ENVIRONMENTAL ASSESSMENT

CYSTORELIN® (gonadorelin)

DATE:	**October 6, 2017**
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ACRONYMS

FTAI	fixed-time artificial insemination
FSH	follicle stimulating hormone
GnRH	gonadotropin releasing hormone
IM	intramuscular
IV	intravenous
LH	luteinizing hormone
NASS	National Agricultural Statistics Service
NAHMS	National Animal Health Monitoring System
$PGF_{2\alpha}$	prostaglandin F2α

1. DESCRIPTION OF PROPOSED ACTION(S) AND NEED

1.1 Proposed Use and Indications

CYSTORELIN (gonadorelin) is currently approved in the U.S. for the treatment of ovarian follicular cysts in cattle (NADA 098-379). Ovarian cysts are non-ovulated follicles with incomplete luteinization which result in nymphomania or irregular estrus. CYSTORELIN initiates release of endogenous LH to cause ovulation and luteinization.

The proposed indication for CYSTORELIN, investigated under this EA, is for use with cloprostenol sodium to synchronize estrous cycles to allow for fixed time artificial insemination (FTAI) in lactating dairy cows and beef cows.

Cloprostenol (as the sodium salt) is approved in the U.S. for intramuscular use to induce luteolysis in beef and dairy cattle. The luteolytic action of cloprostenol can be utilized to manipulate the estrous cycle to better fit certain management practices, to terminate pregnancies resulting from mismatings, and to treat certain conditions associated with prolonged luteal function. For the proposed use, there is no change to the approved indication for cloprostenol.

1.2 Dosage and Route of Administration

For treatment of ovarian follicular cysts, the recommended intravenous or intramuscular dosage of CYSTORELIN (as gonadorelin diacetate tetrahydrate) is 100 mcg (2 mL) per cow.

The recommended intramuscular dosage of CYSTORELIN for synchronization of estrous cycles is 100 mcg (2 mL) per animal. If CYSTORELIN is administered twice, the first IM injection is administered 6 to 8 days prior to administration of prostaglandin and the second IM injection of CYSTORELIN (2 mL) is administered 30 to 72 hours after prostaglandin. Use FDA-approved labeled dosage and administration instructions for the prostaglandin (cloprostenol sodium) to cause luteolysis. Inseminate animals either at detected estrus according to standard herd practices, or at 0 to 24 hours after a second CYSTORELIN injection.

This EA employs the conservative assumption that gonadorelin is administered in two doses.

The dosage of cloprostenol sodium product, for the proposed indication, is 0.5 mg cloprostenol (2 mL) administered once per animal by IM injection. Each mL contains cloprostenol sodium equivalent to 250 mcg cloprostenol (NADA 113-645).

2. IDENTIFICATION OF SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

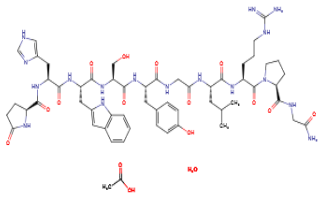
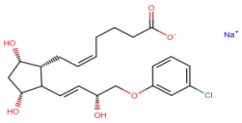
2.1 Description of Active Ingredients

CYSTORELIN contains the active ingredient gonadorelin diacetate tetrahydrate (50 mcg/mL) (see Table 2-1). CYSTORELIN also contains benzyl alcohol (9 mg/mL), sodium chloride (7.47 mg/mL), and water for injection. Gonadorelin is a decapeptide composed of a sequence of amino acids (5-oxoPro-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2-a). It has a molecular weight of 1182.32 g/mol and empirical formula $C_{55}H_{75}N_{17}O_{13}$. The diacetate tetrahydrate ester has a molecular weight of 1374.48 g/mol and empirical formula $C_{59}H_{91}N_{17}O_{21}$. Gonadorelin has a structure identical to the natural

gonadotropin-releasing hormone (GnRH). GnRH is the hypothalamic releasing factor responsible for the release of gonadotropins (e.g., LH, FSH) from the anterior pituitary. Synthetic gonadorelin is physiologically and chemically identical to the endogenous bovine hypothalamic releasing factor.

Cloprostenol sodium is a synthetic prostaglandin analogue, structurally related to prostaglandin F2α ($PGF_{2\alpha}$) (see Table 2-1).

Table 2-1 Physical and chemical properties of gonadorelin diacetate tetrahydrate and cloprostenol sodium

Standard Name	gonadorelin diacetate tetrahydrate	cloprostenol sodium
CAS No.	78308-49-3	55028-72-3
Molecular weight	1374.5 g/mol[1]	446.9 g/mol[2] (MW of cloprostenol = 424.9)[3]
Molecular formula	$C_{59}H_{91}N_{17}O_{21}$[1]	$C_{22}H_{28}ClNaO_6$[3]
Melting point	Not available	68-70 °C[4]
Molecular[1,2] structure		

[1] Chem ID Plus 2014a
[2] Chem ID Plus 2014b
[3] Chem ID Plus 2014c
[4] Chemical Book 2014

2.2 Description of the Mode of Action of the Active Ingredients

Endogenous gonadorelin is synthesized and/or released from the hypothalamus during various stages of the bovine estrus cycle following appropriate neurogenic stimuli. It passes via the hypophyseal portal vessels, to the anterior pituitary to affect the release of gonadotropins [e.g., luteinizing hormone (LH), follicle-stimulating hormone (FSH)]. Pharmaceutical gonadorelin administered intravenously (IV) or IM also causes the release of endogenous LH and FSH from the anterior pituitary.

$PGF_{2\alpha}$ is secreted by uterine endometrial cells of nonpregnant cows. The luteolytic properties of $PGF_{2\alpha}$ and its analogues in cattle are well established; however, corpora lutea are refractory to PGF-induced luteolysis during the early stages of the estrous cycle in cattle (Jackson et al., 1979; Kiracofe et al., 1985). Thus, administration of exogenous PGF to cattle induces regression of corpora lutea that have acquired luteolytic capacity. Cloprostenol sodium is a $PGF_{2\alpha}$ analog that binds to the $PGF_{2\alpha}$ receptor.

When given at a random stage of the estrous cycle, the first treatment of synthetic gonadorelin induces ovulation and causes emergence of a new follicular wave in treated cows or heifers. Treatment with cloprostenol approximately 7 days later regresses the spontaneous and/or GnRH-induced corpora lutea. Finally, the second treatment of synthetic gonadorelin synchronizes the time of ovulation of the dominant follicle of the follicular wave that began

growing after the first injection. In this way, the treatment protocol with synthetic gonadorelin and cloprostenol synchronizes follicular development, luteal regression, and ovulation, such that artificial insemination can be conducted at a fixed time without the need for estrus detection.

3. PRODUCT USE PATTERN

3.1 Description of Proposed Product Use

CYSTORELIN is proposed for use with cloprostenol sodium to synchronize estrous cycles to allow for fixed time artificial insemination (FTAI) in lactating dairy cows and beef cows.

The recommended intramuscular dosage of CYSTORELIN for synchronization of estrous cycles is 100 mcg (2 mL) per animal. If CYSTORELIN is administered twice, the first IM injection is administered 6 to 8 days prior to administration of prostaglandin, and the second IM injection of CYSTORELIN (2 mL) is administered 30 to 72 hours after prostaglandin. Use the FDA-approved labeled dosage and administration instructions for the prostaglandin (cloprostenol sodium) to cause luteolysis. Inseminate animals either at detected estrus according to standard herd practices, or at 0 to 24 hours after a second CYSTORELIN injection.

3.2 Description of the Current Breeding Practices for Dairy and Beef Cattle where the Product Would Be Used

According to the USDA National Agricultural Statistics Service (NASS) (USDA, 2014), as of July 1, 2014, the total number of cattle and calves in the U.S. was 95 million head, which includes 39 million cows and heifers that have calved. Of these, 29.7 million are beef cows and 9.3 million are milk cows.

3.2.1 *Dairy Industry*

Results from the USDA's National Animal Health Monitoring System (NAHMS) national dairy study conducted in 2007 (USDA, 2009a), are discussed below.

Reproductive practices in dairy operations are crucial to maintain a timely calving regime, as well as consistent milk production (USDA, 2009a). At the time of the study, the average age for time at first calving was 25.2 months and the calving interval (time between producing a healthy calf) was 13.2 months. The study, which captured data on 79.5% of the Nation's dairy operations, found that timed artificial insemination programs were used by 58.2% of operations to manage reproduction in at least some of the heifers and/or cows in the operation, and that these operations used timed artificial insemination at a higher percentage for cows (57.6%) than for heifers (25.4%). However, the study also showed that less than 7% of operations used individual timed artificial insemination protocols, such as the use of prostaglandins and GnRH to induce ovulation, for first-service breeding on the majority of females (USDA, 2009a). It is likely that usage has since increased.

3.2.2 *Beef Industry*

According to the NAHMS Beef Cow-calf Studies conducted in 2007–2008, 7.9% of all operations investigated used estrus synchronization to help improve reproductive efficiency, and utilization increased as herd size increased (USDA, 2009b).

4. PHARMACOKINETICS AND ENVIRONMENTAL FATE

4.1 Literature Search

A comprehensive literature search was performed to identify the pharmacokinetic and environmental fate characteristics of gonadorelin and cloprostenol. Publically available databases (e.g., HSDB, DrugBank 4.0) were searched, along with various environmental sciences databases (MEDLINE®, AGRICOLA, AGRIS, CAB ABSTRACTS, Plant Science, BIOSIS Previews®, Aquatic Science & Fisheries Abstracts (ASFA), Water Resources Abstracts, Aqualine, Ecology Abstracts, Environment Abstracts, GEOBASE™, Animal Behavior Abstracts, ToxFile®, Toxicology Abstracts, TOXLINE, PASCAL, Embase®, and Zoological Record Plus), which were searched via the ProQuest DIALOG search service. The following search terms were used in combination for the searches:

- Environmental fate terms: degradation, biodegradation, hydrolysis, transformation, soil adsorption/desorption
- Pharmacokinetic terms: metabolism, excretion, elimination
- Synonyms/other search terms for gonadorelin: (synthetic) GnRH, (synthetic) Gonadotropin Releasing Hormone, GONABREED, Luforan, Lutamin
- Synonyms/other search terms for cloprostenol: ESTROPLAN, Cloprostenolum, Estrofan, ESTRUMATE, $PGF_{2\alpha}$
- Appropriate CAS numbers for gonadorelin and cloprostenol compounds were also included in the search

Results of the literature search are discussed in the sections below.

4.2 Environmental Fate of Gonadorelin Diacetate Tetrahydrate and Cloprostenol Sodium

Only one study (Mesquita, 2003) was identified as relevant to the environmental fate of the substances of interest during the literature search, and it is described below. Most of the environmental fate characteristics, shown in Table 4-1, were obtained from publically available websites, and are typically based on values estimated by modeling. No environmental fate data were available for gonadorelin diacetate tetrahydrate; therefore, values available for gonadorelin are used, because it is assumed that fate properties (such as partition coefficients) would not be affected significantly by the form of the salt moiety.

In the study performed by Mesquita et al. (2003), the partitioning of $PGF_{2\alpha}$ between water and organic components (aggregates of suspended humic substances and negatively charged phospholipid vesicles) in the water column was examined. Concentrations of humic acids used in this study are representative of concentrations found in natural water systems according to Mesquita et al. (2003). The results indicate that $PGF_{2\alpha}$ and analogues of this compound are likely to be removed (in part) from the water column due to partitioning to organic components, although the effects of this for $PGF_{2\alpha}$ were less than for the other two endogenous substances tested. This study did not resemble a standard guideline (OECD 106) study, so the results could not be used to characterize the K_{oc} for use in subsequent calculations.

4.3 Pharmacokinetics

Because the salts dissociate in mammalian systems, the pharmacokinetic information for gonadorelin diacetate tetrahydrate and cloprostenol sodium is the same as for gonadorelin and cloprostenol. This information, presented below, indicates that both substances are metabolized

rapidly. However, as a worst case scenario, these substances are assumed to be completely excreted from treated cattle.

4.3.1 *Pharmacokinetics of Gonadorelin*

Gonadorelin undergoes rapid metabolism by peptidase enzymes into smaller, inactive peptides and amino acids that are excreted in urine. The plasma half-life of gonadorelin in cattle is less than 0.5 hours (Monnoyer et al., 2004).

Peterson and Nett (1976) studied the clearance of synthetic GnRH after IM or IV injections in cattle. A total of 22 Hereford heifers weighing from 170 to 200 kg were divided into four groups. Ten heifers (Group 1) were given 2.5 mg synthetic GnRH IM. Four heifers (Group 2) received 100 mCi ^{125}I-GnRH IM. Four heifers (Group 3) received an injection of 2.5 mg synthetic GnRH IV. Four heifers (Group 4; controls) were administered 5.0 ml sterile saline IM. Maximal levels (186 and 82 pg/mL, respectively) of GnRH were observed at 5 and 15 minutes after dosing via IV and IM routes, respectively. Muscle-tissue levels of GnRH returned to baseline levels within 24 hours, and serum levels returned to baseline after 1.5 hours (IV) or 8 hours (IM).

In another study, 12 lactating cattle, weighing approximately 600 kg, were administered doses of 50, 100, or 200 mcg of gonadorelin diacetate tetrahydrate as a single IM injection (Monnoyer et al., 2004). At 5 hours post-administration, even for the highest dose administered (200 mcg), plasma GnRH fell below the limit of detection. Maximum concentrations measured were 60, 124, and 267 ng/L for the 50, 100, and 200 mcg treatments, respectively. The terminal half-life of GnRH in plasma was found to be 0.46 hour, and the GnRH rate of clearance in plasma was estimated to be 43 mL/kg/min.

Table 4-1 Environmental fate parameters for gonadorelin diacetate tetrahydrate and cloprostenol sodium

Property	Gonadorelin Diacetate Tetrahydrate	Reference	Cloprostenol Sodium	Reference
Solubility in water (mg/L)	5.88×10^{-2} g/L (58.8 mg/L)	DrugBank 2014, for gonadorelin	50 mg/mL (50000 mg/L)	Santa Cruz Biotechnology, 2014a, for cloprostenol
Octanol-water partition coefficient (LogP)	-6.3 to -0.09	DrugBank 2014, for gonadorelin	2.31	ChemSpider, 2015, for cloprostenol
Octanol-water distribution coefficient (LogD)	pH 5.5: -6.41 pH 7.4: -5.44	ChemSpider, 2014, for gonadorelin	pH 5.5: 1.69 pH 7.4: -0.11	ChemSpider, 2015, for cloprostenol
Soil organic carbon-water partitioning coefficient (K_{oc})	pH 5.5: 1 pH 7.4: 1	ChemSpider, 2014, for gonadorelin	pH 5.5: 84.16 pH 7.4: 1.35	ChemSpider, 2015, for cloprostenol
Dissociation constant (pKa)	9.47 to 11.16	DrugBank 2014, for gonadorelin	4.76	Santa Cruz Biotechnology, 2014a, for cloprostenol
Vapor pressure at 25°C (Pa)	Not Evaluated	-	0.0±1.9 mmHg	ChemSpider, 2015, for cloprostenol

4.3.2 *Pharmacokinetics of Cloprostenol*

Pharmacokinetic studies show that cloprostenol is excreted very rapidly, and detectable levels of cloprostenol do not persist in blood, milk, or cattle tissues.

In a study conducted by Reeves (1978), cloprostenol (administered as C^{14} radiolabeled) was administered via IM injection to 16 dairy cows, each weighing approximately 500 kg. The cloprostenol dose (0.5 mg) was preceded by treatment with $PGF_{2\alpha}$, such that eight cows were in the luteal phase and eight in the follicular phase of the estrous cycle at the time of cloprostenol dosing. Milk, urine, and blood, as well as various tissues, were sampled to determine post-dosing levels of cloprostenol. Cloprostenol and its metabolites were excreted very rapidly, with maximum tissue levels occurring about 30 minutes post-dosing and declining thereafter. Forty-eight hours after treatment, tissue residues in cattle were present in only three samples: one injection site, one kidney, and one uterus sample. Elimination was about equally divided between urinary and fecal routes, and the mean half-life of elimination in urine was 2.8 hours.

Bourne et al. (1980a) reported a maximum concentration of 0.270 ng/mL of cloprostenol in cow milk, occurring at 4 hr post-dose administration (0.5 mg via intramuscular injection). It was also reported that increasing the dose from 0.5 mg to 10 mg did not seem to affect the rate at which the cloprostenol was cleared. Bourne et al. (1980b) reported further that, after doses of 0.5 mg and 10 mg of cloprostenol, urinary excretion accounted for 58.2% and 56.3%, respectively, of the administered dose.

4.3.3 *Pharmacokinetics of Endogenous $PGF_{2\alpha}$ and other Synthetic Analogues*

In a metabolic study performed in cattle, ^{3}H-radiolabelled etiproston, a synthetic analogue of $PGF_{2\alpha}$, was administered intramuscularly. At 48 hours post-administration, most of the radioactivity had been eliminated (92.6%); 66% and 26% of the radioactivity was found in the urine and feces, respectively (Benech et al., 1994).

Manns (1975) found maximum plasma and milk concentrations of $PGF_{2\alpha}$ of 2.4 ng/mL and 0.91 ng/mL shortly after IM administration of 30 mg $PGF_{2\alpha}$. Levels in both milk and plasma returned to baseline within 3 hours post-administration.

Lamond et al. (1973) also found rapid clearance of $PGF_{2\alpha}$ after IV and intrauterine administrations in cattle, reporting a return to baseline in a matter of minutes.

4.4 Metabolism

The information, presented below outlines the metabolic schemes for gonadorelin and cloprostenol and indicates that the resulting metabolites are either biologically less active than the parent compound or inactive.

4.4.1 *Metabolism of Gonadorelin*

Gonadorelin undergoes rapid metabolism by peptidase enzymes into smaller, inactive peptides and amino acids that are excreted in urine (Krause *et al.*, 1982). The primary cleavage occurs at the tyrosine[5]-glycine[6] bond (see star); howerver, cleavage may occure anywhere along the peptide chain (Schally *et al*., 1972; Fujino *et al.,* 1972). Evenually the peptide frangments will break down to the individual amino acids (*i.e.*, glycine, leucine, proline, arginine, histidine, glutamic acid, serine, tyrosine and tryptophan) (Krause *et al.*, 1982).

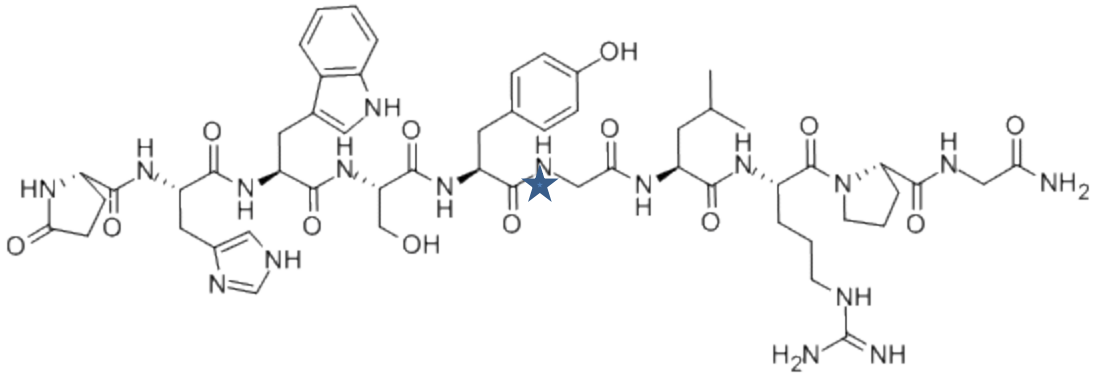


Figure 1. The Structure of Gonadorelin (The star indicates the primary site of cleavage).

Schally *et al*. (1972) and Fujino *et al.* (1972) examined the the biological activity of various fragments of gonadorelin. The N-terminal tripeptide and tetrapeptide fragments of gonadorelin, as well as the C-terminal octapeptide and nonapeptide were shown to be inactive (Schally *et al*., 1972), while alteration of N- or C-terminal amino-acids resulted in a dramatic loss of activity (Fujino *et al*., 1972). Thus, the peptide fragments derived from GnRH metabolism show little-to-no biological activity and are unlikely to have any environmental consquence.

4.4.2 *Metabolism of Cloprostenol*

Cloprostenol is an analogue of the endogenous prostaglandin PGF2. Cloprostenol, following intramuscular administration in cows, was eliminated in the urine as two major components; the unchanged drug and its tetranor-acid, as well as a minor component, the glucuronic acid conjugate of tetranor-cloprostenol (Bourne *et al*., 1980b). Endogenous $PGF_{2\alpha}$ metabolism occurs rapidly via three main pathways: 1) oxidation of the C13-C15 allylic alcohol system by 15-hydroxyprostaglandin-dehydrogenase and 13,14-reductase, 2) β-oxidation of the carboxylic side chain, and 3) hydroxylation and oxidation of the aliphatic side chain. (Nidy and Johnson, 1975; Bourne *et al*., 1980b). However, due to the presence of the aryloxy group in cloprostenol, metabolism by the 1st and 3rd pathways is not possible, and therefore β-oxidation of the carboxylic side chain is the main metabolic pathway (Welburn and Jones, 1978). Cloprostenol is metabolized to the tetranor-acid of cloprostenol via two β-oxidation steps (Figure 1). The major metabolite of cloprostenol is the tetranor acid of cloprostenol, and its biological activity is approximately one hundredth that of cloprostenol (EMEA, 1997). The glucuronic acid conjugate of tetranor-cloprostenol is a result of glucuronidation of the tetranor acid of cloprostenol and is a minor bovine metabolite (Bourne *et al*., 1980b).

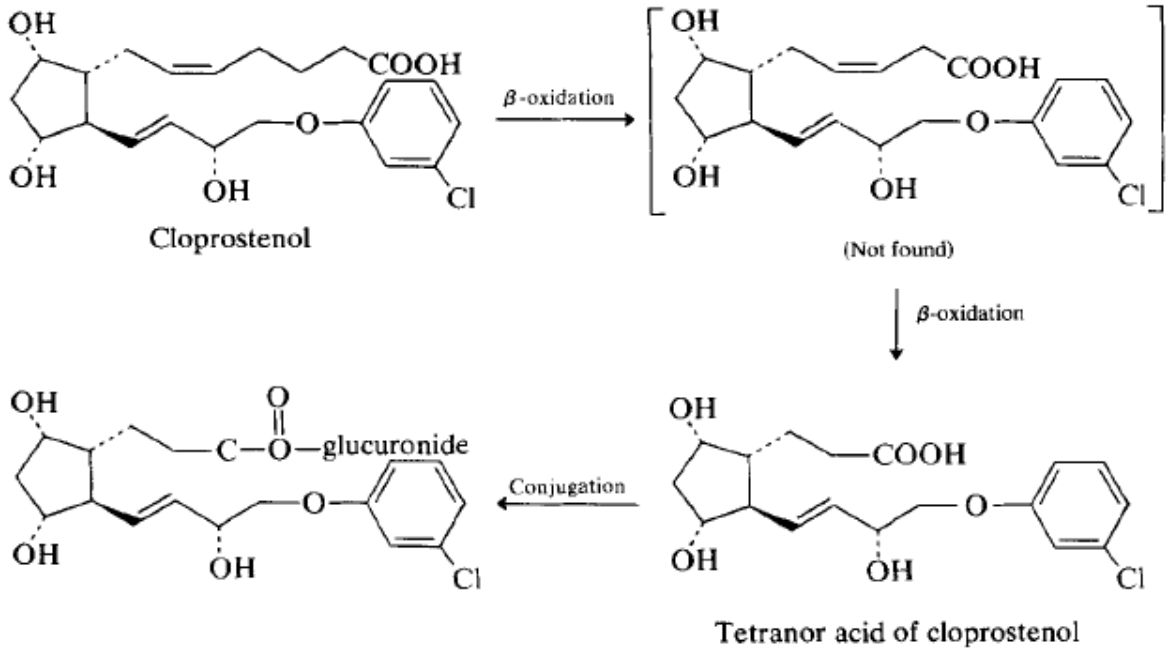


Figure 2. The Metabolism of Cloprostenol in the Cow (Bourne *et al*., 1980b).

5. EXPOSURE ASSESSMENT

The proposed use of CYSTORELIN plus cloprostenol sodium will result in excretion of the subject compounds through the feces and urine of treated cattle. In this manner, the compounds can enter the soil and water compartments of the environment and potentially result in exposure to terrestrial and aquatic plants and animals. These potential exposures are evaluated using conservative assumptions to calculate the predicted environmental concentration (PEC) in soil (PEC_{soil}) and in surface water ($PEC_{surfacewater}$). The approach follows that given in EMEA (2008) and includes calculations for both intensively reared cattle and pastured cattle, because animals potentially treated with the proposed products could be raised under either scenario.

Following the guidance given in FDA/CVM Guidance for Industry #89 (CVM, 2001), the initial PEC_{soil} values, calculated using conservative assumptions, are compared to the Phase I trigger limit of 100 mcg/kg. Where these values are less than the Phase I trigger, a Phase II EA is normally not required, and the assessment stops. However, given the elevated awareness of the potential impact of hormones in the environment, additional evaluation of the $PEC_{surfacewater}$ was performed following Phase II procedures (CVM 2006).

5.1 PEC_{soil} for Intensively Reared Cattle

The initial PEC_{soil} for intensively reared animals is calculated according to the following equation found in EMEA (2008):

$$PEC_{soil\ initial} = \left(\frac{D \times Ad \times BW \times P \times Ns \times Fh}{\rho \times A \times Ds \times Ny \times H}\right) \times CF \qquad \textbf{Eq. 5-1}$$

where PEC_{soil}: predicted environmental concentration in soil [mcg/kg]

D	:	daily dose of the active ingredient [mg/kg bw/d]
Ad	:	number of days of treatment [d]
BW	:	animal body weight [kg]
P	:	animal turnover rate per place per year [/place × year]
Ns	:	nitrogen spreading limit [kg N/ha]
F_h	:	fraction of herd treated [n]
ρ	:	bulk density of dry soil [kg/m^3]
A	:	area of 1 hectare (ha) [m^2/ha]
D_s	:	depth of penetration into soil [m]
Ny	:	Nitrogen produced in one year per place [kg N/place × year]
H	:	Housing factor (either 1 for animals housed throughout the year or 0.5 for animals housed for only 6 months)
CF	:	conversion factor [1000 mcg/mg]

The assumptions included in Table 5-1 are used for the equation described above, per EMEA guidelines.

Table 5-1 Default values for use in calculating the PEC_{soil} for intensively reared animals

Animal type	Number of animals raised per place per year (P)	Bodyweight (BW) [kg]	Nitrogen produced in 1 year per place (Ny) [kg N/place per year]	Housing factor (H)
Dairy cow	1	425	60	0.5
Cattle (>2 years)	1	450	35	0.5

Gonadorelin is administered either as one dose or two doses. This EA employs the conservative assumption of two doses of gonadorelin.

The value for Ns (nitrogen spreading limit) given in the EMEA guidance is applicable to Europe (EMEA, 2008). Therefore, U.S.–specific data were used instead. General nitrogen application standards in the U.S. by region were characterized by Ribaudo et al. (2003), using data from the 2000 Agricultural Resource Management Survey. Average nitrogen application standard rates for dairy operations were 186 lbs N/acre (approximately 210 kg N/ha) in the northern regions and 223 lbs N/acre (approximately 250 kg N/ha) in the southern regions. In a 2009 Report to Congress (USDA, 2009c), the USDA reported that average manure nutrient application rates (2003–2006) for nitrogen (including hog, broiler, and dairy producers), ranged from approximately 30 to130 lbs N/acre (34 to 146 kg N/ha), depending on the crop planted. For the purposes of this analysis, it is assumed that manure will be applied at a rate of 250 kg N/ha as a worst-case estimate.

The values used for the parameters in Equation 5-1 for each compound, for the intensively reared cattle scenario, are presented in Table 5-2, as are the calculated initial PEC_{soil} values.

Table 5-2 Initial PEC_{soil} of gonadorelin and cloprostenol for intensively reared cattle

Parameter	Gonadorelin		Cloprostenol		Reference
	Dairy cattle	Beef cattle	Dairy cattle	Beef cattle	
D, Daily dose (mg), per animal	0.100	0.100	0.5	0.5	Section 1.2
D, Daily dose (mg/kg bw/day)	$2.35x10^{-4}$	$2.22 x10^{-4}$	$1.18x10^{-3}$	$1.11x10^{-3}$	Section 1.2 and Table 5-1
Ad, number of days of treatment	2	2	1	1	Conservatively assessed as two consecutive doses for gonadorelin (Section 1.2)
BW, animal body weight, kg	425	450	425	450	EMEA, 2008 default (Table 5-1)
P, Animal turnover rate (/place x y)	1				EMEA, 2008 default (Table 5-1)
Ns, Nitrogen spreading (kg N/ha)	250				Ribaudo et al., 2003
Fh, fraction of herd treated	1				Worst-case assumption
ρ, bulk density of dry soil, kg/m^3	1500				EMEA, 2008 default
A, area of 1 hectare, m^2/ha	10000				EMEA, 2008 default
Ds, depth of penetration into soil, m	0.05				EMEA, 2008 default
Ny, Nitrogen produced (kg N/place x y)	60	35	60	35	EMEA, 2008 default
H, Housing factor	0.5				EMEA, 2008 default
CF, Conversion factor	1000				EMEA, 2008 default
PEC_{soil}, (mcg/kg)	0.0022	0.0038	0.0056	0.0095	Eq. 5-1

5.2 PEC_{soil} for Pasture Reared Cattle

The initial PEC_{soil} for pasture-reared animals is calculated according to the following equation found in EMEA (2008):

$$PEC_{soil} = \frac{D \times Ad \times BW \times SD \times F_H}{\rho * A * Ds} \times CF \qquad \textbf{Eq. 5-2}$$

where PEC_{soil} : PEC_{soil} in mcg/kg
D : daily dose of the active ingredient [mg/kg bw/d]
Ad : number of days of treatment [d]
BW : animal body weight [kg]
SD : stocking density [animal/ha]
F_H : fraction of herd treated [n]
ρ : bulk density of dry soil [kg/m^3]
A : area of 1 hectare (ha) [m^2/ha]
Ds : depth of penetration into soil [m]
CF : conversion factor [1000 mcg/mg]

The default values for stocking density and animal body weight from EMEA (2008), shown in Table 5-3, were used in the calculations. All values used in solving Equation 5-2, as well as the resulting PECs for pasture-reared cattle for each compound, are presented in Table 5-4.

Table 5-3 Default values for use in calculating the PEC_{soil} for pasture-reared animals

Animal type	Stocking density (animals/ha)	Body weight (BW) [kg]
Dairy cow	3.5	600
Beef Cattle	9.5	330

Table 5-4 Initial PEC_{soil} of gonadorelin and cloprostenol for pasture-reared cattle

Parameter	Gonadorelin		Cloprostenol		Reference
	Dairy cattle	Beef cattle	Dairy cattle	Beef cattle	
D, Daily dose (mg), per animal	0.100	0.100	0.5	0.5	Section 1.2
D, Daily dose (mg/kg bw/day)	$1.67x10^{-4}$	$3.03x10^{-4}$	$8.33x10^{-4}$	$1.52x10^{-3}$	Section 1.2 and Table 5-3
Ad, number of days of treatment	2	2	1	1	Conservatively assessed as two consecutive doses for gonadorelin (Section 1.2)
BW, animal body weight, kg	600	330	600	330	EMEA, 2008 default (Table 5-1)
Fh, fraction of herd treated	1				Assumed max
ρ, bulk density of dry soil, kg/m^3	1500				EMEA, 2008 default
A, area of 1 hectare, m^2/ha	10000				EMEA, 2008 default
Ds, depth of penetration into soil, m	0.05				EMEA, 2008 default
CF, Conversion factor	1000				EMEA, 2008 default
PEC_{soil}, (mcg/kg)	0.00093	0.0025	0.0023	0.0063	Eq. 5-2

5.3 Discussion of initial PEC_{soil} values

The EMEA guidance recommends that, for a combination product, the individual PEC_{soil} values should be summed and compared against the Phase I trigger. The worst-case scenario PEC_{soil} predicted for use in cattle is reflected by adding the highest PECs estimated for gonadorelin plus cloprostenol. The values for use in beef cattle were higher and represent the worst case.

The worst-case PEC_{soil} for intensively reared cattle (Table 5-2) is thus (0.0038 + 0.0095) = 0.0133 mcg/kg. The worst-case PEC_{soil} for pasture-reared cattle (Table 5-4) is thus (0.0025 + 0.0063) = 0.0088 mcg/kg. These values are significantly less than the Phase I trigger value of 100 mcg/kg. Synthetic gonadorelin is physiologically and chemically identical to the endogenous GnRH. Cloprostenol (cloprostenol sodium) is a synthetic analogue of the naturally occurring $PGF_{2\alpha}$. GnRH and $PGF_{2\alpha}$ are hormones that are produced endogenously and metabolized quickly. However, due to the general concern regarding potential environment impacts of the use of steroidal hormones, the $PEC_{surfacewater}$ is also calculated and compared to concentrations observed in natural systems.

5.4 Calculation of $PEC_{groundwater}$ and $PEC_{surfacewater}$

The initial $PEC_{surfacewater}$ is calculated using the equations in the EMEA (2008) guidance. As an initial step, the $PEC_{groundwater}$ is calculated via the following equation:

$$PEC_{groundwater} = \frac{PEC_{soil} \times RHO_{soil}}{K_{soil\ \_\ water} \times CF}$$

Eq. 5-3

where $PEC_{groundwater}$: predicted environmental concentration in groundwater [mcg/L]
PEC_{soil} : predicted environmental concentration in soil, calculated based on mixing depth of 20 cm in soil (0.2 m) rather than 5 cm (0.05 m) (effectively dividing the initial PEC_{soil} by 4) [mcg/kg]
RHO_{soil} : bulk density of fresh soil [1700 kg/m^3]
$K_{soil\text{-}water}$: partition coefficient solids and water in soil (v/v) [m^3/m^3]
CF : conversion factor

The $K_{soil\text{-}water}$ is calculated using the following formula:

$$K_{soil\ water} = (F_{air\ soil} \times K_{air\ water}) + F_{water\ soil} + (F_{solid\ soil} \times \frac{Kp_{soil}}{CF} \times RHO_{solid})$$

Eq. 5-4

where $Fair_{soil}$: fraction of air in soil [0.2 m^3/m^3]
$K_{air\text{-}water}$: partition coefficient air and water in soil [m^3/m^3]
$Fwater_{soil}$: fraction of water in soil [0.2 m^3/m^3]
$Fsolid_{soil}$: fraction of solids in soil [0.6 m^3/m^3]
Kp_{soil} : partition coefficient solids and water in soil (v/w) [L/kg]
CF : conversion factor
RHO_{solid} : density of soil solids [2500 kg/m^3]

$K_{air\text{-}water}$ and Kp_{soil} are calculated using equations 5-5 and 5-6, respectively:

$$K_{air\ \_\ water} = \frac{VP \times MW}{SOL \times R \times TEMP}$$

Eq. 5-5

$$Kp_{soil} = Foc_{soil} \times K_{oc}$$

Eq. 5-6

where VP : vapor pressure [Pa]
MW : molar mass [g/mol]
SOL : water solubility [mg/L]
R : gas constant [8.314 Pa x m^3/mol*K]
TEMP : temperature at air-water interface [285 K]
Foc_{soil} : weight fraction of organic carbon in soil [0.02 kg/kg]
K_{oc} : water/organic carbon partitioning coefficient [L/kg]

The values for the input parameters needed to solve Equations 5-4, 5-5, and 5-6, and the resulting values for $PEC_{groundwater}$ for the intensively reared cattle scenario are presented in Table 5-5. It is noted that the vapor pressure for gonadorelin is considered "negligible," so this value was set to zero. The molar mass (molecular weight) values used were for gonadorelin diacetate tetrahydrate and cloprostenol to be consistent with the dosing information in Section 1.2. The value selected for K_{oc} was the lowest of the available values for each compound, to be conservative (e.g., favoring partitioning to water). Similarly, the values for the input parameters and resulting values for $PEC_{groundwater}$ for the pasture-reared cattle scenario are presented in Table 5-6.

Table 5-5 Initial $PEC_{groundwater}$ of gonadorelin and cloprostenol for intensively reared cattle

Parameter	Gonadorelin		Cloprostenol		Reference
	Dairy cattle	Beef cattle	Dairy cattle	Beef cattle	
PEC_{soil} [mcg/kg]	0.00056	0.00095	0.0014	0.0024	Initial PEC_{soil}, modified to a 20 cm mixing depth
RHOsoil [kg/m^3]	1700				EMEA, 2008 (default)
Ksoil-water [m^3/m^3]	0.23	0.23	0.23	0.23	Calculation, Eq. 5-4
CF	1000				EMEA, 2008 default
$Fair_{soil}$ [m^3/m^3]	0.2				EMEA, 2008 default
Kair-water [m^3/m^3]	0	0	$5.9x10^{-20}$	$5.9x10^{-20}$	Calculation, Eq. 5-5
$Fwater_{soil}$ [m^3/m^3]	0.2				EMEA, 2008 default
$Fsolid_{soil}$ [m^3/m^3]	0.6				EMEA, 2008 default
Kpsoil [L/kg]	0.020	0.020	0.021	0.021	Calculation, Eq. 5-6
RHOsolid [kg/m^3]	2500				EMEA, 2008 default
VP [Pa]	0	0	$1.64x10^{-14}$	$1.64x10^{-14}$	See Table 4-1
MW [g/mol]	1374.5	1374.5	424.9	424.9	See Table 2-1
SOL [mg/L]	58.8	58.8	50000	50000	See Table 4-1
R [Pa x m^3/mol*K]	8.314				EMEA (2008), Constant
TEMP [K]	285				EMEA, 2008 default
Foc_{soil} [kg/kg]	0.02				EMEA, 2008 default
Koc [L/kg]	1	1	1.06	1.06	See Table 4-1
$PEC_{groundwater}$, (mcg/L)	0.0041	0.0070	0.010	0.017	Eq. 5-3

Table 5-6 Initial $PEC_{groundwater}$ of gonadorelin and cloprostenol for pasture-reared cattle

Parameter	Gonadorelin		Cloprostenol		Reference
	Dairy cattle	Beef cattle	Dairy cattle	Beef cattle	
PEC_{soil} [mcg/kg]	0.00023	0.00063	0.00058	0.0016	Initial PEC_{soil}, modified to a 20 cm mixing depth
RHOsoil [kg/m^3]	1700				EMEA, 2008 (default)
Ksoil-water [m^3/m^3]	0.23	0.23	0.23	0.23	Calculation, Eq. 5-4
CF	1000				EMEA, 2008 default
$Fair_{soil}$ [m^3/m^3]	0.2				EMEA, 2008 default
Kair-water [m^3/m^3]	0	0	$5.9x10^{-20}$	$5.9x10^{-20}$	Calculation, Eq. 5-5
$Fwater_{soil}$ [m^3/m^3]	0.2				EMEA, 2008 default
$Fsolid_{soil}$ [m^3/m^3]	0.6				EMEA, 2008 default
Kpsoil [L/kg]	0.020	0.020	0.021	0.021	Calculation, Eq. 5-6
RHOsolid [kg/m^3]	2500				EMEA, 2008 default
VP [Pa]	0	0	$1.64x10^{-14}$	$1.64x10^{-14}$	See Table 4-1
MW [g/mol]	1374.5	1374.5	424.9	424.9	See Table 2-1
SOL [mg/L]	58.8	58.8	50000	50000	See Table 4-1
R [Pa x m^3/mol*K]	8.314				EMEA (2008), Constant
TEMP [K]	285				EMEA, 2008 default
Foc_{soil} [kg/kg]	0.02				EMEA, 2008 default
Koc [L/kg]	1	1	1.06	1.06	See Table 4-1
$PEC_{groundwater}$, (mcg/L)	0.0017	0.0047	0.0043	0.012	Eq. 5-3

According to the EMEA guidance (2008), a reasonable initial estimate of the $PEC_{surfacewater}$ can be calculated under the assumption that one part run-off water will be diluted by two parts receiving water. Therefore, to estimate the concentration reaching the surface water ($PEC_{surfacewater}$), the estimated concentration in the porewater ($PEC_{porewater}$ = $PEC_{groundwater}$) should be divided by three, as shown in the following equations:

$$PECsurfacewater = \frac{PECporewater}{3}$$ **Eq. 5-7**

$$PECporewater = PECgroundwater$$ **Eq. 5-8**

where $PEC_{surfacewater}$: predicted environmental concentration in surfacewater [mcg/L]
$PEC_{porewater}$: predicted environmental concentration in porewater [mcg/L]
$PEC_{groundwater}$: predicted environmental concentration in groundwater [mcg/L]

The initial $PEC_{surfacewater}$ for the intensively reared and pasture-reared scenarios are calculated per equations 5-7 and 5-8 and are shown in Table 5-7 and Table 5-8, respectively.

Table 5-7 Initial $PEC_{surfacewater}$ of gonadorelin and cloprostenol for intensively reared cattle[1]

Parameter	Gonadorelin		Cloprostenol	
	Dairy cattle	Beef cattle	Dairy cattle	Beef cattle
$PEC_{surfacewater}$ [mcg/L]	0.0014	0.0023	0.0034	0.0058

Table 5-8 Initial $PEC_{surfacewater}$ of gonadorelin and cloprostenol for pasture reared cattle[1]

Parameter	Gonadorelin		Cloprostenol	
	Dairy cattle	Beef cattle	Dairy cattle	Beef cattle
$PEC_{surfacewater}$ [mcg/L]	0.00057	0.0016	0.0014	0.0039

Following logic similar to that used in evaluating the PEC_{soil}, the worst-case $PEC_{surfacewater}$ for a combination product would be (0.0023 + 0.0058) = 0.0081 mcg/L for the intensively reared cattle scenario and (0.0016 + 0.0039) = 0.0055 mcg/L for the pasture-reared cattle scenario. While there is no similar Phase I trigger for the $PEC_{surfacewater}$ for terrestrial-use veterinary products, this worst-case $PEC_{surfacewater}$ is well below the 1mcg/L value cited as the Phase I trigger for aquaculture drugs, in Question 11 of the Phase I VICH guidance (CVM, 2001). According to Hubbard et al. (2002), concentrations of pheromones observed under natural conditions are likely to be within a range in magnitude from 10^{-11} to 10^{-8} M, which would equate roughly to concentrations of 0.00425 to 4.25 mcg/L for cloprostenol and 0.0137 to 13.7 mcg/L for gonadorelin. These calculations are based on the molecular weights for the chemical forms used for dosing and to calculate predicted environmental concentrations: cloprostenol and gonadorelin diacetate tetrahydrate (approximately 425 and 1374 g/mol, respectively); an example is shown in Equation 5-9 for the highest concentration of cloprostenol:

$$425 \text{ g/mol} \times 10^{-8} \text{ mol/L} \times 10^{6} \text{ mcg/g} = 4.25 \text{ mcg/L}$$ **Eq. 5-9**

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

6. EFFECTS ASSESSMENT

The standard approach in an EA is to develop predicted no-effect concentrations (PNECs) for comparison to the PECs and calculation of Risk Quotients (RQs). PNECs are based on studies with representative aquatic and terrestrial organisms in which effects of the test substance on ecologically relevant endpoints (e.g., survival, growth, reproduction) are measured.

A comprehensive literature search was performed to locate information on the potential effects to ecological receptors from exposures to gonadorelin and cloprostenol. Publically available databases (e.g., HSDB, ECOTOX) were searched, along with various environmental sciences databases, which were searched via the ProQuest DIALOG search service, as discussed previously (Section 4.1). No studies were located that presented results useful in deriving a PNEC for gonadorelin or cloprostenol. None of the articles measured effects on survival, growth, or reproduction parameters. The majority of the studies were excluded from analysis, because the exposure route was not environmentally relevant (i.e., via injection). There were a few studies that examined exposures of goldfish to $PGF_{2\alpha}$ via water; these are discussed below.

The study by Menningen et al. (2010) focused on investigating the effects of fluoxetine on the reproductive axis in goldfish. In this study, groups of sexually mature goldfish, *Carassius auratus*, were exposed to fluoxetine for 14 days before receiving doses of endogenous sex pheromones ($PGF_{2\alpha}$ or 17,20β-dehydroxy-4-pregnene-3-one [17,20βP]). However, as part of the study design, one group of fish was exposed only to 3 nM PGF2α (nominal concentration). It was found that, at this level of exposure, $PGF_{2\alpha}$ had a significant effect on testosterone levels and expression of the pituitary gene fsh-β, as well as isotocin and vasotocin mRNA expression; milt volume, serum levels of luteinizing hormone, and growth hormone were unaffected. These results are not unexpected, because $PGF_{2\alpha}$ plays a key role in stimulating the reproductive axis (Menningen et al., 2010).

Sorensen et al. (1989) also investigated the effects of $PGF_{2\alpha}$ exposures in goldfish. In this set of studies, groups or individual male goldfish were exposed to low concentrations (1×10^{-9} to 1×10^{-7}M) of $PGF_{2\alpha}$, 15K-$PGF_{2\alpha}$ (a metabolite of $PGF_{2\alpha}$), and 17,20βP, individually or in combination. At the lowest concentration ($1x10^{-9}$ M), the control and $PGF_{2\alpha}$ exposures did not have an effect on swimming activity, whereas swimming activity was significantly increased in all other exposure groups. This level is thought to approximate the olfactory threshold in goldfish (Sorensen et al. 1988; unpublished data referred to in Sorensen *et al*., 1989). At higher concentrations, $PGF_{2\alpha}$ and mixtures containing $PGF_{2\alpha}$ elicited changes in behavior in male goldfish, but affected only levels of gonadotropin when combined with 15K-$PGF_{2\alpha}$, in grouped males. It was also noted by Sorensen et al. (1988) that triggered behavioral responses do not seem to increase with increasing pheromone concentrations, but rather elicit the maximal response once detected. Triggered responses also seem to diminish in a matter of minutes in the absence of a female releasing the cue (Stacey et al., 2003). Findings from this study suggest that the principal action of water-borne $PGF_{2\alpha}$ and metabolites in goldfish is to stimulate male sexual arousal. The lowest concentration of $PGF_{2\alpha}$ eliciting effects in the experiments of Sorensen et al. (1989) would be equivalent to 4.25 mcg/L based on cloprostenol (e.g., 425 g/mol × 10^{-8} mol/L × 10^{6} mcg/g = 4.25 mcg/L;). This is well above the worst-case initial $PEC_{surfacewater}$ for cloprostenol (0.0058 mcg/L) and thus suggests that $PGF_{2\alpha}$ concentrations resulting from CYSTORELIN would not be expected to affect fish behavior.

Although no studies were available to determine definitive effect levels of gonadorelin or cloprostenol exposures in non-target animals, it is not expected that exposures to these compounds would have adverse effects at levels predicted for this use profile.

7. RISK CHARACTERIZATION

The risk characterization typically compares the PEC values to the Predicted No-Effectgs Concentrations (PNECs) for non-target organisms, and risk quotients (RQ = PEC/PNEC) are calculated. However, if RQ cannot be calculated due to a lack of effects data, the risk characterization should focus on the limited exposure of the drugs in the environment, due to their extensive metabolism in the target animal and rapid degradation in the environment.

Predicted environmental concentrations in soil, groundwater, and surface water were calculated for gonadorelin or cloprostenol using guidance provided in EMEA (2008).

Predicted environmental concentrations in soil were calculated for use in both dairy cattle and beef cattle; scenarios of intensively reared and pasture-reared cattle were considered. The worst-case scenario PEC_{soil} values, obtained by adding the highest PEC_{soil} values for gonadorelin plus cloprostenol in beef cattle, were 0.0133 and 0.0088 mcg/kg for intensively reared cattle and pasture-reared cattle, respectively (see data in Tables 5-2 and 5-4). The PEC_{soil} values are significantly less than the Phase I trigger value of 100 mcg/kg, which is expected to be protective of terrestrial receptors such as earthworms, microbes, and plants (CVM 2001).

Predicted environmental concentrations in groundwater and surface water were also calculated for use in dairy cattle and beef cattle under the intensively reared and pasture-reared scenarios. Following the same approach as used with the PEC_{soil}, the worst-case $PEC_{surfacewater}$, obtained by adding the highest PECs for gonadorelin plus cloprostenol in beef cattle, were 0.0081 and 0.0055 mcg/L for intensively reared and pasture-reared scenarios, respectively (see data in Tables 5-7 and 5-8). These values are well below the 1 mcg/L value cited as the Phase I trigger for aquaculture drugs (CVM 2001).

The worst-case initial PEC_{soil} and $PEC_{surfacewater}$ values, which were determined by combining the highest PECs obtained for gonadorelin and cloprostenol, are summarized in Table 7-1. PECs calculated for dairy cattle scenarios were lower.

Table 7-1 Worst-case initial PEC_{soil} and $PEC_{surfacewater}$

Parameter	Intensively reared cattle	Pasture-reared cattle
PEC_{soil} [mcg/kg]	0.0133	0.0088
$PEC_{surfacewater}$ [mcg/L]	0.0081	0.0055

The PECs were calculated using very conservative assumptions, and the resulting values therefore overestimate the actual concentrations that would be expected to occur in environmental compartments due to the proposed use, because of the following factors:

- Assumption that 100% of the dose is excreted unchanged into the environment. However, both compounds undergo rapid metabolism in cattle.

- Assumption of no degradation in manure or in the environment.

Moreover, gonadorelin is structurally identical to a naturally occurring substance, while cloprostenol is a synthetic analog of a naturally occurring compound. The use of gonadorelin and cloprostenol for the purpose of synchronization of estrous cycles to allow for fixed-time artificial insemination in cattle is not expected to result in environmental concentrations that would cause effects in ecological receptors.

8. SUMMARY AND CONCLUSIONS

Treatment with GnRH and $PGF_{2\alpha}$ is a practical method for controlling ovarian follicular and luteal functions and increasing the precision of estrus synchronization in cyclic and acyclic postpartum cows and heifers.

Products with active ingredients of gonadorelin (as the diacetate tetrahydrate) and cloprostenol (as the sodium salt) are currently approved for use in the U.S. in dairy cattle. For the proposed use, there is no change to the approved indication for cloprostenol or the approved dosage of gonadorelin. Approval of the proposed indication of CYSTORELIN would allow for use with cloprostenol to synchronize estrous cycles, allowing for fixed-time artificial insemination in lactating dairy cows and beef cows.

Both substances are naturally occurring and endogenously produced. Gonadorelin is a synthetic analogue of GnRH, and cloprostenol is a synthetic analogue of the prostaglandin $PGF_{2\alpha}$. As naturally occurring substances, they are likely already present in the environment or are degraded rapidly on entry into the environment, such that the concentration or distribution of the substance in the environment is not altered.

Even assuming worst-case scenarios for the calculation of predicted environmental concentrations of these substances, the highest PEC_{soil} is significantly less than the Phase I trigger value of 100 mcg/kg; likewise, the highest $PEC_{surfacewater}$ is well below the 1 mcg/L value cited as the Phase I trigger for aquaculture drugs and is within the range of concentrations of pheromones present in natural systems. Furthermore, actual exposures of ecological receptors from the proposed use is likely to be far less, because the estimated PECs do not take into account the rapid metabolism observed for these substances, nor do they consider any degradation of the substances once in the environment.

In summary, the use of CYSTORELIN (gonadorelin) with cloprostenol for the purpose of synchronization of estrous cycles to allow for fixed time artificial insemination in cattle is not expected to result in environmental concentrations that would cause effects in ecological receptors.

9. MITIGATION MEASURES

Because the proposed action would not have a significant effect on the environment, no mitigation measures will be required.

10. ALTERNATIVES TO THE PROPOSED ACTION

The only alternative to the proposed action is the ‘no action’ alternative, which would be the failure to approve the supplemental new animal drug application for CYSTORELIN (gonadorelin).

11. LIST OF PREPARERS

This document was prepared by Jane P. Staveley (Exponent, Inc.) and James B. Fischer (Merial, Inc.), with contributions from Josie Bamford (Nusz), under the direction of Jane P. Staveley. No other experts were consulted in the preparation of this document.

12. CERTIFICATION

The undersigned official certifies that the information presented in this Environmental Assessment is true, accurate, and complete to the best of their knowledge.

Jane P. Staveley

October 6, 2017

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