without a CIDR™ 1380 Progesterone-Releasing Intravaginal Insert. Pharmacia SR-0847-7926-2002-001, 10 June 2002. Investigating laboratory: Pharmacia Animal Health Study period (years): 2002
Species/strain: Bovine/Holstein No. of animals/sex: 30/lactating female (10 pregnant cows and 20 cows selected from 64 cows treated with Lutalyse and observed to be in estrus 2-4 days following treatment) Dose: 1.38 g Route of administration: Intravaginal Administrations: 1 Duration of treatment: 7 Formulation: Silicon-based insert Analytical method: RIA for progesterone in milk – LOQ = 0.10 ng/mL, LOD = 0.01 ng/mL	Chemical structure: 

Study Day	Milking Time	Treatment for	**Mean Progesterone Concentration in Skim Milk, ng/mL**		
	AM/PM	CIDR Group	**Pregnant Group***	**Control Group†**	**CIDR Group‡**
17	AM	CIDR Implanted	4.37	3.25	2.81
17	PM	(after AM milk)	4.36	3.62	3.83
18	AM		4.74	3.66	3.87
18	PM		4.83	3.69	4.26
19	AM		4.59	3.59	4.04
19	PM		4.40	3.94	3.77
20	AM		4.47	3.18	3.30
20	PM		5.08	3.50	3.92
21	AM		5.40	3.89	3.89
21	PM		4.75	2.86	3.49
22	AM		4.46	2.67	2.70
22	PM		4.38	2.30	2.65
23	AM		4.43	1.93	2.71
23	PM		4.82	2.31	3.05
24	AM	CIDR Removed	4.25	1.95	2.16
24	PM	(after AM milk)	4.93	2.12	1.46
25	AM		4.38	1.76	1.17
25	PM		4.97	1.99	0.93
26	AM		4.19	1.06	0.62
26	PM		4.23	0.88	0.60
27	AM		4.41	0.59	0.51
27	PM		4.88	0.70	0.49

* Progesterone levels in lactating pregnant cows. † Progesterone levels in lactating open estrus-cycling non-treated cows. ‡ For Progesterone levels in lactating estrus-cycling CIDR-implanted cows

Statistical Analysis Results, AUC (day•ng/mL)

Variable	Pregnant Mean (SE)	CIDR* Mean (SE)	Control* Mean (SE)	Δ†	D‡ (SE)	UCL§	Reject H_0¶
Log(AUC)**	3.81 (0.08)	3.33 (0.16)	3.05 (0.16)	0.76	0.28 (0.24)	0.70	YES
Log(AUC)	3.81 (0.08)	3.22 (0.15)	2.86 (0.15)	0.96	0.37 (0.21)	0.74	YES
AUC**	46.33 (3.30)	30.13 (4.10)	24.81 (4.10)	21.53	5.32 (5.97)	15.90	YES
AUC	46.33 (3.30)	27.62 (3.64)	22.92 (3.64)	23.41	4.69 (5.21)	13.82	YES

* CIDR and control means adjusted for pre-treatment differences through analysis of covariance. † Δ = Pregnant Mean – Control Mean. ‡ D= CIDR Mean – Control Mean. § UCL = 95% upper confidence limit on D. ¶ Reject H_0 if UCL < Δ. ** Excludes control cow 8904 and CIDR cow 8917 due to violations of protocol criteria.

Assay method and additional information on conduct of study: The Coat-A-Count® Progesterone RIA kit manufactured by Diagnostic Products Corporation (DPC), Los Angeles, CA was used to assay the skim milk samples collected in this study for the concentration of progesterone. The kit was originally developed and validated by DPC to assay progesterone in human serum and plasma samples, but the assay was validated for bovine milk samples using a modified procedure.

Significant findings: For the log transformed AUC excluding cows 8904 and 8917, the pregnancy group mean was 3.81 day•ng/mL and the adjusted control group mean was 3.05 day•ng/mL. Thus, the increase due to pregnancy, Δ, was calculated as 0.76 day•ng/mL (3.81 – 3.05). This Δ represents an acceptable increase in milk progesterone. The hypothesis tested was to determine if the increase due to use of the CIDR insert increases milk progesterone significantly less than this acceptable increase. The CIDR group mean was 3.33 day•ng/mL, thus the increase due to CIDR was 0.28 day•ng/mL (3.33 – 3.05) compared to the increase due to pregnancy of 0.76 day•ng/mL. Applying a 95% upper confidence interval to the increase due to CIDR gives a value of 0.70 day•ng/mL and because 0.70 day•ng/mL is less than 0.76 day•ng/mL the null hypothesis can be rejected. Therefore, it can be concluded that the use of the CIDR insert causes an increase in milk progesterone that is significantly less (α=0.05) than the increase resulting from pregnancy.

Conclusions: From the statistical analysis it can be concluded that the increase in milk progesterone due to the use of the CIDR intra vaginal progesterone insert is significantly less than the increase in milk progesterone due to pregnancy. These results support the conclusion that use of a CIDR™ 1380 insert in lactating dairy cows should not require a milk discard period.

Study in compliance with GLP: yes X no

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