Veterinary Medicine Research & Development
Kalamazoo, Michigan 49007
United States

Formerly doing business as Pfizer Inc.

Environmental Assessment for Synovex® ONE (Estradiol Benzoate and Trenbolone Acetate Extended Release Implant) Feedlot and Grass for Beef Steers and Heifers

Active Ingredients: Trenbolone Acetate, Estradiol Benzoate

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LIST OF ABBREVIATIONS AND ACRONYMS

The following is a partial listing of the abbreviations and acronyms used throughout this EA. The abbreviations listed may be used alone or in combination with others.

ac	Acre
AED	Androstenedione
AF	Assessment factor
AFO	Animal feeding operation
APPI	Atmospheric pressure photoionization
AR	Applied radioactivity
AU	Animal Unit
BLD	Below limit of detection
BLQ	Below limit of quantitation
BMP	Best Management Practice associated with manure management
bu	Bushels
BW	Body weight
CAFO	Concentrated Animal Feeding Operation
CAS	Registry numbers assigned by the Chemical Abstracts Service
CDL	Cropland Data Layer
CE	Categorical Exclusion
CI	Confidence Interval
CNMP	Comprehensive Nutrient Management Plan
CVM	FDA's Center for Veterinary Medicine
DOC	Dissolved organic carbon
dph	Days post-hatch
DRP	Dissolved reactive phosphorus
DT_{50}	Time to dissipate or degrade to one-half of the initial concentration. The term half-life is also used interchangeably for DT_{50} in this document.
dw	Dry weight
E1	Estrone
17α-E2	17α-Estradiol
17β-E2	17β-Estradiol
E3	Estriol
EA	Environmental Assessment
EB	Estradiol benzoate
EC_{50}	The concentration for which 50% of the test population is affected by a chemical
EC_X	The concentration for which X% of the test population is affected by a chemical
ED	Endocrine disruption
EDC	Endocrine disrupting compound(s)
EE2	17α-Ethinylestradiol
EEQ	Estradiol equivalents
EIA	Enzyme immunoassay
EMEA	European Medicines Agency, the EU regulatory agency for the evaluation of medicinal products
EPA	US Environmental Protection Agency
EQS	Environmental Quality Standard

ESI	Electrospray ionization
EU	European Union
EXAMS	US EPA's Exposure Analysis Modeling System
EXPRESS	US EPA's PRZM/EXAMS Simulation Shell
F0	Parental generation in a reproduction study
F1	First offspring generation in a reproduction study
F2	Second offspring generation in a reproduction study
F3	Third offspring generation in a reproduction study
FDAH	Fort Dodge Animal Health, division of Wyeth, subsidiary of Pfizer Inc.
FHM	Fathead minnow
FW	Fresh water
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GC-MS/MS	Gas chromatography-mass spectrometry/mass spectrometry
GIS	Geographic Information System
GLP	Good Laboratory Practices
GSI	Gonadosomatic index
ha	Hectare (2.471 acre/ha)
HSI	Hepatosomatic index
HPLC	High Performance Liquid Chromatography
HUC	Hydrologic Unit Code (HUC) 12-digit Watershed Boundary Dataset
IR	Index Reservoir
IUPAC	International Union for Pure and Applied Chemistry
K_d	Soil distribution coefficient
K_{OC}	Distribution coefficient or Freundlich adsorption coefficient normalized to the organic carbon content of the soil
K_{OW}	Octanol-water partition coefficient
LC_{50}	The concentration for which 50% of the test population is killed by a chemical
lb	Pound(s)
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
LOEC	Lowest Observed Effect Concentration
LOD	Limit of detection
LOQ	Limit of quantitation
LSC	Liquid scintillation counting
M	Million
MATC	Maximum Acceptable Toxicant Concentration
MASE	Microwave-assisted solvent extraction
MGA	Melengestrol acetate
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
mW	Milliwatt
MW	Molecular weight
n	Number of samples used to estimate means, etc.
NA	Not applicable or not available or not analyzed
NADA	New Animal Drug Application
NASS	USDA National Agricultural Statistics Service
ND	Not determined or not detected
NEPA	National Environmental Policy Act

NMP	Nutrient Management Plan for manure
NMR	Nuclear magnetic resonance
NOEC	No Observed Effect Concentration
NPDES	EPA National Pollutant Discharge Elimination System
NR	Not reported
NRCS	USDA Natural Resources Conservation Service
OECD	Organization for Economic Co-operation and Development
OC	Organic carbon
ONE-F	Synovex® ONE Feedlot
ONE-G	Synovex® ONE Grass
P	Phosphorus
Pa	Pascal
PCA	Percent cropped area
PEC	Predicted Environmental Concentration
PEC_{GW}	Predicted Environmental Concentration in groundwater
PEC_{manure}	Predicted Environmental Concentration in manure
PEC_{soil}	Predicted Environmental Concentration in soil
PEC_{water}	Predicted Environmental Concentration in water
PNEC	Predicted No Effect Concentration
POCIS	Polar organic chemical integrative samplers
PPCPs	Pharmaceuticals and personal care products
ppt	Parts per thousand
PRZM	US EPA's Pesticide Root Zone Model
QC	Quality control
RAM	Radiochemical/Radioactivity detection
RQ	Risk quotient (PEC/PNEC)
SCI-GROW	US EPA's Screening Concentration In GROundWater Model
SD	Standard deviation
SFO	Single first-order
SPE	Solid phase extraction
SSD	Species sensitivity distribution
17α-TB	17α-Trenbolone
17β-TB	17β-Trenbolone
TBA	Trenbolone acetate
TBME	Tert-butyl methyl ether
TEQ	Testosterone equivalents (androgen)
TLC	Thin layer chromatography
UK	United Kingdom
US	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
UV	Ultraviolet
VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products
VMP	Veterinary medicinal product
VTG	Vitellogenin
WWTP	Waste water sewage treatment plant

EXPLANATION OF TERMS

Animal Feeding Operation (AFO)	An AFO is defined under Title 40 Code of Federal Regulations (CFR) 122.23(b)(1) as a lot or facility (other than an aquatic animal production facility) where the following conditions are met: 1) animals have been, are, or will be stabled or confined and fed or maintained for a total of 45 days or more in any 12-month period, and 2) crops, vegetation, forage growth, or post-harvest residues are not sustained in the normal growing season over any portion of the lot or facility. An AFO can describe any size of animal feedlot. For the purposes of this EA, small and medium AFOs as described as having <1000 head of cattle, and large AFOs are described as having >1000 head of cattle.
Aggregate exposure	Aggregate exposure is defined as exposure to a single chemical by multiple pathways and routes of exposure (e.g., runoff from CAFO, pasture, and cropland).
Animal Unit (AU)	For this EA, one AU equals one beef steer or heifer on pasture or in a feedlot.
Asymmetric carbon	An asymmetric carbon atom is one which is attached to four atoms or groups of atoms, no two of which are alike.
Concentrated Animal Feeding Operation (CAFO)	A facility must meet the definition of an AFO before it can be considered a CAFO (see definition of AFO above). According to the Environmental Protection Agency's regulations, 40 CFR 122.23 (b) and (c), CAFOs for beef cattle are defined or designated as follows: • An AFO is defined as a large CAFO if it meets the requirements of an AFO and has ≥1000 beef cattle [40 CFR 122.23(b)(4)]. • An AFO is defined as a medium CAFO if it meets the requirements of an AFO, has 300-999 beef cattle, and meets one of the following conditions: 1) pollutants are discharged into waters of the US through a man-made ditch, flushing system, or other similar man-made device, or 2) pollutants are discharged directly into water of the US which originate outside of and pass over, across, or through the facility or otherwise come into direct contact with animals confined in the operation [40 CFR 122.23(b)(6)]. • An AFO can also be designated as a medium CAFO by a permitting authority if it is found to be a significant contributor of pollutants to the surface waters [40 CFR 122.23(c)]. • An AFO with <300 beef cattle can be designated as a small CAFO by a permitting authority if it is a significant contributor of pollutants to surface waters and if it meets one of the two conditions discussed above under medium CAFOs [40 CFR 122.23(c)].
Cumulative exposure	For this EA, a cumulative exposure is defined as a concurrent exposure by all relevant pathways and routes to multiple agents or stressors with similar mechanism of action (e.g., EDCs).
Endocrine disrupting compound (EDC)	An EDC is defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance of homeostasis, reproduction, development and/or behavior [1].
Epimer	An epimer is either of two stereoisomers that differ in the arrangement of groups on a single asymmetric carbon atom.

Epimerase	Epimerases are enzymes that catalyze the stereochemical inversion of the configuration about an asymmetric carbon atom in a substrate having more than one center of asymmetry, thus interconverting epimers. 17α-TB and 17β-TB are examples of epimers.
Epimerization	Epimerization is the conversion of one epimeric form of a compound into another.
LOEC	The LOEC is the lowest concentration of the toxicant tested that has a significant effect on the organisms exposed to it or, more formally, that yields a statistically significant deviation from a control.
MATC	Maximum Acceptable Toxicant Concentration is reported as the geometric mean of the NOEC and the LOEC.
Maximum average	In the OECD water/sediment studies conducted by Pfizer, the maximum average value is the maximum reported value (from both sediments) of all of the averaged values determined (average of 2 replicates) at each of the time points for each of the transformation products.
Mixed-use watershed	For this EA, a mixed-use watershed is defined as a watershed receiving estradiol and trenbolone metabolites from multiple exposure pathways, including runoff from manured croplands, pasture cattle, AFO with <1000 beef cattle, and application of runoff water collection from a lagoon to cropped fields from CAFOs with $\geq$1000 beef cattle.
NOEC	The NOEC is the highest concentration of the toxicant tested that has no significant observable effect on the organism(s) exposed to it.
Stereoisomers	Stereoisomers are two molecules having the same atoms connected in the same sequence, but the atoms have different three-dimensional orientations.
Surrogate estradiol compound	For the purpose of this EA, surrogate estradiol compound is defined as one estradiol-like compound with the physical-chemical and environmental fate properties that conservatively represents a composite of the primary metabolites of estradiol benzoate, i.e., 17β-estradiol, 17α-estradiol, and estrone (see Figure 8).
Surrogate trenbolone compound	For the purpose of this EA, surrogate trenbolone compound is defined as one trenbolone-like compound with the physical-chemical and environmental fate properties that conservatively represents a composite of the primary metabolites of trenbolone acetate, i.e., 17β-trenbolone, 17α-trenbolone, and trendione (see Figure 9).
Zoetis	Zoetis was formerly known as Pfizer Animal Health but became an independent animal health company and separated from Pfizer in 2013.

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EXECUTIVE SUMMARY

Zoetis is seeking approval of a new animal drug application (NADA) for Synovex ONE [estradiol benzoate (EB) and trenbolone acetate (TBA) extended release implant] Feedlot and Grass for beef steers and heifers. The indications for Synovex ONE are as follows:

- Synovex ONE-Feedlot (ONE-F): for increased rate of weight gain and improved feed efficiency for up to 200 days in steers and heifers fed in confinement for slaughter
- Synovex ONE-Grass (ONE-G): for increased rate of weight gain for up to 200 days in pasture cattle steers and heifers (stocker, feeder and slaughter)

The approval process for a veterinary drug product in the United States (US) entails satisfying certain requirements of the National Environmental Policy Act (NEPA), which are included in the US Food and Drug Administration's (FDA) regulations in Title 21 Code of Federal Regulations (CFR) Part 25. According to 21 CFR 25.15(a), all applications or petitions requesting agency action (e.g., NADA) require the submission of an environmental assessment (EA) or a claim of categorical exclusion from the requirement to prepare an EA. The FDA has determined that an EA is required in support of the proposed action; i.e., approval of the NADA for Synovex ONE Feedlot and Grass.

The purpose of this EA is to assess the fate, effects, and overall impact of the environmentally-relevant metabolites of the active ingredients (EB and TBA) contained in Synovex® ONE. Recommendations from the Center for Veterinary Medicine (CVM) were followed, in addition to those presented in CVM's Guidance for Industry number 166: Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) - Phase II VICH Guideline 38, dated 09 January 2006.

The risk assessment described herein consists of 1) an exposure assessment in which predicted environmental concentrations (PEC) were developed for various scenarios for the primary metabolites of EB and TBA, 2) an effects assessment in which the predicted no effect concentrations (PNEC) were derived for the primary metabolites of concern, and 3) a risk characterization for these metabolites. The risk characterization utilized the risk quotient (RQ) method, which is based on the ratio of a PEC to a PNEC.

EB, TBA, and related metabolites are endocrine disrupting compounds (EDCs) that are known to cause effects on the reproduction of aquatic organisms (i.e., fish) when chronically exposed to low concentrations (parts-per-trillion or ppt). Therefore, in this EA, the aquatic environment was identified as the ecosystem of concern and fish were identified as the sensitive non-target species of concern. Active metabolites of EB and TBA are excreted in cattle manure and have the potential to enter the aquatic environment through runoff or leaching from: 1) the manure contained in feedlots, 2) manure deposited on pastureland, and/or 3) manure applied to crop fields. When all of these sources and exposure pathways exist in a watershed, it could potentially result in an aggregate[a] exposure. In addition, the EB and TBA metabolites entering the environment may also contribute to an already existing load of EDCs, including synthetic and naturally occurring estrogens and androgens; ultimately, resulting in a cumulative[b] exposure. Therefore, in the exposure assessment, the PEC values in water (PEC_{water}) were estimated for both individual farm-scale scenarios and

[a] For the purpose of this EA, aggregate exposure is defined as exposure to a single chemical by multiple pathways and routes of exposure.

[b] For the purpose of this EA, cumulative exposure is defined as aggregate exposures to multiple EDCs.

aggregate watershed-scale scenarios. In addition, the potential contribution of the EB and TBA metabolites from Synovex ONE to the overall cumulative mass of steroid hormones entering the environment was evaluated.

Based on excretion data and field monitoring data from manure storage structures on feedlots, it has been determined that the principal metabolites of Synovex ONE found in beef cattle manure are 17α-estradiol and 17α-trenbolone. However, minor amounts of the other EB and TBA metabolites (17β-estradiol and estrone; 17β-trenbolone and trendione) are also excreted. In addition, 17α-estradiol and 17α-trenbolone are expected to be rapidly transformed in the soil and aquatic environments to estrone and trendione, respectively, and minor amounts of the 17β isomers. Thus, the impacts of the six primary EB and TBA metabolites were evaluated in this assessment. However, preparing a collective environmental exposure and risk assessment on all six of these compounds would be complex and scientifically challenging because certain key data are currently lacking. Therefore, a conservative approach was used. Because the structures and many of the physical-chemical properties of 17α-estradiol, 17β-estradiol, and estrone are quite similar, for the purposes of this assessment it has been assumed that they will be transported, transformed, and degraded similarly in the environment. For the same reasons, the same assumption also was used for 17α-trenbolone, 17β-trenbolone, and trendione. Thus, to account for all potential EB and TBA metabolites in the environment and to simplify the environmental fate modeling and exposure assessment, a single surrogate estradiol compound[c] and a single surrogate trenbolone compound[d] have been defined and modeled in the exposure assessment and used for characterizing risk. This was done rather than modeling each of the six TBA and EB metabolites in the affected environmental compartments for multiple conservative farm- and watershed-scale scenarios.

To model the fate, transport, and exposure of the surrogate compounds in aquatic and terrestrial environments, data on the physical-chemical properties and rates of degradation in soil, water, and sediment were required. The selection of the physical-chemical and environmental fate properties used in the environmental modeling of the surrogate compounds was performed using a conservative approach and assumptions that would ensure that PEC values determined for water would not be underestimated. Selection of the parameters to use in the modeling was accomplished using data obtained from acceptable literature studies and/or Zoetis-owned studies that were conducted in accordance with Good Laboratory Practices (GLP). The physical-chemical properties and environmental fate data for the estradiol and trenbolone metabolites suggest that they will bind moderately to soil and degrade rapidly via microbially-mediated processes in aerobic soils (degradation half-life, DT_{50}, of approximately 3 days). In addition, further binding and degradation of the metabolites is expected in the aerobic and anaerobic water-sediment environment (aerobic DT_{50} of 31 and 53 days and anaerobic DT_{50} of 108 and 191 days for the surrogate estradiol and trenbolone compounds, respectively). However, hydrolysis and photodegradation of the estradiol and trenbolone metabolites were not accounted for in the environmental fate modeling, which leads to an overestimate of exposure.

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[c] For the purpose of this EA, surrogate estradiol compound is defined as one estradiol-like compound with the physical-chemical and environmental fate properties that conservatively represents a composite of the primary metabolites of EB.

[d] For the purpose of this EA, surrogate trenbolone compound is defined as one trenbolone-like compound with the physical-chemical and environmental fate properties that conservatively represents a composite of the primary metabolites of TBA.

The PEC values were estimated using several computer simulation models developed by the US Environmental Protection Agency (EPA) for evaluating the environmental risk of pesticides and herbicides. These models include Screening Concentration in Groundwater (SCI-GROW), Pesticide Root Zone Model (PRZM), Exposure Analysis Modeling System (EXAMS), and the EXAMS-PRZM Simulation Shell (EXPRESS). These models were used to simulate: 1) potential leaching from cropland and feedlots (SCI-GROW and EXPRESS), 2) 51 individual farm-scale scenarios that evaluated the potential runoff from manure applied to cropland (34 till and 17 no-till scenarios; EXPRESS), 3) five pastureland scenarios (PRZM/EXAMS), and 4) five mixed-use watershed-scale scenarios that evaluated an aggregate exposure from all potential sources of Synovex ONE (i.e., runoff from manure and lagoon water applied to cropland, pastureland, and feedlots; modified PRZM and EXAMS). The models employed, as well as the parameters chosen, were designed to provide conservative PEC estimates, resulting in an EA that provides a high margin of environmental safety and an overestimate of risk.

The final component of the environmental fate modeling was the development of a new watershed model (referred to as "mixed-use watershed" throughout the EA) based on US Department of Agriculture (USDA) data on beef cattle numbers and density in five regions of the US (Iowa, Texas, Ohio, Michigan, and Pennsylvania). The regions chosen represent areas of high density of beef cattle as well as diverse weather patterns, topography, and distribution of feedlot sizes and pastureland. This model addresses the aggregate exposure from a watershed receiving estradiol and trenbolone metabolites from several sources, including runoff from 1) manured croplands, 2) pastureland, 3) small and medium animal feeding operations (AFOs) with <1000 beef cattle, and 4) croplands irrigated with runoff water collected in lagoons on concentrated animal feeding operations (CAFOs) with ≥1000 beef cattle. This model is useful for determining chronic exposure within a watershed over a specified period of time. Modeling results for all individual farm-scale and aggregate watershed-scale scenarios are expressed as 90^{th} percentile 21-day moving averages determined from simulations over 30 years. These values are considered to be conservatively representative of reasonable worst-case exposures in the US.

Direct runoff from small and medium AFOs has the greatest effect on the PEC values for the mixed-use watershed scenarios. In modeling direct runoff from feedlots, it was assumed that all CAFOs with ≥1000 head were in compliance with the Clean Water Act and are not discharging to surface water. For small and medium AFOs with <1000 head, we have conservatively assumed that a portion of these AFOs are not in compliance with the Clean Water Act and are directly discharging to surface waters (i.e., they are significant contributors of pollutants to surface waters). Using USDA data from 1997, we have estimated that only approximately 17% of AFOs on a nationwide basis have runoff that directly enters into surface water. Based on this estimate, we have used an assumption of 25% AFOs <1000 head are directly discharging to surface waters to represent what we believe is a typical nationwide scenario. We have also used a highly conservative assumption that 50% of AFOs <1000 head are direct dischargers to represent a reasonable worst-case for local watersheds.

As described above, the RQ values were calculated by comparing the PEC_{water} values to the PNEC values. In the effects assessment, PNEC values were derived for the primary metabolites of concern based on effects on fish reproductive endpoints. Fish are considered a sensitive and sentinel taxonomic group for understanding and evaluating the impact of endocrine disruption (ED) in the aquatic environment. Therefore, reproduction studies in fish were conducted to establish no observed effect concentration (NOEC) values for population-relevant endpoints for 17α-estradiol and 17α-trenbolone. In addition, NOEC and/or EC_{10} (the effects concentration that results in a 10% reduction in fecundity) values were also determined for the more potent 17β isomers of estradiol and trenbolone using published literature data. To derive a conservative PNEC, the NOEC or EC_{10} values from the fish reproduction studies were divided by an assessment factor (AF) of 10 to account for uncertainty in laboratory data; except for 17β-estradiol, for which a smaller AF of 2 was used because a considerable amount of effects data was available. The PNEC values were determined to be 25, 1.4, and 3.2 ng/L for 17α-estradiol, 17β-estradiol, and 17α-trenbolone, respectively. A range of PNEC values (0.25-0.5 ng/L) was derived for 17β-trenbolone due to uncertainty in analytical data from a key study using the most sensitive fish species.

In the risk characterization, the RQ values for the surrogate estradiol compound and surrogate trenbolone compound were estimated using different methods. For the surrogate estradiol compound, it was assumed that the toxicity of the compound was equal to the toxicity of the 17α isomer in order to estimate the RQs for fish reproduction-related endpoints. This comparison was made because, based on excretion and field monitoring data, 17α-estradiol is expected to be the primary metabolite in manure applied to land. Thus, to derive the RQ values for the surrogate estradiol compound, the PEC_{water} for the surrogate estradiol compound was compared to the PNEC of 17α-estradiol. However, it is also expected that a small portion of the surrogate estradiol compound represents 17β-estradiol and estrone. Based on available data, the 17β-estradiol is a more potent endocrine disruptor in fish than 17α-estradiol, and also more potent than estrone. Therefore, in addition to determining RQs based on the PNEC value for 17α-estradiol, we also calculated a separate set of RQs where it was conservatively assumed that the toxicity of the surrogate estradiol compound was equal to the toxicity (i.e., PNEC) of 17β-estradiol.

In contrast, for the surrogate trenbolone compound, a somewhat different approach was used to determine RQ values. Because cattle excretion data are available for trenbolone, these data were used to proportion the PEC_{water} values for the surrogate trenbolone compound based on the relative distribution of the 17α and 17β isomers in manure[e]. The individual PEC_{water} values were multiplied by both 0.20 and 0.80 to attribute a portion of the value to 17β-trenbolone and 17α-trenbolone, respectively[f]. It was assumed that 20% of the surrogate trenbolone compound was 17β-trenbolone because 1) based on the Zoetis-owned excretion study, 1.68% of the TBA metabolites in the feces were determined to be 17β-trenbolone, 2) we conservatively assumed that the entire unidentified radioactivity in the urine was attributed to 17β-trenbolone (16%), and 3) <3% interconversion of 17α-trenbolone to 17β-trenbolone can occur in the terrestrial and aquatic environments. When accounting for these three sources, a maximum of 20% of the PEC_{water} for the surrogate trenbolone

[e] Due to a lack of excretion data, the PEC_{water} values of the surrogate estradiol compound could not be proportioned between the 17α and 17β isomers of estradiol.

[f] It is important to note that the potential risk of trendione was not quantitatively evaluated in this EA because data suggests that 17α-trenbolone and 17β-trenbolone are more potent endocrine disruptors in fish than trendione, and therefore, the approach used herein should be protective of potential exposures to trendione.

compound could be attributed to 17β-trenbolone, the most toxic isomer, although this percentage is likely much lower. The RQ values attributed to 17α-trenbolone and 17β-trenbolone were calculated by dividing the specific PEC_{water} values by their respective PNEC values. In addition, to account for potential additive effects of these isomers, another set of RQ values was calculated, wherein the individual RQ values attributed to 17α-trenbolone and 17β-trenbolone were added together to estimate the final additive RQ values for the surrogate trenbolone compound.

Typically an RQ value of <1 is used as a preliminary screening level to determine if additional analysis and refinement of the risk assessment may be needed. In this EA, because of the many conservative assumptions used throughout the exposure and effects assessment and the many refinements that have been incorporated into the PEC values, we believe that an RQ value in the range of 1 or less indicates that significant environmental effects are highly unlikely at the predicted level of exposure.

The RQs were <1 for the surrogate estradiol compound when compared to the PNEC values for 17α-estradiol (≤0.004) and 17β-estradiol (≤0.08), in all of the following scenarios: 1) pasturelands simulated for five regions in the US, 2) 34 crop scenarios assuming tilled manure application techniques, 3) 17 crop scenarios assuming no-till manure application techniques, and 4) five mixed-use watersheds in various regions of the US with combined inputs from pasture, cropped land, and feedlot runoff to surface water.

Similar results were also found for the surrogate trenbolone compound when specific proportions of the PEC_{water} value was attributed to 17α-trenbolone and 17β-trenbolone. The additive RQ values were <1 for all farm-scale and mixed-use watershed scenarios evaluated, except for the Iowa mixed-use watershed. The Iowa mixed-use watershed scenario produced an RQ slightly above 1 (1.33) when conservatively assuming that 50% of the AFOs are directly discharging to surface waters and using the lowest potential PNEC value for 17β-trenbolone (0.25 ng/L); this is considered a reasonable worst-case scenario at the local scale. For a more representative nation-wide watershed scenario in which it is assumed that 25% of the AFOs are directly discharging to surface waters, the RQ values were all <1.

The PEC_{water} values upon which these RQ values are based are considered to be an overestimation of the potential exposures to 17β-trenbolone from use of Synovex ONE-F and ONE-G for several important reasons. For example, 1) it was assumed that Synovex ONE-F and ONE-G accounted for 100% of the market share (i.e., all implants administered within a modeled watershed), when this would clearly not be the case because there are currently many other approved products available in the marketplace, and 2) it was conservatively assumed that the all of the unidentified metabolites in the urine are attributed to 17β-trenbolone, even though it is expected that a portion of those metabolites, potentially most of them, would be attributable to 17α-trenbolone or less potent metabolites. Furthermore, for the only RQ that exceeded a screening value of 1, the assumption that 50% of AFOs within a watershed are directly discharging to surface waters is an overestimation and is very unlikely to occur. As discussed in Appendix 9, we believe this percentage is in the range of 17% based on USDA Census data from 1997, which brings the RQ value below 1. However, it is expected that the current percentage of feedlots with direct runoff to surface waters is likely reduced from 17% (estimated using 1997 USDA Census of Agriculture data) due to more recent facility upgrades and compliance with the Clean Water Act; however, more current information is not available to update this number.

In addition, to the quantitative assessment described above, we have also considered the potential impacts from cumulative exposure to steroid hormones on a watershed level in the environment due to multiple natural and anthropogenic sources, such as via excretion by humans, livestock and wildlife. Using data available in the published literature, rough estimates were calculated for the mass of estrogens and androgens excreted by humans and livestock that could potentially enter the environment. These estimates were compared to the estimated maximum mass of the estradiol and trenbolone metabolites associated with the use of Synovex ONE to determine the potential contribution of Synovex ONE to the overall load of estrogens and androgens entering the environment. Overall, we were able to determine, based on these rough, but conservative, estimates, that the contribution of estradiol and trenbolone associated with Synovex ONE will be in the range of 1% (or less) compared to the overall load of estrogens and androgens entering the environment from human and livestock sources.

As described in Section 9, many additional mitigating factors that could not be quantified in this risk assessment would also likely further reduce the risk estimates for both the surrogate estradiol and trenbolone compounds, which are all already at an acceptable level. Thus, based on all available information and the RQ values determined herein, we conclude, based on sensitive fish reproductive endpoints, that no significant environmental impacts are expected from the use of Synovex ONE in beef steers and heifers in the US.

1. INTRODUCTION

Zoetis will be requesting approval of a new animal drug application (NADA) for Synovex ONE [estradiol benzoate (EB) and trenbolone acetate (TBA) extended release implant] Feedlot and Grass for beef steers and heifers. The proposed indications for Synovex ONE are as follows:

- Synovex ONE-Feedlot (ONE-F): for increased rate of weight gain and improved feed efficiency for up to 200 days in steers and heifers fed in confinement for slaughter.
- Synovex ONE-Grass (ONE-G): for increased rate of weight gain for up to 200 days in pasture cattle steers and heifers (stocker, feeder and slaughter).

Throughout this EA, the Synovex ONE implant products may be referred to as ONE-F for feedlot cattle and ONE-G for pasture cattle.

The Synovex product line was originally developed by Syntex. Fort Dodge Animal Health (FDAH) acquired the product line and developed an extended-release implant product based upon a patented technology (Synovex ONE). With the purchase of FDAH, Pfizer acquired the Synovex products. In February 2013, Pfizer Animal Health became Zoetis, an independent animal health company, and retained the Synovex product line. Therefore, the study reports summarized in this EA for Synovex One prepared by Zoetis consist of original studies from Syntex, FDAH, Pfizer, and Zoetis. Although the focus of this EA is Synovex ONE, the full Synovex product line is briefly described in this document.

1.1. Purpose and Need

The approval process for a veterinary drug product in the United States (US) requires satisfying the requirements of the National Environmental Policy Act (NEPA), which is codified in the US Food and Drug Administration's (FDA) regulations under Title 21 Code of Federal Regulations (CFR) Part 25. According to 21 CFR 25.15(a), all applications or petitions requesting agency action [e.g., NADA] require the submission of an environmental assessment (EA) or a claim of categorical exclusion from the requirement to prepare an EA. Because Zoetis plans to submit an original NADA pursuant to Section 512 [21 US Code §360b] of the Federal Food Drug and Cosmetic Act for Synovex ONE Feedlot for feedlot steers and heifers and Synovex ONE Grass for pastured heifers and steers, the FDA has determined that an EA is required in support of the proposed action.

1.2. Risk Assessment Approach

This EA evaluates the potential for environmental impacts from the introduction of EB and TBA metabolites into the environment via direct runoff from feedlots, application of manure to cropland, and deposition of manure on pastureland as a result of the proposed use of Synovex ONE-F and ONE-G in feedlot and pasture cattle. The fate, transport, and exposure of the EB and TBA metabolites are estimated using environmental fate modeling to determine the predicted environmental concentrations (PECs) in soil (PEC_{soil}) and water (PEC_{water}) on a farm- and watershed-scale. These values are compared to the predicted no effect concentration (PNEC) values for fish reproduction-related effects determined for the estradiol and trenbolone metabolites to assess if there is a potential for significant environmental impacts from the proposed use of Synovex ONE-F and ONE-G. The assumptions, approaches and methods used in this assessment to predict the environmental risk of the metabolites of EB and TBA are summarized in this section and are illustrated in Figure 1 below.

Scientific literature supports that the principal metabolites of EB and TBA contained in manure of beef cattle treated with Synovex ONE are 17α-estradiol and 17α-trenbolone (see Figure 1 below); therefore, these were the primary metabolites of concern in this EA. However, minor amounts of the other EB and TBA metabolites are also excreted and are expected to be present in the terrestrial and aquatic environments due to transformation and degradation processes. Thus, for EB (or estradiol) metabolites, 17β-estradiol and estrone were also evaluated in this EA. Likewise, for TBA (or trenbolone) metabolites, 17β-trenbolone and trendione were also assessed.

To assess the potential risk of EB and TBA metabolites excreted from cattle implanted with Synovex ONE, the risk quotient (RQ) method was used, which compares the ratio of PEC_{water} values to the PNEC for inhibition in fish reproduction.

A conservative approach was used in the exposure assessment to predict the PEC_{water} values for the EB and TBA metabolites. Because the structures and many of the physical-chemical properties of 17α-estradiol, 17β-estradiol and estrone are quite similar (Table 7 and Section 4.2.4), for the purposes of this assessment, it was assumed that they will be transported, transformed, and degraded similarly in the environment. For the same reasons, the same assumption has also been used for 17β-trenbolone, 17α-trenbolone, and trendione (Table 7 and Section 4.2.6). Thus, rather than individually modeling each of the six primary metabolites, we derived and modeled a single surrogate estradiol compound and a single surrogate trenbolone compound. This allowed us to simplify the environmental fate modeling and exposure assessment while accounting for all potential metabolites of EB and TBA.

The physical-chemical properties and environmental fate data for the surrogate estradiol compound and surrogate trenbolone compound were derived using all available and acceptable published literature and Zoetis-owned study data for each of the metabolites. Conservative derivation methods were used to ensure that the PEC_{water} values would not be underestimated, and in fact, would represent conservative overestimates. The PEC_{water} values for the surrogate estradiol and trenbolone compounds were estimated using several environmental fate models employed by the Environmental Protection Agency (EPA) when conducting risk assessments of pesticides; including the Screening Concentration in Groundwater (SCI-GROW), Pesticide Root Zone Model (PRZM), Exposure Analysis Modeling System (EXAMS), and the EXAMS-PRZM Simulation Shell (EXPRESS). These models were used to simulate: 1) potential leaching from cropland and feedlots (SCI-GROW and EXPRESS), 2) 51 individual farm-scale scenarios that evaluated the potential runoff from manure applied to cropland (34 till and 17 no-till scenarios; EXPRESS), 3) five pasture land scenarios (PRZM/EXAMS), and 4) five mixed-use watershed-scale scenarios that evaluated an aggregate exposure from all potential sources of Synovex ONE (i.e., runoff from manure and lagoon water applied to cropland, pastureland, and feedlots; modified PRZM and EXAMS). These PEC_{water} values are considered to be a very conservative overestimation of the potential environmental concentrations of the surrogate estradiol and trenbolone compounds in surface water.

The PEC_{water} values estimated for the surrogate compounds were then compared to the PNEC values for fish reproduction-related effects determined for the individual estradiol and trenbolone metabolites to predict if there is a potential for significant environmental impacts from the use of Synovex ONE-F and ONE-G. The principal body of scientific literature describing effects of estradiol and trenbolone metabolites on fish reproduction is associated with 17β-estradiol and 17β-trenbolone. However, because the principal metabolites contained in cattle manure applied to land are 17α-estradiol and 17α-trenbolone, effects of both the 17α and 17β isomers on fish reproduction endpoints were evaluated. Acceptable published literature and Zoetis-owned study data were evaluated and used to determine appropriate reproduction no observed effect concentration (NOEC)[9] values for the 17α and 17β isomers. The PNEC values used in the risk characterization were calculated by dividing the NOEC (or EC_{10}, concentration that results in 10% reduction in fecundity) values by an appropriate assessment factor (AF).

In the risk characterization, the RQ values for the surrogate estradiol compound and surrogate trenbolone compound were estimated using different methods (Figure 1). For the surrogate estradiol compound, it was assumed that the toxicity of the compound was equal to the toxicity of the 17α isomer in order to estimate the RQs for fish reproduction-related endpoints. This comparison was made because, based on excretion and field monitoring data, 17α-estradiol is expected to be the primary metabolite in manure applied to land. Thus, to derive the RQ values for the surrogate estradiol compound, the PEC_{water} for the surrogate estradiol compound was compared to the PNEC of 17α-estradiol. However, it is also expected that a small portion of the surrogate estradiol compound represents 17β-estradiol and estrone. Based on available data, the 17β-estradiol is a more potent endocrine disruptor in fish than 17α-estradiol, and also more potent than estrone. Therefore, in addition to determining RQs based on the PNEC value for 17α-estradiol, we also calculated a separate set of RQs where it was conservatively assumed that the toxicity of the surrogate estradiol compound was equal to the toxicity (i.e., PNEC) of 17β-estradiol.

In contrast, for the surrogate trenbolone compound, a somewhat different approach was used to determine RQ values. Because cattle excretion data are available for trenbolone, these data were used to proportion the PEC_{water} values for the surrogate trenbolone compound based on the relative distribution of the 17α and 17β isomers in manure (Figure 1). The individual PEC_{water} values were multiplied by both 0.20 and 0.80 to attribute a portion of the value to 17β-trenbolone and 17α-trenbolone, respectively. Based on excretion and environmental fate data, a maximum of 20% of the PEC_{water} for the surrogate trenbolone compound was attributed to 17β-trenbolone, the most toxic isomer, although this percentage is likely much lower. Subtracting 20% from 100%, the remaining 80% of the PEC_{water} value was attributed to 17α-trenbolone. None of the PEC_{water} value was attributed to trendione or other unknown metabolites. The RQ values attributed to 17α-trenbolone and 17β-trenbolone were calculated by dividing the specific PEC_{water} values by their respective PNEC values. In addition, to account for potential additive effects of these isomers, another set of RQ values was calculated, wherein the individual RQ values attributed to 17α-trenbolone and 17β-trenbolone were added together to estimate the final additive RQ values for the surrogate trenbolone compound.

[9] In one instance, an EC_{10} (concentration that results in 10% reduction in fecundity) was also determined and used to calculate the PNEC (Section 6.2.3).

Typically an RQ value of <1 is used as a preliminary screening level to determine if additional analysis and refinement of the risk assessment may be needed. In this EA, because of the many conservative assumptions used throughout the exposure and effects assessment and the many refinements that have been incorporated into the PEC values, we believe that an RQ value in the range of 1 or less indicates that significant environmental effects are highly unlikely at the predicted level of exposure.

1.3. Study and Data Evaluation

Individual environmental fate and effects parameters were derived using: 1) published data from at least two independent scientific studies conducted with acceptable methods and producing consistent results and/or 2) a Zoetis-owned GLP laboratory study conducted in accordance with Organization for Economic Cooperation and Development (OECD) guidelines or other acceptable guidelines and with available raw data. Several criteria were evaluated to determine whether the data from a published literature study were acceptable for use in deriving an environmental fate parameter, such as whether: 1) the experimental methods and procedures were adequate or similar to OECD and/or other acceptable guidelines, 2) the analytical methods were adequate and the analytical results were within acceptable standards, and 3) the data analysis methods used were adequate to determine the final endpoint of interest. If the published study did not follow acceptable methods or procedures, the study data were not used in the derivation of the final parameters. Additional information on the selection of the environmental fate and effects data is provided in Section 4 and Section 6.

Figure 1. Overview of Assumptions, Approaches and Methods Used in the Risk Assessment

Problem Formulation with Hazard Identification

Use Pattern of Synovex ONE Feedlot and Grass Implants for Beef Steers and Heifers (Section 2)

- Synovex ONE-F: total of 28 mg EB and 200 mg TBA per animal released over 200 days
- Synovex ONE-G: total of 21 mg EB and 150 mg TBA per animal released over 200 days

Primary Compounds of Concern (Section 2 and 4)

- EB and TBA are not excreted from cattle; the primary metabolites in the manure on a feedlot are (Section 4.1 and 8.2):
  1) 17α-estradiol > 17β-estradiol > estrone
  2) 17α-trenbolone (47.2%) > 17β-trenbolone (1.68%) > trendione

Ecosystem at the Site of Introduction (Section 3.3, Figures 2 and 3)

- Highest beef cattle production occurs in the Great Plains and Midwest
- Surface waters were identified as the compartment of concern due to potential impacts on fish (see Section 6)

Exposure Pathways of Concern (Section 3.4, Figure 4)

- Primary sources and exposure pathways evaluated at a farm- and aggregate watershed-scale include runoff/erosion from:
  1) Pastureland containing pasture cattle manure
  2) Cropland with solid manure from feedlot cattle applied as fertilizer
  3) Cropland irrigated with water from feedlot runoff collection lagoons/ponds
  4) Open feedlots without adequate runoff control (i.e., feedlots with <1000 cattle)

Analysis

Characterization of Exposure

Environmental Fate Modeling (Section 5)

- EPA EXPRESS or PRZM/EXAMS models are used to estimate the PEC_{water} values for the four primary exposure pathways
- Total of 56 individual farm-scale scenarios were modeled: 5 pasture, 34 tilled crop, and 17 no-till crop
- Total of 5 aggregate mixed-use watersheds evaluating inputs from all four sources and exposure pathways (TX, IA, OH, PA and MI)
- A single surrogate estradiol compound and a single surrogate trenbolone compound were modeled
- 90th percentile 21-day moving average PEC_{water} values determined from simulations over a 30 year period
- Many conservative assumptions utilized (see Sections 5 and 9)

Selection of Fate Parameters (Section 4.2, Figures 8 and 9)

- Physical-chemical and fate properties for each surrogate compound were derived using methods that ensured the PEC_{water} would not be underestimated (see Tables 9 and 13 for modeling input parameters)
- Each fate parameter was derived from composite data for the EB or TBA metabolites obtained from published literature and/or Zoetis-owned studies

Characterization of Effect

Endpoint Selection (Section 6.2, Tables 35 and 42)

- Assessment endpoint: fish reproduction (e.g., fecundity, embryo fertility, sex ratio) during chronic exposures (≥ 21 days)
- The lowest reproduction NOEC values (or range of NOEC and EC_{10} values) were selected for the α- and β-isomers of estradiol and trenbolone from adequate literature (Section 1.3) and Zoetis-owned studies

Derivation of the PNEC (Section 6.3)

- The PNEC was derived for the α- and β-isomers of estradiol and trenbolone using the selected NOEC/EC_{10} value(s) and an assessment factor (AF)

PNEC = NOEC (or EC_{10}) / AF

Risk Characterization

Assumptions and Approach (Section 7, Tables 51 and 52)

- Risk Quotient (RQ) method where the RQ = PEC/PNEC, where an RQ in the range of 1 or less indicates that significant environmental effects are unlikely at the predicted level of exposure

Surrogate Estradiol Compound

- Because of a lack of manure excretion data, it was assumed that the toxicity of the surrogate estradiol compound modeled in the exposure assessment were equal to the toxicity of the 17α or 17β forms

RQ = 21-day PEC_{water} of the surrogate estradiol compound/21-day PNEC for 17α-E2
RQ = 21-day PEC_{water} of the surrogate estradiol compound/21-day PNEC for 17β-E2

Surrogate Trenbolone Compound

- Manure excretion data was used to proportion the PEC values for the surrogate trenbolone compound based on the relative distribution of 17β- and 17α-trenbolone [20% and 80%, respectively]; RQ values were then determine for each based on the following equations:

$RQ_{\alpha 80}$ = (21-day PEC_{water} of the surrogate trenbolone compound * 0.80) / (21-day PNEC for 17α-TB)
$RQ_{\beta 20}$ = (21-day PEC_{water} of the surrogate trenbolone compound * 0.20) / (21-day PNEC for 17β-TB)

- To account for potential additivity, the individual RQ values attributed to 17α-trenbolone and 17β-trenbolone were added together to estimate the final RQ values for the surrogate trenbolone compound

Additive RQ for surrogate trenbolone compound = $RQ_{\alpha 80}$ + $RQ_{\beta 20}$

2. PRODUCT CHARACTERISTICS

The core of each implant pellet of Synovex ONE is identical to the EB and TBA formulation approved for Synovex Plus under NADA 141-043. However, each Synovex ONE pellet has a porous polymer coating. The porous polymer coating results in a slower *in vivo* release of EB and TBA. The porous polymer coating allows a 200-day payout compared to the 130-day payout characteristic of comparable uncoated pellets in Synovex Plus approved under NADA 141-043 (Appendix 13.4).

2.1. Active Components

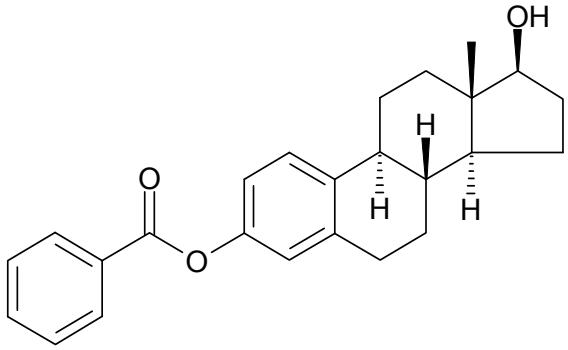
Estradiol benzoate (EB) - The IUPAC name for estradiol benzoate (CAS 50-50-0) is [(8R,9S,13S,14S,17S)-17-hydroxy-13-methyl-6,7,8,9,11,12,14,15,16, 17-decahydrocyclopenta[a]phenanthren-3-yl] benzoate. The molecular weight (MW) is 376.49.

Trenbolone acetate (TBA) - The IUPAC name for trenbolone acetate (CAS 10161-34-9) is [(8S,13S,14S,17S)-13-methyl-3-oxo-2,6,7,8,14,15,16, 17-octahydro-1H-cyclopenta[a]phenanthren-17-yl] acetate. The MW is 312.40.

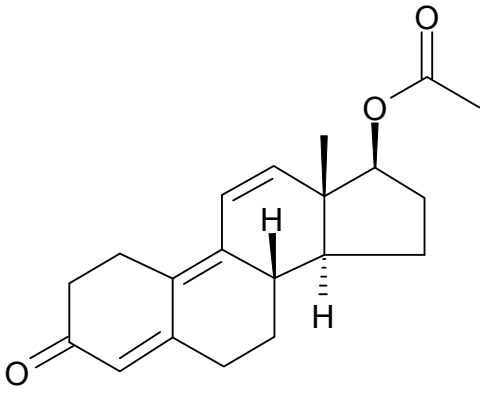
Both EB and TBA have an oxygen on carbon 17 of the ring in the β configuration (Figure 2). When the benzoate group of EB is hydrolyzed in cattle, 17β-estradiol is formed. Likewise, 17β-trenbolone is formed when the acetate of TBA is hydrolyzed in cattle. The chemical structures of EB and TBA are shown in Figure 2.

Figure 2. Chemical Structure of Estradiol Benzoate (EB), Trenbolone Acetate (TBA) and Carbon Numbering of a Steroid

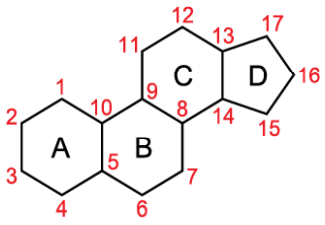
EB



TBA



Carbon Numbering of Ring



2.2. Formulation and Dose for Pasture and Feedlot

ONE-F and ONE-G consist of multiple, precisely formulated pellets each containing 3.5 mg of EB and 25 mg of TBA that are implanted in the ears of cattle in a single subcutaneous injection. ONE-F and ONE-G are identical product formulations. They differ only in the number of pellets per dose. ONE-F contains eight pellets per dose. Therefore, each dose of ONE-F contains 28 mg of EB and 200 mg of TBA. ONE-G contains six pellets per dose. Therefore, each dose of ONE-G contains 21 mg of EB and 150 mg of TBA. The composition of each pellet of the Synovex ONE formulation and pellet film coating are specified in Table 1 and Table 2, respectively.

Table 1. Synovex ONE Pellet Ingredients

Ingredient	Function
Trenbolone acetate	Active ingredient
Estradiol benzoate	Active ingredient
Povidone (K-90), USP	Binder
Polyethylene glycol 8000, NF	Binder, lubricant
Magnesium stearate, NF	Lubricant
Purified water, USP	

Table 2. Synovex ONE Pellet Coating Ingredients

Ingredient	Function
Ethylcellulose aqueous dispersion, NF	Film coating
Dibutyl sebacate, NF	Plasticizer
Polyethylene glycol 8000, NF	Pore former
Purified water, USP	

To achieve the extended release kinetics of the ONE-F and ONE-G implant formulations, each pellet is coated with a porous polymer film that yields a steady release of the active components *in vivo*, effectively providing a 200-day payout time-period.

2.3. Label Indications for Use

Synovex ONE-F is a pelleted implant indicated for increased rate of weight gain and for improved feed efficiency for up to 200 days in steers and heifers fed in confinement for slaughter.

Synovex ONE-G is a pelleted implant indicated for increased rate of weight gain for up to 200 days in pasture cattle steers and heifers (stocker, feeder and slaughter).

2.4. Annual Frequency of Use

Based on efficacy achieved with release for 200 days, each pasture and feedlot animal will be implanted once.

2.5. Disposal of Synovex ONE Implants

MSDS: "To minimize disposal of active ingredients, all attempts should be made to utilize the Synovex product completely, in accordance with its intended use. If this is not possible, unused inserts should be stored in a sealed plastic bag and disposed of following applicable Federal, State, and Local regulations regarding waste disposal."

Label: "Synovex ONE waste materials should be disposed of according to prescribed Federal, State, and Local guidelines."

2.6. Principal Metabolites Excreted from Cattle are 17α Isomers

EB and TBA are not excreted from cattle in the form administered to them. The benzoate and acetate groups are rapidly hydrolyzed in the animal to form 17β-estradiol and 17β-trenbolone, respectively (Section 4.1). Based on available laboratory and field monitoring data (see Sections 4.1 and 8 for a summary of the relevant literature), the principal metabolite identified in cattle excreta[h] following the administration of EB is 17α-estradiol, with additional minor metabolites, 17β-estradiol and estrone. The principal metabolite identified in excreta following administration of TBA is 17α-trenbolone (47.2%) with additional minor metabolites, 17β-trenbolone (1.68%) and trendione (6.2%). Therefore, the major metabolites of Synovex ONE introduced into the environment via manure applied to land, manure deposited on pasture, and effluent discharged from feedlots are assumed to be 17α-estradiol and 17α-trenbolone. The metabolism and excretion profile of EB and TBA are described in Sections 4.1.1 and 4.1.2, respectively. Table 7 presents the structures of the principal and minor metabolites of EB and TBA.

2.7. Molecular Weight Adjustment for Loss of Acetate and Benzoate

The loss of the benzoate and acetate groups as a result of metabolism of EB and TBA in the target animal to estradiol and trenbolone, respectively, requires a MW adjustment to provide the quantity of biologically active material in the implant.

Base activity of EB = MW of estradiol/MW of EB = 272.38/376.49 = 72.35%
Base activity of TBA = MW of trenbolone/MW of TBA = 270.37/312.40 = 86.55%

Therefore, in order to determine potential environmental effects of the EB and TBA metabolites excreted, the implants contain the following:

2.7.1. Synovex ONE-F

28 mg EB x 0.7235 = 20.26 mg of estradiol
200 mg TBA x 0.8655 = 173.1 mg of trenbolone

2.7.2. Synovex ONE-G

21 mg EB x 0.7235 = 15.19 mg of estradiol
150 mg TBA x 0.8655 = 129.83 mg of trenbolone

[h] The "principal metabolite identified in excreta" is not necessarily the same as the "principal metabolite excreted from the target animal" because additional metabolism may occur within the excreta, but it is the most relevant metabolite for the purposes of this assessment. Thus, the focus of the assessment will be on the principal metabolites found in the manure on feedlots.

2.8. Description of all Marketed Synovex Products

There are several different implants in the Synovex product line, each of which is implanted in the ear of beef cattle. Each product contains a combination of EB or 17β-estradiol and progesterone, testosterone propionate, or TBA. Table 65 of Appendix 1 lists each of these products, the quantity of active ingredients, the duration of effect, and labeled indication.

3. ECOSYSTEMS AT THE SITE OF INTRODUCTION OF EB AND TBA METABOLITES INTO THE ENVIRONMENT

Synovex ONE will be administered to beef cattle housed in an animal feeding operation (AFO) or on pastureland. To support modeling of the fate of EB and TBA metabolites in the environment, information on the national population and distribution of pasture and feedlot beef cattle in the US was evaluated. Active pharmaceuticals in treated cattle on AFOs are excreted in manure, which is stored on an AFO and then applied to cropland as fertilizer. Information on all known sources of environmental exposure associated with beef cattle manure containing estradiol and trenbolone are presented. A description of the ecosystems at risk and the potential for aggregate exposure from multiple sources of EB and TBA metabolites released in the environment are also addressed.

3.1. Pasture Cattle

Synovex ONE-G is intended for use in pasture cattle (stockers). Therefore, USDA statistics for the number, types, and locations of beef cattle contained on pasture in the US were evaluated.

3.1.1. Pasture cattle population

The use of USDA agricultural statistics for estimating the total number of pasture cattle, and of those cattle, the number of “stocker” cattle (targeted group for use of Synovex ONE-G), is not straightforward. The group of pasture cattle is made up of “suckling calves” (<500 lb) that are not candidates for EB and TBA implants. Larger stocker animals (>500 lb) are candidates for implants. A description of the stocker cattle group and a US map indicating their population and location extracted from an article by beef economist, Shane Ellis (Beef Magazine, 2010 [2]), is provided in Figure 3.

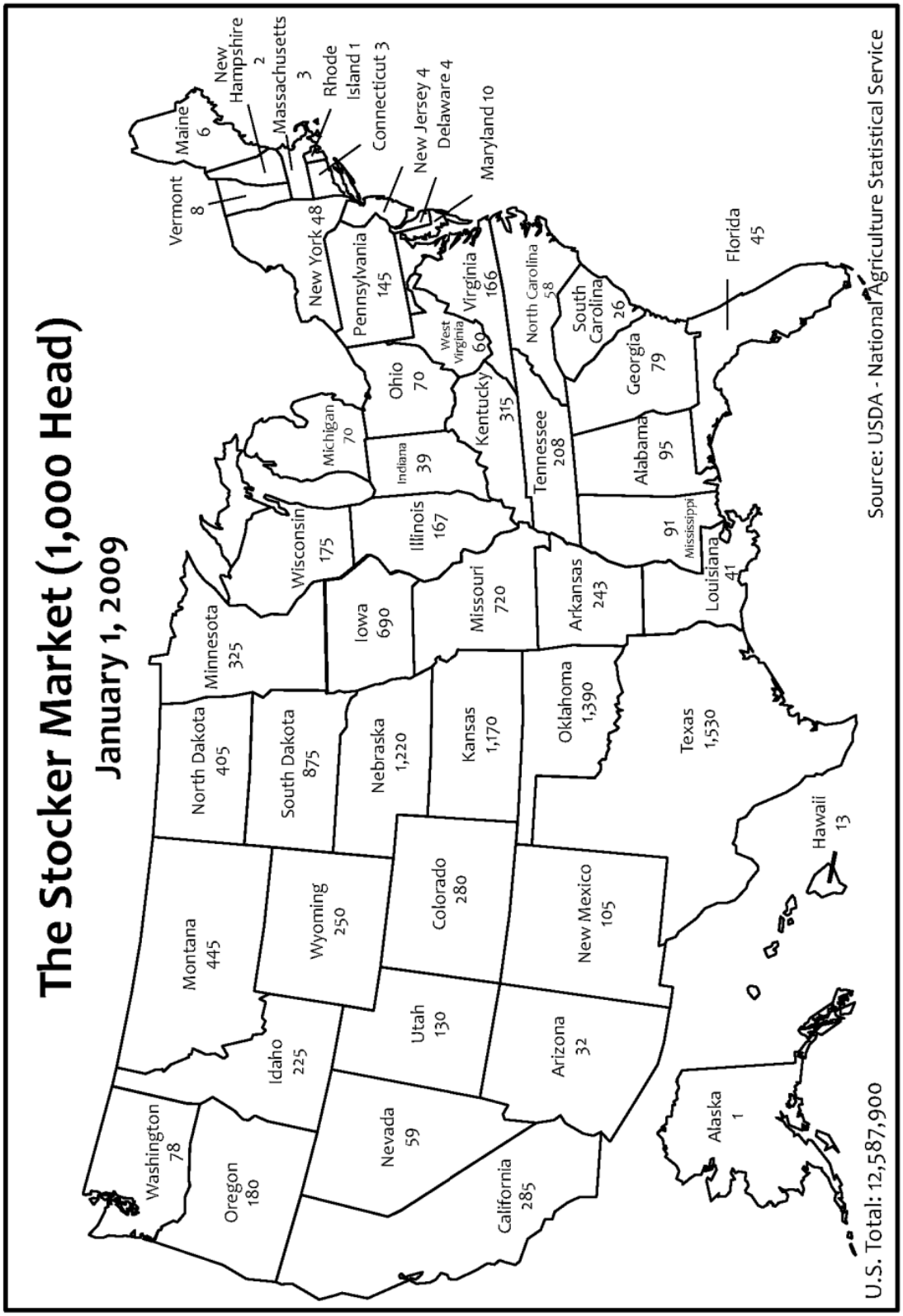


Figure 3. Pasture Cattle (Stocker) Density by State in the US from USDA in 2009

Stockers are defined by the USDA survey as "cattle, weighing over 500 pounds not in a feedlot or being kept for replacements, being grown on high-roughage diets" [2]. These cattle are listed in the yearly January 1st USDA survey as "cattle available for placement outside of feedlots." The map demonstrates that the highest densities of stockers are located in the central plains states. The estimate of stocker cattle from the USDA data in Dr. Ellis's report is 12.588 million (M) [2]. The report also states that "it is nearly impossible to tie down a count of either stocker operations or the cattle they own." According to the report, there are 15.0 M beef/dairy calves <500 lb, excluding replacement heifers. Therefore, an approximation of the total pasture cattle in the US on January 1, 2010, is 27.6 M (12.6 M stocker + 15 M suckling).

The number of stocker, suckling calves, and total pasture cattle can be estimated from the USDA NASS Agricultural Survey data; however, the number must be calculated by subtracting out overlapping cattle groups in the listing. The total US cattle on January 1, 2012, by tracked category from the USDA are shown in Table 3.

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

Cattle Category Class	Number of Cattle
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 lb	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 lb	16,071,500
Bulls ≥500 lb	2,052,000
Calves under 500 lb	14,144,800
Cattle on feed (beef cattle in feedlots)	14,121,400

* Source: USDA NASS Cattle Inventory [3]
† Replacement heifers expected to calve during the year

Calculation of the number of stockers from the USDA data set is as follows:

[Heifers ≥500 lb, Other heifers] + [Steers ≥500 lb] – [Cattle on feed] = Stockers
9.65 M + 16.07 M – 14.12 M = 11.60 M stocker cattle on pasture in US on January 1, 2012

Calculation of total pasture cattle from the USDA data set is as follows:

Stockers + Calves (cattle <500 lb or suckling) = Total cattle on pasture

11.6 M stocker + 14.14 M calves = 25.74 M pasture cattle in US on January 1, 2012

The estimate of 11.6 M stockers in 2012 is similar to the value of 12.6 M presented in the 2010 Beef Magazine article cited above [2]. The estimate of 25.7 to 27.6 M total pasture cattle is very conservative because many feedlot cattle would go to slaughter in the first quarter of the year and then be replaced from the inventory of pasture cattle. As per the USDA, there were 27.539 M pasture cattle on January 1, 2010, and 21.128 M on April 1, 2010 [4], a decline of 6.41 M pasture cattle that were moved into feedlots.

These data for stocker and total pasture cattle estimates are available from the USDA on the national and state level; however, they are not reported at the county level. The lack of county level data on the number of pasture cattle makes it difficult to estimate this cattle group for watershed modeling. A county level estimate of pasture cattle can be determined by estimating the population of all other groups of cattle and subtracting them from the total cattle in the county. This approach is presented in Table 25 (Section 5.7.2) where the percent of dairy and beef replacement heifers relative to the total beef and dairy cattle on the state level is extrapolated to estimate the county level of replacement heifers. The pasture cattle population for the county can then be estimated.

3.1.2. National pasture cattle summary

The spatial and temporal data on pasture cattle presented above are included to provide a summary of pasture cattle demographics in the US. National data were not specifically used in the environmental prediction models presented in this EA. In the models, regional specific information on pastureland and cattle densities was utilized. This is discussed in Sections 5.6 and 5.7 of this EA.

3.2. Feedlot Cattle (Cattle on Feed)

Synovex ONE-F is intended for use in feedlot beef cattle (steers and heifers). EB- and TBA-containing implants have been in use for approximately 27 years. The use of implants has been readily accepted by the beef industry, and they are typically used in most beef cattle. The exception to this is organic beef, which is a small segment of the beef market. This section describes the categories of AFOs and/or CAFOs, the geographic distribution of AFOs and/or CAFOs, and the recent inventories of beef cattle held on AFOs in the US.

3.2.1. Concentrated animal feeding operations (CAFOs)

The Clean Water Act prohibits the discharges of pollutants from point sources into US waters without a National Pollutant Discharge Elimination System (NPDES) permit issued by the EPA. According to Section 502 of the Clean Water Act, concentrated animal feeding operations (CAFOs) are considered point source discharges.

A facility must meet the definition of an AFO before it can be considered a CAFO. An AFO is defined in EPA's regulations as a lot or facility (other than an aquatic animal production facility) where the following conditions are met: 1) animals have been, are, or will be stabled or confined and fed or maintained for a total of 45 days or more in any 12-month period, and 2) crops, vegetation, forage growth, or post-harvest residues are not sustained in the normal growing season over any portion of the lot or facility [40 CFR 122.23(b)(1)]. An AFO can describe any size of animal feedlot; however, a CAFO is a specific type of AFO as defined under EPA's regulations. Under 40 CFR 122.23(b) and (c), CAFOs for beef cattle are defined or designated as follows:

- An AFO is defined as a large CAFO by EPA if it meets the requirements of an AFO and has ≥1000 beef cattle [40 CFR 122.23(b)(4)].
- An AFO is defined as a medium CAFO by EPA if it meets the requirements of an AFO, has 300-999 beef cattle, and meets one of the following conditions: 1) pollutants are discharged into waters of the US through a man-made ditch, flushing system, or other similar man-made device, or 2) pollutants are discharged directly into water of the US which originate outside of and pass over, across, or through the facility or otherwise come into direct contact with animals confined in the operation [40 CFR 122.23(b)(6)].
- An AFO can also be designated as a medium CAFO by a permitting authority if it is found to be a significant contributor of pollutants to the surface waters [40 CFR 122.23(c)].
- An AFO with <300 beef cattle can be designated as a small CAFO by a permitting authority if it is a significant contributor of pollutants to surface waters and if it meets one of the two conditions discussed above under medium CAFOs [40 CFR 122.23(c)].

If a CAFO discharges or proposes to discharge, the owner or operator must seek coverage under an NPDES permit. Any permit issued to a CAFO must include a Comprehensive Nutrient Management Plan (CNMP) that covers requirements listed under 40 CFR 122.42(e)(1) and 40 CFR 412(e)(5).

Two main areas of CAFOs are regulated: 1) the production areas and 2) land application areas. The production area is the part of the farm that includes the animal confinement area, the manure storage area, the raw materials storage area, and the waste confinement area. The exact requirements for runoff control differ from state to state. The production area must be designed, built, operated, and maintained to handle all manure and process wastewater, including, at a minimum, all normal rainfall events up to a 25-year, 24-hour rainfall event[i] [5].

The land application area is any land that is under the control of the AFO operator and to which manure or wastewater from the production area is (or might be) applied [5]. CAFOs must follow best management practices (BMPs) when applying manure to land, including:

- All manure and wastewater applied to land must be in accordance with the developed CNMP, which is used to minimize runoff of nitrogen and phosphorous.
- The nutrient content of manure must be analyzed once per year and cropland soil once every 5 years.
- A setback area must be maintained within 100 feet of any down-gradient surface waters or other conduits to surface waters where manure and other wastewaters are not applied.

[i] A 25-year, 24-hour rainfall event is one that has a probable recurrence interval of 25 years [40 CFR 412.2(h)(i)].

Since the 2003 implementation of the EPA NPDES permits and state enforcement of fines, there have been significant improvements in the control of runoff from these facilities. Based on the CAFO regulations under the Clean Water Act, the following assumptions concerning beef cattle CAFOs were incorporated into this risk assessment:

- NPDES requirements for farms with ≥1000 beef cows include a CNMP for manure management and collection of runoff water from the feedlot.
- Farms with <1000 beef cows do not have any control systems to prevent direct runoff from the feedlot to surface water, which is a conservative assumption.

3.2.2. Inventory of beef cattle in confinement

On January 1, 2012, there were 14.1 M beef cattle on feed out of 90.8 M total cattle in the US (Table 3). Beef cattle in feedlots comprised 16% of all cattle in the US at that point in time. The cattle inventory changes throughout the year; therefore, yearly values were used to conservatively estimate cattle numbers. The maximum potential product usage in beef cattle may be estimated from the last US agricultural census in 2007 [6], which included the yearly total cattle on feed inventory (Table 4) and cattle on feed marketed (Table 5). The USDA conducts yearly surveys but only conducts a census every five years. Cattle on feed are the target population for feedlot use of Synovex ONE-F. "Cattle on feed" is defined in the survey as "cattle and calves that were fed a ration of grain or other concentrates that will be shipped directly from the feedlot to the slaughter market and are expected to produce a carcass that will grade select or better. This category excludes cattle that were pastured only, background feeder cattle, and veal calves."

Based on the most recent census data from 2007, the inventory of cattle on feed in 2007 totaled 16.1 M beef cattle (Table 4) with a total of 27.6 M cattle marketed (Table 5). A comparison of the 2002 and 1997 census data indicates a large decline in total number of cattle marketed from small farms (<500 animals) and a general trend of declining numbers of total cattle marketed (data not shown). A comparison of the total cattle marketed to the total inventory indicates that there are approximately 1.7 cattle cycles per year (27,595,928/16,098,910 = 1.7). However, most of the influence on cycle times being >1 per year comes from the large facilities with >2500 cattle.

Table 4. Total US Cattle on Feed Inventory by Farm Size - 2007 Census of Agriculture

Farms with # Animals (mean of range)	# Farms	Inventory	% of Inventory
1-9 (5)	15,818	65,809	0.41
10-19 (15)	7,072	93,242	0.58
20-49 (35)	9,136	280,083	1.74
50-99 (75)	6,313	426,159	2.65
100-199 (150)	4,375	586,624	3.64
200-499 (300)	3,744	1,118,788	6.95
500-999 (750)	1,997	1,429,215	8.88
1000-2,499 (1750)	780	1,152,679	7.16
2500 or more (>2500)	774	10,946,311	67.99
Total	50,009	**16,098,910**	100.00

2007 Census of Agriculture Table 12 page 19 [6]
www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_US/

Table 5. Total US Cattle on Feed Marketed by Farm Size - 2007 Census of Agriculture

Farms with # Animals (mean of range)	# Farms 2007	Number Marketed 2007	% of Marketed 2007	# Farms 1997†
1-9 (5)	34,543	129,460	0.47	52,448
10-19 (15)	10,574	138,957	0.50	17,240
20-49 (35)	11,736	357,197	1.29	19,295
50-99 (75)	6,579	449,362	1.63	9,052
100-199 (150)	4,710	634,210	2.30	5,424
200-499 (350)	3,975	1,183,536	4.29	3,867
500-999 (750)	1,980	1,376,157	4.99	1,397
1000-2,499 (1750)	1,264	1,872,253	6.78	939
2500-4,999 (3,750)	366	1,248,925	4.53	318
5,000 or more (>5000)	669	20,205,871	73.22	640
Total	76,396	**27,595,928**	100.00	110,620

2007 Census of Agriculture. Marketed Table 13, page 19 [6].
† Data from Table 25 from census 1997 "Cattle fattened on grain and concentrates for slaughter" www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_US/ and www.agcensus.usda.gov/Publications/1997/Vol_1_Chapter_1_U._S._National_Level_Data/index.asp

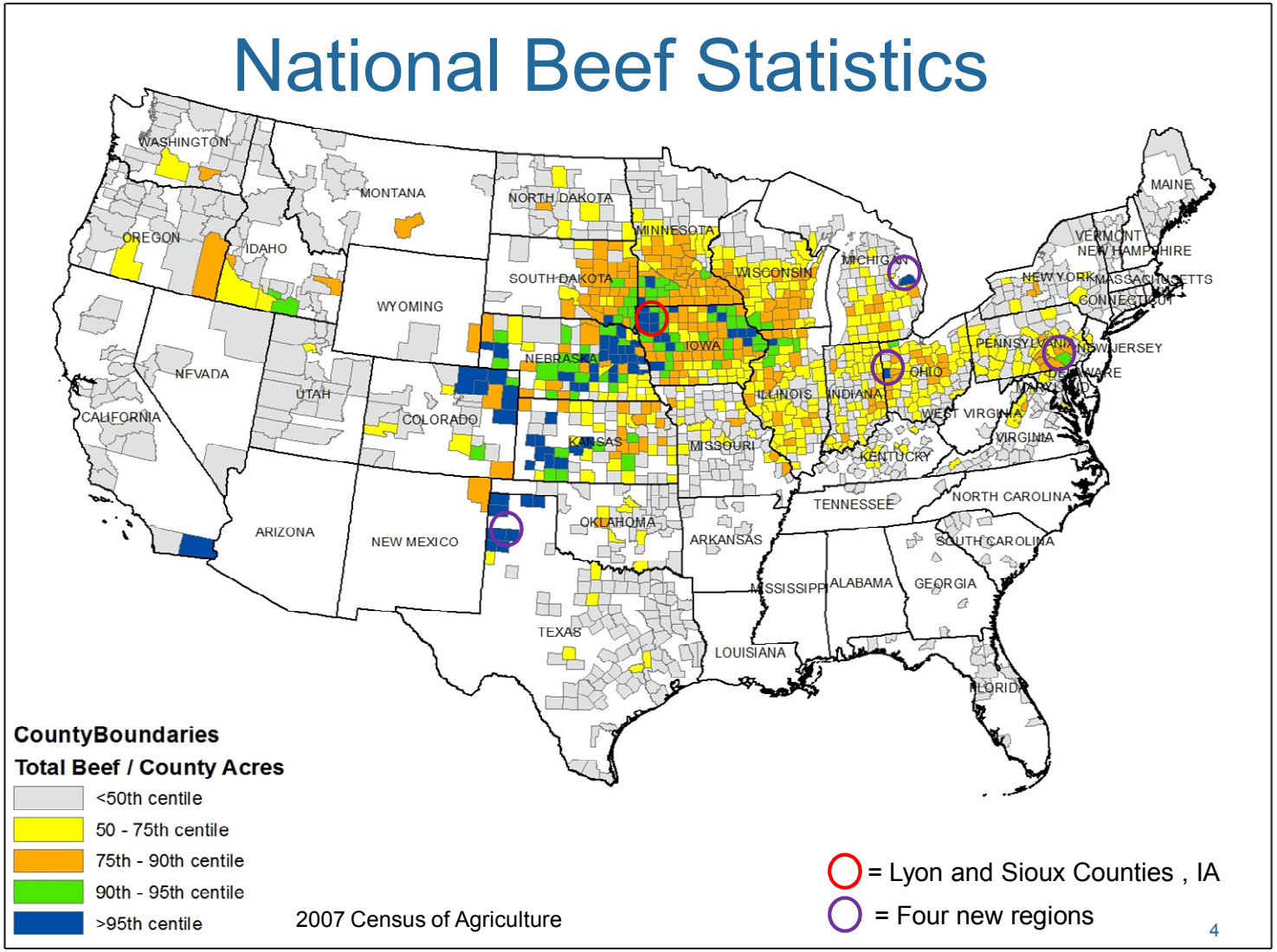
The data in Table 5 indicate that most of the beef cattle (~85%) are produced on large capacity farms with ≥1000 animal units (AU)[j] and are a small percentage of total farms (3%). Therefore, most of the potential ONE-F use will be in large farms ≥1000 AU that are defined as CAFOs. These feedlots are regulated under the Clean Water Act. CAFOs ≥1000 AU are less likely to be polluters of surface water because such operations are required to minimize potential pollution of surface water through the use of runoff containment from the feedlot as well as CNMPs for applying manure to agricultural fields using BMPs (see Section 3.2.1 for additional information on the EPA CAFO Rule). Additional information on national estimates of the number of cattle facilities in compliance with the Clean Water Act can be found in Appendix 9.

3.2.3. Geographic distribution of feedlot beef cattle by density

The map in Figure 4 illustrates the density of feedlot beef cattle per acre. The density per acre was determined by normalizing the beef cattle feedlot population within a county (reported in the USDA 2007 census) by the area of the county. The map illustrates the distribution of these densities and indicates that beef production is concentrated in the central US. This map, along with other maps of cattle facilities of various sizes, were used to identify regions of the US where surface water would be prone to potential contamination from beef cattle manure (Section 5.7 and Attachment 1). Because of the distribution of small, medium, and large facilities in Iowa and the availability of Geographic Information System (GIS) information for the feedlot locations, Lyon and Sioux counties, Iowa (IA) were chosen as the initial watershed to model (red circle on map). The additional four purple circles on the map represent other regions chosen to add to the mixed-use watershed models based on their high density of cattle and diversity in farm size. More detailed information on these mixed-use watershed models is available in Section 5.7, Appendix 8 and Attachment 1.

[j] In this EA, an animal unit (AU) is equal to one beef animal (e.g., 1000 beef cattle (steer, heifer) equals 1000 AU).

Figure 4. Beef Feedlot Cattle Density by County Across the US



3.2.4. Manure applied to agricultural land

According to the 2002 major land use publication from the USDA, the US has a total land area of nearly 2.3 billion acres [7]. Of the land area, cropland accounted for 19.5% of the total. By 2007, cropland was reduced to 406,424,909 acres or approximately 17.7% of total land area (Table 8, page 16 of reference [6]). Acres of cropland treated with manure were 22,096,315, which corresponds to only 5.4% of total agricultural cropland [6].

The percent of cropland to which manure is applied from confined livestock can be approximated from the total fertilizer nutrient content reported in the 2003 manure management survey [8] and is presented in Table 6.

Table 6. 2003 Survey of Confined Livestock of Farms with >35 Animal Units (AU). Total Distribution of Manure Nitrogen and Phosphorus in the US.

Livestock Type	Animal Units	Pounds Manure Nitrogen	Percent	Pounds Manure Phosphorus	Percent
Fattened cattle	**13,193,896**	**1,481,784,875**	**20.9%**	**449,201,459**	**22.9%**
Milk cows	15,448,663	2,235,427,462	31.5%	425,073,626	21.7%
Swine	9,073,203	1,256,177,612	17.7%	375,873,882	19.2%
Turkeys	2,206,628	525,875,015	7.4%	207,734,091	10.6%
Broilers	2,966,935	1,041,747,587	14.7%	305,145,588	15.6%
Layers	1,374,533	398,365,032	5.6%	146,767,400	7.5%
Pullets	209,374	44,011,426	0.6%	16,582,152	0.8%
Confined heifers	26,827	2,962,551	0.0%	882,549	0.0%
Veal	1,182,548	120,000,451	1.7%	33,802,682	1.7%
Total	45,682,607	7,106,352,011	100%	1,961,063,429	100%

Table B-2 of reference [8]

These data suggest that beef cattle account for approximately 21-23% of the total manure used for fertilizer across the US based on nutrient content. Using the higher number, if 23% of nutrients are derived from fattened cattle manure (Table 6) and 5.4% of the total agricultural cropland in the US is manured each year [6], approximately 1.2% (5.4% X 23%) of the total cropland in the US could receive manure from the Synovex ONE-F treated target population each year assuming 100% of beef cattle held on feedlots are treated with Synovex ONE. Beef cattle production, however, is not evenly distributed across the landscape. Therefore, there will be regions of the US with high beef densities where a greater percentage of cropped fields are manured than the national estimates indicate and where cattle manure makes up a greater percentage of the total manure applied. These national statistics do not directly apply to potential usage within a watershed with a high beef cattle density. The mixed-use watershed models presented in this EA employ acres manured based on the cattle population within the watershed and are not based on the national statistics.

3.2.5. National feedlot cattle summary

The spatial and temporal data on feedlot cattle presented above are included to provide a summary of beef cattle demographics in the US. These data were not specifically used in the environmental prediction models presented in this EA. In the models, regional specific information on pastureland and cattle densities was utilized and is discussed in those specific modeling sections of this EA. The national data on manure production indicate that only about 5.4% of all cropland in the US is manured each year [6].

Table 5 shows the changes in beef cattle farm-size between 1997 and 2007. These data are used in Appendix 9 to estimate the redistribution of the number of cattle in AFOs and CAFOs over time and then further used to refine the estimate of the percentage of farms with <1000 AU that are potentially in need of improvements to control feedlot runoff.

3.3. The Ecosystems Potentially at Risk from the Use of Synovex Products are Principally Freshwater Watersheds

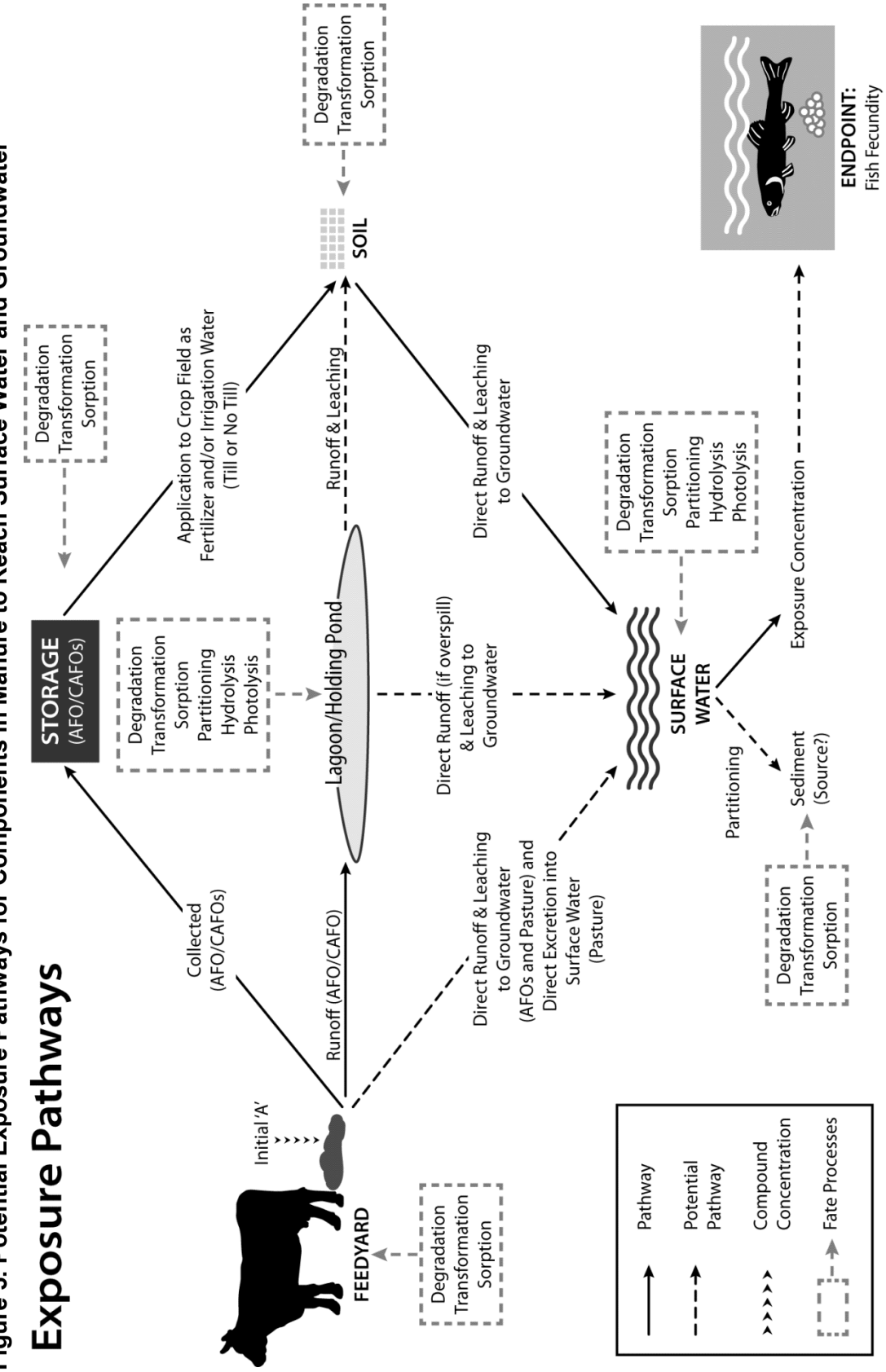
Beef cattle production is principally located inland (Figure 4). Therefore, potential exposure to EB and TBA metabolites will be in soil and surface water receiving runoff from pasture, manured cropland, and feedlot operations. Beef cattle production is generally not located in coastal regions. Thus, significant exposure to the marine environment is not anticipated. There are no specific data to suggest terrestrial organisms, such as earthworms and plants, are sensitive to exposures of steroid hormones (Section 6.1). As a result, the impacts on the terrestrial environment were not considered in this EA. In studies on the effects of EDCs on non-target species, fish and amphibians have been identified as the most sensitive species. Therefore, the EA will focus on the potential for EB and TBA metabolites to migrate to and impact freshwater environments from both individual sources (e.g., feedlots, cropland, and pasture), as well as from multiple sources within a watershed (i.e., mixed-use watersheds as described in Section 3.5 below).

3.4. Potential Introduction of EB and TBA Metabolites into Surface Water

The EB and TBA metabolites, from use of Synovex ONE implants, are expected to be introduced into the environment primarily through: 1) direct runoff from AFOs, 2) application of manure or wastewater to cropland as fertilizer, and 3) deposition of manure on pastureland. In this section, ten potential sources (exposure pathways) are described for the introduction of estradiol and trenbolone into the environment from beef cattle manure. For each of these sources and pathways, an evaluation was conducted of the potential for estradiol and trenbolone to migrate to surface water by: 1) surface runoff, 2) erosion[k], and/or 3) leaching to groundwater and movement of the groundwater through the subsurface to surface waters. The exposure pathways found to be potential contributors are described in Section 3.4.1. Those sources and exposure pathways found to be negligible contributors to surface water concentrations were eliminated from further evaluation in the farm and watershed level modeling and are described in Section 3.4.2. Leaching models estimated that negligible amounts of estradiol and trenbolone would likely migrate to groundwater (Sections 3.4.2.1 and 3.4.2.2). Therefore, the final models employed in this risk assessment for watershed modeling were surface water runoff/erosion models. An illustration of potential exposure pathways is shown in Figure 5.

[k] Erosion is defined as the transport of a chemical via soil particles.

Figure 5. Potential Exposure Pathways for Components in Manure to Reach Surface Water and Groundwater



3.4.1. Exposure pathways analyzed

3.4.1.1. Surface runoff from manure applied to cropped fields from feedlots

In the feedlot, manure will either be stored in the feedyard, stockpiles, or in liquid storage. Although there is evidence that estradiol and trenbolone metabolites may not be stable in certain manure storage conditions (e.g., surface of feedlot; Appendix 12), the conservative assumption was made that 100% of the excreted metabolites remain active in the stored manure, do not degrade, and are applied to cropland soil in manure used as fertilizer.

Once applied to cropland, estradiol and trenbolone metabolites contained in the manure can potentially migrate to surface waters from either direct surface runoff or leaching to groundwater, which will be eventually discharged to surface waters (Figure 5). Runoff from the soil surface will likely be a significant contributor to surface water exposure, but the concentration in surface runoff can be reduced by adsorption to soil, which can decrease the mobility and bioavailability of estradiol and trenbolone metabolites of concern. Degradation by microbes in the soil column can also contribute to reducing this concentration. Additional information on fate and transport of estradiol and trenbolone metabolites in the terrestrial and aquatic environments are discussed in Section 4.2. The evaluation of surface runoff from the application of manure to cropland via modeling is provided in Sections 5.4, 5.6, and 5.7.5.

3.4.1.2. Runoff from fields irrigated with storage lagoon/pond water

As illustrated in the conceptual model (Figure 5), water from feedlot runoff is collected and held in a storage structure (e.g., lagoon or pond), which allows time for components in manure to partition to the organic material at the bottom or in the water column, of the lagoon/pond. Periodically, the water will be used to irrigate land, and the solids portion will be incorporated into land as fertilizer. Field monitoring data indicate that wastewater lagoons/ponds can contain 17α-estradiol, 17β-estradiol, and estrone [9, 10, 11, 12]. Field data, however, do not always indicate the presence of 17α-trenbolone or 17β-trenbolone in the lagoons [12] or in feedlot runoff due to rainfall events [13]. For modeling purposes, it was conservatively assumed that 100% of the metabolites are active in the feedlot runoff and remain active in the lagoons and that they are applied to cropped soil during irrigation. Therefore, it was assumed that no degradation of the estradiol and trenbolone metabolites occurred in the storage lagoon/pond. Additional information on the use of irrigation water in the mixed-use watershed models is provided in Section 5.7.6.

3.4.1.3. Runoff from AFOs <1000 AU directly discharging to surface water

Although smaller AFOs (<1000 cattle) are not legally required to meet the design criteria of CAFOs and their compliance with BMPs (e.g., containment of wastewater, CNMP, etc.) is voluntary, these operations are still legally responsible for pollution discharges (Section 3.2.1). If an AFO has the potential to impact surface waters, then NPDES authorities can designate the AFO as a CAFO and can require the AFO to adhere to the criteria and BMPs that apply to a CAFO. The regulations and BMPs introduced as part of the Clean Water Act have encouraged proper manure management and have reduced the potential for environmental exposures to nutrients and other constituents contained in manure.

It is anticipated that many, if not most, small and medium AFOs (<1000 cattle) are voluntarily employing some of these BMPs, such as a CNMP and runoff controls. At this time, there are no estimates available for the number or percentage of facilities that do not employ such controls. There are, however, USDA estimates of the number of small and medium feedlots that were in need of runoff control improvements. These data were used by Zoetis to estimate a percentage (17%) of small and medium feedlots in the US that may be directly discharging to surface waters (Appendix 9).

These data were based on estimates from 1997 and do not include adjustments for increased voluntary compliance or enforcement of the Clean Water Act and the NPDES program (Section 3.2.1) to date. Therefore, the estimate of 17% is considered to overestimate the number of farms that are directly discharging. Compliance at the state and local level may vary; therefore, this national average may not be predictive of local percentages. As a result, we have estimated PEC values in Section 5.7 using values covering a range of 0 to 100% for this parameter. For example, an assumption of 0% would represent a scenario in which all AFOs had acceptable BMPs in place and were not directly discharging to surface waters. An assumption of 25% of AFOs with <1000 AU directly discharging to surface waters was used in the environmental fate modeling in Section 5 to represent a conservative national average based on the 17% estimation from Appendix 9. Alternatively, an assumption of 50% of AFOs with direct discharge was used in the modeling to represent a reasonable worst-case situation.

3.4.1.4. Pastureland runoff

Pasture cattle will excrete estradiol and trenbolone metabolites onto grasslands via feces and urine. Grazing land has a lower potential than cropland for surface water runoff because the cattle excrete in a discrete pattern and the grass cover aids in water infiltration. Potential runoff from pastureland was included in the mixed-use watershed models (Section 5.7.8).

3.4.2. Exposure pathways excluded from further analysis

3.4.2.1. Subsurface leaching from manure applied to cropped fields from feedlots

As discussed above, when manure from Synovex-treated cattle is applied to cropland, there is a potential for estradiol and trenbolone metabolites to leach to groundwater that is subsequently discharged to surface waters. The potential for these metabolites to leach into soils was evaluated using EPA's Screening Concentration in GROundWater (SCI-GROW) model (Section 5.3) and Exposure Analysis Modeling System (EXAMS)-Pesticide Root Zone Model (PRZM) Simulation Shell (EXPRESS model) (Appendix 7.2). Based on the SCI-GROW model, these metabolites are not expected to significantly impact groundwater. The EXPRESS model found similar results when simulating the potential leaching 1 m below the surface of cropland soils that have had manure applied (Appendix 7.2). These results are not surprising because, based on the physical-chemical properties and environmental fate data for these compounds (Section 4.2.3), estradiol and trenbolone metabolites are expected to bind moderately and degrade rapidly in soil. Based on the available data, concentrations of EB and TBA metabolites in groundwater are expected to be negligible compared to the concentrations in surface runoff; therefore, groundwater transport of estradiol and trenbolone metabolites is not expected to be a major contributor to surface water exposure. Thus, leaching from cropland is excluded from the mixed-use watershed models.

3.4.2.2. Leaching from tile-drained fields from manure applied to cropped fields

Tile-drained fields are used to remove excess water from the soil in order to enhance crop production. Modern tile drains use a perforated plastic pipe that is placed approximately 2 to 4 feet under the soil surface [14]. If the groundwater rises, it is removed by the pipe to a drainage ditch. Also, if the soil becomes excessively moist, the leachate that reaches the drainage pipe can drain from the field to the ditch. Tile drains empty into a circuit of ditches that are considered part of the agricultural infrastructure necessary to maintain the drainage of the land.

The EXPRESS model was used to estimate the PEC_{water} of leachate at 1 m below the soil surface using 34 cropland scenarios (Appendix 7.2). These PEC values simulate the potential concentrations of the surrogate estradiol and trenbolone compounds that could reach tile drains. The concentrations predicted using the EXPRESS model are considered negligible ($<4.1 \times 10^{-12}$ µg/L; Table 19, Section 5.3.2) compared to the conservative terrestrial runoff values to surface water (>0.08 µg/L; Table 21 and Table 22).

These data are supported by a study conducted by Kolodziej et al. [15] that evaluated groundwater samples collected from tile-drains after application of lagoon waste from a dairy farm. 17β-Estradiol, estrone, and estriol were not detected above the limit of detection (LOD) in any of six samples analyzed. Thus, tiled-drains are not expected to be a major contributor of estradiol and trenbolone metabolites to the surface water. Therefore, tile-drained fields were excluded as an exposure route from the mixed-use watershed models.

3.4.2.3. Overspill of runoff storage lagoon/pond

As discussed under Section 3.2.1, defined and designated CAFOs require an NPDES permit and must comply with pollution prevention standards. For example, a CAFO must have a storage structure (e.g., lagoon or pond) that can contain runoff from (at a minimum) a 25-year, 24-hour rainfall event. When rainfall events exceed these volumes, overspill of the holding lagoon can occur. Such an event, although improbable, would result in an acute environmental exposure to contaminants contained in the lagoon/pond. As the focus of this EA is to evaluate potential chronic exposures and how they may affect fish reproduction, modeling of acute exposures resulting from rare and extreme rainfall events are not included in the farm-scale and mixed-use watershed models.

3.4.2.4. Leaching from unlined storage lagoons and ponds

Storage lagoons and ponds are constructed with the intention to retain contaminated wastewater runoff from the production area of an AFO. Based on current construction standards, it is expected that most storage lagoons/ponds would be constructed with an impervious liner or compacted clay layer [16], and as a result little or no leaching will occur. However, there are ponds that were built prior to publication of these standards that may not be constructed with impervious materials. In these cases, the SCI-GROW model results suggest that concentrations of surrogate estradiol and trenbolone compounds leaching into groundwater are expected to be negligible compared to the surface water concentrations (see SCI-GROW results in Section 5.3.2). SCI-GROW was developed to simulate leaching from soil surfaces using conservative assumptions of sandy soils and shallow groundwater [17]. These soil characteristics are not expected at most CAFO sites and for most storage lagoon/ponds; thus, the SCI-GROW results should overestimate the leachate. Because leaching is expected to be negligible, we have excluded this pathway from further analysis in the farm-scale and mixed-use watershed models.

3.4.2.5. Leaching under unpaved small and medium AFO feedlots

The potential leaching of estradiol and trenbolone metabolites under unpaved small or medium AFOs was evaluated using the EPA's PRZM model (Section 5.6.3 of Attachment 1). The PRZM model was modified to simulate feedlot surface conditions, including a high organic carbon content (38%) and a high runoff curve number of 95. When modeling feedlots with <1000 AU, the PRZM model did not predict any leaching under the surface of the feedlot (all concentrations were zero at 1 m). This lack of leaching from the unpaved feedlot surface is likely due to: 1) the impervious nature of the feedlot surface that becomes compacted due to repeated pounding by cattle hooves, and 2) the moderate to high potential to bind to the organic matter-rich surface of the feedlot. Therefore, subsurface transport under these feedlots by groundwater was excluded from evaluation in the farm-scale and mixed-use watershed models.

3.4.2.6. Subsurface leaching under pastures

Pasture cattle will excrete estradiol and trenbolone metabolites onto grasslands via feces and urine. For the same reasons described above for cropland and storage lagoons/ponds, little or no leaching of these metabolites is expected from soil on pasture lands. The EPA SCI-GROW model predicts these metabolites will not be mobile and will not leach through the soil (Section 5.3). Therefore, a leaching component in the pasture model was excluded from the mixed-use watershed models.

3.4.2.7. Direct excretion by pasture cattle into surface waters

While on pasture, cattle implanted with Synovex ONE-G may have direct access to surface waters. This access could result in direct excretion of estradiol and trenbolone metabolites into surface waters by pasture cattle. Although direct excretion is possible, it is not expected to occur on a widespread basis (in terms of time, space, and number of animals) and is not expected to result in chronic exposures in surface waters. As a result, this exposure pathway has not been included in the farm-scale and mixed-use watershed models.

3.5. Potential for Aggregate Exposure within a Watershed

Land use within a watershed is diverse; therefore, the combined exposure from multiple potential sources of Synovex products must be considered. For example, runoff from multiple feedlots may combine with runoff from pastureland and manured cropland, resulting in an aggregate exposure. Therefore, a mixed-use watershed model was developed using cattle population data from individual watersheds to estimate runoff and erosion of surrogate estradiol and trenbolone compounds from these combined sources. Based on the information discussed in Section 3.4, this model included four primary exposure pathways:

1. Surface runoff from pasture cattle manure
2. Surface runoff from feedlot manure applied to cropland
3. Feedlot runoff from AFOs <1000 AU directly discharging to surface water
4. Runoff from cropped fields irrigated with storage lagoon water

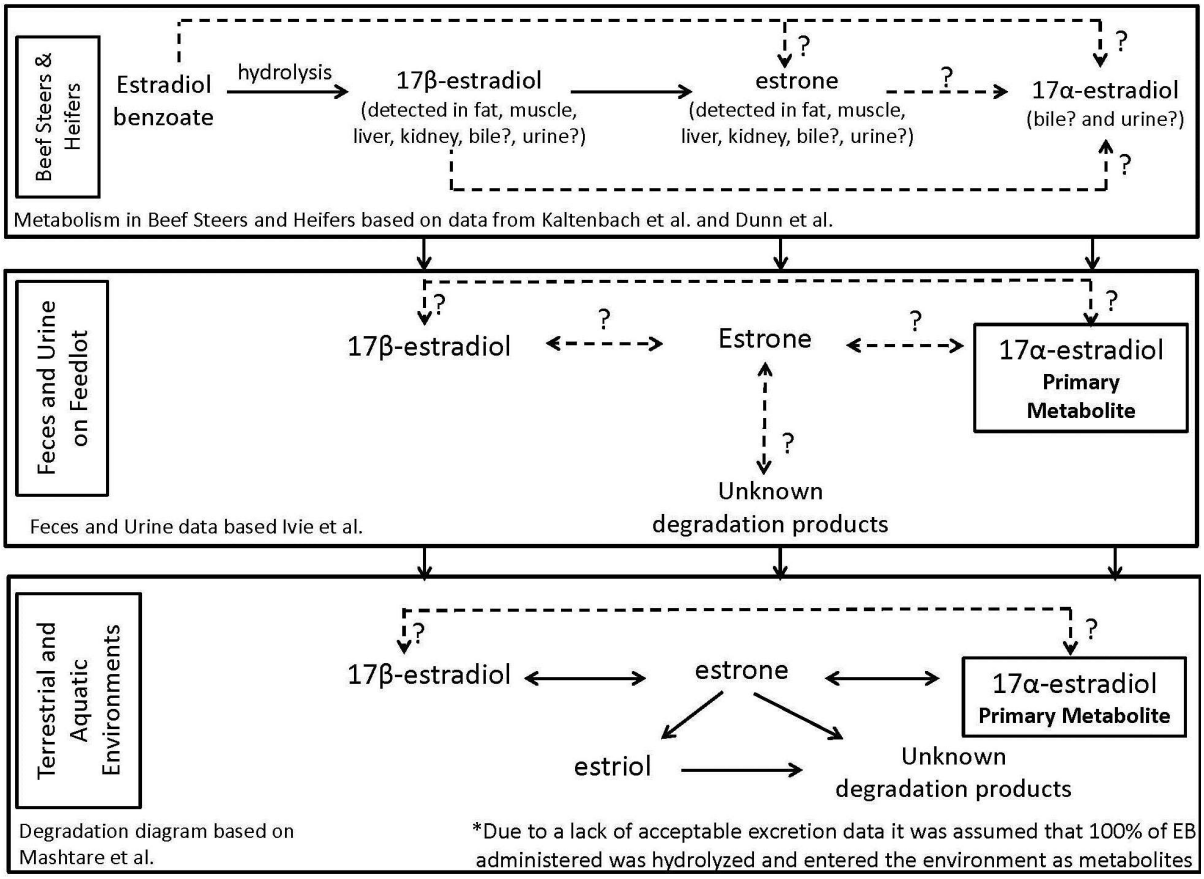
A schematic of this mixed-use model is shown in Figure 20 (Section 5.7.4). The model is discussed in Section 5.7, and the complete modeling report is found in Attachment 1.

4. METABOLISM AND ENVIRONMENTAL FATE

Both EB and TBA are transformed in cattle to produce metabolites that are excreted and further transformed and degraded in the environment. When solubilized from the implant in the target animal, EB and TBA are hydrolyzed to 17β-estradiol and 17β-trenbolone, respectively. The chemical structures of EB and TBA along with their principal metabolites are shown in Table 7. The 17β isomers of estradiol and trenbolone are the compounds collectively responsible for the growth performance enhancement effect observed in cattle. Estradiol and trenbolone metabolites are excreted from cattle in urine and feces (manure). The principal metabolites contained in manure on feedlots, however, are not the 17β isomers; rather, the 17α isomers are the principal metabolites found in manure stored on feedlots.

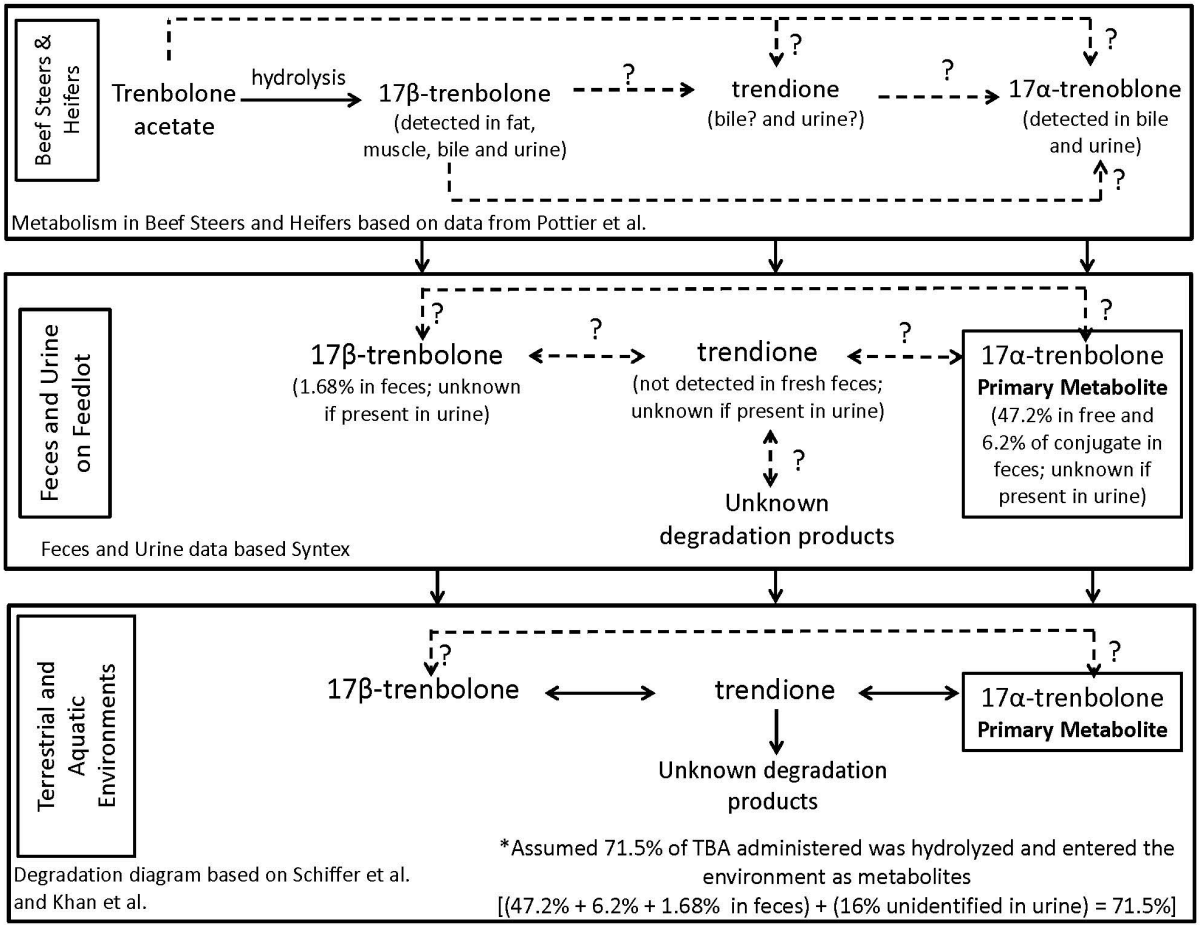
The metabolism of EB and metabolites is shown in Figure 6 and discussed in Sections 4.1.1 and 4.2.3. The metabolism of TBA and metabolites is shown in Figure 7 and discussed in Sections 4.1.2 and 4.2.5. 17β-Trenbolone, and likely, 17β-estradiol are biotransformed *in vivo* in cattle to their 17α isomers; 17α-trenbolone and 17α-estradiol, respectively (Figure 6 and Figure 7). Upon entering the environment in manure, the metabolites of estradiol and trenbolone could be present in manure piles, remain in an open feedlot or pasture, enter a manure storage lagoon, or be applied to agricultural soil. These metabolites can potentially bind to manure or soil and/or be transformed and degraded in these environments. Some metabolites of estradiol and trenbolone have the potential to enter the aquatic environment where additional binding, transformation and degradation can occur. The environmental degradation pathway of these metabolites is similar regardless of whether they enter the terrestrial or aquatic environment (Figure 6 and Figure 7). 17α-Estradiol is transformed principally to estrone, with a minor amount of the 17α-estradiol and/or estrone transformed to 17β-estradiol (a process known as interconversion). 17α-Trenbolone is transformed principally to trendione, with a minor amount of the 17α-trenbolone or trendione transformed to 17β-trenbolone. These metabolites then undergo further degradation to smaller, less potent compounds (ultimately to CO_2) and do not accumulate in the environment.

Figure 6. Transformation and Degradation of EB and Related Metabolites from Beef Steers and Heifers (solid line represents a known metabolic pathway; dotted line and question mark represents an unknown metabolic pathway)



Kaltenbach et al. [18], Dunn at al. [19], Ivie et al. [20], and Mashtare et al. [21].

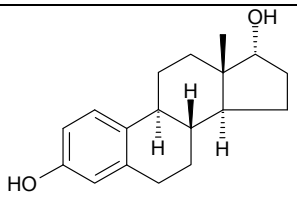
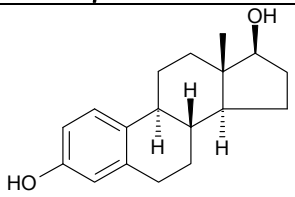
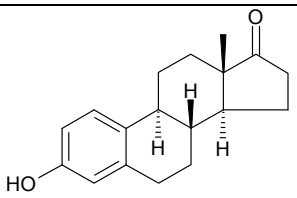
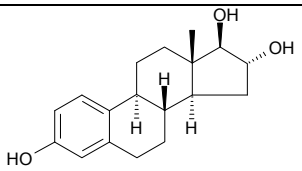
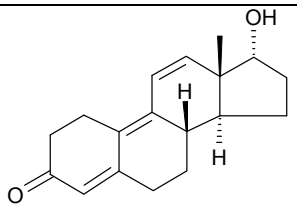
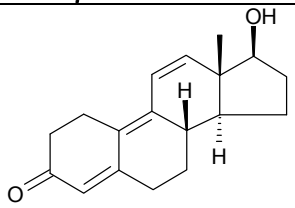
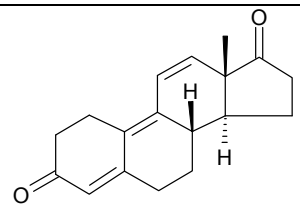
Figure 7. Transformation and Degradation of TBA and Related Metabolites from Beef Steers and Heifers (solid line represents a known metabolic pathway; dotted line and question mark represents an unknown metabolic pathway)



Pottier et al. [22, 23], Syntex [232, 233], Schiffer et al. [24], and Khan et al. [25].

The structure of the principal metabolites of EB and TBA are shown in Table 7.

Table 7. Principal Metabolites of Estradiol Benzoate and Trenbolone Acetate

Name	17α-Estradiol	17β-Estradiol	Estrone
Structure			
CAS Number	57-91-0	50-28-2	53-16-7
Molecular Weight	272.37	272.37	270.37
Name	**Estriol**		
Structure			
CAS Number	50-27-1		
Molecular Weight	288.38		
Name	**17α-Trenbolone**	**17β-Trenbolone**	**Trendione**
Structure			
CAS Number	80657-17-6	10161-33-8	4642-95-9
Molecular Weight	270.37	270.37	268.36

In Section 4.1, the available data on the metabolism of EB and TBA metabolites in cattle are discussed. In Section 4.2, important parameters involved with the transport and fate of these metabolites in the environment are discussed. These parameters are used in the environmental fate models that were used to estimate the PEC values of the metabolites in Section 5.

4.1. Metabolism in Cattle

A review of the suggested metabolic pathways of EB and TBA and related metabolites is provided in Rico [26] and is also discussed further below.

4.1.1. Estradiol benzoate metabolism in cattle

Three published literature studies were identified that discuss the metabolism of EB in cattle and distribution of EB metabolites (17β-estradiol, 17α-estradiol, and estrone) in the bile, urine, and feces. These publications are presented below because they provide information and data relative to the metabolism, transformation, and distribution of EB and its related metabolites. Several additional literature publications on the metabolism of 17β-estradiol

were also obtained and evaluated, but they were found to lack relevant information on the transformation pathways of EB metabolites or data on the distribution of EB metabolites in cattle, and therefore, they were not discussed.

Presentation of Studies

In a study conducted by Kaltenbach et al. [18], six male and six female cattle were injected daily for 11 days with 1 mg 17β-estradiol or EB. These non-radioactive estrogen injections were followed by three daily injections of ^{14}C-17β-estradiol or ^{14}C-17β-EB. Concentrations of the parent compound and metabolites were analyzed in fat, muscle, liver, and kidney. No EB was recovered in tissue. The liver and kidney contained the highest concentrations of radioactivity. In liver and kidney, 17β-estradiol and estrone were present but as conjugated metabolites, principally glucuronides accounting for 85 to 95% of the radioactivity. Steers tended to have higher concentrations of estrogen metabolites in muscle and fat and lower concentrations in liver than heifers. The differences were statistically significant in some instances but were relatively small [18].

Dunn et al. [19] conducted two experiments in cattle. Experiment 1 was similar to the one described above under Kaltenbach et al. In Experiment 2, two steers and two heifers were injected with 1 mg EB once daily for 12 days followed by two daily injections of ^{14}C-17β-EB. Concentrations of the parent compound and metabolites were measured in the same tissues as above and were also measured in the urine and feces on the final day. In muscle, 38-70% of the extracted radioactivity was 17β-estradiol and 17-45% was estrone. The highest concentration of radioactivity, which occurred in the urine, bile, and feces (Table 1 of reference [19]), was at least 100-fold higher in excreta and bile than any other tissue examined; however, the metabolites in these samples were not identified. In the liver and kidney, both free and conjugated metabolites were detected. In Experiment 1, the majority of metabolites were predominantly glucuronides. Following enzyme hydrolysis, these glucuronides yielded mostly 17α-estradiol and to a lesser extent estrone and estriol [19]. In Experiment 2, the free metabolites were predominant in the liver and the free and conjugated metabolites were equally distributed in the kidney. Regardless of whether metabolites were free or conjugated, 17α-estradiol was the predominant isomer, followed by 17β-estradiol, estrone, and estriol. The metabolites in liver and kidney were eliminated in urine and feces.

In a study conducted with three Holstein steer calves by Ivie et al. [20], feces and urine were collected at 24-hour intervals after injection of ^{14}C-17β-estradiol. Of the total dose, 42.1% was eliminated in urine and 57.7% in feces, which accounted for 99.8% of the total radioactivity. 17α-Estradiol, primarily as its glucuronide form, comprised by far the majority of the radiocarbon found in urine. However, both estrone and its glucuronide form were also present. Urinary estrogen metabolites from Holstein steer calves showed a metabolite profile similar to that of sexually mature dairy heifers [18], with 17α-estradiol (90%) as the primary metabolite and estrone (7%) as a secondary metabolite [20]. Neither 17β-estradiol nor its glucuronide form was detected in urine. In feces, 17α-estradiol (64%) was the predominant metabolite, but 17β-estradiol (11%) and estrone (13%) were also present [20]. Because of the route of administration and limited number of animals, the relevance and adequacy of these data is questionable.

Summary of EB Metabolism in Cattle

Based on the information presented above and the field monitoring data discussed in Appendix 12, 17α-estradiol is expected to be the principal metabolite found in manure from cattle treated with EB. Estrone, and to a lesser extent 17β-estradiol, are also expected to be found in cattle manure. The data demonstrate that EB is hydrolyzed to 17β-estradiol in the target animal. 17β-Estradiol is then likely further metabolized to 17α-estradiol; however, because most studies to date were conducted with a limited number of animals and samples, this cannot be definitively concluded at this time. Field monitoring data (discussed in Appendix 12) clearly support that 17α-estradiol and estrone, with a minimal quantity of 17β-estradiol, are the primary metabolites found in cattle waste on a feedlot (whether excreted in this form or whether transformed after excretion). The structures of these metabolites are shown in Table 7. Their possible transformation pathways are shown in Figure 6.

4.1.2. Trenbolone acetate metabolism in cattle

Three published literature studies were identified that discuss the metabolism of TBA in cattle and distribution of TBA metabolites (17β-trenbolone, 17α-trenbolone, and trendione) in the tissues (muscle, liver and kidney) and bile. These publications are presented below because they provide information and data relative to the metabolism, transformation, and distribution of TBA and its related metabolites. In addition, a study was conducted by Syntex (owned by Zoetis) that evaluated the distribution of TBA metabolites in cattle feces and urine. Additional literature publications on the metabolism of TBA and related metabolites were also evaluated, but they were excluded from analysis herein because they lacked distribution data and other useful information.

Presentation of Studies

In 1975, Pottier et al. [22] conducted a residue study in two cows using ^{3}H-TBA to determine plasma, milk, and tissue residue levels. Based on radioactivity, the principal route of excretion was bile (81%), with a minor quantity (<10%) detected in urine. Feces were not analyzed. 17β-Hydroxy-estra-4,9,11-trien-3-one and estra-4,9,11- trien-3,17- dione were tentatively identified in tissue residues. Pottier et al. [23] published a more extensive review of TBA metabolism in 1981. They identified that, in the heifer, 17α-epimerization[I] is the major metabolic pathway. The main metabolite found was 17α-hydroxyestra-4,9,11-triene-3-one. Of the metabolites identified in bovine bile, 90% were 17α-hydroxylated compounds. Pottier et al. [23] stated that the major metabolism pathways in bovine for trenbolone are similar to that of testosterone and 17β-estradiol, which are primarily excreted as their 17α-epimers. Eighty percent of the dose administered to a heifer was recovered in the bile. Of that 80%, 63.7% was extractable as free metabolites (3.5%), glucuronides (30.0%), and sulfates (30.2%). Unchanged TBA was not detected.

After hydrolysis, 44.7% of the metabolites in the bile were found to retain the 3-oxotriene structure with 40.4% of the radioactivity identified as 17α-hydroxy isomers, 1.6% as 17β-hydroxy- and 2.7% as 17-oxo isomers. The remaining compounds were unidentified metabolites (1.7%) retaining the 3-oxotriene structure and an uncharacterized amount of 18.6% [23]. The sum of the percent excreted radioactivity for all 17-substitution compounds was 46.4%, which represents the total of potentially active isomers [23].

[I] Epimerases catalyze the interconversion of epimers. 17α-TB and 17β-TB are examples of epimers. See Explanation of Terms for a complete definition of epimerization.

These data indicate that the TBA parent molecule is not excreted from the bovine and the principal metabolite is 17α-trenbolone excreted through the bile into feces. Pottier et al. [23] only characterized the bile metabolites of trenbolone and reported total urinary radioactivity (not metabolites). In the residue studies conducted by Syntex (now owned by Zoetis) [232, 233] summarized below, the TBA metabolites in tissue and feces were characterized; however, urine was also not profiled.

Evrard et al. [27] evaluated the fate and residues of TBA and related metabolites (17β-trenbolone, 17α-trenbolone, and trendione) in the plasma, liver, kidney, muscle and bile of six calves implanted with 140 mg [^{3}H]TBA (the study also included one control calf that was not implanted with TBA). The plasma was collected intermittently over 50 days after implantation. Residues in the tissues and bile were only determined for samples collected at day 50 after implantation. The tissues were extracted using solvent (ether) followed by water. The radioactivity associated with non-extractable portion was also determined. The plasma was only analyzed for radioactivity associated with TBA; the distribution of TBA metabolites in the plasma was not determined. The concentration of radiolabeled TBA in the plasma remained constant over the 50 days following implantation, which was expected because the implant was designed to release radiolabeled TBA constantly. Only 17β-trenbolone, 17α-trenbolone, and trendione were detected during the analysis of tissue and bile samples. All results for tissues and bile were reported as TBA equivalents (e.g., ng of TBA equivalent/g of tissue = specific activity of [^{3}H]TBA/the amount of TBA applied). The distributions of TBA and related metabolites (17β-trenbolone, 17α-trenbolone and trendione) were reported, except for the fraction extracted with ether from liver and kidney samples. The largest proportion of TBA residues were found to be non-extractable (>50%), and thus, considered to be covalently bound residues. Approximately 7-14% and 6-11% of TBA metabolites were found to be soluble in ether and water, respectively. The highest concentration of TBA equivalents was reported in the bile. The liver and kidney also contained a high concentration of TBA equivalents; whereas, the muscle and fat contained minor concentrations. Only 17β-trenbolone was detected in the muscle; whereas, 17α-trenbolone and 17β-trenbolone were the predominant forms in the liver and kidney.

Syntex conducted a metabolism study in which 24 calves were implanted with pellets containing ^{14}C-TBA (300 mg) and EB (42 mg). The results are summarized in two study reports evaluating the concentrations in edible tissue and feces/urine [232] and another characterizing the metabolites [233]. Study summaries are included in Appendix 13.2 and Appendix 13.3, respectively. The total radioactivity was determined in urine and feces, but the composition of the radioactivity was determined for the feces only. The mean excretion rate of total radioactivity in urine was 16.42% while the primary route of excretion was in feces (83.58%). In the metabolite characterization portion of the study, approximately 50% of the radioactive residue was extractable from feces [233]. The high performance liquid chromatography (HPLC) profiles in feces were consistent from animal-to-animal and from day-to-day. 17α-Trenbolone was the prominent metabolite which accounted for, on average, 47.2% of the total radioactivity recovered and measured using HPLC analysis. 17β-Trenbolone was present in small quantities (mean of 1.68%). TBA was not detected. A metabolite comprising 6.2% of the radioactivity with a retention time of 16 minutes may correspond to 17α-trenbolone glucuronide, a major metabolite in liver and bile. The remaining radioactivity consisted of four (each ≤10%) unidentified metabolites (32.6%) and background radioactivity that could not be resolved into discernible peaks (12.4%).

From the study conducted by Syntex [233], approximately 50% of the radioactivity in the feces was not extractable and, therefore, not identified. It is likely that the non-extractable residues are not bioavailable or readily mobile. Regardless, a conservative approach was taken and the percentage of each metabolite identified in the HPLC profile of the feces extract was applied to the total radioactivity found in the feces. Following this approach, the three primary metabolites (47.2% 17α-trenbolone + 1.68% 17β-trenbolone + 6.2% 17α-trenbolone glucuronide) represent 55.1% of the administered dose. The metabolites in the urine were not profiled; therefore, it was conservatively assumed that the entire radioactivity measured in urine (16.4%) is the major metabolite. By combining urinary and fecal excretion results, we assume throughout the EA that 71.5% of the excreted dose is 17α-trenbolone. Therefore, 71.5% will be used as an estimate of active metabolites in the target animal for the excretion rate of trenbolone metabolites. Because the non-extractable portion from feces (approximately 50%) was included in the 71.5% estimate as well as the 16.4% of uncharacterized urine metabolites, this estimate is conservative.

In a study conducted by Sellin et al. [28], it was suggested that metabolites of trenbolone excreted in cattle urine may not be active. In the study, fathead minnows were exposed to the urine and feces of nine steers implanted with 100 mg TBA and 14 mg EB (Synovex Choice) and four untreated control steers. In the liquid chromatography/mass spectrometry (LC-MS/MS) analysis of the urine from treated cattle, 17α- and 17β-trenbolone were not detected. Also, there was no increase in ED activity of TBA-treated cattle urine above that of control cattle urine; however, fecal slurries from TBA-treated cattle had increased ED activity over fecal slurries from control cattle. These data suggest that the metabolites of trenbolone in urine are not as active as those in the feces, which support that summing all metabolites measured in the urine and feces is a conservative approach.

Summary of TBA Metabolism in Cattle

Based on the information presented above (Section 4.1.2), 17α-trenbolone is expected to be the principal metabolite excreted in manure from cattle treated with TBA. Additional minor metabolites found in manure include trendione and a minimal quantity of 17β-trenbolone. These three metabolites make up 71.5% of the excreted dose. The data demonstrate that TBA is transformed to 17β-trenbolone in the target animal. 17β-Trenbolone is then further metabolized to 17α-trenbolone. Field monitoring data (discussed in Appendix 12; e.g., Durhan et al. [29]) also supports that 17α-trenbolone and trendione, and to a lesser extent 17β-trenbolone, are the primary metabolites found in cattle waste on a feedlot. The structures of these metabolites are shown in Table 7 and their transformation pathways are shown in Figure 7.

4.1.3. Conclusions of EB and TBA metabolism in cattle

Following administration of Synovex ONE, EB and TBA are transformed to metabolites resulting in no excretion of the parent compounds. The principal estradiol-related compounds in manure and those potentially entering the environment from use of Synovex ONE implants in cattle are, in decreasing order of occurrence, 17α-estradiol, estrone, 17β-estradiol and conjugated estrogens (Section 4.1.1; Figure 6). The principal trenbolone-related compounds potentially entering the environment through manure from Synovex ONE treated cattle are 17α-trenbolone and small amounts of 17β-trenbolone (Section 4.1.2; Figure 7). The metabolites of EB and TBA are excreted in both urine and feces and may be present in either a free or conjugated form (e.g., as a glucuronide or sulfate). However, if the metabolites enter the soil in a conjugated form, it is expected that they would be rapidly hydrolyzed back to a free (unconjugated) form. For example, Scherr et al. [30] demonstrated the rapid hydrolysis of 17β-estradiol-3-sulphate in New Zealand pasture soils.

This EA assumes that 100% of the estradiol dose and 71.5% of the trenbolone dose are present as 17α isomers in the manure that is stored on the feedlot and applied later to cropland. Field monitoring data of manure piles, runoff lagoons, and feedlot surfaces discussed in Appendix 12 support that 17α-estradiol and 17α-trenbolone are the principal metabolites excreted into the environment, with conversion of these α metabolites to estrone and trendione, respectively. The transformation process is illustrated in Figure 6 and Figure 7, respectively.

4.2. Environmental Fate Data

The fate of estrogens and androgens in the environment has been extensively reviewed by Young and Borch [31], Kolok and Sellin [32], and Khanal et al. [33]. In order to evaluate the potential fate, transport, and exposure of EB and TBA metabolites in the terrestrial and aquatic environments, extensive environmental fate modeling was conducted as discussed in detail in Section 5 of this EA. Physical-chemical property data and environmental fate data are important input parameters for the environmental fate models in order to estimate the potential partitioning, persistence, and mobility of the compounds in the different environmental compartments. Sections 4.2.1 through 4.2.2 describe the assumptions, approaches, and methods used to select the physical-chemical and environmental fate data used in the modeling of a surrogate estradiol compound[m] and a surrogate trenbolone compound[n]. Summaries of the original key publications containing physical-chemical properties and environmental fate data, and explanations regarding the derivation of these final values are presented in Sections 4.2.4 and 4.2.6. In addition, literature publications discussing environmental parameters and fate data that were not essential for the exposure modeling presented herein are also contained in these sections as supporting information. The conservative parameters used to model the environmental fate of the surrogate estradiol compound and surrogate trenbolone compound are shown in Table 9 and Table 13, respectively.

[m] For the purpose of this EA, this term describes one estradiol-like compound with the physical-chemical and environmental fate properties that conservatively represents a composite of the primary metabolites of EB, i.e., 17β-estradiol, 17α-estradiol, and estrone.

[n] For the purpose of this EA, this term describes one trenbolone-like compound with the physical-chemical and environmental fate properties that conservatively represents a composite of the primary metabolites of TBA, i.e., 17β-trenbolone, 17α-trenbolone, and trendione.

4.2.1. Approach for use of surrogate compounds in the exposure assessments

Although the principal metabolites from Synovex ONE excreted from cattle are 17α-estradiol and 17α-trenbolone, they are expected to be rapidly transformed in the manure, soil, and aquatic environments to estrone and trendione, respectively, and to minor amounts of 17β isomers (Figure 6 and Figure 7). Preparing a collective environmental exposure and risk assessment on all six of these compounds would be extremely complex and scientifically challenging because much of the needed data are currently lacking and/or are difficult to obtain. For example, there is inadequate information available on 1) the proportion of each metabolite excreted in feces and urine, which is need to estimate the application rate of each metabolite in the model, 2) fish reproductive toxicity data for trendione and estrone, and 3) soil adsorption and degradation data for some metabolites. Therefore, a novel approach has been taken herein. Because the structures and many of the physical-chemical properties of 17α-estradiol, 17β-estradiol, and estrone are quite similar (Table 7 and Section 4.2.4) it has been assumed, for the purposes of this assessment, that they will be transported, transformed, and degraded similarly in the environment. The same assumption was used for 17β-trenbolone, 17α-trenbolone, and trendione (Table 7 and Section 4.2.6). Thus, to account for all potential EB and TBA metabolites in the environment and to simplify the environmental fate modeling and exposure assessment, a single surrogate estradiol compound and a single surrogate trenbolone compound have been defined, evaluated, and modeled in the exposure assessment to characterize risk. This was done rather than modeling each of the six EB and TBA metabolites in three environmental compartments for the 61 conservative scenarios (34 tilled crops, 17 no-till crops, 5 pasture, and 5 watersheds) modeled in Section 5. See the conceptual model in Figure 8 and Figure 9 for additional information on the surrogate compounds.

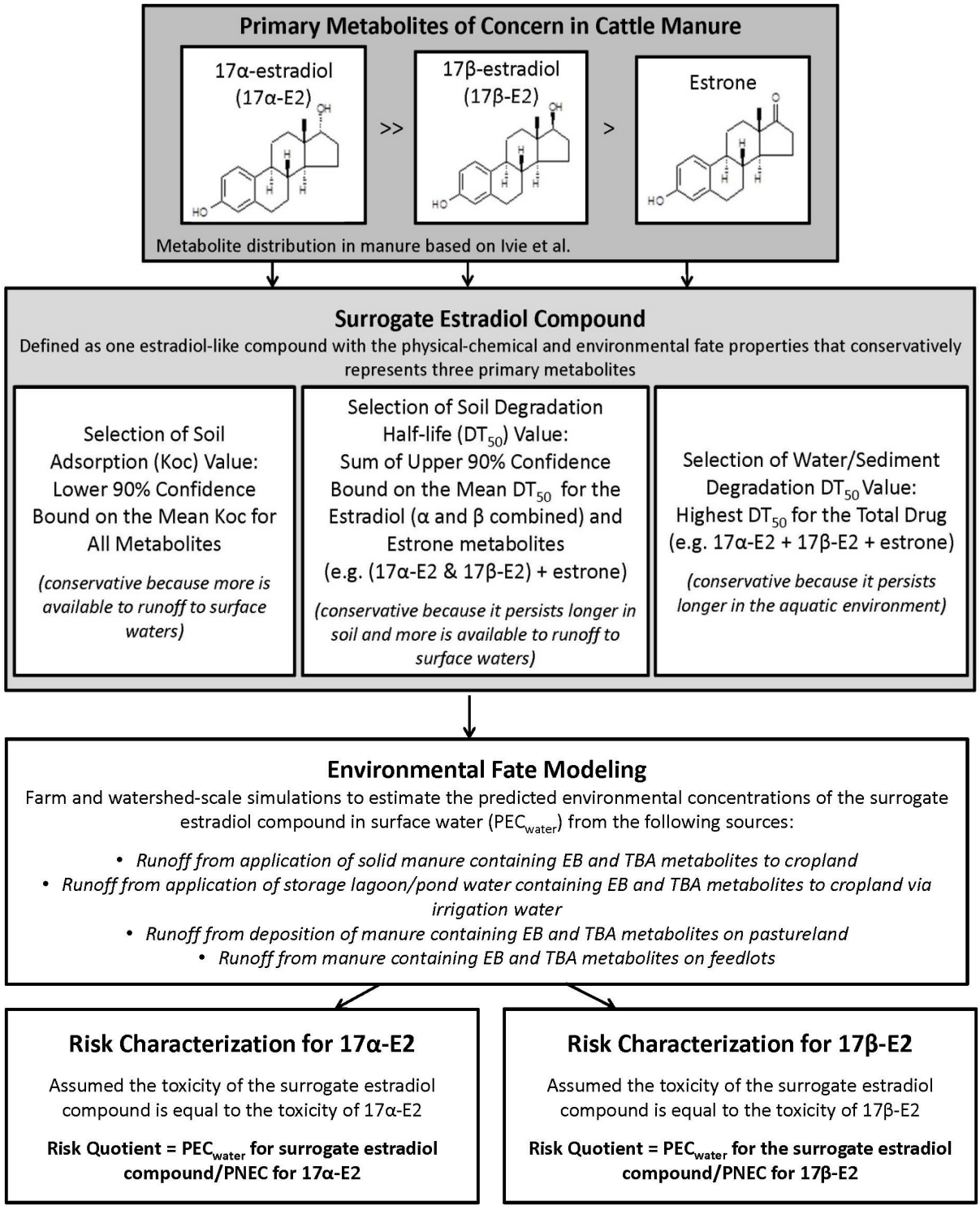
Parameter selection for the surrogate compounds was performed using a conservative approach and assumptions that ensure that PEC values for soil and water would not be underestimated and, in fact, would be overestimated in some cases. Additional details regarding the derivation of conservative physical-chemical and environmental fate values for all active metabolites of EB and TBA are described in Section 4.2.2, and in the conceptual models shown in Figure 8 and Figure 9 below.

To construct a surrogate compound, the following general assumptions and methods were used. Estradiol is used as the example, but the same principles apply to trenbolone.

- A single metabolite was assumed to be transported and metabolized in the environmental matrices mentioned above. As an example, in a feedlot manure hard pack, 17α-estradiol will be present along with its metabolite estrone and a small fraction of 17β-estradiol (Appendix 12). Rather than attempt to attribute portions of the total drug to each metabolite in each of the environmental compartments and then to model the fate of each metabolite individually, the composite data for all of the metabolites were utilized (and in certain cases added together such as for soil half-lives; Figure 8) and one resulting surrogate estradiol compound was modeled. Thus, the environmental concentrations (i.e., PEC values) estimated for the surrogate estradiol compound are considered to be conservative representations of the total residue for all three individual metabolites in the relevant compartment modeled (e.g., leachate, soil, and water/sediment). See Figure 8 and Figure 9 below for additional information.

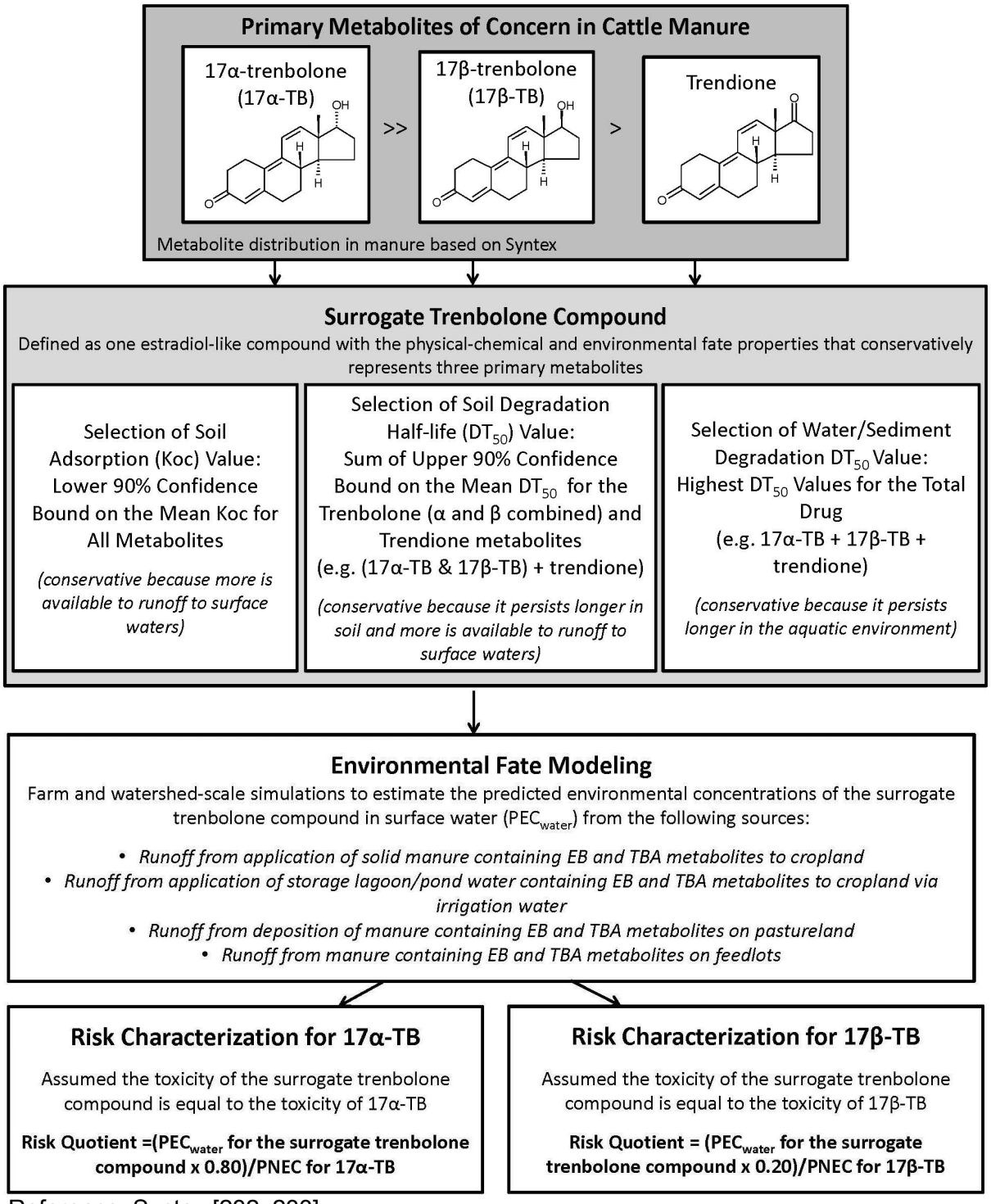
- The physical-chemical properties and environmental fate parameter data for the individual surrogate estradiol compound were used as inputs into the environmental fate modeling programs. Data for all three metabolites (17α-estradiol, 17β-estradiol and estrone) were considered when available. A single parameter value (e.g., soil degradation half-life value) was selected or derived in a manner such that it would be either representative of the entire data set available or conservative with respect to each of the individual metabolites. A conservative value would be one that results in a higher PEC in water (Figure 8 and Figure 9).
- The specific details regarding the derivation or selection of the physical-chemical properties and environmental fate parameters for the surrogate estradiol and trenbolone compounds are presented in Sections 4.2.4 and 4.2.6, respectively. The final input values used in the environmental fate modeling are summarized in Table 9 and Table 13.

Figure 8. Conceptual Model for Use of the Surrogate Estradiol Compound in Exposure Assessment and Risk Characterization



Reference: Ivie et al. [20]

Figure 9. Conceptual Model for Use of the Surrogate Trenbolone Compound in Exposure Assessment and Risk Characterization



Reference: Syntex [232, 233]

4.2.2. Methods used to derive modeling parameters for the surrogate compounds

A large portion of the physical-chemical and environmental fate modeling parameters are available in the scientific literature (see Section 1.3 for criteria regarding selection of adequate literature studies). To minimize bias in assigning conservative values to the literature data when three or more values for a modeling parameter were available, a 90th percentile confidence bound of the mean was determined and either the upper or lower value of the interval was used depending on which would result in a higher PEC for surface water (Figure 8 and Figure 9). This approach results in conservative environmental fate parameters being employed in the modeling of the surrogate compounds. The methodology for determining the conservative 90th percentile confidence bound followed an EPA recommended approach.

On page 23 of reference [34], the EPA recommends using a one-sided 90th percentile confidence bound with the width of the confidence interval (CI) dependent on the number of observations. Table 8 provides the equation to perform this calculation.

Table 8. EPA Recommendation for Calculating the 90th Percentile Confidence Bound on the Mean for Conservative Modeling Parameter Assignments

One-sided student t_{90} confidence bound = Mean $\pm$ $t_{90,n-1}$ X SD / $\sqrt{n}$										
n-1	2	3	4	5	6	7	8	9	10	∞
t_{90}	1.886	1.638	1.533	1.467	1.440	1.415	1.397	1.383	1.356	1.282

Depending on the parameter, either the upper or lower 90th percentile confidence bound on the mean was used to derive a conservative concentration in surface waters (Figure 8 and Figure 9). For example, to ensure the highest concentration of the surrogate compound is estimated to migrate into the surface waters, the lower 90th percentile confidence bound on the mean soil K_{OC} was used, ensuring a greater amount of drug would partition to water and less would be bound to organic matter. In contrast, the upper 90th percentile confidence bound on the mean soil degradation half-life (DT_{50}) value was used ensuring that a greater amount of drug would be present for a longer period of time in soil. This method allowed for the estimation of conservative environmental fate input parameters for the surrogate compounds, which would result in a conservative PEC_{water} value (Figure 8 and Figure 9). Further, when there were only two data values available, the more conservative (i.e., higher or lower) of the two values was used. If only one value was available (which was only the case in one instance), the value was multiplied by a factor of 3, as recommended in EPA's guidance.

4.2.3. Summary of environmental fate modeling parameters for the surrogate estradiol compound

Table 9 below lists the physical-chemical properties and fate values used in the environmental fate modeling to estimate the PEC values for the surrogate estradiol compound. The surrogate estradiol compound (composite of 17β-estradiol, 17α-estradiol, and estrone) was found to have a moderate binding potential and rapid degradation rate in soil. In addition, the surrogate estradiol compound is expected to degrade in water-sediment environments. Thus, the surrogate estradiol compound is expected to be neither highly mobile in the terrestrial environment nor persistent in either terrestrial or aquatic environments. The published literature and Zoetis-owned study data, and methods used to derive the physical-chemical properties and fate values listed in Table 9, are summarized in detail in Section 4.2.4 below.

Table 9. Physical-Chemical and Environmental Fate Parameters used in the Exposure Assessment and Environmental Fate Modeling of the Surrogate Estradiol Compound

Parameter	Value Selected for Modeling	Comments/ Reference
Excretion rate from cattle	100%	Not enough available data to determine excretion rate of EB metabolites in manure; thus, 100% excretion was assumed
Molecular weight adjustment for difference between EB and estradiol	72.35%	MW of estradiol/MW EB = 272.38/376.49
Soil K_{OC}	1259	Section 4.2.4.9
Sediment K_{OC}	1259	Soil K_{OC} value used
Molecular weight	272.4	Section 4.2.4.1
Aqueous solubility (mg/L)	3.9	Section 4.2.4.4
Vapor Pressure (Pa)	1E-8	Section 4.2.4.3
Hydrolysis	Not Used	Assumed no hydrolysis occurs in the environment
Soil DT_{50} (days)	3.1	Estradiol 1.0 day + estrone 2.1 days = 3.1 days Section 4.2.4.11 and Table 11
Aerobic water-sediment DT_{50} (days)	31.1	Sum of (17α-estradiol + 17β-estradiol + estrone) in total water-sediment system, Section 4.2.4.12
Anaerobic water-sediment DT_{50} (days)	107.8	Sum of (17α-estradiol + 17β-estradiol + estrone) in total water-sediment system Section 4.2.4.12
Aquatic direct photolysis DT_{50}	Not Used	Assumed no photodegradation in the environment; however, 0.5 day is reported in Section 4.2.4.7
Feedlot and manure storage degradation rate DT_{50}	Not Used	Assumed no degradation in manure

Additional environmental fate parameters for estradiol and metabolites that were not required for the environmental fate models can be found in Section 4.2.4 and Appendix 12.

4.2.4. Presentation of studies used to derive the environmental fate parameters for the surrogate estradiol compound

This section summarizes the published literature and Zoetis-owned study results for the physical-chemical properties and environmental fate values used in modeling the fate and exposure of the surrogate estradiol compound in the terrestrial and aquatic environments. In addition, the assumptions and methods used to derive the final endpoint values for each parameter are also described. The final input parameters used in the modeling are summarized in Table 9 (Section 4.2.3).

4.2.4.1. Molecular weight (MW)

The MWs of 17α-estradiol, 17β-estradiol, and estrone are 272.37, 272.37, and 270.37, respectively [35, 36]. **For modeling the surrogate estradiol compound, a MW of 272.4 was used.**

4.2.4.2. Melting point

The Merck Index reports melting point values of 220-223, 173-179, and 254-256°C for 17α-estradiol, 17β-estradiol, and estrone, respectively [37]. The melting points for both 17α- and 17β-estradiol listed in the SciFinder® data base for experimental measurements are approximately 173-178°C [36]. The EPIweb software package [35] estimated the melting points for 17α-estradiol, 17β-estradiol, and estrone to be 152.4, 152.4, and 153.1°C, respectively. Although these values differ, a melting point for the surrogate estradiol compound was not determined because it is not an input parameter used in the environmental fate modeling.

4.2.4.3. Vapor pressure

Khanal et al. [33] determined the vapor pressure of estrone to be 3E-5 Pa. Using EPIweb [35], the vapor pressure was estimated to be 2.65E-7, 2.65E-7, and 6.79E-1 Pa for 17α-estradiol, 17β-estradiol, and estrone, respectively. Although the vapor pressure predicted for estrone using EPIweb differs considerably from the measured value from Khanal et al. [33], the vapor pressures are so low that the surrogate estradiol compound is not expected to be volatile; thus, the vapor pressure is not expected to have a large influence on the environmental fate of the surrogate estradiol compound in the modeling.

For modeling purposes, a vapor pressure of 1E-8 Pa was chosen for the surrogate estradiol compound. This value is considered to be conservative because it will result in less partitioning of the surrogate estradiol compound into the air, resulting in higher concentrations in soil and water.

4.2.4.4. Aqueous solubility

Hurwitz and Liu [38] determined the aqueous solubility of 17α-estradiol and estrone to be 3.9 and 0.8 mg/L, respectively. Shareef et al. [39] determined the solubility of 17β-estradiol and estrone to be 1.51 and 1.3 mg/L, respectively. The solubilities were the same between a pH of 4 to 7, but increased at a pH of 10, which is close to the pKa of these molecules. Yu et al. [40] determined the solubility of 17β-estradiol and estrone to be 3.1 and 2.1 mg/L, respectively. Jurgens et al. [41, page 21] reported the aqueous solubility of 17β-estradiol and estrone to be 13 and 12.4 mg/L, respectively. These values are much higher than those determined in the other three studies.

For modeling purposes, a solubility of 3.9 mg/L was chosen for the surrogate estradiol compound (Table 9). This concentration is well above the estradiol concentration that is expected to occur in runoff and waterways (Appendix 3 through Appendix 5); thus, this value is considered conservative because the model will not be limited by the amount of estradiol that can be solubilized in surface runoff.

4.2.4.5. Dissociation constant (pKa)

Hurwitz and Liu [38] determined the pKa of 17α-estradiol and estrone to be 10.46 and 10.38, respectively. These determinations are in agreement with those of Lewis and Archer [42] for 17β-estradiol and estrone of 10.71 and 10.77, respectively. In addition, Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2010 ACD/Labs) from SciFinder® [36] predicted a pKa of 10.27, 10.27, and 10.25 for 17α-estradiol, 17β-estradiol, and estrone, respectively. However, a dissociation constant was not derived for the surrogate estradiol compound because the dissociation constant is not an input parameter used in the environmental fate modeling.

4.2.4.6. Ultraviolet (UV)- absorbance spectra

The UV-Visible (VIS)-spectra of estradiol and estrone are published in numerous studies examining photodegradation. The UV spectrum of 17α-estradiol is shown in Figure 1 of Hurwitz and Liu [38]. The UV spectra of estrone and estradiol are also shown in Liu and Liu [43] and Chowdhury et al. [44]. However, a UV-VIS spectrum was not determined for the surrogate estradiol compound because UV-VIS spectra are not used in the environmental fate modeling.

4.2.4.7. Photodegradation

The photodegradation of 17β-estradiol and estrone was investigated in several studies, which are summarized below.

Presentation of Studies

The photodegradation of estrone was investigated in aqueous solution using natural sunlight (wavelengths of 290-700 nm) produced using a solar simulator [44]. The degradation of estrone followed pseudo-first-order kinetics, with the rate constant decreasing slightly with increasing initial concentration and varying linearly with solar intensity in the region of 25-100 mW cm^{-2}. The DT_{50} of estrone using 1 Sun (100 mW cm^{-2}) was 52.50 minutes under natural conditions and was 122.75 minutes using ¼ Sun (25 mW cm^{-2}). The major intermediate detected in estrone photodegradation was benzeneacetic acid/phenylacetic acid, which also photodegraded. The photodegradation of estrone was also determined in the presence of humic acid (dissolved organic carbon or DOC). As the concentration of humic acid increased (0-10 mg/L), the photodegradation process increased. Therefore, the presence of DOC enhanced degradation.

Caupos et al. [45] studied the photodegradation of estrone under a light with an irradiance approximating that of the sun and with and without DOC. DOC (fulvic acid) extracts isolated from Suwannee River (Georgia), South Platte River (Colorado), and from a pond in a forest near Poitiers, France were used in the study. Pyrex glass vials containing estrone with and without DOC were irradiated with light for up to 8 hours. No changes in estrone concentration were observed in aluminum foil-covered vials, indicating that degradation was due only to exposure to light. The DT_{50} of estrone in purified water (without DOC) was 7.9 hours, whereas the DT_{50} values in water containing 8.4-8.9 mg DOC/L ranged from 3.9-6.8 hours. Similar to the observations of Chowdhury et al. [46], the presence of DOC enhanced photodegradation of estrone.

Chowdhury et al. [46] determined the pseudo-first-order rate constant (k) of 17β-estradiol under 1 Sun irradiation unit to be $0.0652 \pm 0.0033\ h^{-1}$, which is equivalent to a DT_{50} of 10.6 hours. As the light intensity decreased to ¼ Sun, the DT_{50} increased to 21.07 hours. As turbidity in the study was increased, the degradation rate decreased, which indicates that turbidity of some water systems could decrease the degradation rate. Mazellier et al. [47] reported direct phototransformation occurs with 17β-estradiol (without DOC), but with an irradiation device that was not fully representative of the emission of the sun.

Summary of Estradiol Photodegradation

Collectively, there were three studies conducted under approximately 1 Sun unit that reported photodegradation DT_{50} values ranging from 1 to 11 hours for estrone and 17β-estradiol [44, 45, 46]. These data indicate that 17β-estradiol and estrone have the potential to undergo photodegradation from sunlight in clear water systems. Applying an upper 90% confidence bound to all of these data yields a photodegradation DT_{50} estimate of 12 hours (0.5 day). However, it is important to note that these studies were generally conducted in clear water systems and using solar simulators. Fonseca et al. [48] found that the photodegradation of 17β-estradiol and estrone under direct solar radiation (average irradiance of 5.2 kWh/m^2) was much slower at 60 and 55 days, respectively. **Although photodegradation was shown for 17β-estradiol and estrone and the environmental fate models adjust the photodegradation rate for sunlight depth penetration and turbidity in the water column for different regions, a photodegradation value was not used in the modeling of the surrogate estradiol compound in order to be conservative. It was conservatively assumed that no photodegradation occurs.**

4.2.4.8. Octanol-Water partition coefficient (K_{OW})

The K_{OW} can be used to model fate of molecules in the absence of a distribution coefficient (log D) and may also be used to understand the potential for bioaccumulation in fish. Because the metabolites of estradiol occur naturally and have been shown to be metabolized rapidly by mammals and fish, bioaccumulation is not expected to occur for estradiol metabolites. Jurgens et al. [41] reported a log K_{OW} of 3.1 for 17β-estradiol, which is similar to the results reported by Qiao et al. [49] (log K_{OW} = 3.73, 3.76, and 3.53 for 17α-estradiol, 17β-estradiol, and estrone, respectively). In the PRZM and EXAMS models, the log K_{OW} can be used as an estimate of adsorption, in the absence of soil adsorption values (K_{OC}). However, experimental soil adsorption data are available for estradiol metabolites, and the K_{OC} is the preferred parameter to use in the modeling. Therefore, a log K_{OW} value for the surrogate estradiol compound was not used in the environmental fate modeling.

4.2.4.9. Adsorption to soil (soil K_{OC})

Eight published literature studies were selected that evaluated the adsorption of 17β-estradiol, 17α-estradiol, and estrone to soil. The methods used in these studies were similar to OECD Guideline 106 [50], and the results were consistent among all studies. Thus, the data obtained in these studies were used in Table 10 to derive a soil adsorption coefficient (K_{OC}) value for the surrogate estradiol compound. Summaries of these studies are provided below.

Presentation of Studies

Mashtare et al. [51] determined that 17α-estradiol and 17β-estradiol exhibited the same sorption affinities. Seven soils were chosen to represent a range of pH, organic carbon (OC) content, soil texture, cation exchange capacity, and dominant clay types. Sorption isotherms for 17α-estradiol and 17β-estradiol were measured from aqueous 0.005 M $CaCl_2$ solution using four to five solution concentrations ranging from 0.004-0.22 mg/L in duplicate or triplicate. The ratios of soil mass (g) to solution volume (mL) ranged from 1:10 to 1:70 for the different soil types. All soils were wet-autoclaved to minimize biological transformation. Single estrogen solutions containing 17α-estradiol and 17β-estradiol were added to tubes containing soils and capped. Samples were equilibrated for 24 hours at room temperature (22 ± 2°C). Both aqueous and soil phases were centrifuged, extracted, and analyzed for estrone, 17α-estradiol, and 17β-estradiol by HPLC. Little to no degradation occurred for 17α-estradiol and 17β-estradiol. The total mass recoveries from solution and soil phases were 101 ± 8% for 17α-estradiol and 99 ± 10% for 17β-estradiol. Linear sorption isotherms were developed using measured solution and soil phase concentrations (r^2 = 0.81-1.00). The distribution coefficient (Kd) values for 17β-estradiol were greater than 17α-estradiol for all but one soil (Oakville-24). A paired t-test confirmed that the log K_{OC} values for 17α-estradiol (2.92, 3.19, 3.00, 2.77, 3.08, 2.93, and 2.92) were significantly different (lower) ($p<0.05$) than 17β-estradiol (3.13, 3.47, 3.15, 2.96, 3.11, 3.12, and 3.06). The best correlation was between Kd and OC with an r^2 of 0.98-0.99, which suggests hydrophobic partitioning is the main influence of sorption.

Yu et al. [40] investigated the sorption of estrogenic steroids in one soil and six sediments. Sorption rates were measured for single-solute systems (17β-estradiol or estrone) at two different initial concentrations, and the potential concentration effects on the rates were determined. Experiments were conducted under both rate-limiting and equilibrium conditions in batch experiments. The soil log K_{OC} values determined in this study were 3.14, 3.55, 3.71, and 5.38 for four 17β-estradiol concentrations and 3.30, 3.67, 3.81, and 5.25 for four estrone concentrations in the Chelsea soil. The log K_{OC} values at a soil to solution ratio of 0.02, were 3.71 and 3.81 for 17β-estradiol and estrone, respectively, and are used in Table 10.

Lee et al. [52] used batch equilibrium studies to examine the sorption of 17β-estradiol by four soils and one freshwater sediment from the Midwestern US. After equilibration, the soil and aqueous phases were extracted, and 17β-estradiol and estrone (the primary transformation product) were quantified for each phase by HPLC. Two soils, named EPA 1 and Drummer 1, were used in the batch equilibrium experiments. The log K_{OC} values measured for 17β-estradiol in the EPA1 and Drummer 1 soils were 3.21 and 3.46, respectively. The log K_{OC} values measured for estrone in the EPA1 and Drummer 1 soils were 3.19 and 3.22, respectively.

Casey et al. [53] examined the sorption, mobility, and transformation of estrogens in natural soils. The authors used improved batch and continuous flow column experiments to study the fate and transport of 17β-estradiol and estrone in soil. Kinetic and equilibrium batch experiments were conducted with radiolabeled 17β-estradiol and estrone. Soil samples were analyzed via liquid scintillation counting (LSC) for radioactivity and thin layer chromatography (TLC) to detect metabolites in extracts. The log K_{OC} values for 17β-estradiol and estrone were determined to be 2.94 and 2.99, respectively. Within the first 24-48 hours, sorption kinetics were more significant than degradation, and sorption reached equilibrium between 5 and 24 hours.

Casey et al. [53] performed a re-analysis of the data presented in reference [54]. A series of laboratory batch sorption and miscible-displacement experiments were conducted using [^{14}C]17β-estradiol and five native soils. The concentrations of 17β-estradiol chosen were similar to those found in manures that are applied to field soils. The batch experiments were used to determine the sorption of 17β-estradiol to each soil, and the miscible-displacement experiments were used to determine the mobility of 17β-estradiol. Samples were analyzed via LSC for radioactivity and TLC to determine transformation products. The sorption affinity appeared to be associated with the surface area and/or cation exchange capacity in the soil. The Kd values for all five soils ranged from 0.086-6.67 L/g. These values were used to transform the Kd values in reference [54] into K_{OC} estimates (Table 2, page 1376 of reference [53]). The log K_{OC} values at 48 hours were determined to be 3.2, 3.2, 4.1, 3.0, and 2.7 for the five soils (as reported in Casey et al., 2005 [53]).

Stumpe and Marschner [55] evaluated the effects of long-term field application of organic soil amendments and the short-term effects of 14 different organic amendments to agricultural soil on mineralization and sorption of [^{14}C]17β-estradiol and [^{14}C]estrone. Mineralization and batch sorption experiments were conducted using 11 soils and several organic amendments, such as manures, biosolids, or wastewater. Manure was collected from six farms (cattle, swine, and poultry). Each soil and manure mixture was supplemented with 17β-estradiol or estrone. Radioactivity was analyzed by LSC. Long-term organic waste applications resulted in increasing soil organic carbon contents and increased sorption of 17β-estradiol and estrone. Short-term organic waste amendments directly increased the mineralization of 17β-estradiol up to 70% in treated soil compared to untreated soil. Sorption of 17β-estradiol increased with the amount of incorporation of organic waste, but the log K_{OC} values (3.1-3.2) were lower than the untreated soil (K_{OC} of 3036 from Table 4 of reference [55]), equal to log K_{OC} of 3.48). The log K_{OC} of 3.48 for 17β-estradiol was used in Table 10.

Caron et al. [56] determined K_{OC} values of 17β-estradiol, estrone, and estriol in 121 soil samples collected throughout the Province of Alberta, Canada and related these values to variations in soil properties, soil landscape position, soil great groups and ecoregions. Soil great groups are subdivisions according to similar kind, arrangement, and diagnostic horizons. Forty-one agricultural soils located across seven ecoregions were considered in the study. Surface soil samples (0-15 cm) were collected from three soil-landscape positions (upper slopes, mid-slopes, and lower slopes) in each of the 41 fields. All soils were air-dried and sieved. Batch equilibrium experiments were conducted in the dark in duplicate to measure sorption of 17β-estradiol and estrone in soils. All soils were sterilized via autoclaving to reduce microbial degradation during the experimental period. Preliminary equilibrium studies were conducted to determine the duration of the experiments. Equilibrium times of two hours for 17β-estradiol, 72 hours for estrone, and 96 hours for

estriol were used with tritium-radiolabeled compounds. At termination of the experiment, all replicates were centrifuged and the supernatants were analyzed for radioactivity via LSC. The OC content of the soils ranged from 6.3-137.9 g C/kg/soil. Clay content ranged from 8.6-55.6% and the sand content ranged from 12.4-76.6%. Overall, a large range of OC, clay and sand contents were present in the soils as recommended in standard testing methods. The K_{OC} of 17β-estradiol, estrone, and estriol were reported by region, soil great groups, and landscape positions. The log K_{OC} values for the soil great groups for 17β-estradiol were 3.11, 3.10, 3.04, 2.94, 2.94, and 3.00; values for estrone were 3.26, 3.25, 3.20, 3.10, 3.12, and 3.18.

Karnjanapiboonwong et al. [57] evaluated the sorption of 17β-estradiol and estrone in two soil types, a sandy loam (74% sand, 10% silt, 16% clay, 1.3% OC) and a silt loam (34% sand, 54% silt, 12% clay, 2.5% OC). Sand was used as a conservative sorption medium given that it had a 0.1% OC content. Sorption tests were conducted in a batch equilibrium experiment. A preliminary study found that the estrogens reached equilibrium within 24 hours. Each treatment contained four replicates. Control samples (no soil or sand) were also included to account for sorption to the test container. After the termination of the adsorption studies, desorption was evaluated over 24 hours by placing clean control solution in each vessel with just the soil. All replicates were centrifuged for 15 minutes and the supernatant was analyzed by HPLC. There was no significant loss due to degradation within the 24-hour experimental period. The log K_{OC} values for 17β-estradiol in the sand, sandy loam, and silt loam soils were 3.58, 3.95, and 3.90, respectively. For estrone, the values were 3.63, 3.72, and 3.69, respectively.

Summary of Estradiol Metabolite K_{OC}

The log K_{OC} values from the eight pertinent studies are presented in Table 10. These values were used to derive a single log K_{OC} value for the surrogate estradiol compound for use in fate modeling. As described in Section 4.2.2, this value was determined by calculating the lower 90% confidence bound on the mean K_{OC} value for all estradiol metabolites. The lower confidence bound was conservatively selected for the K_{OC} because a lower K_{OC} would result in a more mobile compound. This would predict a higher concentration of the surrogate estradiol compound entering the surface water through runoff, ultimately resulting in higher PEC_{water} estimates. **Thus, the mean lower 90th percentile confidence bound of 3.10 was used as a conservative estimate of the log K_{OC} (K_{OC} of 1259) for the surrogate estradiol compound**.

Table 10. A Summary of Published Log K_{OC} values for 17α-Estradiol, 17β-Estradiol, and Estrone and the Calculation of the Lower 90th Percentile Confidence Bound on the Mean Log K_{OC} for the Surrogate Estradiol Compound

Hormone	Log K_{OC}	Mean	SD	n-1	T_{90}	Lower 90% Confidence Bound on the Log K_{OC}
17α-Estradiol	(2.92, 3.19, 3.00, 2.77, 3.08, 2.93 2.92)[a]	2.97	0.134	6	1.440	2.90
17β-Estradiol	(3.13, 3.47, 3.15, 2.96, 3.11, 3.12, 3.06)[a] 3.71[b], (3.21, 3.46)[c], 2.94[d] (3.2, 3.2, 4.1, 3.0, 2.7)[d,e] 3.48[f], (3.11, 3.10, 3.04, 2,94, 2.94, 3.00)[g] (3.58, 3.95, 3.90)[h]	3.25	0.351	25	1.316	3.16
Estrone	3.81[b], (3.19, 3.22)[c], 2.99[d] (3.26, 3.25, 3.20, 3.10, 3.12, 3.18)[g] (3.63, 3.72, 3.69)[h]	3.34	0.273	12	1.383	3.23
Log K_{OC} mean 90th percentile lower confidence bound						**3.10**
Soil K_{OC} used for modeling						**1259**

[a] Mashtare et al. [51]; [b] Yu et al. [40]; [c] Lee et al. [52]; [d] Casey et al. [53]; [e] Re-analysis of Casey et al. 2003 [54] data by Casey et al. 2005 [53]; [f] Stumpe and Marschner (K_{OC} of 3036 in Table 4 of reference [55]); [g] Soil Great Groups in Table 3 of Caron et al. [56]; [h] Karnjanapiboonwong et al. [57].

4.2.4.10. Adsorption to sediment (sediment K_{OC})

The adsorption of compounds to sediment differs from that of soil due to the differences in organic matter content. Jurgens et al. [41] conducted a study to investigate the binding of 17β-estradiol to suspended sediment in aquatic systems. The log K_{OC} of 17β-estradiol to suspended sediment was determined to be 3.46 for a Calder sediment and 3.76 for an Aire sediment (page 34 of reference [41]). As described in Section 4.2.4.9 above, Yu et al. [40] evaluated the potential binding of 17β-estradiol and estrone in one soil and six sediments. The log K_{OC} values ranged from 3.14-5.38 and 3.30-5.25 in Chelsea soil for four 17β-estradiol and four estrone concentrations, respectively. The log K_{OC} values for sediment were determined to range from 3.35-6.24 for four 17β-estradiol concentrations in three sediment types, and 3.23-5.19 for four estrone concentrations in six sediment types. Note that Yu et al. [40] used extrapolated values for the highest concentration reported in their study (5 ng/L). In addition, Takigami et al. [58] determined the mean 17β-estradiol log K_{OC} for sediment to be 4.30.

The range for sediment K_{OC} values is similar to soil K_{OC} values. **Therefore, the more conservative soil log K_{OC} of 3.10 (K_{OC} of 1259) was used in the environmental fate modeling**. Using a lower value is considered more conservative because it will result in a higher estimated concentration of the surrogate estradiol compound in the water phase (i.e., a higher PEC_{water}), which is the compartment of interest for potential endocrine disrupting effects.

4.2.4.11. Transformation and degradation in soil (soil DT_{50})

Six published literature studies were selected that evaluated the potential transformation and degradation of 17β-estradiol and estrone in aerobic soils. Although many of these studies only assessed degradation in one soil type, the methods used were similar to OECD Guideline 307 [59]. All six studies used differing soil types, and the results were consistent across all studies. Thus, the data obtained in these studies were collectively used to derive a DT_{50} value for the surrogate estradiol compound in soil (Table 11). The degradation studies are summarized below.

Presentation of Studies

As part of a study to measure the effects of agricultural antibiotics on the persistence of 17β-estradiol, Chun et al. [60] measured the disappearance of 17β-estradiol and its primary metabolite estrone in a loam soil from Tennessee having a total carbon content of 7.9 mg/kg and pH of 6.9. The soil was passed through a 2-mm mesh screen, and the moisture level was maintained at 70% of field capacity, 0.3 bar, and pre-incubated for two days for equilibration. 17β-Estradiol was added to the soil at a level of 2000 µg/kg and then incubated for seven days at 37°C. Following extraction, the concentrations of 17β-estradiol and estrone were determined by HPLC/MS at Days 1, 2 and 7 (n = 3). The authors found the transformation of 17β-estradiol to be rapid, declining from 172 µg/kg soil on Day 1 to 20 µg/kg on Day 7 (see 17β-estradiol control in Table 1 of Chun et al. [60]). The formation and disappearance of estrone was also rapid, with 286 µg/kg appearing after one day of incubation and then declining to 99 µg/kg at Day 7. This study demonstrates that degradation of 17β-estradiol in soil occurs readily, with a DT_{50} of 17β-estradiol in soil of ≤3 days. The study also evaluated the degradation of 17β-estradiol in combination with antibiotics. However, the data for the antibiotics were not used in the derivation of a DT_{50} value. The potential effect of antibiotics on the degradation of 17β-estradiol is described at the end of this section.

Fan et al. [61] modeled the transport and degradation of [^{14}C]17β-estradiol in soil column experiments. Cores of sandy, mixed, frigid typic endoaquolls (Hamar) soils were collected. They had the following physical properties: bulk density of 1.54 g/cm^3, porosity of 0.42, organic matter of 2.23%, and composition of 14.0% clay, 19% silt, and 67% sand. Batch sorption experiments were conducted in triplicate at room temperature using a ratio of 1.6 g of sieved Hamar soil to 8 mL 0.01 M $CaCl_2$ at concentrations of 0.138, 0.069 and 0.015 mg/L 17β-estradiol. Test vials were placed on a rotating shaker and triplicate radioactivity measurements of the liquid phase were made at 0.5, 1, 5, 24, 48 and 168 hours by LSC. To evaluate photodegradation, controlled experiments were conducted in clear and amber vials with autoclaved sterile soil. For miscible-displacement experiments, soil columns were inverted and saturated with 0.01 M $CaCl_2$. After saturation, pulse experiments using 0.05 M $CaCl_2$ were conducted to determine the transport of the non-sorbing solute (Cl^-) in the Hamar soil. Following the breakthrough experiments with Cl^-, pulse experiments were conducted with 17β-estradiol. Fractions of effluent were collected periodically and analyzed for radioactivity using LSC. 17β-Estradiol metabolites in the fractions were determined using TLC. Modifications of the pulse experiments were made in an effort to trap any volatile residues and increase ^{14}C mass recovery. Results of batch experiments indicated that photodegradation (clear vs. amber vial, p = 0.9937) was not a significant factor in explaining the relative concentrations compared to sterility (p=0.008) and time (p=0.0001). The authors indicated this also supported the relative importance of biological processes for the fate of 17β-estradiol. Approximately 6% of ^{14}C was recovered

from the column effluents during pulse experiments. TLC indicated that the primary metabolites recovered were estrone (~25%) and a higher polarity unidentified metabolite (~70%). No 17β-estradiol was recovered in the effluent. Trapping experiments indicated 17β-estradiol was resistant to mineralization (0.01% recovered as $^{14}CO_2$). Incomplete ^{14}C recovery in pulse experiments (78-79%) was attributed to incomplete soil matrix combustion or unaccounted 17β-estradiol mineralization. Results from these studies were modeled to quantify the fate and transport of 17β-estradiol. The results indicated that 17β-estradiol was quickly degraded to estrone and a polar metabolite. Further analysis of soil columns indicated ^{14}C was 47-51% irreversibly and 22-25% reversibly sorbed. The TLC analysis of the resident soil extracts from the reversible sorption sites indicated that 55%, 20%, and <2% of ^{14}C were recovered as the polar metabolite, estrone, and 17β-estradiol, respectively. The calculated DT_{50} of 17β-estradiol was <5 hours, and then its concentration declined to nearly zero. These data support a DT_{50} of estradiol in soil of ≤0.5 days.

In another soil biodegradation study by Fan et al. [62], the persistence and fate of 17β-estradiol was measured in four different soil microcosms: 1) native soil under aerobic conditions, 2) native soil under anaerobic conditions, 3) sterilized (autoclaved) soil under aerobic conditions, and 4) sterilized soil under anaerobic conditions. Samples of Hamar soil (sandy, mixed, frigid typic endoaquolls series) were collected from the surface horizon for the experiments. [^{14}C]17β-Estradiol was added to the moisture-adjusted soils at a concentration which had been found in animal manures applied to agricultural fields (75 µg of 17β-estradiol per 200 g of soil in approximately 50 mL of 0.01 M $CaCl_2$). The aerobically and anaerobically incubated soils were maintained under a flow of air and helium, respectively. In the aerobic and anaerobic systems, $^{14}CO_2$ was trapped in outflow flasks containing NaOH. In the anaerobic systems, a Porapak column was used to trap ^{14}C-labeled volatile organic compounds (except for $^{14}CH_4$), and an additional trap was used to collect $^{14}CH_4$. Samples of the trapping solutions were taken periodically during the 132-hour study, at which time the experiment was stopped for analysis for ^{14}C. Following five days of incubation, 70-73% and 50-67% of the ^{14}C in native and sterile soils, respectively, were non-extractable. Approximately 12-19% of 17β-estradiol was extractable from the native soil, and 24-28% was extractable from the sterilized soil. Analysis of the trapping solutions and extracts of the native soils indicated that 17β-estradiol was transformed anaerobically to 28% estrone and 61% unidentified polar metabolites after five days (these percentages only relate to the portion of extractable compounds). In native soils under aerobic conditions, 63% of 17β-estradiol was transformed to an unidentified polar metabolite. In autoclaved soil incubated aerobically or anaerobically, ≤12% of the 17β-estradiol was transformed to unidentified polar compounds. In native soils under aerobic and anaerobic conditions, 6% and 0.9% of the ^{14}C-radiolabeled 17β-estradiol carbon was mineralized to CO_2, respectively; whereas, 0.2% of 17β-estradiol was mineralized in the sterile microcosms. 17β-Estradiol was not degraded to $^{14}CH_4$ in any incubation vessel. These data support an aerobic and anaerobic soil DT_{50} of estradiol of ≤5 days.

Colucci et al. [63] measured the persistence and pathways of dissipation of 17β-estradiol and estrone in soil in laboratory microcosms. Three agricultural soils (loam, sandy loam and silt loam) were supplemented with [^{14}C]17β-estradiol and [^{14}C]estrone and incubated at a range of temperatures and moisture levels in the microcosm systems that included vessels containing NaOH to trap $^{14}CO_2$. Parallel soils were autoclaved. The authors reported approximately 56-91% of applied radioactivity as nonextractable from three soils after a 3-day incubation and 50% degradation of 17β-estradiol after <12 hours of incubation.

Estrone was a major metabolite in autoclaved and nonsterile soils. However, estrone was stable in autoclaved soil suggesting the microbiota were responsible for its removal. The degradation rate constant (k) for 17β-estradiol was calculated to be 1.45, 3.12, and 2.37 day^{-1} for the silt loam, sandy loam, and loam soils, respectively. Using the rate constant, the DT_{50} can be calculated using $DT_{50} = ln(2)/k$ (see example calculation in footnote[o]). The resulting DT_{50} values were 0.48, 0.22, and 0.29 days for silt loam, sandy loam, and loam soils, respectively. For estrone, k was calculated to be 1.13, 0.41, and 0.75 day^{-1} for the three soils tested, which corresponds to DT_{50} values of 0.61, 1.69, and 0.92 days, respectively. Mineralization of estradiol to $^{14}CO_2$ reached 11% at 19°C, and bound residues were slowly mineralized. These data support DT_{50} values of estradiol in soil of ≤0.5 days and estrone of ≤2 days.

The degradation of 17β-estradiol and estrone were examined in loam soils pre-exposed or unexposed to estrogens sourced from city secondary treatment wastewater [64]. The degradation rates were determined under both aerobic and reduced oxygen conditions. The aerobic 17β-estradiol DT_{50} was determined to be 2.3 and 2.1 days for pre-exposed and unexposed, soils, respectively. The aerobic estrone DT_{50} was determined to be 0.6 and 1.1 days for pre-exposed and unexposed soils, respectively. Under anaerobic conditions, the 17β-estradiol DT_{50} was determined to be 1.9 and 1.6 days for pre-exposed and unexposed conditions, respectively. The anaerobic estrone DT_{50} was determined to be 6.3 and 3.4 days for pre-exposed and unexposed soils, respectively. The values for the unexposed soil were used in the derivation of a single DT_{50} value for the surrogate estradiol compound (Table 11). The degradation rate parameters for anaerobic soils are not an input parameter used in the environmental fate models; however, the results are reported in Table 11 to present all available data considered in the exposure assessment.

In a study examining the degradation of estrogenic hormones in a silt loam soil with a 15% moisture content at 25°C, 17α-estradiol degradation was compared to that of 17β-estradiol (Table 3 of reference [65]). Because this study was conducted in 20% non-sterilized soil rather than 100% non-sterilized soil, the half-lives are lower than would be expected with whole soil. Indeed, the DT_{50} of 17β-estradiol in 100% non-sterilized soil (i.e., 0.17 day) was less than $1/5^{th}$ of that in 20% non-sterilized soil (0.92 day). In the study, the DT_{50} values of 17α-estradiol (1.9 days) and 17β-estradiol (0.92 days) in 20% non-sterilized soil were both rapid. The results for the degradation of 17β-estradiol in the non-sterilized soil was used in the DT_{50} determination. The authors also measured the effects of different temperature and moisture levels on the degradation of 17β-estradiol in the 20% non-sterilized soil. 17β-Estradiol degraded optimally in the 20% non-sterilized soil at a moisture level of 20% and a temperature of 25°C. An increase in the moisture level to a saturation level resulted in a longer DT_{50}. However, when the temperature was increased to 35°C, only a marginal enhancement of 17β-estradiol degradation occurred. The potential effect of antibiotics on the degradation of 17β-estradiol, which was a separate part of the study, is described at the end of this section.

Summary of Estradiol Metabolite Soil DT_{50} Estimates

The data from the six acceptable literature studies discussed above were used to determine a DT_{50} for 17β-estradiol and estrone. The conservative upper 90^{th} percentile confidence bound DT_{50} estimates for the 17β-estradiol and estrone in soil were determined to be

[o] $DT_{50} = ln(2)/k = 0.693/1.45\ day^{-1} = 0.48$ days

1.0 and 2.1 days, respectively (Table 11). The use of the upper confidence bound to derive the DT_{50} values is conservative because it assumes the surrogate estradiol compound is available for a longer period of time to runoff into surface waters. Further, to determine the soil DT_{50} value for the surrogate estradiol compound, the final DT_{50} values for 17β-estradiol and estrone were summed to ensure that dissipation of all potential estradiol metabolites was represented. **Thus, a soil DT_{50} value of 3.1 days [1.0 day + 2.1 days = 3.1 days] for the surrogate estradiol compound was used in the environmental fate modeling.**

At the time the DT_{50} values in Table 11 were derived, there were insufficient data published for the degradation of 17α-estradiol in soil. However, since that time, a study was published by Mashtare et al. in 2013 [21]. The results of that publication are described in detail following Table 11, under "Additional Supporting Information," along with other data available in the published literature that evaluated the degradation of estradiol metabolites in varying environmental conditions (i.e., under varying temperatures and in the presence of wastewater and manure). Briefly, Mashtare et al. [21] found that the degradation of 17α-estradiol, 17β-estradiol and estrone was rapid in two soils (DT_{50} <1.8 days) and that the DT_{50} values for 17α- and 17β-estradiol were not significantly different, indicating that the two compounds likely degrade at similar rates. These results agree with the data shown in Table 11 and with the conservative DT_{50} value of 3.1 days derived for the surrogate estradiol compound.

Table 11. A Summary of Published DT_{50} Values for 17β-Estradiol and Estrone and the Calculation of the Upper 90th Percentile Confidence Bound on the Mean DT_{50} for the Surrogate Estradiol Compound in Aerobic and Anaerobic Soils

Hormone	DT_{50} Days	Mean	SD	n-1	T_{90}	Upper DT_{50} 90th percentile confidence bound
Aerobic 17β-Estradiol	2.1[a], (0.48, 0.22, 0.29)[c] 0.2[e], 0.17[f]	0.577	0.755	5	1.476	1.0 day
Aerobic Estrone	1.1[a], 3[b], (0.61, 1.69, 0.92)[c]	1.46	0.944	4	1.533	2.1 days
Aerobic soil half-life estradiol + estrone						**1.0 + 2.1 = 3.1 d**
Anaerobic 17β-estradiol	1.6[a], 5[d]	3.3	2.40	1	-	5 days*
Anaerobic Estrone	3.4[a]	3.4	-	0	-	§

[a] Carr et al. [64], [b] Chun et al. [60], [c] Colucci et al. [63], [d] Fan et al. [62], [e] Fan et al. [61], [f] Xuan et al. [65]

* It is not appropriate to estimate a variance with only two data points; therefore, the highest half-life was used. However, it is important to note that the anaerobic soil DT_{50} is not used in any modeling in this assessment.

§ A standard deviation cannot be determined with only one observation and was not used in the determination of the PEC values because an anaerobic soil DT_{50} value was not used in the modeling.

Additional Supporting Information

Although not used to derive the DT_{50} value for the farm and watershed scale modeling, the following studies support the rapid degradation of estradiol metabolites, both free and conjugated, in soil (including in the presence of wastewater and manures and under varying temperatures).

17α-Estradiol degradation data

Mashtare et al. [21] determined the biotransformation of 17α- and 17β-estradiol in two aerobic soils: 1) silty clay loam (Drummer, D46) and 2) sandy loam (Coloma, C45). After collection, the soils were passed through a 2-mm sieve and stored at 4°C in the dark. Soil (10 g dry weight basis) was transferred to sterilized amber glass bottles. Soil moisture was maintained at 75% and 84.6% of field capacity for C45 and D46, respectively. Soil microcosms were capped and allowed to re-acclimate in the dark at room temperature (21 ± 2°C) for seven days prior to hormone addition. Sterile controls were prepared by autoclaving and were used to differentiate between microbial and abiotic processes. After seven days of incubation, hormones were added (0.184 µmol/kg soil) using a talc carrier (not to exceed 1% of soil weight), shaken, and recapped. Incubations were conducted in the dark. Biotic and abiotic microcosms were sacrificed in triplicate at nine time points over approximately 19 days. Soils were extracted and analyzed by HPLC-electrospray ionization (ESI)-MS/MS. Pseudo first-order exponential decay models were used to establish biotransformation rates. Both 17β- and 17α-estradiol exhibited rapid degradation in unsterile soil with similar degradation rates for both soils. The results support the interpretation that microbial processes were primarily responsible for degradation. The DT_{50} values for 17α-estradiol were 0.44 and 0.16 days for the sandy loam (C45) and silty clay loam (D46) soils, respectively. The DT_{50} values for 17β-estradiol were 0.45 and 0.23 days for the sandy loam (C45) and silty clay loam (D46) soils, respectively. Estrone was formed in both microcosms and a DT_{50} was determined to be 0.78 and 0.14 days in the 17α-estradiol C45 and D46 microcosms and 1.77 and 0.26 days in the 17β-estradiol C45 and D46 microcosms, respectively. The authors found that the pseudo first-order decay model was a moderately good fit (r^2 of 0.88-0.96), but the model was poor after two to three half-lives and did not predict the persistent residuals observed at later times. 17α-Estradiol was not observed in the 17β-estradiol microcosm and vice versa; however, it was suggested that estrone is the first primary metabolite formed for both 17β- and 17α-estradiol. Thus, the DT_{50} was rapid for estradiol metabolites in both soils (DT_{50} ranged from 0.2 to <2 days), which agrees with the data displayed in Table 11 and also the DT_{50} value of 3.1 days derived for the surrogate estradiol compound. Further, the study determined that the 17α- and 17β-estradiol DT_{50} values were not significantly different, indicating that the two compounds likely degrade at similar rates.

Degradation of Estradiol metabolites in wastewater and manure amended soils

As mentioned previously, Carr et al. [64] showed that the DT_{50} for 17β-estradiol was higher for both aerobic soils and anaerobic soils that were previously exposed to wastewater effluent containing pharmaceuticals and personal care products (PPCPs) (including 17β-estradiol and estrone) than in soils unexposed to PPCPs. For estrone, the DT_{50} values were lower in the aerobically incubated exposed and unexposed soils. The aerobic DT_{50} values for the exposed and unexposed soils were 0.6 and 1.1 days, and 2.3 and 2.1 days for estrone and 17β-estradiol, respectively. The anaerobic DT_{50} values for the exposed and unexposed soils were 6.3 and 3.4 days, and 1.9 and 1.6 days for estrone and 17β-estradiol, respectively. Regardless of whether 17β-estradiol and estrone were incubated aerobically or anaerobically or in soil exposed or unexposed to PPCPs, the DT_{50} values were all <6.5 days. As described in another article continuing their work [66], the authors examined the rates of transformation of 17β-estradiol and estrone in soil exposed or unexposed to PPCPs, during periods of super-saturation or with standing water, and the period immediately after the soils were drained. The DT_{50} of estrone was found to be 33.4, 27.5, 56.8, and 38.1 days for the drained samples in exposed soil, drained samples in unexposed

soils, saturated samples in exposed soil, and saturated samples in unexposed soils, respectively. The DT_{50} of 17β-estradiol was found to be 4, 2.6, 1.5, and 3.4 days for the drained samples in exposed soil, drained samples in unexposed soils, saturated samples in exposed soil, and saturated samples in unexposed soils, respectively. Regardless of the treatment employed, the DT_{50} of 17β-estradiol was calculated to be considerably lower than that of estrone. There was no loss of 17β-estradiol and estrone in autoclaved soils; thus, the degradation of these two compounds was attributed to the soil microflora.

Stumpe and Marschner [55] studied the mineralization of [^{14}C]17β-estradiol, [^{14}C]estrone and [^{14}C]17α-ethinylestradiol in agricultural soil and in inert sand amended with solid and liquid manures from cattle, swine, poultry and biosolids. They examined the effects of long-term and short-term field application of organic soil amendments on mineralization using 11 soils and several organic amendments, such as manures, biosolids, or wastewater. Manure was collected from six farms (cattle, swine, and poultry). Each soil and manure mixture was supplemented with radiolabeled drug, and radioactivity was analyzed by LSC. Short-term organic waste amendments increased the estradiol mineralization up to 70% in treated soil compared to untreated soil; therefore, the authors were able to demonstrate that addition of organic wastes to soils serves to stimulate the mineralization of estrogens in the short term.

Laboratory microcosm experiments were conducted with and without [^{14}C]17β-estradiol and [^{14}C]testosterone over a range of soil types, organic amendment concentrations, and temperatures [67]. Three soils, with varying pH, organic matter and textural classification, and two types of organic amendments (slurry from commercial swine-producing farms and sewage sludge biosolids from a municipal WWTP) were used. Parallel soils and organic amendments were sterilized by autoclaving. The microcosms were incubated in the dark at 30°C. Soils were sampled at 0, 6, 24, 48, and 144 hours, extracted with organic solvents, and then analyzed via LSC and HPLC. Sewage biosolids and liquid swine slurry accelerated the mineralization of 17β-estradiol after 96 hours. HPLC fractionation of the radioactivity in extracts taken immediately after the start of the incubation revealed that 24% of the radioactivity in extracts from unamended soil and approximately 74% of the radioactivity in the soil amended with biosolids and swine manure slurry was associated with estrone. 17β-Estradiol was quickly converted to estrone, and its DT_{50} was less than the length of the 6-day study. Furthermore, it was shown that the microbiota in the organic amendments can convert 17β-estradiol to estrone and that mineralization of [^{14}C]17β-estradiol requires a viable soil microbial population. Although liquid swine slurry was used in this study and not cattle manure, it is reasonable to assume that organic material in cattle manure would also enhance the degradation of 17β-estradiol.

Degradation of conjugated estradiol metabolites at varying temperatures

Estrogen sulfates are conjugates of estrogen excreted into the environment by cattle. In a study by Scherr et al. [30], three pasture soils (clay loam, silt loam and sandy loam) were treated with non-radiolabeled 17β-estradiol-3-sulfate. The loss of parent and formation of other metabolites was followed in aerobically incubated microcosms in laboratory experiments at three different incubation temperatures (7.5, 15, and 25°C). The incubations were conducted in the dark, and soil samples were collected and extracted from the microcosms at several time points over 8-10 days. Autoclaved sterile controls were also prepared. The initial transformation of 17β-estradiol-3-sulfate was biologically mediated and very rapid, with DT_{50} values of parent measured in hours. The primary metabolite identified

was estrone-3-sulfate and ranged from approximately 55% to 100% of the parent. The degradation of 17β-estradiol-3-sulfate in the three soils was rapid, and the DT_{50} values decreased with increasing temperatures. The DT_{50} values for 17β-estradiol-3-sulfate in soils ranged from 1.07-7.69 hours at 7.5°C, 0.77-3.25 hours at 15°C, and 0.424-1.84 hours at 25°C. Estrone-3-sulfate also degraded rapidly. Some free estrone accumulated, and then was greatly reduced by 80 to 200 hours at 7.5 and 15°C, and almost completely disappeared by 40 to 200 hours at 25°C (visually estimated from Figure 1 of the article). Minimal quantities of free 17β-estradiol were detected, but these too were rapidly degraded in <40 hours in all soils. By 240 hours (Day 10), the presence of estrogenic material was observed in only one of the soils at ≤2% of the parent. Based on these data, it was concluded that conjugated estrogens are rapidly transformed initially through reduction of the estradiol (-OH) to the estrone (=O), followed by hydrolysis of the conjugate to form free estrone. The authors concluded that arylsulfatase activity originating in the soils is the key to the degradation of 17β-estradiol-3-sulfate. These data support the half-lives of conjugated 17β-estradiol of <8 hours in the clay loam, silt loam, and sandy loam. In addition, these results also demonstrate that the degradation of estradiol metabolites in soil is temperature dependent; the DT_{50} decreases with increasing temperature.

Effects of antibiotics on the degradation of estradiol metabolites

Chun et al. [60] tested the effects of three agricultural antibiotics (sulfamethazine, tylosin, and chlortetracycline) on the persistence and transformation of 17β-estradiol in a loam soil. 17β-Estradiol was mixed with the soil at a 2 mg/kg level. For each treatment, 10 g of soil was transferred to amber vials to which the antibiotics were added as 70% methanol/30% water (v/v) stock solutions such that the final concentrations of each antibiotic were 2 and 200 mg/kg soil. To one of the treatments, the same volume of 70% methanol/30% water (v/v) only was added; this treatment served as a control. Treatments were incubated for seven days at 37°C, and 17β-estradiol and estrone were measured at Days 1, 2, and 7 by HPLC coupled with electrospray and tandem mass spectrometry following extraction. Rapid transformation of 17β-estradiol to estrone occurred in all treatments. The concentrations of estrone were lower in all the antibiotic treatments, which implies the presence of the antibiotics may have slowed the transformation of 17β-estradiol in the soil. However, the study results did not show a prolonged persistence of 17β-estradiol in the presence of the antibiotics.

As part of a study designed to measure the degradation kinetics of 17β-estradiol, Xuan et al. [65] also investigated the effects of the presence of three veterinary antibiotics (sulfadimethoxine, oxytetracycline and tylosin) on the rate of degradation of 17β-estradiol. 17β-Estradiol was added to 20% non-sterilized soil at a concentration of 4.0 µmol/kg dry weight (equivalent to 1.09 mg/kg). The soils were incubated with various concentrations of the three antibiotics at 25°C for five days with daily sampling. For the sulfadimethoxine-treated soil, there was no significant difference between the degradation rates of 17β-estradiol in soils containing 0 or 8 µmol/kg (0 or 2.48 mg/kg) of the antibiotic. However, the rate of degradation of 17β-estradiol was significantly decreased in the presence of ≥40 µmol/kg of the antibiotic. In the presence of 40 µmol oxytetracycline or tylosin per kg soil (equivalent to 18.4 and 36.6 mg/kg, respectively), no significant decrease in the degradation rate of 17β-estradiol was observed.

It is important to note that the antibiotics used in the Chun et al. [60] and Xuan et al. [65] studies were tested at very high concentrations (mg/kg) and are not representative of environmentally relevant values. Therefore, the predictive value of these data is unclear.

4.2.4.12. Aerobic and anaerobic water-sediment transformation

There are limited published literature studies that evaluated the partitioning, transformation, and degradation of 17β-estradiol, 17α-estradiol, and estrone in water-sediment systems. Further, none of these studies followed methods similar to OECD Guideline 308 [68] (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems). Thus, Pfizer Inc. conducted two water-sediment system degradation studies with 17α-estradiol using the methods recommended under OECD Guideline 308. The results of these Zoetis-owned studies were used to derive the aerobic and anaerobic water-sediment DT_{50} values for the surrogate estradiol compound shown in Table 12. These studies are summarized below. In addition, other available literature studies evaluating the degradation of estradiol metabolites in the water or sediment environments are summarized following Table 12 (under "Additional Supporting Information").

Zoetis-owned OECD Guideline 308 studies

Two GLP OECD 308 studies were conducted by Pfizer using [^{14}C]-17α-estradiol under both aerobic (reference [239] and Appendix 13.9) and anaerobic (reference [240] and Appendix 13.10) conditions. Each study used two water-sediment systems, Taunton River and Weweantic River, representing high and low organic content, respectively. 17α-Estradiol was added to the water at 1 mg/L, a higher concentration than would be found naturally, to provide sufficient analytical sensitivity for determining degradation products. The test vessels were incubated at 20 ± 2°C. The disappearance of 17α-estradiol and the production and disappearance of three known metabolites (17β-estradiol, estrone, and estriol) were measured in both the water and sediment phase. In addition, mineralization of 17α-estradiol was also measured as production of $^{14}CO_2$. In both the aerobic and anaerobic incubations, 17α-estradiol was converted primarily to estrone and a small quantity of 17β-estradiol. Both estrone and 17β-estradiol were found in the sediment phase. Very small amounts of these metabolites were detected in the water phase. From the production of these transformation products, the DT_{50} of estrone and 17β-estradiol were determined, along with, the DT_{50} of parent 17α-estradiol. The DT_{50} values for 17α-estradiol, 17β-estradiol, estrone and the total drug (17α-estradiol + 17β-estradiol + estrone) are summarized in Table 12. Additional details on the methods and results of these studies are available in Appendix 13.9 and Appendix 13.10.

Table 12. Degradation Half-lives (DT_{50}) of [^{14}C]-17α-Estradiol, 17β-Estradiol, Estrone, and the Total Drug in Aerobic and Anaerobic Water-Sediment Systems following OECD Guideline 308

Aqueous Phase + Sediment Phase	DT_{50} (days)			
	17α-Estradiol	Total Drug*	17β-Estradiol	Estrone
Aerobic Incubation				
Taunton River	8.5	**31.1**	11.0	10.2
Weweantic River	16.3	25.5	13.6	2.9
Anaerobic Incubation				
Taunton River	60.6	**107.8**	39.5	15.7
Weweantic River	62.8	103.5	26.7	22.1

* Total Drug = sum 17α-estradiol + 17β-estradiol + estrone. Note that the half-life of total drug is not the sum of the individual DT_{50} values.

For modeling environmental fate of the surrogate estradiol compound, the DT_{50} values from the total system (aqueous + sediment phase concentrations) were used. To represent a more conservative approach, the system total drug DT_{50} was calculated from the sum of the transformation products at each time point. Further, for the two river systems, the highest DT_{50} value was chosen to be used in the fate modeling instead of the mean. This method of selecting a DT_{50} value is conservative because the total drug DT_{50} accounts for the transformation, and ultimate degradation, of all EB metabolites over time in both the aqueous and sediment phase. **Therefore, the DT_{50} for the surrogate estradiol compound used for modeling aerobic degradation in water-sediment systems was 31.1 days and the anaerobic DT_{50} was 107.8 days.**

Additional Supporting Information

Additional published literature whose authors investigated degradation of estradiol metabolites in water or sediment environments is described below:

The degradation of estrogens was studied in microcosm experiments using column water and sediments from three English rivers (Aire, Calder, and Thames) and two estuaries (Tees and Tyne) [41]. 17β-Estradiol (equivalent to 500 µg/L) was added to a series of flasks containing 50 mL of river water samples. Half of the flasks were incubated anaerobically and the other half were incubated aerobically at 20°C. For each river water sample, autoclaved flasks were included to serve as controls. The flasks were sampled at the start of the experiment and at 10 additional times during the 49-day study to measure the loss of parent 17β-estradiol. A similar experiment was conducted using 500 µg/L of 17β-estradiol (of which 10 µg/L was radiolabeled) to monitor the evolution of $^{14}CO_2$ from the phenol ring of the 17β-estradiol. Each flask was connected to an apparatus designed to collect evolved $^{14}CO_2$ and was incubated at 20°C. No decrease in 17β-estradiol was observed in sterile controls throughout the 49-day incubation. Under aerobic incubations, the formation of estrone corresponded with decreasing concentrations of 17β-estradiol, which suggested that 17β-estradiol was converted to estrone and then further degraded. The anaerobic test system was described as imperfect because it allowed the entry of air during sampling. As observed under aerobic conditions, degradation of 17β-estradiol to estrone was rapid. Under anaerobic conditions, the estrone produced appeared to be much more persistent. In the aerobically incubated Aire and Calder River water samples, 17β-estradiol yielded degradation half-lives of <3 days; samples from the Thames River water samples yielded a DT_{50} of 4 days. The degradation half-lives for 17β-estradiol were higher in the estuary

microcosms at 6 and 27 days. The authors suggested that the higher salt content of the estuary system may have inhibited microbial activity, resulting in slower degradation compared to the freshwater systems.

In a similar series of experiments, Jurgens et al. [69] further investigated the biodegradation rate of 17β-estradiol using river water samples collected from the three rivers discussed above [41] at six different sites. The top few cm of sediment samples were collected from two of the rivers, the Calder (at Methley Bridge) and the Thames (at Wallingford). The sediment samples were then sieved (2 mm screen) and stored at 4°C until use. As described for earlier experiments (above), 17β-estradiol concentrations declined with a corresponding formation of estrone. The biotransformation of 17β-estradiol to estrone followed first-order decay kinetics. The aerobic water degradation DT_{50} of 17β-estradiol ranged from 0.2-8.7 days in experiments covering a pH range between 7.1 and 8.4, an OC content between 2.9 and 10.3 mg/L, and suspended sediments between 5.2 and 83 mg/L (Table 1 of reference [41]). The aerobic water degradation DT_{50} of estrone ranged between 0.1 and 10.9 days. The degradation was determined to be temperature dependent, with half-lives roughly two times longer at lower temperatures (10°C versus 20°C). In experiments comparing spiked concentrations of 17β-estradiol at 100 µg/L and 0.1 µg/L, the degradation rate was similar, though slightly faster at the lower concentrations, with the generation of estrone as the first metabolite. Under anaerobic conditions, 17β-estradiol rapidly converted to estrone, with a complete conversion within two days. Bed sediment samples and river water taken from the same respective sites were placed into 100 mL conical flasks to make up a total of 5 g solids with 10 mL river water. The flasks were incubated under a nitrogen flow to maintain anaerobic conditions. 17β-Estradiol (5 µg) was added to the flasks to yield a final concentration of 0.5 mg/L then the flasks were incubated at 20 ± 2°C. Autoclaved samples were used as sterile controls. HPLC/MS was used to analyze the steroids. The anaerobic sediment degradation DT_{50} for 17β-estradiol and estrone in two bed sediments ranged from 0.37-0.66 days and 11.5-14.3 days, respectively. Under aerobic conditions in one bed sediment, the DT_{50} was notably shorter for 17β-estradiol (0.11 days) and estrone (0.42 day). Microbial cleavage of the steroid ring system in water samples from River Aire was demonstrated by release of $^{14}CO_2$ from the aromatic ring of estradiol (position 4 in the A ring). The authors also determined the photolytic degradation of 17β-estradiol and reported a DT_{50} of 124 hours during continuous radiation, which the authors deemed equivalent to 10 days of bright sunshine per day.

In an examination of hormones in stream sediment, Bradley et al. [70] measured the biodegradation of [4-^{14}C]-labeled 17β-estradiol, estrone, and testosterone in oxic sediment from Fourmile Creek near Ankeny, IA; Boulder Creek near Boulder, CO; and South Platte River near Denver, CO. For each sediment source, the top 10 cm of sediment were collected in 250-mL amounts from four locations evenly distributed upstream and downstream from WWTPs. Sediments were prepared under aerobic conditions in microcosms. Water samples were collected midstream at mid-depth. Autoclaved controls and a single sediment-free control were prepared for each sediment type. The microcosms were amended with 0.04 µCi of [4-^{14}C]-labeled substrate so that the sediment concentration was 30 ng/g dry weight. All of the microcosms were incubated in the dark at 23°C for 32-34 days. In sediments upstream from the WWTP, [4-^{14}C]-17β-estradiol was mineralized to approximately 30%, 38%, and 40% of the applied radioactivity in these respective sediments after 32 days (based on Figure 2 in the report). [4-^{14}C]-17β-Estradiol mineralization in sediments immediately downstream from the WWTPs was more than twice that of upstream sediment. [4-^{14}C]-Estrone was mineralized to approximately 38%, 45% and

90% of the applied radioactivity in these respective upstream sediments after 33 days (Figure 3 in the report). In downstream sediments, estrone mineralization was less than upstream sediments. The mineralization rates of these compounds in water microcosms without sediment was reduced compared to microcosms containing both water and sediment. No significant $^{14}CO_2$ was observed in both the autoclaved or sediment-free controls. Because only mineralization and not parent loss or metabolite formation were measured, the degradation rate of 17β-estradiol and estrone in the water-only systems cannot be determined. However, the rate of mineralization suggests that the degradation rates for 17β-estradiol and estrone were relatively rapid.

A field study was conducted on a 10-km stretch on the Redwood River in Minnesota using 17β-estradiol at a starting concentration of 10 ng/L and a tracer dye or sodium bromide to allow for measurement of dilution in the river [71]. The dye was added to the river at a WWTP site to establish the basic hydrological flow conditions and transit times measured at 2.1 and 10 km downstream. A second tracer test was conducted with sodium bromide, 4-nonylphenol, and 17β-estradiol added at a sufficient concentration to ensure measurement but to minimize environmental risk. It was not stated what the mass of sodium bromide added to the river was, but the masses of 4-nonylphenol and 17β-estradiol were approximately 2 g and 0.009 g, respectively. Preliminary estimated concentrations of the two compounds in the river were 1000 and 5 ng/L, respectively, assuming that the river was completely mixed 600 m downstream. The study was conducted during the nighttime to minimize losses due to photolysis. Four locations along the river were sampled: 1) the river 100 m upstream from a WWTP outfall, 2) at the WWTP outfall, 3) on the river 2.1 km downstream from the WWTP outfall, and 4) on the river 10 km downstream from the WWTP outfall. A sample of the river water was taken from the WWTP outfall immediately prior to addition of the tracer. The attenuation and transformation of 17β-estradiol to estrone was rapid. The estradiol first-order decay constant for 17β-estradiol was -3.2 ± 1 day^{-1} and the production constant for estrone was 0.6 ± 0.8 day^{-1}. A DT_{50} of 5.2 hours for 17β-estradiol was calculated in a natural river system using the degradation rate [$DT_{50} = \ln(2)/k = \ln(2)/3.2$ = 0.22 day or 5.3 hours]. Because 17β-estradiol has a low vapor pressure and because the study was conducted at night, it was concluded that the primary removal mechanisms were biodegradation and sorption. The production rate constant for estrone was 28 hours [ln(2)/0.6 = 1.16 day].

The potential for degradation of 17β-estradiol and estrone in a wastewater effluent recharged aquifer system was assessed using lab scale microcosms (Lim et al. [72]). The aquifer materials (aquifer water and sediment) used in this study were collected from a test site for aquifer storage and recovery. The ultrafiltered secondary effluent (UFE) water was obtained from a WWTP and passed through a filter, which created an abiotic environment by removing all bacteria. A series of experiments was conducted to assess: 1) the biodegradability of 17β-estradiol in the absence and presence of aquifer materials and UFE, 2) the biodegradability in the presence of aquifer materials alone (aquifer water and sediment alone), 3) the biodegradability in aquifer materials and UFE in both biotic and abiotic conditions, and 4) the effect of degradation in the presence of methanol. Samples were analyzed by liquid chromatography (LC) equipped with a triple quad tandem mass spectrometer. This study determined that microorganisms play a major role in the biotransformation of estrogens. The DT_{50} of 17β-estradiol in the UFE water alone was 16.6 ± 4 days. In contrast, the DT_{50} in UFE water and sediment was <3 days and estrone was no longer detectable after five days. The DT_{50} of 17β-estradiol in the biotic environment in the presence of methanol was 5.6 days compared to 17.8 days in the abiotic environment with methanol. These data support DT_{50} values for estradiol and estrone of ≤6 days.

The DT_{50} of 17β-estradiol was determined by Liu et al. [73] in river water samples collected from the Huayuankou site in the middle reach of the Yellow River (China). A 200 µL aliquot of 17β-estradiol stock solution in methanol was added to a series of 500 mL flasks and evaporated in a fume hood, followed by the addition of 200 mL of autoclaved or non-autoclaved water samples. Final concentrations of 17β-estradiol were approximately 1000 µg/L. Incubations were carried out in a rotary incubator-shaker at 200 rpm at 20°C and 30°C for 14 days under aerobic conditions. Additional experiments were conducted to evaluate the impact of salts, easily degraded organics (i.e. glucose), and municipal waste on the degradation rate. Samples were collected at various time intervals and analyzed by LC-MS/MS. 17β-Estradiol was readily degraded in water samples from the Yellow River with DT_{50} values of 6 days and 3 days at 20°C and 30°C, respectively. Biodegradation did not occur in autoclaved samples, and the authors concluded that 17β-estradiol degradation was attributable to bacteria. At 20°C, approximately 90% of the 17β-estradiol was degraded in Yellow River water at a relatively constant rate during a 14-day period. However, in the presence of nutrient salts (NH_4Cl and K_2HPO_4), 17β-estradiol was completely degraded. The addition of glucose had no apparent effect on the degradation rate of 17β-estradiol; however, in the presence of waste material, 17β-estradiol was more rapidly and completely degraded. Estrone was the only identified metabolite of 17β-estradiol. Both the presence of salts and waste material greatly increased the degradation of estrone. The authors concluded that temperature, nutrient salts, and the presence of bacteria will greatly impact the degradation of 17β-estradiol and estrone.

The literature data presented above support an aerobic DT_{50} of ≤8.7 days and ≤11 days for 17β-estradiol and estrone, respectively, in water only and river systems. The literature data above also support an anaerobic DT_{50} of <1 day and ≤14 days for 17β-estradiol and estrone, respectively.

4.2.5. Summary of environmental fate modeling parameters for the surrogate trenbolone compound

Table 13 below lists the physical-chemical properties and fate values used in the environmental fate modeling to estimate the PEC values for the surrogate trenbolone compound. The surrogate trenbolone compound (composite of 17β-trenbolone, 17α-trenbolone, and trendione) was found to have a moderate binding potential and a rapid degradation rate in soil. In addition, the surrogate trenbolone compound is expected to degrade in water-sediment environments (Table 13). Thus, the surrogate trenbolone compound is expected to be neither highly mobile in terrestrial systems nor persistent in either the terrestrial or aquatic environments. The published literature and Zoetis-owned study data used to derive the physical-chemical properties and fate values listed in Table 13 are summarized in detail in Section 4.2.6.

Table 13. Physical-Chemical and Environmental Fate Parameters Used in the Exposure Assessment and Environmental Fate Modeling of Surrogate Trenbolone Compound

Parameter	Value Selected for Modeling	Comments/ Reference
Excretion rate from cattle	71.5%	Section 4.1.2
Molecular weight adjustment for difference between TBA and trenbolone	86.55%	Base activity of TBA = MW of trenbolone/MW TBA = 270.37/312.40
Soil K_{OC}	912	Section 4.2.6.9 and Table 14
Sediment K_{OC}	912	Soil K_{OC} value used
Molecular weight	270.4	Section 4.2.6.1
Aqueous solubility (mg/L)	360	Section 4.2.6.4
Vapor pressure (Pa)	1E-7	Section 4.2.6.3
Hydrolysis	Not Used	Assumed no hydrolysis occurs in the environment
Soil DT_{50} (days)	3	0.5 day for trenbolone + 2.5 days for trendione = 3 days Table 15
Aerobic water-sediment DT_{50} (days)	53.3	Sum of (17α-trenbolone + 17β-trenbolone + trendione) in total water-sediment system, Section 4.2.6.12
Anaerobic water-sediment DT_{50} (days)	191	Sum of (17α-trenbolone + 17β-trenbolone + trendione) in total water-sediment system, Section 4.2.6.12
Aquatic direct photolysis DT_{50} (days)	Not Used	Assumed no photodegradation in the environment; however, 0.5 days is reported in 1 non-GLP study in Section 4.2.6.7
Feedlot and manure storage degradation rate DT_{50}	Not Used	Assumed no degradation in manure

Additional environmental fate parameters for trenbolone and metabolites not required for the environmental fate models can be found in Section 4.2.6 and Appendix 12. Summary tables of these additional parameters are not provided because there was no need to calculate conservative 90% confidence bounds for these parameters.

4.2.6. Presentation of studies used to derive the environmental fate parameters for the surrogate trenbolone compound

This section summarizes the published literature and Zoetis-owned study data for the physical-chemical properties and environmental fate values used in modeling the fate and exposure of trenbolone in the terrestrial and aquatic environments. Summaries of the studies and the methods used to derive the input parameters are provided below. The final input parameters used in modeling are summarized in Table 13.

4.2.6.1. Molecular weight (MW)

The MWs of 17α-trenbolone, 17β-trenbolone, and trendione are 270.37, 270.37, and 268.35 [35, 36], respectively. **For modeling the surrogate trenbolone compound, a MW of 270.4 was used**.

4.2.6.2. Melting point

The melting points of 17α-trenbolone, 17β-trenbolone and trendione were determined to be 145.6, 145.6, and 146.3°C, respectively, using EPIweb [35]. The Merck Index [37] reports a melting point of approximately 183-186°C for 17β-trenbolone. Syntex, the previous owner of the Synovex product line, also conducted a melting point study and reported a melting point for 17α-trenbolone of 92-94.3°C (page 74 of Syntex 1995 EA [74]). However, melting point is not an input parameter used in the environmental fate modeling; thus, a representative value was not selected.

4.2.6.3. Vapor pressure

The vapor pressures of 17α-trenbolone, 17β-trenbolone and trendione were determined to be 2.48E-06, 2.48 E-06 and 2.82E-04 Pa, respectively, using EPIweb [35]. **However, trenbolone is not expected to be a volatile compound; thus, for modeling, a conservative low vapor pressure of 1E-7 Pa was used for modeling the fate of the surrogate trenbolone compound.** This is considered conservative because less of the surrogate trenbolone compound would be partitioned into the air, therefore resulting in a higher concentration in soil and water.

4.2.6.4. Aqueous solubility

Syntex, the previous owner of the Synovex product line, conducted a water solubility study and reported a water solubility for 17α-trenbolone of 360 mg/L (page 74 of Syntex 1995 EA [74]). In addition, the aqueous solubilities of 17α-trenbolone, 17β-trenbolone, and trendione were estimated to be 324, 324, and 281 mg/L, respectively, using EPIweb [35]. **For modeling purposes, a water solubility value of 360 mg/L was used for the surrogate trenbolone compound.** This concentration is well above the concentration of trenbolone that is expected to be applied to the soil in manure (Appendix 3 through Appendix 5); thus, this value is considered conservative because the model will not be limited by the amount of trenbolone that can be solubilized in surface runoff.

4.2.6.5. Dissociation constant (pKa)

17α- and 17β-Trenbolone have a single -OH group at the 17 position of the steroid ring that is not easily dissociated. The predicted pKa for 17α- and 17β-trenbolone is 14.73, which was calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2010 ACD/Labs) from within SciFinder® [36]. Therefore, 17α- and 17β-trenbolone are

not expected to be dissociated at environmentally relevant pH values. Trendione does not have a dissociable group, and therefore no pKa value can be estimated. Because the predicted pKa was estimated to be high relative to environmental pH values and a pKa value is not used in the environmental fate modeling, a pKa study using 17α-trenbolone was not conducted.

4.2.6.6. UV-absorbance spectra

The UV-VIS-spectra of 17β-trenbolone is shown in the photolysis study conducted by Gryglik et al. [75]. The absorption peaks at approximately 250 and 350 nm closely match the absorption spectra for 17α-trenbolone determined in a photolysis study conducted by Syntex [235] (see Appendix 13.5 for the executive summary). UV-VIS spectra are not used in the environmental fate modeling.

4.2.6.7. Photodegradation

There are several published and Zoetis-owned studies that investigated the photodegradation of the trenbolone metabolites. These studies are summarized below.

Presentation of Studies

Syntex conducted a photolysis study for 17α-trenbolone that followed the OECD 316 guideline for the methods, conduct, and analysis of the study. However, the study was not monitored or inspected, and therefore was not conducted under GLP compliance. The study used a three-phase approach (following the logic tree in Figure 1 of the OECD guideline) to develop the definitive study where the final quantum yield and photolysis rate constants were determined. 17α-Trenbolone was shown to be rapidly transformed by sunlight. After adjustment of the quantum yield with a known actinometer standard, the photodegradation DT_{50} of 17α-trenbolone was determined to be 0.169, 0.355 and 0.608 days in summer, fall and winter, respectively, in sunlight at a latitude of 40°N (Table 14 of reference [235]). The quantum yields were determined to be 3.3E-04, 3.1E-04, and 2.9E-04, respectively. Additional information on the methods and results of this study is found in Appendix 13.5.

Qu et al. [76] conducted simulated photolysis in the daylight (12 hours) and dark (12 hours) over 72 hours at varying pH and temperatures. Samples were collected during daylight and night-time hours to determine photoproduct stability across a range of conditions in surface waters. Samples were analyzed for 17α-trenbolone and 17β-trenbolone by liquid chromatography-high resolution tandem mass spectroscopy (LC-HRMS/MS) and NMR to characterize resulting product structures and the potential for persistent bioactivity. The authors found that 17α-trenbolone decayed during daylight, but a portion regenerated during the 12-hour dark period (approximately 15% of initial mass). A similar response was found with 17β-trenbolone but at a reduced reversion (approximately 1% of initial mass). The transport of trenbolone metabolites from the photic zone in surface waters to the darker regions of a water body (e.g., sediments) was also simulated by photolyzing solutions with 17α-trenbolone, 17β-trenbolone, and trendione and then storing the waters in the dark for 5 days at 25°C. After 120 hours, 17α-trenbolone exhibited substantial reversion to over 60% of the initial mass, for which 17β-trenbolone and trendione yields were much lower at 10% reversion of the initial mass. The rate and total reversion were highly dependent on pH and temperature, with higher reversion occurring in mildly acidic or alkaline waters (increase in reversion at pH 5 and 9, but much greater increase at pH 2 and 12) and at warmer temperatures (30-fold increase in reversion when temperatures are increased from

5 to 35°C). Finally, the authors examined the reversion in agricultural waters by dosing a small collection pond on a rangeland with manure from TBA implanted cattle. 17α-Trenbolone concentrations decreased approximately 50% during the daylight. Samples were collected and stored in the dark at 35°C in the laboratory. Reversion was observed in samples stored at 35°C (from 7 to 20 ng/L), whereas there was no difference in samples stored in the dark at 1°C. When samples stored at 1°C were warmed to 35°C after 24 hours, 17α-trenbolone concentrations increased (reversion to 15 ng/L). This study demonstrates that photodegradation of trenbolone metabolites may be somewhat reversible. See additional comments regarding the potential for reversion in Section 4.2.7.

The rapid photolysis of 17β-trenbolone in water was also demonstrated in two other photodegradation studies; however, these were not conducted under natural sunlight irradiance (1 Sun unit).

- Gryglik et al. [75] investigated the photodegradation kinetics of boldenone and 17β-trenbolone in aqueous solutions at 254 nm irradiation and at pH values of 5, 7, and 10. Quartz tubes containing $5x10^{-6}$ to $1x10^{-4}$ M trenbolone were exposed to a photon fluence rate from 11.8 to 29.1 W/m^2, equivalent to 0.012 to 0.029 Sun units, assuming 1 Sun unit = 1000 W/m^2 [44]. The concentration of 17β-trenbolone at the higher fluence rate was halved in approximately 30 minutes and displayed only a very weak or no influence of pH. It was determined that exposure to a level of UV irradiation considerably lower than a full Sun unit nevertheless lead to the rapid destruction of 17β-trenbolone.
- Bledzka et al. [77] measured the elimination of 17β-trenbolone from aqueous solutions by photolysis at 254 nm irradiation. For the direct photolysis test, the concentration range of 17β-trenbolone was 0.51-10 x 10^{-5} M. The reaction solutions were exposed to a xenon arc lamp, which simulated solar radiation. The initial concentration of 17β-trenbolone was halved after 26 minutes of the reaction. As was the case in the Gryglik et al. [75] study, the decomposition of 17β-trenbolone was virtually independent of the pH of the reaction solutions.

Summary of Photodegradation

These data collectively support the rapid phototransformation of trenbolone in aquatic systems. Although the turbidity and depth of some water bodies can limit the effect of photodegradation, this process could be a significant removal path in shallow streams and creeks with clear water. **Although photodegradation was shown for 17α-trenbolone and 17β-trenbolone and the environmental fate models adjust the photodegradation rate for sunlight depth penetration and turbidity in the water column for different regions, a photodegradation value was not used in the modeling of the surrogate trenbolone compound to be conservative. It was conservatively assumed that no photodegradation occurs.**

4.2.6.8. Octanol/Water partition coefficient (K_{OW})

Khan et al. [78] determined the log K_{OW} of 17α-trenbolone, 17β-trenbolone and trendione to be 2.72, 3.08 and 2.63, respectively (mean of 2.81). The log K_{OW} values are <4, suggesting that the surrogate trenbolone compound is not expected to bioaccumulate (VICH Phase II Guidance [79]). In the PRZM and EXAMS models, the log K_{OW} can be used as an estimate of adsorption; however, the soil K_{OC} is known and is a preferred parameter to use instead of K_{OW}. Therefore, a K_{OW} value was not used in the modeling.

4.2.6.9. Adsorption to soil (soil K_{OC})

There was only one literature study on the adsorption of trenbolone metabolites published. Thus, Pfizer Inc. conducted two soil degradation studies in accordance with OECD Guideline 106 [50] using 17α-trenbolone and trendione. The published literature and the Zoetis-owned soil adsorption studies are summarized below.

Presentation of Studies

Pfizer Animal Health, currently known as Zoetis, conducted two studies following the OECD Guideline 106 (Adsorption-Desorption Using a Batch Equilibrium Method) to determine the soil K_{OC} of 17α-trenbolone [237] and trendione [238] in five varying soil types. The adsorption and desorption isotherm experiments for 5-[^{14}C]-17α-trenbolone and 5-[^{14}C]-trendione were conducted in duplicate at five nominal concentrations of 0.010, 0.050, 0.100, 0.503 and 1.02 mg/L using the batch equilibrium method. The properties of the test soils are listed in Appendix 13.7. The experiments were conducted at an optimal soil to solution ratio (1:5) at equilibrium (4 hours for adsorption and 24 hours for desorption determined during an adsorption kinetics study). The log K_{OC} of 17α-trenbolone was determined to be 2.78, 2.61, 2.44, 2.69 and 2.71 in clay, sandy clay loam, sandy clay loam, sandy loam, and clay loam, respectively [237]. The log K_{OC} of trendione was determined to be 3.42, 3.15, 3.18, 3.36 and 3.09 in clay, sandy clay loam, sandy clay loam, sandy loam, and clay loam, respectively [238]. These studies are described in more detail in Appendix 13.7 and Appendix 13.8 and include soil desorption data. The potential for 17α-trenbolone and trendione to desorb was also evaluated in these studies [237, 238]. The mean percent desorption values for 17α-trenbolone and trendione were 9.35% and 6.64%, respectively. The model used in this EA (PRZM-3) to predict environmental concentrations does not use a desorption parameter; however, desorption from soils is expected to be low.

Khan et al. [78] determined the K_{OW} and K_{OC} of 17α-trenbolone, 17β-trenbolone and trendione in five sterilized soils using methods similar to those recommended in OECD Guideline 106 [50]. Sorption of 17α-trenbolone, 17β-trenbolone and trendione was measured by independently quantifying the concentrations in the solution and sorbed phases. Concentrations were quantified using LC with electrospray mass spectrometry. Log K_{OC} values for 17α-trenbolone in the five soils were 2.65, 2.92, 2.68, 2.87, and 2.74. Log K_{OC} values for 17β-trenbolone in the five soils were 2.99, 3.22, 3.05, 3.15, and 2.99. Log K_{OC} values for trendione in the five soils were 3.63, 3.5, 3.36, 3.29, and 3.13. Khan et al. [78] determined that hydrophobic partitioning to the organic carbon was the major sorption mechanism of the trenbolone metabolites.

Summary of Soil K_{OC}

The log K_{OC} values from Kahn et al. [78] and Pfizer [237, 238] are presented in Table 14 and were used to derive a log K_{OC} value to input into the environmental fate modeling for the surrogate trenbolone compound. The lower confidence bound was conservatively selected for the K_{OC} because a lower K_{OC} would result in a higher concentration of the surrogate trenbolone compound entering the surface water through runoff, ultimately resulting in higher PEC_{water} estimates. **Thus, the mean lower 90th percentile confidence bound of 2.96 was used as a conservative estimate of the log K_{OC} (K_{OC} of 912).**

Table 14. A Summary of Log K_{OC} Values for 17α-Trenbolone, 17β-Trenbolone, and Trendione and the Calculation of the Lower 90th Percentile Confidence Bound on the Mean Log K_{OC} for the Surrogate Trenbolone Compound

Hormone	Log K_{OC}	Mean	SD	n-1	T_{90}	Lower Log K_{OC} 90th percentile confidence bound
17α-Trenbolone	(2.65, 2.92, 2.68, 2.87, 2.74)[a] (2.78, 2.61, 2.44, 2.69, 2.71)[b]	2.71	0.135	9	1.383	2.65
17β-Trenbolone	(2.99, 3.22, 3.05, 3.15, 2.99)[a]	3.08	0.102	4	1.533	3.01
Trendione	(3.63, 3.5, 3.36, 3.29, 3.13)[a] (3.42, 3.15, 3.18, 3.36, 3.09)[c]	3.31	0.176	9	1.383	3.23
Log K_{OC} mean lower 90th percentile confidence bound						**2.96**
Soil K_{OC} used for modeling						**912**

[a] Data From Kahn et al., 2009 [78], [b] Pfizer [237], [c] Pfizer [238].

Additional Supporting Information

In a study evaluating the difference in binding and partitioning coefficients of 17α and 17β isomers of trenbolone and estradiol to commercial DOC, Qiao et al. [49] reported the 17α isomers of both trenbolone and estradiol exhibited lower binding coefficients than the 17β isomers. This trend is also evident for the K_{OC} estimates presented in Table 10 for estradiol metabolites and Table 14 for trenbolone metabolites, respectively.

4.2.6.10. Adsorption to sediment (sediment K_{OC})

The sorption properties of a compound may be different in sediment and soil due to variations in their organic matter compositions. It is expected that more binding will occur in sediment than in soil; thus, the EPA EXPRESS software documentation recommends using 2X the soil K_{OC} to estimate the sediment K_{OC} (page 29 of reference [80]). **However, for modeling purposes, the soil K_{OC} (912) reported in Table 14 was conservatively used because the sediment K_{OC} for trenbolone was not experimentally determined.** Using the soil K_{OC} is considered more conservative because it will assume more of the surrogate trenbolone compound is available in the water phase (i.e., a higher PEC_{water}), which is the compartment of interest for potential endocrine disrupting effects.

4.2.6.11. Transformation and degradation in soil (soil DT_{50})

Two published literature studies were found that evaluated the potential transformation and degradation of 17β-trenbolone, 17α-trenbolone, and trendione in aerobic soils. The methods used in these studies were similar to OECD Guideline 307 [59] (Aerobic and Anaerobic Transformation in Soil). The results obtained in the two studies were consistent; thus, the data shown in Table 15 were used to derive a DT_{50} value for the surrogate trenbolone compound in soil. The two degradation studies are summarized below.

Presentation of Studies

Khan et al. [25] investigated the degradation of 17β-trenbolone, 17α-trenbolone, and trendione at varying concentrations in three soils with varying characteristics: two were clay loam (Drummer) and one was a sandy soil (Coloma). The authors also investigated the potential degradation of trenbolone metabolites in autoclaved (abiotic soils) and with the addition of manure. Approximately 5 g of soil were placed in 40 mL or 120 mL glass flasks, and filter-sterilized deionized water was added to adjust the soil moisture content to field

capacity. Moist soils were preincubated at 22°C for 72 hours prior to adding the test chemical. Trenbolone metabolites were added to soils with ethanol as a carrier solvent (3-5 µL) and manual mixing of soil to reach approximately 0.05, 0.1, 1.0, 7.0, and 10 mg/kg soil for 17β- and 17α-trenbolone and 0.04, 3.0, and 3.5 mg/kg soil for trendione. Both 17β-trenbolone and 17α-trenbolone rapidly degraded in soil by similar pathways with DT_{50} estimates of <12 hours [25]. Less than 5% and 3% of 17β-trenbolone and 17α-trenbolone, respectively, were remaining at Day 3. The degradation rate of 17β- and 17α-trenbolone decreased with increasing metabolite concentrations (10-fold decrease between 0.1 and 10 mg/kg); however, the DT_{50} was still <2 days. Trendione, whether produced from trenbolone degradation or directly added to soil, persisted longer than trenbolone with a DT_{50} of one to three days in soil. 17β- and 17α-Trenbolone were also transformed to trendione in autoclaved soil, but degradation did not occur for three days. The authors suggested that the onset of degradation in the abiotic controls was likely due to new microbial growth from microbes inadvertently introduced during hormone addition. The slower initial degradation in the autoclaved soils suggests that the degradation of trenbolone metabolites is mainly microbial. The study also assessed the potential interconversion of isomers and found that <1.5% of the trendione was converted back to 17β-trenbolone. No conversion of 17β-trenbolone to 17α-trenbolone was observed; however, <1% of 17α-trenbolone was converted to the 17β isomer. The addition of manure did not affect the degradation rate of 17α-trenbolone, but the addition of manure enhanced the subsequent degradation of trendione. These data support a DT_{50} in soil of ≤0.5 days for 17α- and 17β-trenbolone and ≤3 days for trendione.

Khan and Lee [81] examined the effects of temperature (5-35°C) and soil moisture content (3-26%) on the degradation of 17α-trenbolone, 17β-trenbolone and trendione in two soils (clay loam and sandy loam) amended with manure. The degradation rates in soil under optimal conditions (25°C and 5-22% moisture) were consistent with the previous study [25]. Degradation rates decreased with a reduction in moisture content and temperature because these conditions limit the microbial activity in the soil and manure. However, even under less optimal conditions (low temperatures and moisture content), it was demonstrated that 17α-trenbolone, 17β-trenbolone, and trendione still degraded relatively quickly (<3 days). The degradation rates estimated for manured soil in this study were consistent with the degradation rates estimated in unmanured soil (Table 15). Soil DT_{50} values for 17α-trenbolone and 17β-trenbolone ranged from <1 to *ca*. 2 days (range 4-50 hours). Soil DT_{50} values for trendione were <3 days at 22-35°C; however, the DT_{50} values in the colder soil incubations at 5°C resulted in a longer DT_{50} ranging from approximately 6-9 days (total range of DT_{50} values for trendione in all incubations tested 10-225 hours). Additionally, the authors found that <1% of 17α-trenbolone interconverted to 17β-trenbolone. Overall, the degradation rates in this study were similar to the previous study [25], with DT_{50} values <3 days under most conditions.

Summary of Degradation in Soil

Using a weight of evidence approach, all summarized literature data were used to determine DT_{50} values for 17α-trenbolone, 17β-trenbolone, and trendione. Data for cold and dry conditions from Khan and Lee [81] were excluded (Table 15) because these conditions represent extremes not typically included in a standard soil biodegradation study. The environmental fate models used in this EA to estimate PEC concentrations adjust the degradation rates in soil to changes in temperature and moisture. In addition, the DT_{50} data from Khan et al. [25] where the initial concentration was excessively high (≥7 mg/kg) were

not used in the derivation of a DT_{50} value for the surrogate trenbolone compound because these concentrations are much higher than what would be found for the anticipated application rates in manure. However, the degradation rates from the soil concentrations of ≤1 mg/kg trenbolone were included.

The conservative upper 90th percentile confidence bound DT_{50} estimates for trenbolone and trendione in soil are 0.5 and 2.5 days, respectively (Table 15). The use of the upper confidence bound to derive the DT_{50} values is conservative because it assumes the surrogate trenbolone compound is available for a longer period of time to runoff into surface waters. Further, to determine the soil DT_{50} value for the surrogate trenbolone compound, the final DT_{50} values for 17β-trenbolone, 17α-trenbolone, and trendione were summed to ensure that dissipation of all potential trenbolone metabolites were represented. Thus, a soil DT_{50} value of 3.0 days for the surrogate trenbolone compound was used in the environmental fate modeling [0.5 days + 2.5 days = 3.0 days].

Table 15. Parameter Assignments for Trenbolone and Trendione Degradation Rates in Soil

Data from Khan et al. 2008 [25], excluding trenbolone concentrations ≥7 mg/kg of soil						
Soil	**Hormone**	**Initial conc., mg/kg**	**Trenbolone Half-Life (h)**	**Mean (h)**	**Trendione Half-life (h)**	**Mean (h)**
D30	17β-TB	0.1	4.4	7.14	16.6	40.7
		1	9.3, 8.9		75, 35	
	17α-TB	0.1	4.2		13.1	
		1	8.9		64	
D36	17α-TB	0.05	4, 3.8	3.9	15, 10	12.5
C32	17β-TB	0.1	7.4	9.9	49	65.2
		1	12, 11		48, 87	
	17α-TB	0.1	8		42	
		1	11		100	
D30	Trendione	0.04			24	74
		3			100	
		3.5			98	
Data from Khan and Lee 2010 [81]: only those data from 25°C and -0.1 to -0.5 MPa moisture are used						
D36	17α-TB	0.05	7, 8	7.5	29, 71	50
C39	17α-TB	0.05	11,19	15	12, 38	25
			Mean	8.69 (0.36 d)	**Mean**	44.6 (1.86 d)
			SD	4.12	SD	23.4
			n-1	4	n-1	5
			T_{90}	1.533	T_{90}	1.476
DT_{50} Upper 90th percentile confidence bound				**11.5 h (0.5 d)**		**59 h (2.5 d)**
Conservative DT_{50} upper 90th percentile confidence bound for TBA soil metabolites = 0.5 days + 2.5 days = 3.0 days						

Based on published data, two studies [25, 81] consistently demonstrated rapid biodegradation of 17α-trenbolone and 17β-trenbolone to trendione and subsequent degradation of trendione, even under less optimal conditions (low and high temperatures and moisture contents). These studies also demonstrated similar degradation rates for the 17α and 17β isomers, and minimal interconversion. The pathway and rate of degradation of trenbolone and its metabolites in soil are consistent with the pathway and rate of estradiol in soil (Section 4.2.4.11). Additional environmental variables such as the presence of antibiotics could slow degradation, and the presence of manure (in the case of estradiol) could stimulate degradation.

Additional Supporting Information

In a series of soil column elution studies, 17α-trenbolone and 17β-trenbolone were shown to oxidize rapidly to trendione with a small amount of epimerization from the 17β to the 17α isomers [24]. The trenbolone metabolites absorbed strongly to soil; however, a K_{OC} value was not reported. The high affinity of trenbolone for organic matter resulted in a high impediment to movement from the upper layers of the soil column. This study agrees with the SCI-GROW estimates which determined that minimal leaching of trenbolone from soils is expected to occur (Section 5.3).

4.2.6.12. Aerobic and anaerobic water-sediment transformation

There are no published literature studies that evaluated the partitioning, transformation, and degradation of 17α-trenbolone, 17β-trenbolone, and trendione in water-sediment systems. Thus, Pfizer Inc. conducted an aerobic water-sediment system degradation study with 17α-trenbolone using the methods recommended under OECD Guideline 308 [68] (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems). The results of this Zoetis-owned study were used to derive the aerobic water-sediment DT_{50} values for the surrogate trenbolone compound shown in Table 16. Due to a lack of degradation data for the trenbolone metabolites in anaerobic water-sediment systems, the aerobic DT_{50} value was multiplied by 3.6 to determine an acceptable anaerobic DT_{50} value for the total system. This method agrees with EPA's guidance for selecting input parameters for the EXAMS model (Section 4.2.2). An explanation of the methods used to determine an anaerobic DT_{50} value is provided below under "Derivation of Anaerobic Degradation Half-Life."

Summary of Pfizer Aerobic Degradation Study

A GLP OECD Guideline 308 study was conducted by Pfizer using [^{14}C]-17α-trenbolone under aerobic conditions [241] (Appendix 13.11). The study used two water sediment systems from Massachusetts (Taunton River and Weweantic River), representing a high and low organic content, respectively. Trenbolone was dosed in the water at 1 mg/L, which was a higher concentration than would be found naturally. This provided sufficient analytical sensitivity to determine degradation products. The disappearance of [^{14}C]-17α-trenbolone and the production of [^{14}C]-17β-trenbolone and [^{14}C]-trendione were measured in both the water and sediment phase. In addition, the mineralization of 17α-trenbolone was also measured by production of $^{14}CO_2$. 17α-Trenbolone was primarily converted to trendione and a small quantity of 17β-trenbolone (maximum average[p] percentage of 1.89%). Both trendione and 17β-trenbolone were primarily found in the sediment phase, with very little of these transformation products detected in the water phase. From the production of these

[p] The 'maximum average' value is the maximum reported value (from both sediments) of all of the averaged values determined (average of 2 replicates) at each of the time points for each of the transformation products.

transformation products, the DT_{50} values for trendione and 17β-trenbolone were determined, along with the DT_{50} of parent 17α-trenbolone. The DT_{50} values for 17α-trenbolone, 17β-trenbolone, trendione and the total drug (17α-trenbolone + 17β-trenbolone + trendione) are summarized in Table 16 below. Additional details for the methods and results of this study are available in Appendix 13.11.

Table 16. Degradation Half-lives (DT_{50}) of [^{14}C]-17α-Trenbolone, 17β-Trenbolone, Trendione, and the Total Drug in Aerobic Water-Sediment Systems Following OECD Guideline 308

Aqueous Phase + Sediment Phase	DT_{50} (days)			
	17α-Trenbolone	Total Drug*	17β-Trenbolone	Trendione
Aerobic Incubation				
Taunton River	21.2	34.7	9.1	6.6
Weweantic River	46.5	**53.3**	14.0	2.8

* Total Drug = sum α-trenbolone + β-trenbolone + trendione. Note that the DT_{50} of total drug is not the sum of the individual DT_{50} values, Reference [241].

For modeling the environmental fate of the surrogate trenbolone compound, the DT_{50} values from the total system (aqueous + sediment phase concentrations) were used. To represent a more conservative approach, the total drug DT_{50} of the system was calculated from the sum of the transformation products at each time point. Further, for the two river systems, the highest DT_{50} value (53.3 days) was chosen for the fate modeling instead of the mean (Section 4.2.5). This method of selecting a DT_{50} value is conservative because the total drug DT_{50} accounts for the transformation, and ultimate degradation, of all trenbolone metabolites over time in both the aqueous and sediment phases. **Therefore, a DT_{50} of 53.3 days was used for modeling aerobic degradation of the surrogate trenbolone compound in water-sediment systems.**

Derivation of Anaerobic Degradation Half-Life

An acceptable GLP anaerobic OECD Guideline 308 study for 17α-trenbolone was not available for use in the environmental fate modeling. The EPA guidance for assigning parameters to the models instructs that in the absence of data, multiplication factors can be used to estimate parameters. For example, the anaerobic soil degradation rate can be approximated by multiplying the aerobic soil DT_{50} by 2, and the sediment K_{OC} approximated by multiplying the soil K_{OC} by 2 [34]. Therefore, in the absence of an anaerobic water-sediment DT_{50}, an appropriate multiplier was determined.

Three references were identified to support the determination of an appropriate multiplier to estimate an anaerobic DT_{50} from an aerobic DT_{50} in aquatic-sediment systems. In all three of these studies, the aerobic and anaerobic sediment samples were sampled and stored according to recommendations in OECD Guideline 308. In Table 17, the anaerobic/aerobic DT_{50} ratios of 17α-estradiol, 17α-methyl testosterone, and exemestane[q] are shown. The upper 90th percentile confidence bound of this ratio was determined to be 3.6X. The upper confidence bound is a more conservative value to estimate DT_{50} values because it will result in a longer exposure to trenbolone metabolites. Therefore, the aerobic water-sediment DT_{50}

[q] Exemestane is a steroidal aromatase inhibitor (which blocks the synthesis of estrogen) that is used to treat breast cancer in humans. Exemestane was included in this survey because has steroidal properties and is structurally similar to androstenedione.

(53 days) was multiplied by 3.6X as a conservative approach to estimate the anaerobic DT_{50} of the surrogate trenbolone compound (191 days) for use in the environmental fate modeling.

Table 17. Ratio of Anaerobic/Aerobic Water-Sediment DT_{50} of Steroid Compounds

Steroid	Sediment	DT_{50} (days)*		Ratio Anaerobic/Aerobic
		Aerobic	Anaerobic	
17α-Estradiol	Taunton River	31.1	107.8	3.47
	Weweantic River	25.5	103.5	4.06
17α-Methyl testosterone	Sand	2.1	8.9	4.24
	Clay	2.8	5.3	1.89
Exemestane	Choptank River	18.7	33	1.76
	Turkey Creek	26.7	36.5	1.37
			Mean	2.80
			SD	1.27
			T_{90}	1.467
			Upper bound of confidence interval	3.6X

* References: 17α-estradiol (Appendix 13.9 and Appendix 13.10), 17α-methyl testosterone [82], Personal communication [231] with author of Exemestane publication [83]. See Section 4.2.2 for calculation of upper confidence bound.

In the EXAMS water transformation model used in this EA, the aerobic DT_{50} had a larger impact on the PEC_{water} results than the anaerobic DT_{50}. To illustrate this, Zoetis conducted a sensitivity analysis to determine the impact of the anaerobic water-sediment DT_{50} value by comparing the PEC_{water} values for the EXPRESS farm pond and Index Reservoir water bodies using an anaerobic DT_{50} of 191 days and 1000 days. The change in anaerobic DT_{50} only increased the 21-day chronic concentration by 4% in the farm pond water body and estimated no impact (0%) in the Index Reservoir water body (data not shown). This demonstrates that the anaerobic water-sediment DT_{50} has little influence on the PEC_{water} values. **Therefore, aerobic and anaerobic water-sediment DT_{50} values of 53.3 and 191 days, respectively, for the surrogate trenbolone compound were used in the environmental fate modeling.**

4.2.7. Potential for reversion or interconversion of metabolites in the terrestrial and aquatic environments

Recent data demonstrate that the both estradiol and trenbolone metabolites can undergo 'interconversion' in various environmental compartments and that trenbolone metabolites can also undergo 'reversion' under certain environmental conditions. For the purpose of this EA, 'interconversion' is defined as the process where the 17α isomer is converted to the 17β isomer and vice versa. In contrast, 'reversion' is defined as the process where a transformation product is converted back to the initial parent compound (e.g., 17β-trenbolone converted to trendione in daylight and then back to 17β-trenbolone in the dark). Because the environmental fate modeling conducted for this EA does not account for the reversion or interconversion processes, the potential for reversion and interconversion was assessed qualitatively herein.

Qu et al. [76] observed that 17β-trenbolone, 17α-trenbolone, and trendione transform when exposed to sunlight, but are able to regenerate in the dark (15-60% for 17α-trenbolone and 1-10% for 17β-trenbolone and trendione). The rate and degree of reversion was highly

dependent on pH and temperature (see description of study under Section 4.2.6.7). The environmental fate modeling conducted for this EA assumed that no photodegradation occurred. In addition, the soil and water-sediment systems degradation studies described in this EA and used in the environmental fate modeling for the surrogate compounds were conducted entirely in the dark (i.e., no light penetration of the microcosms) and over a longer time period than 72 hours (3 days up to 150 days). Thus, any reversion that occurred during this period of time should be integrated into the degradation rates from these studies. Based on the above information, reversion of surrogate compounds was likely accounted for during the studies used to derive the DT_{50} values for the surrogate estradiol and trenbolone compounds.

Interconversion has also been demonstrated to occur between 17α-trenbolone and 17β-trenbolone, as well as with 17α-estradiol and 17β-estradiol in soil degradation studies (Sections 4.2.4 and 4.2.6). Figure 6 and Figure 7 illustrate this potential pathway. This process was observed in the water-sediment studies conducted by Pfizer with 17α-estradiol [239] and 17α-trenbolone [241] (Sections 4.2.4.12 and 4.2.6.12, respectively). Both studies were initiated with the 17α isomer only; however, a minor amount of the 17β isomer was detected in the systems. Approximately 1.89% of 17α-trenbolone was interconverted to 17β-trenbolone; whereas, 1.4% and 5.8% of 17α-estradiol was interconverted to 17β-estradiol in the water and sediment, respectively. This process was also observed in soils for the trenbolone metabolites. Khan et al. [25] reported that <1% of 17α-trenbolone interconverts to 17β-trenbolone, while no interconversion of 17β-trenbolone to 17α-trenbolone was found. Khan and Lee [81] found similar results with approximately 1% of 17α-trenbolone interconverted to 17β-trenbolone. However, interconversion of the 17α and 17β isomers of estradiol was not observed in the soil studies reported in Section 4.2.4.

Although reversion and interconversion may occur in the soil, water, and sediment systems, these process should be reflected in the degradation rates derived in Sections 4.2.4 and 4.2.6 because a total drug approach was used.

4.3. Conclusions of Fate Data

A summary of the physical-chemical properties and environmental fate data used in the environmental fate modeling of the surrogate estradiol compound and surrogate trenbolone compound are presented in Table 9 and Table 13, respectively. The studies discussed in Section 4.2 (Fate Data) have demonstrated that estradiol metabolites (17α-estradiol, 17β-estradiol, estrone) and trenbolone metabolites (17α-trenbolone, 17β-trenbolone, trendione) bind moderately to soils and are rapidly degraded in soil, with conservative half-lives of <3 days. In the degradation studies conducted in soil, both 17α-estradiol and 17α-trenbolone were principally converted respectively to estrone and trendione prior to further environmental degradation. In addition, all degradation studies demonstrated that the degradation of estradiol and trenbolone metabolites was microbially-mediated. Many studies in the literature have also identified the potential for interconversion and reversion of trenbolone and estradiol metabolites in the environment. The degradation studies (both in soil and water-sediment systems) presented in this EA report that only a minor amount of the 17α isomers were converted to the 17β isomers (<3%) in soil. Any interconversion or reversion that may occur in the terrestrial or aquatic environments should be accounted for in the degradation rates from these studies because a total drug approach was used.

Although numerous fate variables are listed in Table 9 and Table 13, not all of these influence the environmental fate modeling. Because the concentrations of the surrogate compounds introduced into the environment are well below their solubility limits, solubility has little influence on their transport in environmental fate models. In addition, the surrogate estradiol and trenbolone compounds are not known to be volatile, so vapor pressure will not influence environmental loss. The parameters exerting the greatest influence on the environmental fate are the soil K_{OC} and the degradation or disappearance rates (expressed as DT_{50} values) in soil and water. The soil K_{OC} values for surrogate estradiol and trenbolone compounds indicate that they will bind moderately and partition to soil and sediment. The DT_{50} in soil for surrogate estradiol and trenbolone compounds was relatively rapid (approximately three days), indicating that EB and TBA metabolites contained in manure or feedlot runoff are expected to be degraded rapidly when they reach the soil system. However, the transformation rate in aerobic water/sediment systems (31-53 days) was appreciably slower than in soil, but still relatively rapid.

The values listed in Table 9 and Table 13 for the surrogate estradiol and trenbolone compounds, respectively, were used as input parameters for the environmental fate modeling discussed in Section 5.

5. ANALYSIS: EXPOSURE ASSESSMENT

The potential exposures of the surrogate estradiol and trenbolone compounds in terrestrial and aquatic environments were determined using: 1) traditional simple calculation methods that do not take into account most environment fate or transport processes, except adsorption to soils (Section 5.2), and 2) more realistic and complex leaching and surface runoff and erosion models available from EPA and independently developed (Sections 5.3 through 5.7) that simulate farm-scale and watershed-scale scenarios using environmental fate properties presented in Table 9 and Table 13 (Section 4.2).

As stated in Section 3.3, the primary compartment of concern for this exposure assessment was surface waters because fish and amphibians have been identified as the most sensitive non-target organisms for EDCs (see Section 6 for additional information on effects on fish and amphibians). The potential sources of the surrogate estradiol and trenbolone compounds contributing to surface water concentrations are outlined in Section 3.4, and a conceptual model is illustrated in Figure 5. The potential exposure pathways that were evaluated in this exposure assessment include:

1. **Feedlots:** EB and TBA metabolites contained in the manure excreted by beef cattle on feedlots may migrate to a receiving water body through surface runoff and erosion from feedlots with <1000 AU that do not have adequate runoff controls in place.
2. **Croplands:**
    a. Manure from the feedlots is collected and stored for application to the fields to be used as fertilizer. The manure can be applied to the surface as broadcast (i.e., no-till) or tilled into the land. Runoff and erosion from fields applied with manure is a source for EB and TBA metabolites to enter a receiving water body.
    b. Feedlots with >1000 AU (CAFO) are required to contain the runoff from the feedlots utilizing runoff control structures such as holding ponds or lagoons. Runoff collected in these holding ponds or lagoons is mixed with irrigation water and applied to croplands. Runoff and erosion from such croplands is also a source for EB and TBA metabolites to enter a receiving water body.
3. **Pasture:** Beef cattle grazing on the pasture excrete manure on the surface of the pastureland. Runoff and erosion from surface of the pasture is also a source for EB and TBA metabolites to enter a receiving water body.
4. **Leaching:** When manure is applied to soil, EB and TBA metabolites have the potential to leach through the soil layers to reach the groundwater. The following sources have the potential for EB and TBA metabolites to leach: cropland amended with manure, tile drained cropland amended with manure, unlined storage ponds, unpaved AFOs, and pastureland.

In order to evaluate the potential for the surrogate estradiol and trenbolone compounds to enter the terrestrial and aquatic environment, the potential concentrations of drug excreted in cattle manure were estimated in Section 5.1. These drug concentrations were used in the traditional calculations in Section 5.2 and the environmental fate modeling in Sections 5.3 through 5.7 to estimate the PEC_{water} values.

Traditional mathematical models and three EPA models were used to simulate the fate and exposure of the surrogate estradiol and trenbolone compounds in the terrestrial and aquatic environments at an individual farm-scale and an aggregate watershed-scale from the use of Synovex ONE. The methods used to estimate the PEC values for each exposure pathway are summarized below and are presented in detail in Sections 5.2 through 5.7.

1. **Traditional Mathematical Models (Section 5.2):** The initial conservative PEC estimates were obtained using traditionally-accepted calculations, including a subset that considered the nutritional needs of the crop to be manured (i.e., based on nutrient management planning). These calculations do not account for the environmental fate and mobility of the surrogate estradiol and trenbolone compounds, except for adsorption to soil; thus, additional environmental fate modeling was necessary.
2. **Leaching (Section 5.3):** The EPA SCI-GROW and PRZM-3 models were used to estimate the potential leaching of the surrogate estradiol and trenbolone compounds from the soil surface (i.e., a feedlot or cropland) to groundwater (PEC_{GW}).
3. **Runoff from Manure Amended Soils (Section 5.4):** The EPA EXPRESS model was used to assess the erosion and runoff of the surrogate estradiol and trenbolone compounds from manure applied to a field to be planted with a crop. In all, 34 till and 17 no-till crop scenarios were included.
4. **Leaching and Runoff from Tile-Drained Fields (Section 5.5):** The EPA EXPRESS model was used to estimate the potential leaching of surrogate estradiol and trenbolone compounds in tile-drained cropland by determining the leachate concentration at a 1-m depth below the soil.
5. **Runoff from Pastureland (Section 5.6):** The EPA PRZM-3 and EXAMS models were used to estimate the potential erosion and runoff from cattle pastureland.
6. **Aggregate Exposure in Five Watersheds (Section 5.7):** The EPA PRZM-3 model was modified to estimate the potential aggregate exposure due to multiple sources of erosion and runoff within a mixed-use watershed (i.e., surface runoff from pastureland, cropland, and feedlots). The daily PRZM-3 results were imported into EXAMS to estimate the watershed PEC_{water} values. Mixed-use watersheds in five states (i.e., Iowa, Texas, Michigan, Ohio, and Pennsylvania) were simulated to assume that there are simultaneous aggregate exposures of the surrogate estradiol and trenbolone compounds entering the surface water. Additional information on assumptions and methods used for the watershed modeling of an aggregate exposure are also provided in Modeling Report contained in Attachment 1.

The results of the model simulations are reported below; these results were used in the risk characterization to determine the potential risk associated with the use of Synovex ONE in steers and heifers. See Section 7 for additional details regarding the risk characterization of the surrogate estradiol and trenbolone compounds. The conceptual models in Figure 8 and Figure 9 provide additional information on the approach to the exposure assessment.

As stated above, the methods and assumptions for modeling each of the potential exposure pathways are described in detail in Sections 5.2 through 5.7. However, there were conservative high-level assumptions that were used when modeling all exposure pathways:

- A single surrogate estradiol compound and a single surrogate trenbolone compound were modeled in the exposure assessment. Rather than attempt to attribute portions of

the total drug to each metabolite in each of the environmental compartments and then to model the fate of each metabolite individually, the composite data for all of the metabolites were utilized (and in certain cases added together such as for half-lives; Figure 8 and Figure 9) and one surrogate compound was modeled. Thus, the environmental concentrations (i.e., PEC values) estimated for the surrogate estradiol compound and the surrogate trenbolone compound are conservative representations of the total residue for all three individual metabolites for each compound in the relevant compartment (e.g., leachate, soil, and water/sediment). See Section 4.2.1 for a detailed explanation of the surrogate estradiol and surrogate trenbolone compounds.

- Environmental fate parameters of the surrogate estradiol and surrogate trenbolone compounds contained in Table 9 and Table 13, respectively, were used. See Section 4.2.2 for an explanation of the methods used to derive the modeling input parameters.
- It was assumed that no degradation of the surrogate compounds occurred in manure storage systems or on the feedlot surface.
- It was assumed that every beef feedlot animal in the watershed was implanted with the Synovex ONE-F product for 365 days a year.
- It was assumed that every pasture beef animal was implanted with the Synovex ONE-G at the beginning of the pasture season and was removed from pasture when the implants were depleted.

5.1. Excretion of Drug from Cattle

To estimate the PEC values using both the traditional mathematical method and environmental fate modeling, the concentrations of the surrogate estradiol and surrogate trenbolone compounds excreted by beef cattle are needed. In this EA, two different release rates of the drug from cattle were used:

1. For the calculation of the PEC_{manure} values using the traditionally accepted method, it was assumed that 100% of the active compound was excreted from a 300 kg beef steer or heifer over a 130-day manure holding period. This method conservatively results in a higher concentration of estradiol and trenbolone over a shorter period of time (130 days) than what is expected based on the actual release period of the product (200+ days). See Appendix 3 for assumptions and calculations of the drug concentrations in manure.
2. For the environmental fate models, the daily excretion rate was estimated for feedlot cattle (Appendix 6.2) and pasture cattle (Section 5.7.8) using the known release rate of the pellets from an explant study conducted by FDAH. Details about the study are provided in Appendix 13.4. It was assumed that 100% of the amount of EB and TBA released from the implant daily was excreted as metabolites; thus, the daily excretion rates of the metabolites were calculated based on the daily release rate of EB and TBA. The known daily excretion rates of the surrogate estradiol and trenbolone compounds used for farm-scale and watershed modeling were estimated to be:
    - **Daily amounts of EB and TBA released: ONE-F** (see Appendix 6.2 for calculations)
      Estradiol is released at 0.0759 mg estradiol per feedlot animal per day
      Trenbolone is released at 0.5858 mg trenbolone per feedlot animal per day

- **Daily amounts of EB and TBA released: ONE-G** (see Section 5.7.8 for calculations)
  Estradiol is released at 0.0569 mg estradiol per grazing animal per day
  Trenbolone is released at 0.4394 mg trenbolone per grazing animal per day

5.2. PEC Calculations from Manured Crops Using Traditional and Nutrient Requirement Methodology

The initial PEC values for manure applied to cropland were calculated: 1) using traditionally accepted methods for a screening level approach and 2) based on Comprehensive Nutrient Management Planning (CNMP) requirements (application of manure based on the phosphorous needs of the crop). The $PEC_{soil\ initial}$ and $PEC_{water\ refined}$ estimates are summarized in Table 18 below. The assumptions used in these calculations are overly conservative because they do not account for most environmental fate processes. All assumptions and calculations are presented in detail in Appendix 3 and Appendix 4.

Table 18. PEC_{water} Values Using a Traditional Screening Methodology, CNMP Based on P_2O_5 Requirements of the Crops, and the Farm Scale EXPRESS Model. These Values only Represent Surrogate Estradiol and Trenbolone Compound Concentrations in Surface Waters Due to Runoff from Contaminated Manure Applied to Cropland.

Method of Estimating PEC values	Estradiol		Trenbolone	
	$PEC_{soil\ initial}$[a] (µg/kg soil)	$PEC_{water\ refined}$[b] (ng/L)	$PEC_{soil\ initial}$ (µg/kg soil)	$PEC_{water\ refined}$ (ng/L)
Traditional Screening Methodology (Appendix 3)	0.17	0.81	1.46	5.9[d]
CNMP Methodology based on Phosphorous Requirements (Appendix 4.2.1)	0.13	NA[c]	1.12	NA
EXPRESS Modeling (Section 5.4)	NA	0.08	NA	0.63

[a] $PEC_{soil\ initial}$ is the concentration of the compounds in the soil without refinement using physical-chemical and/or environmental fate data.
[b] $PEC_{water\ refined}$ is the concentration of compounds in the surface water with adjustments using the potential for the surrogate estradiol and trenbolone compounds to adsorb to organic carbon in sediment (log K_{OC}).
[c] NA represents PEC values that were not determined using the specific methodology.
[d] The trenbolone PEC_{water} is refined for 71.5% excretion in manure.

The traditional screening and the CNMP methods resulted in similar $PEC_{soil\ initial}$ values. The calculations assumed the entire amounts of EB and TBA in the implant pellets were excreted over a 130-day period (traditionally accepted methodology), whereas EB and TBA are actually released for ≥200 days (Appendix 13.4). Therefore, this resulted in a conservative overestimation because at 130 days following administration of Synovex, 51.3% of EB and 38.5% of TBA still remain in the implant pellets. Therefore, further refinement based on measured release rates of EB and TBA over the 200-day treatment period was used in the environmental fate modeling (Appendix 6).

The $PEC_{soil\ initial}$ values in Table 18 were used for comparison purposes only. They are not used in the calculation of the risk quotients in Section 7 because this is a traditional screening-level approach. It was determined that a refined exposure assessment approach using environmental fate modeling was needed due to the effects of EDCs at low

concentrations in surface waters (ng/L or parts per trillion range). Based on the need for a refined exposure assessment and the need to assess an aggregate exposure, the $PEC_{water\ refined}$ was estimated using the EPA's EXPRESS or PRZM and EXAMS models. Methods, assumptions, results, and conclusions from environmental fate modeling are presented in Sections 5.4 to 5.7.9. The $PEC_{water\ refined}$ estimates are also reported in Table 18.

The $PEC_{water\ refined}$ estimates support that the traditional screening methodology is more conservative than EXPRESS modeling methodology, which takes into account physical-chemical and environmental fate properties of these compounds. For example, the PEC_{water} values estimated using the traditional mathematical model were much higher than those determined by the EXPRESS modeling (0.81 vs. 0.08 ng/L for the surrogate estradiol compound and 5.9 vs. 0.63 ng/L for the surrogate trenbolone compound, respectively, Table 18).

5.3. Leaching to Groundwater (PEC_{GW}) Estimate Using EPA SCI-GROW

5.3.1. SCI-GROW model background

EPA's SCI-GROW (Screening Concentration in Groundwater) model was used to predict potential leaching to groundwater [17]. This model is currently used by the EPA for estimating the potential pesticide impact on groundwater in the US as a Tier-1 screening tool. SCI-GROW is expected to represent conservative values because the model is based on ten groundwater monitoring studies that were conducted by applying the pesticide at maximum allowed rates and frequency to hydrogeologically vulnerable sites with sandy soils and shallow groundwater. The input parameters for the model include: 1) application rate, 2) number of applications per year, 3) the soil K_{OC}, and 4) the soil DT_{50}. The individual parameters are shown in Table 19.

5.3.2. SCI-GROW model results

Based on the PEC_{GW} estimated by the SCI-GROW model, the surrogate estradiol and trenbolone compounds are expected to be negligible in the groundwater (PEC_{GW}) compared to the surface water (PEC_{water}), and are not expected to impact groundwater or significantly contribute to the surface water exposure through leaching (see Table 19 below).

Table 19. Leaching Estimates for the Surrogate Estradiol and Trenbolone Compounds Using Manure Application Rates, Soil Adsorption Coefficients, and Degradation Estimates for Soil

	SCI-GROW Parameters			Leaching models PEC_{GW} (ng/L)		Surface runoff models PEC_{water} (ng/L)
Surrogate Compound	Application rate (lb/acre)	Soil K_{OC} Table 10 and Table 14	Soil DT_{50} (days) Table 11 and Table 15	SCI-GROW	Highest 90th percentile EXPRESS Table 81	Highest 90th percentile EXPRESS Table 53 and Table 55
Estradiol	0.000341[a]	1259	3	0.000561	4.1×10^{-12}	0.08
Trenbolone	0.00292[b]	912	3.1	0.00479	1.9×10^{-13}	0.63

[a] Equivalent to 155.3 mg/acre
[b] Equivalent to 1326.5 mg/acre (Appendix 3.3)

Because leaching was also determined with the EXPRESS model (Appendix 7.2), the values from the EXPRESS model are shown in Table 19 along with the estimated surface water values. Both SCI-GROW and EXPRESS models estimate that leaching of the surrogate estradiol and trenbolone compounds through the soil would be a negligible addition to the surface runoff estimates. For example, PEC_{water} values for the surface runoff from manure applied to cropland for surrogate estradiol and trenbolone compounds are 0.08 and 0.63 ng/L, respectively, whereas leaching estimates from SCI-GROW were more than two-orders of magnitude below that. Leaching is not expected to be a major source of surrogate estradiol and trenbolone compounds to surface waters because both compounds have low DT_{50} values in soil and a moderate binding capacity to OC, indicating limited mobility in soils. Because the leaching of the surrogate compounds is expected to be negligible compared to the surface runoff, the leaching component was excluded from the mixed-use watershed models.

5.4. PEC_{water} from Manure Applied to Cropland Using the EPA EXPRESS Model

The fates of the surrogate estradiol and trenbolone compounds in agricultural soil and surface waters were modeled using the EPA Tier-2 pesticide assessment model, EXPRESS. The EXPRESS software interfaces with the soil fate model, PRZM-3, and the water fate model, EXAMS II. The EXPRESS model is currently used by EPA for aquatic risk assessments of pesticides. The modeling output includes predictions of both acute and chronic surface water concentrations. The rationale for choosing EXPRESS over other available fate and transport models is discussed in Attachment 1.

The EXPRESS model was used in this exposure assessment to simulate the farm-scale runoff and erosion of the surrogate estradiol and trenbolone compounds from manure applied to agricultural crops from beef cattle in confined feedlots. Because EXPRESS cannot simulate direct runoff from feedlots or pastureland, different versions of the PRZM and EXAMS models were required to model these individual and aggregate scenarios. See Section 5.7 and Attachment 1 for additional information. This section provides information on the methods, assumptions, results, and conclusions of the EXPRESS modeling of the surrogate estradiol and trenbolone compounds.

5.4.1. EXPRESS model background

The EXPRESS model was used to assess the erosion and runoff on a farm-scale from manure containing the surrogate estradiol and trenbolone compounds applied to a field to be planted with a crop. The EXPRESS model uses PRZM-3 to simulate the surface erosion and runoff from the terrestrial component of the model to the water body. The edge of field loadings estimated by PRZM are then input into the EXAMS model. The EXAMS model simulates the fate (i.e., partitioning and degradation) in the aquatic compartment to estimate exposure concentrations in surface water (i.e., PEC_{water}).

The following information on the EPA EXPRESS model (PRZM-3 and EXAMS II) is from the EPA Center for Exposure Assessment Modeling (CEAM) website [84] and the user manual [80].

PRZM-3

PRZM-3 simulates both transport and transformation of an organic chemical through the crop root and unsaturated zone and transport of surface water runoff and soil erosion.

- The model estimates the mobility and fate of a chemical in a cropped field on a day-to-day basis.
- The following factors are used in the model: daily rainfall over a 30-year period from actual weather station data for the region, temperature, sunlight, planting and growing season of regional specific crops, and how and when the chemical is applied to the field.
- There are over 80 potential crop scenarios available in EXPRESS. Each crop modeling scenario represents a unique combination of climatic conditions, crop specific management practices, soil properties, site specific hydrology, and chemical application and dissipation processes. For this assessment, 34 till and 17 no-till crop scenarios were simulated. Each crop scenario represents real soil and weather conditions of the area where the crop is grown.
- PRZM-3 allows the user to consider pulse loads and predict peak runoff events.
- The principal parameters used in this terrestrial model include soil type, slope of soil, runoff characteristics of cropped and fallow field, application depth of chemical, biodegradation rate in soil, solubility, vapor pressure and soil K_{OC}.
- The model assumes that the chemical is applied at the same time, the same concentration, and the same way every year for 30 years. However, multiple applications within a year can occur.
- The model takes into account the soil temperature to adjust the degradation rate.
- The hydrological chemical daily load data from PRZM-3 are imported into the EXAMS II model to estimate surface water concentrations.

EXAMS II

EXAMS II is used to assess the fate, exposure, and persistence of organic chemicals in aquatic ecosystems.

- It accounts for volatilization, sorption, hydrolysis, biodegradation, and photolysis of the chemical in two water bodies (farm pond and Index Reservoir).
- The model takes into account the water temperature, turbidity, and light penetration to adjust UV degradation rates.
- EXAMS is a steady-state model, with the water bodies having a constant volume.
- The EXPRESS model can simulate exposure in two water bodies: a farm pond and a small reservoir within a watershed (Index Reservoir). The farm pond is a 1-ha by 2-m deep water body and the Index Reservoir is a 640-m long x 82-m wide x 2-m deep water body in a 427 acre watershed [80]. The watershed was modeled with properties and characteristics that make it prone to contamination by agricultural chemicals. When the index reservoir and the farm pond water bodies are modeled with different crop scenarios in EXPRESS, the results represent the 90^{th} percentile of runoff vulnerability for organic chemical transport to surface waters [80].

- The standard pond scenario is typically used for ecological risk assessment, whereas the Index Reservoir is more conservative and is used for drinking-water assessment.
- Multiple-year chemical concentrations in the water column are calculated from the simulations as the yearly daily peak, maximum yearly 96-hour average, maximum yearly 21-day average, maximum yearly 60-day average, and annual average. The upper 90th percentile concentrations are reported for the 30-year simulation.

Additional information is available in the EXPRESS user's manual [80].

The physical-chemical properties and environmental fate values used in the EXPRESS modeling are presented in Table 9 and Table 13.

5.4.2. EXPRESS model input parameters

The EXPRESS model was used to estimate the PEC_{water} for the surrogate estradiol and trenbolone compounds by assuming that the compounds are applied to land (in a manure matrix) similar to the way a pesticide is applied. The EXPRESS model was run for a selection of available crop scenarios in different locations using the following input parameters:

1. Specific crop scenarios to be simulated (34 crop scenarios were selected in EXPRESS, Table 20)
2. The application date, application rate of the surrogate estradiol and trenbolone compounds based on the P_2O_5 requirements of the crop, and incorporation depth into soil (Appendix 4.2)
3. The specific environmental fate parameters for the surrogate estradiol and trenbolone compounds (Table 9 and Table 13)

5.4.2.1. Selection of crop scenarios

The crop scenarios in the EXPRESS model were built by EPA to be a conservative representation of scenarios in which a pesticide may be applied to a cropped field. In this assessment, it was assumed that instead of a pesticide, the surrogate estradiol and trenbolone compounds were applied to simulate what would occur when manure containing these compounds was applied to a field as fertilizer. The crop scenarios consist of a specific crop type (e.g., corn, cotton), a location (e.g., Huron County, MI), the soil texture class (e.g., clay), and the hydrologic soil group (HSG; e.g., C and D soil classes), among other variables.

The crop scenarios modeled were the field crops associated with cattle production available as part of the EXPRESS package, including corn, cotton, alfalfa, wheat, sugar beet, potato, dry bean, sorghum, and soybean. A total of 34 crop scenarios with varying locations, soil properties, and weather patterns were selected in EXPRESS to simulate erosion and runoff of the surrogate estradiol and trenbolone compounds in manure applied to cropland. The diverse range of regions ensured that the surrogate estradiol and trenbolone compound exposures were estimated under various conditions found in cattle production regions in the US. All 34 crop scenarios are listed in Table 20 and additional specifics regarding the region, slope, soil type, weather, and yearly runoff are listed in Table 71 through Table 73 (located in Appendix 7.1).

5.4.2.2. Application date, quantity, and incorporation depth of surrogate estradiol and trenbolone compounds applied to soil

The application rates for the surrogate estradiol and trenbolone compounds (Table 74) were determined from:

1. Phosphate (P_2O_5) nutrient requirement of the crop (see Table 67 in Appendix 4.2 for derivation of phosphate requirements),
2. Daily release rate of EB and TBA metabolites per kg P_2O_5 excreted in manure (see Appendix 6.3 for assumptions and calculations), and
3. Historic regional crop yields from USDA data (see Table 71 through Table 73 for historic yield statistics)

The methods used to determine the surrogate estradiol and trenbolone application rates are outlined in Appendix 6.3 (Table 69 and Table 70).

All 34 crop scenarios assumed a single yearly manure application was applied to the surface of the soil and incorporated (tillage) to a depth of 15 cm. See Appendix 2 for an explanation of tillage depth chosen. The EXPRESS crop codes, abbreviations, and day of application prior to planting and post-harvest for the 34 crops are listed in Table 20. The application date of a chemical to cropped fields can have a big influence on the amount of the chemical in runoff. If there is a major rainfall event right after application, the PEC_{water} would be high. To assure that an appropriate value was chosen for each of the 34 crop scenarios, a sensitivity analysis was conducted using five application dates prior to planting and five dates after harvest (Appendix 7.1.1). An upper 90% confidence interval on the mean was calculated for the PEC values resulting from the 10 application dates. The application date that resulted in a PEC value that was closest to the upper 90% confidence bound on the mean PEC_{water} was chosen as the conservative date to use in the individual crop scenarios. The determination of the application date is presented in Table 80 (Appendix 7.1.1).

Surface application of manure from livestock, also known as 'no-till' application, is becoming a popular manure application technique used in the US. Thus, a total of 17 no-till crop scenarios were developed in addition to the till scenarios to ensure that all common manure application practices were considered. These 17 scenarios included crops that are the principal crops grown using no-till practices [85]: corn, wheat, cotton, soybean and sorghum. The no-till scenarios assumed an incorporation depth of 5 cm. See Appendix 2 for an explanation regarding no-till incorporation depths. These no-till crop scenarios were simulated using PRZM and EXAMS modeling programs because EXPRESS could not be modified for this purpose. In order to simulate a no-till situation, the surface roughness, water infiltration and surface water runoff variables were modified in PRZM and EXAMS. An explanation regarding the selection of crop scenarios to be modeled using no-till techniques is provided in Appendix 7.3, along with a list of the no-till crop scenarios selected. The parameters used in PRZM and EXAMS to simulate no-till modeling are available in the study report from the mixed-use watershed modeling (Attachment 1).

Information on manure application techniques to tilled, no-till, and pastureland typically used in the US is provided in Appendix 2. Detailed information on the individual crop model scenarios used in this assessment and the surrogate estradiol and trenbolone compounds application rates to both tilled and no-till models are described in Appendix 7 (Table 71 through Table 74).

Table 20. Express Crop Codes and Abbreviations Used in Plots and Tables

EXPRESS Code used for data presentation	Crop	State	Soil % Slope	Manure Application Day ‡	Applications per year
IL1 Corn MLRA-108	Corn	IL	6	Day -28	1
MS1 Corn MLRA-134	Corn	MS	6	Day -14	1
NC1 Corn - E MLRA-153A	Corn	NC	6	Day -28	1
NC2 Corn - W MLRA-130	Corn	NC	1	Day -14	1
ND1 Corn MLRA-56	Corn	ND	1.5	Day +21	1
TX1 Corn MLRA-86/87	Corn	TX	6	Day +7	1
TX2 Corn MLRA-83D	Corn	TX	6	Day +28	1
CA1 Corn MLRA-17	Corn	CA	0.5	Day -21	1
OH1 Corn MLRA-111	Corn	OH	4.5	Day +28	1
PA1 Corn MLRA-148	Corn	PA	6	Day -14	1
MS1 Soybean MLRA-134	Soybean	MS	2	Day -21	1
ND1 Wheat MLRA-56	Wheat	ND	1.5	Day +28	1
OR1 Wheat MLRA-2	Wheat	OR	6	Day -7	1
TX2 Wheat MLRA-86/87	Wheat	TX	3	Day -21	1
KS2 Sorghum MLRA-112	Sorghum	KS	4	Day -7	1
TX1 Sorghum MLRA-86/87	Sorghum	TX	6	Day -7	1
CA1 Alfalfa MLRA-17	Alfalfa	CA	2	Day -1	1
IL1 Alfalfa MLRA-108	Alfalfa	IL	12	Day +21	1
MN1 Alfalfa MLRA-56	Alfalfa	MN	1.5	Day -14	1
NC1 Alfalfa MLRA-136	Alfalfa	NC	6	Day +14	1
PA1 Alfalfa MLRA-148	Alfalfa	PA	12	Day +14	1
TX1 Alfalfa MLRA-86/87	Alfalfa	TX	1.8	Day +21	1
WA1 Beans MLRA-7/8	Beans	WA	6	Day +14	1
MI1 Beans MLRA-99	Beans	MI	1	Day +7	1
CA1 Cotton MLRA-17	Cotton	CA	2.5	Day +7	1
MS1 Cotton MLRA-134	Cotton	MS	6	Day -1	1
NC1 Cotton MLRA-133A	Cotton	NC	6	Day -1	1
TX1 Cotton MLRA-83D	Cotton	TX	0.5	Day +21	1
TX2 Cotton MLRA-86/87	Cotton	TX	5	Day +7	1
CA1 Sugarbeet MLRA-17	Sugar beet	CA	2	Day -21	1
MN1 Sugarbeet MLRA-56	Sugar beet	MN	1.5	Day -1	1
ID1 Potato MLRA-11B	Potato	ID	1	Day +14	1
ME1 Potato MLRA-146	Potato	ME	6	Day -7	1
WA1 Potato MLRA-7/8	Potato	WA	6	Day +28	1

‡ Negative manure application values are days prior to planting, positive are days after harvest. Additional information on these EXPRESS crops and the specifics of soil type, crop yields and precipitation can be found in Table 71, Table 72, and Table 73. Application rates of the surrogate estradiol and trenbolone compounds can be found in Table 74.

5.4.2.3. Environmental physical-chemical and fate parameters

The physical-chemical and environmental fate parameters input into the EXPRESS and PRZM and EXAMS model are listed in Table 9 and Table 13. In addition, descriptions of the supporting studies and derivation methods used to determine these parameters are available in Sections 4.2.4 and 4.2.6.

5.4.3. Presentation of EXPRESS modeling results

Results of the EPA EXPRESS, PRZM-3, and EXAMS II models, are presented in terms of the upper 10th percentile derived from a set of modeling results for multiple simulations covering an extended period of time, typically 30 years. In this EA, the term "90th percentile" will be used because it more effectively communicates that 90% of the results will be less than or equal to the reported value. The modeling results include predictions of both acute and chronic 90th percentile PEC_{water} values for yearly single-day peak concentrations and maximum moving average concentrations across the following time intervals: 96-hour, 21-day, 60-day, 90-day, and 365-day (annual). Use of the 90th percentile was chosen because it is the EPA recommended parameter to use from the EXPRESS model for regulatory environmental decisions on safety and is considered a conservative value. The 90th percentile is the only percentile that is available from the EXPRESS program. Moving average concentrations are calculated by stepping ahead one day and calculating the average across the new time interval. Moving averages are used to approximate chronic exposure concentrations. Although all modeled PEC values are reported, only the 21-day average PEC values were summarized and used in the risk characterization in Section 7. The 21-day PEC_{water} was chosen because the predicted no effect concentration (PNEC) used in the risk characterization was calculated using the results of 21-day fish reproduction studies.

5.4.4. PEC_{water} results for the surrogate estradiol compound due to runoff from manure applied to cropland

A summary of the highest 90th percentile moving average PEC_{water} values for the surrogate estradiol compounds is presented in Table 21 for different periods of time and covering both till and no-till scenarios. The PEC results for each of the tilled crop scenarios are illustrated in Figure 10 and Figure 11; the complete data set can be found in Table 75 and Table 76 of Appendix 7. The PEC results for each of the no-till crop scenarios are illustrated in Figure 12 and Figure 13; the complete data set can be found in Table 83 and Table 84 of Appendix 7.

For the 34 tilled and 17 no-till crop scenarios, including for both the farm pond and the index reservoir water bodies, the highest 90th percentile 21-day PEC for the surrogate estradiol compound was 0.08 and 0.07 ng/L, respectively. The highest PEC was estimated in the Index Reservoir water body for the tilled Mississippi (MS) corn scenario (0.08 ng/L).

Table 21. Summary of Highest 90th Percentile PEC_{water} Values[a] (ng/L) for the Surrogate Estradiol Compound in the Farm Pond and Index Reservoir Water Body from Modeled EXPRESS Scenarios

Peak	Moving Average Period				
	96-Hour	21-Day	60-Day	90-Day	Annual
Across all 34 Tilled Crop Scenarios Farm Pond					
0.08	0.07	0.06	0.04	0.03	0.01
Across all 34 Tilled Crop Scenarios Index Reservoir					
0.11	0.10	0.08	0.05	0.01	<0.01
Across all 17 No-till Crop Scenarios Farm Pond					
0.08	0.07	0.06	0.03	0.03	0.01
Across all 17 No-till Crop Scenarios Index Reservoir					
0.10	0.09	0.07	0.04	0.03	0.01

[a] These PEC_{water} values represent the 90th percentile of the distribution of the yearly maximum moving average concentration values.

In Figure 10 through Figure 13, “Limnetic Concentration” refers to the concentration of the representative surrogate estradiol compound in the water column, not in the sediment. The coding system for the crops in these figures can be found in Table 20.

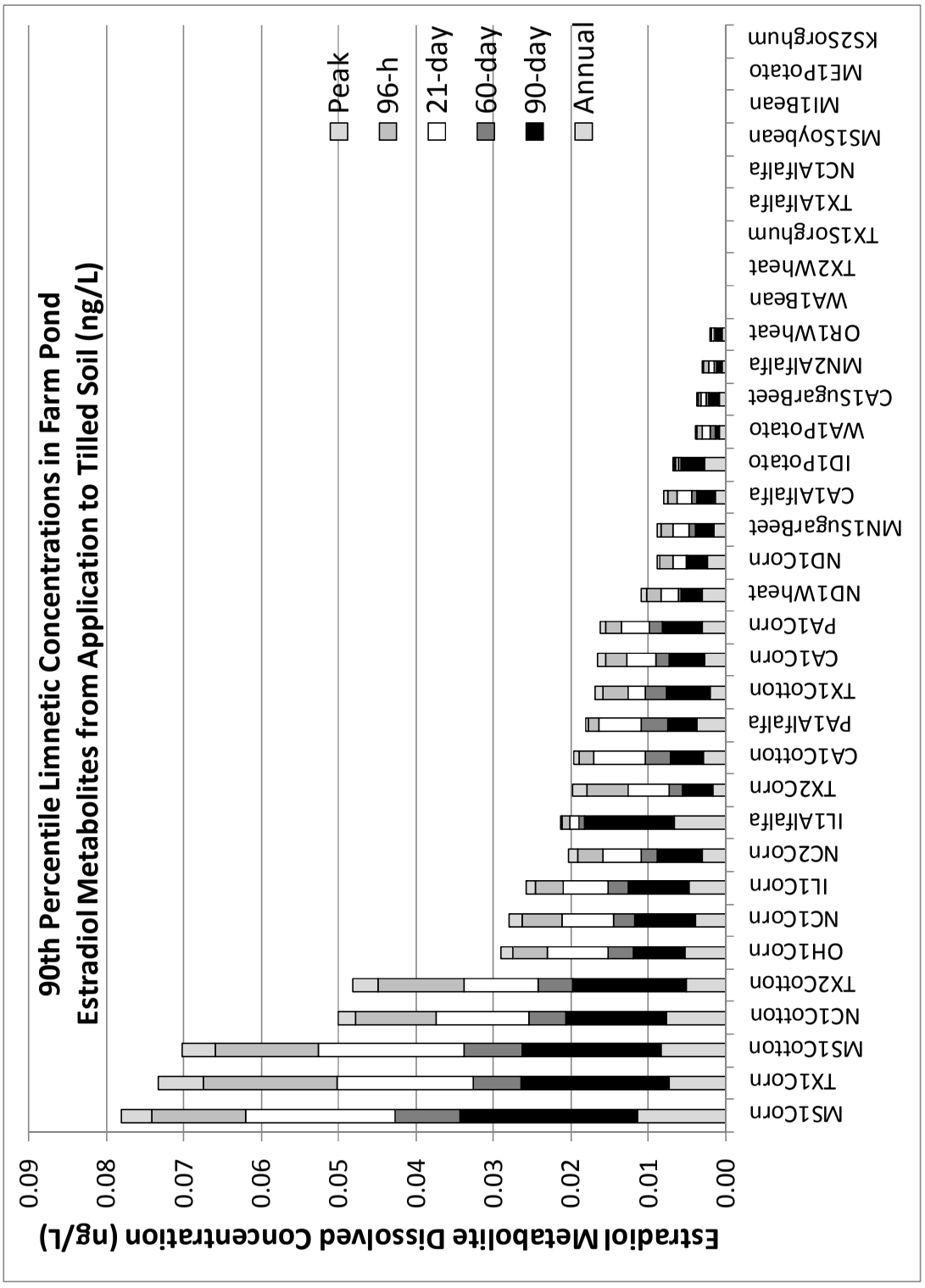
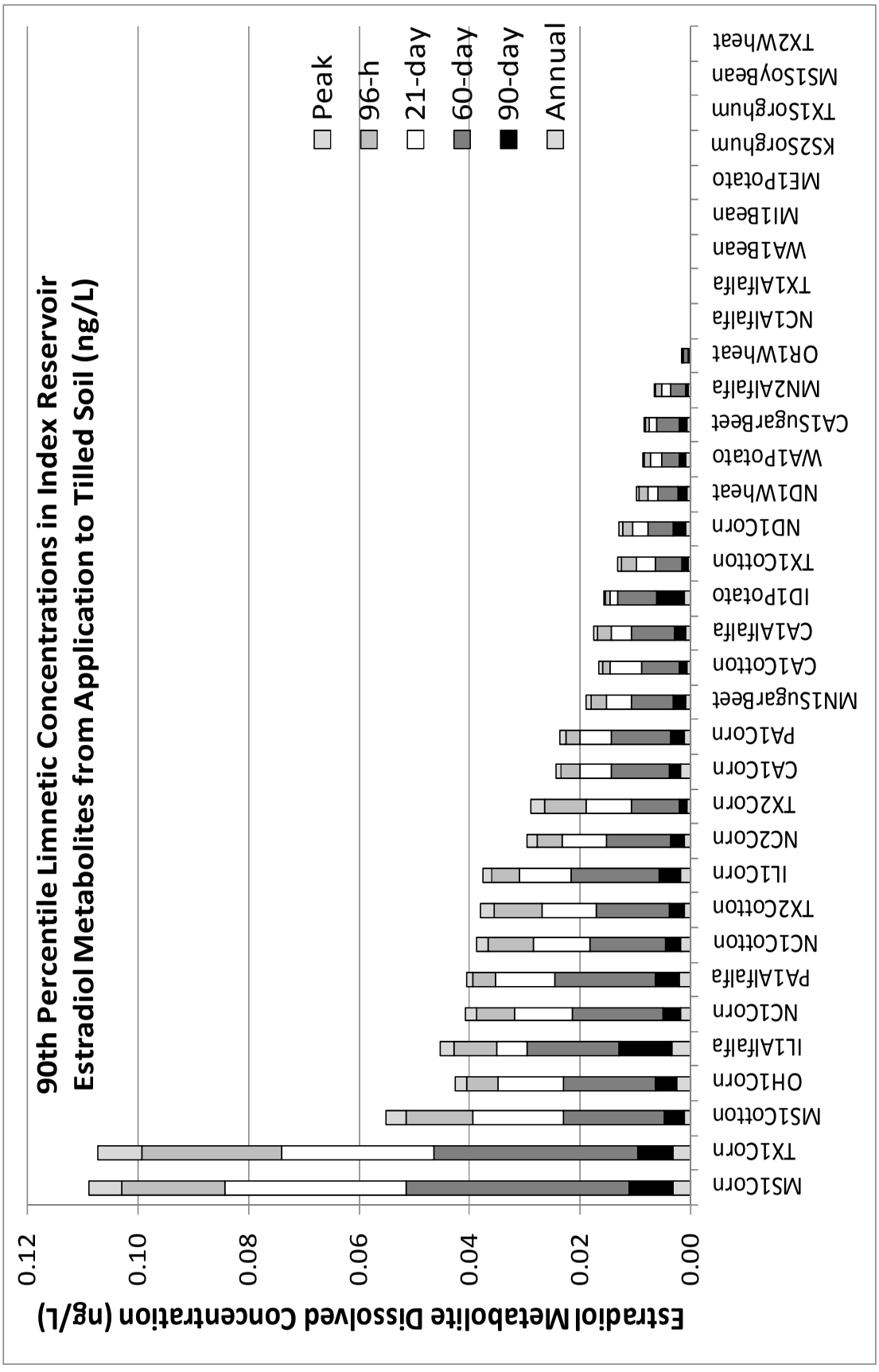


Figure 10. Acute and Chronic PEC_{water} Values for the Surrogate Estradiol Compound in 34 Tilled Soil Scenarios for the Farm Pond

Figure 11. Acute and Chronic PEC_{water} Values for the Surrogate Estradiol Compound in 34 Tilled Soil Scenarios for the Index Reservoir



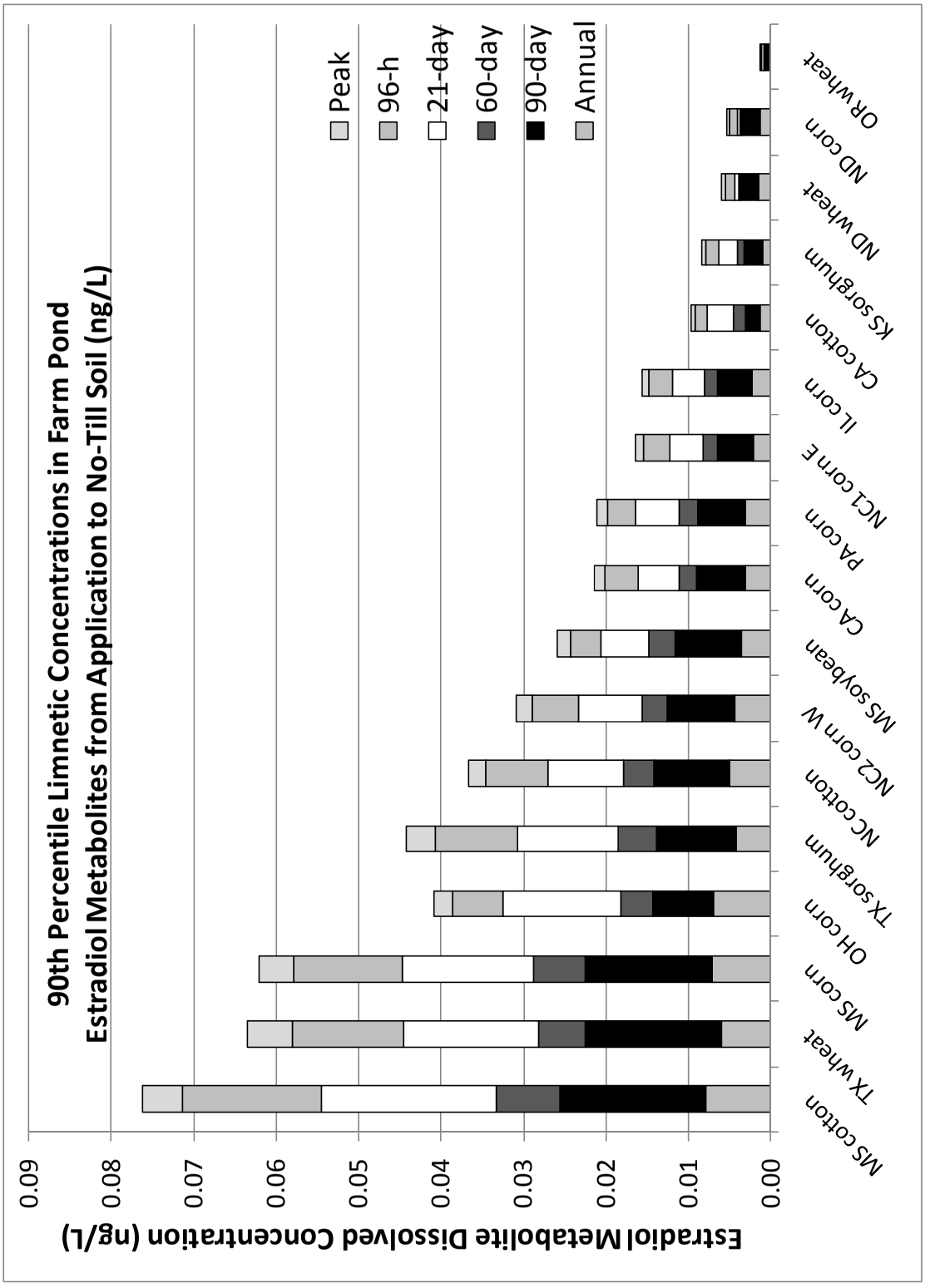
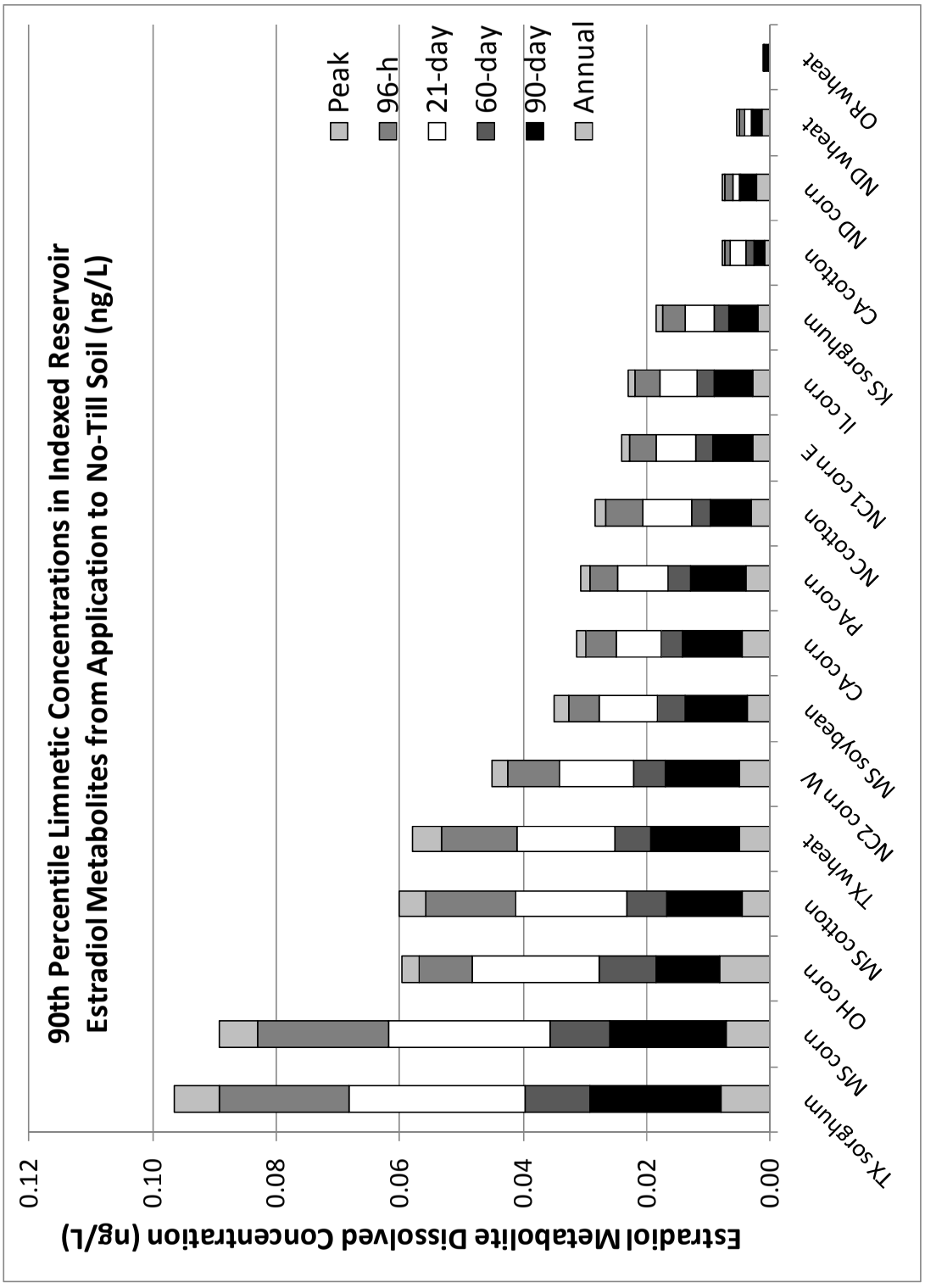


Figure 12. Acute and Chronic PEC_{water} Values for the Surrogate Estradiol Compound in 17 No-Till Soil Scenarios for the Farm Pond

Figure 13. Acute and Chronic PEC_{water} Values for the Surrogate Estradiol Compound in 17 No-Till Soil Scenarios for the Index Reservoir



5.4.5. PEC_{water} results for the surrogate trenbolone compound due to runoff from manure applied to cropland

A summary of the highest 90th percentile moving average PECwater values for the surrogate trenbolone compounds is presented in Table 22 for a range of different average periods of time and covering both till and no-till scenarios. The PEC results for each of the tilled crop scenarios are illustrated using stacked bar graphs in Figure 14 and Figure 15. The complete data set can be found in Table 77 and Table 78 of Appendix 7. The PEC results for each of the no-till crop scenarios are illustrated in Figure 16 and Figure 17; the complete data set can be found in Table 85 and Table 86 of Appendix 7.

For the 34 tilled and 17 no-till crop scenarios, including for both the farm pond and the index reservoir water bodies, the highest 90th percentile 21-day PEC for the surrogate estradiol compound was 0.62 and 0.63 ng/L, respectively. The highest PEC was estimated in the Index Reservoir water body for the no-till Mississippi (MS) corn scenario (0.63 ng/L).

Table 22. Summary of Highest 90th Percentile PEC_{water} Values[a] (ng/L) for the Surrogate Trenbolone Compound in the Farm Pond and Index Reservoir Water Body from Modeled EXPRESS Scenarios

Peak	Moving Average Period				
	96-Hour	21-Day	60-Day	90-Day	Annual
Across all 34 Tilled Crop Scenarios Farm Pond					
0.56	0.55	0.48	0.37	0.31	0.13
Across all 34 Tilled Crop Scenarios Index Reservoir					
0.77	0.73	0.62	0.41	0.10	0.04
Across all 17 No-till Crop Scenarios Farm Pond					
0.70	0.67	0.55	0.38	0.31	0.11
Across all 17 No-till Crop Scenarios Index Reservoir					
0.86	0.81	0.63	0.42	0.32	0.10

[a] These PEC_{water} values represent the 90th percentile of the distribution of the yearly maximum moving average concentration values.

In Figure 14 through Figure 17, “Limnetic Concentration” refers to the concentration of the surrogate trenbolone compound in the water column, not in the sediment. The coding system for the crops in these figures can be found in Table 20.

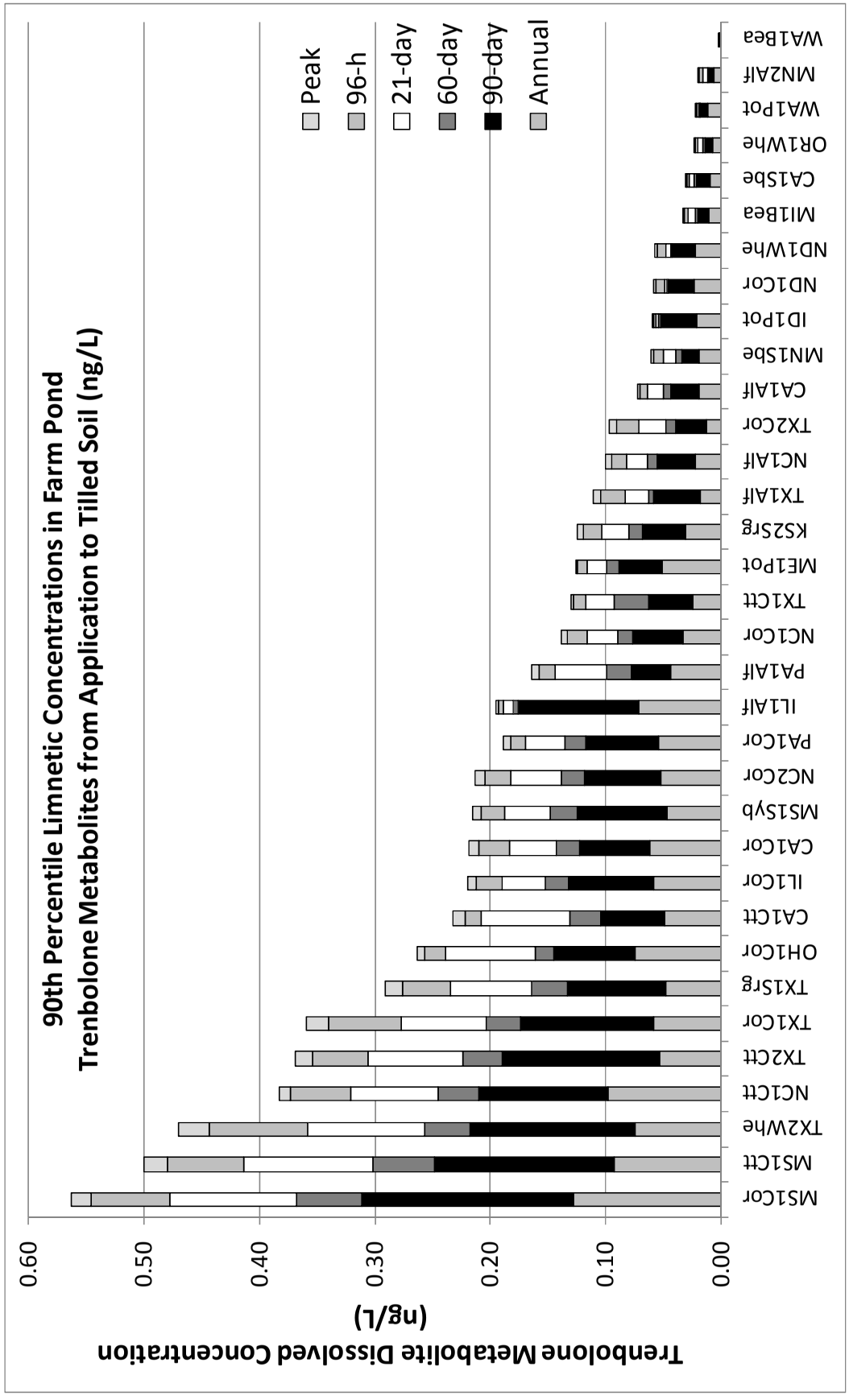


Figure 14. Acute and Chronic PEC_{water} Values for the Surrogate Trenbolone Compound in 34 Tilled Soil Scenarios for the Farm Pond

Figure 15. Acute and Chronic PEC_{water} Values for the Surrogate Trenbolone Compound in 34 Tilled Soil Scenarios for the Index Reservoir

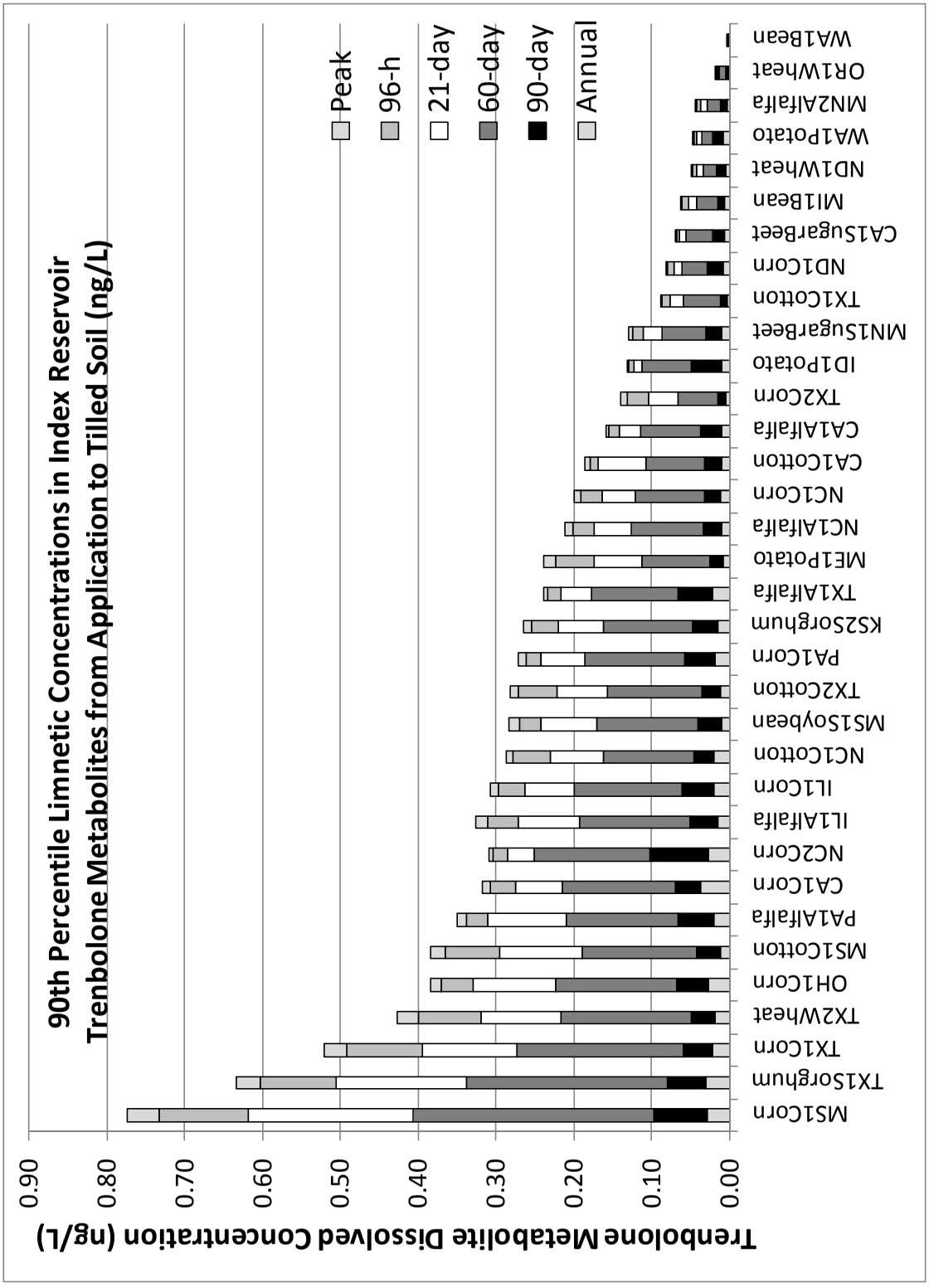
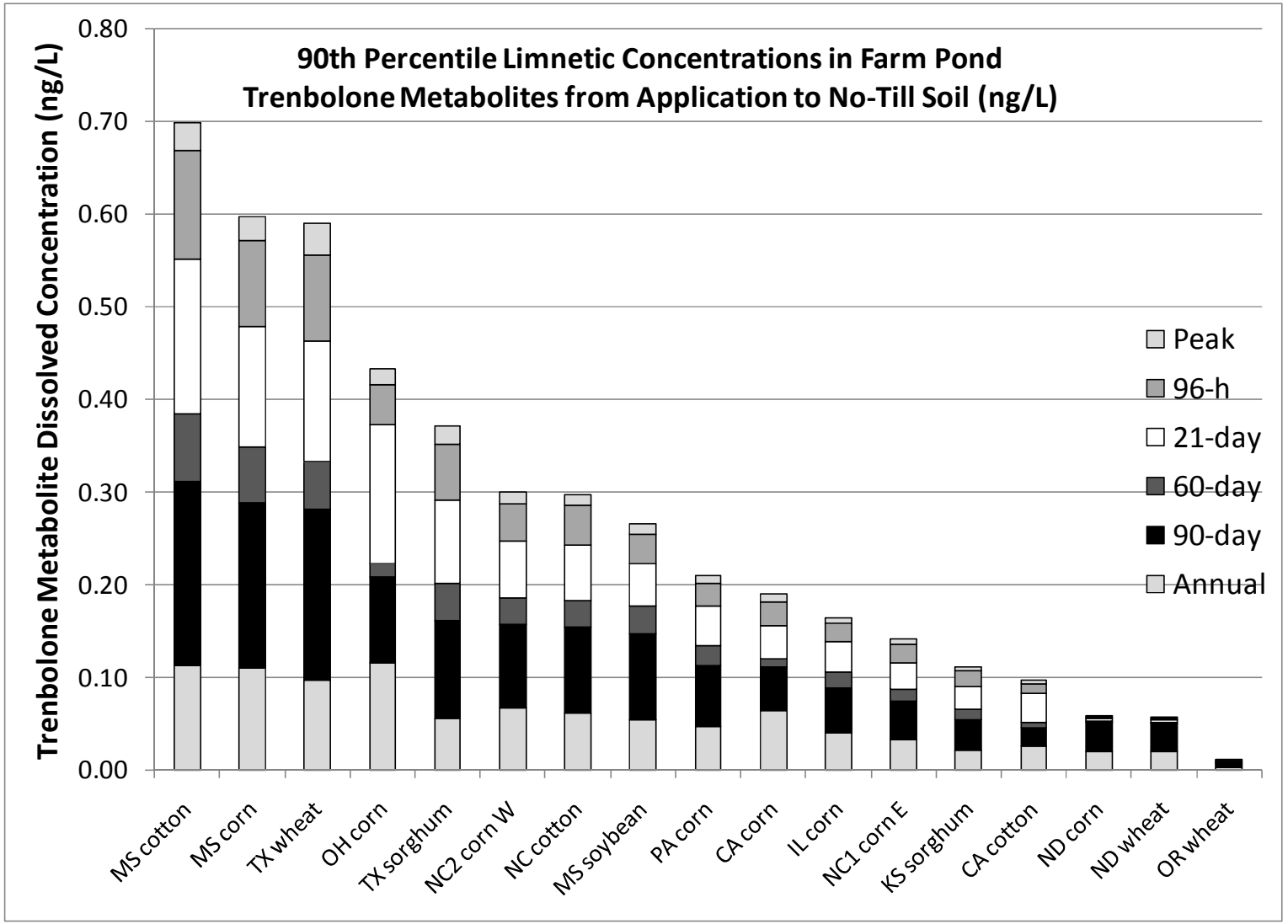


Figure 16. Acute and Chronic PEC_{water} Values for the Surrogate Trenbolone Compound in 17 No-Till Soil Scenarios in the Farm Pond



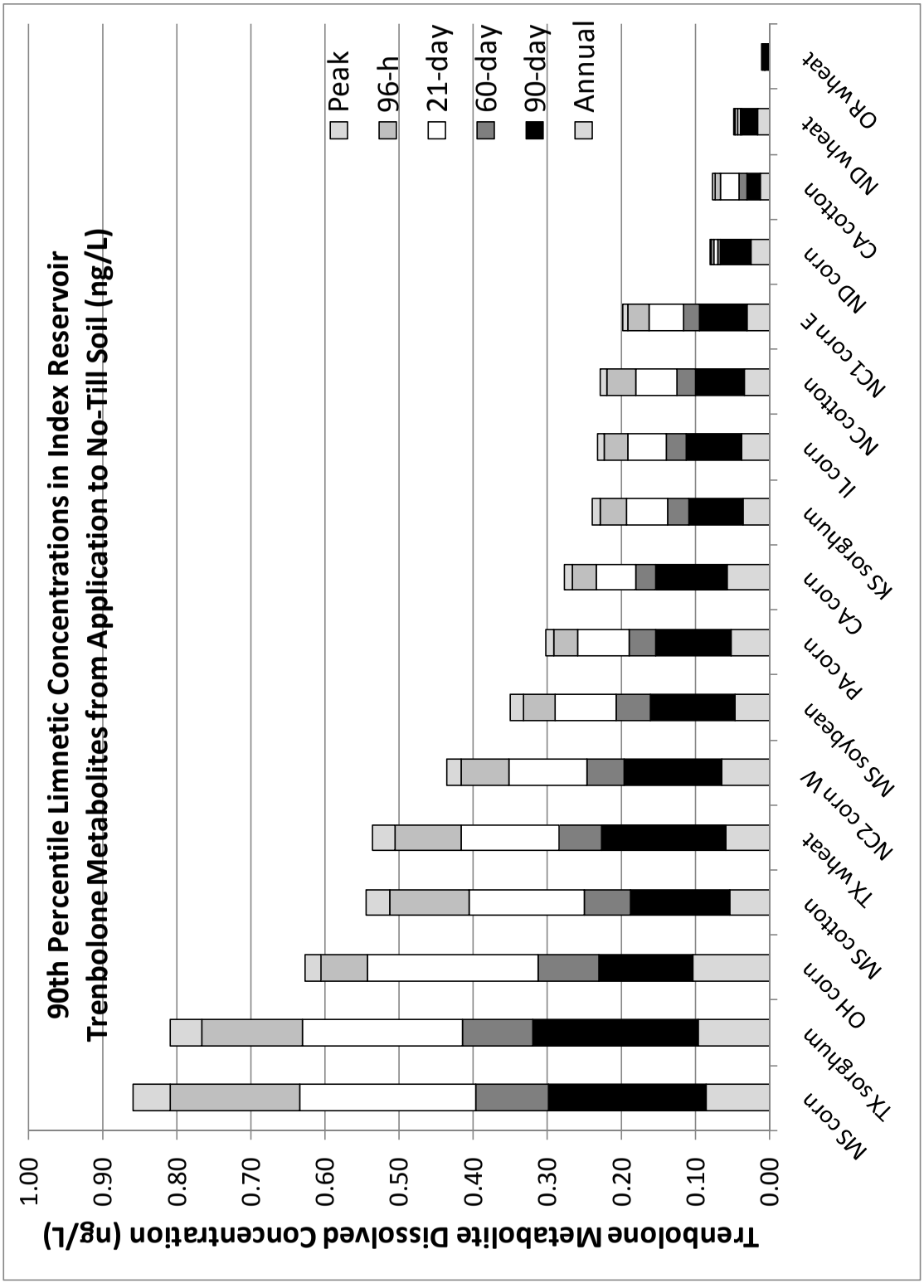


Figure 17. Acute and Chronic PEC_{water} Values for the Surrogate Trenbolone Compound in 17 No-Till Soil Scenarios in the Index Reservoir

5.5. PEC_{water} from Tile-Drained Cropped Fields Using the EXPRESS Model

Background information on tile-drained fields is provided in Section 3.4.2.2. Based on their physical-chemical properties and biodegradation parameters, the potential for surrogate estradiol and trenbolone compounds to reach tile drains was determined in the 34 tilled soil EXPRESS scenarios by estimating the leachate concentration at 1 m depth below the soil. Although tile drains can be placed at different depths in a field, EXPRESS reports the concentration at 1 m below the soil, which typically represents the bottom of the soil column. It is also reported that tile drains are typically placed 2-4 feet (or 0.61 to 1.2 m) below the surface [14]; therefore, 1 m is an appropriate and representative depth for tile drains.

The highest surrogate estradiol compound concentration was 4.1×10^{-12} µg/L and the highest surrogate trenbolone compound concentration was 1.9×10^{-13} µg/L (Table 81 in Appendix 7.2). The data for each scenario is presented in Appendix 7.2. The data agree with the low leachate concentrations predicted by the SCI-GROW model in Section 5.3 and indicate a very low potential for either surrogate estradiol or trenbolone compounds to leach into groundwater or reach tile drains and significantly contribute to surface water concentrations from movement through the subsurface. Therefore, a tile drain component was not included in the aggregate mixed-use watershed model presented in Section 5.7.

5.6. PEC_{soil} and PEC_{water} from Pastureland

Similar to Section 5.2 and Appendix 3, the initial PEC_{soil} values for pasture cattle receiving ONE-G was calculated using the traditionally accepted screening method approach:

$PEC_{soil\ initial}$ = 0.158 µg/kg soil for 17α-estradiol metabolite
$PEC_{soil\ initial}$ = 1.35 µg/kg soil for 17α-trenbolone metabolite

These values were calculated without adjustment for physical-chemical or environmental fate data. The assumptions and calculations to estimate the initial PEC_{soil} values are outlined in detail in Appendix 5.

The EXPRESS model does not have a scenario that represents the discrete excretion pattern that occurs on pasture. Thus, in order to refine the PEC values using physical-chemical and environmental fate data, the PEC_{water} values for the surrogate estradiol and trenbolone compounds resulting from runoff from pasture were also determined using the modified PRZM-3 and EXAMS II models that were used to simulate the mixed-use watershed in Section 5.7. Thus, pasture runoff was modeled in five regions of the US, including Texas, Iowa, Ohio, Michigan, and Pennsylvania (Appendix 8.3). The mixed-use model was run as a single-use model setting pastureland to 100% and all other uses to 0%. PEC_{water} data for runoff from pastureland are presented in Table 23 and Table 24. Details on the modeling methods and results are discussed in the study report on mixed-use watershed models in Attachment 1.

For the surrogate estradiol compound, the highest 90th percentile of the yearly maximum 21-day moving average PEC_{water} value for pasture cattle, across all five regions, was 0.03 ng/L for Ohio. For the surrogate trenbolone compound, the highest 90th percentile of the yearly maximum 21-day moving average PEC_{water} value for pasture cattle across all five regions was 0.30 ng/L for Ohio.

Table 23. Pasture Cattle 90th Percentile PEC_{water} Values[a] for the Surrogate Estradiol Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models

Study Region	Estradiol PEC_{water} (ng/L)					
	Peak	96-Hour	21-Day	60-Day	90-Day	Annual
IA	0.03	0.03	0.03	0.02	0.01	0.01
TX	0.03	0.03	0.02	0.01	0.01	0.01
PA	0.02	0.02	0.01	0.01	0.01	0.01
OH	0.03	0.03	0.03	0.02	0.02	0.01
MI	0.03	0.03	0.02	0.02	0.01	0.01
Maximum	**0.03**	**0.03**	**0.03**	**0.02**	**0.02**	**0.01**

[a] PECs represent the 90th percentile and assume 100% of the watershed is an area with pasture cattle.

Table 24. Pasture Cattle 90th Percentile PEC_{water} Values[a] for the Surrogate Trenbolone Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models

Study Region	Trenbolone PEC_{water} (ng/L)					
	Peak	96-Hour	21-Day	60-Day	90-Day	Annual
IA	0.32	0.31	0.28	0.23	0.20	0.11
TX	0.27	0.26	0.22	0.15	0.14	0.06
PA	0.18	0.17	0.15	0.12	0.10	0.06
OH	0.34	0.33	0.30	0.23	0.21	0.13
MI	0.29	0.28	0.25	0.21	0.19	0.11
Maximum	**0.34**	**0.33**	**0.30**	**0.23**	**0.21**	**0.13**

[a] PECs represent the 90th percentile and assume 100% of the watershed is an area with pasture cattle.

In the farm-scale and mixed-use watershed modeling of runoff from pastureland, it was assumed 100% pasture cattle are treated with Synovex ONE implants at the beginning of the pasture season (March or April 1) and that cattle are maintained on pasture until the implants are actually exhausted (211 days for trenbolone and 270 days for estradiol; data based on an Explant Study described in Appendix 13.4). This approach is conservative because pasture cattle are raised with minimal handling, allowing for the potential for some pasture cattle within the watershed to not be implanted.

5.7. Aggregate PEC_{water} from Mixed-Use Watershed Models

Within a watershed there can be many different sources (i.e., runoff from cropland, feedlots and pastureland) and exposure pathways for surrogate estradiol and trenbolone compounds to enter surface waters. All of these sources could simultaneously contribute to the surface water runoff and PEC values at the watershed level. This is considered an aggregate exposure scenario and is reflective of what could occur in a typical agricultural watershed. A mixed-used watershed was developed and modeled to assess the potential risks of an aggregate exposure of the surrogate compounds at a watershed-scale.

5.7.1. Mixed-use watershed modeling

The EXPRESS model cannot be used to simulate the potential aggregate exposure within a watershed through multiple, independent, and simultaneous inputs of EB and TBA metabolites. Therefore, in order to do so, a new model was developed by modifying the PRZM and EXAMS models (see Attachment 1) to determine chronic concentrations in a mixed-use watershed, including inputs from surface erosion/runoff from:

1. Pastureland containing pasture cattle manure
2. Cropland with solid manure from feedlot cattle applied as fertilizer
3. Cropland irrigated with water from feedlot runoff collection lagoons/ponds
4. Open feedlots without adequate runoff control (i.e., feedlots with <1000 cattle)

In Section 5.3, leaching was found to be negligible compared to surface runoff. Therefore, leachate was not evaluated in the watershed aggregate exposure.

The following high-level assumptions were used in the mixed-use watershed modeling:

- A single surrogate estradiol compound and single surrogate trenbolone compound were modeled in the mixed-use watershed modeling. See Section 4.2.1 and Figure 8 and Figure 9 for a detailed explanation of the surrogate estradiol and trenbolone compounds.
- Environmental fate parameters of the surrogate estradiol and trenbolone compounds contained in Table 9 and Table 13, respectively, were used. See Section 4.2.2 for an explanation of the methods used to derive the modeling input parameters.
- The assumption was made that 90% of the manure from confined cattle would be collected as solid manure in the feedlot pen and directly applied to fields. The remaining 10% would be collected in the runoff lagoons and applied to cropland via irrigation. An explanation for these percentages is provided in Section 5.7.6.
- It was assumed that 100% of surrogate estradiol and trenbolone compounds excreted on a feedlot are available to runoff from the feedlot surface and that 100% are available (following a holding period) in the manure to be applied to manure amended cropland. Thus, the proportion of surrogate compounds excreted from feedlot animals was not attributed to a specific source. For example, 100% of the surrogate compounds were assumed to be present in the manure and irrigation water applied to cropland, and at the same time, 100% of EB and TBA metabolites were assumed to be present at a constant concentration on the feedlot surface. In real-world circumstances, it would be expected that some EB and TBA metabolites will runoff from the feedlot manure pack, leaving <100% of the EB and TBA metabolites in the manure applied to cropland. Thus, these assumptions increased the overall mass of surrogate compounds entering the watershed. This very conservative assumption was made because data was not available to determine the specific percent contributions from each source.

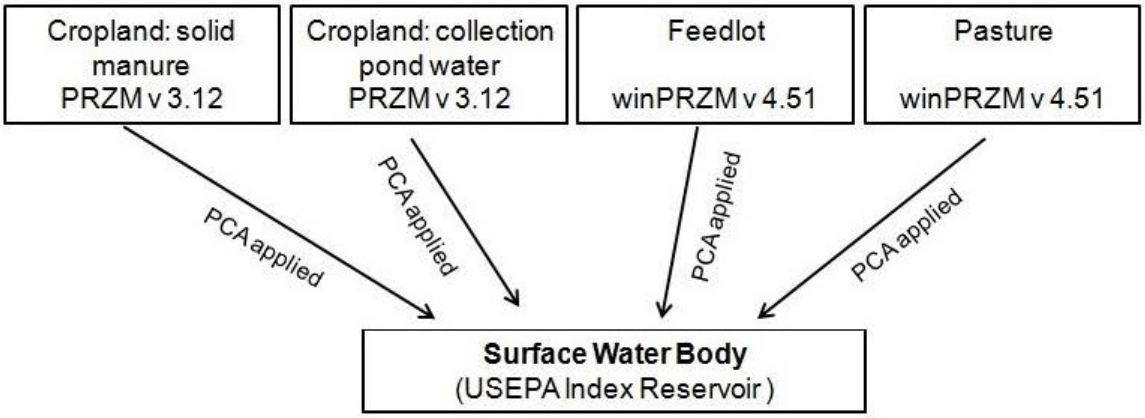
- It was assumed that no degradation of surrogate estradiol and trenbolone compounds occurred during storage in solid or liquid manure. This assumption is considered conservative because several monitoring studies suggest that EB and TBA metabolites have the potential to degrade in manure storage systems (Appendix 12).
- This model takes into account the watershed characteristics from beef cattle production areas prone to runoff within diverse regions of the country noted for their high density of feedlot and pasture cattle. Available GIS data from the watershed areas were used to determine the essential information needed for incorporation into the model (spatial distribution of feedlots, size, and number of animals in the watershed, pastureland, etc.).

A summary of the methodology used in the mixed-use watershed modeling is provided below. Additional details specific to each exposure pathway are provided in Sections 5.7.4 through 5.7.8 and can be found in the study report provided in Attachment 1.

1. A national geospatial analysis was conducted to identify regions of high potential vulnerability of surrogate estradiol and trenbolone compounds to runoff or erosion into surface waters by spatially overlaying areas of high beef cattle density, feedlots, and normal annual precipitation. Based on this information, five regions were selected: Iowa (IA), Texas (TX), Ohio (OH), Michigan (MI), and Pennsylvania (PA) (see Section 5.7.2 and Figures 1-9 of the study report in Attachment 1).
2. Specific watersheds were selected or developed within each of these study regions to represent a 90th percentile watershed in terms of exposure potential of trenbolone to impact surface waters, also known as an Exposure Index (see Section 5.7.3 and the study report in Attachment 1). The watershed characteristics for each of the five regions were identified using county- and state-level GIS and agricultural statistics data (Section 5.7.3).
3. For each region selected, the soil scenarios for the appropriate crop and pastureland were developed and the nearest weather station was used for weather data such as rain, temperature, etc. (Attachment 1).
4. The four sources of manure containing the surrogate estradiol and trenbolone compounds were modeled with individual model runs. Modeling for cropland, for both solid manure and collection pond water, was conducted using PRZM v 3.12. Modeling for feedlot and pasture were conducted using WinPRZM v 4.51 (Figure 18).
5. Once the results were obtained from the PRZM model, a percent cropped area (PCA) was applied to the daily edge-of-field loadings generated by PRZM (Figure 18). As part of the model, each of the land uses (feedlot, pasture cattle, manured crops, and irrigated crops) was assigned a PCA based on values from the frequency distributions for the GIS survey. The PCA approach essentially accounts for each land use source (feedlot, pasture cattle, manured crops) as a percent of the watershed area (Table 27). The concept and development of the PCA approach used for watershed modeling in screening for pesticide assessments are detailed in an EPA publication [86]. The use of PCAs in this evaluation is aligned with the EPA approach.
6. The daily edge-of-field loadings from feedlot, pasture cattle, manured crops, and irrigated crops were combined after application of the PCA factors (Figure 18).

7. The sum of the total edge-of-field loadings from all sources was entered into the EXAMS Index Reservoir water body model to determine chronic PEC_{water} values (Figure 18). The EPA Index Reservoir water body was selected because it represents a small watershed prone to agricultural runoff and could be configured to represent various proportions of land use for feedlot, pasture, and cropland (Section 5.4). This model is highly conservative in that it assumes any runoff will reach a water body without any reduction in concentration from encountering buffer strips, sedimentation or infiltration into the ground as it migrates to surface water.

Figure 18. Methodology for Modeling an Aggregate Exposure within a Watershed Using EPA's PRZM and EXAMS Models



5.7.2. Regions in the US selected for mixed-use watershed modeling

A GIS survey indicated that northeast Nebraska/northwest Iowa regions contain a dense beef cattle population with adequate rainfall to be considered a region with high potential for EB and TBA metabolite exposure to the environment, and therefore represents a conservative situation for the US (Figure 4). The area of Lyon and Sioux Counties in Iowa was selected because the GIS database was available for Iowa, and it listed the location and population of animal production facilities, including farms with <1000 AU. This location served as the primary site to characterize properties of individual small watersheds within a dense beef production area. To ensure a conservative, comprehensive, and representative analysis, four additional counties in other cattle-producing areas of the country were considered. Each of these areas has different geographic, agricultural, and climatic conditions associated with it (e.g., differing soil types, weather patterns, AFO sizes). The counties selected for modeling were: 1) Huron County, MI; 2) Mercer County, OH; 3) Lancaster County, PA; and 4) Castro County, TX (areas circled in Figure 4). Together, these five areas represent beef production areas with considerable diversity between the sites. The cattle populations within these areas are listed in Table 25 and are shown on the US map in Figure 4.

Table 25. 2007 USDA Ag Census Data for the Five Study Areas and Estimates of Pasture Cattle and Cattle in Feedlots <1000 AU

	MI Huron County	OH Mercer County	PA Lancaster County	TX Castro County	IA Lyon + Sioux Counties
Acres	317,161	303,801	629,314	582,814	869,295
Total Cattle	105,734	79,058	270,577	530,890	471,331
Beef cows	1,321	2,091	6,289	11,458	26,784
Estimate of Beef Replacement Heifers. number (% of beef cows)	404‡ (30.6%)	439 (21.0%)	1,679 (26.7%)	1,696 (14.8%)	3,990 (14.9%)
Milk Cows	27,237	21,515	109,653	28,702	32,647
Estimate of Dairy Replacement heifers. number (% of milk cows)	11,249‡ (41.3%)	8,240 (38.3%)	53,839 (49.1%)	12,830 (44.7%)	18,641 (57.1%)
Cattle on feed (Beef feedlot cattle)	45,367	28,448	43,349	341,694	303,244
Percentage of beef cattle in feedlots <1000 head	46% §	96.7% §	93.8% §	1.18% §§	34.0%¶
Pasture cattle (subtraction from total cattle) †	20,156	18,325	55,768	134,510	86,025
Estimate of pasture cattle (GIS satellite image data layer) ††	39,958	21,372	45,574	153,751	101,905

Data from Tables 8, 11, 42 and 45 of 2007 Census of Agriculture – County Data and State level estimates of beef and dairy replacement heifers are from the USDA interactive web site [87].

‡ Beef and dairy replacement heifer data are not available on a county level. Therefore, the state level data for beef cows and beef replacement heifers were used to determine the percentage of replacement heifers in relation to cows and the statewide data extrapolated to the county level. For example, in MI there are 33,000 beef replacement heifers and 108,000 beef cows (30.6%) [87]. This factor was then used to estimate county level beef replacement heifers from the number of beef cows (1,321 beef cows X 30.6% = 404 replacement heifers). This same procedure was used for milk cows and dairy replacement heifers.

§ The percentage of beef cattle in feedlots <1000 AU was estimated from data from individual counties' websites giving the number of cattle in the county in CAFOs. County-level CAFO data for Huron, Mercer, and Lancaster Counties are provided in references [88, 89, 90].

§§ Castro county feedlots <1000 AU were estimated from county data of 0.654% in lots <500 AU + state level estimate of 0.524% in lots 500-999 AU [91].

¶ Values for feedlots <1000 AU of the actual watershed modeled in Lyon/Sioux Counties were obtained from GIS information obtained from the report by Waterborne Environmental (Attachment 1).

† The number of pasture cattle was determined by subtraction [total cattle – (cows + cattle on feed + replacement heifers)]. The estimate of pasture cattle also includes bulls and stags; therefore, this estimate is conservative for the number of pasture cattle. Also, not all pasture cattle will be treated with Synovex ONE because it is only for stocker cattle not suckling cattle.

†† The number of pasture cattle was determined by the GIS data layer for pastureland in the 2007 Census of Agriculture. Each acre was multiplied by 3.15 cattle per acre to determine the number of pasture cattle (Attachment 1). The GIS layer was used to determine the number of cattle in individual watersheds in the mixed-use models and is relatively comparable to the agricultural census estimate determined by subtraction.

Table 25 was developed for several reasons: 1) to report the typical cattle distribution within each county, 2) to determine the pastureland within a specific watershed, and 3) to determine the percentage of feedlot cattle in lots <1000 AU, the latter of which is needed to model direct runoff from the feedlot for each region.

The placement of the five study regions in the national distribution for each of the variables examined (i.e., number of feedlots, number of pasture cattle, acreage of crop and pastureland, and rainfall) is displayed in Table 26. The data in Table 26 support that the regions selected are representative of areas in the US with high densities of large (>1000 head cattle) and small/medium (<1000 head cattle) feedlots located in both dry and wet climates. For example, the counties contain a large density of cattle on feedlots (both

small/medium and large), and are in the 97th percentile of counties in the nation for acres treated with manure. In addition, all counties are in the 95th percentile in the national distribution for feedlot cattle density (large and small/medium AFOs combined). Thus, these regions should conservatively represent beef cattle production areas in the US with a high potential for exposure to EB and TBA metabolites. Additional information on study site selection is provided in Attachment 1.

Table 26. US National Percentile Ranking by Density of Beef Cattle Per County Acre and Manured Cropland per Acre in Counties Chosen to Model as Mixed-Use Watersheds

State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Cropland Manured Density Rank	Pasture Cattle Density Rank	Annual Rainfall Rank	March Rainfall Rank	October Rainfall Rank
Iowa	Lyon	98.9%	96.8%	99.7%	99.6%	92.5%	23.0%	22.6%	20.2%
Iowa	Sioux	99.5%	98.4%	99.8%	99.9%	97.5%	23.3%	24.0%	20.8%
Michigan	Huron	95.5%	89.6%	98.1%	97.9%	75.3%	29.7%	31.6%	33.1%
Ohio	Mercer	96.0%	88.4%	99.4%	99.8%	88.4%	39.8%	40.5%	29.5%
Pennsylvania	Lancaster	94.9%	72.8%	99.6%	100.0%	94.9%	56.1%	52.9%	68.4%
Texas	Castro	99.9%	99.6%	74.1%	95.4%	99.7%	11.1%	5.1%	15.0%

Data provided from GIS survey (Attachment 1).

5.7.3. Selection of the specific watershed to be modeled in each region

After the study regions were chosen, the specific watershed within each study region had to be defined. A watershed selection process was conducted in the IA (Lyon and Sioux Co.) and TX (Castro Co.) study regions to identify the 90th percentile watershed in terms of exposure potential of surrogate estradiol and trenbolone compounds to surface waters. The IA and TX study regions were chosen for this process because they represent the 98th percentile or greater in terms of beef cattle density at the county level. In addition, GIS and county-level statistics were available for both of these study regions (this type of data was not available for PA, MI, or OH) and this approach agrees with guidance from EPA for the PRZM modeling [92].

In order to select a 90th percentile watershed from the distribution of all watersheds in the IA and TX study regions, an 'exposure potential of trenbolone' to surface waters, also known as an Exposure Index, was computed and ranked. The Exposure Index was computed for 42 watersheds in Lyon/Sioux Counties and 35 watersheds in Castro County. Essentially, the Exposure Index was comprised of PRZM and EXAMS simulations to determine the actual mass loading of trenbolone from each potential source. Additional details on the calculations used are provided in the study report in Attachment 1. Upon calculating the Exposure Index for every watershed in the IA and TX study regions, the indices were ranked and the watershed that closely represented the 90th percentile distribution for potential environmental exposure to the surrogate trenbolone compound was selected. The summary statistics of these rankings are provided in Tables 2 and 3 of the study report in Attachment 1. Additional details of this selection process and ranking are also provided in Attachment 1.

Once the IA and TX watersheds were selected, the watershed characteristics were defined, including: the density of feedlots (feedlots with <1000 cattle and >1000 cattle), the density of manure amended cropland, and the density of lands for pasture or grazing cattle. Agricultural statistics data obtained from the Iowa Department of Natural Resources (DNR) AFO GIS Database, the Texas Commission on Environmental Quality (CEQ) CAFO GIS Database, the 2007 USDA Census of Agriculture, and the USDA NASS Quick Stats Database[r] were used to define these characteristics. The watershed characteristics are outlined in Tables 5 and 6 of the study report in Attachment 1. Once the density for each contributing source was determined, the number of beef cattle and acres manured could be calculated. These calculations are too complex to summarize in the EA; however, they are described in detail in the study report in Attachment 1 (Sections 4.3 through 4.5 of Attachment 1). The beef cattle and land use characteristics (i.e., PCAs) for the IA and TX watersheds are presented in Table 27 below.

Due to the lack of GIS and county-level data, an alternative approach was required to define the watershed characteristics for PA, MI, and OH (i.e., feedlot density and manure amended crop/pastureland density). For these study regions, state and county regulatory agencies do not prepare publically available databases of AFO locations and characteristics, but according to the 2007 USDA Census of Agriculture, there are a substantial number of feedlots of all sizes in these states. In addition, the USDA agricultural statistics databases report the total number of feedlot and pasture cattle for each of these study regions. It was assumed that these regions would have land use characteristics more similar to IA, with a larger number of small feedlots and pastureland, rather than TX, which consists primarily of large feedlots with >1000 cattle. Therefore, the watershed characteristics for the density of feedlots and manure-amended cropland for the IA watershed were used to scale these watershed characteristics for PA, MI, and OH. Because GIS information on grass/pasture/hay spatial data for PA, MI, and OH was available from USDA, it was used to calculate the actual pasture densities in these watersheds. The specific methods and calculations used to estimate the watershed characteristics for PA, MI, and OH are described in detail in the final study report in Attachment 1. The beef cattle and land use characteristics (i.e., PCAs) for PA, MI, and OH watersheds are provided in Table 27 below.

[r] www.nass.usda.gov/QuickStats/

Table 27. Characteristics of Individual Mixed-use Watersheds for Size, Beef Cattle Numbers and Percent Cropping Areas (PCA)

State Counties	MI Huron County	OH Mercer County	PA Lancaster County	TX Castro County	IA Lyon+Sioux Counties
Watershed Area (acres)	10,708	6,737	16,051	27,073	21,128
# Beef Cattle in <1000 AU feedlots	935	1,233	1,822	247	5,373
# Beef Cattle in ≥1000 AU feedlots	1,513	167 round up to 1000 AU*	142 round up to 1000 AU*	20,965	10,410
# Beef Pasture Cattle Suckling + Stocker	4,029	1,198	1,026	3,720	1,525
Principal Crop Modeled	Corn for Grain	Corn for Grain	Corn for Silage	Corn for Grain	Corn for Grain
Tillage	Tilled	No-till	No-till	Tilled	Tilled
	Percent Cropping Area (PCA) of 90th Percentile Watershed				
Feedlot <1000 AU	0.032%	0.068%	0.04%	0.003%	0.094%
Cropped Land Manured	20.10%	29.13%	9.41%	53.59%	56.62%
Beef Pastureland	4.05%	2.93%	2.67%	4.36%	2.29%

Data provided from GIS survey (Attachment 1).

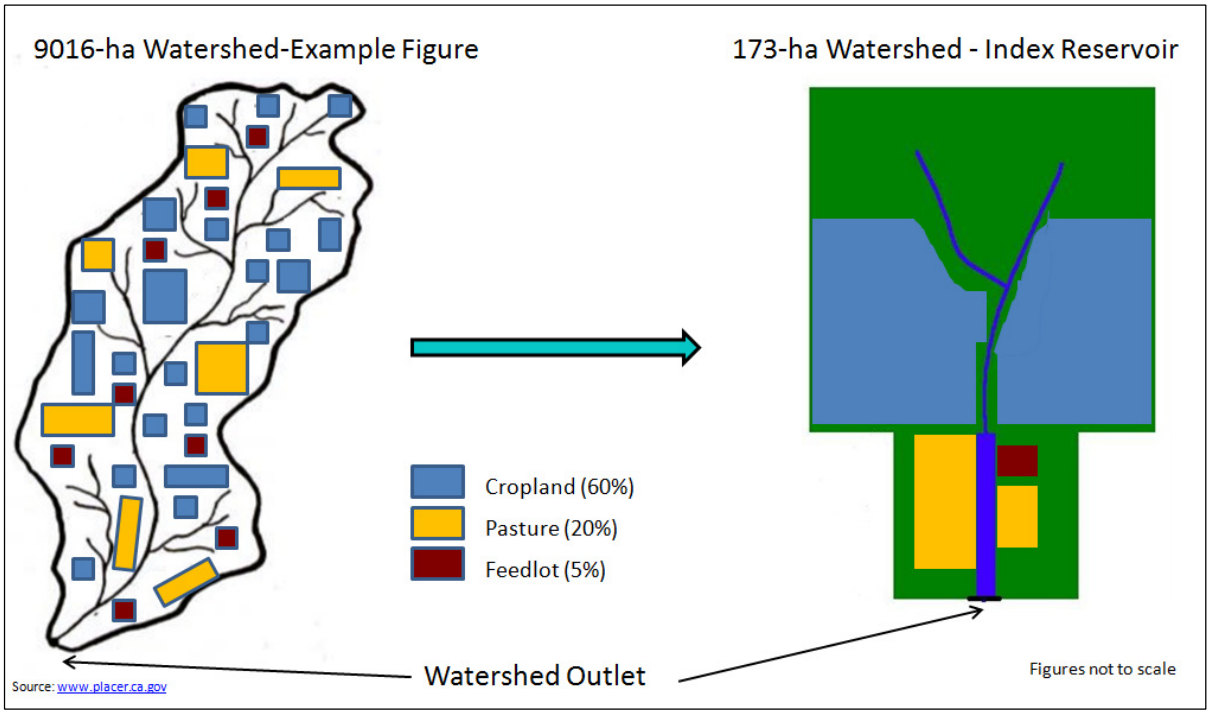
* As a result of extrapolation of county-level data to the smaller watershed level, there were not enough cattle in lots ≥1000 head. Therefore, it was assumed that at least one CAFO was located in the watershed and the value rounded up to 1000 AU. Note that in Table 25 for Mercer County, OH, there are only 3.3% of all the cattle on feed in lots ≥1000 AU in the entire county, or 1024 AU total for the county. Therefore, rounding up from 167 AU to 1000 AU to calculate the PCA is a conservative assumption because a >1000 AU feedlot cannot contain <1000 AU.

As stated above, the PCA factors (also referred to as watershed density factors) are used by the EPA to scale the final PRZM-estimated concentrations to represent the area in the watershed that actually contributes to the chemical concentrations in runoff and erosion. In this case, PCA factors were used to scale the watershed area that contributed to concentrations from cropland, pasture and feedlot. For example, in Table 27, the IA watershed was 21,128 acres in size. Using the PCA factor of 0.094% for area of watershed that represents feedlots <1000 AU, the actual acres modeled were as follows:

21,128 acres X 0.094% = 19.8 acres of feed lot surface in the IA watershed.

The acres of manured cropped land, feedlot, and pastureland available for grazing are represented by these PCA factors. The schematic in Figure 19 illustrates how individual land use patterns within a large watershed were scaled to a smaller representative watershed using the PCA factors.

Figure 19. Schematic of Modeling Using EPA Tier-2 Approach

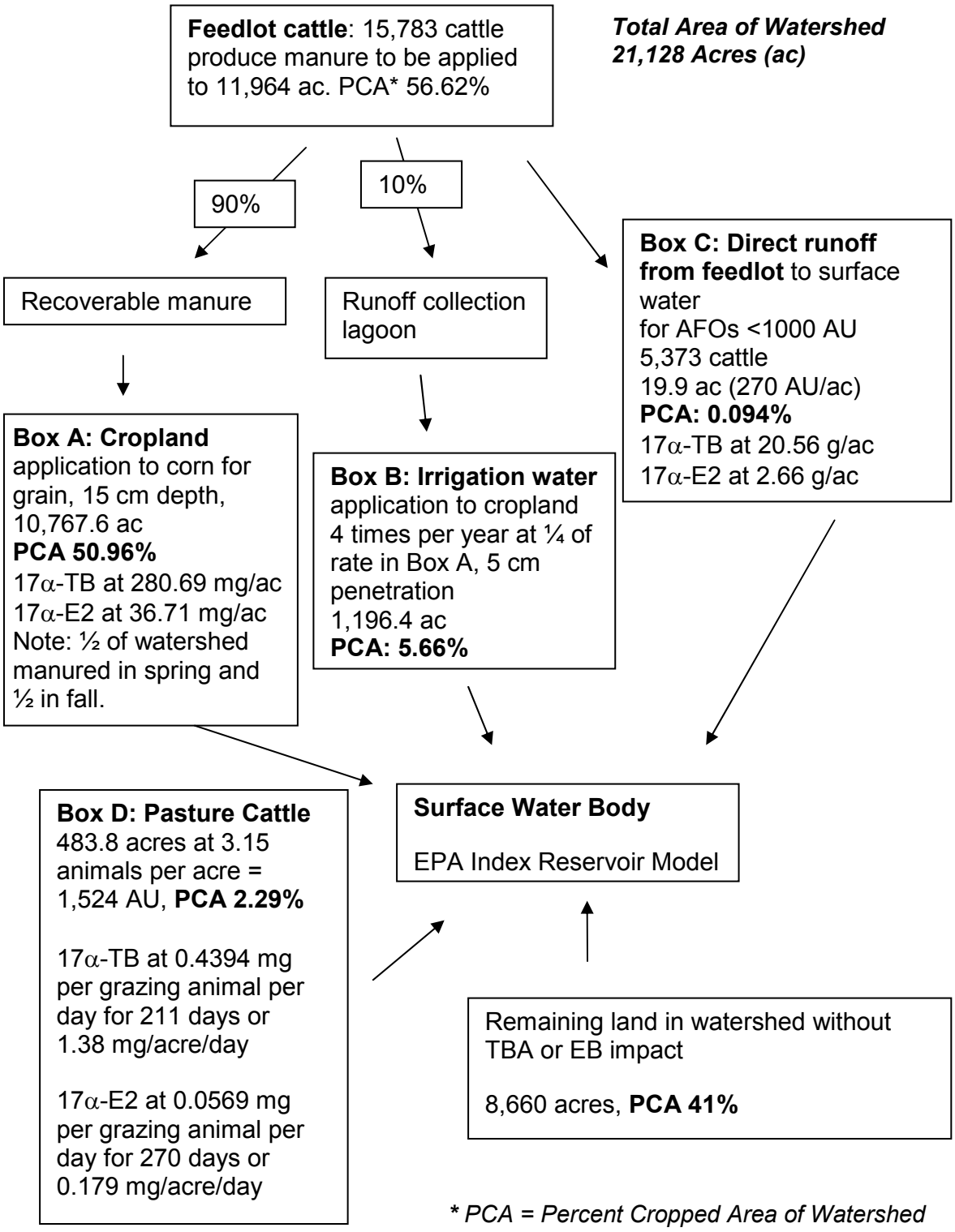


5.7.4. Example mixed-use watershed: Watershed in Lyon/Sioux County, IA

To better explain the assumptions and methods used in the mixed-use watershed, the modeling approach for the Lyon/Sioux Co, IA watershed is outlined below.

Figure 20 provides a visual representation (conceptual model) to illustrate the flow of manure runoff and transport of manure solids from potential sources modeled in the 90th percentile watershed in IA. The actual watershed in Iowa used to derive cattle numbers and land characteristics is shown in Figure 21. The assumptions outlined in Sections 5.7.5 through 5.7.8 for each exposure pathway (feedlot, pasture and manure applied to cropland) were incorporated. The size of the watershed was 21,128 acres and contained 15,783 beef cattle in feedlots, of which 5,373 cattle were in feedlots <1000 AU with a greater propensity for inadequate runoff control (Table 27, Section 3.2.1, and Appendix 9). A full explanation of assumptions and methods is provided in Attachment 1.

Figure 20. Conceptual Model of Iowa Watershed



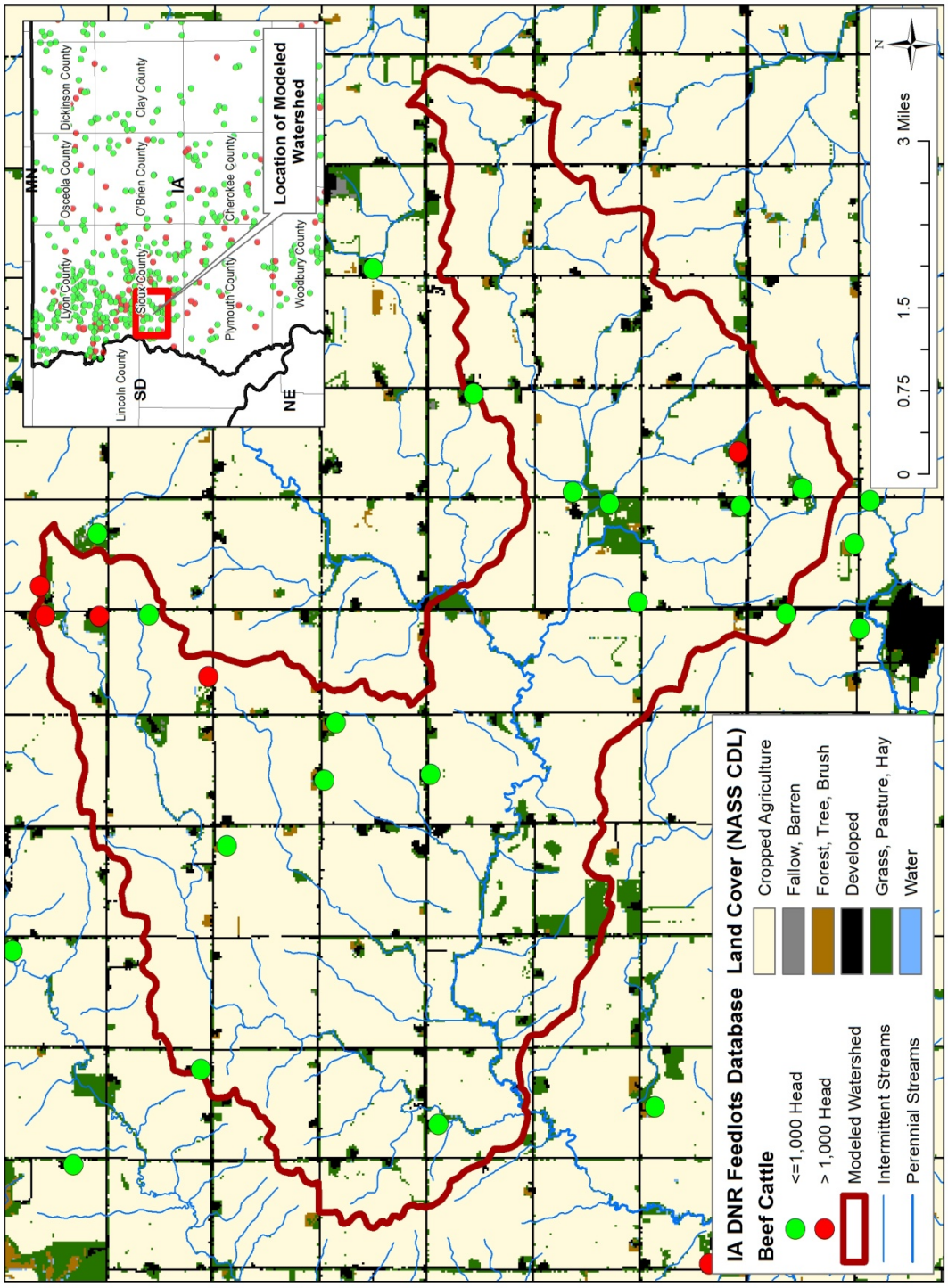


Figure 21. Actual Watershed in Iowa that was Modeled as a Mixed-Use Watershed

5.7.5. Assumptions, methods, and input parameters used to model runoff from solid manure amended cropland in the watershed model (Box A of Figure 20)

In order to simulate the runoff of the surrogate estradiol and trenbolone compounds from cropland amended with manure and to estimate the surface water concentrations, the following modeling input parameters are needed:

1. The selection of specific crop scenario(s) to be simulated
2. The application date, application rate of the surrogate estradiol and trenbolone compounds based on the P_2O_5 requirements of the crop, and depth of incorporation into soil
3. The specific environmental fate parameters for the surrogate estradiol and trenbolone compounds (Table 9 and Table 13)

5.7.5.1. Selection of crop scenario, application rate, and depth of incorporation into soil

The principal crops grown in the five modeled counties are corn, wheat for grain, soybeans, and alfalfa for hay (Attachment 1). In Michigan, sugar beets and dry beans are also prominent crops, and in Texas, sorghum and cotton are significant crops (Attachment 1). The historical crop yields for these counties were determined from USDA agricultural surveys and are presented in Table 89 in Appendix 8.2. The methodology used to obtain these yields was identical to that described in Appendix 7 for the EXPRESS scenarios. Briefly, the highest regional yield data for the individual crops were transferred to Table 90 (Appendix 8.2) to calculate the amount of phosphorus (P_2O_5) from manure required for each of the crop types. From the amount of P_2O_5 required by the crop per acre, the amounts of the surrogate estradiol and trenbolone compounds applied per acre with the manure were determined.

In all regions, corn silage required the greatest amount of manure based on the crop's expected yield and P_2O_5 requirement to achieve the specified yield (Table 90 in Appendix 8.2). In Pennsylvania, corn silage is the dominant crop type with 40% of the corn acreage harvested as silage (Table 89 in Appendix 8.2). In the other regions, the prominent crop type was corn for grain, not silage. Therefore, for PA, the application rate for corn silage from Table 90 (Appendix 8.2) was used as the conservative application rate. For MI, OH, IA and TX, the application rate for corn for grain was used. These application rates are shown in bold text in Table 90 (Appendix 8.2). In Lancaster County, PA and Mercer County, OH, no-till is a common agricultural practice [93]. Therefore, the corn crop was modeled as no-till in these two counties with a 5-cm incorporation depth. All other regions were modeled as tilled crops with a 15-cm incorporation depth.

5.7.5.2. Selection of manure application date

Manure from a farm is removed from feedlots and typically stored in close proximity to the feedlot for a period of time. This manure can be used throughout the year, but is generally applied either prior to planting in the spring or after harvest in the fall. Unlike pesticides, which are applied at relatively predictable times, application timing for manure is difficult to predict for individual crops. The predicted runoff from the model will vary depending upon the time of application relative to rainfall events (before or after rainfall as well as the amount of rainfall in an event). The same application days (one in the fall and one in spring) were used for all 30 years of the model simulation. For each watershed, conservative, yet realistic, application times were selected based on annual rainfall data from a 30-year weather dataset. The methodology is described in Appendix 7.1.1 and Attachment 1. It was also assumed that manure was divided, with 50% being applied to half of the manured watershed in the spring prior to planting and the remaining 50% applied to the other half of the manured watershed in the fall after harvest (Attachment 1).

5.7.5.3. IA example calculation in Box A of Figure 20

The methods used to calculate the values presented in Box A of Figure 20 are presented below:

- In a watershed with 21,128 acres, 15,783 cattle produce enough manure to be applied to 11,964 acres (0.758 acres/AU from Table 90).
- As stated in Section 5.7.1, it was assumed that 90% of manure went directly to cropland as solid manure application and that 10% went to the runoff collection lagoon, which was applied as irrigation water.
- 11,964 x 90% = 10,767.6 acres received solid manure potentially containing EB and TBA metabolites. This acreage and a depth of 15-cm uniform incorporation were used as model inputs for IA. See Appendix 2 for discussion of till and no-till application practices and incorporations depths.
- The PCA treated with manure (10,767.6 acres) was 50.96% of the total 21,128 acre watershed area as shown in the example for the IA watershed (Figure 20).

5.7.6. Assumptions, methods, and input parameters used to model runoff from cropland irrigated with storage lagoon/pond water (Box B of Figure 20)

To determine the manure contributing to runoff in collection lagoons from feedlots, the amount of erosion of the feedlot to the lagoon was estimated based on a publication by the University of Nebraska Cooperative Extension [94]. In a study with penned cattle (4% slope within the facility), it was found that 6.2% of organic matter in pens was lost to runoff in the summer months and 1.9% was lost during the winter months. When these totals are summed, it results in a yearly total of approximately 8.1%. Therefore, a conservative value of 10% was used to account for manure erosion from feedlots into runoff collection lagoons.

The same input parameters needed to model runoff from the solid manure application in Section 5.7.5 are required to simulate the runoff from cropland irrigated with storage lagoon/pond water. In the model, surrogate estradiol and trenbolone compounds in manure collected in lagoons were applied to cropped fields via the irrigation water during the growing period of the crop. Four application intervals were used throughout the growing season. The rate for each of the four applications was ¼ the rate applied to manured

cropped fields (Box B). It was assumed that the irrigation water was surface-applied. An incorporation depth of 5 cm was used, assuming uniform distribution due to surface roughness and bioturbation from soil organisms. See Appendix 2 for additional information on surface application assumptions. The fields where irrigation water was applied are different than those where solid manure was applied.

5.7.6.1. IA example calculation in Box B of Figure 20

The methods used to calculate the values presented in Box B of Figure 20 are presented below:

- In a watershed with 21,128 acres, 15,783 cattle produce enough manure to be applied to 11,964 acres (0.758 acres/AU from Table 90).
- As stated in Section 5.7.1, it was assumed that 90% of manure went directly to cropland as solid manure application and that 10% went to the runoff collection lagoon, which was applied as irrigation water.
- 11,964 x 10% = 1,196.4 acres receiving manure potentially containing EB and TBA metabolites from the runoff collection lagoon.
- The PCA treated with irrigation water (1,196.4 acres) was 5.66% of the total 21,128 acre watershed area.

5.7.7. Direct runoff from feedlots to surface water (Box C of Figure 20)

CAFOs (i.e., lots with ≥1000 head) are required by EPA regulations to have feedlot runoff collection lagoons or other designs to prevent surface water contamination. Unlike AFOs, CAFOs are also required to obtain a government-issued (NPDES or state-equivalent) permit and are subject to inspection. Therefore, in modeling direct runoff from feedlots, it was assumed that all CAFOs with >1000 head were in compliance with the Clean Water Act and are not discharging to surface water. See Section 3.2.1 for additional information on the regulatory requirements of CAFOs.

Many small and medium AFOs with <1000 head will not fall within the criteria to be defined as a CAFO and may not be designated by the state authorities as a CAFO, and therefore, may not need to follow management requirements that apply to CAFOs (e.g., control of wastewater runoff). But, regardless, we have conservatively assumed that a portion of these AFOs are not in compliance with the Clean Water Act and are directly discharging to surface waters (i.e., they are significant contributors of pollutants to surface waters). For modeling purposes, we have used the entire range of possible values for small and medium AFOs <1000 head that may be directly discharging to surface waters (0, 25, 50, 75, 100% of AFOs with direct discharge were evaluated). In Appendix 9, we have estimated that only approximately 17% of AFOs on a nationwide basis have runoff that directly enters into surface water. Based on this estimate, we have used an assumption of 25% AFOs <1000 head are directly discharging to surface waters to represent what we believe is a typical nationwide scenario. We have also used an assumption of 50% of AFOs <1000 head are direct dischargers to represent a reasonable worst-case for local watersheds. Because many of these AFO feedlots are not in direct proximity to surface water, and those close to a water body are likely to be regulated for pollution control, use of this 50% assumption in this modeling is expected to result in a conservative overestimation of PEC values.

In order to simulate the runoff of the potential surrogate estradiol and trenbolone compounds from the surface of feedlots with <1000 cattle, the following parameters are needed:

1. The typical cattle stocking density on a feedlot in the US
2. Maximum estimated concentrations of surrogate estradiol and trenbolone compounds on a feedlot surface
3. Feedlot surface characteristics, such as depth, bulk density, organic carbon concentration, and field capacity
4. Additional modifications to the WinPRZM default values to simulate a feedlot environment such as runoff curve number and soil erosion equation
5. The specific environmental fate parameters for surrogate estradiol and trenbolone compounds (Table 9 and Table 13)

5.7.7.1. Stocking density of feedlot cattle

In order to determine the acres of land associated with feedlots in a watershed, a typical cattle stocking density on a feedlot was determined. In a feedlot composition study by Cole et al. [95], the average density of cattle in the earthen feedlots studied was 15 m^2 per animal. In another publication on modeling runoff from feedlots, the author modeled densities of 15 and 20 m^2 per animal [96]. Thus, for modeling runoff from feedlots in this EA, a cattle density of 15 m^2 per animal for earthen feedlots was used. Because there are 4046.9 m^2 per acre, a 1000 animal feedlot would typically be 3.7 acres in size, which corresponds to 270 AU per acre.

5.7.7.2. IA example calculation in Box C of Figure 20

The methods used to calculate the values presented in Box C of Figure 20 are presented below:

- There are 5,373 cattle in lots <1000 AU and, therefore, 19.9 acres of feedlot are required in the watershed to accommodate this number of cattle.
- Therefore, the PCA for feedlot (19.9 acres) was 0.094% of the total 21,128 acres.

5.7.7.3. Maximum concentrations in feedlot manure pack

When modeling the feedlot scenario, a constant surrogate estradiol and trenbolone compound concentration was assumed for the feedlot manure pack to a depth of 10 cm (see Appendix 8.1 for supporting literature; [97, 98]). Table 87 and Table 88 (Appendix 8.1) contain calculations to estimate the concentrations of the surrogate estradiol and trenbolone compounds on the feedlot surface; 6.6 and 50.8 µg/kg, respectively (or 2.66 and 20.56 g/acre, respectively). These concentrations were used for all regions modeled. Additional information on these calculations can be found in Appendix 8.1.

5.7.7.4. Model modifications to simulate feedlot runoff

To simulate a feedlot with the PRZM model, it was necessary to modify the program code of WinPRZM model version 4.51 (Attachment 1). The WinPRZM model is a version of the PRZM model used in the European Union (EU) for pesticide risk assessment.

The soil profile for a feedlot was represented in a different manner than for a cropland. The top 10-cm layer of feedlot soil profile was assumed to be manure. The next 10 cm was assumed to be "interface layer," which is a mixture of manure and soil. The top 20 cm of a feedlot was collectively simulated as "manure pack." To model the feedlot environment, a constant concentration of the chemical was maintained in the top 10 cm of the feedlot manure pack with a slope of 4%. The bulk density, organic carbon content, field capacity, and crop wilting point for each 10 cm horizon were also modified to simulate a feedlot hard pack in the top 10 cm. Appendix 8.1 contains an explanation and supporting information regarding the selection of the feedlot characteristics and depth.

No applications of the chemical were made for the feedlot simulation, as was done in the manure amended cropland scenario. Rather, to maintain a constant concentration in the feedlot manure pack, any drug lost through erosion and runoff was replaced daily to maintain the constant concentration of 6.6 µg/kg and 50.8 µg/kg for the surrogate estradiol and trenbolone compounds, respectively (as reported above and in Table 87 and Table 88, respectively, in Appendix 8.1). Also, the manure erosion equation and the runoff curve numbers used in WinPRZM were modified so the terrestrial portion of the model would mimic a feedlot rather than a cropped field. Descriptions of the modifications made to WinPRZM are provided in Section 5.3 of Attachment 1.

It should be noted that the constant daily replacement of surrogate estradiol and trenbolone compounds effectively increased the total quantity of surrogate estradiol and trenbolone compounds excreted in feedlots within the watershed, which resulted in an overestimation of runoff from those feedlots. In addition, the total watershed PEC values were also overestimated because it was assumed that 100% of surrogate estradiol and trenbolone compounds excreted on a feedlot are available to runoff from the feedlot surface and 100% are available (following a holding period) in the manure to be applied to manure amended cropland. Thus, the proportions of EB and TBA metabolites excreted from feedlot animals were not attributed to a specific source. For example, 100% of the surrogate estradiol and trenbolone compounds from ONE-F were assumed to be present in the manure and irrigation water applied to cropland (Boxes A + B in Figure 20), and 100% of surrogate estradiol and trenbolone compounds were assumed to be present at a constant concentration in the feedlot runoff model (Box C, AFOs <1000 AU). Typically, in real-world circumstances, some metabolites would be expected to runoff from the feedlot manure pack, leaving <100% of the metabolites in the manure applied to cropland. Thus, these assumptions conservatively increased the overall mass of metabolites entering the watershed in the models.

5.7.8. Pasture cattle (Box D of Figure 20)

Pasture density relates to the percentage of the watershed comprised of lands potentially used for pasturing or grazing cattle. To estimate the pasture density at the watershed level in the five study regions, the grass and pasture/hay land use types from the NASS Cropland Data Layer (CDL) program (remotely sensed geospatial data) were used in combination with the Census of Agriculture figures on pasturing acres to locate lands in the watershed with the potential for pasturing and grazing of beef cattle. This approach uses the best source of pastured land area (the Census of Agriculture) and estimates its location within the watershed using the location information from the NASS CDL remote sensing spatial data. The density of pastureland in each of the five watersheds is reported in Table 27. More information on the methods used to determine the acres of pastureland is available in Attachment 1.

5.7.8.1. IA example calculation in Box D of Figure 20

The methods used to calculate the values presented in Box D of Figure 20 are presented below:

- 483.8 acres were identified by GIS as pasture. With a stocking density of 3.15 AU/acre, there are 1,524 pasture cattle in the IA example watershed.
- Therefore, the PCA for pastureland in the watershed is 2.29% (483.8 acres) of the 21,128 acre watershed.

In order to simulate the runoff of the potential surrogate estradiol and trenbolone compounds from the surface of beef cattle pastureland, the following parameters are needed:

1. The typical cattle stocking density on pasture in the US
2. Concentrations of EB and TBA metabolites excreted daily from pasture cattle
3. Manure application start and end date
4. Pasture surface characteristics, such as depth, bulk density, organic carbon concentration, and field capacity,
5. Additional modifications to the WinPRZM default values, such as incorporation depth, runoff curve number, and the soil erosion equation to simulate a pasture environment
6. The specific environmental fate parameters for the surrogate estradiol and trenbolone compounds (Table 9 and Table 13)

5.7.8.2. Daily excretion rates of EB and TBA metabolites from grazing cattle

Application rates of surrogate estradiol and trenbolone compounds to pasture were estimated based on daily release rate and cattle stocking density for pasture of 3.15 head/acre. See Appendix 5 for pasture cattle density. The application rate for pasture is presented as a daily application rate for the number of days it takes for EB and TBA to be completely released from the Synovex ONE-G implant. It was assumed that every pasture steer and heifer in each watershed was implanted with ONE-G in spring, which released EB and TBA metabolites at the known rate and duration described below; the duration is described in Section 5.7.8.3 below.

- 0.0759 mg estradiol/day x 100% excretion = 0.0759 mg estradiol/day (Appendix 6.2)
- 0.8193 mg trenbolone/day x 71.5% excretion = 0.5858 mg trenbolone/day

The implant for pasture cattle contains 6 slow release pellets identical to the 8 pellets used in the release rate study; therefore, the release rate for pasture cattle was adjusted by a factor of 0.75 (6/8).

- 0.0759 mg estradiol/day x 0.75 = 0.0569 mg EB metabolite per grazing animal/day
- 0.5858 mg trenbolone/day x 0.75 = 0.4394 mg TBA metabolite per grazing animal/day

5.7.8.3. Application dates of surrogate estradiol and trenbolone compounds to pastureland

To model cattle grazing on pasture, daily loadings or applications of the compound excreted in the form of manure can be modeled with user-specified start and stop dates. It was assumed that cattle began grazing on pastureland on April 1st each year in IA, PA, OH, and MI and on March 1st each year in TX. Excretion end times from grazing cattle were estimated based on the number of days it would take to completely exhaust EB and TBA from the Synovex ONE-G implant, i.e., 211 days for TBA and 270 days for EB (Appendix 13.4). Therefore, the release of TBA and EB from the implants (and subsequent excretion of the EB and TBA metabolites) from pasture cattle ends on October 28th and December 26th, respectively, for IA, PA, OH, and MI. For TX, the release of TBA and excretion of its metabolites from pasture cattle ends on September 27th and on November 26th for the release of EB and excretion of its metabolites. Additional information concerning the selection of start and end dates for excretion of EB and TBA metabolites from pasture cattle are provided in Section 5.3.2 of the study report in Attachment 1.

5.7.8.4. Pastureland model modifications

To simulate a pasture environment with the WinPRZM model, the program code of WinPRZM model version 4.51 was modified (see comments regarding the WinPRZM program in Section 5.7.7 and Attachment 1). The incorporation depth into soil was uniformly 5 cm due to surface roughness and bioturbation by soil organisms (Appendix 2). To model daily excretion rate for 211-270 days, PRZM code was modified to simulate daily loadings at user-specified dates (Attachment 1). Pasture scenarios were developed by changing erosion and runoff parameters to simulate less runoff than cropped land due to the increased infiltration of water into untilled grassland (Attachment 1).

5.7.9. PEC_{water} results from five mixed-use watershed study regions

The acute and chronic 90th percentile exposure concentrations (PEC_{water}) for the surrogate estradiol and trenbolone compounds generated from the five regions modeled in the aggregate mixed-use watershed are presented in Table 91 through Table 100 in Appendix 8.3. Tabular data are presented for the following: 1) conservative scenarios where it was assumed that 25 and 50% of small and medium AFOs with <1000 AU directly discharge to surface water, and 2) occurrences where 100% of the watershed is pastureland (PCA = 100%). The tables and plots presented in this section were produced using these data.

5.7.9.1. Contribution of individual sources of EB and TBA metabolites to 21-day PEC_{water} estimates

An analysis was conducted to estimate the contribution of each source (i.e., feedlot, manured cropland, irrigated cropland, and pastureland) in a watershed (Appendix 8.3.6). The Iowa study region and the surrogate trenbolone compound were selected for this analysis because the highest surface water concentrations were predicted for this case. The mixed-use watershed model was run four times, each time with a single contributing source: feedlot, cropland with solid manure, cropland with collection pond water, and pasture. The PCA used for each contributing source was 0.094%, 50.96%, 5.66%, and 2.29%, respectively. A PCA factor of zero was used for other sources to represent a non-contributing source. The runoff from feedlot surfaces was, by far, the largest contributor to the PEC_{water} values. The 90th percentile 21-day PEC_{water} values for feedlot runoff (2.32 ng/L) was more than an order of magnitude greater than those for manured cropland (0.19 ng/L)

and grazed pasture (0.006 ng/L). These results are not unexpected given that feedlots are highly prone to surface losses through runoff and erosion because of bare (uncropped) feedlot surface covered with loose uncompacted manure pack containing a higher amount of trenbolone and estradiol (50.8 g/ha) compared to cropland and pasture. Additional information on the methods and results of this analysis are presented in Appendix 8.3.6 and in the study report in Attachment 1.

Although the modeling found that direct runoff from AFOs had the greatest influence on the PEC values, at this time, there is no specific data available to determine the percentage of feedlots with direct runoff in the five mixed-use watersheds modeled. In order to cover the entire range of possibilities, all five mixed-use watershed models were run assuming different percentages of AFOs with direct runoff, including 0, 25, 50, 75, and 100%. A percentage of 25% AFOs with direct discharge is expected to conservatively represent a typical nationwide situation (see paragraph below). These percentages were modeled by changing the feedlot PCA factor to 0, 25, 50, 75, and 100% of the feedlot PCA for each watershed. All PEC_{water} data for the aggregate exposure in a watershed are presented in Table 91 through Table 100 in Appendix 8.3 and are illustrated in Figure 22 and Figure 23.

In Appendix 9, it was estimated by Zoetis that 17% of feedlots on a national scale are potentially in need of runoff and erosion control improvements, and therefore, are likely to directly discharge to surface waters. However, this is an estimate based on national statistics and cannot necessarily be extrapolated to all regions of the country. The percentage is likely to differ regionally based on local factors (e.g., weather, enforcement, etc.). Therefore, an assumption of 25% was used to represent a conservative national average based on the 17% estimation from Appendix 9. In addition, an assumption of 50% was also used to represent a reasonable worst-case for a local scenario. The PEC values assuming 50% and 25% of AFOs are directly discharging are presented below and are used in the risk characterization in Section 7.

5.7.9.2. Watershed aggregate PEC_{water} values assuming 50% of AFOs <1000 AU discharge directly to surface waters

In the risk characterization (Section 7), the 21-day NOEC values for disruption in fish reproduction endpoints were compared to 21-day PEC_{water} values. The yearly maximum 21-day moving average 90th percentile PEC_{water} values for the surrogate estradiol and trenbolone compounds are presented in Table 28 below. These values represent the assumption that 50% of AFOs discharge directly into surface waters (see results for 25% of AFO discharge in Section 5.7.9.3). The 90th percentile 21-day PEC_{water} values for the surrogate estradiol compound were 0.11, 0.05, 0.09, 0.04, and 0.02 ng/L for IA, PA, OH, MI, and TX, respectively. The 21-day 90th percentile PEC_{water} values for the surrogate trenbolone compound were 1.26, 0.54, 0.92, 0.40, and 0.23 ng/L for IA, PA, OH, MI, and TX, respectively. The full results are presented in the study report in Attachment 1.

Table 28. 90th Percentile PEC_{water} Values for the Surrogate Estradiol and Trenbolone Compounds Assuming 50% of Feedlots with <1000 AU Discharge Directly to Surface Waters

		90th percentile concentrations in water (ng/L)					
Region	**Drug**	**Peak**	**96-Hour**	**21-Day**	**60-Day**	**90-Day**	**Annual**
Iowa	Trenbolone	1.33	1.31	**1.26**	1.18	1.12	0.91
	Estradiol	0.12	0.12	**0.11**	0.10	0.09	0.07
Pennsylvania	Trenbolone	0.58	0.57	**0.54**	0.51	0.50	0.42
	Estradiol	0.06	0.05	**0.05**	0.05	0.05	0.04
Ohio	Trenbolone	0.98	0.96	**0.92**	0.89	0.85	0.71
	Estradiol	0.09	0.09	**0.09**	0.08	0.08	0.06
Michigan	Trenbolone	0.42	0.41	**0.40**	0.37	0.36	0.30
	Estradiol	0.04	0.04	**0.04**	0.03	0.03	0.03
Texas	Trenbolone	0.28	0.27	**0.23**	0.16	0.14	0.05
	Estradiol	0.03	0.03	**0.02**	0.02	0.01	<0.01

5.7.9.3. Watershed aggregate PEC_{water} values assuming 25% of the AFOs <1000 AU discharge directly to surface waters

In Table 29, the 90th percentile PEC_{water} values are reported assuming 25% of feedlots with <1000 AU discharged directly to surface waters. The 21-day 90th percentile PEC_{water} values for the surrogate estradiol compound were 0.06, 0.03, 0.04, 0.02, and 0.02 ng/L for IA, PA, OH, MI, and TX, respectively. The 21-day 90th percentile PEC_{water} values for the surrogate trenbolone compound were 0.70, 0.27, 0.46, 0.21, and 0.22 ng/L for IA, PA, OH, MI, and TX, respectively. The full results are presented in the study report in Attachment 1.

Table 29. 90th Percentile PEC_{water} Values for the Surrogate Estradiol and Trenbolone Compounds Assuming 25% of Feedlots with <1000 AU Discharge Directly to Surface Waters

		90th percentile concentrations in water (ng/L)					
Region	**Drug**	**Peak**	**96-Hour**	**21-Day**	**60-Day**	**90-Day**	**Annual**
Iowa	Trenbolone	0.74	0.72	**0.70**	0.67	0.62	0.48
	Estradiol	0.07	0.07	**0.06**	0.05	0.05	0.04
Pennsylvania	Trenbolone	0.30	0.29	**0.27**	0.26	0.25	0.22
	Estradiol	0.03	0.03	**0.03**	0.02	0.02	0.02
Ohio	Trenbolone	0.50	0.49	**0.46**	0.45	0.43	0.36
	Estradiol	0.05	0.05	**0.04**	0.04	0.04	0.03
Michigan	Trenbolone	0.22	0.22	**0.21**	0.19	0.19	0.15
	Estradiol	0.02	0.02	**0.02**	0.01	0.02	0.01
Texas	Trenbolone	0.28	0.26	**0.22**	0.16	0.13	0.04
	Estradiol	0.03	0.03	**0.02**	0.02	0.01	<0.01

Figure 22 and Figure 23 illustrate the PEC_{water} model concentrations in each of the five regions when the compliance of AFOs with the Clean Water Act is varied from 0% to 100%. Although national averages and regional values will likely differ, approximately 17% of feedlots may require improvements to reduce the potential for runoff discharges, as estimated in Appendix 9.

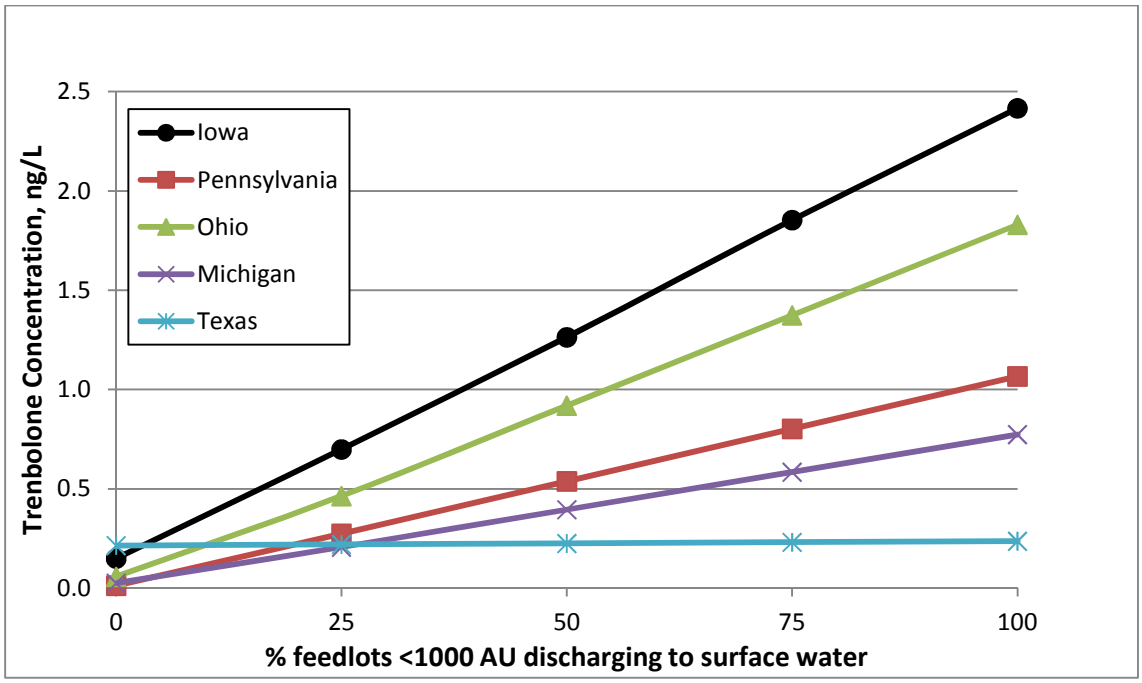
5.7.10. Discussion and conclusions of results of mixed-use watershed PEC_{water} values

The PEC_{water} values for the surrogate compounds are shown in Figure 22 and Figure 23 for the five watersheds modeled (at different percentages of AFOs directly discharging). Of those modeled, Iowa had the highest 21-day chronic PEC_{water} values. When assuming 50% of AFOs are directly discharging, the PEC values for the surrogate estradiol and trenbolone compounds were 0.111 and 1.264 ng/L, respectively (Table 91 and Table 92). When assuming 25%, the PEC values were 0.060 and 0.699 ng/L, respectively (Table 91 and Table 92, respectively). These PEC values were used in Section 7 to characterize the risk for environmental impacts.

Because of their compacted soil, lack of vegetation, and other factors, feedlots have a high runoff and erosion potential which results in a higher amount of drug transport to surface waters compared to other sources (i.e., manured cropland and grazed pastures). Of the watersheds modeled, Iowa had the highest relative percentage of acres attributed to feedlots with <1000 head (PCA factor = 0.094%, Table 27) and also the highest PEC values. The source contribution analysis discussed in Appendix 8.3.6 indicates that this is due to runoff from these feedlots.

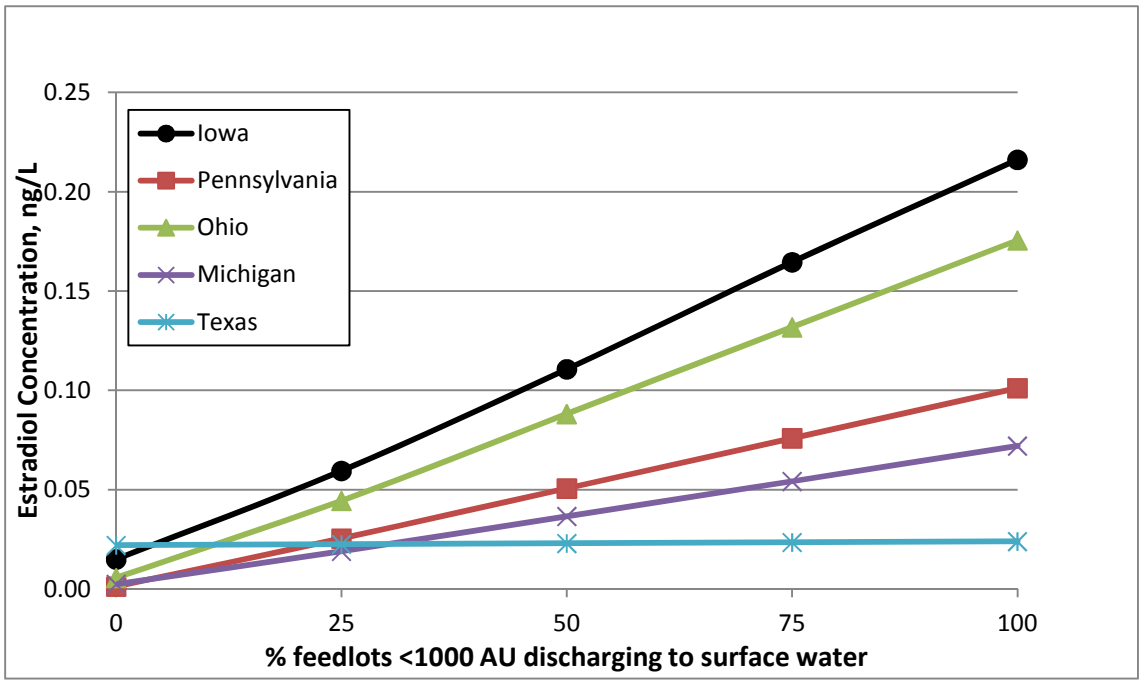
In contrast, Texas had the lowest PCA factor for feedlots with <1000 head, and the lowest PEC values. The Texas region had a relatively high PCA factor for cropland (53.6%) compared to a feedlot PCA of 0.003%. In Texas, most cattle are in CAFOs >1000 AU and are required to have a CNMP that includes runoff control measures such as holding ponds. In Texas, slope, soil type, and curve number are more sensitive parameters because cropland is the major contributor of mass loadings to the water body. When looking at the slope of the surrogate trenbolone and estradiol compound concentration lines in Figure 22 and Figure 23 for the Texas watershed, it is apparent that changing the percentage of feedlots with <1000 AU discharging has very little effect in comparison to the other watersheds. This is because most of the cattle in the Texas panhandle are in lots >1000 AU. Therefore, the PEC_{water} concentrations are principally driven by runoff from manured crop land and pastures. In Table 25, Castro County, TX has only 1.18% of cattle in lots <1000 AU but Huron County, MI has 46%. Therefore, changing this variable will have a greater effect in MI than in TX.

Figure 22. 21-Day 90th Percentile Chronic Surface Water Concentrations (PEC_{water}) of the Surrogate Trenbolone Compound in all Five Mixed-use Watersheds at Different Percentages of AFOs (<1000 head) with Direct Runoff



Note that the scale of the trenbolone plot (Figure 22) is ten times higher in concentration than the estradiol plot (Figure 23).

Figure 23. 21-Day 90th Percentile Chronic Surface Water Concentrations (PEC_{water}) of the Surrogate Estradiol Compound in all Five Mixed-use Watersheds at Different Percentages of AFOs (<1000 head) with Direct Runoff



Note that the scale of the trenbolone plot (Figure 22) is ten times higher in concentration than the estradiol plot (Figure 23).

6. ANALYSIS: EFFECTS ASSESSMENT

Due to the use pattern of hormone implants and the potential for multiple ED-contributing sources within a watershed, there is the potential for chronic exposure in the environment. These products will be implanted into most or all cattle contained in an AFO for up to 365 days a year. The extended-release characteristics of Synovex ONE have the potential to result in trenbolone, estradiol, and their metabolites entering the environment on a continuous or semi-continuous basis, and therefore, potentially contributing to the estrogen and androgen load already present in the environment from other sources (e.g., pesticides, human wastewater, wildlife, plant estrogens).

The overall goal of this risk assessment is to evaluate potential effects on aquatic ecosystems using measureable endpoints in single species toxicity tests that can be used to predict potential population-level impacts. Based on their endogenous physiological role, steroid hormones, such as estradiol and trenbolone, are expected to have effects on the endocrine system of vertebrates, which regulates growth and reproduction. Therefore, chronic endpoints that measure effects on growth and reproduction in vertebrates are more relevant and sensitive indicators of estradiol and trenbolone exposures than acute endpoints.

Based on the expected mechanism of action of steroid hormones and the body of literature on their environmental fate and effects, fish reproduction parameters (e.g., fecundity, embryo fertility, sex ratio) are the most pertinent endpoints to assess potential ecosystem level impacts, resulting from chronic exposures of estradiol and trenbolone.

6.1. Acute and Chronic Effects

Acute effects of xenobiotics are evaluated over a short exposure period, typically 2-4 days. The typical endpoints in acute tests are either an effect concentration (EC_{50}) or lethal concentration (LC_{50}) in which 50% of the test population is affected by the xenobiotic. There are only a few acute effects studies for trenbolone and estradiol in the scientific literature. A 48-hour acute *Daphnia magna* toxicity study was conducted with 17α-trenbolone [236]. Based on immobility, the LC_{50} was estimated to be 340,000 ng/L (Appendix 13.6), which is approximately five orders of magnitude higher than the 17α-trenbolone PEC_{water} value associated with the use of Synovex ONE (Section 7.2). However, chronic exposures to 17α-trenbolone have been found to produce effects at concentrations much lower than those observed following acute exposures. For example, reproduction (fecundity) was reduced when fish were exposed over 14-21 days to 94-120 ng/L of 17α-trenbolone (Section 6.2.3.3). These concentrations are at least three orders of magnitude lower than the acute LC_{50} for *D. magna* (Table 30).

A similar trend was also found for estradiol. LC_{50} values of 2,970,000 and 2,870,000 ng/L were reported by Hirano et al. [99] and Brennan et al. [100], respectively, for *D. magna* exposed to 17β-estradiol over 48 hours (Table 30), whereas chronic fish reproduction endpoints were found to be much more sensitive, with lowest observed effect concentrations (LOEC) ranging from 8.66-669 ng/L for 17β-estradiol (Table 35). Further, data available in the literature demonstrate that chronic endpoint values, such as reproduction, result in lower effects concentrations than acute endpoint values for similar steroid hormones, e.g., 17α-ethinylestradiol (EE2) [101, 102].

As discussed above, chronic exposures are more relevant for assessing the potential impact of estradiol and trenbolone on the environment due to their effects on reproduction. Chronic tests evaluate the potential effects of long-term exposures to lower concentrations of xenobiotics, which allows for the observation of effects beyond the simple lethality endpoint used in acute studies, e.g., growth and reproduction. The goal of a chronic study is to establish a No Observed Effect Concentration (NOEC) and a LOEC. Over the years, scientific research has focused on fish as the most sensitive species to EDCs, specifically assessing reproduction to understand the potential chronic effects caused by androgens and estrogens in the environment. Further support for this is demonstrated by the species sensitivity distributions (SSD) for EE2 and 17β-estradiol that have been published by Caldwell et al. [102]. In general, exposure to estrogens has resulted in feminization of male fish, while masculinization of female fish has been associated with exposure to androgens. These phenotypic changes can adversely affect the reproductive capability of these fish, and if sustained over time, could ultimately result in a decline in their populations.

The effects of 17β-estradiol and 17β-trenbolone on aquatic species have been extensively studied and reported in the scientific literature. However, there is little to no literature available on the chronic effects of 17α-estradiol and 17α-trenbolone. Thus, a selection of available effects data for plants, invertebrates, amphibians, and fish exposed to estradiol and trenbolone metabolites are described below to demonstrate that fish reproduction-related effects are the most sensitive endpoints for estrogens and androgens.

Plants

Based on their physiological role, estradiol and trenbolone are expected to affect the endocrine system of vertebrates; as such there is an abundance of publications on the effects of estradiol and trenbolone on fish and fish reproduction. Not unexpectedly, few studies have been conducted with microbes and plants because little evidence exists in these taxa for a physiological role for vertebrate sex steroids [103]. In addition, Caldwell et al. [101, 102] concluded that algae and aquatic macrophytes are insensitive to estradiol. Data on the effects of estradiol and trenbolone on terrestrial plants do not exist; however, manure containing estradiol and trenbolone has been applied as fertilizer to agricultural crops over the years with no reported observed adverse effects.

Invertebrates

There are several published studies demonstrating that chronic exposures of aquatic invertebrates to 17β-estradiol do not result in significant effects at concentrations <400,000 ng/L (Table 30 below). For example, Kashian and Dodson [104] found that exposures of 1,000, 10,000, and 100,000 ng/L of 17β-estradiol over 6 days did not affect sex determination, reproduction, or growth in *D. magna*. Brennan et al. [100] found no effects on *D. magna* reproduction or their ability to moult in the first generation (F0) when exposed to 17β-estradiol at concentrations up to 1,000,000 ng/L over 21 days. There were no significant effects on survival at 200,000 ng/L. However, effects were observed on survival in the 400,000, 600,000, 800,000, and 1,000,000 ng/L treatments over 21 days in both the F0 and F1 generations. In addition, in the F0 generation, a maximum mortality of 40% was observed at 1,000,000 ng/L, while 100% mortality was observed in the F1 generation at concentrations ≥800,000 ng/L. Jukosky et al. [105] did not find any effects on the survival or reproduction of another daphnid species, *Ceriodaphnia dubia*, exposed to 5,000, 50,000, 500,000, and 1,000,000 ng/L of 17β-estradiol over 7 days. Jukosky et al. also noted that the hormones tested, including 17β-estradiol, EE2, progestin, and

medroxyprogesterone, had no effects on reproduction or survival of *C. dubia* at 10^6 times the concentrations at which reproductive effects have been documented in several fish species. The acute and chronic data for invertebrates exposed to 17β-estradiol are summarized in Table 30.

Table 30. Acute and Chronic Effects of 17β-Estradiol and Acute Effects of 17α-Trenbolone on Aquatic Invertebrates

Species	Duration	Endpoint[a]	Endpoint	ng/L	Reference
17β-Estradiol					
D. magna	48 hours	Immobilization	LC_{50}	2,970,000	Hirano et al. [99]
A. bahia[d]	96 hours	Survival	LC_{50}	890,000	
D. magna	48 hours	Immobilization	EC_{50}	2,870,000	Brennan et al. [100]
		Moulting frequency	EC_{50}	2,040,000	
D. magna	6 days	Sex determination, reproduction, and growth	LOEC	>100,000 (F0)	Kashian and Dodson [104]
D. magna	21 days	Survival	LOEC	400,000 (F0)[c]	Brennan et al. [100]
		Reproduction and ability to moult	LOEC	>1,000,000 (F0)[b]	
			NOEC	600,000 (F1)[b]	
C. dubia	7 days	Survival and reproduction	LOEC	1,000,000[b]	Jukosky et al. [105]
17α-Trenbolone					
D. magna	48 hours	Immobilization	EC_{50}	340,000	Pfizer [236]

[a] The effect observed at the lowest concentration was reported. There may have been additional effects not reported in this table at higher concentrations.
[b] No effects in F0 were observed at the concentrations tested. F1 could not be evaluated at >600,000 ng/L due to 100% mortality observed at ≥800,000 ng/L.
[c] LOEC is listed based on mortality observed in F0 (20%) and F1(30%); however, statistical significance of these effects was not reported.
[d] *Americamysis bahia*, mysid shrimp

Amphibians

Adverse effects on metamorphosis has been observed in amphibians exposed to steroid hormones. Several studies published in the literature demonstrate the effects of 17β-estradiol on amphibians (Table 31); however, amphibian data are limited for 17α-estradiol and 17α-trenbolone. Lutz et al. [106] exposed larvae of the African clawed frog (*Xenopus laevis)* to 17β-estradiol at concentrations ranging from 200-6,000 ng/L. Exposures occurred from 6-8 days post-fertilization through the completion of metamorphosis at 82 days post-fertilization. An EC_{50} of 120 ng/L of 17β-estradiol was proposed based on effects on development and sexual differentiation. Wolf et al. [107] also exposed *X. laevis* to 200, 1500, and 6,000 ng/L of 17β-estradiol from eight days post-fertilization through completion of metamorphosis at Nieuwkoop-Faber (NF) stage 66 or at 82 days post-fertilization, whichever came first. This study was performed in two different laboratories to confirm reproducibility. Gonadal histology confirmed that at concentrations ≥200 ng/L, the sex ratio was skewed towards phenotypic females. An increase in mixed sex gonads were observed at concentrations >200 ng/L. In addition, histological findings indicated an increase in dilated testicular tubules, dividing gonocytes in the testis, and

dilated ovarian cavities in phenotypic ovaries. These results were confirmed by Kloas et al. [108], who observed gonadal feminization and delayed onset of metamorphosis in *X. laevis* exposed to 200 ng/L of 17β-estradiol. Sharma and Patino [109] also exposed *X. laevis* to 17β-estradiol from fertilization to metamorphosis (NF stage 66 or day 75, whichever came first). Exposure to 1,000 ng/L of 17β-estradiol resulted in skewed sex ratios toward phenotypic females, and the onset of metamorphosis was delayed.

Table 31. Chronic Effects of 17β-Estradiol on Amphibians

Species	Duration	Endpoint[a]	Endpoint	ng/L	Reference
X. laevis	82 days post-fertilization	Development and sexual differentiation	EC_{50}	120	Lutz et al. [106]
X. laevis	82 days post-fertilization	Skewed sex ratio toward females	NOEC	<200	Wolf et al. [107]
X. laevis	82 days post-fertilization	Gonadal feminization and delayed onset of metamorphosis	NOEC	<200[b]	Kloas et al. [108]
X. laevis	75 days	Skewed sex ratio toward females and delayed onset of metamorphosis	NOEC	<1000	Sharma and Patino [109]

[a] The effect observed at the lowest NOEC was reported. There may have been additional effects not reported in this table at higher concentrations
[b] Based on exposure to only a single concentration

There is limited information on the effects of trenbolone metabolites on amphibian growth, development and metamorphosis (Table 32 below). Finch et al. [110] exposed *X. laevis* to 10, 100, and 500 ng/L of 17α-trenbolone from two days post-hatch (dph) through 60 dph. Total body mass, total length, snout-vent length, and stage of development were reduced at 500 ng/L of 17α-trenbolone, resulting in a NOEC of 100 ng/L for 17α-trenbolone. No effects on tail length, survival or swimming behavior were observed. Omstead et al. [111] exposed *X. tropicalis* to 78, 100, 310, 1,250, and 5,000 ng/L of 17β-trenbolone in Experiment 1 and to 3.7, 11, 33, and 100 ng/L in Experiment 2. Exposures occurred from <48 hours until metamorphosis in both experiments, with an additional 6-week grow-out period in clean water included in Experiment 1 only. Larval survival in post-NF stage 58 tadpoles was reduced at concentrations ≥100 ng/L. Nuptial pad presence (male secondary sex characteristic) was induced in both male and female tadpoles at 100 ng/L. Although there were no effects on time to complete metamorphosis or body size observed up to 100 ng/L in Experiment 2, body mass and length six weeks post-metamorphosis were reduced in grow-outs placed in clean media at concentrations ≥78 ng/L. Evaluations of sex ratio were equivocal, skewed towards males in Experiment 1 at 78 ng/L and no effects observed up to 100 ng/L in Experiment 2. Histological assessment of gonads showed half with normal male phenotypes and half with mixed-sex phenotypes at 100 ng/L. Based on these results, a NOEC of 33 ng/L was reported for the secondary sex characteristic of nuptial pad prevalence and NOECs of 78 or 100 ng/L were established for the remaining endpoints evaluated following larval exposure to 17β-trenbolone, as outlined in Table 32.

Table 32. Chronic Effects of 17β-Trenbolone and 17α-Trenbolone on Amphibians

Species	Isomer	Duration	Endpoint[a]	Endpoint	ng/L	Reference
X. laevis	17α-TB	60 days	Body mass, total length, snout-vent length, and stage of development	NOEC	100	Finch et al. [110]
X. tropicalis	17β-TB	82 days post-fertilization	Survival and gonadal histology	NOEC	78	Omstead et al. [111]
			Body length and mass, time to metamorphosis		100[b]	
			Secondary sex characteristic		33	

[a] The effect observed at the lowest NOEC was reported. There may have been additional effects not reported in this table at higher concentrations

[b] LOEC of 78 ng/L was reported for body mass and length following 6 week grow-out period in clean media, post-metamorphosis

Summary

Based on information presented above, 17β-estradiol appears to have no significant effects on aquatic invertebrates at concentrations <400,000 ng/L, while effects on the sex ratio and onset of metamorphosis in amphibians can occur at concentrations ≤200 ng/L. As will be presented in Section 6.2.3.2, a LOEC associated with fish reproduction endpoints was reported at 8.66 ng/L of 17β-estradiol (Table 35). Reported effects on invertebrates, amphibians, and fish following exposures to trenbolone indicate a similar order of increasing species sensitivity. An EC_{50} of 340,000 ng/L was reported for *D. magna* following exposure to 17α-trenbolone. Effects on growth and development in amphibians following exposure to 17β-trenbolone were observed at concentrations of ≥78 ng/L, while the reported effects on fish reproduction were observed at concentrations of ≥26 ng/L (Table 42).

Therefore, based on available literature data, fish are the most sensitive sentinel taxonomic group of aquatic organisms to evaluate the effects of exposure to estradiol and trenbolone. Further, because trenbolone and estradiol are known to affect the endocrine system, reproduction-related endpoints (e.g., fecundity, sex ratio, embryo fertilization, etc.) are the most sensitive endpoints available indicative of potential population-level impacts. As discussed in Section 6.2.1, biomarkers, such as vitellogenin (VTG), are often sensitive measures of exposure, but these endpoints are not necessarily predictive of reproduction- and/or population-level effects at any particular concentration. This risk assessment will therefore focus on the potential for effects on fish reproductive-related endpoints following chronic exposure to the active metabolites of concern excreted from cattle following the use of Synovex ONE: 17β-estradiol, 17α-estradiol, 17β-trenbolone, and 17α-trenbolone.

6.2. Chronic Fish Reproductive Effects for Estradiol and Trenbolone Metabolites

There are many publications on the potential ED effects on fish, which typically include survival, growth, reproduction (fecundity), sex ratio, vitellogenin (VTG), gonadosomatic index (GSI), hepatosomatic index (HSI), secondary sex characteristics, embryo fertility, embryo hatchability, larvae survival and development, and gonadal histology, etc. Significant effects observed in biomarker endpoints, such as VTG, are considered evidence of exposure and may indicate the potential for a compound to behave as an endocrine disruptor. However, significant effects on fecundity and survival, for example, are indicative of population-relevant effects. For the purposes of this EA, VTG data were used to compare the relative potency of the estradiol metabolites (17α-estradiol, 17β-estradiol, and estrone) and the TBA metabolites (17α-trenbolone, 17β-trenbolone, and trendione) in Section 6.2.1. Reproduction related effects data (i.e., fecundity, fertility, etc.) in Sections 6.2.2 and 6.2.3 were used to determine the predicted no effect concentrations (PNECs) for use in the risk characterization in Section 7. Data on the effects of estradiol and TBA metabolites (including 17β-estradiol, 17α-estradiol, 17β-trenbolone, and 17α-trenbolone) on fish reproduction from the published literature, as well as Zoetis-owned studies conducted by Pfizer Animal Health, are presented in Sections 6.2.2 and 6.2.3.

6.2.1. Vitellogenin-Based Relative Potency of Estradiol Metabolites and Trenbolone Metabolites

VTG, an egg yolk protein precursor, is commonly referred to as a biomarker of exposure for EDCs, and therefore, may be considered a valuable tool for understanding relative potencies of endocrine disruptors such as estradiol and trenbolone. The VTG data presented below were used to characterize the relative potencies of the 17β isomers of estradiol and trenbolone and the principal metabolites of EB and TBA found in cattle manure, specifically 17α-estradiol and 17α-trenbolone.

6.2.1.1. VTG data for estradiol metabolites

In general, estrogens excreted from cattle are principally 17α-estradiol and estrone, along with low levels of 17β-estradiol (Section 4.1.1). 17α-Estradiol, the principal metabolite of EB, and 17β-estradiol are transformed in soil and water to estrone, but do not accumulate (Sections 4.2.4.11 and 4.2.6.11). Based on cell binding assays, biomarker data (e.g., VTG), and reproduction data, the largest body of work published on estrogens has been on 17β-estradiol. According to a review of published estrogen biomarker data, Caldwell et al. [102] proposed that the order of activity (from highest to lowest) of estradiol metabolites is: 17β-estradiol > estrone > estriol; however, the authors did not evaluate 17α-estradiol. To help understand the effects of exposure to estradiol on VTG and then apply these data to understand relative potency of the potential estradiol metabolites, several published studies on VTG levels in fish exposed to estradiol metabolites were reviewed and summarized in Table 33.

Table 33. Summary of Effects of Estradiol Metabolites on VTG in a Several Male Fish Species

Species	Substance	Duration	VTG[a]	Endpoint	ng/L	Ref
Juvenile Rainbow Trout	17β-Estradiol	14 days	Plasma	LOEC	19-26	Thorpe et al. [112]
	Estrone				60	
Fathead minnow	17β-Estradiol	21 days	Plasma	LOEC	18	Shappell et al. [113]
	17α-Estradiol				151	
Fathead minnow	17α-Estradiol	21 days	Plasma	LOEC	250	Pfizer [242]
Medaka	17β-Estradiol	21 days	Liver	LOEC	21.9	Ministry of Japan [114]
Medaka	17β-Estradiol	21 days	Liver	LOEC	8.94	Seki et al. [115]
Fathead minnow		14 days	Plasma		28.6	
Zebrafish		21 days	Plasma		85.9	
Fathead minnow	17β-Estradiol	21 days	Plasma	LOEC	10	OECD [116]
Medaka			Liver		10-32	
Zebrafish			Plasma		32-100	
Medaka	17β-Estradiol	101 days	Liver	LOEC	8.66	Seki et al. [117]
Medaka	17β-Estradiol	25 days	Liver	LOEC	29.3	Kang et al. [118]

[a] Only the effects of estradiol metabolites on adult male fish plasma and liver VTG were included. There were other studies conducted during early life stages and on induction of VTG messenger ribonucleic acid (mRNA) that are not included in the table, but are discussed below.

Summary of Estradiol Metabolite Effects on VTG

The VTG results summarized in Table 33 for the estradiol metabolites support the following conclusions:

- **Relative potency of estrone to 17β-estradiol:** The data reported by Thorpe et al. [112] indicate that the potency of estrone to induce VTG is approximately 1/3rd that of 17β-estradiol. However, this value is based on only one study.
- **Relative potency of 17α-estradiol to 17β-estradiol:** The data reported by Shappell et al. [113] indicate that the potency of 17α-estradiol to induce VTG is approximately 1/8th that of 17β-estradiol[s]. This conclusion is also supported by the fathead minnow study by Pfizer [242] for 17α-estradiol (250 ng/L) and the fathead minnow studies by OECD [116] and Seki et al. [115] for 17β-estradiol (10 ng/L and 28.6 ng/L, respectively). These studies indicate that the potency of 17α-estradiol to induce plasma VTG is approximately 1/25th to 1/9th that of 17β-estradiol[t]. Also, EC_{50} estimates of VTG, choriogenin H (CHG-H), and estrogen receptor "α" (ERα) in the livers of male medaka indicate that 17α-estradiol is approximately 11-17 times less potent than 17β-estradiol [119].
- **Conclusions for VTG:** The data in Table 33 have been limited to direct comparisons between estradiol metabolites, either within a study or for common species recommended in standard OECD guidelines. Expanding the table to include VTG observations to non-standard test species would likely increase variability. However, data from this table suggest that: 1) 17β-estradiol is more potent than 17α-estradiol, and 2) the fathead minnow is a more sensitive species than medaka and zebrafish. In addition, the results from the OECD inter-laboratory validation study [116] demonstrate

[s] 18 ng/L divided by 151 ng/L equals 1/8th

[t] 10 ng/L divided by 250 ng/L equals 1/25th and 28.6 ng/L divided by 250 ng/L equals 1/9th

that VTG concentrations are variable both within and between species and can be inconsistent between laboratories.

6.2.1.2. VTG data for trenbolone metabolites

17α-Trenbolone, the principal metabolite of TBA found in the manure of cattle treated with Synovex products [47.2% in feces and 6.2% in urine (Section 4.1.2)], is transformed in soil and water to trendione, and to a lesser extent to, 17β-trenbolone. Minimal data have been published on 17α-trenbolone in comparison to 17β-trenbolone; however, the effects on VTG, along with other *in vitro* data, may be applied to understand relative potency. Some available data are summarized in Table 34.

Table 34. Effect of 17α- and 17β-Trenbolone on VTG in Several Female Fish Species

Species	Substance	Duration	VTG	Endpoint	ng/L	Ref.
Fathead minnow	17β-Trenbolone	21 days	Plasma	LOEC	4060	Seki et al. [115]
Medaka			Liver		40	
Zebrafish			Plasma		351	
Fathead minnow	17β-Trenbolone	21 days	Plasma	LOEC	50-500[a]	OECD [116]
Medaka			Liver		50-500[a]	
Zebrafish			Plasma		500-5000	
Fathead minnow	17α-Trenbolone	21 days	Plasma	LOEC	>120	Pfizer [243]
Medaka	17α-Trenbolone	21 days	Liver	LOEC	10	Pfizer [244]
Fathead minnow	17β-Trenbolone	21 days	Plasma	LOEC	26	Ankley et al. [120]
Fathead minnow	17α-Trenbolone	21 days	Plasma	LOEC	32	Jensen et al. [121]

[a] Reference [116] includes data from 4 laboratories. Only one or two laboratories reported effects at 50 ng/L.

Summary of Trenbolone Metabolite Effects on VTG

Based on the published data presented in Table 34, both 17β-trenbolone and 17α-trenbolone can reduce female VTG levels in fish species at concentrations ranging from 10 to 5000 ng/L. The effects were highly variable between laboratories and even within species (e.g., fathead minnow). The *in vivo* VTG data for 17α-trenbolone and 17β-trenbolone appear to be much more variable than the estradiol data presented in Table 33. It is therefore difficult to calculate a relative potency using VTG data, as was presented for estrogens in Section 6.2.1.1.

6.2.1.3. Use of VTG data to support PNEC

Arguments in Miller et al. [122] are made to support a link between biomarker data (i.e., molecular, biochemical, or histological endpoints) and effects in individuals and populations. At this time, there is a general consensus in the literature that VTG is acceptable as a biomarker of exposure in fish for certain endocrine disrupting compounds. However, VTG is not yet considered a predictive biomarker of effect because effects on this endpoint have not yet been directly related to individual or population-relevant impacts (i.e., a decrease in survival, reproduction, fertility, etc.). In addition, data presented in this EA demonstrate that VTG is generally not a more sensitive endpoint to estradiol and trenbolone exposure than fish reproductive endpoints (e.g., fecundity, fertilization, etc.). Therefore, because we are concerned about potential population level impacts in this EA, we have used fish survival and reproduction endpoints as the primary regulatory assessment endpoints.

6.2.2. Estradiol metabolite fish reproduction-related effects

The overall goal of this assessment is to evaluate the potential for significant effects on ecosystems using endpoints that are indicative of population-level impacts. Based on the information presented above, the best indicators of true population-relevant impacts are the effects on fish reproduction endpoints. This is also supported by Mills and Chichester [123]. Primary among these endpoints is fecundity; however, other endpoints such as fertility and offspring sex ratio can also be important for predicting population-level impacts. Thus, chronic effects values based on reproduction-related endpoints were the primary basis for establishing the PNEC values in this EA.

A chronic effects value (or in some cases a range of values) for the estradiol metabolites was derived from studies selected using the criteria described under Section 1.3. In addition to these criteria, the effects study also needed to meet validation criteria described in OECD guideline 229 [124] or other acceptable guidelines. The published literature and Zoetis-owned study data for effects of 17β- and 17α-estradiol on fish reproduction endpoints that met these criteria are discussed below.

6.2.2.1. Summary of Estradiol Metabolite Effects on Fish Reproduction

The reproduction-related effects in fish with the lowest NOEC and LOEC values are summarized in Table 35. The studies referenced in Table 35 are presented in detail in Sections 6.2.2.2 and 6.2.2.3.

Table 35. Summary of Effects of 17α- and 17β-Estradiol on Fish Reproduction Endpoints (i.e., Fecundity, Embryo Survival/Fertility/Hatchability, Sex Ratio)

Species	Salinity (ppt)	Duration	Endpoint[a]	NOEC (ng/L)	LOEC (ng/L)	Reference
17β-Estradiol Studies						
Japanese Medaka (F0 and F1 generation)	FW[b]	101 days post-fertilization	Embryo fertility	2.86	8.66	Seki et al. [117]
Sheepshead minnow	20	280 days (F0-F3)	Fecundity	189-F0 36-F1 36-F2	290-F0 82-F1 82-F2	Cripe et al. [129]
Sand Goby	30	8 months	Fecundity	97	669	Robinson et al. [131]
Java-medaka	33.8	6 months	Fertility	9.5	16	Imai et al. [132]
Japanese Medaka	FW	21 days	Fecundity and fertility	227	463	Kang et al. [118]
17α-Estradiol Studies						
Fathead minnow	FW	21 days	Fecundity, Embryo survival/fertility/ hatchability, sex ratio	250	>250	Pfizer [242]

[a] The fish reproduction endpoints with the lowest NOEC or LOEC were reported in the table. Effects on other reproduction endpoints observed at higher concentrations are not reported here. In addition, the effects on other endpoints (e.g., gonadal histology, GSI) are summarized in Section 6.2.1.1 but are not reported in this table.

[b] FW=freshwater

The following conclusions for estradiol metabolite effects on fish reproduction were made based on the studies reviewed in Section 6.2.2.2 and Section 6.2.2.3 (Table 33 and Table 35):

- **17α-Estradiol:** Based on the lack of published fish reproduction data for 17α-estradiol (the principal EB metabolite in manure held on feedlots), a chronic (21 day) reproduction study in the fathead minnow was conducted under GLP conditions following OECD Guideline 229 [242]. The measured exposure concentrations ranged from 2.5 to 250 ng/L and resulted in no significant effects ($p>0.05$) on survival, reproduction (fecundity), embryo fertilization, and F1 survival and development. Although, the NOEC for male VTG was 80 ng/L (Table 41), the most relevant endpoints in this study for assessing population-level impacts are reproduction-related endpoints. The NOEC and LOEC for all reproduction endpoints (fecundity, sex ratio, embryo fertilization and hatching success, and embryo and larval survival and development) were 250 ng/L and >250 ng/L, respectively (reference [242] and Appendix 13.12). Thus, a NOEC of 250 ng/L was used to derive the PNEC value for 17α-estradiol in Section 6.3.2.
- **17β-Estradiol:** The NOEC values from four long duration (160-280 days) reproduction studies with 17β-estradiol ranged from 2.86 ng/L (based on embryo fertility in freshwater medaka) to 227 ng/L (fecundity in medaka). These data are summarized in Table 35. The LOEC values ranged from 8.66 to 669 ng/L of 17β-estradiol. In addition, Caldwell et al. [102] estimated a NOEC for 17β-estradiol using an SSD analysis. The SSD analysis was conducted using 21 reproductive NOEC values reported in 19 chronic reproduction studies on 17β-estradiol using eight fish species. The authors proposed a NOEC value of 4.3 ng/L (95% CI of 1.5 and 4.5 ng/L), which represents the 5th percentile from the SSD analysis. Based on the acceptable data in Table 35, a conservative NOEC value of 2.86 ng/L was chosen to derive the PNEC value for 17β-estradiol in Section 6.3.2. While Caldwell et al. used data that did not always meet FDA's acceptability criteria, the NOEC value derived for 17β-estradiol in that publication (4.3 ng/L) is very closely aligned with the NOEC value selected for this EA (2.86 ng/L), and supports that the NOEC value chosen is conservative.
- **Estrone and Estriol:** A PNEC was not derived for estrone or estriol because the evaluation of 17β- and 17α-estradiol should be conservatively predictive of any effects from exposure to estrone and estriol. There have been three chronic fish reproduction studies conducted with estrone [125, 126, 127] and only one for estriol [125]. No adverse effects on fish reproduction were observed in these studies at ≥98 ng/L. Limited VTG data suggest that estrone is 1/3rd as potent as 17β-estradiol and also that the activity of estrone may be similar to 17α-estradiol [112] (Section 6.2.1.1). In addition, Metcalfe et al. [125] suggests that estrone is 1/15th as potent as 17β-estradiol, and estriol is about four times less potent than estrone and about 1/30th as potent as 17β-estradiol. However, Caldwell et al. suggests that estriol is likely less potent than estimated by Metcalfe et al. and proposes that estriol is about 1/300th as potent as 17β-estradiol.

6.2.2.2. Presentation of Fish Reproduction Studies for 17β-Estradiol

In this section, five published literature articles that investigated the effects of 17β-estradiol on fish reproduction endpoints are reviewed. The reproduction-related effects on fish NOEC and LOEC values described in the studies are summarized in Table 35 above.

Estimating a predicted no effects concentration for 17β-estradiol, estrone, and estriol using a species sensitivity distribution (SSD) [Caldwell et al.]

Caldwell et al. [102] developed an SSD for 17β-estradiol in order to derive a PNEC for fish. An SSD was developed using a total of 21 NOEC values obtained from chronic (14 to 280 days) fish reproduction studies conducted with eight fish species. The SSD was constructed by fitting a distribution to the NOECs obtained from the 21 studies. An HC5,50 (a hazard concentration for which 5%[u] of all the species tested are affected and the 50% confidence interval) was derived from the reproduction data, which was found to be the most sensitive endpoint. The resulting HC5,50 for fish reproduction was 4.3 ng/L (95% CI = 1.5 and 4.5). The authors determined the PNEC by applying an AF of 2 to account for the absence of a long-duration study exposing the Chinese rare minnow, a species that appears to be the most sensitive to estrogens, to 17β-estradiol. Thus, a PNEC value of 2 ng/L was proposed for 17β-estradiol (PNEC = 4.3 ng/L / 2 = 2 ng/L). The authors noted that the PNEC for fish reproduction was lower than the effects values for induction of VTG in male fish.

An SSD could not be developed for estriol or estrone because insufficient reproduction data were available. Instead, the authors used *in vivo* VTG induction studies to determine the relative ability of estrone and estriol to induce VTG and then compared that to the results of 17β-estradiol to derive the respective PNEC values. Based on three studies evaluating the induction of VTG in fish exposed to estrone, it was determined that estrone is approximately 1/3rd less potent than 17β-estradiol. The PNEC for estrone was derived by applying this factor to the PNEC for 17β-estradiol; thus, the PNEC of 6 ng/L was proposed for estrone.

For estriol, the authors discussed the results from two studies: one literature study published by Metcalfe et al. [125] that evaluated the effect of estriol on the induction of VTG, and a thesis publication by Routledge [128] that tested the estrogenic activity of estriol using a yeast estrogen screen. Metcalfe et al. suggested that estriol is 1/4th as potent as estrone and 1/30th as potent as 17β-estradiol; whereas, Routledge determined that estriol is approximately 1/100th as potent as estrone. After further evaluation of the Metcalfe et al. data, Caldwell et al. found that Metcalfe et al. may have had a typographical error and concluded that the relative potency for estriol should be 1/300th as potent as 17β-estradiol (not 1/30th as potent as proposed by Metcalfe et al.). Thus, using the method described above, Caldwell et al. suggests that the PNEC value for estriol is 60 ng/L, which was derived using an AF of 10.

[u] According to the authors, selecting the 5th percentile of the SSD means that as long as concentrations of 17β-estradiol are less than or equal to the HC5, 95% of the species tested will not display adverse effects associated with exposure to 17β-estradiol.

Fish full life-cycle testing for effects of 17β-estradiol on Japanese medaka (*Oryzias latipes*) [Seki et al.]

Seki et al. [117] conducted a two-generation study examining the chronic effects of 17β-estradiol on reproduction in medaka (*Oryzias latipes*). Parental generation (F0) embryos were exposed from 12 hours post-fertilization to 101 days. First generation (F1) embryos were exposed through 59 days post hatch. Mean measured concentrations of 17β-estradiol delivered under flow-through conditions were 0.939, 2.86, 8.66, 27.9, and 92.4 ng/L. The control group was exposed to dechlorinated tap water only. The assessment endpoints for F0 and F1 generations included survival, embryo development, hatching success, post hatch survival and growth, sexual differentiation, reproduction (F0 only) and hepatic VTG.

In the F0 generation, no significant differences in mortality, abnormal behavior and appearance were observed between treatment groups and the controls. Although growth (length and body weight) in the F0 generation was significantly different ($p<0.05$), with an increase observed in the 2.86 and 8.66 ng/L treatment groups compared to the controls, no significant difference in growth was observed in 27.9 and 92.4 ng/L treatments when compared to the controls. The sex ratio, based on secondary sex characteristics, was significantly different ($p<0.01$) and was skewed toward female characteristics at ≥27.9 ng/L when compared to the controls (Table 36). Gonadal histology confirmed a significant difference ($p<0.01$), which was associated with a decrease in the number of fish with testis and a significant increase in number of fish with ovaries and testis-ova in the 27.9 and 92.4 ng/L treatments compared to the controls. Specifically, all fish in the 92.4 ng/L treatment groups had ovaries, indicating complete sex reversal, while seven of the 20 fish in the 27.9 ng/L treatment group had testis-ova gonads (Table 36). Male VTG concentrations were significantly higher in fish exposed to 8.66 ng/L ($p<0.05$) and 27.9 ng/L ($p<0.01$) when compared to the controls. Egg production in the 27.9 ng/L treatment group was significantly different ($p<0.01$), with fewer eggs produced when compared to the controls, whereas the 92.4 ng/L treatment group did not produce any eggs during the reproductive phase. Fertility and VTG induction were observed to be the more sensitive endpoints with NOEC and LOEC values of 2.86 ng/L and 8.66 ng/L, respectively (Table 36). There were no significant differences noted for the F1 generation exposed at ≤8.66 ng/L. However, the F1 generation was not produced at concentrations greater than 8.66 ng/L; thus, effects on the F1 generation could not be assessed at concentrations >8.66 ng/L.

Table 36. Reproduction Effects of 17β-Estradiol on Parental (F0) and First Generation (F1) of Japanese Medaka (*O. latipes*)

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Fecundity	8.66	27.9
Embryo fertility	2.86	8.66
VTG induction in males	2.86	8.66
Skewed sex ratio toward females/secondary sex characteristics[a]	8.66	27.9

[a] Histology: testis-ova gonads were observed in males at 27.9 ng/L. Complete sex reversal was observed in males at 92.4 ng/L.

Reference Seki et al. [117].

Reproduction effects in a multigenerational exposure of sheepshead minnow (*Cyprinodon variegatus*) to 17β-estradiol [Cripe et al.]

The EPA protocol for assessing chronic multigenerational reproduction effects in sheepshead minnow (*Cyprinodon variegatus*) was used by Cripe et al. [129] to conduct a 280-day study with 17β-estradiol to assess reproduction and development effects on the parental (F0) generation and three subsequent (F1, F2, and F3) generations. The study was initiated with the F0 generation consisting of reproductively active fish. Replicates included spawning groups of three females and two males. Following a 10-day acclimation and an 8-day pre-exposure spawning response period, 17β-estradiol exposures were initiated along with seawater control and solvent control groups. Following 18-21 days of exposure, a subset of normal embryos from each spawning group was collected and incubated, which consisted of the F1 generation. After 21 days, the adult fish were sexed, weighed, and samples were collected for plasma VTG and histology. The F1 generation was continuously exposed through maturation and spawning. A subset of 60 eggs was again collected from each spawning pair and grown until spawning occurred (F2 generation). The mean measured exposure concentrations for F0 and F1 were 12, 36, 82, 189, and 290 ng/L, ranging from 68 ± 25% to 153 ± 64% of nominal. F2 and F3 generations were exposed to 200 ng/L or less.

In the study, the most consistent endpoint was reproduction rate, which was determined for each 7-day interval of the three week spawning period (i.e., three consecutive 7-day spawning intervals), resulting in a NOEC of 36 ng/L and a LOEC of 82 ng/L for all three generations. However, egg production was variable across all three generations. For example, the cumulative egg collection in the first 7-day interval for the F0 generation resulted in a NOEC of ≥290 ng/L, whereas the second and third 7-day intervals resulted in NOEC values of 82 and 189 ng/L, respectively. Egg production in the F1 generation was more consistent, resulting in a NOEC of 82 ng/L for all three 7-day intervals. The F2 generation had inconsistent reproduction, resulting in NOEC values of 82, 36, and 82 ng/L for the three 7-day intervals (Table 37). The 21-day cumulative egg production NOEC values were determined to be 189, 36, and 36 ng/L for the F0, F1, and F2 generations, respectively. There was a significant ($p \leq 0.05$) but inconsistent difference observed in egg fertility in the F1 and F2 generations at 36 ng/L (LOEC), resulting in a NOEC of 12 ng/L, whereas the NOEC for egg fertility for the F0 generation was 290 ng/L (Table 37). It should be noted that spawning in the 290 ng/L treatment group could not be assessed because all fish appeared to be phenotypic females. The mean GSI values of female fish exposed to 290 ng/L in the F0 and F1 generations were significantly lower ($p<0.0001$) when compared to the controls (Table 37). No significant effects on HSI were determined in the males in either generation or in females in the F0 generation. The HSI of females in the F1 generation exposed to 36, 189, and 290 ng/L treatments were significantly different ($p<0.001$), being lower when compared to the solvent control (Table 37).

Based on the data from this multigenerational study in sheepshead minnow, Cripe et al. [129] ranked the endpoints from most to least sensitive as production of abnormal (F2) or infertile embryos (F1 and F2), reproduction rate (F0 and F2), cumulative embryo production (F1 and F2), changes in ovarian tissue histopathology (F0 and F1), VTG (F1), and female GSI (F0 and F1). Changes in standard length were inconsistent across treatment levels and generations tested. Therefore, production of infertile eggs appears to be the most sensitive endpoint following exposure of sheepshead minnow to 17β-estradiol under the conditions of this study.

Table 37. Reproduction Effects of 17β-Estradiol on Sheepshead Minnow (*Cyprinodon variegatus*) Over Multiple Generations

	Generation LOEC and NOEC (ng/L)					
	F0		F1[c]		F2[c]	
Endpoint	**NOEC**	**LOEC**	**NOEC**	**LOEC**	**NOEC**	**LOEC**
Growth	NA	NA	290	>290	NA	NA
Cumulative embryo production (over 21 days)	189	290	36	82	36	82
Reproduction rate (# normal embryos/female/day) (for each interval 2-4)[a]	36[b]	82[b]	36	82	36	82
Abnormal embryos	290	>290	290	>290	12	36
Infertile eggs	290	>290	12	36	12	36
Ovarian histology	82	189	82	189	NA	NA
GSI	189	290	189	290	NA	NA
HSI	290	>290	12	36	NA	NA
Vitellogenin	290	>290	82	189	NA	NA

[a] Interval 1: pre-exposure F0, not included in the analysis; Interval 2: days 1-7; Interval 3: days 8-14; and interval 4: days 15-21
[b] Data for intervals 3 and 4. Interval 2: NOEC 189 ng/L, LOEC 290 ng/L
NA = not measured
GSI = gonadosomatic index; HSI = hepatosomatic index
[c] All fish were phenotypic females at 290 ng/L; thus, no spawning occurred at 290 ng/L.
Reference Cripe et al. [129].

Population-level effects in a multigenerational modeling of sheepshead minnow (*Cyprinodon variegatus*) to 17β-estradiol [Raimondo et al.]

In a companion paper to Cripe et al. [129], Raimondo et al. [130] used population modeling to determine how the measured effects of 17β-estradiol on sheepshead minnow (*Cyprinodon variegatus*) would potentially alter natural populations of fish. Although effects on reproduction rate were observed at 82 ng/L during the laboratory study, these effects were not expected to result in a significant difference at the population level because, based on the population modeling results, the sex ratio was skewed towards more females which compensated for the loss of reproductive output. The authors concluded that population-level effects were significantly different ($p \leq 0.0038$) following chronic exposures to 17β-estradiol at 200 ng/L in the F2 generation, resulting in a population-relevant NOEC of 82 ng/L in the sheepshead minnow. The authors [129] stated that the overall LOEC of 82 ng/L reported by Cripe et al. [129] for the F1 generation (also LOEC for interval 3 and 4 for reproduction rates of F0 generation) would be protective of sheepshead minnow populations exposed to estradiol for two generations and could provide a conservative estimate for a screening-level assessment.

Bioindicators and reproductive effects of prolonged 17β-estradiol exposure in a marine fish, the sand goby (*Pomatoschistus minutus*) [Robinson et al.]

Robinson et al. [131] investigated the effects of 17β-estradiol on survival, growth rates, sexual maturation, hepatic VTG mRNA expression, and reproductive success during an 8-month waterborne exposure of a marine fish, the sand goby (*Pomatoschistus minutus*). The study was initiated with juvenile sand gobies collected in early autumn from an estuary in northeastern Scotland. The fish were acclimated for three weeks and transferred to

experimental aquaria. Exposures were initiated mid-October and continued through early June, with an 8-week breeding experiment conducted during April-May. Fish were exposed to a solvent control, dilution water, and 16, 97, and 669 ng/L of 17β-estradiol. In addition, during late May/early June, a two-week between-treatment breeding experiment was conducted in clean water by breeding female fish exposed to untreated (dilution) water with males exposed to 669 ng/L of 17β-estradiol, and *vice versa*.

Nesting behavior and reproductive output for these breeding pairs were compared to a control group of males and females exposed to dilution water alone. The exposure to 17β-estradiol at 669 ng/L resulted in significantly different ($p<0.001$) mortality rates and reduced growth in both male and female fish. At various time points throughout the study, nuptial coloration was significantly different ($p<0.01$-0.001) based on impaired scores for fish exposed to 97 and 669 ng/L, when compared to the controls. In addition, males exposed to 669 ng/L showed no significant testicular development, a lack of development of sperm duct glands, and significantly different GSI values ($p<0.01$-0.001) based on reduced indices from December to March, when compared to the controls. Fish exposed to 669 ng/L showed a significant difference (males, $p<0.01$ and females, $p<0.001$) in seasonal liver growth, which were reduced when compared to controls. GSI increased in both males and females between October and March; however, in January, females exposed at all concentration levels had significantly different GSI values ($p<0.05$), which were reduced when compared to the controls. The higher VTG levels in males exposed to ≥97 ng/L were significantly different ($p<0.001$) when compared to control fish, while no significant effects on VTG levels were observed in the female fish.

The two-week between-treatment breeding experiment resulted in no pairs breeding and no eggs produced/brood for water-alone treated males paired with 669 ng/L of 17β-estradiol-exposed females and for water-alone treated females paired with 669 ng/L of 17β-estradiol-exposed males. In addition, a significant difference ($p<0.001$) was observed in a lower percentage of males nested when compared to the controls in the spawning group of females exposed to water alone and males exposed to 669 ng/L of 17β-estradiol.

Robinson et al. [131] concluded that exposure of males to 669 ng/L of 17β-estradiol prevented or reversed male maturation, exposure of males to 97 ng/L delayed and reduced male maturation, and exposure to 16 ng/L did not adversely affect male maturation. At the population level, sand gobies exposed to 97 ng/L of 17β-estradiol produced fertile eggs at a significantly slower rate than the solvent control fish ($p<0.05$), due to smaller brood size and lower fertility. There was also a delay in the time fish started to spawn. Because of this, coupled with the slower reproduction rate, the 97 ng/L-exposed population produced 12% fewer eggs than the solvent control. However, based on the design of the study, specifically to detect a reduction of ≥50%, reproductive behavior and breeding success were not significantly different in the 97 ng/L population versus the solvent controls using an analysis of variance (ANOVA). Sand goby showed large variations in normal reproductive output, as demonstrated by the average brood size of untreated fish in this study of 1764 ± 144 eggs. Similar variability observed in laboratory studies using sand goby was also observed and presented in other publications cited by Robinson and co-authors. Therefore, Robinson et al. [131] suggested that the small reduction (12%) in fecundity observed in this study following exposure to 97 ng/L of 17β-estradiol may be within the normal range of sand goby reproductive success. Under the conditions of the study, the NOEC and LOEC for reproductive behavior and breeding success in the sand goby were 97 ng/L and 669 ng/L, respectively (Table 38).

Table 38. Reproduction Effects of 17β-Estradiol on a Marine Fish, the Sand Goby (*Pomatoschistus minutus)*

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Mortality	97	669
Growth rate	97	669
Sexual maturation[a]	16	97
Hepatic VTG mRNA expression	16	97
Fecundity	97	669
Reproductive behavior and breeding success[b]	97	669
Egg fertility	97	669

[a] GSI, sperm duct gland somatic index (SDGSI) and nuptial coloration.
[b] % paired males nested, % pairings bred, total eggs/brood, % egg fertility, number fertile eggs/brood, population production of fertile eggs, and number eggs/breeding pair
Reference Robinson et al. [131].

Effects of 17β-estradiol on the reproduction of Java-medaka (*Oryzias javanicus*) [Imai et al.]

Imai et al. [132] conducted a study to understand the reproductive effects on the estuarine Java-medaka (*Oryzias javanicus*) following a 6-month exposure to 17β-estradiol at mean measured concentrations equal to 9.5, 16, 68, 159, and 243 ng/L with seawater as the control. Concentrations were measured at 0 and 24 hours after preparation and were maintained at 55-95% (mean = 75%) of newly prepared solutions. Java-medaka from Penang Island, Malaysia were collected and maintained in laboratory culture for >4 years prior to study initiation. A spawning group of three males and six females was placed in a 10 L aquarium of seawater. The eggs were collected and randomly separated into six replicates (108 eggs total) for the seawater control and eight replicates (150 eggs total per treatment level) for each exposure group. Exposures were initiated within 24 hours of fertilization with test seawater changed daily throughout the study.

No significant differences were observed between control and exposure groups for either hatching rate or mean hatch time. At 187 days post hatch, significant differences ($p<0.05$) were observed in lower body weights for exposure groups dosed at ≥9.5 ng/L. Shorter body lengths resulted in groups dosed at ≥16 ng/L, when compared to the controls. Fecundity was significantly different ($p<0.05$) from controls based on the higher egg production observed at 68 ng/L (Figure 1 of reference [132]), and the remarkably small number of eggs produced at ≥159 ng/L. Fertility rates of eggs were significantly different ($p<0.05$) at 16 ng/L and 68 ng/L, based on the reduced fertility observed when compared to the controls (Figure 2 of reference [132]). HSI values were significantly different ($p<0.05$) from controls, based on higher indices for males and females in the 234 ng/L exposure group. No significant difference was observed in GSI values for males and females when compared to controls (Figure 3 of reference [132]). VTG concentrations were significantly different ($p<0.05$) from controls, based on elevated levels in the livers of male fish exposed to ≥68 ng/L and females exposed to 243 ng/L (Figure 4 of reference [132]). Less than 2.8% of the fish exposed to >68 ng/L displayed male secondary sexual characteristics. Specifically, more than 63% of the fish had unidentifiable secondary sex characteristics at 89 days after hatching, and no male fish were found in the 159 and 243 ng/L exposure groups at 138 dph. Histological observations at the termination of the exposure found 50% and 29.4% of the fish were males in the 159 and 243 ng/L exposure groups, respectively. Further, 33% and 60% of the males in the 159 and 243 ng/L exposure groups, respectively, had developed

testis-ova (those fish showing testis-ova were categorized as males). This study supports fecundity and male VTG NOEC and LOEC values of 16 ng/L and 68 ng/L, respectively, and fertility NOEC and LOEC values of 9.5 ng/L and 16 ng/L, respectively (Table 39).

Table 39. Reproduction Effects of 17β-Estradiol on Java-medaka (*Oryzias javanicus*)

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Length	9.5	16
Body weight	<9.5	9.5
Hatching rate	243	>243
Time to hatch	243	>243
Fecundity	16	68
Embryo fertility	9.5	16
Liver VTG induction in males	16	68
Liver VTG induction in females	159	243
HSI males and females	159	243
GSI males and females	243	>243
Skewed sex ratio toward females/secondary sex characteristics	68	159

Reference Imai et al. [132].

Effect of 17β-estradiol on the reproduction of Japanese medaka (*Oryzias latipes*) [Kang et al.]

Kang et al. [118] evaluated the effects of 17β-estradiol on the reproduction of Japanese medaka (*Oryzias latipes*) exposed to mean measured concentrations of 29.3, 55.7, 116, 227, and 463 ng/L for 21 days. From a broodstock maintained in the laboratory for three years, sexually mature fish were selected four months post-hatch to form 59 mating pairs. Each mating pair was placed in a 1-L chamber of tap water maintained under flow-through conditions for a three-week acclimation period. Based on the number of spawned eggs during the last week of the acclimation period, 48 mating pairs were chosen for the exposure test. During the exposure period, eggs were collected each morning for 21 days and fertilized eggs were counted. At the end of 21 days, the females were sacrificed for histological evaluation and determination of hepatic VTG levels. Males were sacrificed for histological evaluation and determination of hepatic VTG levels at day 25. During the last three days of exposure, all fertilized eggs from each mating pair were collected and assessed daily for 14 days for developmental effects and mortality. Hatched larvae were counted. Fifteen larvae from each chamber were maintained in clean tap water under flow-through conditions for 60 days and assessed daily for mortality, abnormal development and behavior.

The total number of eggs spawned, and the fertility of the eggs produced during the third week, were significantly different (p=0.005 and p=0.023, respectively), with reductions observed in the 463 ng/L exposure group when compared to controls (Table 40). Fertility in the remaining treatment groups exceeded 90% with no significant differences observed. GSI in male medaka was significantly different (p=0.034) based on reduced indices in the 463 ng/L treatment group when compared to controls. No significant effects were observed in female GSI or male and female HSI values when compared to controls (Table 40). Hepatic VTG levels were significantly different in males exposed at ≥55.7 ng/L (p<0.05) and in females exposed to 463 ng/L (p=0.002) based on elevated values when compared to

controls. No histological abnormalities of ovaries were observed. Induction of testis-ova was observed in males exposed to ≥29.3 ng/L; however, spermatogenesis was normal. As a result, no significant decrease in egg production or fertility was observed at concentrations <463 ng/L. No adverse effects were observed in hatchability and time-to-hatch of F1 embryos (Table 40). In addition, no effects were observed in mortality, growth or sex ratio in offspring maintained in clean tap water through 60 dph.

Table 40. Reproduction Effects of 17β-Estradiol on Japanese Medaka (*Oryzias latipes*)

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Hatching rate	463	>463
Time to hatch	463	>463
Fecundity[a]	227	463
Embryo fertility[a]	227	463
Liver VTG induction in males	29.3	55.7
Liver VTG induction in females	227	463
GSI males	227	463
HSI males and females, GSI females	463	>463

[a] Based on data from the third week of the exposure (days 14 to 21)
Reference Kang et al. [118].

6.2.2.3. Presentation of fish reproduction study exposing fathead minnows (*Pimephales promelas)* to 17α-estradiol

Due to a lack of published data on the effects of 17α-estradiol on fish reproduction, a short-term reproduction study to investigate the effects of 17α-estradiol on adult survival, fecundity (number of eggs/female/day), GSI, nuptial tubercle score, plasma VTG concentration, embryo fertilization rate, embryo hatching success, percent normal fry at hatch, and percent normal fry following yolk sac absorption in the fathead minnow (*Pimephales promelas*) was conducted by Pfizer (now owned by Zoetis) according to OECD Guideline 229 (reference [242] and Appendix 13.12).

The fathead minnows used during this Zoetis-owned study were obtained from a laboratory supply of reproductively mature animals in spawning condition. Fish were exposed to a water control, a solvent control, and mean measured concentrations of 2.5, 7.2, 25, 80, and 250 ng/L of 17α-estradiol. Fifty-three spawning groups, each consisting of four females and two males, were evaluated during a 14-day pre-exposure period. The suitability for testing was established when regular spawning occurred in each replicate chamber at least twice in the immediate seven-day period preceding exposure initiation and when production of greater than 10 eggs/female/day/replicate was achieved. Following the 14-day pre-exposure period, 28 spawning groups with the greatest number of eggs/female/day were randomly added to the exposure system for the definitive study. Once the spawning groups were distributed, the exposure was initiated and maintained for 21 days in the adult population. Egg production was monitored daily to establish fecundity and fertility. Once per week during the 21-day study, all embryos produced in each replicate of each treatment level were pooled and a subsample of 50 embryos was indiscriminately selected and placed into an incubation cup to serve as a representative population for that treatment level. The cup containing the eggs was placed in an incubation chamber within the exposure vessel at the respective dose and allowed to incubate until hatching began. Once all embryos hatched, newly hatched larvae were observed for general physical appearance and behavior until yolk sac absorption was complete. Following the 21-day exposure of the adults, the fish were sacrificed.

Statistical significance of the reproduction endpoints and endocrine biomarkers were established based on comparison of each treatment level with the pooled controls and reported based on mean measured concentrations of 17α-estradiol. A summary of the results is reported in Table 41. The NOEC and LOEC values for each of the endpoints evaluated in the study were 250 ng/L and >250 ng/L, respectively, with the exception of male VTG. A significant difference (p=0.0466) was observed with increased plasma VTG levels observed in males exposed to 250 ng/L when compared to the controls. Therefore, the NOEC and LOEC values for induction of plasma VTG in males were 80 ng/L and 250 ng/L, respectively [242]. Additional details on the methods and results are presented in Appendix 13.12.

Table 41. Survival and Reproduction Effects of 17α-Estradiol on Fathead Minnows (*Pimephales promelas*)

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Survival	250	>250
Fecundity		
GSI		
Nuptial tubercle score		
Plasma VTG induction in males	80	250
Embryo fertilization rate	250	>250
Embryo hatching success		
Percent normal fry at hatch		
Percent normal fry following yolk sac absorption		

Reference Pfizer Study Number 1A73N-60-11-785 [242].

6.2.3. Trenbolone metabolite fish reproduction-related effects

The published literature and Zoetis-owned study data for effects of 17β- and 17α-trenbolone on fish reproduction endpoints are reviewed below. Similar to the estradiol metabolites, acceptable study data for 17β- and 17α-trenbolone were selected based the criteria outlined in Section 1.3.

6.2.3.1. Summary of trenbolone metabolite effects on fish reproduction

The reproduction-related effects in fish with the lowest NOEC and LOEC values in these studies are summarized in Table 42. The studies referenced in Table 42 are presented in detail in Sections 6.2.3.2 and 6.2.3.3.

Table 42. Summary of Effects of 17α- and 17β-Trenbolone on Fish Reproduction Endpoints

Species	Salinity (ppt)	Duration (days)	Endpoint[a]	NOEC (EC_{10}) (ng/L)	LOEC (ng/L)	Ref
17β-Trenbolone Studies						
Fathead minnow	FW[b]	21	Fecundity	<26[c] (2.5-5)[d]	26	Ankley et al. [120]
Sheepshead minnow	20	280	Fecundity	130 (F0) 27 (F2) 7 (F3)	870 (F0) 130 (F2) 27 (F3)	Cripe et al. [133]
Mosquito fish	FW	28	Anal fin length	100	300	Sone et al. [134]
			Sexual differentiation	300	1000	
17α-Trenbolone Studies						
Fathead minnow	FW	21	Fecundity	94[e]	>94[e]	Jensen et al. [121]
		last 14 (i.e., days 15-21)		32	94	
Fathead minnow	FW	21	Fecundity	35	120	Pfizer [243]
Medaka	FW	21	Fecundity	110	>110	Pfizer [244]

[a] Only the fish reproduction endpoints with the lowest NOEC or LOEC are reported in the table. Effects on other reproduction endpoints at higher concentrations reported in the summaries in Section 6.2 are not reported here. In addition, effects on other endpoints (e.g., gonadal histology, GSI) are summarized in Section 6.2.1.2 but not reported in this table.

[b] FW=freshwater

[c] The measured concentration was reported as 1.5 ng/L; however, there is analytical uncertainty around the measured value; therefore, this value was reported as less than the next highest measured concentration (see text under description of Ankley et al. in Section 6.2.3.2 below).

[d] Due to the analytical uncertainty in the lowest treatment group, an EC_{10} was calculated both with and without the data from this group. Therefore, a range of EC_{10} values were reported in this table. (See text under description of Ankley et al. in Section 6.2.3.2 below.)

[e] The authors did not report a NOEC value [121]; values reported herein were from a reanalysis of the raw data conducted as per OECD statistical methods.

The following conclusions for trenbolone metabolite effects on fish reproduction were made based on the studies reviewed in Section 6.2.3.2 and Section 6.2.3.3 and the information in Table 33 and Table 42:

- **17α-Trenbolone:** The NOEC values for reproduction in fathead minnow were 32 and 35 ng/L for 17α-trenbolone in Jensen et al. [121] and Pfizer [243], respectively. In addition, the NOEC value for medaka exposed to 17α-trenbolone was 110 ng/L [244]. In comparing the NOEC values following 21-day exposures to the fathead minnow (32 and 35 ng/L) and the medaka (110 ng/L), fathead minnow reproduction was determined to be more sensitive to 17α-trenbolone (Table 42) and was used as the basis for the PNEC. The two fathead minnow reproduction NOEC values were almost identical (32 and 35 ng/L). The lower of the two values (32 ng/L) was selected to be most conservative and this value was used in Section 6.3.3 to determine a PNEC.
- **17β-Trenbolone:** The fish reproduction LOEC values for 17β-trenbolone were 26-870 ng/L. Fathead minnow appears to be the most sensitive species of those tested (Table 42). A NOEC could not be determined with confidence from the data reported in Ankley et al. [120] due to analytical issues that caused uncertainty in the measured concentration of the lowest treatment group. Because of this uncertainty, we have analyzed the fecundity data both with and without the data from the lowest treatment group in order to estimate the EC_{10} values as a surrogate for the NOEC (see discussion under Ankley et al. in Section 6.2.3.2 below). The EC_{10} values were determined to be 5 and 2.5 ng/L, respectively. This range of values was used to derive the PNEC in Section 6.3.3.
- **Trendione:** At this time, data is lacking on the potency and toxicity of trendione. However, there is no information to suggest that trendione would be more toxic than 17β- and 17α-trenbolone. In general, metabolites are equivalent to, or less potent/toxic than, their precursors. Therefore, because the evaluation of 17β- and 17α-trenbolone should be conservatively predictive of any effects from exposure to trendione, a PNEC was not derived for trendione.
- **Relative potency of 17β- and 17α-trenbolone:** Based on a comparison of the LOEC data for fecundity in fathead minnows exposed to 17β- and 17α-trenbolone (26 ng/L and 94 to 120 ng/L, respectively; Table 42), 17β-trenbolone is approximately four times more toxic than 17α-trenbolone.

6.2.3.2. Presentation of fish reproduction studies for 17β-trenbolone

In this section, three published literature studies on the effects of 17β-trenbolone on fish reproduction endpoints are reviewed. The NOEC and LOEC values associated with reproduction-related effects in fish from these studies are summarized in Table 42 above. These data were used to determine a PNEC value for each metabolite to assess environmental risk in Section 7.

Effects of 17β-trenbolone on fecundity and reproduction of the fathead minnow (*Pimephales promelas*) [Ankley et al.]

Ankley et al. [120] published the results of a 21-day study assessing the effects of 17β-trenbolone on fathead minnow (*P. promelas*) reproduction under flow-through conditions. Sexually mature fathead minnows from an on-site culture maintained under continuous flow of Lake Superior water at 25±1°C were used in this study. Following a

three-week pre-exposure period, groups of four females and two males per tank were exposed continuously to 17β-trenbolone over 21 days to nominal concentrations of 0, 5, 50, 500, 5000, and 50,000 ng/L; measured concentrations were 0, 1.5, 26, 270, 4400, 41,000 ng/L, respectively. Samples were collected for analytical measurements on days 1, 5, 8, 12, 15, and 20; except for the lowest concentration in which samples were only collected on days 1, 12, 15, and 20. The following endpoints were monitored over the exposure period: survival, growth (body weight), fecundity, fertility, hatching success, secondary sex characteristics, and plasma VTG.

Measured concentrations were approximately 80% of nominal concentrations at the two highest concentrations (5000 and 50,000 ng/L), but only about 50% of nominals at 50 and 500 ng/L. For the lowest treatment group, the mean measured concentration (1.5 ng/L) was only 30% of the nominal (5.0 ng/L). There is considerable uncertainty in the 1.5 ng/L mean value because of several significant analytical issues associated with the lowest treatment group. It is important to note that the analytical procedure for this group required a concentration step, which was not required for the other treatments tested; this additional step may have contributed to these analytical issues. First, samples for this group were collected on only four of the six sampling days (days 1, 12, 15, and 20). This sampling regimen resulted in a 10 day period (i.e., almost half of the exposure period) where the exposure tanks for the lowest treatment group were not analyzed and no measured concentrations were reported. Second, samples were only collected from one of the three exposure tanks on days 1, 12, and 15 (on day 20 samples were collected from all three exposure tanks). Although not published at the time of this research, OECD Guideline 229 [124] recommends that the actual test chemical concentrations be measured in all tanks at the start of the test and at weekly intervals thereafter. In contrast, the sampling regimen of the other five treatment groups was similar to these recommendations; all three exposure tanks were sampled six times over the 21 days. Third, of the four measured concentrations reported in Table 1 of reference [120], the day 1 concentration (1 ng/L) was below the LOD (1.2 ng/L) and the concentrations on days 12, 15, and 20 (2, 1.4, and 1.4 ng/L, respectively) were below the likely LOQ. An LOQ was not reported in the publication; however, based on recommendations in CVM GFI #64[v], we would expect the LOQ to be approximately 3.3 times the LOD, or 4 ng/L, which is well above all the measured values reported for the lowest treatment group. Fourth, the SD of the mean measured values from the lowest treatment group (recovery ± SD = 87% ± 25%; n = 4) did not meet the acceptance criteria of ± 20% of the mean measured concentrations as stated in OECD Guideline 229[w]. However, the SD of the mean measured values for the four highest treatment groups met this acceptance criteria (recovery ± SD = 91% ± 6.5%; n = 10). Based on the reasons listed above, we believe the analytical results for the lowest treatment group are potentially unreliable. Although it was clear there were no reproductive effects at this level (see Figure 24 below), it is unclear as to what the actual exposure concentration was for this group. Therefore, the analyses were conducted with and without the data for the lowest treatment group.

[v] CVM GFI #64 "Validation of Analytical Procedures: Methodology" (1999); http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052379.pdf (Accessed May 7, 2014)

[w] OECD Guideline 229 (2009) [124], Paragraph 13, page 3, states "evidence should be available to demonstrate that the concentrations of the test substance in solution have been satisfactorily maintained within ±20% of the mean measured values"

Table 43 lists the NOEC and LOEC values for the effects endpoints assessed in this study based on actual measured concentrations. There were no statistically significant differences in any of the endpoints measured in the lowest treatment group. However, as previously discussed, because of the analytical issues, it is unclear what level of 17β-trenbolone this treatment group was actually exposed to. Therefore, we cannot report a specific NOEC value with confidence for those endpoints where the NOEC would have been the lowest treatment group (i.e., fecundity, VTG and tubercle scores; Table 43). When this occurred, we reported it as <26 ng/L, the next highest concentration tested.

No treatment-related mortalities were observed during the exposure period, but effects on fish weight and fecundity were reported. The males exposed to 41,000 ng/L were approximately 10% lower in body weight than the controls, while the females demonstrated a significant difference ($p \leq 0.05$) in weight based on the dose dependent increase at ≥270 ng/L when compared to the controls. A reduction in female VTG levels and fecundity were observed at concentrations ≥26 ng/L (Figure 24 below for fecundity data). In addition, the authors concluded that the concentration-response curves for a subset of endpoints (for body weight, testosterone, estradiol, and VTG) suggested to them subtle, although not significant, responses could have occurred even at the lowest concentration tested.

As stated above, specific NOEC values could not be determined for fecundity, VTG and tubercle scores (Table 43), due to analytical issues. However, there was a fairly consistent dose response for fecundity at the higher concentrations, which allowed for use of an alternative approach to estimate a PNEC value that would be useful for risk assessment. The study data were obtained from the primary author and the fecundity data was analyzed to determine effects concentration (EC) values for a specific percent reduction in egg production; e.g., EC_{10} is equivalent to a 10% reduction in egg production. Because of the uncertainty around the lowest measured concentration value, the data were analyzed both with and without this treatment group. Only the fecundity data were analyzed because, based on the data presented (Table 43), it was the most significant and sensitive biological endpoint for assessing population level impacts (compared to VTG and tubercle scores; Table 43). The fecundity data were fit to the Hill model assuming a Poisson distribution for eggs/female/day using the SAS NLMixed procedure. The model provides a reasonably good fit to the means.

The EC_5, EC_{10}, and EC_{50} values were 2.8, 5.0, and 26.7 ng/L, respectively when the data from the lowest treatment group was included in the analysis (Table 44 below). The values were 1.2, 2.5 and 20.9 ng/L, respectively, when the data from the lowest treatment group was excluded from the analysis (Table 44 below). The range of EC_{10} values (2.5 to 5 ng/L) were used as surrogates for the NOEC in Section 6.3.3 to derive a range of PNEC values for 17β-trenbolone. It is important to note that this range (0.25 to 0.5 ng/L) covers the lowest measured concentration reported in Ankley et al. (1.5 ng/L) where no effects on reproduction endpoints were observed.

Table 43. Survival and Reproduction Effects of 17β-Trenbolone on Fathead Minnow (*Pimephales promelas*)

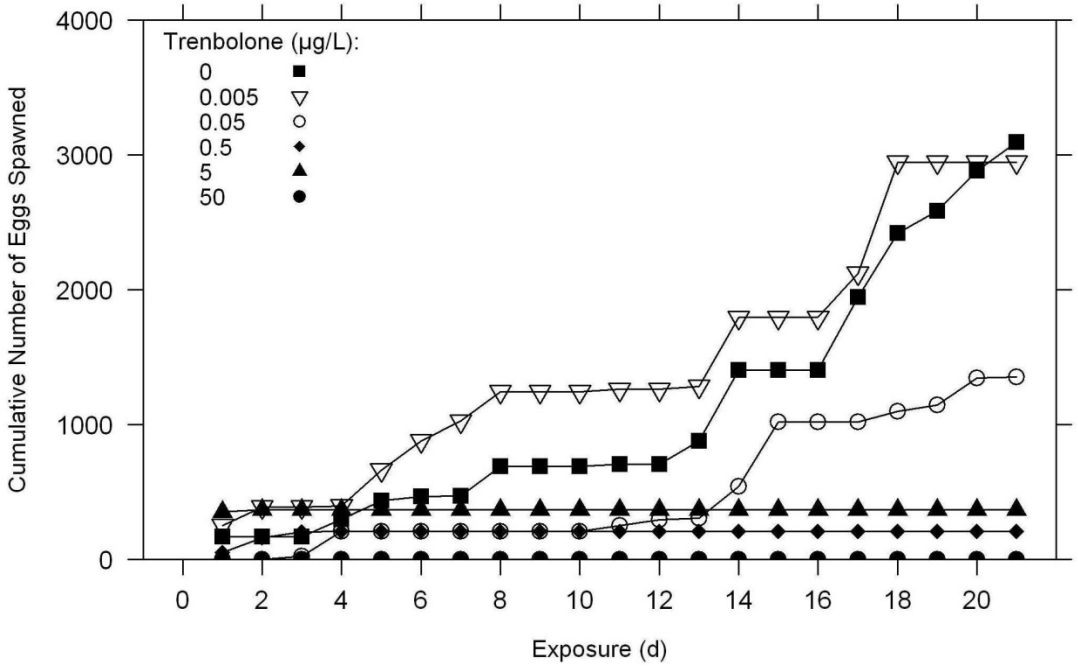
Endpoint	NOEC (ng/L)	LOEC (ng/L)
Survival	41,000	>41,000
Growth - males	41,000	>41,000
Growth - females	26	270
Fertility	26	>26
Hatching Success	26	>26
Fecundity	<26[a]	26
Nuptial tubercle score - females	<26[a]	26
Plasma VTG - females	<26[a]	26
Plasma testosterone - females	26	270
Plasma 17β-estradiol - females	26	270
GSI	41,000	>41,000

[a] As noted in the study description above, due to analytical uncertainty in the lowest treatment group, these data were not used when estimating the effects endpoints.
Reference Ankley et al. [120].

Table 44. EC values for Reproduction Effects of 17β-Trenbolone on Fathead Minnow (*Pimephales promelas*)

Effects Endpoint (EC_x value)	With Lowest Treatment Group		Without Lowest Treatment Group	
	EC value (ng/L)	95% Confidence Interval	EC value (ng/L)	95% Confidence Interval
EC_5	2.8	0-9.3	1.2	0-4.7
EC_{10}	5.0	0-14.7	2.5	0-8.6
EC_{50}	26.7	0-54.0	20.9	0-47.3

Figure 24. Cumulative Mean Number of Eggs/Female Over 21 Days When Exposed to 17β-Trenbolone (concentrations represent nominal values)



This figure was reproduced using data obtained from EPA (and is similar to Figure 3 in Ankley et al. [120]).

The concentration-response data from Ankley et al. [120] were used to project population-level changes for a fathead minnow population exposed to varying concentrations of 17β-trenbolone in Miller and Ankley [135]. The model combined a Leslie population projection matrix and logistic equation, which projects population growth over time using fecundity and survival rates of individual age classes within the population. Based on the reproduction data from Ankley et al. [120], it was determined that populations at carrying capacity exposed to concentrations of 27 ng/L of 17β-trenbolone would exhibit a 51% projected decrease in average population size after two years of constant exposure. In addition, when exposed to concentrations of 266 ng/L of 17β-trenbolone, the population size would exhibit a 93% projected decrease in average population size after two years.

Effects on survival, development, and reproduction of three generations of the estuarine sheepshead minnow (*Cyprinodon variegatus*) exposed to 17β-trenbolone [Cripe et al.]

Cripe et al. [133] conducted a 42-week, multigenerational reproduction study with 17β-trenbolone to evaluate the reproduction effects of the androgen receptor agonist on multiple generations of sheepshead minnow (*C. variegatus*). The fish used in the study were estuarine sheepshead minnow maintained in aquaria receiving a continuous flow of aerated, filtered seawater at 20‰ (parts per thousand) salinity [133]. The study was initiated with reproductively active fish (F0) and continued through hatching of the F3 generation for a total study duration of approximately 294 days. The F0 generation was exposed to a seawater control, solvent control, and mean measured concentrations of 7, 27, 130, 870, and 4100 ng/L of 17β-trenbolone. In addition to the seawater control and solvent control, the F1 generation was exposed to treatment levels ≤870 ng/L of 17β-trenbolone, the F2 generation to treatment levels ≤130 ng/L of 17β-trenbolone, and the F3 generation to treatment levels ≤27 ng/L of 17β-trenbolone. Mean measured concentrations ranged from 68 ± 20% to 91 ± 14% of nominal over the duration of the study. The endpoints evaluated were survival, growth, reproduction, daily reproductive rate, fertility, hatching success, abnormal embryos, sex ratio, plasma VTG, GSI, and HSI. NOEC and LOEC values were determined for each endpoint in each generation. The study was conducted using four replicate spawning groups per treatment level, each consisting of three females and two males. The fish were acclimated in the spawning chambers for 12 days prior to the seven-day pre-exposure period designed to assess reproduction within the individual spawning groups. The F0 generation was exposed for 21 days with eggs collected for initiation of the F1 generation. The F1 generation was continually exposed from hatch through maturation and spawning. At 100 dph, a subset of the spawning fish from the F1 generation were placed in spawning groups of three females and two males, and acclimated for 10 days prior to initiation of egg collection. Following day 16 of egg collection, embryos were removed for grow-out of the F2 generation. Similarly, a subset of eggs produced from the F2 generation were collected for the F3 generation and evaluated for hatch and survival.

The effects on survival were limited to embryo hatching, as no effects were observed in larval, juvenile, or adult survival following exposure to 17β-trenbolone. Embryo hatching success was significantly different in the F1 and F2 generations when compared to their respective solvent controls (Table 45). A reduction in hatching was observed in the F1 generation at 4100 ng/L ($p<0.001$). In the F2 generation, hatching success was reduced at ≥130 ng/L ($p<0.001$) and increased at 7 ng/L ($p=0.035$) as compared to the control. No effect on embryo hatching was observed in the F3 generation exposed to ≤27 ng/L. Because hatching success was reduced in the F2 generation at ≥130 ng/L, there were no surviving fish to initiate the F3 generation at >27 ng/L, and as a result, effects at concentrations >27 ng/L were not assessed in the F3 generation. Abnormal embryos observed in the F0, F1, and F2 generations resulted in NOEC values of 870, 27, and 7 ng/L of 17β-trenbolone, respectively, for embryo hatching (Table 45).

The NOEC for female plasma VTG in the F0 generation was 130 ng/L 17β-trenbolone (Table 45). GSI and HSI were affected by exposure to 17β-trenbolone; however, the changes observed were not dose-dependent, and therefore, may not be considered the most reliable endpoints for assessing 17β-trenbolone exposure in the sheepshead minnow. Phenotypic male characteristics were observed after nine days in the F0 generation at ≥130 ng/L of 17β-trenbolone (i.e., exhibited a black caudal fin border). In the F1 generation, all fish in the 130 ng/L treatment group identified as female by the rotund body form had either faint or ambiguous male phenotypic characteristics. In the F1 generation, all fish in the 870 ng/L treatment group appeared to be phenotypic males. Histological examinations confirmed that 14 out of the 15 fish were males, demonstrating a significantly different ($p<0.001$) sex ratio when compared to the average of 56% males observed in the control fish. Atypical ovaries and/or intersex in the F1 generation were limited to the 130 and 870 ng/L treatment groups, which also resulted in significant differences ($p<0.05$) when compared to the controls, as noted by reduced reproduction, increased numbers of abnormal embryos and infertile eggs, and reduced hatch of offspring. In the F2 generation, no significant differences in sex ratio were observed between the controls and any remaining treatments ≤27 ng/L. The NOEC values for sex ratio were 27, 27, and >27 ng/L for the F0, F1, and F2 generations, respectively (Table 45).

The highest concentrations of 17β-trenbolone with reproducing populations at the end of the F0, F1 and F2 generations were 4100, 870, and 27 ng/L, respectively. The percentages of abnormal embryos in the F0, F1, and F2 generations were significantly different ($p\leq0.05$) from the controls at 4100, 130, and 27 ng/L, respectively (Table 45). Reproduction in the F0, F1, and F2 generations was significantly different ($p\leq0.05$) from the controls based on the reduction in cumulative egg production, reproduction rate (number of normal embryos spawned per female per day) and infertile eggs at 870, 7-27, and 27 ng/L of 17β-trenbolone, respectively. Fecundity NOECs of 130, 27, and 7 ng/L and LOECs of 870, 130, and 27 ng/L for 17β-trenbolone were reported for the F0, F1, and F2 generations, respectively (Table 45).

Although observations of phenotypic male characteristics in female fish appear to be indicative of exposure to androgens, these observations did not coincide with adverse effects on reproduction. Therefore, the authors concluded that adverse impacts on the reproduction of sheepshead minnow following exposure to 17β-trenbolone appeared to be related to the conditions of the ovaries, as well as the duration of the exposure. Based on the data presented, reproduction was the most sensitive measure of androgen affects with sensitivity increasing for the key endpoints of embryo production, reproduction rate, abnormal embryos, and infertile eggs with each subsequent generation.

Table 45. Reproduction Effects of 17β-Trenbolone on Sheepshead Minnow (*Cyprinodon variegatus*) Over Multiple Generations

Endpoint	Generation, LOEC and NOEC (ng/L)					
	F0		F1		F2	
	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
Survival	NA	NA	870	4100	27	130
Growth	NA	NA	<7	7	<7	7
Sex ratio	27	130	27	130	27	>27
Cumulative embryo production (over 21 days, and intervals 2, 3 and 4)	130	870	27	130	7	27
Reproduction rate (# normal embryos/female/day) (for each interval 2-4)[a]	130	870	7[b]	27[b]	7	27
Hatching success	NA	NA	870	≥4100	27	≥130
Abnormal embryos	870	4100	27	130	7	27
Infertile eggs	130	870	27	130	7	27
GSI	130[b]	870[c]	27	130	NA	NA
HSI	4100	>4100	7[c]	27[c]	NA	NA
Histology	130	870	27	130	NA	NA
Vitellogenin	130	870	870	>870	NA	NA

[a] Interval 1: pre-exposure F0, not included in the analysis; Interval 2: days 1-7; Interval 3: days 8-14; interval 4: days 15-21
[b] Data for interval 3 and 4. Interval 2, NOEC 27, LOEC 130
[c] Reported in Table 3, Cripe et al. [133]; however, for the GSI and HSI data presented in Table 2 of Cripe et al., there were no significance differences noted at higher concentrations, supporting the interpretation of a lack of dose response.
NA = not analyzed
GSI = gonadosomatic index; HSI = hepatosomatic index
Reference Cripe et al. [133].

Effects of 17β-trenbolone on masculinization of mosquitofish (*Gambusia affinis*) [Sone et al.]

Sone et al. [134] conducted a 28-day study with newborn mosquitofish *(Gambusia affinis)* to evaluate the effects of 17β-trenbolone on relative anal fin length. Newly released Day 0 fry were exposed to nominal concentrations of 100, 300, 1000 or 10,000 ng/L of 17β-trenbolone. At 28 days, no effects on body length were observed; however, fry exposed to ≥300 ng/L exhibited elongation of the anal fin similar to that seen in mature males. In addition, exposures at 300 ng/L had no effect on the differentiation of the testis and ovary. However, at concentrations ≥1000 ng/L, ovotestes developed in all females and an acceleration of testicular development was observed in males. Therefore, a NOEC of 100 ng/L and a LOEC of 300 ng/L were determined for anal fin length, while a NOEC of 300 ng/L and LOEC of 1000 ng/L were established for sexual differentiation.

Trenbolone masculinization of zebrafish (*Danio rerio*) [Morthorst et al.]

Morthorst et al. [136] conducted a study, generally following the OECD guideline 210 (Fish, Early-life Stage Toxicity Test), to investigate the reversibility of the masculinizing effects of 17β-trenbolone on zebrafish (*Danio rerio*). Fish from a local supplier were acclimated to laboratory conditions for several weeks. Newly fertilized eggs were collected and divided

into groups of 100 eggs. One day post-fertilization, unfertilized eggs were removed and replaced with fertilized eggs from a reservoir. A total of 100 eggs were then transferred to each exposure tank to initiate the study. Replicates were exposed to clean water, a solvent control, and 9.2, 15.5, and 26.2 ng/L of 17β-trenbolone under flow-through conditions. Eggs were exposed through 60 dph, at which time 15-20 fish per replicate were sacrificed for histological evaluation and VTG analysis, and 20-25 fish per replicate were transferred to new aquaria with clean water for a depuration period of 170 days. The remaining fish were maintained under exposure conditions until 230 dph. Sex ratios in all groups treated with 17β-trenbolone were significantly different ($p<0.05$) from the control (Table 46). Following exposure to 17β-trenbolone, 100% male populations were found in both the 15.5 and the 26.2 ng/L treatment groups. Following exposure to 9.2 ng/L, the reduction in the number of females was determined to be significantly different ($p=0.036$).

The sex-ratio effect observed by Morthorst et al. [136] in zebrafish varied from the observations made by Orn et al. [137] in which no effects were observed in either the medaka or the zebrafish at 10 ng/L of 17β-trenbolone. At 50 ng/L, Orn et al. [137] observed a 100% male population in zebrafish only, resulting in a significant effect ($p<0.05$) on sex ratio. Morthorst et al. also evaluated VTG levels and reported no significant difference between control fish and exposed males and females at 60 dph (Table 46). Orn et al. reported a similar NOEC of 10 ng/L and observed a significant difference ($p<0.05$) in VTG in both zebrafish and medaka following exposure to 50 ng/L of 17β-trenbolone. At 230 dph, fish placed in depuration for 170 days following the 60-day exposure to 15.5 and 26.2 ng/L remained as 100% male populations. The reduction in females in the 9.2 ng/L treatment group at 60 dph remained significantly different ($p=0.011$) following 170 days of depuration. Fish exposed to 17β-trenbolone continuously for 230 days maintained 100% male populations in both the 15.5 and 26.2 ng/L treatment levels; however, the sex ratio in the 9.2 ng/L treatment group was not significantly different ($p>0.05$) from the controls (Table 46). No significant differences were observed in VTG concentration in males and females after depuration or following the lifetime exposure of males in all treatment groups (through 230 dph) when compared to the control. However, VTG concentrations in females exposed to 9.2 ng/L were significantly different ($p=0.015$). Overall, Morthorst et al. [136] demonstrated irreversible effects on sexual development in the zebrafish following exposure to ≥15.5 ng/L of 17β-trenbolone.

Table 46. Sexual Development Effects of 17β-Trenbolone on Zebrafish *(Danio rerio*)

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Survival	26.2	>26.2
Sex ratio 60 dph	<9.2	9.2
Sex ratio 230 dph (60 day exposure/170 day depuration)	<9.2	9.2
Sex ratio 230 dph (lifetime exposure)	9.2	15.5
Male and female VTG 60 dph	26.2	>26.2
Male and female VTG (60 day exposure/170 day depuration)	26.2	>26.2
Male VTG 230 dph (lifetime exposure)	26.2	>26.2
Female VTG 230 dph (lifetime exposure)	<9.2	9.2

Reference: Morthorst et al. [136].

6.2.3.3. Presentation of fish reproduction studies for 17α-trenbolone

One published study and two Zoetis-owned studies on the effects of 17α-trenbolone metabolites on fish reproduction endpoints are reviewed in this section. The lowest NOEC and LOEC values determined in these studies were presented in Table 42.

Effects 17α-trenbolone on reproductive endocrinology of the fathead minnow (*Pimephales promelas*) [Jensen et al.]

There is one published literature study on the effects of 17α-trenbolone on fish reproduction endpoints, which was conducted by Jensen et al. from the EPA [121]. Because a NOEC was not reported in the publication, FDA's CVM obtained the tabulated data from the primary author, Dr. Kathleen Jensen, and reanalyzed the data to determine effect concentrations for fecundity. A summary of the results reported in the study, as well as the effects concentrations estimated by FDA's CVM, are discussed below.

In the Jensen et al. study [121], two experiments were conducted by exposing sexually-mature fathead minnows (*P. promelas*) for 21 days to mean measured concentrations of 130, 660, 1300, 2800, and 7100 ng/L 17α-trenbolone (Experiment 1) and 3.5, 9.7, 32, and 94 ng/L (Experiment 2). Fish from an on-site culture were held in the test system for at least two weeks prior to exposure initiation. During the pre-exposure periods, fecundity was evaluated daily. The 17α-trenbolone concentrations chosen for Experiment 1 were based on competitive binding assays with mammalian androgen receptors, which indicated that 17α-trenbolone was likely an order of magnitude less potent than 17β-trenbolone.

In Experiment 1, spawning groups consisting of four females and two males were exposed in duplicate to each test concentration and a control for 21 days. Significant differences from the control mean ($p \leq 0.05$) were observed in all treatment levels, with virtually no egg production in any of the 17α-trenbolone exposures ≥130 ng/L. In addition, nuptial tubercles were observed in all treatment levels, indicating masculinization of the female fish.

Based on the data from Experiment 1, the 17α-trenbolone concentrations in Experiment 2 were reduced in order to estimate the NOEC and LOEC and the concentration-response relationships for the endpoints of interest. In addition, a modification from spawning groups to spawning pairs (one male and one female) was made in Experiment 2. This design included exposure of eight pairs of fish per treatment level and control for 21 days following the acclimation period. The endpoints assessed in Experiment 2 included: survival, fecundity, fertility, hatching success, secondary sex characteristics and VTG.

Whole fish tissue concentrations of 17β- and 17α-trenbolone were reported from both experiments. There were no treatment-related mortalities observed in either experiment. In Experiment 2, Jensen et al. reported reduced fecundity in a concentration- and time-dependent manner; however, an effect level (NOEC, LOEC, or EC_x) for fecundity was not reported for the entire 3-week exposure period. Based on cumulative fecundity over the last two weeks (days 15-21) of the study, an EC_{50} of 11 ng/L was estimated [95% confidence interval (CI): 7-16 ng/L]. Female fish developed male-like nuptial tubercles in the 32 and 94 ng/L of 17α-trenbolone treatments (two of eight and five of eight fish, respectively), and VTG was reduced in female fish at concentrations ≥32 ng/L. No effects were observed on fertility and hatching success at any of the concentrations tested.

The authors concluded that 17α-trenbolone is not an order of magnitude less potent than 17β-trenbolone and provided two potential explanations for the unanticipated degree of reproduction/endocrine toxicity of 17α-trenbolone observed: 1) the fathead minnow androgen receptor does not have a greater affinity for β- than α-trenbolone, and 2) 17β-trenbolone was measured in the plasma of the fish, which indicates conversion from 17α-trenbolone *in vivo*. Thus, the converted 17β-trenbolone may be predominantly responsible for the observed effects.

Summary of FDA's CVM reanalysis of the data obtained for Jensen et al.

As stated previously, Jensen et al. [121] did not report a NOEC value for any effects endpoint evaluated in the study. Zoetis and FDA's CVM independently obtained the tabulated effects data from the authors of the publication. CVM conducted additional statistical analyses to determine NOEC and EC values based on daily egg count and assuming that egg count follows a negative binomial distribution. To estimate the NOEC, an analysis of variance was performed in which average egg count for each concentration was compared to the control. Assuming a monotonic dose response, regression analysis and confidence intervals were used to estimate EC_x values. The results of the NOEC and LOEC analyses are summarized in Table 47 and were independently corroborated by Zoetis.

Table 47. Survival and Reproduction Effects Levels for Fathead Minnow (*Pimephales promelas*) Exposed to 17α-Trenbolone (the fecundity data are based on reanalysis of the raw data for the last 14 days of the study)

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Survival[a,b]	94	>94
Fecundity – full 21 days[b]	94	>94
Fecundity – last 14 days[c]	32[d]	94
Fertility[b]	94	>94
Hatching success[b]	94	>94

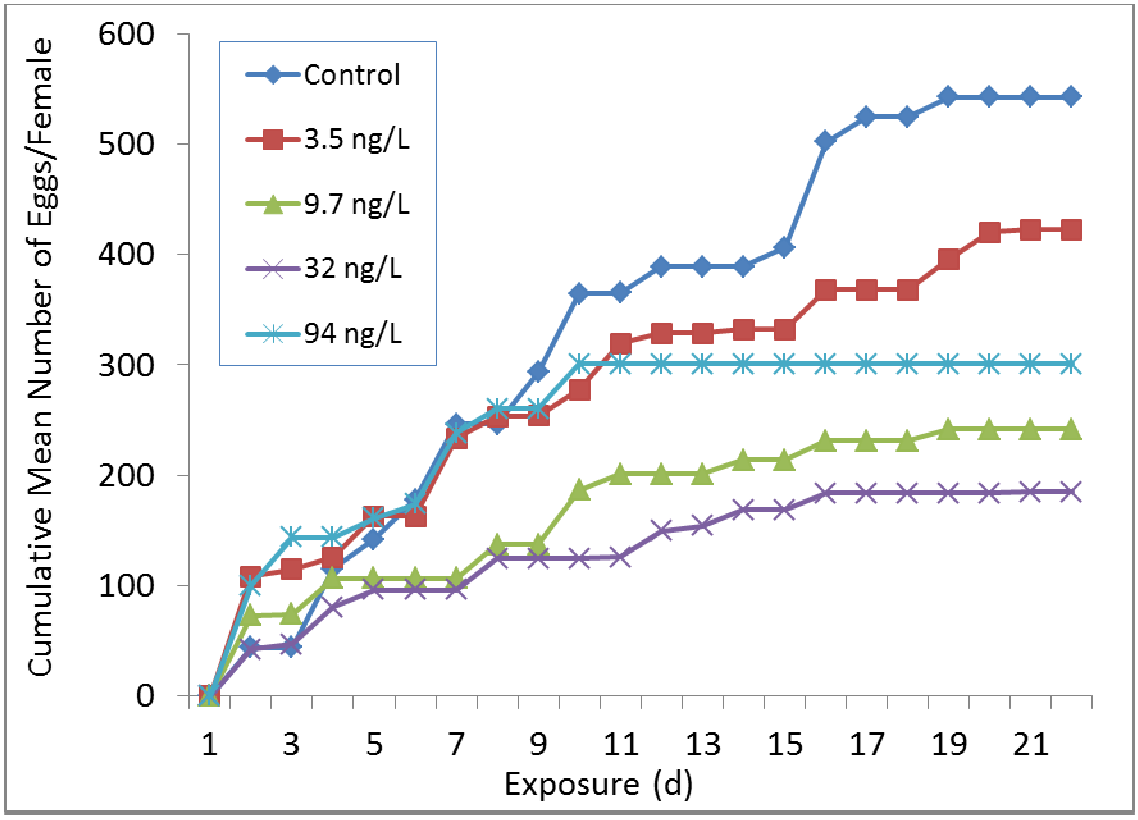
[a] No mortality was observed in Experiment 1, NOEC 7100 ng/L and LOEC >7100 ng/L. See the text of the EA for additional details on the statistical analysis conducted.
[b] Because there were no significant differences on survival, fertility and hatching success from the control mean, the NOEC and LOEC values for these endpoints were based on the highest concentration tested.
[c] Data from days 15-21 were used for this analysis.
[d] The dose-response for the NOEC was found to be monotonic.
Reference Jensen et al. [121].

There was high variability among replicates in the 21-day reproduction data from the Jensen et al. [121] study primarily due to inconsistent reproduction that occurred in the initial seven days of the exposure period (see Figure 25 below). The inconsistent reproduction is likely an indirect result of the use of paired spawning (one male: one female) in this study, which reduced the number of females in each replicate. With fewer females per replicate, there are more likely to be days when no spawning occurs because fathead minnows lay their eggs in batches and there may be one or more days between batches [138]. OECD Guideline 229 (published in 2009 subsequent to the Jensen et al. study) recommends using four females per replicate (female to male ratio of 4:2) to ensure that reproduction is adequate and consistent throughout the entire testing period[x]. The 4:2 female to male ratio recommended in OECD Guideline 229 was used in the Pfizer-study described below in Section 6.2.3.3.

[x] Based on conversations with Drs. Kathleen Jensen and Gary Ankley, a 1:1 sex ratio was used in Jensen et al. [121] in order to facilitate the determination of parentage and geneomic analysis.

In the Jensen et al. [121] publication, the authors reported an EC_{50} value of 11 ng/L (95% CI = 7-16 ng/L) for the last 14 days of the study. When the tabulated data from the study were re-evaluated for this EA, a decision was made to only report results based on data for the last 14 days of the study because of the high variability in the data from the full 21-day study. EC_x values determined using the full 21-day data are not considered reliable because the slope of the regression line was not significantly different from zero ($p \leq 0.05$). However, when using the last 14-day data from the Jensen et al. study, the slope was found to be different from zero.

Figure 25. Cumulative Mean Number of Eggs/Female Over 21 Days When Exposed to 17α-Trenbolone



This figure was reproduced using data obtained from EPA (and is similar to Figure 1 in Jensen et al. [121]).

Thus, the EC_5, EC_{10}, EC_{50}, and NOEC values (95% CI) estimated from the last 14 days of reproduction data were determined to be 2.8 (0-33), 5.8 (0-37), 38 (2-93), and 32 ng/L, respectively. The EC_{50} value (38 ng/L) determined by FDA's CVM differs greatly from the EC_{50} value of 11 ng/L reported in Jensen et al. [121]. It is unclear why these values differ. It is highly unusual for the EC_{50} to be lower than the NOEC, which is the case for the EC_{50} reported by Jensen et al. (11 ng/L) in comparison to the NOEC of 32 ng/L determined by FDA's CVM. A logistic regression analysis was used by Jensen et al. to estimate the EC_{50}. Analyzing the data assuming alternative distributional assumptions or using specialized software might explain the differences between these values. The wide confidence intervals for the EC_X values suggests there is considerable uncertainty in these values; e.g., the EC_5 value of 2.8 ng/L falls within the EC_{50} confidence bounds (2 and 93 ng/L).

17α-Trenbolone reproduction assay with fathead minnow (*Pimephales promelas*) following OECD Guideline 229 [Pfizer]

Because only one literature reference contained information for 17α-trenbolone effects on fish fecundity, Pfizer sponsored short-term reproduction studies with Japanese medaka (*O. latipes*; discussed below) and fathead minnow (*P. promelas*) following OECD Guideline 229 using 17α-trenbolone [243, 244]. The studies were conducted under flow-through conditions in compliance with GLP at nominal exposure concentrations of 1.3, 3.9, 12, 38, and 120 ng/L of 17α-trenbolone.

A 21-day reproduction study was conducted by Pfizer [243] to investigate the effects of 17α-trenbolone on adult survival, fecundity (# eggs/female/day), GSI, nuptial tubercle score, plasma VTG concentration, embryo fertilization rate, embryo hatching success, percent normal fry at hatch, and percent normal fry following yolk sac absorption in the fathead minnow. Fathead minnows were obtained from a laboratory supply of reproductively-mature animals in spawning condition. Fish were exposed to a water control, a solvent control, and mean measured concentrations of 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone. A 14-day pre-exposure period was conducted to evaluate 53 spawning groups, each consisting of four females and two males. The suitability for testing was established when regular spawning occurred in each replicate chamber at least twice in the immediate 7-day period preceding exposure initiation and production of >10 eggs/female/day/replicate was achieved. Following the pre-exposure period, 28 spawning groups with the greatest number of eggs/female/day were randomly added to the exposure system for the definitive study. Once the spawning groups were distributed, the exposure period was initiated and maintained for 21 days in the adult population. Egg production was monitored daily to establish fecundity and fertility. Once a week during the 21-day study, all embryos produced in each replicate of each treatment level were pooled and a subsample of 50 embryos was indiscriminately selected and placed into an incubation cup to serve as a representative population for that treatment level. The cup containing the eggs was placed in an incubation chamber within the exposure vessel at the respective dose and allowed to incubate until hatching began. Once all embryos hatched, newly hatched larvae were observed for general physical appearance and behavior until yolk sac absorption was complete. Following the 21-day exposure of the adults, the fish were sacrificed. Statistical significance of the reproduction endpoints and endocrine biomarkers were established based on comparison with the pooled controls and reported based on mean measured concentrations.

The only endpoints that were significantly affected by the exposure of fathead minnow to 17α-trenbolone were female GSI ($p=0.0296$) and fecundity ($p=0.0166$), which resulted in a NOEC of 35 ng/L for both endpoints. Therefore, the NOEC, LOEC, and Maximum Acceptable Toxicant Concentration (MATC) values established for the short-term reproduction effects (fecundity) of 17α-trenbolone on fathead minnow were 35 ng/L, 120 ng/L, and 65 ng/L, respectively (Appendix 13.13).

Table 48. Survival and Reproduction Effects of 17α-Trenbolone for Fathead Minnow

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Survival	120	>120
Fecundity	35	120
GSI females	35	120
GSI males	120	>120
Nuptial tubercle score		
Plasma VTG induction in males and females		
Fertilization rate		
Hatching success		
Percent normal fry at hatch		
Percent normal fry following yolk sac absorption		

Reference Pfizer Study Number A5Y3N-US-12-001 [243].

17α-Trenbolone reproduction assay with medaka (*Oryzias latipes*) following OECD Guideline 229 [Pfizer]

A 21-day reproduction study was conducted by Pfizer [244] to investigate the effects of 17α-trenbolone on adult survival, fecundity (# eggs/female/day), GSI, liver VTG concentration, secondary sex characteristics (papillary processes), embryo fertilization and hatching success, percent normal fry at hatch, and percent normal fry following yolk sac absorption in the medaka (*O. latipes*). Medaka were originally obtained from a commercial supplier, purchased as embryos and reared to sexual maturity at the testing facility. Fish were exposed to a water control, a solvent control and mean measured concentrations of 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone. A 14-day pre-exposure period was conducted to evaluate 28 spawning groups, each consisting of five females and five males. The suitability for testing was established when an egg production rate of >10 eggs/female/day was achieved. Following the 14-day pre-exposure period, the 14 spawning groups with the greatest number of eggs/female/day were randomly added to the exposure system for the definitive study. Once the spawning groups were distributed, the exposure period was initiated and maintained for 21 days in the adult population. The exposure continued for an additional eight days to collect F1 embryo data. Egg production was monitored daily to establish fecundity and fertility. Once a week during the 21-day study, all embryos produced in each replicate of each treatment level were pooled and a subsample of 50 embryos were randomly selected and placed into an incubation cup to serve as a representative population for that treatment level. The egg cup was placed in an incubation chamber within the exposure vessel at the respective dose and allowed to incubate until hatching began. Once all embryos hatched, larvae were observed for general physical appearance and behavior until yolk sac absorption was complete. Following the 21-day exposure of the adults, the fish were sacrificed. Statistical significance of the reproduction endpoints and endocrine biomarkers were established based on comparison with the pooled controls and reported based on mean measured concentrations.

Female VTG levels were significantly different when compared to the pooled controls at 10 ($p=0.0366$), 33 ($p=0.0204$), and 110 ($p=0.0026$) ng/L of 17α-trenbolone. In addition, some female medaka exposed to 110 ng/L were masculinized as indicated by the development of papillary processes ($p=0.0142$). The hatching success of the F1 generation was also reduced at 33 ng/L ($p=0.0162$) and 110 ng/L ($p=0.0014$) of 17α-trenbolone, but was only reduced to 85-89% compared to 97% in the pooled controls. This is considered a

minor reduction and is not consistent with NOEC values established for hatching success in the fathead minnow of 94 ng/L of 17α-trenbolone as reported by Jensen et al. [121] and 120 ng/L of 17α-trenbolone from the Pfizer fathead minnow study [243], or in the F2 generation for sheepshead minnow (NOEC 27 ng/L of 17β-trenbolone) reported by Cripe et al. [133]. The reduction in percent normal larvae at hatch was significantly different (p=0.0100) at 110 ng/L; however, all fish recovered and appeared normal by yolk absorption (no significant difference p>0.05). Thus, because the effect on hatching success in medaka was minor and inconsistent with other reported studies, fecundity is considered the most population-relevant endpoint. The NOEC, LOEC and MATC values established for the 21-day reproduction effects (fecundity) of 17α-trenbolone in medaka were 110 ng/L, >110 ng/L, and ≥110 ng/L, respectively (Appendix 13.14).

Table 49. Survival and Reproduction Effects of 17α-Trenbolone on Japanese Medaka

Endpoint	NOEC (ng/L)	LOEC (ng/L)
Survival	110	>110
Fecundity		
Secondary sex characteristics[a] in females	33	110
Secondary sex characteristics[a] in males	110	>110
GSI females		
GSI males		
Liver VTG induction in females	4.2	10
Liver VTG induction in males	110	>110
Hatching success	10	33
Fertilization success	110	>110
Percent normal fry at hatch	33	110
Percent normal fry following yolk sac absorption	110	>110

[a] Papillary processes
Reference: Pfizer Study Number 1A73N-60-11-786 [244].

6.3. Predicted No Effect Concentration (PNEC) for Estradiol and Trenbolone Metabolites

A PNEC is typically derived by dividing an effects value (e.g., NOEC or EC value) by an appropriate assessment factor (AF). The PNEC is then compared to the PEC value(s) in the risk characterization (Section 7) to determine whether the risk to the environment is acceptable (i.e., no significant impacts are expected), or if additional analyses are needed. Table 51 below lists the effects values (NOEC and/or EC_{10}), the AF, and the resulting PNEC for the 17α and 17β isomers of estradiol and trenbolone. These PNEC values were used in the risk characterization in Section 7.

The PNEC values for the major metabolites of EB and TBA are derived below. Although 17α-estradiol and 17α-trenbolone are expected to be the primary metabolites present in cattle manure stored on a feedlot and applied to land, minor amounts of the 17β isomers, estrone and trendione are also expected to be present (see discussion in Section 4.1). In addition, the 17β isomers, estrone and trendione are also known to be present in terrestrial and aquatic environments, likely due to transformation and degradation in the environment (see discussion in Section 4.2). Therefore, it is expected that some portion of the surrogate estradiol compound estimated in Section 5 also represents 17β-estradiol and estrone; likewise, a portion of the surrogate trenbolone compound also represents 17β-trenbolone

and trendione. Because the 17β isomers are expected to be more potent endocrine disruptors in fish, and are expected to be more potent than estrone and trendione, it is appropriate to also include an evaluation of the 17β isomers in the risk characterization. Therefore, PNEC values for 17β-estradiol and 17β-trenbolone have also been calculated below.

In contrast, we have not derived PNECs values or characterized the individual risks for estrone and trendione because the α and βisomers of estradiol and trenbolone are expected to be much more potent, and therefore, sufficiently address the potential risk posed by estrone and trendione. See Section 7 for additional information on the risk characterization.

6.3.1. Safety assessment factors (AF) for determining PNEC

CVM GFI #166 recommends that NOEC values obtained from chronic effects studies for aquatic organisms be divided an AF of 10 to estimate the PNEC [79]. The AF is intended to address uncertainties in the data, such as intra- and inter-laboratory and species variation, life-stage sensitivities, the need to extrapolate results from laboratory studies to the field, etc. The AF is applied to assure adequate protection of sensitive species and populations and is always applied when limited endpoint data are available. In some cases, a smaller AF can be used if there sufficient data to address uncertainties normally accounted for by the AF, e.g., effects data is available to address inter- and intra-species differences.

Because there is limited chronic reproduction data available for 17α-estradiol, 17α-trenbolone, and 17β-trenbolone, an AF of 10 was applied to the NOEC values for fish reproduction studies to establish PNEC values. Whereas, a lower AF is considered sufficient for 17β-estradiol because the NOEC was selected from the results of five chronic fish reproduction studies with four different fish species (Table 35). In addition, four of these chronic fish reproduction studies were greater than 100 days and one evaluated effects over multiple generations. In 2012, Caldwell et al. [102] proposed an AF of 2 for 17β-estradiol based on an SSD analysis of 21 NOEC values obtained from 19 studies evaluating eight different fish species. This AF was selected to account for the absence of a long-term study for 17β-estradiol using the Chinese rare minnow, which, of all the fish species tested to date, was the one most sensitive to estrogenic effects based on data for EE2. Because of the extensive chronic reproduction data set available for 17β-estradiol, we believe an AF of 2 is appropriate and protective to determine the PNEC for 17β-estradiol.

6.3.2. PNEC for 17α-estradiol and 17β-estradiol

17α-Estradiol: For 17α-estradiol, the principal metabolite from EB excreted in cattle manure, the reproduction NOEC and LOEC values are 250 ng/L and ≥250 ng/L, respectively (Section 6.2.2.1 and Table 35). Using an AF of 10, the corresponding PNEC for 17α-estradiol is 25 ng/L. See Table 51 below.

17β-Estradiol: As described above in Section 6.2.2 and Section 6.3.1, a NOEC value of 2.86 ng/L and an AF of 2 were selected for 17β-estradiol. These values are supported by the SSD analysis conducted by Caldwell et al. [102]. Based on these values, the PNEC value for 17β-estradiol is 1.4 ng/L. This PNEC is considered conservative and was used to characterize the risk of 17β-estradiol in Section 7. See Table 51 below.

6.3.3. PNEC for 17α-trenbolone and 17β-trenbolone

17α-Trenbolone: For 17α-trenbolone, the principal metabolite from TBA excreted in cattle manure, the reproduction NOEC is 32 ng/L and the LOEC is 94 ng/L (Section 6.2.3.1 and Table 42). The NOEC and LOEC values from the Jensen et al. study [121] are in good agreement with the NOEC and LOEC values determined in the Pfizer study [243] (Table 50 below). With an AF of 10, the PNEC for 17α-trenbolone is 3.2 ng/L. See Table 51 below.

Table 50. Effect of 17α-Trenbolone on Reproduction in Fathead Minnow (*Pimephales promelas*)

	17α-Trenbolone (ng/L)	
NOEC	32	35
LOEC	94	120
Reference	Jensen et al. [121]	Pfizer [243]

Data summarized from Table 42 above

17β-trenbolone: Based on the data discussed in Section 6.2.3.1 (Table 42) and 6.2.3.3, the range of EC_{10} values for reproductive effects in fish exposed to 17β-trenbolone is 2.5 to 5 ng/L. Because of the uncertainty in the data for the most sensitive species, we chose to use a range of EC_{10} values, rather than a single value. Applying an AF of 10 results in a PNEC range from 0.25 to 0.5 ng/L. See Table 51 below.

Table 51. NOEC or EC_{10}, AF, and PNEC Values for the 17α and 17β Isomers of Estradiol and Trenbolone

Metabolite	NOEC or EC_{10} (ng/L) value(s)	AF	PNEC (ng/L)
17α-estradiol	250	10	25
17β-estradiol	2.86	2[a]	1.4
17α-trenbolone	32	10	3.2
17β-trenbolone	2.5-5.0	10	0.25-0.5

[a] An AF of 2 was selected for 17β-estradiol because there were results of five chronic fish reproduction studies with four different fish species (Table 35). In addition, four of these chronic fish reproduction studies were greater than 100 days and one evaluated effects over multiple generations.

6.4. Effects in Fish Due to Mixtures of Estrogens and Androgens

Effects in both male and female fish have been observed following exposure to endocrine disruptors. Data indicate female fish are more susceptible to reproduction effects from exposure to androgenic compounds, as demonstrated by observed masculinization. In a similar way, male fish tend to be more susceptible to the reproduction effects of estrogenic compounds, as demonstrated by the greater hormonal changes observed in male fish following exposure to estrogens. When conditions are such that fish may be exposed to both androgenic and estrogenic compounds simultaneously, the effects can be additive, antagonistic, or synergistic. Predicting the effects of mixtures of endocrine disruptors *in vivo*, whether present from endogenous or exogenous sources, is highly challenging due to many factors. For example, 1) multiple biological systems could be effected; 2) differences in sensitivities are expected at various fish life cycle stages; 3) compound-to-compound variations in absorption, distribution, metabolism, and excretion are expected; and 4) differences in binding affinities and capacities at the androgen and estrogen receptors (including many subtypes), which can be influenced by in vivo concentrations. In

addition, because they act at different receptors, estrogenic and androgenic compounds, if present at the same time, could potentially have opposing effects on the endocrine system, which makes it difficult to predict the overall biological effects of a mixture.

Limited research has been published regarding the potency and effects of androgen and estrogen mixtures on fish. The following are summaries of the research published by Thorpe et al., Blake et al. and Velasco-Santamaría et al. [112, 139, 140].

Mixtures of Various Estrogens

Thorpe et al. [112] published a study that measured the estrogenic activity of 17β-estradiol, estrone and EE2, including the combined effects of EE2 and 17β-estradiol on VTG induction in a 14-day *in vivo* juvenile rainbow trout (*Oncorhychus mykiss*) screening assay. Median effect concentrations for VTG induction, relative to 17β-estradiol, were determined for estrone and EE2. Relative estrogenic potency of each was also calculated. EE2 was 11 to 27 times more potent than 17β-estradiol, and 17β-estradiol was 2.3 to 3.2 times more potent than estrone. These potency data were then used to predict the combined effect of 14.4 ng/L of 17β-estradiol plus 0.6 ng/L of EE2. Using a model of concentration addition and relative potency, it was shown that the activity of the mixture could be predicted, specifically indicating that mixtures of estrogens can be additive. Similar results were observed by Petersen and Tollefsen [141] in a study assessing VTG induction following exposure of a mixture of 17β-estradiol, estrone, estriol and diethylstilbestrol in a rainbow trout primary hepatocyte cell culture. Concentration addition and independent action models were used to assess the combined effects of estrogen receptor agonists. The combined effects at lower relative mixture concentrations followed prediction models, while combined effects at higher relative mixture concentrations appeared to be less than additive.

Mixtures of Various Androgens

In 2010, Blake et al. [139] published a study testing the hypothesis that androgen receptor agonists cause additive responses in a mixture. In the study, the MDA-kb2 cell line, a human breast cancer cell line, was used with endogenous androgen receptors to quantify the androgenic activity of seven natural and synthetic androgens including 17β- and 17α-trenbolone, dihydrotestosterone, methyl testosterone, testosterone, trendione and androstenedione. Observed activities of various combinations of these seven androgenic compounds were compared to expected activity based on a concentration additive model. The analysis supported the hypothesis that androgen receptor agonists cause additive responses in a mixture. Blake et al. concluded that the androgen response MDA-kb2 cell line should produce reliable estimates of androgen activity in complex environmental matrices, such as CAFO wastes. The potencies of the various androgens tested, relative to 17β-trenbolone, are presented in Table 52.

Table 52. Relative Potency of Androgens in MDA-kb2 Cells

Androgen	Relative potency
17β-Trenbolone	1.00
Dihydrotestosterone	0.562
Methyl testosterone	0.260
Testosterone	0.194
Trendione	0.050
17α-Trenbolone	0.021
Androstenedione	0.0018

Reference Blake et al. [139]

Based on these data, when compared to 17β-trenbolone, 17α-trenbolone is 48 times less active and trendione (the principal degradant of 17α-trenbolone in both soil and water) is approximately 20 times less active compared to 17β-trenbolone. These data represent androgen receptor agonist affinity (relative potency). The data are not supported by actual fecundity measurements in chronic fish reproduction studies; thus, it is unclear how predictive this assay would be for *in vivo* effects in fish.

Mixtures of Trenbolone with Estradiol

Blake et al. also included an assay of a binary mixture of 17β-trenbolone and 17β-estradiol. 17β-Estradiol was found to induce androgenic activity, but only at concentrations 600-fold greater than those found in the environment. It was hypothesized that 17β-estradiol activation of the androgen receptor in MDA-kb2 cell line was due to the structural homology between the estrogen and androgen receptors. A combination study was conducted by Velasco-Santamaría et al. [140] to investigate the hypothesis that the estrogenic effects of EE2 on male eelpout, *Zoarces viviparus* (a marine eel-like fish), could be counteracted in the presence of 17β-trenbolone. In the study, fish were treated with EE2 and 17β-trenbolone, individually and in combination at 5 and 20 ng/L, respectively. No significant antagonistic action of 17β-trenbolone on the EE2 effects was observed despite the fact that 17β-trenbolone is recognized as a potent androgenic compound. The lack of an observed combined effect at the concentrations tested led the authors to conclude that 17β-trenbolone in concentrations lower than 20 ng/L were unable to counteract the estrogenic effects of EE2 in this simultaneous exposure. However, no definitive conclusion could be drawn regarding the ability of 17β-trenbolone to minimize the effects of EE2 if present at higher exposure concentrations.

Summary

Based on the limited *in vitro* data available, there is evidence that effects from estrogen mixtures alone and androgen mixtures alone may be additive. At this time, there is little or no *in vivo* data regarding the potential for additive effects in fish from exposure to mixtures of androgens and estrogens. Therefore, we cannot predict if (and at what concentrations) a mixture of estradiol and trenbolone would result in additive (or synergistic or antagonistic) effects on population-relevant endpoints.

6.5. Conclusion

Fish reproductive endpoints were chosen to assess potential population level effects from exposures to estradiol and trenbolone metabolites. Reproduction data demonstrates that the 17α-estradiol and estrone are less toxic than the 17β-estradiol, which is consistent with data for induction of VTG. In addition, available fish reproduction data also supports that 17α-trenbolone is less toxic than 17β-trenbolone. NOECs or EC_{10} values were selected to derive the PNEC for the 17α and 17β isomers of estradiol and trenbolone. The PNEC values have been used in Section 7 to characterize the risk of these metabolites.

7. RISK CHARACTERIZATION FOR FISH REPRODUCTION EFFECTS

The risk characterization herein is based on the risk quotient (RQ) method. This method calculates an RQ by dividing the PEC for a particular exposure scenario by the PNEC for a sensitive species; i.e., RQ = PEC/PNEC. Typically an RQ value of <1 is used as a preliminary screening level to determine if additional analysis and refinement of the risk assessment may be needed. In this EA, because of the many conservative assumptions used throughout the exposure and effects assessment and the many refinements that have been incorporated into the PEC values, we believe that an RQ value in the range of 1 or less indicates that significant environmental effects are highly unlikely at the predicted level of exposure. In Section 7.1 and Section 7.2 below, the RQs for the surrogate estradiol and trenbolone compound, respectively, were calculated using the highest 90th percentile 21-day PEC values from the farm- and watershed-scale exposure scenarios modeled in Section 5 and the conservative chronic PNEC values for fish reproduction determined in Section 6. Although the environmental fate models estimate PEC_{water} values for a range of time periods (e.g., 96-hour, 21-day, 60-day, etc.), the 21-day PEC_{water} values were chosen to calculate the RQs because the reproduction studies used to estimate the PNEC were generally conducted with an exposure period of 21 days (Section 6).

For the surrogate estradiol compound, it was assumed that the toxicity of the compound was equal to the toxicity of the 17α form in order to estimate the RQs for fish reproduction-related endpoints. This comparison is made because, based on excretion and field monitoring data, 17α-estradiol is expected to be the primary metabolite in manure applied to land (Section 4.1 and Appendix 12). Thus, to derive the RQ values for the surrogate estradiol compound, the PEC_{water} for the surrogate estradiol compound was compared to the PNEC of 17α-estradiol. However, it is also expected that a small portion of the surrogate estradiol compound represents 17β-estradiol and estrone (Section 4.1 and Appendix 12). Based on available data, the 17β-estradiol is more potent endocrine disruptor in fish than 17α-estradiol, and also more potent than estrone (Section 6). Therefore, in addition to determining RQs based on the PNEC value for 17α-estradiol, we also calculated a separate set of RQs where it was conservatively assumed that the toxicity of the surrogate estradiol compound was equal to the toxicity (i.e., PNEC) of 17β-estradiol.

In contrast, for the surrogate trenbolone compound, a somewhat different approach was used to determine RQ values. Because cattle excretion data are available for trenbolone (Section 4.1.2), these data were used to proportion the PEC_{water} values for the surrogate trenbolone compound based on the relative distribution of the 17α and 17β isomers in manure[y]. The individual PEC_{water} values were multiplied by both 0.20 and 0.80 to attribute a portion of the value to 17β-trenbolone and 17α-trenbolone, respectively. It was assumed that 20% of the surrogate trenbolone compound was 17β-trenbolone because 1) based on the Zoetis-owned excretion study, 1.68% of the TBA metabolites in the feces were determined to be 17β-trenbolone (Section 4.1.2), 2) we conservatively assumed that the entire unidentified radioactivity in the urine was attributed to 17β-trenbolone (16%; Section 4.1.2), and 3) <3% interconversion of 17α-trenbolone to 17β-trenbolone can occur in the terrestrial and aquatic environments (Section 4.2). When accounting for all of these sources, a maximum of 20% of the PEC_{water} for the surrogate trenbolone compound could

[y] Due to a lack of excretion data, the PEC_{water} values of the surrogate estradiol compound could not be proportioned between the 17α and 17β isomers of estradiol.

be attributed to 17β-trenbolone, the most toxic isomer, although this percentage is likely much lower. The RQ values attributed to 17α-trenbolone and 17β-trenbolone were calculated by dividing the specific PEC_{water} values by their respective PNEC values. In addition, to account for potential additive effects of these isomers, another set of RQ values was calculated, wherein the individual RQ values attributed to 17α-trenbolone and 17β-trenbolone were added together to estimate the final additive RQ values for the surrogate trenbolone compound.

Because of the inherent conservative assumptions used when deriving the PEC and PNEC values, the RQs calculated below are believed to conservatively over-represent the potential risk for environmental impacts in the US due to use of Synovex ONE implants in feedlot and pasture beef cattle.

7.1. Risk Quotients (RQs) for Surrogate Estradiol Compound

Table 53 and Table 54 summarize the highest 21-day PEC_{water} values and the resulting RQs for the surrogate estradiol compound across a range of exposure scenarios modeled (Section 5), including aggregate exposures in five mixed-use watersheds. The full range of PEC_{water} values for the surrogate estradiol compound for all farm-scale scenarios (and time-weighted averages) are presented in Table 75 and Table 76 (Appendix 7.1), and Table 83 and Table 84 (Appendix 7.3). These values are illustrated in Figure 10 through Figure 13 (Section 5.4). In addition, the full range of PEC_{water} values for all the mixed-use watershed scenarios are presented in Table 91, Table 93, Table 95, Table 97, and Table 99 (Appendix 8.3), and are illustrated in Figure 23 (Section 5.7). The PEC_{water} values for pasture are also reported in the tables presented in Appendix 8.3.

Of the five pasture scenarios modeled, Ohio had the highest PEC_{water} value for the surrogate estradiol compound (0.03 ng/L; Table 53). Of the 34 tilled crop and 17 no-till crop scenarios that were modeled, the tilled Mississippi corn scenario had the highest PEC_{water} value (0.08 ng/L; Table 53). In the five mixed-use watersheds, Iowa had the highest PEC_{water} values when assuming both 25 and 50% of AFOs directly discharge to surface waters (0.06 and 0.11 ng/L, respectively; Table 53).

RQs Attributed to 17α-Estradiol

The PNEC value presented in Table 53 (25 ng/L) is based the NOEC for 17α-estradiol (Section 6.3). When the PNEC is compared to the PEC_{water} values presented in Table 53 below, all RQs for the surrogate estradiol compound were well below 1 (≤0.004), indicating that environmental impacts are not expected from introduction of 17α-estradiol into surface waters from the proposed use of EB in Synovex ONE. The full range of RQ values attributed to 17α-estradiol for all scenarios are listed in Table 103 and Table 104 in Appendix 10.

Table 53. Highest 21-day PEC_{water} Values[a] (assuming 100% attributed to 17α-Estradiol) and Highest Risk Quotients for the Surrogate Estradiol Compound

Exposure Scenarios Modeled	PNEC for 17α-E2 (ng/L)	Highest PEC_{water} (ng/L)	Highest $RQ_{\alpha 100}$	Source of PEC_{water} Reference
Pasture cattle at landscape level (PCA[b] 100%)	25	0.03	0.001	Table 23
34 manured and tilled cropped fields (EXPRESS[c])		0.08	0.003	Table 21
17 manured and no-tilled cropped fields (EXPRESS)		0.07	0.003	Table 21
5-Regional mixed-use watersheds with **25%** of feedlots <1000 AU[d] discharging to surface water		0.06	0.002	Table 29
5-Regional mixed-use watersheds with **50%** of feedlots <1000 AU discharging to surface water		0.11	0.004	Table 28

[a] These PEC_{water} values represent the highest 90th percentile of the distribution of the 21-day moving average concentrations for the surrogate estradiol compound over 30 modeled years.
[b] Percent cropped area
[c] EXPRESS is US EPA's PRZM/EXAMS simulation shell modeling tool.
[d] Animal unit

RQs Attributed to 17β-Estradiol

There is a general consensus in the published literature that the primary metabolite contained in manure from cattle administered EB is 17α-estradiol (Section 4.1.1). However, some portion of EB metabolites will also be excreted as 17β-estradiol, estrone, and estriol (Section 4.1.1). Further, 17α-estradiol can be converted to 17β-estradiol and estrone in terrestrial and aquatic environments. Because a small portion of the surrogate estradiol compound is attributable to 17β-estradiol, and because 17β-estradiol is expected to be a more potent endocrine disruptor than 17α-estradiol and estrone, the potential impacts of 17β-estradiol were also indirectly evaluated below by assuming that the toxicity of the surrogate estradiol compound was equivalent to the 17β isomer.

Note that we did not use the same approach that was used for trenbolone (see additional information in Section 7.2 below) for refining and adding the RQ values because 1) adequate data was not available to determine the potential proportion of the 17α and 17β isomers in the manure or environment, and 2) even when assuming a worst-case (i.e., 100% of the PEC_{water} value is 17β-estradiol, the more potent metabolite), all of the RQ values for the surrogate estradiol compound were <0.1; therefore, even if the RQs for both the 17α and 17β isomers were added together they would still be well below 1 ($RQ_{\alpha 100}$ + $RQ_{\beta 100}$).

Using an extensive set of data on the effects of 17β-estradiol on fish reproduction endpoints, the PNEC for 17β-estradiol was determined to be 1.4 ng/L (Section 6.3.2). When the PEC_{water} values for the surrogate estradiol compound (Table 54) are compared to the PNEC for 17β-estradiol, the RQs are <1 for all scenarios (≤0.08). Even if one were to use the most conservative PNEC value available for 17β-estradiol (i.e., NOEC of 2.86 ng/L[z] divided by an AF of 10 equals a PNEC of 0.286 ng/L), the RQs would still be <1 (≤0.39). In addition, all of the RQs are also <1 (≤0.28) when compared to the proposed EU environmental quality standard (EQS) of 0.4 ng/L for 17β-estradiol [142]. Thus, collectively these results demonstrate that environmental impacts are not expected from the introduction of 17β-estradiol into surface waters due to the proposed use of EB in Synovex ONE implants.

[z] 2.86 ng/L for reduction in embryo fertility in medaka; Table 35, Section 6.2.2

Further, the potential risks are considered to be overestimated because only a small percentage of the administered dose of EB is expected to be excreted as 17β-estradiol; however, it has assumed herein that the 100% of the excreted dose will be 17β-estradiol. The full range of RQ values attributed to 17β-estradiol for all scenarios are listed in Table 105 and Table 106 in Appendix 10.

Table 54. Highest 21-day PEC_{water} Values[a] (assuming 100% attributed to 17β-Estradiol) and Highest Risk Quotients for Surrogate Estradiol Compound

Exposure Scenarios Modeled	PNEC for 17β-E2 (ng/L)	Highest PEC_{water} (ng/L)	Highest $RQ_{β100}$	Source of PEC_{water} Reference
Pasture cattle at landscape level (PCA[b] 100%)	1.4	0.03	0.02	Table 23
34 manured and tilled cropped fields (EXPRESS[c])		0.08	0.06	Table 21
17 manured and no-tilled cropped fields (EXPRESS)		0.07	0.05	Table 21
5-Regional mixed-use watersheds with **25%** of feedlots <1000 AU[d] discharging to surface water		0.06	0.04	Table 29
5-Regional mixed-use watersheds with **50%** of feedlots <1000 AU discharging to surface water		0.11	0.08	Table 28

[a] These PEC_{water} values represent the highest 90th percentile of the distribution of the 21-day moving average concentrations for the surrogate estradiol compound over 30 modeled years
[b] Percent cropped area
[c] EXPRESS is US EPA's PRZM/EXAMS simulation shell modeling tool.
[d] Animal unit

7.2. Risk Quotients (RQs) for Surrogate Trenbolone Compound

As stated previously, a different approach was used to estimate the RQ values for the surrogate trenbolone compound where we attributed a proportion of the PEC_{water} values to the 17α and 17β isomers. The PEC_{water} values were multiplied by both 0.20 and 0.80 to attribute a portion of the value to 17β-trenbolone and 17α-trenbolone, respectively (Table 55 and Table 56 below). These proportions were determined as follows. It was assumed that 20% of the surrogate trenbolone compound was 17β-trenbolone because 1) based on the Zoetis-owned excretion study, 1.68% of the TBA metabolites in the feces were determined to be 17β-trenbolone (Section 4.1.2), 2) we conservatively assumed that the entire unidentified radioactivity in the urine was attributed to 17β-trenbolone (16%; Section 4.1.2), and 3) <3% interconversion of 17α-trenbolone to 17β-trenbolone can occur in the terrestrial and aquatic environments (Section 4.2). When accounting for all three of these sources, a maximum of 20% of the PEC_{water} for the surrogate trenbolone compound could be attributed to 17β -trenbolone, the most potent isomer, although this percentage is likely much lower. Subtracting 20% from 100%, the remaining 80% of the PEC_{water} value was attributed to 17α-trenbolone. None of the PEC_{water} value was attributed to trendione or other unknown metabolites. The RQ values attributed to 17α-trenbolone ($RQ_{α20}$) and 17β-trenbolone ($RQ_{β80}$) were calculated by dividing the specific PEC_{water} values by their respective PNEC values (Table 55 and Table 56). In addition, to account for potential additive effects of these isomers, another set of RQ values was calculated, wherein the individual RQ values attributed to 17α-trenbolone ($RQ_{α80}$) and 17β-trenbolone ($RQ_{β20}$) were added together to estimate the final additive RQ values for the surrogate trenbolone compound ($RQ_{α80} + RQ_{β20}$; Table 57 below).

Table 55 and Table 56 summarize the highest 21-day PEC_{water} values for the surrogate trenbolone compound across a range of exposure scenarios modeled (Section 5), including aggregate exposures in five mixed-use watersheds. The full range of PEC_{water} values for the surrogate trenbolone compound for all farm-scale scenarios (including all time periods) are presented in Table 77 and Table 78 (Appendix 7.1), and Table 85 and Table 86 (Appendix 7.3), and are illustrated in Figure 13 through Figure 16 (Section 5.4). In addition, the full range of PEC_{water} values for all of the mixed-use watershed scenarios are presented in Table 92, Table 94, Table 96, Table 98, and Table 100 (Appendix 8.3). These values are illustrated in Figure 25 (Section 5.7). The PEC_{water} values for pasture are also reported in the tables presented in Appendix 8.3.

Of the five pasture scenarios modeled, Ohio had the highest PEC_{water} value for the surrogate trenbolone compound (0.30 ng/L; Table 55). Of the 34 tilled crop and 17 no-till crop scenarios, the no-till Mississippi corn scenario had the highest PEC_{water} value (0.63 ng/L). In the five mixed-use watersheds, Iowa had the highest PEC_{water} values when assuming both 25 and 50% of AFOs directly discharge to surface waters (0.70 and 1.26 ng/L, respectively; Table 55).

RQs Attributed to 17α-Trenbolone

Table 55 presents 1) the PNEC value for 17α-trenbolone for inhibition of fish reproduction (3.2 ng/L; Section 6.3), 2) the highest 21-day PEC_{water} values when either 100% or 80% is attributed to 17α-trenbolone, and 3) the RQ values when 100% ($RQ_{\alpha 100}$) or 80% ($RQ_{\alpha 80}$) of the PEC_{water} is attributed to 17α-trenbolone. When the PNEC is compared to the highest PEC_{water} values presented in Table 55 below, all RQs for the surrogate trenbolone compound were <1 (≤0.40) indicating that environmental impacts are not expected from the introduction of 17α-trenbolone into surface waters from the proposed use of TBA in Synovex ONE. The range of RQ values for all scenarios are listed in Table 107, Table 108, Table 111, and Table 112 in Appendix 11.

Table 55. Highest 21-day PEC_{water} Values[a] (assuming 100% or 80% attributed to 17α-Trenbolone) and Highest Risk Quotients Attributed to 17α-Trenbolone

Exposure Scenarios Modeled	PNEC for 17α-TB (ng/L)	Highest PEC_{water} Assuming 100% 17α-TB (ng/L)	Highest $RQ_{\alpha 100}$	Highest PEC_{water} Assuming 80% 17α-TB (ng/L)	Highest $RQ_{\alpha 80}$	Source of PEC_{water} Reference
Pasture cattle at landscape level (PCA[b] 100%)	3.2	0.30	0.09	0.24	0.07	Table 24
34 manured and tilled cropped fields (EXPRESS[c])		0.62	0.19	0.50	0.16	Table 22
17 manured and no-tilled cropped fields (EXPRESS)		0.63	0.20	0.51	0.16	Table 22
5-Regional mixed-use watersheds with **25%** of feedlots <1000 AU[d] discharging to surface water		0.70	0.22	0.56	0.18	Table 29
5-Regional mixed-use watersheds with **50%** of feedlots <1000 AU discharging to surface water		1.26	0.39	1.01	0.32	Table 28

[a] These PEC_{water} values represent the highest 90th percentile of the distribution of the 21-day moving average concentrations for the surrogate trenbolone compound over 30 modeled years
[b] Percent cropped area
[c] EXPRESS is US EPA's PRZM/EXAMS simulation shell modeling tool.
[d] Animal unit

RQs Attributed to 17β-Trenbolone

As previously described in Sections 6.2.2 and 6.3, because of uncertainty in the analytical data from a key study with the most sensitive fish species, a range of PNEC values was derived for 17β-trenbolone (0.25 to 0.5 ng/L), rather than a single value than as is typically done. Therefore, in this section we have calculated RQ values based on this range, rather than on a single PNEC value.

Table 56 presents 1) the range of PNEC values for 17β-trenbolone for inhibition of fish reproduction (0.25-0.5 ng/L; Section 6.3), 2) the highest 21-day PEC_{water} values when either 100% or 20% is attributed to 17β-trenbolone, and 3) the range of RQ values when 100% ($RQ_{\beta 100}$) or 20% ($RQ_{\beta 20}$) of the PEC_{water} is attributed to 17β-trenbolone. When the range of PNEC values are compared to the highest PEC_{water} values across all scenarios and assuming that 100% is attributed to 17β-trenbolone, most $RQ_{\beta 100}$ values are above 1. However, as previously described, it is expected that at most only 20% of the surrogate trenbolone compound would be attributed to 17β-trenbolone (1.68% in feces + 16% in urine + <3% interconversion in the environment), and perhaps much less. In order to refine the RQ values based on the 17β isomer, 20% of the PEC_{water} values for the surrogate trenbolone compound were attributed to 17β-trenbolone. When these PEC_{water} values are compared to the range of PNECs, only a single $RQ_{\beta 20}$ value exceeded a value of 1 (1.01; Iowa mixed-use watershed scenario and a PNEC of 0.25 ng/L). The range of RQ values for all scenarios are listed in Table 109, Table 110, Table 113, and Table 114 in Appendix 11.

Table 56. Highest 21-day PEC_{water} Values[a] (assuming 100% or 80% attributed to 17β-Trenbolone) and Highest Risk Quotients Attributed to 17β-Trenbolone

Exposure Scenarios Modeled	PNEC for 17β-TB (ng/L)	Highest PEC_{water} Assuming 100% 17β-TB (ng/L)	Highest $RQ_{β100}$	Highest PEC_{water} Assuming 20% 17β-TB (ng/L)	Highest $RQ_{β20}$	Source of PEC_{water} Reference
Pasture cattle at landscape level (PCA[b] 100%)	0.25-0.5	0.30	0.59-1.18	0.06	0.12-0.24	Table 24
34 manured and tilled cropped fields (EXPRESS[c])		0.62	1.24-2.48	0.12	0.25-0.50	Table 22
17 manured and no-tilled cropped fields (EXPRESS)		0.63	1.27-2.54	0.13	0.25-0.51	Table 22
5-Regional mixed-use watersheds with **25%** of feedlots <1000 AU[d] discharging to surface water		0.70	1.40-2.80	0.14	0.28-0.56	Table 29
5-Regional mixed-use watersheds with **50%** of feedlots <1000 AU discharging to surface water		1.26	2.52-5.04	0.25	0.504-1.01	Table 28

[a] These PEC_{water} values represent the highest 90th percentile of the distribution of the 21-day moving average concentrations for the surrogate trenbolone compound over 30 modeled years
[b] Percent cropped area
[c] EXPRESS is US EPA's PRZM/EXAMS simulation shell modeling tool.
[d] Animal unit

Additive RQs for the Surrogate Trenbolone Compound ($RQ_{α80} \pm RQ_{β80}$)

To account for potential additive effects of the 17α and 17β isomers, another set of RQ values was calculated (Table 57). The individual RQ values determined by attributing 80% and 20% of the PEC_{water} values to 17α-trenbolone ($RQ_{α80}$) and 17β-trenbolone ($RQ_{β20}$), respectively, were added together to calculate an 'additive' RQ value for the surrogate trenbolone compound; i.e., $RQ_{α80} + RQ_{β20}$. The highest additive RQ values for the surrogate trenbolone compound for all 56 farm-scale scenarios (runoff from pasture and cropland) were <1 (≤0.51), indicating significant impacts are not expected at the landscape farm-level.

For the five regional mixed-use watershed scenarios, the highest additive RQ values were all <1 (≤0.67) for the surrogate trenbolone compound (when calculated with either a PNEC of 0.25 or 0.5 ng/L) where it was assumed that 25% of AFOs were directly discharging to surface waters. This scenario is expected to conservatively represent the typical nationwide scenario in which it has been estimated that approximately 17% of small and medium AFOs are in need of runoff control improvements (Appendix 9). For what we believe is the reasonable worst-case for a nation-wide scenario, i.e., 50% of AFOs are directly discharging, the additive RQ values are also <1 (≤0.82) for four of the five mixed-use watershed scenarios (Texas, Ohio, Michigan, and Pennsylvania; when calculated with either a PNEC of 0.25 or 0.5 ng/L). The lone exception was the Iowa mixed-use watershed with an RQ of slightly greater than 1 (1.33); this RQ was calculated with the lowest PNEC of 0.25 ng/L. All of the additive RQ values for the surrogate trenbolone compound for all mixed-use watersheds are <1 when calculated using a PNEC value of 0.5 ng/L. The range of additive RQ values for all scenarios are listed in Table 115 and Table 116 in Appendix 11.

Table 57. Highest Additive RQ Values for Surrogate Trenbolone Compound (assuming 80% and 20% PEC_{water} attributed to 17α and 17β isomers, respectively)

Exposure Scenarios Modeled	Highest $RQ_{\alpha 80}$	Highest $RQ_{\beta 20}$	Highest Additive RQ Value for Surrogate Trenbolone Compound ($RQ_{\alpha 80} + RQ_{\beta 20}$)
Pasture cattle at landscape level (PCA[a] 100%)	0.07	0.12-0.24	0.19-0.31
34 manured and tilled cropped fields (EXPRESS[b])	0.16	0.25-0.50	0.41-0.66
17 manured and no-tilled cropped fields (EXPRESS)	0.16	0.25-0.51	0.41-0.67
5-Regional mixed-use watersheds with **25%** of feedlots <1000 AU[c] discharging to surface water	0.18	0.28-0.56	0.46-0.74
5-Regional mixed-use watersheds with **50%** of feedlots <1000 AU discharging to surface water	0.32	0.50-1.01	0.82-1.33

[a] Percent cropped area
[b] EXPRESS is US EPA's PRZM/EXAMS simulation shell modeling tool.
[c] Animal unit

There were several potential mitigating factors that could not be quantified in this risk assessment, but if it were possible to account for these factors, it is expected they would appreciably reduce the RQ values reported herein for the surrogate trenbolone compound. These factors include:

- It was assumed that the entire portion of unidentified radioactivity in beef cattle urine (16%) was attributed to 17β-trenbolone, when in all likelihood, a portion of that radioactivity would consist of 17α-trenbolone and/or other metabolites with lower potency.
- As described in Section 9, many conservative assumptions were employed in this EA that would result in lower PEC_{water} values if additional refinements were used in the exposure assessment. For example:
  - It was assumed that Synovex ONE-F and ONE-G accounted for 100% of the market share for growth-promoting implants used in their respective categories of beef cattle; i.e., all implants administered within a modeled watershed. This is highly unlikely because there are many other approved implant products available.
  - The PEC_{water} values do not account for potential degradation of the compounds in manure or on feedlot surfaces. In addition, adsorption in manure was also not accounted for in the modeling.
  - The PEC_{water} values are overestimated because it was assumed that 100% of the EB and TBA metabolites present in the manure and irrigation water were applied to cropland, and at the same time, we also assumed that 100% of these metabolites were present at a constant concentration on the feedlot surface and available for runoff. In real-world circumstances, it is expected that less than 100% of the EB and TBA metabolites would remain in the manure applied to cropland because of losses due to runoff.
  - The EA did not account for the use of vegetative buffer strips or any other BMPs typically used on farms.

Of the many RQ values calculated for the additive surrogate trenbolone compound in Table 57 above, there was only one scenario that resulted in a RQ of >1, and the RQ in this case (1.33) was only marginally above a value of 1. However, because of all of the conservative assumptions listed above and discussed in Section 9 that have been incorporated into these analyses, we believe that these RQ values greatly overestimate the actual risks for reproductive effects on fish populations. As a result, we believe significant environmental impacts will not occur from the introduction of 17β- and 17α-trenbolone into surface waters due to the use of TBA in Synovex ONE implants.

7.3. Conclusion of Risk Characterization

This risk assessment included a landscape-scale assessment of five grazed pasturelands, 34 tilled field crops, 17 no-till field crops and five mixed-use watersheds with aggregate exposures. The PEC_{water} estimates determined by the environmental fate models for these scenarios, and used to derive the RQ values above, represent the 90th percentile 21-day moving averages determined from simulations over a 30-year period and, thus, predict reasonable worst-case exposures on a nationwide basis. In addition, the five mixed-use watersheds modeled have among the highest per county acre densities of beef cattle in the US, and they also represent varying and conservative climatic (high and low rainfalls), hydrographic (steep slopes), and geologic conditions (C and D soil classifications). Thus, we believe that these RQs reported herein represent reasonable worst-case risk estimates for the use of Synovex ONE-F and ONE-G in the US.

When the PEC_{water} values for the surrogate estradiol compound were compared to the PNEC values for 17α-estradiol or 17β-estradiol, the RQs for all scenarios were ≤0.08 (Table 53), which is more than a factor of 10 below the screening level value of 1 used for evaluating risks. These results clearly indicate that significant environmental effects are unlikely from the introduction of 17α- and 17β-estradiol into surface waters due to the use of Synovex ONE.

Similar results were also found for the surrogate trenbolone compound when specific proportions of the PEC_{water} value were attributed to the 17α-trenbolone and 17β-trenbolone. The additive RQ values were <1 for all farm-scale and mixed-use watershed scenarios evaluated in Table 57 above, except for the Iowa mixed-use watershed which produced an RQ slightly above 1 (1.33) when assuming that 50% of the AFOs are directly discharging to surface waters and using the lowest potential PNEC value for 17β-trenbolone (0.25 ng/L).

The PEC_{water} values upon which these RQ values are based are considered to be an overestimation of the potential exposures to 17β-trenbolone from use of Synovex ONE-F and ONE-G for several important reasons. For example, 1) it was assumed that Synovex ONE-F and ONE-G accounted for 100% of the market share (i.e., all implants administered within a modeled watershed), when this would clearly not be the case because there are currently many other approved products available in the marketplace, and 2) it was conservatively assumed that the all of the unidentified metabolites in the urine are attributed to 17β-trenbolone, even though it is expected that a portion of those metabolites, potentially most of them, would be attributable to 17α-trenbolone or less potent metabolites. Furthermore, for the only RQ that exceeded a screening value of 1, the assumption that 50% of AFOs within a watershed are directly discharging to surface waters is an overestimation and is very unlikely to occur. As discussed in Appendix 9, we believe this percentage is in the range of 17% based on USDA Census data from 1997, which brings the RQ value below 1. However, it is expected that the current percentage of feedlots with

direct runoff to surface waters is likely reduced from 17% due to more recent facility upgrades and compliance with the Clean Water Act; however, more current information is not available to update this number. As described in Section 9, many additional mitigating factors that could not be quantified in this risk assessment would also likely further reduce the risk estimates for both the surrogate estradiol and trenbolone compounds. Thus, based on all available information and the RQ values determined herein, we conclude, based on sensitive fish reproductive endpoints, that no significant environmental impacts are expected from the use of Synovex ONE in beef steers and heifers in the US.

8. STEROID HORMONES IN THE ENVIRONMENT

An endocrine disruptor is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance of homeostasis, reproduction, development and/or behavior [1]. Endocrine disrupting compounds (EDCs) can enter the terrestrial and aquatic environments from a variety of sources and can affect the endocrine function of many organisms. A thorough explanation of how the endocrine system functions, how EDCs can disrupt endocrine function, and examples of endocrine disruption in wildlife is provided in the recent report from the United Nations Environment Programme and the World Health Organization (WHO), titled "State of the Science of Endocrine Disrupting Chemicals – 2012" [143].

Steroid hormones, such as estrogens and androgens, are known EDCs. Steroid hormones can be introduced into the terrestrial and aquatic environments from many natural and anthropogenic sources, such as 1) wash off and breakdown of plant matter that contains phytoestrogens, 2) excretion of natural hormones from wildlife, domestic animals, and humans, and 3) excretion of synthetic hormones from domestic animals and humans. Once approved, the use of Synovex ONE (which contains EB and TBA) in beef steers and heifers could be one potential source of estrogens and androgens entering the environment. In addition, other compounds (i.e., EDCs) entering the environment, such as biocides, plasticizers, and pharmaceuticals, can also interfere with natural hormone actions by exerting direct action on hormone receptors and receptor function, as well as exerting direct actions controlling hormone delivery to the receptor [143].

Recent research has focused on the potential exposure and effects of natural and synthetic steroid hormones in the environment as a potential cause to endocrine disruption observed in wildlife, including some fish species [144, 145, 146, 147, 148, 149]. However, at this time, establishing a direct causal link between the observed endocrine disruption and specific sources is extremely difficult because of the complex chemical (e.g., mixtures of EDCs) and physical (e.g., fate and distribution of compounds) interactions occurring in the environment. If estradiol and trenbolone metabolites from Synovex ONE enter the aquatic environment in a high enough concentration and exist for a sufficient duration, or if they add to an already present mixture of EDCs, this exposure could potentially cause endocrine disrupting effects in fish and amphibians in waterways located near AFOs.

Section 8 of this EA will focus on the potential for a cumulative exposure to steroid hormone compounds in the aquatic environment, including an evaluation of the contributions of steroid hormones entering the environment from various sources in comparison to the maximum estimated contribution from use of Synovex ONE. In addition, other sources of EDCs (e.g., biocides, plasticizers, etc.) that could contribute to a cumulative exposure of endocrine disruptors in environment and endocrine disrupting effects in wildlife will be included when relevant to the discussion.

8.1. Cumulative Impact Assessment of Steroid Hormones in the Environment

Estrogens and androgens are naturally occurring in all vertebrates and are typically found in the terrestrial and aquatic environments, even in pristine areas not impacted by contaminant inputs from agricultural or urban areas. However, synthetic sources of estrogens and androgens (e.g., prescription drugs), as well as other EDCs (e.g., pesticides), can contribute

to the overall estrogenic and androgenic activity in US waterways and to the risk of endocrine disrupting effects in aquatic organisms. Thus, a cumulative exposure will likely occur in aquatic environments due to a combination or mixture of steroid hormones and/or EDCs entering the environment from several different sources.

This section of the EA examines the types and sources of steroid hormones entering the environment, including those from Synovex ONE, and the potential relative contribution of each source to the aquatic exposure. Sources and exposures to other (non-steroidal) EDCs will be discussed when relevant; however, the potential for a cumulative exposure of estrogens and androgens will be the main focus of this discussion.

Evaluating cumulative exposures[aa] and impacts of steroid hormones in the aquatic environment can be difficult and complex due to the many factors that affect these exposures, such as 1) the spatial proximity of sources to one another and to waterways, 2) the frequency and/or time of year the steroid hormones are introduced from each of the sources, 3) the mass or concentration of steroid hormones introduced into the environment from each source, 4) the mobility, environmental fate, and bioavailability of each steroid hormone compound, 5) the removal rates in a wastewater treatment plant (WWTP), and 6) the toxicity of each steroid hormone. Because many of these variables are unknown for some of these compounds, it is impossible to quantitatively estimate the potential cumulative exposure of steroid hormones that is occurring in the aquatic environment and to estimate the potential risk to non-target organisms. However, there are some data available on the mass of estrogens and androgens entering the environment. The mass of other estrogens and androgens entering the environment can be compared to the estimated mass of estradiol and trenbolone contributions entering the environment from the use of Synovex, to provide insight into the relative contribution of Synovex ONE to the cumulative exposure of estrogens and androgens in the environment.

Therefore, in this section of the EA, we have semi-quantitatively estimated 1) the potential contributions from the primary sources of steroid hormones using available published literature data on the mass entering the environment, and 2) the proportion of the total mass entering the environment that is attributed to the use of Synovex ONE. In addition, when relevant, the sources and contributions of other EDCs have also been presented.

8.1.1. Sources of Steroid Hormones and Other Endocrine Disrupting Compounds in the Environment

Natural and synthetic steroid hormones, as well as other chemicals that mimic natural steroids and/or disrupt endocrine functions in humans and animals, are introduced into the environment from a variety of sources. These sources are depicted in Figure 26 below (adapted from Wise et al. [150]), which illustrates how EDCs may enter the environment from: 1) household products (e.g., human prescription drugs, personal care products, cleaning products, etc.), 2) industry, 3) agriculture (e.g., livestock and crop production), and 4) natural sources from wildlife and plants. The sources of EDCs are discussed below in detail.

[aa] To clarify, for the purposes of this EA, a cumulative exposure is defined as the analysis of aggregate exposures to multiple agents or stressors, and an aggregate exposure is defined as the analysis of exposure to a single chemical by multiple pathways and routes of exposure.

Steroid Hormones

Based on the extensive published literature on natural and synthetic steroid hormones (including data from fielding monitoring studies, Appendix 12), we consider that steroid hormones in the environment primarily originate from human and livestock sources [151, 152]. As depicted in Figure 26, natural steroid hormones (e.g., 17β-estradiol and testosterone) are excreted and enter the environment from livestock (such as swine, poultry, dairy cattle, and beef cattle), humans, and wildlife. Synthetic steroid hormones can also be excreted and enter the environment from livestock (e.g., TBA) and humans (e.g., EE2).

As discussed in Section 3.4 of this EA, natural and synthetic steroid hormones contained in livestock waste can enter the environment through runoff and leaching from 1) an AFO, 2) manured cropland, and/or 3) manure deposited onto pasture land. Although there are many BMPs in place to control runoff from the feedlot and agricultural land, if these practices are not followed or the BMPs are not operating correctly, steroid hormones can enter the surface waters from livestock and farming operations. In addition, natural and synthetic steroid hormones contained in human waste can enter the surface waters from 1) WWTP discharges, 2) leaching or leaking from septic systems, and 3) leaching and runoff following the application of biosolids to land. A partial list of some natural and synthetic steroid hormones excreted by livestock and humans is presented in Table 58.

Although the primary sources contributing to a cumulative exposure of steroid hormones are human (e.g., WWTPs) and livestock (e.g., CAFOs) wastes, these two sources are generally not located in close proximity to one another. In addition, the timing of the introduction of steroid hormones from these two sources can vary considerably. WWTPs discharge effluent containing steroid hormones on a daily and almost continuous basis (considered pseudo-persistent exposures); whereas, leaching and runoff from feedlots and cropland typically occurs only during rainfall events (considered pulsed or intermittent exposures). Further, leaching from feedlots is not expected to be a major source because of the impervious nature of feedlot surfaces and runoff from most feedlots is expected to be limited due to the EPA regulations requiring CAFOs to maintain their wastewater and the many BMPs employed by farmers (Section 3.2). Based on estimates in this EA (Appendix 9), it is expected that approximately 15% of AFOs (<1000 head) in the US are not employing BMPs, but as this is a national average, the local percentage could be lower or higher than this value. Moreover, livestock manure containing steroid hormones is typically applied to land only two times a year (before planting in the spring and after harvest in the fall); it is not expected to contribute daily to the exposure in the waterways. However, the spring application could potentially occur during the typical reproduction period of most fish and amphibians, which is a life-cycle stage that is known to be sensitive to EDCs.

Natural steroid hormones can also enter the environment from wildlife and plants as well; however, it is expected that these sources would contribute much less to the cumulative exposure of steroid hormones in most scenarios due to lower mass of these hormones entering the environment and/or their lower overall potencies compared to the steroid hormones excreted by livestock and humans (see Section 8.1.2.3 below for more information on phytoestrogens and wildlife).

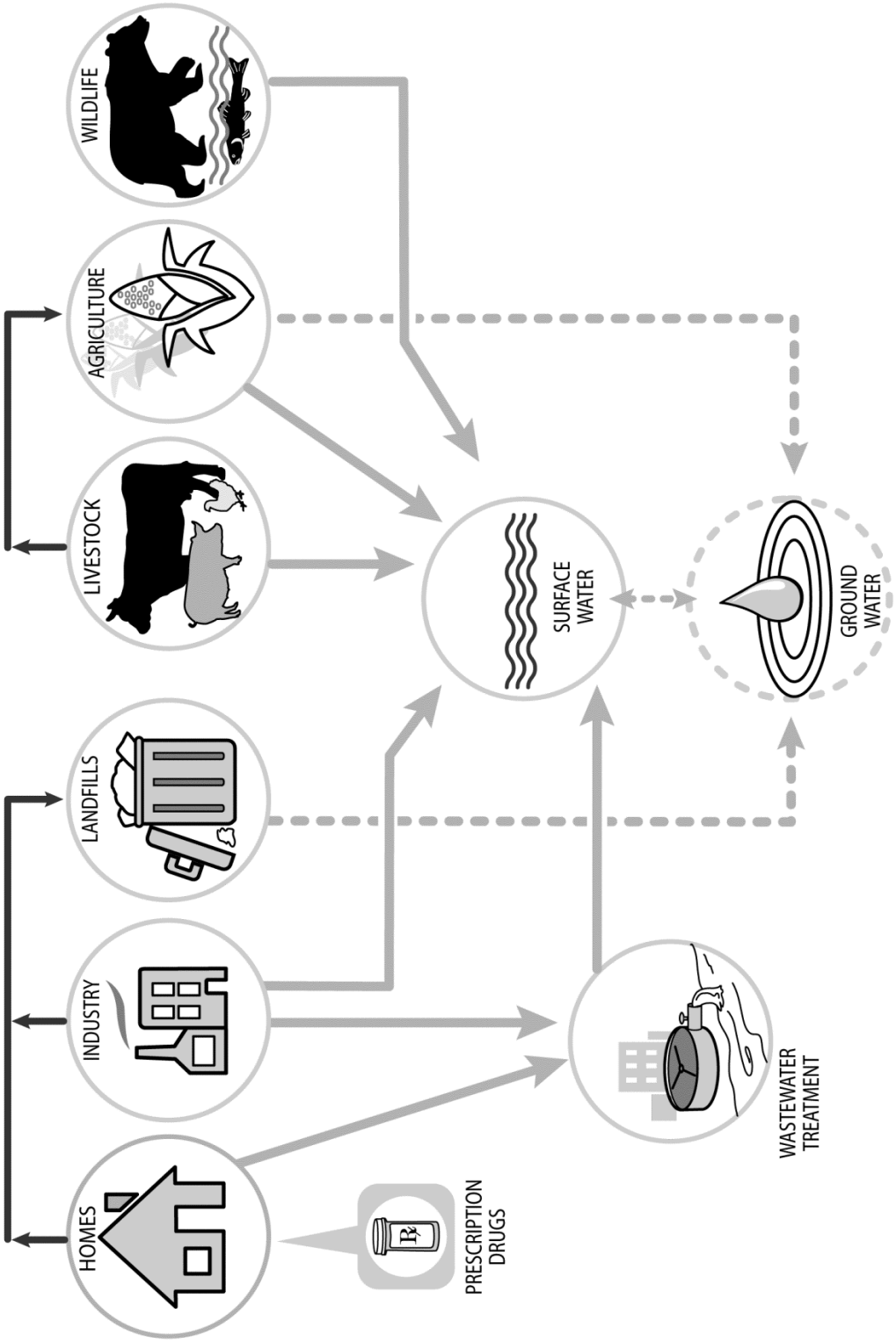


Figure 26. Conceptual Diagram of Sources of Natural and Synthetic Hormone Introduction to Surface Water and Groundwater. The solid black lines represent the movement of solid waste, solid grey lines represent movement of steroid hormones in water, and dashed grey lines represents leaching of steroid hormones to groundwater. (Adapted from Wise et al. [150])

Table 58. Partial List of Natural and Synthetic Estrogens and Androgens Potentially Excreted by Livestock and Humans

Class	Origin	Compound*
Estrogen	Natural	17α-Estradiol
		17β-Estradiol
		Estrone
		Estriol
	Synthetic	α-Zearalenone
		Zearalenone
		Zearalanone
		17α-Ethinylestradiol
Androgen	Natural	Testosterone
		5α-androstan-17β-ol-3-one
		Androsterone
		5α-androstane-3,17-dione
		4-androstene-3,17-dione
		Boldenone
		Nandrolone
	Synthetic	17α-trenbolone
		17β-trenbolone
		Trendione

* The compounds listed are provided based on the knowledge of experts in the field and on the expertise of the authors of this EA.

Other Endocrine Disrupting Compounds

Many other compounds can affect the endocrine system through 1) mimicking estrogens and androgens by directly interacting with hormone receptors (i.e., agonists), 2) interfering with hormone receptors and receptor function (antagonists), and/or 3) interfering with actions controlling hormone delivery to the receptor (i.e., anti-estrogenic and anti-androgenic compounds). Known and suspected EDCs can be introduced into the environment from industrial practices, household products, pharmaceuticals and personal care products, and agricultural and household pesticides. In addition, there are still many compounds that have the potential to interfere with the endocrine system, but have not yet been identified due to a lack of testing. See Section 8.1.2.3 below regarding the potential environmental exposures due to other EDCs.

8.1.2. Contributions of Steroid Hormones from Humans and Livestock

Based on the data reviewed below in Section 8.1.2 and Appendix 12, we consider that, in most areas of the US, the primary sources of natural and synthetic steroid hormones entering the environment originate from human and livestock waste associated with WWTPs and CAFOs [151, 152]. When feminization effects were first noted in the mid-1990s in natural fish populations downstream of WWTPs [144, 153], researchers hypothesized that the potent estrogenic substances discharged from WWTPs may be responsible. Among the estrogens discharged, EE2, a synthetic human contraceptive, was the primary focus of research because of its high potency and it is more recalcitrant to metabolism than natural hormones, which allows it to pass through WWTPs relatively unaffected. Like humans, livestock (cattle, swine, poultry, etc.) also release many types of natural hormones, as well as synthetic hormones (such as TBA) into the environment. Therefore, when androgenic or

estrogenic effects were observed in fish in agricultural environments, where WWTPs were not located, research also centered on these hormones and their sources as a potential cause of endocrine disruption in wild fish populations [149, 154, 225].

Estimates of the mass of natural and synthetic estrogens and androgens excreted by humans and livestock obtained from the published literature are presented below (Sections 8.1.2.1 and 8.1.2.2, respectively). In addition, we also estimate the potential maximum mass of estradiol and trenbolone entering the environment associated with the use of Synovex ONE in beef steers and heifers (Section 8.1.2.4). Although, the mass of estrogens and androgens excreted by humans and livestock allows for a comparison of the contributions of potential estrogens and androgen entering the environment, it does not directly equate to potential environmental exposure concentrations. As described above, there are many factors that affect entry of, and ultimately, the exposure to steroid hormones in the environment (e.g., spatial proximity, frequency and timing of discharge, environmental fate, etc.), which makes it impossible to accurately and quantitatively estimate the cumulative exposure. However, using the available data on mass of natural and synthetic steroid hormones excreted, we are able to estimate the potential relative contribution from Synovex ONE to the overall load of estrogens and androgens entering the US environment from human and livestock sources (see Section 8.1.3 below). Additional information on measured exposure concentrations of estradiol and trenbolone metabolites in US waterways is discussed in Appendix 12.

8.1.2.1. Human sources of estrogens and androgens

Natural and pharmaceutical steroid hormones contained in human urine and feces are introduced to surface waters via WWTP effluent, and these introductions are considered to be pseudo-persistent because these effluents are introduced on a continuous basis [155]. In addition to EE2, WWTP discharges can also contain over 15 natural estrogenic compounds and more androgenic compounds, in the free and conjugated form [151, 156, 157]. The most well-known, and potent, of these estrogens are 17β-estradiol, estrone, and estriol. In addition, human excreta can also contain natural androgenic substances, such as testosterone, dihydrotestosterone (DHT), and androsterone (AD). When discharged from WWTP, these estrogenic and androgenic substances can potentially result in effects on sensitive environmental receptors at very low concentrations (i.e., ng/L).

Published data on the amount and types of estrogens and androgens excreted in human urine and feces are presented below. There are fairly extensive data published on the amount of estrogens and androgens excreted in the urine of women of varying age classes (pre-menopausal, post-menopausal, and pregnant) and by men in urine. However, the data are limited for excretion of estrogens in human feces, and no data could be located on excretion of androgens via human feces. In addition, the results of a recent analysis of the potential chronic exposures and effects of natural and pharmaceutical estrogens discharged from WWTPs (including EE2) are also discussed.

Natural and Pharmaceutical Estrogens Excreted by Humans

Recently, Laurenson et al., 2014 [155] published a comprehensive review and analysis of current data on the long-term ecological exposure and effects of EE2 and other estrogens discharged from WWTPs. This analysis examined the mass of natural and pharmaceutical estrogens excreted by humans, the potential environmental exposures in waterways downstream of WWTPs using advance computer modeling (i.e., P*h*ate model), and the potential risks to non-target organisms (i.e., fish) from these exposures.

Laurenson et al. summarized the mass of human pharmaceutical estrogens sold in the US in recent years and determined the proportion of pharmaceuticals that contributes to the overall estrogenic load excreted by humans [155]. EE2 is one of the most commonly used active pharmaceutical ingredients (API) in the US. It is used by approximately 10.7 million females aged 15-44 between 2006 and 2008 (relating to approximately 17% of females 15-44 years old). However, the mass of EE2 sold is relatively low (approximately 100 kg/year sold in 2011) compared to other commonly used APIs (e.g., approximately 1 million kg/year of amoxicillin). The mass of 17β-estradiol sold in the US is higher with approximately 497 kg sold in 2011 to treat symptoms of menopause and hypoestrogenism. In addition, approximately 378 and 21 kg of conjugated and esterified estrogens, respectively, were produced in 2011 for management of symptoms associated with vulvar and vaginal atrophy, motor deficits associated with menopause, hypoestrogenism, and management of post-menopausal symptoms.

Using data from the published literature, Laurenson et al. [155] reported the average excretion per person per day for four common estrogens (estrone, 17β-estradiol, EE2, and estriol), and the fraction of this load that is attributed to prescriptions (original source of data was Anderson et al. [158]). These values are reported in Table 59 below. In addition, the authors also presented the 17β-estradiol (E2) equivalents (E2-eq) of these compounds. The potency of estrogens is typically measured in relation to 17β-estradiol and is based on the results of receptor binding assays. The E2-eq of an estrogen is determined by comparing the potency of binding of specific estrogens to the potency of binding for 17β-estradiol.

Table 59. Primary Urinary and Fecal Metabolites of Prescription and Natural Estrogens Excreted by Humans. Based on data reported in Laurenson et al., 2014 [155] and Anderson et al., 2012 [158].

Metabolite	Average excretion (µg/day/person)[a]	Fraction from prescriptions	E2 equivalence (E2-eq)	E2-eq average excretion from prescriptions (µg/day/person)
Estrone	19	0.2	0.33	1
17β-estradiol	7.7	0.1	1	0.8
EE2	0.41	1	20	8
Estriol	81	0.00075	0.033	0.002
Total	**108.1**			9.802

[a] Excreted mass of naturally produced estrogens was estimated by identifying age- and gender-specific excretion rates and weighting those rates according to the fraction of the total population.
[b] Estimated using ratios of prescribed estrogens to dietary and naturally produced estrogens from Table I of Caldwell et al. [159], and reported as overestimates due to the omission of phytoestrogen contributions.

In a review article, Liu et al. [151] reported estimates of the average excretion rates of natural estrogens (17β-estradiol, estrone, estriol and other estrogens) in the urine of specific groups: men, pre-menopausal women, post-menopausal women, and pregnant women. The urinary excretion data reported from Liu et al. [151] were gathered from 20 published literature studies and the average excretion rates for natural estrogens were reported (*note:* the data from Anderson et al. [158] were not used and human pharmaceutical estrogens were not considered). In addition, Liu et al. also determined the estrogen equivalents (E2-eq) for the reported data. These values are reported in Table 60 below.

Liu et al. [151] found that pregnant women excrete substantially more estrogen than pre-menopausal women, post-menopausal women, and men. For example, the E2-eq excretion rate by pre-menopausal women was just 0.23% that of pregnant women. The excretion rates published by Anderson et al. [158] and Liu et al. [151] cannot be compared. Anderson et al. determined the estimated mean mass by identifying age- and gender-specific excretion rates and weighting those rates according to the fraction of the total population. Whereas, Liu et al. only reported the mass expected to be excreted daily by a specific group and did not normalize these rates to the total population. In addition, the data reported in Liu et al. only accounted for excretion via urine; whereas, the data reported in Laurenson et al. also included excretion via feces (although, fecal excretion of estrogens only represents about 5 to 10% of total estrogens excreted [160, 161]). It is also unknown whether the excretion data reported by Liu et al. also includes estrogens originating from pharmaceuticals.

It is important to note that 17β-estradiol, estrone, and estriol contributed about 66 to 82% of the total E2-eq in the four different groups; whereas, the other estrogens contributed about 18 to 34% of the total E2-eq. The authors state that, generally, 17β-estradiol, 17α-estradiol, estrone, and estriol are the primary estrogens of concern in field monitoring studies; however, the data in Table 60 demonstrate that some other natural estrogens may also exist in wastewater with relative high concentrations or estrogenic activities.

Table 60. Average Estimated Excretion Rates of Natural Estrogens in Human Urine. Data obtained from Liu et al., 2009 [151].

Metabolite	Average µg metabolite/person/day (the E2-eq[b] is provided in parentheses)			
	Men	Pre-menopausal Women	Post-menopausal Women	Pregnant Women[c]
Estrone	3.90 (0.39)	10.73 (1.07)	5 (0.5)	1194 (119.4)
17β-estradiol	1.50 (1.50)	4.71 (4.71)	2.78 (2.78)	347 (347)
Estriol	1.50 (0.17)	8.12 (0.9)	2.78 (0.31)	24078 (2649)
Other Estrogens[a]	9.42 (1.05)	25.60 (3.25)	6.07 (0.79)	5110 (1221.8)
Total per person per day	16.32 (3.11)	49.16 (9.93)	16.63 (4.38)	30,729 (4337.2)

[a] Other Estrogens: 2-hydroxyestrone, 4-hydroxyestrone, 16α-hydroxyestrone, 2-hydroxyestradiol, 4-hydroxyestradiol, 4-methoxyestrone, 17-epiestriol, 2-methoxyestrone, 2-methoxyestradiol, 4-methoxyestradiol, 16-epiestriol, and 16-ketoestradiol

[b] E2-eq- Estradiol equivalence to 17β-estradiol

[c] The authors for this study did not report E2-eq for breastfeeding mothers; therefore, the total estimate of E2-eq is low because it is expected that estrogen levels in lactating women would be lower than pregnant women but higher than pre-menopausal women.

Using the data in Table 59, Laurenson et al. [155] assessed the potential environmental exposure and effects of EE2 and other estrogens discharged from WWTPs. Laurenson et al. stated that assessing the exposure, and potential impacts, of pharmaceutical estrogens in the environment is a complex process due to the multiple estrogenic substances arising from various sources, the difficulties of developing toxicity profiles for the various substances and organisms, and the unknown multifaceted aspects of human metabolism, wastewater treatment, and environmental fate. The authors analyzed the potential risks of natural and pharmaceutical estrogens excreted by humans using a similar risk assessment process as was used in this EA (e.g., probabilistic risk assessment comparing the exposures estimates determined by advance computer modeling to the potential chronic effects on fish

reproduction). The analysis indicated that the mean-flow long-term PEC_{water} values for EE2 were lower than the estimated PNEC of 0.1 ng EE2/L for fish reproduction in 99% or more of the US surface water segments downstream of WWTPs. Those environments in which the PEC would exceed the PNEC are expected to be in localized, effluent dominated streams. Similar results were found for other human pharmaceutical estrogens. In addition, Laurenson et al. also examined the potential contribution of EE2 to the total cumulative estrogenic load entering the environment from all sources, including other human pharmaceutical estrogens, endogenous estrogens, natural environmental estrogens, and industrial chemicals. The authors determined that the cumulative exposure was highly uncertain and variable, but based on the analysis, it appears that the contribution of EE2 (based on E2-eq) to the total estrogenic load in the environment is relatively low. The authors found that this analysis agrees with most studies that have examined the exposure of pharmaceutical estrogens in drinking water in the U.S.

Natural and Pharmaceutical Androgens Excreted by Humans

There are limited data available regarding the excretion of natural or pharmaceutical androgens by humans, and most of these data are focused on concentrations of natural androgens in urine. No data could be located in the published literature on the mass of androgens excreted in human feces. However, it is believed that the amount excreted in feces is much less than that excreted via urine [160, 161]; e.g., Aldercreutz and Jarvenpaa [160] determined that fecal excretion of estrogens represented about 5 to 10% of total estrogens excreted by women and men.

Like the estrogen values reported in Table 59 above, the average excretion rates of natural androgens in the urine of men and pre-menopausal women was reported by Liu et al. [151]. In addition, Liu et al. also reported the data in terms of testosterone equivalents (T-eq). These values are reported in Table 61 below. As expected, the urinary excretion rate of androgens was much greater for men than for women (approximately 10 times higher). In addition, dihydrotestosterone, 5β-androstanediol, androstenediol, and androsterone were also excreted by men and were noted by the authors to be the natural androgens with highest androgenic activities that may exist in wastewater. Moreover, an additional seven natural androgenic metabolites also contributed to the total rates of androgens excreted daily (noted as “other androgens” in Table 61); however, they were considered by the authors to be less potent androgenic compounds.

The data reported in Table 61 do not account for potential excretion of androgens in feces (although, this is expected to be low compared to that excreted in the urine) or pharmaceutical androgens; thus, the values reported in Table 61 are considered to be underestimated.

Table 61. Average Estimated Excretion Rates of Natural Androgens in Human Urine. Data obtained from Liu et al. (2009) [151].

Metabolite	Average µg metabolite/person/day (the T-eq[b] is provided in parentheses)		
	Men	Women	Average for Men and Women
Testosterone	56.65 (56.65)	6.78 (6.78)	32
Dihydrotestosterone (DHT)	14.1 (21.5)	--	7
5β-androstanediol (β-ADL)	115.7 (13.21)	--	58
Androstenediol (ANL)	435.8 (4.94)	203.3 (2.29)	320
Androsterone (AD)	3340 (2.77)	1570 (1.30)	2455
Other androgens[a]	1813.2 (3.33)	868.3 (1.26)	1341
Total	5775 (102.4)	2648 (11.63)	4212

[a] Other androgens: 4-androstenedione, epitestosterone, 5α-androstanedione, androstanediol, epiandrosterone, dehyroepiandrosterone, and cortisol
[b] T-eq- Testosterone equivalents

Natural Estrogen and Androgen Conjugates Excreted by Humans

Neither Laurenson et al. [155] nor Liu et al. [151] evaluated the excretion of estrogen and androgen conjugates due to a lack of data on these compounds. Natural estrogens and androgens are thought to be mainly excreted as sulfate and glucuronide conjugates, and then are transformed to their unconjugated forms in the environment [151]. Estrogen conjugates are known to exist in effluent of WWTP, and although the estrogenic potencies of conjugates are believed to be negligible compared to 17β-estradiol and estrone, the potencies could potentially change if they are biotransformed in the environment. Thus, it is possible these conjugates may also contribute indirectly to the overall estrogenic activity in waterways.

8.1.2.2. Livestock sources of estrogens and androgens

Naturally produced estrogens and androgens excreted from livestock, such as cattle, swine, poultry, etc., are another potential source that could contribute to a cumulative exposure of steroid hormones in the environment. Livestock excrete both free and conjugated forms of estrogens and androgens, including 17β-estradiol, 17α-estradiol, and estrone, as well as testosterone. Further, cattle implanted with growth-promoting veterinary pharmaceuticals also excrete potent synthetic androgenic substances: 17β-trenbolone, 17α-trenbolone and trendione. The form, route and amount of estrogens and androgens excreted by livestock vary depending on the species and environmental factors [152, 162, 163]. For example, cattle excrete ≥90% of estrogens as free and conjugated forms of 17α-estradiol, 17β-estradiol and estrone, with 17α-estradiol being much more prevalent than 17β-estradiol [162, 163]. Conversely, free and conjugated 17β-estradiol, estrone and estriol are the primary estrogens found in swine and poultry excreta; however, 17α-estradiol is rarely found. Further, cattle excrete estrogens mostly in feces (approximately 58%); whereas, swine and poultry excrete estrogens mostly in urine (96% and 69%, respectively) [163].

Estimates of the total mass of estrogens and androgens introduced into the environment from cattle, pigs, chickens, and sheep are found in publications by Shore and Shemesh [162], Hanselman et al. [163], Johnson et al. [164], and Lange et al. [152]. These publications reported estimates of the mass of estrogens and/or androgens excreted by

each type of livestock species (e.g., cattle), and some of these break this down further and report estimates for specific animal classes within each species (e.g., calves, pregnant cows, bulls, etc.). However, these estimates are incomplete because data were lacking for some livestock species and classes for certain steroid hormones. In addition, overall, there were more extensive data on the quantity of estrogens excreted; whereas, data on androgens were generally limited.

The excretion rate data reported by Lange et al. [152] are the most comprehensive and complete available at this time, as such, these data have been used for calculations throughout the EA. Lange et al. presented estimates of the daily and yearly excretion rates of estrogens and androgens for cattle, swine, chickens, and sheep in the US obtained from published literature. The data were presented for the entire population of each species and for specific animal classes, e.g., calves, cycling cows, pregnant cows, and bulls. The daily and yearly estrogen and androgen excretion rates (i.e., mass per day or year) for each category are reported in Table 62 and Table 63 below; however, these rates are reported solely in terms 'estrogens' and 'androgens', and are not attributed to specific estrogenic or androgenic compounds (e.g., 17β-estradiol, 17α-estradiol, testosterone, etc.) or equivalents (e.g., E2-eq or T-eq). In addition, some census data were missing for certain animal classes; thus, some of the total yearly estimates of estrogens and androgens for a livestock population were likely underestimated.

The excretion data reported in Shore and Shemesh [162], Hanselman et al. [163], and Johnson et al. [164], while informative for certain purposes, could not be used for computational analyses in the EA because of the following limitations: 1) Shore and Shemesh had major data gaps and reported their data on a concentration basis (e.g., µg estrogen/kg manure) which does not allow easy comparison to the excretion rate reported in Lange et al., 2) Hanselman et al. presented their data on a 1000 kg live mass basis, which cannot be compared to the 'per animal' units reported in Lange et al., and 3) Johnson et al. only reported estimates for the UK, which are not necessarily applicable to the US because excretion of these compounds are dependent upon management and husbandry conditions, such as feed type and veterinary drugs administered. Overall, all four references were in general agreement that cattle excrete a greater mass of estrogens than other livestock species (principally because they weigh more), and that pregnant or cycling animals excrete a much greater amount of estrogens than non-cycling and non-pregnant animals.

Estrogens and androgens excreted from cattle

Lange et al. [152] estimated that cattle in the US excreted approximately 45,502 kg/year (45 tonnes[bb]/year) of estrogens in 2000 (Table 62 below). The individual contributions of estrogens from each cattle type (e.g., calves, cycling cows, pregnant cows, and bulls) varied greatly, ranging from 270 to 43,000 kg/year. Pregnant cows contributed the greatest amount of estrogens compared to all other cattle; approximately 43,000 kg/year.

Lange et al. [152] also estimated the total androgens excreted from 98 million cattle in the US to be 1900 kg/year (1.9 tonnes/year) in the year 2000. Of the total androgens, 17 million male calves and 2.3 million bulls contributed 1000 and 900 kg/year (1.0 and 0.9 tonnes/year), respectively. Lange et al. [152] did not report the total androgens excreted for cycling cows, pregnant cows, or feedlot steers; thus, the values for the total androgens excreted are underestimated.

[bb] A tonne is equivalent to a metric ton or 1,000 kg.

Estrogens and androgens excreted from swine

Johnson et al. [164] concluded that, for estrogens, urine was the predominate excretion route for swine (70 to 99%), with estrone being the major steroid present. This is different from ruminants whereby estrogens are predominantly excreted via feces (Lange et al. [152]). Lange et al. estimated that swine in the US excreted approximately 830 kg/year (0.83 tonnes/year) of estrogens in 2000; of the total estrogens, pregnant sows and boars contributed 400 and 430 kg/year, respectively. The authors were unable to estimate yearly excretion amounts for the total population of cycling sows in the US because there were no census data for that class of swine. Based on the yearly excretion rate of 0.070 kg per pregnant sow and 0.043 kg per cycling sow, it is estimated that the total amount of estrogens would increase substantially if a yearly excretion estimate for the population of cycling sows could be made and included in the total. In addition, Hanselman et al. [163] found that the amount of estrogens excreted by pregnant sows was much higher than by nonpregnant sows and, similar to dairy cattle, there was a greater amount of estrogens excreted in the urine of pregnant sows compared to the feces.

Further, the yearly total androgens excreted for swine is estimated to be 350 kg/year, which is based on excretion rates from boars [152]. Androgen excretion rates for female swine were not reported by Lange et al.

Estrogens and androgens excreted by chickens

Based on the reviews by Johnson et al. [164], Lange et al. [152], and Hanselman et al. [163], laying hens account for the majority of natural estrogens excreted by chickens. Lange et al. estimated that chickens in the US excreted approximately 2700 kg/year of estrogens in 2000. Laying hens contributed 2400 kg estrogens/year, whereas, female and male broilers contributed 230 and 48 kg estrogens/year. Lange et al. [152] also reported that chickens in the US excreted approximately 2100 kg/year of androgens; of which, 1100 kg/year was attributed to laying hens and 480 kg/year was attributed to both female and male broilers. However, total yearly estrogen and androgen estimates for cocks were not reported by Lange et al. because there were no census data available for this category of chickens. Based on the yearly excretion of estrogens and androgens by a cock (1.2 kg estrogens/year/cock and 8.9 kg androgens/year/cock), the total estrogens and androgens for the entire population chickens would likely be greatly increased.

Estrogens and androgens excreted by sheep

Lange et al. [152] estimated that the US sheep population excreted a total of 92 kg of estrogens in 2000. Pregnant ewes contributed the greatest amount of estrogen with 87 kg/year and rams contributed 5 kg/year. However, the total yearly estrogen estimates for cycling ewes were not reported by these authors, likely because there were no census data available for this category of sheep. Based on the yearly estrogen excretion data for a cycling ewe (8.4 mg/year/cycling ewe), the total mass of estrogens excreted by sheep is expected to be much higher than was estimated. No data could be located to estimate the mass of androgens excreted by sheep.

Table 62. Summary of Amounts of Estrogens[a] Excreted from US Livestock, Reported in Lange et al. [152]

Species	US Population in 2000 (Millions per year)[c]	Mean Estrogens in Feces (µg per day)	Mean Estrogens in Urine (µg per day)	Total Daily Excretion of Estrogens (µg per day)	Total Yearly Excretion of Estrogens (mg per year)[d]	Total Estrogens Excreted for the US Population[b] (kg per year)
Cattle	**98**					**45,502**
Calves	17	30	15	45	16	272
Cycling Cows	20	200	99	299	110	2200
Pregnant[b]	43				990	42570
Steers	17					
Bulls	2.3	360	180	540	200	460
Swine	**59**					**830**
Piglets, young pigs	51					
Cycling Sows		14	100	120	43	
Pregnant[b]	5.7				70	400
Barrows						
Boars	0.52	270	2000	2300	830	430
Others	2.6					
Sheep	7.7					**92**
Lambs	2.5					
Cycling Ewes		20	3	23	8.4	
Pregnant[b]	4.6				19	87
Rams	0.58	22	3	25	9.1	5
Chickens	**1816**					**2678**
Female Broilers	691				0.34	230
Male Broilers	691				0.07	48
Laying Hens	332				7.1	2400
Cocks					1.2	
Total for All Livestock Species						**49102**

Note: Blank boxes indicate where data were not reported by Lange et al.

[a] Estrogens mainly consist of estrone, 17α-estradiol, and 17β-estradiol; however, the specific metabolite ratio was not reported in Lange et al. These values do not include excretion of pharmaceutical estrogens administered to livestock.

[b] Daily excretion rates of pregnant animals could not be calculated because the mass is dependent on the stage of pregnancy, but total excretion during pregnancy was determined by integrating the area under a curve describing a time course of fecal estrogen concentrations.

[c] Estimates are based on USDA NASS Census data from 2000.

[d] These values were determined by multiplying the total estrogens excreted per day by the number of animals held for 365 days a year; except for pregnant livestock, in which the total excretion for the year was based on both the periods they were pregnant and the periods they were not pregnant. The duration of pregnancy was set as 280 days for cows, 115 days for swine, and 149 days for sheep, assuming one pregnancy for cattle and sheep and two for swine.

Table 63. Summary of Amounts of Androgens[a] Excreted from US Livestock, Reported in Lange et al. [152].

Species	US Population in 2000 (Millions per year)[b]	Total Yearly Excretion of Androgens (mg per year)[c]	Total Androgens Excreted for the US population[b] (kg per year)
Cattle	**98**		**1900**
Calves	17	120	1000 (male)
Cycling Cows	20		
Pregnant	43		
Steers	17		
Bulls	2.3	390	900
Swine	**59**		**350**
Piglets, young pigs	51		
Cycling Sows			
Pregnant	5.7		
Barrows			
Boars	0.52	670	350
Others	2.6		
Sheep	7.7		
Lambs	2.5		
Cycling Ewes			
Pregnant	4.6		
Rams	0.58		
Chickens	**1816**		**2100**
Female Broilers	691	0.7	480
Male Broilers	691	0.7	480
Laying Hens	332	3.4	1100
Cocks		8.9	
Total for All Livestock Species			**4350**

Note: Blank boxes indicate where data were not reported by Lange et al. In addition, the mass of androgens was not attributed to excretion route in Lange et al.; only yearly excretion rates of androgens was available.

[a] Androgens mainly consist of 17α-testosterone, 17β-testosterone, or androstenedione; however, the specific metabolite ratio was not reported in Lange et al. These values do not include excretion of pharmaceutical androgens administered to livestock.

[b] Estimates are based on USDA NASS Census data from 2000.

[c] These values were determined by multiplying the total estrogens excreted per day by the number of animals held for 365 days a year; except for pregnant livestock, in which the total excretion for the year was based on both the periods they were pregnant and the periods they were not pregnant. The duration of pregnancy was set as 280 days for cows, 115 days for swine, and 149 days for sheep, assuming one pregnancy for cattle and sheep and two for swine.

8.1.2.3. Data Gaps in Contributions of Steroid Hormones and Other Endocrine Disrupting Compounds

As described above, there are data available that allow for a rough estimation of the mass of estrogens and androgens excreted from humans and most livestock; however, the estimates reported in the literature can be highly variable. In addition, there were several data gaps identified during the analysis of the mass of hormones excreted by humans and livestock, including a lack of sufficient data on the excretion of estrogens and androgens in human feces and a lack of any data for some specific animal classes (e.g., lack of androgen excretion data for sheep, and cycling and pregnant cows, etc.). Further, data are also lacking for several other sources of steroid hormones and EDCs that could potentially enter the environment and contribute to a cumulative exposure.

The data gaps identified during the preparation of this EA are listed below. However, it is important to note that this list outlines only those data gaps could be readily identified at the time this EA was prepared. This list is not intended to be an exhaustive account of all potential sources of steroid hormones and/or EDCs.

1. *Phytoestrogens:* There is no available information regarding the mass of estrogens originating from plants that could potentially enter the environment [1]. Phytoestrogens are produced by various plant matter, such as nuts and legumes, and can enter surface waters from industrial and municipal effluents (i.e., excretion from humans) [165, 166, 167, 168] and from agricultural non-point sources (wash off from plant and plant debris and runoff from manured cropland) [168, 169, 170]. Concentrations of phytoestrogens detected in surface waters are highly variable, both spatially and temporally. Elevated concentrations of phytoestrogens have been detected downstream of industrial and WWTP discharges [166, 168]. Phytoestrogens can be present in high concentrations near soy-processing facilities and other plant-based industries (e.g., biodiesel plants) [165]. For example, a recent study of 19 industrial wastewater streams in Minnesota and Iowa found elevated levels of two phytoestrogens, genistein and daidzein, near industrial facilities (e.g., 127 to 250 µg/L in soy oil and soy milk production effluents, 1.3 to 22.5 µg/L in biodiesel refinery effluent, and 39.9 µg/L from dairy effluents) [165]. In addition, WWTP effluents have been found to contain phytoestrogens (e.g., zearalenone, formononetin, genistein, equol, and daidzein) at concentrations ranging from <1 to 1300 ng/L due to excretion by humans following ingestion of plant material [165, 168, 171]. In contrast, concentrations of the same phytoestrogens detected in agricultural waters are typically <50 ng/L [169, 170].

   Although data on the mass of phytoestrogens entering the environment are lacking, it is known that many of these phytoestrogens (i.e., genistein and daidzein) degrade rapidly in water due to microbial processes [172] and anywhere from 23 to 100% of the phytoestrogen can be removed during wastewater treatment depending on the phytoestrogen (see Table 3 of [166]). In addition, most phytoestrogens are known to be much less potent (i.e., typically orders of magnitude less potent) than 17β-estradiol as determined by *in vitro* relative binding assays [Table 1 of 168, 173, 174] and *in vivo* studies with fish (observing development, behavior, vitellogenin, and reproduction) [166, 172, 175]. Many researchers agree that phytoestrogens may make a significant contribution to the total estrogenic activity in the aquatic environment in localized areas due to high concentrations found in some industrial, municipal and agricultural effluents, but at this time, the information on the mass of phytoestrogens entering the environment is too limited to determine their relative contribution compared to other known estrogenic inputs on a widespread basis [166, 168, 170].

2. *Domesticated Animals:* As described above, steroid hormones can be excreted by domestic animals and potentially enter the environment from agricultural management of animal wastes (e.g., storage on a feedlot or application to cropland). Some estimates of estrogens and androgens excreted by livestock, such as cattle, swine, chickens, and sheep, are described under Section 8.1.2.2. However, there were many data gaps that likely resulted in an underestimation of the estrogens and androgens excreted from these animals. For example, data are not available for all domestic animal species; specifically, data are not available for excretion of estrogens and androgens from turkeys, horses, goats, rabbits, dogs, cats, zoo animals, etc. These sources are expected to excrete relatively minor amounts of

estrogens and androgens compared to cattle, swine, and chickens; however, the overall contribution from all of these sources will influence the total mass entering the environment. In addition, many domesticated animals, such as dogs, cats and zoo animals, are expected to be located in primarily urban areas and are not expected to contribute significantly to exposures in agricultural watersheds.

Further, there were data gaps in the estrogen and androgen excretion data for several of the livestock species in Table 62 and Table 63 above. For example, there were no androgen data available for 1) cycling and pregnant cows, 2) cycling and pregnant sows, and 3) all classes of sheep. Thus, the estimates for estrogen and androgen excretion from livestock in the US are considered to be underestimates.

3. *Wildlife:* There is little information regarding the amount of estrogens and androgens excreted by wildlife. Some researchers have suggested that wildlife could contribute to the steroid hormone concentrations detected in terrestrial and aquatic environments [155, 162]. However, in general most wildlife species are expected to be present at relatively low densities in agricultural areas, such as near feedlots and cropland; thus, these sources are expected to contribute relatively minor amounts of estrogens and androgens to exposures in agricultural areas.
4. *Aquaculture*: There is little information available to estimate the concentration of estrogens and androgens excreted by aquaculture facilities. Kolodziej et al. [15] found that effluents from a fish hatchery contained concentrations of testosterone, androstenedione, and estrone ranging from 0.1 to 0.8 ng/L. When excretion rates are normalized to animal mass, Kolodziej et al. suggested that fish could excrete steroid hormones at rates (µg/mass of animal/day) similar to livestock. In addition, Shore and Shemesh [162] noted a study that measured concentrations of 17β-estradiol in fish aquariums at concentrations ranging from 3.5 to 15 ng/L, and a study that measured estrogens and testosterone at concentrations of 5 to 7 ng/L in fish ponds over four years. However, there are relatively few aquaculture facilities in the US (<4400, [176]) compared to the number of AFOs (76,396 in 2007) and the biomass in these facilities is much smaller on average. In addition, these aquaculture facilities tend to be widely dispersed around the country (except for catfish ponds in the southeastern US, which usually discharge water only on an intermittent basis). Thus, aquaculture facilities may contribute to localized exposures, but are not expected to contribute a significant amount to exposures of estrogens and androgens on a watershed or broader scale.
5. *Human urine and feces*: As described under Section 8.1.2.1, there is little information available in the published literature regarding the concentrations of estrogens and androgens excreted in the feces of men and women. However, it is thought that the amount excreted by human in feces is much lower than that in urine. Aldercreutz and Jarvenpaa [160] determined that fecal excretion of estrogens represented about 5 to 10% of total estrogens excreted by women and men. In contrast, feces are the primary route of excretion of estrogens by cattle (Table 62).

   In addition, there is a lack of data on the mass of estrogen and androgen conjugates (e.g., glucuronide and sulfate conjugates) that are excreted by humans and livestock, and discharged from WWTPs. Although the estrogenic potency of these conjugates are considered to be negligible compared to the unconjugated forms, the potencies of the conjugates could change if they are biotransformed in the environment, which could contribute indirectly to the total estrogenic activity in waterways.

6. *Pharmaceuticals:* Many pharmaceuticals are intentionally developed and marketed to have an endocrine-related effect in humans, such as estrogen and androgen replacement therapies, contraceptives, and cancer treatments. The masses of some estrogen and androgen APIs sold in human pharmaceuticals in the US are reported in Section 8.1.2.1; however, this EA does not report the masses of all pharmaceuticals that may have some estrogenic or androgenic activity because these data are not known at this time.
7. *Pesticides:* Some pesticides are known or suspected to have estogenic, anti-estrogenic, or anti-androgenic properties (e.g., organochlorines) [1, 143]. It is generally expected that a high amount of pesticides are used in agricultural regions to protect crops from pests. These compounds could enter the aquatic environments during rainfall events and could potentially contribute to the overall exposure and effects of EDCs in agricultural watersheds. At the time this EA was prepared, most active and inert ingredients in pesticides have not been tested yet for their endocrine disrupting potential; however, in the upcoming years many of these compounds will be tested under EPA's Endocrine Disruptors Screening Program (EDSP) to determine their endocrine disrupting potential.[cc] Thus, at this time, the data are incomplete on the number of pesticides that are hormonally active, the mass of these compounds entering the environment, and the contribution of these pesticides to the estrogenic, anti-estrogenic and anti-androgenic activity in the terrestrial and aquatic environment.
8. *Industry*: Some industrial chemicals have been identified or suspected as endocrine disrupting compounds [1], and there are likely more that are hormonally active, but have not yet been identified. Industrial chemicals can enter waterways through point sources like manufacturing facilities, domestic and industrial wastewater effluents, runoff from urban areas, and leaching from landfills [150]. Wise et al. [150] stated that there is a great deal of uncertainty regarding the number of industrial chemicals with estrogenic activity since many chemicals are untested for this effect. As noted above for the pesticides, many industrial chemicals will also likely be tested for their endocrine disrupting potential in the upcoming years as part of EPA's EDSP. Thus, the data are also incomplete on the number of industrial chemicals found in US surface and drinking waters that are hormonally active, the mass of these compounds entering the environment, and the contribution of these chemicals to the estrogenic, anti-estrogenic and anti-androgenic activity in the terrestrial and aquatic environment.

[cc] http://www.epa.gov/scipoly/oscpendo/index.htm (accessed March 6, 2014)

8.1.2.4. Mass of Estradiol and Trenbolone Excreted by Beef Steers and Heifers Implanted with Synovex ONE

The estimated masses presented in Section 8.1.2.2 (Table 62 and Table 63) do not account for excretion of estrogens and androgens resulting from the use of growth-promoting drugs approved in the US, including Synovex ONE. Therefore, in this section, we calculate the total yearly mass of estradiol and trenbolone excreted from beef cattle administered Synovex ONE[dd]. These data will then be used to make comparisons to other sources and to determine the potential contribution of Synovex ONE to the overall potential estrogen and androgen loads entering the environment (Section 8.1.3).

Calculation of Maximum Masses of Estradiol and Trenbolone Excreted from Beef Cattle Implanted with Synovex ONE

The theoretical maximum use of TBA and EB in beef cattle has been calculated by conservatively assuming that 100% of beef cattle marketed in the US are treated once per year with Synovex ONE-F and that 100% of stocker cattle are treated once per year with Synovex ONE-G. In addition, the following assumptions were also made: 1) 27.6 M beef cattle are marketed yearly (Section 3.2.2 Table 5), 2) 11.6 M pasture stocker cattle are marketed yearly (Section 3.1.1), and 3) 71.5% (or 0.715) of the TBA administered to cattle is excreted as trenbolone metabolites (Section 4.1.2). Also, due to a lack of data, it was assumed that 100% of EB is excreted as estradiol metabolites (Section 4.1.1). Therefore, the maximum mass of estradiol and trenbolone metabolites excreted from beef cattle following the approved label use of Synovex ONE implants was calculated as follows:

Synovex ONE-F (Section 2.7.1)

20.26 mg of estradiol X 27.6 M cattle X 1 X 1E-6 kg/mg = 559 kg
173.1 mg of trenbolone X 27.6 M cattle X 0.715 X 1E-6 kg/mg = 3,416 kg

Synovex ONE-G (Section 2.7.2)

15.19 mg of estradiol X 11.6 M cattle X 1 X 1E-6 kg/mg = 176 kg
129.83 mg of trenbolone X 11.6 M cattle X 0.715 X 1E-6 kg/mg = 1,077 kg

Amounts of Estradiol and Trenbolone Entering the Environment as a Result of Combined Pasture + Feedlot Use of Synovex Products

Estradiol: 559 kg + 176 kg = 735 kg
Trenbolone: 3,416 kg + 1,077 kg = 4,493 kg

According to the assumptions and calculations presented above, if all beef cattle (both feedlot and pasture cattle) in the US were implanted with a Synovex ONE product, the maximum potential mass of estradiol and trenbolone entering the environment from the use of these implants would be 735 kg estradiol/year and 4,493 kg trenbolone/year. At this time, it is unknown what portion of this mass would be attributed to specific metabolites of estradiol (e.g., 17β-estradiol, 17α-estradiol, estrone, estriol) due to a lack of data. However, available data discussed in Section 4.1 of the EA suggest that the majority of estradiol found in the excreta of cattle would be 17α-estradiol, with a minor amount excreted as 17β-estradiol and estrone. There is information available from a Zoetis-owned study to support that the majority of trenbolone excreted would be in the form of 17α-trenbolone

[dd] It is important to note that Synovex ONE-F and ONE-G are only proposed for use in beef steers and heifers; not calves.

(53.4% in feces) and a minor amount would be excreted as 17β-trenbolone (1.68% in feces); it is unknown if any would be excreted as trendione because analysis for this metabolite was not performed in the study.

The masses reported above are believed to be overestimated because they are based on the assumptions that 1) 100% of beef cattle marketed in the US will be administered a growth promoting implant each year, and 2) that every implant administered will be Synovex ONE, i.e., Synovex ONE accounts for the full market share of growth promoting implants. It is highly unlikely that 100% of beef cattle in the US would be administered Synovex ONE because it is not approved for use in all beef cattle classes and is only one of many growth-promoting products on the market. According to the USDA Animal and Plant Health Inspection Service (APHIS) [177], the use of growth-promoting implants in beef steers and heifers varies depending on the size of the feedlot and the size and sex of the animal. In 2011, approximately 84% and 86% of large CAFOs (>1000 head) implanted beef heifers and steers weighing less than 700 lbs, respectively. Approximately 91.3% and 93% of large CAFOs implanted beef heifers and steers, respectively, weighting more than 700 lbs. In addition, as stated above, beef cattle could be administered a different growth-promoting implant that could potentially consist of a lower dose of estradiol and/or trenbolone.

8.1.3. Discussion of Findings and Conclusions Regarding Cumulative Exposures of Steroid Hormones in the Environment

As discussed above, steroid hormones can enter the environment from both natural and anthropogenic sources, such as via excretion by humans, livestock and wildlife, which could result in a cumulative exposure in the aquatic environment particularly on a watershed scale due to multiple inputs. As a result, once approved, Synovex ONE could potentially add to the already existing cumulative exposure in the environment. At the present time, due to a lack of data or in some cases because of uncertainty in the existing data for many of the variables needed to predict a cumulative exposure (e.g., contributions from all sources, spatial proximity, frequency and timing of discharge, environmental fate, potency, etc.), it is impossible to accurately estimate the potential cumulative exposure of steroid hormones in the aquatic environment on a quantitative basis. As an alternative, using the data available in the published literature, rough estimates have been provided for the mass of estrogens and androgens excreted by humans and livestock that could potentially enter the environment (Sections 8.1.2.1 and 8.1.2.2). These estimates will now be compared to the estimated maximum mass of the estradiol and trenbolone metabolites associated with the use of Synovex ONE (Section 8.1.2.4) to determine the potential contribution of Synovex ONE to the overall load of estrogens and androgens entering the environment.

It is important to note that these estimates were only on a yearly mass (kg) basis in terms of potential entry into the environment. These mass estimates do not necessarily directly predict potential environmental exposures because these exposures will depend on many additional factors that influence the environmental fate and distribution of the compounds (e.g., half-life, water solubility, adsorption to soil/sediment). Exposures, particularly those at a watershed or regional scale, will also be highly influenced by both the spatial distribution and the temporal nature of the sources/inputs. For example, the exposures will depend on whether these inputs are continuous or intermittent, and whether they are widespread, or limited and localized. Exposures in urban areas would be expected to be influenced most by inputs from WWTPs, while those in agricultural areas would be influenced more by inputs from non-point sources such as runoff.

In addition, as previously mentioned, although there are some data available on the masses of natural and synthetic estrogens and androgens entering the environment from humans and livestock sources, these estimates vary widely, resulting in considerable uncertainty in the accuracy of these estimates. Further complicating the situation, there is limited information, and in some cases no information, on the contribution of natural estrogens from certain livestock species (e.g., turkeys, fish), wildlife and plants, and the data on the contribution of androgens from human and livestock are also lacking. Although data gaps and uncertainty exists, we were able to make rough comparisons that indicate that the contribution of Synovex ONE to the overall load of estrogens and androgens entering the environment will be minor compared to the mass entering from human and livestock sources.

Table 64 below summarizes the yearly estimated mass of estrogens and androgens entering the environment from each potential source examined in Section 8.1 (humans, livestock, and Synovex ONE), and the percent contribution of each source to the overall load of estrogens and androgens entering the environment. The yearly maximum mass of estradiol and trenbolone metabolites excreted from cattle administered a Synovex ONE implant was estimated to be 735 kg/year and 4493 kg/year for estradiol and trenbolone, respectively. The mass of estradiol excreted from beef cattle implanted with Synovex ONE is very small compared the overall mass of estrogens excreted from cattle in the US (45,502 kg/year; Table 62) and the overall mass of estrogens from all livestock sources in the U.S. (49,102 kg/year; Table 62). In addition, the mass of estradiol excreted from beef cattle implanted with Synovex ONE is also extremely small compared to the estimated amount of natural and pharmaceutical estrogens excreted by the entire US population (12,467 kg/year). Thus, based on these rough estimates, it was determined that the estradiol associated with Synovex ONE would consist of approximately 1.19% of all the estrogen entering the environment including all three sources.

In contrast, the yearly maximum mass of trenbolone excreted from beef cattle implanted with Synovex ONE (4493 kg/year) is much higher than the total mass of natural androgens estimated to be excreted from cattle (1900 kg/year; Table 63) and is similar to the total overall mass of natural androgens entering the environment from all livestock in the US (4350 kg/year; Table 63). Thus, the trenbolone excreted from cattle with Synovex ONE implants could be a major contributor to the overall mass of androgens entering the environment from livestock sources each year. However, similar to estrogens, the maximum mass of trenbolone excreted from beef cattle implanted with Synovex ONE was also very small in comparison to the total contributions of natural androgens excreted by the entire US human population (485,800 kg/year). Thus, based on these rough estimates, the trenbolone associated with Synovex ONE would consist of approximately 0.9% of all the androgens entering the environment from each of these three sources.

Table 64. Summary of the Yearly Estimated Mass (kg) and Percent Contribution of Estrogens and Androgens Entering the Environment from Humans, Livestock, and Synovex ONE

Source	Estrogens		Androgens		Relevant EA Section[f]
	Yearly Estimated Mass (kg)	Percent Contribution to Overall Load[c]	Yearly Estimated Mass (kg)	Percent Contribution to Overall Load[c]	
Humans	12,467[a,b]	20.0	485,900[d]	98.2[e]	8.1.2.3, Table 59 and Table 61
Livestock	49,102	78.8	4350	0.9	8.1.2.2, Table 62 and Table 63
Synovex ONE	735	1.19	4493	0.9	8.1.2.4
Total	62,304		494,743		

[a] This value was calculated by multiplying the total estrogens excreted/person/day from Table 59 (108.1 µg estrogens/person/day) by the number of days in a year (365 days) and the most recent census data for the US [316 M estimated in 2013 (http://quickfacts.census.gov/qfd/states/00000.html)].
[b] The mass of estrogens excreted by humans includes the mass of estrogens from pharmaceutical estrogens, including EE2.
[c] This value was calculated by dividing the mass attributed to an individual source by the sum of the mass of estrogens or androgens from the three sources. E.g., 735 kg estradiol from Synovex ONE / 62304 kg total estrogens or 4493 kg trenbolone from Synovex ONE / 494,643 kg total androgens.
[d] This value was calculated by multiplying the total average mass of androgens excreted/person/day from Table 61 (4213 µg androgens/person/day) by the number of days in a year (365 days) and the most recent census data for the US (316 M estimated in 2013).
[e] The mass of androgens excreted by humans does not take into account the mass of androgens from human pharmaceuticals.
[f] See the Sections listed in this table for specific calculations to determine the yearly mass.

There is some level of uncertainty associated with these rough estimates, and it is known that the masses of naturally produced estrogens and androgens excreted by cattle, all livestock, and humans were underestimated. As discussed in Section 8.1.2.3, the available estimates of estrogens and androgens excreted from humans and livestock were variable and some key data were lacking, which prevents calculation of an accurate estimate of the total estrogens and androgens excreted by humans and livestock. For example, the mass of androgens estimated for the US population does not include those androgens in human feces or administered in pharmaceuticals or illicit uses of androgens by humans (e.g., illegal use of trenbolone to build muscle mass), and there were a lack of data available on the mass of androgens excreted by some classes of livestock (i.e., all classes of sheep and cycling and pregnant cows and sows).

Further, the maximum masses of estradiol and trenbolone entering the environment from the use Synovex ONE are appreciably overestimated because they are based on the assumption that 100% of cattle in the US would be administered Synovex ONE (i.e., that Synovex ONE would account for 100% of the market share of growth-promoting implants). However, this assumption conservatively overestimates the potential use of Synovex ONE because 1) it is known that approximately 84 to 93% of beef steers and heifers in the US are administered some type of hormone implant [177], and 2) we assumed that the implant administered to all beef cattle was Synovex ONE even though some of these beef cattle could be administered a different growth-promoting implant that consists of a lower dose of estradiol and/or trenbolone.[ee] Thus, the maximum mass of estradiol and trenbolone

[ee] Of all growth promoting implants approved, Synovex ONE will contain the highest dose of estradiol, and will be equivalent to the highest dose of trenbolone already approved in other products.

associated with the use of Synovex ONE would be much less than estimates of 735 and 4493 kg/year, respectively. Because the estimated maximum mass of estradiol and trenbolone entering the environment from the use of Synovex ONE is overestimated, and the estimated masses of naturally produced estrogens and androgens entering the environment from human and livestock waste is underestimated, then the overall contribution of Synovex ONE is expected to be even lower than estimated (i.e., less than 1.19% for estrogens and 0.9% for androgens).

These estimates only represent the total mass excreted each year; they do not represent the potential environmental exposure concentrations of steroid hormones in the aquatic environment. The exposure concentrations of steroid hormones would be impossible to estimate with a high degree of accuracy because of multiple factors that affect exposures in the environment, such as 1) the spatial proximity of sources to one another and to waterways, 2) frequency and/or time of year the steroid hormones are introduced from each of the sources, 3) the mass or concentration of steroid hormones introduced into the environment from each source, and 4) the mobility, environmental fate, and bioavailability of each steroid hormone compound. In addition, the amounts entering the aquatic environment will in some cases be significantly reduced by removal in WWTPs and degradation in manure and soils, and the relative ratio of metabolites will also be altered due to biotransformation in WWTPs and the environment.

Furthermore, the load of estrogens and androgens entering the environment is only one part of the data needed to complete a risk assessment. The potency (e.g., relative E2-eq and T-eq) of each steroid hormone and the relative amounts of each metabolite is ultimately needed to calculate the individual risks of these compounds, as well as any comparative and/or cumulative risks. Although the potency data are generally available [151], the necessary metabolite data are not available. Therefore, in this assessment, our analysis was limited to using mass as a surrogate to estimate the potential risk of Synovex ONE compared to the overall load entering the environment from all primary known sources.

Overall, we were able to determine, based on rough, but conservative, estimates, that the contribution of estradiol and trenbolone associated with Synovex ONE will be in the range of 1% compared to the overall load of estrogens and androgens entering the environment from human and livestock sources. At this time, as previously discussed, it is impossible to quantitatively estimate the cumulative exposure of steroid hormones in the environment. Likewise, it is impossible to accurately estimate the contribution of the estradiol and trenbolone metabolites from Synovex ONE to the overall load of EDCs in the environment due to a lack of data and uncertainty in the identity, mass, and potency of all EDCs. Because these other EDCs will add to the overall load of endocrine disruptors in the environment (esp., aquatic), which includes steroid hormones, the relative contribution from Synovex ONE should be smaller than what has already been estimated (i.e., approximately 1% or less for the aquatic environment).

9. SOURCES OF UNCERTAINTY AND CONSERVATIVE ASSUMPTIONS

In conducting a risk assessment and designing models to predict environmental concentrations, some level of uncertainty is inevitable. When possible, conservative assumptions were made in order to account for uncertainty or for a lack of data. A list of uncertainties and conservative assumptions in this EA are provided below.

9.1. Sources of Uncertainty

Potential sources of uncertainty in the modeling and assessment include:

- Lack of information on the composition (identity and relative amount) of certain TBA and EB metabolites, including conjugates, in beef cattle manure.
- Lack of information on the effects of estradiol and trenbolone metabolites on fish survival, development and reproduction over several generations.
- The EXPRESS, PRZM, and EXAMS models have not been formally validated, but have been widely used by EPA for pesticide risk assessment for many years.
- Large CAFOs (>1000 AU) out of compliance with the Clean Water Act were not evaluated.
- Potential illegal use of the drug (i.e., exceeding the indicated dose, administering the drug to unapproved animals, etc.) was not evaluated.
- Farmers applying manure at a higher rate than CNMP recommendations were not evaluated.
- The impact of the presence of antibiotics, which may be present in manure of implanted cattle, on the degradation rate of estradiol and trenbolone in soil is not well characterized.
- Lack of information on the effects of mixtures of estradiol and trenbolone metabolites on fish reproductive endpoints.
- Lack of information to determine cumulative exposures of steroid hormones and EDCs in the aquatic environment (see Section 8 for additional information).

9.2. Conservative Assumptions

Conservative assumptions made in this assessment are as follows:

Exposure Assessment

- The PEC_{water} estimates determined by the environmental fate models represent the 90^{th} percentile 21-day moving averages determined from simulations over a 30-year period. These values are considered to be conservatively representative of reasonable worst-case exposures in the US.
- It was assumed that every beef steer and heifer held on feedlots in the watersheds modeled had a Synovex ONE-F implant 365 days a year (i.e., 100% of the market share for growth-promoting implants used in these feedlots is attributed to Synovex ONE-F).

- On a watershed level, it was assumed that 100% of the pasture cattle are treated with Synovex ONE-G (i.e., 100% of the market share for growth-promoting implants used in pasture cattle is attributed to Synovex ONE-G). This assumption is also considered a conservative overestimate because only stocker pasture cattle would be treated; suckling cattle should not be implanted. On a national level, there are 27.6 M pasture cattle; however, only 12.6 M of the pasture cattle are stockers (Section 3.1).
- Because there was a lack of data on the amount of estradiol metabolites excreted from cattle administered EB, it was assumed 100% of the estradiol administered was excreted as estradiol metabolites. See Section 4.1 for additional information.
- Although estradiol and trenbolone are believed to degrade to some extent in feedlot soil, stored manure, and feedlot runoff collection lagoons, due to a lack of available data, no degradation was assumed in these three matrices (Section 4.2).
- Several studies published in the literature demonstrate that both trenbolone and estradiol are photosensitive and degraded rapidly when exposed to sunlight (Section 4.2); however, photodegradation DT_{50} values were not used in the environmental fate models.
- When three or more acceptable values were available for a fate parameter, a 90^{th} percentile confidence bound of the mean was determined and either the upper or lower value of the interval was used depending on which would result in a higher PEC for surface water. When only two values were available, the more conservative value (either higher or lower) was chosen. If only one value was available (which was only the case in one instance), the value was multiplied by a factor of 3, as recommended in EPA's guidance. These methods are considered conservative for this assessment because they would result in a higher PEC_{water} value, which is the main compartment of concern. See Section 4.2 for additional details.
- The EPA crop scenarios were developed in such a manner as to represent realistic sites within a given geographic region where specific crops would be grown and that would be vulnerable to surface runoff. These scenarios were designed to have conservative properties that would result in greater contaminant runoff and leaching, including soil types prone to erosion and runoff (soil classifications of C and D) and steep slopes (0.5-12%). See Section 5 for information on the EPA models.
- It was assumed that 25 and 50% of AFOs with <1000 AU directly discharge their feedlot runoff into surface water without consideration of any designs to retain runoff water (drainage basin, lagoon or pond, storage tank, etc.). These assumptions are considered conservative because, using USDA data, it has been estimated that only 17% of all AFOs in the US are in need of controls for runoff discharge (Appendix 9). See section 5.7 for additional information.
- In the watershed modeling, it was assumed that EB and TBA metabolites on a feedlot surface are maintained at a constant concentration and mass (i.e., mass is not reduced due to runoff or degradation; Section 5.7). This assumption resulted in an increase in the total quantity of EB and TBA metabolites contained on feedlot surfaces within the watershed, which resulted in an overestimation of runoff from those feedlots.

- It was assumed that 100% of EB and TBA metabolites excreted on a feedlot are present to runoff from the feedlot surface and that 100% are present (following a holding period) in the manure to be applied to manure amended cropland (Section 5.7). Thus, the proportions of EB and TBA metabolites excreted from feedlot animals were not attributed to different sources (i.e., feedlot or cropland). For example, 100% of the EB and TBA metabolites from ONE-F were assumed to be present in the manure and irrigation water applied to cropland, and at the same time, we also assumed that 100% of EB and TBA metabolites were present at a constant concentration on the feedlot surface. In real-world circumstances, it would be expected that some EB and TBA metabolites will runoff from the feedlot manure pack, leaving <100% of the EB and TBA metabolites in the manure applied to cropland. Thus, these assumptions increased the overall mass of EB and TBA metabolites entering the watershed.
- In fields routinely manured in animal production areas, phosphorus can build up and limit the amount of manure that can be applied. Herein, it was conservatively assumed all soils had a phosphorus content of zero (Section 5); thereby the only factors that limited the amount of manure that could be applied to fields was the phosphorous content in the manure and the phosphorous needs of the crop.
- The five regions modeled have among the highest per county acre densities of beef cattle in the US, and they also represent varying and conservative climatic (high and low rainfalls), hydrographic (steep slopes), and geologic conditions (C and D soil classifications). See Section 5.7 for additional information.
- When using the EXPRESS and PRZM models, manure is applied on the same day each year over 30-year time period. This date was conservatively selected to predict the upper 90th percentile confidence bound on the mean PEC_{water} based on a sensitivity analysis (Appendix 7.1.1).
- Typical BMPs for application of manure to cropland (e.g., vegetative buffer strips, field slope, or distance of manure application to waterway) were not considered in the modeling to determine the PEC values, even though many of these practices are commonly used by farmers.

Effects Assessment

- There were multiple chronic (≥21 day exposures and some multi-generational) studies used in the analysis to derive the PNEC values for the 17α and 17β isomers (Section 6).
- When deriving the PNECs for 17α-estradiol, 17α-trenbolone, and 17β-trenbolone, an AF of 10 was applied to account for the uncertainties associated with laboratory studies (i.e., inter- and intra-species differences, inter-laboratory variation, extrapolation from laboratory to field, etc.; Section 6.3). In addition, an AF of 2 was used to derive the PNEC for 17β-estradiol, even though a large data set was available (21 NOEC values from 19 studies covering 8 fish species).

Risk Characterization

- It was conservatively assumed in the risk characterization that the toxicity of the surrogate estradiol compound was equal to the toxicity of 17α-estradiol and 17β-estradiol. Based on limited information in the literature, it appears that the primary metabolite contained in beef cattle manure is 17α-estradiol, and only minor amounts of 17β-estradiol is contained in the excreta. In addition, data in Section 4.2 and Appendix 12 support that the 17α and 17β isomers are further degraded in the environment to estrone, which is expected to be less potent.

10. SUMMARY AND CONCLUSIONS

This EA evaluated the potential for environmental impacts from the introduction of EB and TBA metabolites into the environment via direct runoff from feedlots, cropland, and pastureland as a result of the use of Synovex ONE-F and ONE-G in feedlot and pasture cattle. To assess the potential risk of EB and TBA metabolites excreted from cattle implanted with Synovex ONE, the RQ method was used, which is based on the ratio of PEC_{water} values to the PNEC value(s) for inhibition in fish reproduction. Typically an RQ value of <1 is used as a preliminary screening level to determine if additional analysis and refinement of the risk assessment may be needed. In this EA, because of the many conservative assumptions used throughout the exposure and effects assessment and the many refinements that have been incorporated into the PEC values, we believe that an RQ value in the range of 1 or less indicates that significant environmental effects are highly unlikely at the predicted level of exposure.

The PEC_{water} values were estimated for both the individual farm-scale and aggregate watershed-scale using environmental fate modeling. The models simulated the runoff from 1) 51 different crop scenarios (34 tilled and 17 no-till), 2) five pastureland scenarios, and 3) five mixed-use watershed scenarios (i.e., aggregate exposure due to runoff from cropland, feedlot and pastureland) in Iowa, Texas, Ohio, Michigan, and Pennsylvania. These PEC values represent the 90th percentile 21-day moving averages determined from simulations over a 30-year period and, thus, predict reasonable worst-case exposures on a nationwide basis.

The PNEC values were estimated for both the 17α and 17β isomers of estradiol and trenbolone using the NOEC or EC_{10} values for fish reproductive endpoints and a conservative AF to account for uncertainty in laboratory data and other factors. The RQ values were calculated by assuming that the toxicity of the surrogate estradiol (or trenbolone) compound was equivalent to that of the 17α isomer or the 17β isomer. Then the PEC_{water} values for the surrogate estradiol compound were divided by the PNEC values for the 17α or 17β isomer to determine the potential risk attributed to 17α-estradiol and 17β-estradiol. A different approach was used to estimate the RQ values for the surrogate trenbolone compound where we attributed a proportion of the PEC_{water} values to the 17α and 17β isomers. The PEC_{water} values were multiplied by both 0.20 and 0.80 to attribute a portion of the value to 17β-trenbolone and 17α-trenbolone, respectively. In addition, to account for potential additive effects of these isomers, another set of RQ values was calculated, wherein the individual RQ values attributed to 17α-trenbolone and 17β-trenbolone were added together to estimate the final additive RQ values for the surrogate trenbolone compound.

Across all 51 crop scenarios, five pasture scenarios, and five mixed-use watersheds modeled over a 30-year period, all RQs were well below the screening value of 1 for 17α-estradiol ($\leq$0.004) and 17β-estradiol ($\leq$0.08), indicating that significant environmental effects are highly unlikely at the predicted level of exposure.

Similar results were also found for the surrogate trenbolone compound when specific proportions of the PEC_{water} value were attributed to the 17α-trenbolone and 17β-trenbolone. The additive RQ values were <1 for all farm-scale and mixed-use watershed scenarios evaluated, except for the Iowa mixed-use watershed, which produced an RQ slightly above 1 (1.33) when conservatively assuming that 50% of the AFOs are directly discharging to

surface waters and using the lowest potential PNEC value for 17β-trenbolone (0.25 ng/L); this is considered a reasonable worst-case scenario at the local scale. For a more representative nation-wide watershed scenario in which it is assumed that 25% of the AFOs are directly discharging to surface waters, the RQ values were all <1.

The PEC_{water} values upon which these RQ values are based are considered to be an overestimation of the potential exposures to 17β-trenbolone from use of Synovex ONE-F and ONE-G for several important reasons listed in the bullets below and Section 9. Furthermore, for the only RQ that exceeded a screening value of 1, the assumption that 50% of AFOs within a watershed are directly discharging to surface waters is an overestimation and is very unlikely to occur. As discussed in Appendix 9, we estimated that this percentage is actually in the range of 17% based on USDA Census data from 1997; however, we used a conservative estimate of 25% for modeling, which resulted in an RQ value well below 1 (<0.60). Additionally, it is expected that the current percentage of feedlots with direct runoff to surface waters is likely reduced from 17% due to more recent facility upgrades and compliance with the Clean Water Act; although, more current information is not available to update this number. Thus, based on all available information and the RQ values determined, we conclude that no significant impacts are expected from the use of EB and TBA in Synovex ONE.

The risk estimates determined in this EA demonstrate that impacts are unlikely on sensitive aquatic receptors and endpoints (fish reproduction) in the environment due to the use of Synovex ONE-Feedlot and ONE-Grass in beef steers and heifers. These risk estimates are believed, if anything, to be highly conservative and unlikely to be underestimated. A partial list of the conservative assumptions and methods used in the risk analyses is presented below. Additional conservative practices employed in the EA are listed in Section 9.

Exposure Assessment

- Synovex ONE-F and ONE-G accounted for 100% of the market share for growth-promoting implants used in their respective categories of beef cattle; this would clearly not be the case because there are currently many other approved products available in the marketplace.
- The PEC_{water} estimates determined by the environmental fate models represent the 90^{th} percentile 21-day moving averages determined from simulations over a 30-year period. These values represent reasonable worst-case exposures in the US.
- The mixed-use watershed was developed specifically to simulate an aggregate exposure due to runoff and erosion from multiple sources and pathways (i.e., runoff from feedlot, cropland, and pastureland) in a watershed.
- The five mixed-use watersheds that were modeled have among the highest per county acre densities of beef cattle in the US, and they also represent varying and conservative climatic (high and low rainfalls), hydrographic (steep slopes), and geologic conditions (C and D soil classifications).
- The crop scenarios modeled were designed to result in greater contaminant runoff and leaching, including the incorporation of soil types prone to erosion and runoff and steep slopes.

- PEC_{water} values for the five aggregate watersheds were estimated assuming that 25 and 50% of AFOs with <1000 AU directly discharge their feedlot runoff into surface water. These assumptions are conservative because it was estimated that only 17% of all AFOs in the US are in need of controls for runoff discharge.
- EB and TBA metabolites on a feedlot surface are maintained at a constant concentration and mass, which increases the total quantity of EB and TBA metabolites in runoff in the watershed model.
- In the watershed model, it was assumed that 100% of the EB and TBA metabolites from ONE-F were present in the manure and irrigation water applied to cropland, and at the same time, we also assumed that 100% of EB and TBA metabolites were present at a constant concentration on the feedlot surface. These assumptions increased the overall mass of EB and TBA metabolites entering the watershed in the modeling.
- It was assumed that beef cattle excreted 100% of the estradiol administered.
- An assumption was made that no degradation or transformation occurs in manure, thus maximizing the amounts of the drugs entering the environment.
- It was assumed that no photodegradation occurs in the modeled terrestrial or aquatic environments.
- Environmental fate parameter values (e.g., K_{OC}, DT_{50}) used for modeling were derived in such a manner to ensure a high PEC_{water} value.
- It was conservatively assumed all soils modeled had a phosphorus content of zero; thereby, increasing the amount of manure and drug applied to cropland.
- Typical BMPs for application of manure to cropland (e.g., vegetative buffer strips, field slope, or distance of manure application to waterway) were not considered in the modeling to determine the PEC values, even though many of these practices are commonly used by farmers.

Effects Assessment

- There were multiple chronic ($\geq$21 day exposures and some multi-generational) studies used in the analysis to derive the PNEC values for the 17α and 17β isomers.
- When deriving the PNECs for the 17α and 17β isomers, a conservative effects endpoint (i.e., reproductive NOEC or EC_{10}) and AF values were used to account for potential uncertainties associated with laboratory studies.

In summary, this EA supports the conclusion that the use of Synovex ONE-Feedlot and ONE-Grass implants in beef cattle are not expected to result in significant environmental impacts.

11. ALTERNATIVES TO THE PROPOSED USE

Synovex ONE is proposed for increased rate of weight gain and improved feed efficiency for up to 200 days in steers and heifers fed in confinement for slaughter, and for increased rate of weight gain for up to 200 days in pasture cattle. The only alternative to the proposed action is the 'no action' alternative, which would be the failure to approve the New Animal Drug Application (NADA) for Synovex ONE Feedlot and Grass. In this case, the use of other currently approved steroid hormone implants would continue. However, this would also be the case if the NADA is approved, except that Synovex ONE would be expected to take a portion of the market from the already approved implants. In this EA, we have conservatively assumed that 100% of beef cattle in the US would be administered Synovex ONE, and based on the expected level of exposure, it was determined that the use of this product is not expected to result in significant environmental impacts. Therefore, we do not believe that the risk would be substantially different than for the "no action alternative."

12. APPROVAL

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13. ACKNOWLEDGMENTS

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Appendix 1. Synovex Full Product Line

Table 65. List of Products in the Synovex Line of Implants for Use in Cattle and their Active Ingredients, Duration of Effectiveness, and Indication of Use

Synovex Product	EB	E2	P	TP	TBA	Approx. Effective Days	Approved Animals	USA	Indications
Synovex C	10 mg		100 mg			Suckling Period	S & H	✓	For use in suckling beef calves up to approximately 400 lb of body weight. Synovex C is also recommended for improvement in rate of weight gain in steers weighing greater than 400 lb and fed in confinement for slaughter when used as part of a re-implant program in which an initial Synovex C implant is followed at approximately 70 days by Synovex S.
Synovex S	20 mg		200 mg			120	S >400 lb	✓	For increased rate of weight gain and improved feed efficiency in steers weighing 400 lb or more. For additional improvement in rate of weight gain in steers fed in confinement for slaughter, SYNOVEX S may be used as part of a re-implant program where an initial SYNOVEX C or SYNOVEX S implant is followed by SYNOVEX S at approximately 70 days.
Synovex H	20 mg			200 mg		120	H >400 lb	✓	For increased rate of weight gain and improved feed efficiency in heifers weighing 400 lb or more.
Synovex Choice	14 mg				100 mg	120	FL, S	✓	For increased rate of weight gain in steers fed in confinement for slaughter.
Synovex Choice	14 mg				100 mg	120	FL, H	CVM = H	For increased rate of weight gain in steers fed in confinement for slaughter, and for increased rate of weight gain and improved feed efficiency in heifers fed in confinement for slaughter.
Synovex Plus	28 mg				200 mg	130	FL, S & H	✓	For increased rate of weight gain and improved feed efficiency in steers and for increased rate of weight gain in heifers fed in confinement for slaughter.
Synovex ONE Feedlot	28 mg				200 mg	200	FL, S & H	CVM	For increased rate of weight gain and improved feed efficiency for up to 200 days in steers and heifers fed in confinement for slaughter.
Synovex ONE Grass	21 mg				150 mg	200	S & H	CVM	For increased rate of weight gain for up to 200 days in pasture steers and heifers (stocker, feeder, and slaughter).
Synovex T -120		24 mg			120 mg	130	FL, S	✓ Not sold	For use in feedlot steers to increase average daily gain (ADG) and feed efficiency. Steer-only product, generic of Revalor-S.
Synovex T – 80		16 mg			80 mg	110	FL, S	✓ Not sold	For use in feedlot steers to increase ADG and feed efficiency. Steer-only product, generic of Revalor-IS.
Synovex T – 40		8 mg			40 mg	100	Stockers	✓ Not sold	For use in pasture cattle (steers and heifers) to increase ADG. Steer and heifer product, generic of Revalor-G.

EB = Estradiol Benzoate; E2 = 17β-Estradiol; P = Progesterone; TP = Testosterone Propionate; TBA = Trenbolone Acetate
S = Steer; H = Heifer; FL-Feedlot; CVM = under review with Center for Veterinary Medicine; ✓ = Approved

Appendix 2. Application Depth of Manure to Cropped Fields and Pastureland Used in Modeling

An incorporation depth of manure is a required input parameter in the PRZM model. Incorporation depth can vary depending upon the exposure pathway (e.g., collection on a feedlot followed by application to cropland or direct deposition onto pastureland) and manure application method (e.g., till or no-till practices on cropland). An explanation is provided below regarding the information used to select the incorporation depths for tilled crop scenarios, no-till crop scenarios, pasture, and application of irrigation water.

Appendix 2.1. Tilled crops

Tillage, plowing the land for weed and pest control and to prepare for seeding, is a common practice used when applying manure to cropland [85]. Due to the high variability in farming practices, the depth of manure incorporation into soil can vary considerably depending on region, crop, and manure application methods. When manure is incorporated into cropland two methods are generally used: 1) manure can be applied to the surface of soil during a dry period and then is tilled into the soil within a few days following application, or 2) liquid manure can be injected directly into the soil at the time of application. For the calculation of PEC_{soil} values and modeling runoff from manured fields, a depth of manure incorporation into soil needs to be specified. Traditional calculation methods in the US have used a depth of 15 cm (6 inches). In the EU's EMEA Guidelines for Environmental Impact Assessment for Veterinary Medicinal Products, the recommended incorporation depth is 20 cm (ca. 8 inches) [178]. In a study measuring the incorporation of different tillage practices for manure incorporation efficiency after spreading, the authors used 1) a heavy tandem disk at 15 cm, 2) chisel plow with spikes at 15 cm, 3) chisel plow with sweeps at 12.5 cm, and 4) a heavy harrow at 41 cm [179]. In an agricultural extension publication, liquid manure or a manure slurry was applied at the same time the field was tilled and the injection depths were listed as: 1) 10-20 cm (or 4-8 inches) for chisels and sweeps, 2) 7-15 cm (or 3-6 inches) for disk applicators, and 3) 10-20 cm (or 4-8 inches) for Coulter applicators [180].

This information indicates manure can be incorporated at a number of different depths, with a majority falling between 10 and 20 cm. Therefore, throughout this risk assessment, 15 cm was used as a representative incorporation depth for manure applied to tilled cropped fields. This depth of incorporation does not take into account additional movement of the manure due to the bioturbation of earthworms and insects after incorporation.

Appendix 2.2. Pasture

For pasture cattle scenarios, manure will fall to the surface of the pasture and be incorporated into the soil by insects and worms. This incorporation due to soil-dwelling organisms can be very rapid in some pasture locations. In a study in Oklahoma, dung beetles rapidly consumed pasture manure and burrowed deeper than 18 inches into the soil [181]. In a study evaluating the contribution of both dung beetles and earthworms to dung degradation in pastures, the two organisms were found to work in unison to facilitate incorporation with each of these organisms contributing approximately 50% to the disappearance of dung pats [182].

For modeling purposes and the calculation of PEC_{soil} values for pasture cattle, a depth of incorporation is a model input even for surface applied agricultural chemicals. When simulating the surface application of chemicals to soil, the EPA models (such as EXPRESS) use a default value of 4 cm for an incorporation depth [80]). In the VICH Phase I guidance,

5 cm is recommended as the incorporation depth for pasture animals for estimating a conservative PEC_{soil} [183]. The action of dung beetles and earthworms is expected to provide an incorporation depth much greater than 5 cm for pasture manure; however, to be conservative, the VICH value of 5 cm was used for the incorporation depth of pasture cattle manure in the environmental fate modeling.

Appendix 2.3. No-till crops

No-till farming (also called zero tillage) is a method for growing crops from year to year without disturbing the soil through tillage. To reduce potential runoff and nutrient loss from cropped fields, no-till cropping practices are becoming more common [184]. Manure can be either surface applied or injected into no-till fields. In a map available from the USDA National Resources Conservation Service (NRCS) [93] depicting the percent of no-till crops across the US by county, no-till practices are primarily used in the South (Tennessee, Mississippi, Alabama, Kentucky), Midwest (Ohio, Indiana, Illinois, Michigan, Wisconsin, South Dakota, and southern Iowa), and the mid-Atlantic regions (South Carolina, North Carolina, Maryland, and parts of Pennsylvania). For surface applied manure under no-till practices, the manure incorporation takes place through rainfall events (into soil micropores) and activity of soil organisms. Generally, it is assumed that incorporation of manure after application conserves nutrients and improves soil physical properties; whereas, surface application of manure would result in greater runoff of nutrients and solids from the manure. However, the weight of evidence in the published literature suggests that no-till surface application results in equal to or less runoff of phosphorous, nitrogen, and solids than manure incorporated by tillage methods.

A study was conducted in Wisconsin to assess the quantity of phosphorus (P) in runoff from no-till plots with non-incorporated dairy manure applications versus chisel-plowed plots with incorporated manure applications [185]. The researchers found higher concentrations of dissolved P in the runoff from the no-till plots versus the chisel plowed plots; however, the total quantity of P lost was lower for the no-till versus the chisel plow method (see Figure 5 of reference [185]). Although there was a reduction in manure P erosion from the no-till plots, the difference was not significant. The authors stated that the increased infiltration of water in the no-till plots lowered the sediment loss and reduced the total P load in runoff.

In a no-till versus tilled study looking at sorghum and wheat plots in Nebraska, the total erosion (solid materials transported in runoff) from tilled sorghum (8 cm disk plow) was significantly higher (4.5x) than no-till sorghum, and 12.7x higher for tilled wheat (Table 1 of reference [186]). In the sorghum portion of the study, manure was surface applied at either 11.5 Mg[ff]/ha to meet corn P requirements plus 104 kg nitrogen (N) /ha or 49.4 Mg/ha to meet corn nitrogen (N) requirements. The highest manure application, “Manure N” is compared to the control “Check” from Table 3 of the reference. For the no-till situation, there was no difference in erosion between the manure N and control in the wet erosion run, which were 1.5 and 1.4 Mg/ha, respectively. This indicates that the manure applied to the no-till plot did not readily erode even under the extreme simulated rainfall of the test. In the tilled plots, there was a significant difference in erosion between the manure N and control in the wet erosion run, which were 12.1 and 5.3 Mg/ha, respectively. Therefore, the tilled manured sorghum had 6.8 Mg/ha (12.1-5.3) more erosion than the manured no-till plot, presumably from the manure. This represents 14% of the applied manure. In the tilled wheat plots (Table 4 of reference [186]), there was no significant difference between the

[ff] Mg = 10^6 g; 2.25 Mg/ha = 1 ton/acre

tilled manure and control. However, there was a numeric trend for greater erosion from the tilled plots. These data indicate erosion is greatest from tilled soil and the sorghum no-till produced significantly less manure erosion when surface applied than from manure tilled into the soil. The authors concluded erosion from surface applied manure and compost from no-till plots was minimal in comparison to tilled under the extreme rainfall conditions used in the study.

Another study also examined the effects of tillage on manure erosion for no-till plots [187]. In Table 4 of the study, the runoff losses of total P (potential indicator of manure runoff) from manure broadcasted plots were not different from plots in which manure was incorporated into the soil (~ 20 cm mixing depth). Although this study did not demonstrate that no-till was superior to tilling to reduce manure erosion, the results indicate that surface application of manure to no-till plots is no worse than tilling manure into the soil after application.

Allen and Mallarino [188] compared dissolved reactive P (DRP) and total P in surface applied swine manure and incorporated manure by disking 10 to 15 cm into the soil to surface application without incorporation. The authors did not report if these were established no-till plots or just harvested fields tilled the prior year and simply not-tilled at manure application. In runoff generated after 24 hours, DRP and total P concentrations were greater from the manure unincorporated into soil than where the manure was incorporated into soil. However, these differences decreased dramatically for the second rainfall event conducted after 15 days. For a rainfall event six months after the treatments were applied, DRP losses from the two treatments were almost identical; however, total P losses in the runoff were significantly greater for the incorporated versus the unincorporated, most likely due to crust formation and surface sealing. Overall, if a rainfall event occurred within 24 hours of manure application, then runoff (total P) was increased without tillage; however, if the rainfall event was delayed 15 days, the differences were minimal.

The results of these studies ([185, 186, 187, 188]) collectively show opposite effects on total P loss than one might have expected from surface-applied non-incorporated manure in no-till plots versus tilling manure into soil. These studies concluded that tilling can decrease surface residue coverage, which causes increased erosion potential; whereas, no-till surface application increases water infiltration. These studies used phosphorous, nitrogen and solids as indicators of erosion and runoff. At this time, there are no studies comparing the potential runoff of estradiol, trenbolone or their metabolites in tilled versus surface application situations. Although field data indicate no-till is a better conservation practice than tilled, in this risk assessment, 17 no-till models from the EPA EXPRESS scenarios (Appendix 2.3) were developed and included in the exposure assessment in order to assess the influence of no-till on the surface runoff of estradiol and trenbolone from manured cropland. In addition, because Ohio and Pennsylvania are two of the five high beef production regions modeled as watersheds in this EA where no-till was more common (Appendix 8.2), these mixed-use watersheds also assumed a surface application. The incorporation default depth in the EXPRESS shell is 4 cm for surface application of pesticides. For the models used in the EA, 5 cm was used for incorporation depth for the same reasons outlined in Appendix 2.2 on pastureland.

Appendix 2.4. Manure applied in irrigation water

Feedlot runoff water from collection lagoons is diluted with clean water prior to irrigating cropland. The irrigation water is typically applied to the surface of the soil; thus, an incorporation depth of 5 cm was assumed for modeling the penetration of the irrigation water into soil.

Appendix 3. Initial PEC_{soil} and PEC_{water} Calculations for Confined Animals Following Traditionally Accepted Methods

For the initial screening-level approach, values traditionally accepted by CVM and consistent with agricultural practices were used to calculate the initial PEC_{soil} and PEC_{water}. For beef cattle confined in a feedlot, it is assumed that a 300 kg beef steer or heifer produces 27.3 kg manure/day (conservative assumption because the target beef animal will be larger), over a 130-day production period. The amount of manure applied to 1 acre of land is assumed to be 27,200 kg. For these calculations, a soil bulk density of 1500 kg/m^3 (910,575 kg/acre at 15 cm) was used as recommended by the CVM Guidance for Industry number 89 (VICH Phase I [183]).

Appendix 3.1. PEC initial assumptions for beef cattle

- Manure holding time: 130 days
- Fraction of animals treated: 100%
- Weight of animal: 300 kg
- Daily manure produced: 27.3 kg
- Total manure produced in holding period = 130 days x 27.3 kg manure/day = 3,549 kg
- Manuring rate: 27,200 kg/acre
- Weight of soil: CVM Phase I GL6 document, Question number 17 (soil bulk density 1500 kg/m^3). At 15 cm, this equals 0.15 m x 1500 kg/m^3 x 4047 m^2/acre or 910,575 kg/acre
- All of the dose is released in the 130-day manure collection period.

Appendix 3.2. Drug concentration in manure (Synovex ONE-F)

The initial concentration of estradiol or trenbolone in manure was calculated by dividing the amount of estradiol and trenbolone excreted (Section 2.7.1) by the total manure produced during the holding period, as follows:

20.26 mg estradiol / 3,549 kg manure = 0.00571 mg estradiol/kg manure
173.1 mg trenbolone / 3,549 kg manure = 0.04877 mg trenbolone/kg manure

Appendix 3.3. Amount of drug applied to one acre of land

The amount of estradiol and trenbolone applied per acre was initially calculated by multiplying the concentration of estradiol or trenbolone per kg manure by the manure application rate:

0.00571 mg estradiol/kg manure X 27,200 kg manure/acre = 155.3 mg estradiol/acre
0.04877 mg trenbolone/kg manure X 27,200 kg manure/acre = 1326.5 mg trenbolone/acre

Appendix 3.4. Calculation of Initial PEC_{soil} for confined animals following previously accepted (traditional) methods

The initial PEC_{soil} was calculated by dividing the concentration of estradiol or trenbolone per acre by the amount of soil per acre:

(155.3 mg estradiol/acre x 1000 µg/mg) / 910,575 kg soil/acre = **0.17 µg estradiol/kg**
(1326.5 mg trenbolone/acre x 1000 µg/mg) / 910,575 kg soil/acre = **1.46 µg trenbolone/kg**

These PEC_{soil} estimates are conservative because at 130 days there would still be 51.3% of the estradiol and 38.5% of the trenbolone remaining in the implant. See Appendix 6 for release rates for these two active ingredients.

100% x 1 - (0.0759 mg estradiol released per day x 130 days / 20.26 mg/implant) = 51.3%
100% x 1 - (0.8193 mg trenbolone released per day x 130 days / 173.1 mg/implant) = 38.5%

Appendix 3.5. Calculation of initial PEC_{water} for confined animals following previously accepted (traditional) methods

It is assumed that an extreme rainfall event occurs that causes 1% of the total drug per acre applied to 10 acres of soil to move into a 1 acre pond which is 2 m deep. The equation used to calculate this value is as follows:

$$PEC_{water\ max} = \frac{PEC_{soil} \text{ x } (9.1 \text{x} 10^5 \text{ kg/acre}) \text{ x } 0.01 \text{ x } 10 \text{ acres}}{\text{Volume of water in pond } (8.1 \text{ x } 10^6 \text{ L})}$$

Appendix 3.5.1. Estimated surrogate estradiol compound concentrations in surface water

$PEC_{water\ max}$ of the surrogate estradiol compound $= \frac{PEC_{soil} \text{ x } (9.1 \text{ x } 10^5 \text{ kg/acre}) \text{ x } 0.01 \text{ x } 10}{8.1 \text{ x } 10^6 \text{ L x } 1 \text{ kg/L}}$

$= 0.17$ µg/kg x 0.011 kg/L = 0.00187 µg/L

$=$ **1.87 ng/L**

Consistent with the previously accepted methods, this value was further refined by considering adsorption to sediment because the K_{OC} for the estradiol metabolites are known (Average K_{OC} = 1259). See Section 4.2.4.9 for K_{OC} estimates.

$PEC_{water\ refined}$ of the surrogate estradiol compound $= \frac{8.3 \text{ x } 10^6 \text{ x } PEC_{water\ max}}{8.1 \text{ x } 10^6 + (3.0 \text{ x } 10^5 \text{ x } K_d)}$

Where: K_d = the partition coefficient (units of mL of soil water per g of soil) for this compound, which equals 0.029 x average K_{OC} = 0.029 x 1259 = 36.51, assuming equilibration of the compound in water within the top 5 cm of sediment

$PEC_{water\ refined}$ of the surrogate estradiol compound $= \frac{8.3 \text{ x } 10^6 \text{ x } 1.87 \text{ ng/L}}{8.1 \text{ x } 10^6 + (3.0 \text{ x } 10^5 \text{ x } 36.51)}$

$=$ **0.81 ng/L**

The calculations from above are for an acute exposure from a single rainfall event.

Appendix 3.5.2. Estimate of the surrogate trenbolone compound concentration in surface water

$PEC_{water\ max}$ of the surrogate trenbolone compound $= \frac{PEC_{soil} \times 9.1 \times 10^5 \text{ kg/acre} \times 0.01 \times 10}{8.1 \times 10^6 \text{ L} \times 1 \text{ kg/L}}$

$= 1.46 \text{ µg/kg} \times 0.011 \text{ kg/L} =$ **0.016 µg/L**

Consistent with the previously accepted methods, PEC_{water} was further refined by considering adsorption to sediment because the K_{OC} for the trenbolone metabolites are known (average K_{OC} = 912). See Section 4.2.6.9 for K_{OC} estimates.

$PEC_{water\ refined}$ of the surrogate trenbolone compound $= \frac{8.3 \times 10^6 \times PEC_{water\ max}}{8.1 \times 10^6 + (3.0 \times 10^5 \times K_d)}$

Where: K_d = the partition coefficient (units of mL of soil water per g of soil) for this compound, which equals 0.029 x average K_{OC} = 0.029 x 912 = 26.45, assuming equilibration of the compound in water within the top 5 cm of sediment.

$PEC_{water\ refined}$ of the surrogate trenbolone compound $= \frac{8.3 \times 10^6 \times 0.016 \text{ µg/L}}{8.1 \times 10^6 + (3.0 \times 10^5 \times 26.45)}$

$= 0.0083 \text{ µg/L} =$ **8.3 ng/L**

The $PEC_{water\ refined}$ can be further refined using excretion data from the animal ($PEC_{water\ excr}$), because only 71.5% of the administered drug is excreted as trenbolone metabolites.

Surrogate trenbolone compound $PEC_{water\ excr}$ (adjusted for excretion) $=$ 8.3 ng/L x 71.5% excreted

$=$ **5.9 ng/L**

The calculations from above are for an acute exposure from a single rainfall event. Also, 17α- and 17β-trenbolone degradation in manure, soil and water could further reduce these estimates.

Appendix 3.6. Summary of PEC_{water} initial calculations using previously accepted (traditional) methods

$PEC_{water\ refined}$ calculations for surrogate estradiol and trenbolone compounds are 0.81 and 5.9 ng/L, respectively.

Appendix 4. Calculation of PEC_{soil} for Confined Animals Representative of US Nutrient Management Practices for Phosphorus

The PEC_{soil} values for estradiol and trenbolone were also determined based on CNMP requirements for the application of manure to cropland. Typically, manure is applied to cropland based on the nutrient requirements of the crop to be grown. Most regions of the US apply manure based on the phosphorous needs of the crop to ensure minimal surface runoff of both phosphorous and nitrogen. The PEC_{soil} based on the phosphorous application rate was calculated using similar assumptions for cattle weights and manure collection period as were used in the traditional method (Appendix 3); however, the manure application rate to cropland was based on BMPs and the phosphorus requirement associated with the crop planted. The crops modeled in EXPRESS were corn silage, alfalfa, wheat, sorghum, soybean, sugar beet, dry bean, cotton, and potato. The highest PEC_{soil} modeled was for corn silage (PEC_{soil} values for each crop are reported in Table 68).

Appendix 4.1. PEC values using CNMP methodologies

Many states require cattle feeding operations to have a CNMP for storage, deposition and runoff control of the manure generated. The manure application to cropland as fertilizer can be managed based on the nutrient requirements of the crop to be grown and the combined nutrient content of the manure and soil (nitrogen, phosphorus, and potassium). Often the nutrient that limits the amount of manure that can be applied to soil is phosphorus [189, 190] because it can accumulate in soils that are repeatedly manured. In these instances, manure is applied and supplemental chemical fertilizer is used to increase the soil nitrogen to the required amount for the crop.

The moisture content and nitrogen content of manure can vary widely and can be influenced by storage conditions [189, 190]; however, the phosphorus content is more stable than the nitrogen content. Thus, the amount of drug applied to the land can be determined based on the amount of phosphorous applied, which is independent of the manure storage method. Linking the drug application rate to the phosphorus application rate (1:1 ratio) is more realistic for current US agricultural practices and will also provide a potential tool for predictive modeling of VMPs in the environment. To determine the amount of drug applied to the land, the amount of phosphate in the manure of beef cattle must be known.

The USDA-NRCS provides data on manure characteristics [191]. In its guidance, data from four databases were used to determine average excretion rates from various livestock species in the US. The data from the USDA-NRCS in Table 66 below is consistent with the value provided by the American Society of Agricultural Engineers (ASAE) on manure characteristics [192].

Table 66. USDA-NRCS Manure Component Excretion Rates for Beef Cattle*

	kg excreted per day for a 1000 kg animal†			
	Nitrogen N	**Phosphorus P**	**Phosphorus as P_2O_5‡**	**Daily Manure Production**
Steer, Bull, Calf	0.318	0.098	0.224	58
Cow	0.345	0.120	0.274	63
Mean	**0.3315**	**0.109**	**0.249**	**60.5**

* USDA-NRCS website on manure: http://www.nrcs.usda.gov/technical/NRI/pubs/nlapp1b.html
† Average values were used from Tables A-3, A-4 and A-5 of reference [191]. Note that kg/1000 kg BW is equivalent to lb/1000 lb BW in the tables.
‡ To convert fertilizer P_2O_5 equivalents to total manure P, multiply by 0.437 [191].

Appendix 4.1.1. Concentration of estradiol or trenbolone per kg manure and manure phosphorus

The dose excreted / total phosphorus produced in a 130-day manure collection window for 300 kg animal is estimated below. For the following calculations, the daily P_2O_5 excretion is expressed per kg body weight (BW). The following assumptions were used:

- The daily excretion rate of P_2O_5 from a 300 kg BW animal is 0.0747 kg P_2O_5/day [0.249 kg P_2O_5 x 300 kg/1000 kg BW].
- The daily manure production is 18.15 kg [60.5 kg manure/1000 kg BW x 300 kg BW].
- The amounts of estradiol and trenbolone contained in the implants are derived in Section 2.7.1.

Appendix 4.1.2. Drug concentration in manure (assuming all drug excreted in 130 days)

The concentration of estradiol or trenbolone in manure was calculated by dividing the amount of estradiol and trenbolone excreted (Section 2.7.1) by the total manure produced during the 130-day holding period:

Estradiol: 20.26 mg/(60.5 kg manure/1000 kg BW x 130 days x 300 kg BW) = 0.00859 mg/kg manure
Trenbolone: 173.1 mg/(60.5 kg manure/1000 kg BW x 130 days x 300 kg BW) = 0.0734 mg/kg manure

Appendix 4.1.3. Drug per kg P_2O_5 (assuming all drug excreted in 130 days and no metabolism)

The amount of estradiol or trenbolone per kg P_2O_5 (assuming a 1:1 ratio of drug to P_2O_5) was calculated by dividing the amount of estradiol or trenbolone by the total P_2O_5 produced during the 130-day holding period:

Estradiol: 20.26 mg/(0.249 kg P_2O_5/1000 kg BW x 130 days x 300 kg BW) = 2.09 mg/kg P_2O_5
Trenbolone: 173.1 mg/(0.249 kg P_2O_5/1000 kg BW x 130 days x 300 kg BW) = 17.8 mg/kg P_2O_5

Appendix 4.2. Application rates of manure to cropped fields and list of crops to be modeled

The amount of manure applied to a field is dependent on the crop to be grown, the nutrient requirement of the crop for the anticipated crop yield, and the nutrients present in the soil and manure. Although crop yields are regional, the highest anticipated yields across the US of each crop to be modeled will be used to estimate the initial conservative manuring rates based on the phosphorus requirement of the crop. It was also assumed that the soil is low in phosphorus and does not limit the manure application. In other sections of this assessment, where specific regions are modeled, regional yield data specific to the crop modeled was utilized when available. The crops modeled for PEC_{soil} calculations will be the same as the crop scenarios modeled in the Tier II EPA EXPRESS model described in Appendix 7. These modeled crops are corn, wheat, alfalfa, cotton, dry beans, potatoes, sorghum, soybeans and sugar beets. These were chosen in part from the principal crops associated with cattle production areas and the crops available to model in the EPA EXPRESS program.

The maximum yield for the crops of interest were determined from county-level plots of yields for the continental US available from the USDA [193]. The maximum yields are listed below and in Table 68:

- Corn = 200 bushels (bu)/acre (the maximum plot scaling indicates 175+ bu/acre with increments of 25 bu/acre, this increment of scaling indicates 200 bu as the maximum amount plotted)
- Spring and winter wheat = 90 bu/acre
- Soybeans = 60 bu/acre
- Sorghum = 100 bu/acre
- Alfalfa hay = 6.9 tons/acre (in irrigated regions of the southwest)
- Dry beans = 2700 lb/acre
- Sugar beets = 50 tons/acre
- Pima cotton = 1850 lb/acre (this is a higher maximum yield than upland cotton)
- Potatoes = 19.3 tons/acre (Note: The USDA plots [193] did not contain a yield plot for potatoes; thus, the estimated yields from Appendix 7, Table 73 were used).

To calculate the amount of phosphorus used to manure a specific crop, the amount of phosphorus contained in the harvested crop and, therefore, removed from the field needs to be known. This information is available in agricultural extension bulletins for fertilizer recommendations for farmers. Nine extension service bulletins (i.e., manure management guides) were used to determine the phosphate requirements for each of the crops listed above (see Table 67 below). There were only minor differences between the phosphorous requirements reported in the nine extension service bulletins for each crop listed. Throughout this risk assessment, when multiple estimates are available from the literature, a 90% confidence bound was generated for the mean and the more conservative value of the upper confidence bound was used (Section 4.2.2). Therefore, the upper 90% confidence bound was used for the estimate of the P_2O_5 requirement because it results in a higher manuring rate to the soil. The upper 90% confidence bound calculated for each crop is shown in Table 67.

Corn can be harvested for grain and the residue left on the ground or the plant can be harvested for corn silage removing a larger amount of nutrients from the field. In Table 67, both values of corn for grain and corn for silage are listed. This is also the case for wheat which can also have the straw harvested, removing more nutrients from the soil. The plant material harvested is called “stover” in the agricultural extension surveys. In the 2007 USDA Census of Agriculture, the percentage of acres of corn harvested for corn silage in the cattle production areas surveyed for watershed modeling were: 5.8% and 8.4% for Lyon and Sioux Counties, IA, respectively; 14.5% for Castro County, TX; 40.1% for Lancaster County, PA; 20.4% for Huron County, MI; and 13.6% for Mercer County, OH (Table 89). With the exception of Lancaster County, PA (which has 40% of corn harvested as silage) corn is principally harvested for grain.

Table 67. Fertilizer P_2O_5 Nutrients Removed for a Given Yield of Harvested Crop (lb/unit of yield) and 90th Percentile Calculation

Crop	Publication									Upper 90th Percentile CI[P]
	MI[a]	OH[b]	MD[c]	MN[d]	IA[e]	NC[f]	PPI[g]	Mosaic[j]	NRCS[k]	
Corn for grain (bushel)	0.37	0.37	0.40	0.34	0.375	0.44	-	0.38	0.353*	**0.39**
Corn for silage , grain + stalks (per bushel grain)¶	0.60[n]†	-	0.54†	§	0.55	0.69†¶	-	0.54†	-	**0.63**
Alfalfa (US ton)	13	13	15	11	12.5	14.8	-	12	10.8*	**13.5**
Wheat (bushel)	0.63	0.63	0.56	0.53	0.6	0.5	-	0.6	0.513*	**0.60**
Wheat grain + straw (per bushel grain)¶	0.73[n]†	0.72	0.67†	0.64	0.72[n]†	0.63	-	0.76	0.63*	**0.71**
Sorghum (bushel)	0.39	0.39	0.42	-	-	0.45	-	0.39	0.403*	**0.42**
Soybean (bushel)	0.8	0.8	1.0	0.82	0.8	0.8	-	0.84	0.820*	**0.87**
Dry edible bean (lb)	0.012	-	0.011	0.01	-	-	-	-	0.011*[m]	**0.012**
Cotton lint/seed (lb)[L]	-	-	0.0135	-	-	0.0096	0.028[h]	0.029	0.0086*	**0.025**
Cotton lint/seed/stalk/leaf (per lb of lint/seed)¶	-	-	§	-	-	0.016†¶	0.060[h]	0.048¶	-	**0.066**
Sugar beets (US ton)	1.3	2	1.8	2.2	-	-	-	-	-	**2.1**
Potato (US ton)	2.6		2.8	2.8	-	3	-	2.4		**2.9**

[a] MI-Michigan, Table 1 of Appendix A of reference [190]; [b] OH-Ohio, Table 6 of reference [189]; [c] MD-Maryland [194]; [d] MN-Minnesota Table 5 of reference [195]; [e] IA-Iowa Table 2 of reference [196]; [f] NC-North Carolina [197]; [g] PPI-Potash and Phosphate Institute [198]; [h] based on 14 lb P_2O_5 removed per bale and 30 lb for entire plant; [j] Mosaic [199]; [k] NRCS [200]. [L] Assumes a bale of cotton weighs 500 lb [200]; [m] Mean for navy and kidney beans; [n] Assumes a bushel of corn weighs 56 lb and wheat 60 lb [201]: Example calculation for wheat (8.2 lb/ton x 1 ton/2000 lb x 56 lb/bu; Corn = 0.23 (Stover) + 0.37 (grain) = 0.60 lb/bu. † Calculated by adding stover (straw, stalk and leaves) to grain lint/seed values. [P] See Section 4.2.2 for calculation of confidence intervals. § Value was not expressed on yield of wheat, cotton or corn but on yield of stalk so total for plant could not be calculated based on yield. ¶ Value was based on yield of corn grain or cotton lint/seed, not on the yields of the plant material (stalk leaves). * To convert P to P_2O_5 NRCS P values were multiplied by 2.29 [195].

The P_2O_5 application rates in Table 68 represent sustainable application rates, where phosphorus will not build up in the soil. The amount of phosphorus applied is based on the maximum expected yield and the amount of phosphorus that yield of plant material would remove from the field by harvesting. Of the crops presented, corn silage (corn + stalk) has the highest P_2O_5 requirement (57.27 kg/acre) and, therefore, will yield the highest initial PEC_{soil} values for both trenbolone and estradiol.

Table 68. Phosphorus Application Rates and $PEC_{soil\ Initial}$ Based on Nutrient Needs of Crop Using the Maximum Yields Obtained Across the Entire Continental US for Each Crop Assuming All Drug is Excreted in a 130-Day Manure Collection Period

Crop	Maximum Yield [193]	P_2O_5 Requirement From Table 67	Maximum P_2O_5 Application Rate to Cropped Field (kg/acre)	Trenbolone † PEC_{soil} (µg/kg)	Estradiol † PEC_{soil} (µg/kg)
Corn	200 bu/acre	0.39 lb/bu	35.45 §	0.693 *	0.081
Corn +Stalk	200 bu/acre	0.63 lb/bu	57.27	1.12	0.131
Alfalfa	6.9 tons/acre	13.5 lb/ton	42.34	0.828	0.097
Wheat	90 bu/acre	0.60 lb/bu	24.55	0.480	0.056
Wheat + Straw	90 bu/acre	0.71 lb/bu	29.05	0.568	0.067
Sorghum	100 bu/acre	0.42 lb/bu	19.09	0.373	0.044
Soybean	60 bu/acre	0.87 lb/bu	23.73	0.464	0.054
Sugar Beet	50 tons/acre	2.1 lb/ton	47.73	0.933	0.110
Dry Bean	2700 lb/acre	0.012 lb/lb	14.73	0.288	0.034
Cotton	1850 lb/acre	0.025 lb/lb	21.02	0.411	0.048
Cotton + Plant	1850 lb/acre	0.066 lb/lb	55.5	1.08	0.127
Potato	19.3 tons/acre	2.9 lb/ton	25.44	0.497	0.058

† Application rate of trenbolone 17.8 mg/kg P_2O_5 and estradiol 2.09 mg/kg P_2O_5 applied to agricultural fields from Appendix 4.1.3, calculation is not adjusted for the actual release rate of the drug from implants or for metabolism.

§ Example calculation: (200 bu/acre X 0.39 lb P_2O_5/bu) / 2.2 lb/kg = 35.45 kg P_2O_5

* Example PEC_{soil} calculation:
(35.45 kg/acre x 17.8 mg trenbolone/kg x 1000 µg/mg)/910,575 kg soil/acre = 0.693 µg trenbolone/kg soil.
The soil weight assumes a 15 cm depth.

Appendix 4.2.1. $PEC_{soil\ initial}$ Summary

Using assumptions similar to those used in traditionally accepted methods [202], but basing the manure application rate on the phosphorus requirement for corn silage, $PEC_{soil\ initial}$ values of 0.13 and 1.12 µg/kg soil for estradiol and trenbolone, respectively, (Table 68) were estimated. These estimates are similar to the traditionally accepted guidance PEC_{soil} values of 0.17 and 1.46 µg/kg soil, respectively, calculated in Appendix 3.4.

Appendix 5. US-Specific Calculation of PEC_{soil} Values for Grazing Animals

On pasture, cattle will typically spend time both with and without implants. Also, in the mid- to northern US, they will spend winter off of pasture in feedlots. The cattle are returned to pasture in the spring. Therefore, the assumption was made that pasture cattle are implanted once with Synovex ONE-G containing 150 mg TBA and 21 mg EB and that all of the drug is released onto the pasture. To calculate the initial PEC_{soil} for pastureland, the dose, stocking density of treated animals, and soil incorporation depth are required. A soil depth of 5 cm, as defined for pasture animals in CVM Guidance for Industry number 89 (VICH GL 6), was used (see Appendix 2.2 for further justification of depth).

Animal stocking densities for the US are generally less than in Europe. In the EMEA Technical Guidance Document, a stocking density of 9.5 animals/ha (9.5/2.47 acres/ha = 3.8 animals/acre) weighing 330 kg is recommended [178]. In a University of Kentucky publication on grazing cattle, examples of typical management of cattle for rotational grazing are provided [203]. For example, a 40-acre farm typically would have 100 steers grazing eight paddocks of about five acres each. They would be stocked at 100 head per paddock (100/5 = 20 animals/acre). However, they would spend a short period of time grazing each paddock and would be moved (i.e., rotational grazing). On average, the stocking density across the farm would be 2.5 head/acre (100 steers/40 acres). For the calculation of PEC values, the average stocking density for the EU and US of 3.15 head/acre was used [(3.8+2.5)/2]. Because most of the grazing cattle in the US are found in the more arid parts of the country, west of the Mississippi River, the stocking density would be lower than that for the Midwest; therefore, the stocking density of 3.15 head/acre is conservative.

Appendix 5.1. Amount of drug applied to one acre of land

In order to calculate the amount of estradiol or trenbolone applied to one acre of land, the percent of active drug ingredient (i.e., without the benzoate or acetate) needs to be determined:

Base activity of EB = MW of estradiol/MW EB = 272.38/376.49 = 72.35%
Base activity of TBA = MW of trenbolone/MW TBA = 270.37/312.40 = 86.55%

The amount of estradiol or trenbolone can be calculated by multiplying the amount of administered drug product (active ingredient with benzoate or acetate) by the percent of active drug ingredient and the stocking density (per acre):

Estradiol: 21 mg/head x 72.35% x 3.15 head/acre = 47.85 mg/acre
Trenbolone: 150 mg/head x 86.55% x 3.15 head/acre = 409.0 mg/acre

Appendix 5.2. Calculation of initial PEC_{soil} for pasture cattle

The initial PEC_{soil} was calculated by dividing the concentration of estradiol or trenbolone per acre by the amount of soil per acre (assuming a 5 cm incorporation depth):

Weight of 5 cm soil: 0.05 m x 1500 kg/m^3 x 4047 m^2/acre = 303,525 kg/acre

Pasture PEC_{soil} = (mg drug/acre) / (kg of soil/acre)

Estradiol: 47.85 mg/acre / 303,525 kg/acre = 0.000158 mg/kg = **0.158 µg/kg soil**
Trenbolone: 409.0 mg/acre / 303,525 kg/acre = 0.00135 mg/kg = **1.35 µg/kg soil**

Note: See Appendix 3.1 for weight of soil at 15 cm (reduced to 5 cm for calculation above), and Section 2.7.2 for the amounts of estradiol and trenbolone in insert.

Appendix 6. Release Rates of Estradiol and Trenbolone from Cattle Treated with Synovex ONE-F and ONE-G used for Landscape and Watershed Modeling

For the traditional PEC_{soil} and PEC_{water} calculations, it was assumed that the entire drug was released from the implant and excreted in a 130-day period. However, a coating on the Synovex ONE (long-acting) pellets extends the release of TBA and EB to a period of approximately 200-day. A study was conducted by FDAH to determine the release rates of EB and TBA for both Synovex Plus and Synovex ONE-F during a 200-day period [234] (details of the study are summarized in Appendix 13.4). This study demonstrated that TBA and EB are released slowly over the 200-day period. Additionally, the study reported the average daily release rates of TBA and EB from the Synovex ONE implants to be 0.9466 mg/day and 0.1049 mg/day, respectively. For the Synovex Plus implant, the release rates of TBA and EB were 1.7073 mg/day and 0.1980 mg/day, respectively.

For the initial PEC_{soil} and $PEC_{\mathbf{water}}$ calculations determined using traditionally accepted methods, it was assumed that all of the metabolites for Synovex ONE were released from the implant and excreted in a 130-day period. However, for landscape and watershed modeling, the actual release rate of TBA and EB from the implants was used. For the purpose of this EA, it is assumed that 100% of the amount of estradiol and trenbolone released from the implant daily will also be excreted; thus, the daily excretion rate is equal to the daily release rate. The release rates of EB and TBA from Appendix 13.4 are presented below:

Appendix 6.1. Synovex ONE molecular weight adjustment

Because TBA is hydrolyzed to 17β-trenbolone and EB is hydrolyzed to 17β-estradiol in the animal prior to excretion, the release rates are adjusted for the difference in molecular weight and metabolism in the animal as follows (the percent molecular weight adjustment was calculated in Section 2.7):

EB is released at 0.1049 mg/day X 72.35% = 0.0759 mg estradiol per day
TBA is released at 0.9466 mg/day X 86.55% = 0.8193 mg trenbolone per day

Appendix 6.2. Synovex ONE metabolism adjustment

In Section 4.1.2, it was determined that 71.5% of the trenbolone is excreted from cattle in manure (see excretion study summary in Appendix 13.3). An estimate of estradiol metabolism was not available, so it was conservatively assumed that 100% of the dose was excreted.

Estradiol is released at 0.0759 mg/day X 100% = 0.0759 mg estradiol per day
Trenbolone is released at 0.8193 mg/day X 71.5% = 0.5858 mg trenbolone per day

Appendix 6.3. Application rates to cropped fields based on daily release rate of active drug ingredient per kg P_2O_5 excreted

The daily P_2O_5 excreted (0.249 kg) in manure from a 1000 kg beef animal was obtained from the USDA-NRCS (Table 66). For a 300 kg animal, 0.0747 kg P_2O_5 would be excreted per day [(0.249 kg/1000 kg BW) X 300 kg BW]. Therefore, the concentration of estradiol or trenbolone per kg P_2O_5 in manure is calculated by dividing the daily drug release rate by the daily P_2O_5 excretion rate:

Estradiol: (0.0759 mg estradiol/day)/(0.0747 kg P_2O_5/day) = **1.02 mg Estradiol/kg P_2O_5**
Trenbolone: (0.5858 mg trenbolone/day)/(0.0747 kg P_2O_5/day) = **7.8 mg Trenbolone/kg P_2O_5**

In landscape and watershed models, where an application rate to soil needs to be determined for manure from confined animals, the daily drug release rate per kg P_2O_5 was used. Thus, the application rate of estradiol and trenbolone to cropland can be calculated by multiplying the concentration of the drug excreted per P_2O_5 by the P_2O_5 application rate. But, first, the P_2O_5 application rate must be determined by multiplying the expected crop yield by the amount of P_2O_5 removed per yield.

An example is provided below using corn silage (corn grain + stalk), which will be planted at an expected yield of 175 bushels per acre with a P_2O_5 requirement of 0.63 lb per bushel harvested (Table 67). Therefore, the crop would require:

(175 bu/acre X 0.63 lb P_2O_5/bu) / (2.2 lb/kg) = 50.1 kg P_2O_5 per acre

And the amount of estradiol and trenbolone applied to the field to be planted would be:

1.02 mg estradiol/kg P_2O_5 X 50.1 kg P_2O_5 per acre = 51.1 mg estradiol/acre
7.8 mg trenbolone/kg P_2O_5 X 50.1 kg P_2O_5 per acre = 390.8 mg trenbolone/acre

Therefore, if manure from confined cattle was applied to a cropped field where the crop was corn silage at an anticipated harvest of 175 bu of grain/acre, 51 mg estradiol and 391 mg of trenbolone would be applied per acre every year in the model.

The measured release rate of the active ingredients from the implant was used to determine the drug application rates used in EXPRESS and mixed-use watershed modeling (Table 74; Appendix 7.1). These values are conservative because they are derived from the assumption that a small animal (BW of 300 kg) is used. Also, the excretion and application rates presented in Appendix 7.1 below assume that the feedlot is at full capacity 365 days of the year and that all the cattle have an implant at all times, which increases the quantity of drug released into the environment in these models. The calculations are shown in more detail in Table 69 and Table 70 for estradiol and trenbolone, respectively. The acres required to use the manure from one AU produced in one year for the crop of interest is also shown in Table 70. Acres/AU is not a required variable for the EXPRESS scenarios (Section 5.4) but was used in the mixed-use watershed models (Section 5.7) to determine how many acres receive manure for a particular crop based on the number of animals within a watershed. For example, if a farm had 2000 head of cattle, it would require 1,080 acres of corn silage planted (2000 AU X 0.54 Acres/AU) to use the amount of manure produced annually.

Table 69. Example Calculation for Application of Manure Containing 17α-Estradiol (17α-E2) from Confined Animals to Corn Silage Cropped Fields with an Anticipated Yield of 175 bu Grain/Acre, and Acres Required per Animal Unit (AU)

Daily release rate estradiol (17α-E2)	0.0759 mg 17α-E2/day*		
Metabolism adjustment (100%)	0.0759 mg 17α-E2/day x 100%	=	0.0759 mg 17α-E2/day
Animal weight	300 kg BW		
Daily P_2O_5† excreted	(0.249 kg/1000 kg BW) X 300 kg BW	=	0.0747 kg P_2O_5/day
17α-E2/P_2O_5	(0.0759 mg 17α-E2/day)/(0.0747 kg P_2O_5/day)	=	1.02 mg 17α-E2/kg P_2O_5
P_2O_5 application rate	(175 bu/acre X 0.63‡ lb P_2O_5/bu) / 2.2 lb/kg		50.1 kg P_2O_5 per acre
17α-E2 Application rate	(1.02 mg 17α-E2/kg P_2O_5) x 50.1 kg P_2O_5/acre	=	**51.1 mg 17α-E2/acre**
Yearly acres needed per AU	365 day x 0.0747 kg P_2O_5/day) / 51.1 kg P_2O_5/acre	=	**0.54 acres/AU**

* Daily release rate of drug from implant from Appendix 6.1. Because each implant contains an equivalent of 20.25 mg estradiol it would take 270 days to release all of the estradiol from the implant.
† Daily P_2O_5 produced from beef cattle (0.249 kg/1000 kg BW) was obtained from Table 66.
‡ lb P_2O_5/bu of crop harvested is from Table 67.

Table 70. Example Calculation for Application of Manure Containing 17α-Trenbolone (17α-TB) from Confined Animals to Corn Silage Cropped Fields with an Anticipated Yield of 175 bu of Grain/Acre, and Acres Required per Animal Unit (AU)

Daily release rate trenbolone (17α-TB)	0.8193 mg 17α-TB/day*		
Metabolism adjustment (71.5%)	0.8193 x 71.5%	=	0.5858 mg 17α-TB/day
Animal weight	300 kg BW		
Daily P_2O_5† excreted	(0.249 kg/1000 kg BW) X 300 kg BW	=	0.0747 kg P_2O_5/day
17α-TB/P_2O_5	(0.5858 mg 17α-TB/day)/(0.0747 kg P_2O_5/day)	=	7.8 mg 17α-TB/kg P_2O_5
P_2O_5 application rate	(175 bu/acre X 0.63‡ lb P_2O_5/bu) / 2.2 lb/kg		50.1 kg P_2O_5 per acre
17α-TB Application rate	(7.8 mg 17α-TB/kg P_2O_5) x 50.1 kg P_2O_5/acre	=	**390.8 mg 17α-TB/acre**
Yearly acres needed per AU	(365 day x 0.0747 kg P_2O_5/day) / 50.1 kg P_2O_5/acre	=	**0.54 acres/AU**

* Daily release rate of drug from implant from Appendix 6.1. Because each implant contains an equivalent of 173.1 mg trenbolone, it would take 211 days to release all of the trenbolone from the implant at this release rate.
† Daily P_2O_5 produced from beef cattle (0.249 kg/1000 kg BW) was obtained from Table 66.
‡ lb P_2O_5/bu of crop harvested is from Table 67.

Appendix 7. Results of EPA's Standard Cropping Scenarios Using the EXPRESS Shell

Appendix 7.1. Tilled crop scenarios using EPA's EXPRESS shell

The crops that a beef cattle producer would typically manure are a rotation of grains (corn, soybean and wheat) along with alfalfa hay [189]. These are the principal crops that were modeled in this EA because they represent the predominant crops in beef cattle production areas. In the various sections of this assessment where regional landscape and small watersheds were modeled, a crop survey indicated that in Texas (Castro County) cotton, wheat, and sorghum for grain were also important crops in beef production areas (Attachment 1). In Michigan (Huron County), sugar beets and dry beans were also grown in the beef production areas. Therefore, modeling scenarios using these additional crops (cotton, sugar beets, sorghum and dry beans) were added to crops previously chosen to model (corn, soybean, wheat, alfalfa). The surveys of Mercer County, OH, Lancaster County, PA, and northwest IA did not indicate any additional crops of significance. Potatoes were also included as a scenario to model. The data on crops just described were generated by Waterborne Environmental Inc. in their regional assessment of the cattle production areas identified above (Attachment 1). Other crop scenarios (e.g., asparagus, coffee, nut trees) are provided in the EXPRESS shell, but they were not modeled because they are not specifically associated with beef cattle production.

The characteristics of each scenario modeled with regional yields and soil types are presented in Table 71 through Table 73. The historic crop yield data for a particular crop within a county was obtained from the USDA Quick Stats database or calculated from the 2007 agricultural census from acres harvested and total weights harvested [204]. On several occasions, the specific county of a crop scenario was not identified in the program code; however, regional information was available (e.g., southern MS valley uplands, District 70). Therefore, when only the region was known, the highest yield within the region was selected. Also, the USDA maintains county-level maps of yields for a select group of crops [193]. These maps were used to visualize counties adjacent to the area being modeled and, if the yield was higher in an adjacent county, the higher yield value was used. Using the highest historic yield and information on the P_2O_5 requirement for a particular crop based on yield, the application rate of P_2O_5 per acre for each EXPRESS scenario was then determined and this information is shown in Table 71 through Table 73. Yield data for potatoes in Florida and dry beans in Illinois could not be located; therefore, these two crops were excluded from modeling, and 34 of the 36 available crops were used for the EXPRESS scenario modeling.

The amount of surrogate estradiol and trenbolone compound applied to cropland (i.e., the drug application rate) can be determined for each crop scenario by multiplying the P_2O_5 application rates derived in Table 71 through Table 73, and the concentration of trenbolone or estradiol metabolites in manure (7.8 mg/kg P_2O_5 for trenbolone and 1.02 mg/kg P_2O_5 for estradiol derived in Appendix 6.3). The drug application rates for each crop scenario in EXPRESS are listed in Table 74. For each crop scenario, the application date varied and was determined from a sensitivity analysis of application time. This analysis is shown at the end of this section in Appendix 7.1.1.

For models that simulated tilled application methods, the following input parameters were used for the EXPRESS model:

- An incorporation depth of 15 cm was used (see Appendix 2 for selection of incorporation depth)
- A uniform incorporation in the soil (CAM = 4)

The physical-chemical properties and environmental fate parameters (soil DT_{50}, $K_{OC,}$ etc.) from Table 9 for the surrogate estradiol compound and Table 13 for the surrogate trenbolone compound

In the small watershed Index Reservoir model, the program has default values for the percent of the watershed that contains the crop (PCA). The EPA guidance document on use of PCAs was modified in March 2012, so the PCA values in EXPRESS were modified to the national level guidance recommended in Table 1 of reference [205]. For each crop, PCA Index Reservoir values used were as follows: corn 61%, soybean 57%, wheat 38%, sorghum 91%, alfalfa 91%, sugar beets 91%, potato 91%, cotton 33%, and dry bean 91%.

The 90^{th} percentile acute and chronic PEC_{water} values for the surrogate estradiol and trenbolone compounds from the EXPRESS scenarios modeled are summarized in Table 75 through Table 78. These data are also presented in stacked bar graphs in Figure 10 and Figure 14.

Table 71. EXPRESS Scenarios for Corn, Soybean, Wheat and Sorghum, along with Soil Type, Historic Regional Yield Data (bu/acre) and Application Rates of P_2O_5 Based on Crop Nutrient Removal Rates

EXPRESS Code [a] (Plot Code)	Soil Type	Slope %	Soil Region Modeled	Weather Station	Yearly Runoff (cm)	% Corn Silage or Straw harvested [c]	*Years* Historic Yield [d] bu/acre Regional Map- Yield [193]	Highest Expected Yield [e] (bu/acre)	P_2O_5 Removal Rate by Crop [f] (lb/yield)	P_2O_5 Application Rate [g] (kg/acre)
IL Corn MLRA-108 (IL1Cor)	Adair Clay Loam	6%	McLean County, IL	Peoria IL	616.8	0.4%	*2006-2010* 182,196,190,186,170 Region-175	196	0.39	34.7
MS Corn MLRA-134 (MS1Cor)	Grenada Silt	6%	Southern MS Valley Uplands, District 70 **[h]**	Jackson MS	1436	<1% for major counties in District 70	*2006-2010* 127,162,121,115,133 Region-175	175	0.39	31.0
NC Corn MLRA-153A (NC1Cor)	Craven silt loam	6%	Pitt County	Raleigh and Durham, NC	599.1	Pitt- NA **[i]** Adjacent counties <1%	*2006-2010* 109, 86, 34, 116, 37 Region-125	125	0.39	22.2
NC Corn MLRA-130 (NC2Cor)	Chewacla loam	1%	Henderson County	Asheville NC	707.2	38%	*2006-2010* 148,126, 129, 138, 145 Region-150	150	0.63 **[j]**	43.0
ND Corn MLRA-56 (ND1Cor)	Bearden Silty Clay Loam	1.5%	Pembina County	Fargo ND	210	1.5%	*2006-2010* 102,118,124,116,126 Region-150	150	0.39	26.6
OH Corn MLRA-111 (OH1Cor)	Cardington Silt Loam	6%	Darke and Pickaway Counties	Dayton OH	533.7	5.6%, 1.2%	*2010*-170,169 *2009*-185,185 *2008*-141,127 *2007*-152,147 2006-163, 168 Region-200	200	0.39	35.5
TX Corn MLRA-86/87 (TX1Cor)	Axtell Sandy Loam	6%	Milam County	Austin TX	749.4	Milam-NA Adjacent counties <1%	*2006-2010* 79, 93, 64, 42, 74 Region-116	116 **[L]**	0.39	20.6
TX Corn MLRA-83D (TX2Cor)	Harlingen Clay	0.5%	Cameron County	Brownsville TX	674	0.12%	*2006-2010* 79, 117, 74, NA, NA Region-125	125	0.39	22.2
CA-Corn MLRA-17 (CA1Cor)	Madera loam	4.5%	Stanislaus and San Joaquin Counties	Sacramento CA	276.9	96%, 45.7%	*2010*-NA, 193 *2009*-NA, 179 *2008*-NA, 191 *2007*-174, 167 2006-177, 161 Region-200	200	0.63 **[j]**	57.3
PA Corn MLRA-148 (PA1Cor)	Hagerstown Silt Loam	6%	Lancaster County	Harrisburg PA	547.5	40.1%	*2006-2010* 160,160,169,178, 159 Region-175	178	0.63 **[j]**	51.0
MS Soybean MLRA-134 (MS1Syb)	Loring Silt Loam	2%	Yazoo County	Jackson MS	1104	NR	*2006-2010* 28, 40, 40, 35, 38 Region-50	50	0.87	19.8

EXPRESS Code [a] (Plot Code)	**Soil Type**	**Slope %**	**Soil Region Modeled**	**Weather Station**	**Yearly Runoff (cm)**	**% Corn Silage or Straw harvested [c]**	***Years* Historic Yield [d] bu/acre Regional Map- Yield [193]**	**Highest Expected Yield [e] (bu/acre)**	**P_2O_5 Removal Rate by Crop [f] (lb/yield)**	**P_2O_5 Application Rate [g] (kg/acre)**
ND Wheat MLRA-56 Spring wheat (ND1Whe)	Bearden silty clay loam	1.5%	Cass County	Fargo ND	244.5	NR	*2006-2010* 49, 36, 62, 55, 56 Region-70	70	0.71 **[k]**	22.6
OR Wheat MLRA-2 Winter wheat (OR1Whe)	Bashaw Clay	6%	Willamette Valley (Marion County)	Salem OR	358.5	NR	*2006-2010* 85, 90, 109, NA, 110 Region-90	110	0.71 **[k]**	35.5
TX Wheat MLRA-86/87 Winter wheat (TX2Whe)	Crockett fine sandy loam	3%	Blacklands prairie Milam County	Austin TX	746.1	NR	*2006-2010* 25, 50, 50, 23, 46 Region-60	60	0.71 **[k]**	19.4
KS Sorghum MLRA-112 (KS2Srg)	Dennis Silt Loam	4%	Osage County	Topeka KS	570	NR	*2006-2010* 65, 68, 72, NA, 55 Region-70	72	0.42	13.7
TX Sorghum MLRA-86/87 (TX1Srg)	Axtell Sandy Loam	6%	Milam County	Austin TX	705.3	NR	*2006-2010* 56, 37, 74, 41, 83 Region-90	90	0.42	17.2

Data for soil type, slope, soil region and weather station were obtained from examination of the Fortran program code within the EXPRESS program. The program code is not provided as a reference. **[a]**-The EXPRESS program uses the following abbreviations in plots Cor-Corn, Whe-Wheat, Syb-Soybeans, Alf-Alfalfa, Ctt-Cotton, Pot-Potato, Bea-Dry Beans, Sbe-Sugar Beet, P-Pond, R-Index Reservoir. Therefore, TX1Ctt-P indicates cotton in the first Texas scenario for the farm pond model. **[b]**-is not used. **[c]**-The percentage of corn crop harvested as corn silage was estimated from the acres of corn for grain and silage from the USDA 2007 agricultural census Table 25 (Selected Crops Harvested) [204], NR- Not Reported indicates that the census does not report straw or whole plant harvesting. When no data were listed for the county of interest, data from surrounding counties were used if available. **[d]**- Historic yields for the crop within the region are from the USDA database or the 2007 crop survey [204] when data were not available. In addition to county level data, the USDA US county level yield maps [193] were examined for yields of adjacent counties. If these data indicated that higher yields were achieved in adjacent counties then the higher Region yield was used. If data were not available from these sources, the 2007 agricultural census data were used to calculate a yield for a county from acres harvested and total weight of harvest. †- Calculated from total harvest yield for county divided by acres harvested from Table 25 or 26 of USDA 2007 agricultural census [204]. **[e]**-Highest yield value from Historic Yield column. **[f]**- The amount of P_2O_5 per unit of yield crop will remove from soil from harvesting from Table 67. **[g]**- Application rate is calculated by multiplying the expected yield by the P_2O_5 removal rate and converting lb to kg. **[h]**-The highest yearly yields reported for all counties in District 70 were used. **[i]**- NA-Not Available. **[j]**- Corn Silage harvest was significant in this area, so the P_2O_5 removal rate for corn silage (whole plant) was used instead of corn for grain. **[k]**- All wheat harvested assumed straw was removed with the grain. **[L]**- Regional yield map for 2010 corn was not correctly colored for counties adjacent to Milam County TX. The USDA Quick Stats data for 2010 reports Burlington County having the highest corn yield of 116 bu/acre.

Table 72. EXPRESS Scenarios for Dry Beans and Cotton, along with Soil Type, Historic Regional Yield Data (lb/acre) and Application Rates of P_2O_5 Based on Crop Nutrient Removal Rates

EXPRESS Code [a] (Plot Code)	Soil Type	Slope %	Soil Region Modeled	Weather Station	Yearly Runoff (cm)	*Years* Historic Yield [d] lb/acre Regional Map-Yield [193]	Highest Expected Yield [e] (lb/acre)	P_2O_5 Removal Rate by Crop [f] (lb/yield)	P_2O_5 Application Rate [g] (Kg/acre)
WA Beans MLRA-7/8 (WA1Bea)	Ekrub fine sand	6%	Grant County	Yakima, WA	34.2	*2008-2010* 1550, 2540, 2400, 2290, 2310 Region-2600	2600	0.012	14.2
IL Beans MLRA-108 (IL1Bea)	Varna silt loam	6%	McLean County	Peoria, IL	771.6	No Data Available	No Data Available	0.012	No Data Available
MI Beans MLRA-99 (MI1Bea)	Toledo silt clay	1%	Huron County	Flint, MI	463.2	*2006-2010* 2180, 1740, 2030,1880,1890 Region- 2100	2180	0.012	11.9
CA Cotton MLRA-17 (CA1Ctt)	Twisselman Clay	2.5%	Fresno County	Fresno, CA	350.7	*2006-2010* 1200, 1484 1255,1466,1224 Region-1500	1500	0.066 [L]	45.0
MS Cotton MLRA-134 (MS1Ctt)	Loring Silt Loam	6%	Yazoo County	Jackson, MS	1295	*2006-2010* 1000, 989, 1052, 708, 1094 Region-1000	1094	0.066 [L]	32.8
NC Cotton MLRA-133A NC1Ctt)	Boswell sandy loam	6%	Nash County	Raleigh/ Durham, NC	846.3	*2007*-688 † Region-1200	1200	0.066 [L]	36.0
TX Cotton MLRA-83D (TX1Ctt)	Harlingen Clay	0.5%	Cameron County	Brownsville, TX	654	*2006-2010* 839, 707, NA, 831, 836 Region-800	836	0.066 [L]	25.1
TX Cotton MLRA-86/87 (TX2Ctt)	Crockett fine sandy loam	5%	Milam County	Austin, TX	654	*2006-2010* 626, 611, 513, 337,844 Region-1000	1000	0.066 [L]	30.0

See Table 71 for explanation of footnotes **[a]** – **[k]**. **[L]**- Assumes entire cotton plant is harvested. Assumes a bale of cotton weighs 500 lb, unless reference indicates a different weight [200]. †- Calculated from total harvest yield for county divided by acres harvested from Table 25 or 26 of USDA 2007 agricultural census [204].

Table 73. EXPRESS Scenarios for Alfalfa, Sugar Beet and Potato, along with Soil Type, Historic Regional Yield Data (tons/acre) and Application Rates of P_2O_5 Based on Crop Nutrient Removal Rates

EXPRESS Code [a] (Plot Code)	Soil Type	Slope %	Soil Region Modeled	Weather Station	Yearly Runoff (cm)	*Years* Historic Yield [d] tons/acre Regional Map-Yield [193]	Highest Expected Yield [e] (tons/acre)	P_2O_5 Removal Rate by Crop [f] (lb/yield)	P_2O_5 Application Rate [g] (kg/acre)
CA Alfalfa MLRA-17 (CA1Alf)	Sacramento clay	2%	California central valley, San Joaquin County	Fresno, CA	290.1	*2007* 6.4 † Region-NA	6.4	13.5	39.3
IL Alfalfa MLRA-108 (IL1Alf)	Varna silt loam	12%	McLean County	Peoria, IL	497.4	*2006- 2010* 4.9, 4.6, 4.1, 4.2, 4.3 Region-4.9	4.9	13.5	30.1
MN Alfalfa MLRA-56 (MN2Alf)	Bearden silty clay loam	1.5%	Polk County	Fargo, ND	130.8	*2006-2010* 2.3, 2.5, 2.9, 2.6, 3.2 Region-3.9	3.9	13.5	23.9
NC Alfalfa MLRA-136 (NC1Alf)	Helena sandy loam	6%	Western North Carolina (Buncombe County)	Asheville, NC	588.6	*2007* 1.5 † Region-NA	1.5	13.5	9.2
PA Alfalfa MLRA-148 (PA1Alf)	Glenville silt loam	12%	York County	Harrisburg, PA	482.4	*2006-2010* 3.3, 4.0, 2.5, 3.9, 3.1 Region-4.9	4.9	13.5	30.1
TX Alfalfa MLRA-86/87 (TX1Alf)	Lufkin Sandy Loam	1.8%	Texas Claypan Area, Milam County	Austin, TX	687	*2007* 2.6 † Region-NA	2.6	13.5	16.0
CA Sugarbeet MLRA-17 (CA1Sbe)	Ryde clay loam	2%	Fresno County	Fresno, CA	90.6	*2006-2010* 31, 31,32, NA, NA Region-40	40	2.1	38.2
MN Sugarbeet MLRA-56 (MNSbe)	Bearden silty clay loam	1.5%	Southeast Red River Valley. Kittson County	Fargo, ND	208.8	*2006-2010* 23, 20, 25,19, 22 Region-30	30	2.1	28.6
FL Potato MLRA-155 (FL1Pot)	Placid fine sand	1%	St. John's County	Jacksonville, FL	1347	No Data Available	No Data Available	2.9	No Data Available
ID Potato MLRA-11B (ID1POT)	Malm fine sandy loam	4%	Bingham County	Pocatello, ID	206.1	*2006-2010* 18, 17, 18, 19.3, 18 Region-NA	19.3	2.9	25.4
ME Potato MLRA-146 (ME1POT)	Conant Silt Loam	6%	Aroostook County (state yield, county level not available)	Caribou, ME	474.3	*2010* 14.6 Region-NA	14.6	2.9	19.2
WA Potato MLRA-7/8 (WA1Pot)	Skoon silt loam	6%	Yakima County	Yakima, WA	54	*2006-2010* 21, 21, 14, 15, NA Region-NA	21	2.9	27.7

See Table 71 for explanation of footnotes **[a]** – **[k]**. †- Calculated from total harvest yield for county divided by acres harvested from Table 25 or 26 of the USDA 2007 agricultural census [204].

Table 74. Application Rates of Surrogate Estradiol and Trenbolone Compound Contained in Beef Cattle Manure Applied to Agricultural Fields Based on Regional P_2O_5 Requirements of the EXPRESS Crop Scenario Modeled in Table 71 through Table 73

EXPRESS Code and Crop	Manure App. Day ‡	P_2O_5 App. Rate* (kg/acre)	Application Rate Based on Daily Release Rate of Drug from Implant †			
			Trenbolone 7.8 mg/kg P_2O_5		Estradiol 1.02 mg/kg P_2O_5	
			mg/acre	lb/acre ¶	mg/acre	lb/acre
IL Corn MLRA-108	Day -28	34.7	270.66 §	5.95E-04	35.39	7.79E-05
MS Corn MLRA-134	Day -14	31.0	241.8	5.32E-04	31.62	6.96E-05
NC1 Corn - E MLRA-153A	Day -28	22.2	173.16	3.81E-04	22.64	4.98E-05
NC2 Corn - W MLRA-130	Day -14	43.0	335.4	7.38E-04	43.86	9.65E-05
ND Corn MLRA-56	Day +21	26.6	207.48	4.56E-04	27.13	5.97E-05
TX1 Corn MLRA-86/87	Day +7	20.6	160.68	3.53E-04	21.01	4.62E-05
TX2 Corn MLRA-83D	Day +28	22.2	173.16	3.81E-04	22.64	4.98E-05
CA-Corn MLRA-17	Day -21	57.3	446.94	9.83E-04	58.45	1.29E-04
OH Corn MLRA-111	Day +28	35.5	276.91	6.09E-04	36.21	7.97E-05
PA Corn MLRA-148	Day -14	51.0	397.8	8.75E-04	52.02	1.14E-04
MS Soybean MLRA-134	Day -21	19.8	154.44	3.40E-04	20.20	4.44E-05
ND Wheat MLRA-56	Day +28	22.6	176.28	3.88E-04	23.05	5.07E-05
OR Wheat MLRA-2	Day -7	35.5	276.9	6.09E-04	36.21	7.97E-05
TX Wheat MLRA-86/87	Day -21	19.4	151.32	3.33E-04	19.79	4.35E-05
KS Sorghum MLRA-112	Day -7	13.7	106.86	2.35E-04	13.97	3.07E-05
TX Sorghum MLRA-86/87	Day -7	17.2	134.16	2.95E-04	17.54	3.86E-05
CA Alfalfa MLRA-17	Day -1	39.3	306.55	6.74E-04	40.08	8.82E-05
IL Alfalfa MLRA-108	Day +21	30.1	234.77	5.16E-04	30.70	6.76E-05
MN Alfalfa MLRA-56	Day -14	23.9	186.42	4.10E-04	24.38	5.36E-05
NC Alfalfa MLRA-136	Day +14	9.2	71.76	1.58E-04	9.38	2.06E-05
PA Alfalfa MLRA-148	Day +14	30.1	234.78	5.17E-04	30.70	6.75E-05
TX Alfalfa MLRA-86/87	Day +21	16.0	124.8	2.75E-04	16.32	3.59E-05
WA Beans MLRA-7/8	Day +14	14.2	110.76	2.44E-04	14.48	3.19E-05
MI Beans MLRA-99	Day +7	11.9	92.82	2.04E-04	12.14	2.67E-05
CA Cotton MLRA-17	Day +7	45.0	351	7.72E-04	45.9	1.01E-04
MS Cotton MLRA-134	Day -1	32.8	255.84	5.63E-04	33.456	7.36E-05
NC Cotton MLRA-133A	Day -1	36.0	280.8	6.18E-04	36.72	8.08E-05
TX1 Cotton MLRA-83D	Day +21	25.1	195.78	4.31E-04	25.60	5.63E-05
TX2 Cotton MLRA-86/87	Day +7	30.0	234	5.15E-04	30.6	6.73E-05
CA Sugarbeet MLRA-17	Day -21	38.2	297.96	6.56E-04	38.96	8.57E-05
MN Sugarbeet MLRA-56	Day -1	28.6	223.08	4.91E-04	29.17	6.42E-05
ID Potato MLRA-11B	Day +14	25.4	198.12	4.36E-04	25.91	5.70E-05
ME Potato MLRA-146	Day -7	19.2	149.76	3.29E-04	19.58	4.31E-05
WA Potato MLRA-7/8	Day +28	27.7	216.06	4.75E-04	28.25	6.22E-05

* The P_2O_5 application rates were derived in Table 71 through Table 73.

† The concentrations of trenbolone or estradiol metabolites (7.8 mg/kg P_2O_5 for trenbolone and 1.02 mg/kg P_2O_5 for estradiol) were derived in Appendix 6.3.

§ Example calculation: 34.7 kg/acre X 7.8 mg trenbolone / kg P_2O_5 = 270.66 mg trenbolone / acre.

¶ Values were converted from mg/acre to lb/acre to be compatible with the input requirements of the EPA EXPRESS program [1 mg = 2.2E-06 lb]. For example, for IL Corn: 270.66 mg X 1E-6 kg/mg X 2.2 lb/kg = 5.95E-04 lb/acre.

‡ Negative manure application values are days prior to planting; positive are days after harvest. See Table 80.

Table 75. Surrogate Estradiol Compound Acute and Chronic 90th Percentile Limnetic Concentrations from EPA EXPRESS Farm Pond from Application of Manure to Tilled Soil (ng/L)

Scenario	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
CA1Alfalfa	0.0079	0.0076	0.0062	0.0044	0.0037	0.0014
CA1Corn	0.0165	0.0156	0.0128	0.0090	0.0074	0.0027
CA1Cotton	0.0196	0.0190	0.0170	0.0105	0.0071	0.0029
CA1SugarBeet	0.0036	0.0035	0.0032	0.0025	0.0022	0.0008
ID1Potato	0.0068	0.0067	0.0065	0.0061	0.0059	0.0028
IL1Alfalfa	0.0214	0.0212	0.0202	0.0190	0.0183	0.0067
IL1Corn	0.0258	0.0246	0.0211	0.0152	0.0126	0.0047
KS2Sorghum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ME1Potato	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MI1Bean	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MN1SugarBeet	0.0089	0.0084	0.0068	0.0047	0.0039	0.0015
MN2Alfalfa	0.0030	0.0028	0.0022	0.0015	0.0011	0.0004
MS1Corn	0.0780	0.0741	0.0620	0.0428	0.0343	0.0115
MS1Cotton	0.0703	0.0660	0.0526	0.0339	0.0263	0.0084
MS1Soybean	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
NC1Alfalfa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
NC1Corn	0.0280	0.0264	0.0212	0.0145	0.0118	0.0040
NC1Cotton	0.0500	0.0478	0.0375	0.0254	0.0207	0.0077
NC2Corn	0.0203	0.0191	0.0159	0.0109	0.0088	0.0031
ND1Corn	0.0089	0.0084	0.0069	0.0052	0.0049	0.0024
ND1Wheat	0.0109	0.0103	0.0083	0.0061	0.0058	0.0030
OH1Corn	0.0290	0.0275	0.0230	0.0152	0.0120	0.0053
OR1Wheat	0.0019	0.0019	0.0019	0.0015	0.0013	0.0005
PA1Alfalfa	0.0181	0.0177	0.0165	0.0110	0.0075	0.0038
PA1Corn	0.0163	0.0155	0.0135	0.0098	0.0081	0.0030
TX1Alfalfa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TX1Corn	0.0733	0.0675	0.0503	0.0326	0.0265	0.0073
TX1Cotton	0.0169	0.0159	0.0126	0.0105	0.0076	0.0021
TX1Sorghum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TX2Corn	0.0198	0.0180	0.0127	0.0074	0.0056	0.0016
TX2Cotton	0.0482	0.0449	0.0339	0.0243	0.0197	0.0051
TX2Wheat	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
WA1Bean	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
WA1Potato	0.0040	0.0038	0.0031	0.0021	0.0014	0.0009
Maximum	**0.0780**	**0.0741**	**0.0620**	**0.0428**	**0.0343**	**0.0115**

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

The maximum values from above are entered in the summary table in Section 5.4.4.

Table 76. Surrogate Estradiol Compound Acute and Chronic 90th Percentile Limnetic Concentrations from EPA EXPRESS Index Reservoir from Application of Manure to Tilled Soil (ng/L)

Scenario	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
CA1Alfalfa	0.0174	0.0168	0.0144	0.0107	0.0029	0.0008
CA1Corn	0.0244	0.0234	0.0200	0.0144	0.0039	0.0018
CA1Cotton	0.0167	0.0159	0.0145	0.0089	0.0020	0.0007
CA1SugarBeet	0.0083	0.0081	0.0075	0.0062	0.0020	0.0006
ID1Potato	0.0156	0.0154	0.0146	0.0132	0.0061	0.0011
IL1Alfalfa	0.0453	0.0428	0.0350	0.0297	0.0130	0.0034
IL1Corn	0.0377	0.0360	0.0309	0.0217	0.0057	0.0019
KS2Sorghum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ME1Potato	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MI1Bean	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MN1SugarBeet	0.0190	0.0181	0.0153	0.0108	0.0031	0.0009
MN2Alfalfa	0.0066	0.0063	0.0053	0.0036	0.0010	0.0003
MS1Corn	0.1089	0.1029	0.0842	0.0516	0.0112	0.0033
MS1Cotton	0.0552	0.0515	0.0393	0.0230	0.0047	0.0012
MS1Soybean	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
NC1Alfalfa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
NC1Corn	0.0408	0.0388	0.0318	0.0214	0.0050	0.0017
NC1Cotton	0.0387	0.0368	0.0284	0.0182	0.0046	0.0019
NC2Corn	0.0295	0.0279	0.0232	0.0152	0.0036	0.0010
ND1Corn	0.0129	0.0123	0.0104	0.0078	0.0033	0.0009
ND1Wheat	0.0097	0.0093	0.0078	0.0058	0.0023	0.0007
OH1Corn	0.0425	0.0406	0.0348	0.0229	0.0063	0.0025
OR1Wheat	0.0015	0.0014	0.0014	0.0012	0.0004	0.0001
PA1Alfalfa	0.0404	0.0393	0.0353	0.0247	0.0064	0.0020
PA1Corn	0.0236	0.0226	0.0200	0.0143	0.0037	0.0011
TX1Alfalfa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TX1Corn	0.1072	0.0992	0.0741	0.0464	0.0096	0.0032
TX1Cotton	0.0131	0.0125	0.0097	0.0064	0.0015	0.0004
TX1Sorghum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TX2Corn	0.0289	0.0264	0.0188	0.0106	0.0021	0.0006
TX2Cotton	0.0380	0.0355	0.0268	0.0170	0.0038	0.0012
TX2Wheat	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
WA1Bean	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
WA1Potato	0.0086	0.0083	0.0073	0.0052	0.0021	0.0008
Maximum	**0.1089**	**0.1029**	**0.0842**	**0.0516**	**0.0130**	**0.0034**

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

The maximum values from above are entered in the summary table in Section 5.4.4.

Table 77. Surrogate Trenbolone Compound Acute and Chronic 90th Percentile Limnetic Concentrations from EPA EXPRESS Farm Pond from Application of Manure to Tilled Soil (ng/L)

Scenario	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
CA1Alfalfa	0.0722	0.0700	0.0633	0.0503	0.0437	0.0193
CA1Corn	0.2179	0.2098	0.1835	0.1424	0.1225	0.0611
CA1Cotton	0.2323	0.2217	0.2074	0.1311	0.1043	0.0485
CA1SugarBeet	0.0305	0.0298	0.0278	0.0234	0.0209	0.0098
ID1Potato	0.0584	0.0580	0.0565	0.0539	0.0522	0.0217
IL1Alfalfa	0.1949	0.1933	0.1885	0.1804	0.1753	0.0718
IL1Corn	0.2190	0.2120	0.1898	0.1522	0.1324	0.0581
KS2Sorghum	0.1242	0.1193	0.1034	0.0799	0.0678	0.0312
ME1Potato	0.1261	0.1241	0.1161	0.0985	0.0888	0.0506
MI1Bean	0.0326	0.0314	0.0281	0.0224	0.0201	0.0106
MN1SugarBeet	0.0602	0.0580	0.0503	0.0388	0.0335	0.0187
MN2Alfalfa	0.0201	0.0193	0.0163	0.0119	0.0103	0.0067
MS1Corn	0.5632	0.5457	0.4773	0.3674	0.3110	0.1280
MS1Cotton	0.4997	0.4799	0.4137	0.3021	0.2479	0.0927
MS1Soybean	0.2157	0.2078	0.1879	0.1477	0.1241	0.0468
NC1Alfalfa	0.0997	0.0951	0.0822	0.0634	0.0554	0.0225
NC1Corn	0.1380	0.1329	0.1158	0.0891	0.0762	0.0334
NC1Cotton	0.3826	0.3735	0.3207	0.2450	0.2104	0.0977
NC2Corn	0.2135	0.2047	0.1817	0.1385	0.1179	0.0524
ND1Corn	0.0583	0.0562	0.0490	0.0465	0.0455	0.0236
ND1Wheat	0.0569	0.0548	0.0478	0.0438	0.0427	0.0226
OH1Corn	0.2631	0.2572	0.2386	0.1609	0.1446	0.0749
OR1Wheat	0.0227	0.0226	0.0200	0.0159	0.0141	0.0073
PA1Alfalfa	0.1638	0.1573	0.1441	0.0992	0.0773	0.0430
PA1Corn	0.1883	0.1824	0.1698	0.1356	0.1172	0.0539
TX1Alfalfa	0.1103	0.1040	0.0826	0.0630	0.0588	0.0184
TX1Corn	0.3589	0.3398	0.2775	0.2032	0.1741	0.0583
TX1Cotton	0.1297	0.1278	0.1175	0.0927	0.0630	0.0248
TX1Sorghum	0.2911	0.2765	0.2339	0.1638	0.1335	0.0476
TX2Corn	0.0964	0.0906	0.0716	0.0483	0.0393	0.0125
TX2Cotton	0.3683	0.3537	0.3062	0.2236	0.1899	0.0534
TX2Wheat	0.4699	0.4432	0.3582	0.2573	0.2177	0.0741
WA1Bean	0.0011	0.0011	0.0010	0.0009	0.0009	0.0006
WA1Potato	0.0221	0.0214	0.0193	0.0184	0.0177	0.0115
Maximum	**0.5632**	**0.5457**	**0.4773**	**0.3674**	**0.3110**	**0.1280**

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

The maximum values from above are entered in the summary table in Section 5.4.5.

Table 78. Surrogate Trenbolone Compound Acute and Chronic 90th Percentile Limnetic Concentrations from EPA EXPRESS Index Reservoir from Application of Manure to Tilled Soil (ng/L)

Scenario	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
CA1Alfalfa	0.1592	0.1554	0.1415	0.1144	0.0375	0.0107
CA1Corn	0.3171	0.3074	0.2747	0.2155	0.0700	0.0375
CA1Cotton	0.1857	0.1797	0.1695	0.1077	0.0322	0.0108
CA1SugarBeet	0.0696	0.0680	0.0646	0.0560	0.0221	0.0074
ID1Potato	0.1310	0.1300	0.1237	0.1128	0.0500	0.0102
IL1Alfalfa	0.3260	0.3112	0.2712	0.1932	0.0510	0.0153
IL1Corn	0.3067	0.2963	0.2636	0.2002	0.0611	0.0206
KS2Sorghum	0.2645	0.2546	0.2203	0.1627	0.0472	0.0157
ME1Potato	0.2390	0.2235	0.1747	0.1134	0.0258	0.0091
MI1Bean	0.0632	0.0608	0.0536	0.0419	0.0154	0.0063
MN1SugarBeet	0.1289	0.1245	0.1113	0.0866	0.0306	0.0098
MN2Alfalfa	0.0441	0.0427	0.0376	0.0285	0.0111	0.0040
MS1Corn	0.7739	0.7327	0.6192	0.4068	0.0964	0.0281
MS1Cotton	0.3843	0.3656	0.2953	0.1894	0.0434	0.0110
MS1Soybean	0.2837	0.2696	0.2424	0.1714	0.0402	0.0108
NC1Alfalfa	0.2119	0.2022	0.1735	0.1266	0.0348	0.0098
NC1Corn	0.1997	0.1918	0.1646	0.1204	0.0328	0.0115
NC1Cotton	0.2863	0.2776	0.2300	0.1617	0.0466	0.0197
NC2Corn	0.3100	0.3046	0.2850	0.2505	0.1021	0.0279
ND1Corn	0.0826	0.0803	0.0713	0.0607	0.0284	0.0080
ND1Wheat	0.0500	0.0482	0.0430	0.0347	0.0174	0.0048
OH1Corn	0.3838	0.3709	0.3289	0.2233	0.0678	0.0278
OR1Wheat	0.0164	0.0163	0.0156	0.0143	0.0048	0.0015
PA1Alfalfa	0.3504	0.3385	0.3114	0.2102	0.0665	0.0198
PA1Corn	0.2707	0.2617	0.2421	0.1861	0.0571	0.0185
TX1Alfalfa	0.2399	0.2346	0.2172	0.1767	0.0669	0.0221
TX1Corn	0.5213	0.4915	0.3949	0.2732	0.0604	0.0215
TX1Cotton	0.0892	0.0864	0.0761	0.0596	0.0122	0.0034
TX1Sorghum	0.6345	0.6025	0.5056	0.3382	0.0808	0.0298
TX2Corn	0.1405	0.1322	0.1038	0.0671	0.0149	0.0045
TX2Cotton	0.2820	0.2710	0.2225	0.1578	0.0351	0.0125
TX2Wheat	0.4264	0.4001	0.3192	0.2173	0.0489	0.0184
WA1Bean	0.0024	0.0024	0.0021	0.0019	0.0011	0.0004
WA1Potato	0.0479	0.0467	0.0427	0.0363	0.0216	0.0077
Maximum	**0.7739**	**0.7327**	**0.6192**	**0.4068**	**0.1021**	**0.0375**

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

The maximum values from above are entered in the summary table in Section 5.4.5.

Appendix 7.1.1. Sensitivity analysis of manure application day in EXPRESS crop scenarios

The EXPRESS model assumes that the manure is applied on the same date each year over the 30-year iteration. However, manure management BMPs recommend that manure is not applied to directly prior to a large rainfall event. Thus, to select a conservative application date for each crop scenario in EXPRESS, a sensitivity analysis was conducted using the EXPRESS model where the PEC_{water} values were estimated for five different application times before planting in spring (1, 7, 14, 21, and 28 days prior to planting) and five application times in fall (1, 7, 14, 21, and 28 days after harvest). From these 10 data points, the upper 90% confidence bound on the mean was determined according to the procedure in Section 4.2.2, and the application date with a PEC_{water} value that was numerically closest to this upper bound was chosen as a conservative application date (Table 80). This procedure minimized extreme outliers where, unrealistically, manure was applied directly before a large rainfall event but is still a conservative application time because it represents the upper 90% confidence bound of the PEC_{water} value. The application amounts and the application date chosen from the sensitivity analysis are shown in Table 74. For each run of the model, all crops received the same amount of drug and all parameters were kept constant except for application day.

All EXPRESS input parameters were the same as those used for final modeling of representative TBA metabolites from Table 13 except for the application rate and anaerobic DT_{50} value. These differences are shown in the following table.

Table 79. Trenbolone Parameter Differences for Manure Application Timing Sensitivity Analysis

Parameter	Value Selected for Modeling	Comments/Reference
Application rate (lb/acre)	0.00292	The same application rate was used for all crop scenarios and is the same value used for SCI-GROW (Table 19)
Anaerobic water-sediment DT_{50} (days)	131.8	A preliminary estimate was used for the sensitivity analysis.
Application depth	15 cm	Appendix 2
Chemical application method	CAM 4	Appendix 7.1

Table 80. Application Day Sensitivity Analysis of PEC_{water} when Manure is Applied Before Planting (-) and after Harvest (+)

Model	Apply	Day -28	Day -21	Day -14	Day -7	Day -1	Day +1	Day +7	Day +14	Day +21	Day +28	Upper 90% CI
CA1Alf	Day -1	1.48E-01	2.92E-01	1.33E-01	1.99E-01	**2.81E-01**	1.56E-01	2.21E-01	4.71E-01	1.72E-01	2.37E-01	2.77E-01
CA1Cor	Day -21	6.38E-01	**5.45E-01**	9.14E-01	5.93E-01	8.30E-01	2.32E-02	9.28E-02	5.03E-02	5.84E-02	2.64E-01	5.59E-01
CA1Ctt	Day +7	5.56E-01	4.08E-01	4.61E-01	1.05E+00	5.39E-01	3.86E-01	**7.61E-01**	7.11E-01	1.19E+00	3.08E-01	7.71E-01
CA1Sbe	Day -21	5.37E-02	**1.30E-01**	3.11E-01	8.88E-02	2.68E-01	4.81E-06	1.93E-05	9.69E-05	4.88E-04	2.46E-03	1.39E-01
ID1Pot	Day +14	1.68E-01	7.12E-02	1.78E-01	6.16E-01	1.93E-02	1.09E-01	1.56E-01	**3.69E-01**	9.84E-02	1.49E-01	2.74E-01
IL1Alf	Day +21	8.37E-01	5.03E-01	7.64E-01	2.87E-01	7.21E-01	2.99E-01	4.95E-01	2.07E+00	**1.16E+00**	5.15E-01	1.01E+00
IL1Cor	Day -28	**8.81E-01**	5.02E-01	1.20E+00	1.01E+00	9.01E-01	3.25E-01	4.33E-01	7.10E-01	7.62E-01	4.28E-01	8.48E-01
KS2Srg	Day -7	9.63E-01	2.79E-01	6.28E-01	**1.14E+00**	8.25E-01	7.70E-01	1.97E+00	5.93E-01	4.88E-01	1.29E+00	1.12E+00
ME1Pot	Day -7	5.77E-01	3.91E-01	3.99E-01	**9.23E-01**	7.84E-01	5.59E-01	4.40E-01	1.38E+00	3.59E-01	1.26E+00	8.79E-01
MI1Bea	Day +14	2.21E-01	4.71E-01	3.63E-01	2.92E-01	5.68E-01	9.25E-01	4.55E-01	**6.07E-01**	7.16E-01	2.12E-01	5.88E-01
MN1Sbe	Day -1	4.09E-01	2.84E-01	2.19E-01	1.19E-01	**2.76E-01**	3.27E-01	1.05E-01	2.79E-01	2.22E-02	6.00E-03	2.67E-01
MN2Alf	Day -14	8.62E-02	8.07E-02	**1.07E-01**	7.50E-02	6.91E-02	2.82E-01	8.02E-02	7.81E-02	3.38E-01	6.94E-02	1.72E-01
MS1Cor	Day -14	1.14E+00	3.27E+00	**2.59E+00**	2.34E+00	3.76E+00	8.50E-01	1.99E+00	2.23E+00	1.23E+00	2.02E+00	2.57E+00
MS1Ctt	Day -1	1.98E+00	3.37E+00	4.98E+00	1.82E+00	**2.26E+00**	9.05E-01	2.18E+00	1.36E+00	1.89E+00	1.30E+00	2.75E+00
MS1Syb	Day -21	2.41E+00	**1.54E+00**	1.39E+00	2.52E+00	2.54E+00	1.43E+00	1.11E+00	1.26E+00	6.79E-01	1.38E+00	1.92E+00
NC1Alf	Day +14	1.15E+00	1.20E+00	2.16E+00	7.12E-01	6.83E-01	4.05E-01	1.37E+00	**1.35E+00**	1.26E+00	9.72E-01	1.35E+00
NC1Cor	Day -28	**9.36E-01**	8.22E-01	6.47E-01	4.40E-01	6.02E-01	8.48E-01	1.25E+00	1.32E+00	1.18E+00	7.37E-01	1.01E+00
NC1Ctt	Day -1	7.92E-01	9.82E-01	9.12E-01	1.75E+00	**1.49E+00**	1.52E+00	1.59E+00	8.71E-01	5.80E-01	7.54E-01	1.32E+00
NC2Cor	Day -14	5.19E-01	9.02E-01	**7.46E-01**	5.44E-01	1.63E-01	7.25E-01	7.04E-01	6.91E-01	6.28E-01	8.60E-01	7.44E-01
ND1Cor	Day +21	2.30E-01	5.37E-01	2.25E-01	1.06E-01	1.59E-01	1.48E-01	3.93E-01	2.33E-01	**3.20E-01**	3.51E-01	3.31E-01
ND1Whe	Day +28	4.77E-01	3.14E-01	2.38E-01	1.38E-01	3.16E-01	3.08E-01	2.59E-01	4.60E-01	2.98E-01	**3.89E-01**	3.66E-01
OH1Cor	Day +28	9.67E-01	1.13E+00	8.84E-01	7.23E-01	1.80E+00	6.14E-01	8.74E-01	1.30E+00	7.06E-01	**1.22E+00**	1.19E+00
OR1Whe	Day -7	3.88E-03	8.99E-03	3.27E-02	**9.36E-02**	1.82E-01	4.16E-02	5.47E-02	1.24E-01	9.65E-04	4.88E-03	8.27E-02
PA1Alf	Day +14	1.01E+00	5.67E-01	6.21E-01	5.17E-01	3.47E-01	5.29E-01	9.48E-01	**7.86E-01**	5.35E-01	1.15E+00	8.19E-01
PA1Cor	Day -14	8.73E-01	5.20E-01	**5.55E-01**	4.88E-01	3.21E-01	6.16E-01	4.39E-01	3.55E-01	4.25E-01	2.76E-01	5.66E-01
TX1Alf	Day +21	6.12E-01	6.01E-01	5.96E-01	4.19E-01	1.43E+00	1.48E+00	8.61E-01	2.66E-01	**9.04E-01**	5.55E-01	9.60E-01
TX1Cor	Day +7	2.22E+00	3.64E+00	1.59E+00	6.70E-01	3.75E-01	1.75E+00	**2.28E+00**	2.95E+00	2.11E+00	3.24E+00	2.56E+00
TX1Ctt	Day +21	4.01E-01	9.39E-02	4.44E-01	4.69E-02	8.53E-02	8.30E-01	1.64E+00	6.21E-01	**7.69E-01**	7.16E-01	7.85E-01
TX1Srg	Day -7	1.36E+00	2.36E+00	1.74E+00	**2.57E+00**	1.76E+00	1.39E+00	1.36E+00	3.65E+00	4.35E+00	2.09E+00	2.73E+00
TX2Cor	Day +28	1.78E-01	2.46E-01	3.18E-01	7.42E-02	2.97E-01	8.25E-01	9.28E-01	3.96E-01	4.92E-01	**5.90E-01**	5.62E-01
TX2Ctt	Day +7	6.88E-01	6.97E-01	5.18E-01	5.30E-01	1.02E+00	1.34E+00	**1.68E+00**	1.21E+00	2.44E+00	2.11E+00	1.53E+00
TX2Whe	Day -21	1.98E+00	**2.94E+00**	3.88E+00	1.86E+00	3.31E+00	1.11E+00	2.70E+00	1.61E+00	3.92E+00	2.63E+00	3.03E+00
WA1Bea	Day +7	5.05E-05	2.66E-04	1.35E-03	2.04E-03	3.82E-03	6.83E-03	**2.74E-02**	1.01E-02	3.19E-02	7.65E-02	2.71E-02
WA1Pot	Day +28	2.17E-02	1.31E-02	2.88E-02	1.57E-01	6.64E-02	1.59E-01	1.70E-01	2.67E-02	1.45E-01	**1.22E-01**	1.21E-01

Application day for trenbolone is shown in bold and was selected for use in the modeling based on the concentration nearest the upper 90% confidence bound. These values are not actual predicted values because they use higher application rates than the final values modeled in this EA, and are only comparable for application times; not environmental concentrations.

Appendix 7.2. PRZM leachate concentrations from 34 EPA EXPRESS tilled scenarios for simulating tile drains

The potential for a compound to reach groundwater or a tile drain below the soil was assessed using the PRZM output that lists the yearly maximum concentration in leachate under the soil column at 1 m. Section 3.4.2.2 and reference [14] discuss the justification for using a value of 1 m for an assessment of potential to reach tile drains. The reported values are the same for both the farm pond and Index Reservoir models so only one value will be reported. Of the 34 crop scenarios modeled in PRZM, 28 resulted in concentrations of 0 µg/L for trenbolone. Six of the crop scenarios modeled resulted in a trace amount of trenbolone. Only two crop scenarios modeled resulted in trace concentrations for estradiol. The other 32 crop scenarios modeled resulted in concentrations of 0 µg/L for the surrogate estradiol compound. The highest leachate concentration was 4.1×10^{-12} µg/L for estradiol and 1.9×10^{-13} µg/L for trenbolone. These data indicate a very low potential for TBA or EB metabolites to leach to groundwater or to reach tile drains at a concentration that could contribute to surface water concentrations from movement through the subsurface.

Table 81. Highest Leachate Concentration from PRZM EXPRESS Runs with Values Greater than Zero

Scenario	Surrogate Trenbolone Compound µg/L	Surrogate Estradiol Compound µg/L
NC1Alfalfa	2.5×10^{-22}	0
WA1Bean	6.4×10^{-17}	0
NC2Corn	6.8×10^{-21}	0
PA1Corn	1.4×10^{-18}	1.4×10^{-21}
WA1Potato	1.9×10^{-13}	4.1×10^{-12}
TX1Sorghum	1.3×10^{-22}	0

The modeled leachate estimates for estradiol and trenbolone in Table 81 appear to be in direct conflict with the data reported in Appendix 12 by Gall et al. [206] on hormone detection in ditches attached to tile drain fields at Purdue University. In tile drain fields, there are several factors dealing with the macrostructure of the drain field that can influence water passage to the tile drains, and these are not accounted for in subsurface transport models. The authors list eight publications (references 22-30 cited in the Gall et al. [206] publication) that discuss several reasons these results may differ. These publications include a discussion on preferential flow and application induced discharge as opposed to precipitation-induced discharge. Kladivko et al. [207] state that the partitioning of chemical losses between surface and subsurface flows will be affected by tile drainage, and that the hydrology of the site should be understood before evaluating the chemical transport effects. These authors concluded that the half-life and sorption to soil are the most important chemical properties affecting the fate and potential off-site transport of the chemicals. Though their discussion focuses on agricultural pesticides, the basic principles of chemical transport are the same. Chemicals with a long half-life will have a greater potential for off-site movement, while soil sorption determines if the off-site transport mechanism is through sediment, or with surface runoff water or with leaching water (subsurface drainage or tile flow) [207]. The authors also discuss the importance of the soil infiltration rate and water holding capacity as important hydrological conditions affecting transport. Although tile drains may be 1 m deep there are times of the year where cracking of the soil may occur from the surface to the tile drain allowing water to pass directly from the surface to the drain. Macro structures in soil, such as worm holes, also allow water to quickly migrate from the

surface to the drain. Hardie et al. [208] demonstrated the impact of antecedent soil moisture content on the depth and rate of dye filtration into soils. The authors reported contrasting results compared to other studies using different soil types and concluded that, in addition to soil type, soil moisture content had a profound effect. This is a logical conclusion if one considers manure applications on soils with low, moderate, or high antecedent moisture content followed by rainfall events over time. These conditions could have profound effects on the concentrations of VMPs from manures observed in any individual tile drain effluent, even in tile drains from the same field. These types of rapid transport mechanisms are not modeled by subsurface leaching models.

Although Gall et al. [206] detected hormones from animal production in the ditches of tile drained fields, it is hard to assess the contribution from drains versus surface runoff, and what the impact on a watershed versus an agricultural drainage ditch would be. During rainfall events, the concentrations in the ditches will be influenced by drainage from the subsurface tile drains plus direct runoff to ditches from the surface of the field. Also, some tile drain fields have drains installed at the surface to remove ponded water from the poorly drained soil. Therefore, the reported concentrations may be a mixture of these factors. In Figure SI-9 (synthetic androgens) of the supplemental information for the publication by Gall et al. [209], many of the higher concentrations of total synthetic androgens occur during periods where there appears to be zero flow in the drains (sites S1, D4, and S3). From Figure S1-9 of the supplemental information, total synthetic androgens ranged between approximately 5 and 35 ng/L, when detected, with a corresponding tile drain flow rate at or near 0 mm/day. It appears these high measurements at times with no flow account for the majority of detection events at these sites. However, sites D1, D3, and S2 had levels of total synthetic androgen at similar levels with a similar frequency (3.0-6.8%>LOQ; according to Table 1 of Gall et al. [206]), and these appear to coincide with higher flow rates. These data demonstrate the complex nature of hormone dynamics within an agricultural site which makes it difficult to put in context infrequent detection events in tile drain effluents, as with TBA metabolites, and the correlation with chronic PEC values and potential effects to the water body they could potentially flow to.

Appendix 7.3. EPA EXPRESS no-till scenarios

Because manure from livestock can potentially be surface applied to no-till crops, 17 no-till crop scenarios were developed for corn, wheat, cotton, soybean and sorghum. These are the principal crops grown under no-till methods [85] and are available as crop scenarios in EXPRESS. Alfalfa, potato, bean, and sugar beet crop scenarios were excluded from no-till modeling because they were not listed by USDA as major no-till crops in the US [85]. Additionally, some of these crop scenarios were excluded from modeling where the region generally did not employ surface application methods (i.e., no-till methods were typically not used). If the practice of no-till was <10%, the region was eliminated from evaluation for no-till in EXPRESS. Therefore, the following crop scenarios were excluded: TX1 Corn MLRA-86/87, TX2 Corn MLRA-83D, CA Cotton MLRA-17, TX1 Cotton MLRA-83D, TX2 Cotton MLRA-86/87. This is because <10% of these specific regions employ no-till application methods.

This leaves the following 17 scenarios to model as no-till from the original 34 tilled models.

Table 82. EXPRESS Code for No-Till Scenarios Modeled

EXPRESS Code For No-Till Scenarios Modeled			
IL Corn	MLRA-108	ND Wheat	MLRA-56
MS Corn	MLRA-134	OR Wheat	MLRA-2
NC1 Corn - E	MLRA-153A	TX Wheat	MLRA-86/87
NC2 Corn - W	MLRA-130	KS Sorghum	MLRA-112
ND Corn	MLRA-56	TX Sorghum	MLRA-86/87
CA Corn	MLRA-17	CA Cotton	MLRA-17
OH Corn	MLRA-111	MS Cotton	MLRA-134
PA Corn	MLRA-148	NC Cotton	MLRA-133A
MS Soybean	MLRA-134		

The scenarios for each crop were modified by changing the runoff curve numbers (CN) and the "C" factor of the erosion equation from tilled to no-till values following standard recommendations for each crop from the USDA Agricultural Handbook. More details on the assumptions, modeling methods and results can be found in Attachment 1.

The manure application day, amount of trenbolone and estradiol applied per acre and environmental fate parameters were all the same as those used for the tilled models described in Table 74. However, for the no-till cropping scenarios, the application depth was reduced to 5 cm (see Appendix 2.3 for an explanation of incorporation depth for no-till application). The scenarios were modeled using PRZM and EXAMS, external to the EXPRESS shell because EXPRESS is only able to model tilled crop scenarios. The results are in Attachment 1 and 90th percentile acute and chronic concentrations are summarized in Table 83 through Table 86. These data are also presented in stacked bar graphs in Figure 12 and Figure 16.

Table 83. Surrogate Estradiol Compound Acute and Chronic 90th Percentile Limnetic Concentrations from EPA Farm Pond Models from Application of Manure to No-Till Soil (ng/L)

Scenario	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
CA corn	0.0214	0.0201	0.0161	0.0111	0.0090	0.0032
CA cotton	0.0098	0.0092	0.0077	0.0046	0.0031	0.0013
IL corn	0.0157	0.0148	0.0119	0.0081	0.0064	0.0023
KS sorghum	0.0084	0.0079	0.0062	0.0041	0.0032	0.0011
MS corn	0.0621	0.0580	0.0448	0.0289	0.0225	0.0071
MS cotton	0.0763	0.0713	0.0546	0.0334	0.0255	0.0079
MS soybean	0.0259	0.0243	0.0207	0.0148	0.0117	0.0037
NC cotton	0.0367	0.0346	0.0271	0.0179	0.0142	0.0050
NC1 corn E	0.0165	0.0155	0.0123	0.0082	0.0065	0.0022
NC2 corn W	0.0309	0.0290	0.0233	0.0157	0.0126	0.0044
ND corn	0.0053	0.0050	0.0041	0.0038	0.0036	0.0014
ND wheat	0.0059	0.0056	0.0044	0.0039	0.0037	0.0015
OH corn	0.0404	0.0382	0.0322	0.0180	0.0142	0.0068
OR wheat	0.0012	0.0011	0.0009	0.0006	0.0005	0.0002
PA corn	0.0211	0.0199	0.0164	0.0111	0.0089	0.0031
TX sorghum	0.0442	0.0407	0.0307	0.0185	0.0139	0.0042
TX wheat	0.0635	0.0580	0.0445	0.0282	0.0226	0.0060
Maximum	**0.0763**	**0.0713**	**0.0546**	**0.0334**	**0.0255**	**0.0079**

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration. The maximum values from above are shown in the summary table in Section 5.4.4.

Table 84. Surrogate Estradiol Compound Acute and Chronic 90th Percentile Limnetic Concentrations from EPA Index Reservoir Models from Application of Manure to No-Till Soil (ng/L)

Scenario	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
CA corn	0.0314	0.0299	0.0250	0.0177	0.0142	0.0046
CA cotton	0.0078	0.0074	0.0064	0.0039	0.0026	0.0009
IL corn	0.0229	0.0218	0.0178	0.0119	0.0092	0.0027
KS sorghum	0.0184	0.0173	0.0139	0.0090	0.0068	0.0021
MS corn	0.0892	0.0830	0.0617	0.0355	0.0259	0.0071
MS cotton	0.0600	0.0557	0.0413	0.0231	0.0168	0.0046
MS soybean	0.0349	0.0326	0.0276	0.0183	0.0137	0.0038
NC cotton	0.0284	0.0267	0.0206	0.0128	0.0098	0.0030
NC1 corn E	0.0240	0.0228	0.0184	0.0121	0.0093	0.0028
NC2 corn W	0.0451	0.0425	0.0342	0.0221	0.0169	0.0050
ND corn	0.0077	0.0074	0.0061	0.0050	0.0047	0.0021
ND wheat	0.0053	0.0051	0.0042	0.0031	0.0029	0.0014
OH corn	0.0590	0.0563	0.0478	0.0273	0.0182	0.0081
OR wheat	0.0010	0.0010	0.0008	0.0006	0.0005	0.0001
PA corn	0.0307	0.0292	0.0247	0.0165	0.0128	0.0039
TX sorghum	0.0965	0.0892	0.0681	0.0398	0.0291	0.0081
TX wheat	0.0580	0.0532	0.0410	0.0252	0.0193	0.0049
Maximum	**0.0965**	**0.0892**	**0.0681**	**0.0398**	**0.0291**	**0.0081**

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration. The maximum values from above are shown in the summary table in Section 5.4.4.

Table 85. Surrogate Trenbolone Compound Acute and Chronic 90th Percentile Limnetic Concentrations from EPA Farm Pond Models from Application of Manure to No-Till Soil (ng/L)

Scenario	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
CA corn	0.1904	0.1824	0.1561	0.1203	0.1124	0.0650
CA cotton	0.0976	0.0937	0.0832	0.0524	0.0467	0.0261
IL corn	0.1645	0.1584	0.1390	0.1054	0.0896	0.0401
KS sorghum	0.1118	0.1069	0.0907	0.0656	0.0541	0.0215
MS corn	0.5980	0.5714	0.4791	0.3492	0.2883	0.1106
MS cotton	0.6993	0.6683	0.5515	0.3842	0.3115	0.1138
MS soybean	0.2657	0.2548	0.2239	0.1772	0.1482	0.0553
NC1 corn E	0.1416	0.1359	0.1165	0.0880	0.0747	0.0326
NC2 corn W	0.3000	0.2873	0.2471	0.1855	0.1568	0.0672
NC cotton	0.2975	0.2862	0.2438	0.1829	0.1542	0.0616
ND corn	0.0584	0.0579	0.0562	0.0534	0.0521	0.0202
ND wheat	0.0565	0.0560	0.0543	0.0516	0.0504	0.0211
OH corn	0.4170	0.4010	0.3590	0.2150	0.2010	0.1110
OR wheat	0.0111	0.0106	0.0092	0.0073	0.0065	0.0037
PA corn	0.2097	0.2013	0.1781	0.1344	0.1135	0.0474
TX sorghum	0.3713	0.3512	0.2912	0.2011	0.1614	0.0566
TX wheat	0.5902	0.5560	0.4631	0.3338	0.2818	0.0976
Maximum	**0.6993**	**0.6683**	**0.5515**	**0.3842**	**0.3115**	**0.1138**

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration. The maximum values from above are shown in the summary table in Section 5.4.5

Table 86. Surrogate Trenbolone Compound Acute and Chronic 90th Percentile Limnetic Concentrations from EPA Index Reservoir Models from Application of Manure to No-Till Soil (ng/L)

Scenario	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
CA corn	0.2766	0.2674	0.2351	0.1817	0.1532	0.0572
CA cotton	0.0772	0.0745	0.0674	0.0424	0.0304	0.0133
IL corn	0.2328	0.2242	0.1920	0.1392	0.1124	0.0383
KS sorghum	0.2391	0.2288	0.1936	0.1385	0.1099	0.0363
MS corn	0.8601	0.8101	0.6344	0.3976	0.2995	0.0862
MS cotton	0.5442	0.5132	0.4049	0.2506	0.1884	0.0543
MS soybean	0.3506	0.3328	0.2901	0.2071	0.1611	0.0477
NC cotton	0.1994	0.1915	0.1629	0.1171	0.0946	0.0312
NC1 corn E	0.4357	0.4162	0.3518	0.2470	0.1969	0.0640
NC2 corn W	0.2296	0.2196	0.1815	0.1256	0.1004	0.0338
ND corn	0.0807	0.0798	0.0763	0.0700	0.0662	0.0259
ND wheat	0.0487	0.0471	0.0442	0.0404	0.0381	0.0164
OH corn	0.6030	0.5820	0.5220	0.3000	0.2220	0.0990
OR wheat	0.0097	0.0093	0.0081	0.0063	0.0054	0.0017
PA corn	0.3026	0.2914	0.2591	0.1900	0.1546	0.0527
TX sorghum	0.8095	0.7658	0.6303	0.4154	0.3194	0.0962
TX wheat	0.5366	0.5050	0.4165	0.2846	0.2278	0.0589
Maximum	**0.8601**	**0.8101**	**0.6344**	**0.4154**	**0.3194**	**0.0990**

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration. The maximum values from above are shown in the summary table in Section 5.4.5

Appendix 8. Mixed-Use Watershed Models

Appendix 8 describes the assumptions, data, calculations, and results of the mixed-use watershed modeling. Appendix 8.1 describes assumptions, methods, and calculations used to determine the amount of the surrogate estradiol and trenbolone compounds contained in feedlot manure pack. Appendix 8.2 describes the assumptions, data, methods, and calculations used to determine the application rate of the surrogate estradiol and trenbolone compounds applied to cropland. Appendix 8.3 presents the PEC_{water} results for the five mixed-use watersheds simulated. The mixed-use models are described in Section 5.7.

Appendix 8.1. Application rate to feedlot manure pack

To calculate conservative concentrations of EB and TBA metabolites deposited in the feedlot, it was assumed that 100% of the cattle have an implant 365 days of the year and the feedlots are stocked to capacity at all times. The daily release rates of estradiol and trenbolone from the implants were used for this calculation because they are the best estimates of daily release rates into cattle feces and urine. In order to use the estimates of concentration in the manure in the feedlot model, an application rate per acre at a specified depth and density must first be determined.

For the calculation of application rate, a manure depth of 10 cm [97, 98] was assumed. Because runoff and erosion will occur principally at the top layer of the manure where the density is the lowest, a density of 1.0 g cm^{-3} [97] was used. The fixed concentrations of estradiol and trenbolone were determined by the amount of drug excreted in 130 days from cattle stocked at a density of 270 head per acre (Section 5.7.7.1).

In a study by Mielke et al. [97], the physical properties of feedlot soil were studied. There are essentially three layers: the top organic layer, a mineral interface and the underlying soil. The authors found that the feedlot soil surface becomes compacted by cattle movement and water infiltration is essentially zero. The mixing of organic matter from manure was generally limited to the first few centimeters of soil. The bulk density at the surface of the feedlot was generally <0.8 g cm^{-3} and had a depth of approximately 10 cm, but the density increased with depth (see Figure 2 of reference [97]). The interface layer was 1.7 g cm^{-3} and the soil layer was 1.4-1.6 g cm^{-3}. The density of the top organic matter (manure) layer ranged from 0.75-0.93 g cm^{-3} (from three Nebraska feedlots) [97]. In another study of a Nebraska feedlot, the top layer of feedlot soil had a mean density of 1.04 g cm^{-3} [98], the interface layer was 1.42 g cm^{-3}, and the soil layer was 1.45-1.62 g cm^{-3}. The depth of the top manure layer was approximately 9 cm as shown in Figure 1 of reference [98].

The concentrations of estradiol (6.6 µg/kg) and trenbolone (50.8 µg/kg) in feedlot manure pack calculated in Table 87 and Table 88, respectively, represent conservative estimates of potential concentrations because degradation in the feedlot and feedlot soils were not factored into the calculations. These values were used as the input parameters for the concentration of estradiol and trenbolone on the feedlot surface in the mixed-use watershed models. For the modeling, it was assumed that a constant concentration of drug was deposited onto the feedlot surface each day; therefore, any drug lost through erosion runoff was replaced daily to maintain the same concentration.

Table 87. Estradiol Concentration in Feedlot Manure Pack Surface and Application Rate per Acre of Feedlot

Daily release rate 17α-E2/AU	0.0759 mg 17α-E2/day/AU *		
Metabolism adjustment 100%	0.0759 x 100%	=	0.0759 mg 17α-E2/day/AU
Days between manure collection	130 days		
Cattle per acre	270 AU/acre		
Total drug excreted per acre in 130 days	0.0759 mg 17α-E2/day/AU x 130 days x 270 AU/acre	=	2,664 mg/acre
17α-Estradiol per acre		**=**	**2.66 g/acre**
Estradiol concentration in manure pack			
Bulk density manure pack	1.0 g/cm^3 = 1.0 x 106 g/m^3	=	1000 kg/m^3
Manure depth	10 cm	=	0.10 m
Volume of 1 acre feedlot at 10 cm depth	0.10 m x 4047 m^2/acre	=	404.7 m^3
Weight of 1 acre feedlot at 10 cm depth	1000 kg/m^3 x 404.7 m^3	=	404,700 kg
17α-E2/kg manure pack	2,664 mg/acre/404,700 kg	=	0.0066 mg/kg
		=	6.6 µg/kg

* Daily release rate of drug from implant from Appendix 6.1.

Table 88. Trenbolone Concentration in Feedlot Manure Pack Surface and Application Rate per Acre of Feedlot

Daily release rate 17α-TB/AU	0.8193 mg 17α-TB/day/AU *		
Metabolism adjustment 71.5%	0.8193 x 71.5%	=	0.5858 mg 17α-TB/day/AU
Days between manure collection	130 days		
Cattle per acre	270 AU/acre		
Total drug excreted per acre in 130 days	0.5858 mg 17α-TB/day/AU x 130 days x 270 AU/acre	=	20,562 mg/acre
17α-Trenbolone per acre		**=**	20.56 g/acre
Trenbolone concentration in manure pack			
Bulk density manure pack	1.0 g/cm^3 = 1.0 x 106 g/m^3	=	1000 kg/m^3
Manure depth	10 cm	=	0.10 m
Volume of 1 acre feedlot at 10 cm depth	0.10 m x 4047 m^2/acre	=	404.7 m^3
Weight of 1 acre feedlot at 10 cm depth	1000 kg/m^3 x 404.7 m^3	=	404,700 kg
17α-TB/kg manure pack	20,562 mg/acre/ 404,700 kg	=	0.0508 mg/kg
		=	50.8 µg/kg

* Daily release rate of drug from implant from Appendix 6.1.

Appendix 8.2. Manure application rate to cropland in mixed-use watershed models

The major crops in each of the five regions modeled in the mixed-use watershed were identified in the GIS survey of these areas and are available in Attachment 1. As described in Appendix 4.2, the manure application rates are calculated based on 1) the crop to be grown, 2) the nutrient requirement of the crop and anticipated crop yield, and 3) the nutrient concentration present in the manure and soil. Specific crop yields were obtained for each crop in the five regions to be modeled in the mixed-use watershed. These crops and their historic county yields are summarized in Table 89. The highest yield for that area is in bold text in the table and was the yield value used in Table 90 to calculate the manure application rate based on the P_2O_5 requirement of the crop. The highest yield was used because its use would result in more manure containing the surrogate estradiol and trenbolone compounds being applied per acre, and therefore, is more conservative. The acres per animal required to dispose of the yearly manure produced are also reported in Table 90. In each region, corn was the most conservative of all the crop scenarios evaluated because it required the most manure per acre. Therefore, in the mixed-use watershed models, corn was used as the crop to determine the manure application rate. In Iowa, Michigan, Texas and Ohio, the application rates of corn for grain were used because corn for silage is not a major crop in that region (Table 89). In Pennsylvania, corn for silage was used because it is the dominant crop. The manure application rates used in the modeling are shown in bold in Table 90.

Table 89. Historic Crop Yields from USDA Database, 2007 Census of Agriculture, and 2008 or 2010 Regional Yield Maps

Crop	Michigan Huron County	Ohio Mercer County	Pennsylvania Lancaster County	Texas Castro County	Iowa Lyon, Sioux Counties
Corn (bushel/acre)	*2006-2010*¶ 167, 147, 174, 168, 173 Region-**175**§	*2006-2010* 160, 148, 143, 152, 156 Region-**175**	*2006-2010* 160, 160, 169, **178**, 159 Region-175	*2006-2010* 203, 213, 221, 208, **225** Region-200	*2006*-164, 163 *2007*-164, 157 *2008*-190, 195 *2009*-194, **203** *2010*-195, 197 Region-200
% Acres corn harvested as Silage	20.4% †	13.6% †	40.1% †	14.5% †	5.8%, 8.4% †
Alfalfa (ton/acre)	*2007*-**4.1**† Region-NA*	*2007*-3.7 † 2010- 4.2 Region-**4.9**	2006-2010 4.2, 4.2, **4.2**, 3.7, 3.7 Region-3.9	*2007*-**4.2** † Region-NA	*2006*-4.8, 4.4 *2007*-4.1, 4.8 *2008*-NA, NA *2009*-NA, 4.5 *2010*-NA, 4.2 Region-**4.9**
Winter wheat (bushel/acre)	*2006-2010* 86, 80, 88, 88, 86 Region-**90**	*2006-2010* 72, 61, 71, **76**, 55 Region-70	*2006-2010* 76, 66, 79, 69, 85 Region-**90**	*2006-2010* 38, 45, 46, **49**, 37 Region-NA	*2006*, *2009-10*- NA *2007*-47, 54 † *2008*-75, 78 Region-NA
Sorghum (bushel/acre)	NA	NA	2007-**103**† Region-NA	2006-2010 60, 61, 52, 68, 60 Region-**100**	NA
Soybean (bushel/acre)	*2006-2010* 50, 45, 49, 40, 46 Region-**50**	*2006-2010* 49, 50, 38, 42, 52 Region-**60**	*2006-2010* 43, 49, 45, **56**, 50 Region-55	*2007-2010*-NA Region-**40**	*2006*-55, 56 *2007*-55, 54 *2008*- 51, 55 *2009*-54, 55 *2010*-55,57 Region-**60**
Sugar beet (tons/acre)	*2006-2010* 25, 25, **29**, 26, 29 Region-28.9	NA	NA	NA	NA
Dry edible bean (lb/acre)	*2006-2010* **2180**, 1740, 2030, 1880, 1890 Region-2100	NA	NA	NA	NA
Cotton (lb/acre)	NA	NA	NA	*2006-2010* 1075, **1225**, 740, 988, 1076 Region-1,200	NA

¶ Year is in italics. Normal font text is yield. Bold font text is highest yield and is the value used in subsequent calculations * NA- Data were either not available on USDA Quick Stats web site or in USDA county surveys. Data for years 2006-2010 were first searched in USDA Quick stats web site, but if they were unavailable, they were calculated from other survey tables of USDA agricultural census of 2007, or other tables available on USDA web site. Data from Table 25 of 2007 USDA census are marked with †. Supporting documents are supplied [210]. § Regional data are from the USDA maps of counties [193].

Table 90. Application Rates of Trenbolone and Estradiol Contained in Beef Cattle Manure Applied to Agricultural Fields Based on P_2O_5 Requirements and Acres of Land Required Per Animal for the Mixed-use Watershed Models and Crops in Section 5.7.

State	Crop (yield)	Expected Yield Table 89	P_2O_5 Removal by Crop (lb/yield) Table 67	P_2O_5 Application Rate (kg/acre)	Trenbolone Application Rate† (mg/acre)	Estradiol Application Rate† (mg/acre)	Acres Required Per Feedlot Animal Unit AU
IA	**Corn Grain (bu)**	**203**	**0.39**	**35.99***	**280.69**	**36.71**	**0.758 §**
	Corn Silage (bu)	203	0.63	58.13	453.43	59.29	0.469
	Wheat + Straw (bu)	78	0.71	25.17	196.35	25.68	1.083
	Soybean (bu)	60	0.87	23.73	185.07	24.20	1.149
	Alfalfa (tons)	4.9	13.5	30.07	234.53	30.67	0.907
PA	Corn Grain (bu)	178	0.39	31.55	246.13	32.19	0.864
	Corn Silage (bu)	**178**	**0.63**	**50.97**	**397.59**	**51.99**	**0.535**
	Wheat + Straw (bu)	90	0.71	29.05	226.55	29.63	0.939
	Soybean (bu)	56	0.87	22.15	172.73	22.59	1.231
	Alfalfa (tons)	4.2	13.5	25.77	201.03	26.29	1.058
	Sorghum (bu)	103	0.42	19.66	153.38	20.06	1.387
OH	**Corn Grain (bu)**	**175**	**0.39**	**31.02**	**241.98**	**31.64**	**0.879**
	Corn Silage (bu)	175	0.63	50.11	390.89	51.12	0.544
	Wheat + Straw (bu)	76	0.71	24.53	191.31	25.02	1.112
	Soybean (bu)	60	0.87	23.73	185.07	24.20	1.149
	Alfalfa (tons)	4.9	13.5	30.07	234.53	30.67	0.907
MI	**Corn Grain (bu)**	**175**	**0.39**	**31.02**	**241.98**	**31.64**	**0.879**
	Corn Silage (bu)	175	0.63	50.11	390.89	51.12	0.544
	Wheat + Straw (bu)	90	0.71	29.05	226.55	29.63	0.939
	Soybean (bu)	50	0.87	19.77	154.23	20.17	1.379
	Alfalfa (tons)	4.1	13.5	25.16	196.24	25.66	1.084
	Dry Bean (lb)	2180	0.012	11.89	92.75	12.13	2.293
	Sugar Beets (tons)	29	2.1	27.68	215.92	28.24	0.985
TX	**Corn Grain (bu)**	**225**	**0.39**	**39.89**	**311.11**	**40.68**	**0.684**
	Corn Silage (bu)	225	0.63	64.43	502.57	65.72	0.423
	Wheat + Straw (bu)	49	0.71	15.81	123.35	16.13	1.724
	Soybean (bu)	40	0.87	15.82	123.38	16.13	1.724
	Alfalfa (tons)	4.2	13.5	25.77	201.03	26.29	1.058
	Sorghum (bu)	100	0.42	19.09	148.91	19.47	1.428
	Cotton (lb)	1225	0.025	13.92	108.58	14.20	1.959
	Cotton + Stover (lb)	1225	0.066	37.75	286.65	37.49	0.742

* Example calculation: (203 bu x 0.39 lb P_2O_5/bu) / 2.2 lb/kg = 35.99. † See Table 69 and Table 70 for example calculations of application rates. The concentration of trenbolone or estradiol metabolites in manure (7.8 mg/kg P_2O_5 for trenbolone and 1.02 mg/kg P_2O_5 for estradiol derived in Appendix 6.3). § An example of the acreage requirement from the yearly production of manure from one AU is given in Table 70 and here (365 days x 0.0747 kg P_2O_5/day) / 35.99 kg = 0.758 acres/AU.

Appendix 8.3. PEC_{water} results of simulations of beef cattle watersheds in five regions of the US (see Attachment 1 for full report)

The methodology used to simulate mixed-use watersheds in five regions of the US was presented in Section 5.7. The watersheds had simultaneous runoff inputs from feedlots (containing <1000 head), pastureland, manured crop land, and cropland irrigated with feedlot runoff pond water. A schematic of a representative watershed is shown in Figure 20. The data presented below are summary tables of peak and chronic PEC_{water} values of the surrogate estradiol and trenbolone compounds in the regions that were modeled (Table 91 through Table 100).

The PEC_{water} values are also reported for various percentages of AFOs (with <1000 AU) out of compliance with EPA regulations (i.e., 0, 25, 50, 75, and 100% of AFOs <1000 AU directly discharging to surface water). It was assumed that all large CAFOs would be in compliance with EPA's regulations. The data summarized in Table 91 through Table 100 demonstrate the influence that direct discharge from AFOs with <1000 AU has on the PEC_{water} values. The PEC_{water} values for 25% and 50% of AFOs with direct discharge to surface water were used in the risk characterization (Section 7). In addition, the PEC_{water} results for runoff from pastureland containing Synovex ONE-implanted pasture cattle are reported in Table 91 through Table 100 as well. The full report and methodology used in these simulations is found in Attachment 1. All assumptions used in the simulations are detailed in Section 5.7 and Attachment 1.

Appendix 8.3.1. Iowa mixed-use watershed model

Table 91. Predicted Environmental Concentrations in Water (PEC_{water}) for the Surrogate Estradiol Compound in the Iowa Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90th Percentile PEC_{water} (ng/L) for the Surrogate Estradiol Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	0.235	0.228	0.216	0.195	0.182	0.139
75% of Feedlots	0.180	0.174	0.165	0.148	0.139	0.105
50% of Feedlots	0.122	0.119	0.111	0.099	0.094	0.070
25% of Feedlots	0.067	0.065	0.060	0.054	0.050	0.037
0% of Feedlots	0.017	0.017	0.015	0.012	0.009	0.003
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.032	0.030	0.025	0.019	0.016	0.007

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

Table 92. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Trenbolone Compound in the Iowa Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90th Percentile PEC_{water} (ng/L) for the Surrogate Trenbolone Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	2.550	2.510	2.417	2.265	2.143	1.769
75% of Feedlots	1.951	1.920	1.854	1.731	1.645	1.345
50% of Feedlots	1.330	1.309	1.264	1.182	1.123	0.905
25% of Feedlots	0.737	0.724	0.699	0.666	0.623	0.484
0% of Feedlots	0.164	0.159	0.150	0.120	0.102	0.048
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.324	0.314	0.277	0.228	0.203	0.107

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

Appendix 8.3.2. Ohio mixed-use watershed model

Table 93. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Estradiol Compound in the Ohio Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90th Percentile PEC_{water} (ng/L) for the Surrogate Estradiol Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	0.183	0.181	0.176	0.160	0.155	0.120
75% of Feedlots	0.138	0.136	0.132	0.120	0.116	0.090
50% of Feedlots	0.092	0.091	0.088	0.080	0.078	0.060
25% of Feedlots	0.047	0.046	0.044	0.041	0.040	0.031
0% of Feedlots	0.007	0.007	0.006	0.004	0.003	0.001
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.032	0.031	0.026	0.019	0.018	0.011

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

Table 94. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Trenbolone Compound in the Ohio Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90th Percentile PEC_{water} (ng/L) for the Surrogate Trenbolone Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	1.943	1.901	1.831	1.763	1.696	1.418
75% of Feedlots	1.461	1.429	1.375	1.325	1.275	1.066
50% of Feedlots	0.979	0.958	0.920	0.888	0.852	0.713
25% of Feedlots	0.498	0.487	0.464	0.450	0.430	0.361
0% of Feedlots	0.069	0.067	0.058	0.042	0.032	0.014
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.343	0.331	0.295	0.231	0.208	0.132

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

Appendix 8.3.3. Michigan mixed-use watershed model

Table 95. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Estradiol Compound in the Michigan Mixed-Use Watershed

% of Feedlots <1000 AU that Discharge to Surface Water	90th Percentile PEC_{water} (ng/L) for the Surrogate Estradiol Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	0.080	0.078	0.072	0.068	0.065	0.051
75% of Feedlots	0.060	0.059	0.054	0.051	0.049	0.039
50% of Feedlots	0.040	0.040	0.037	0.034	0.033	0.026
25% of Feedlots	0.021	0.021	0.019	0.017	0.017	0.013
0% of Feedlots	0.003	0.003	0.002	0.002	0.001	0.001
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.026	0.025	0.021	0.016	0.014	0.008

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

Table 96. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Trenbolone Compound in the Michigan Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90th Percentile PEC_{water} (ng/L) for the Surrogate Trenbolone Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	0.825	0.813	0.774	0.731	0.722	0.595
75% of Feedlots	0.622	0.613	0.585	0.550	0.543	0.448
50% of Feedlots	0.421	0.414	0.396	0.368	0.364	0.301
25% of Feedlots	0.221	0.217	0.207	0.189	0.185	0.154
0% of Feedlots	0.030	0.029	0.026	0.021	0.016	0.011
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.293	0.282	0.248	0.207	0.186	0.106

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

Appendix 8.3.4. Pennsylvania mixed-use watershed model

Table 97. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Estradiol Compound in the Pennsylvania Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90th Percentile PEC_{water} (ng/L) for the Surrogate Estradiol Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	0.110	0.106	0.101	0.097	0.093	0.073
75% of Feedlots	0.082	0.080	0.076	0.073	0.070	0.055
50% of Feedlots	0.055	0.054	0.051	0.048	0.047	0.037
25% of Feedlots	0.028	0.027	0.025	0.024	0.023	0.019
0% of Feedlots	0.002	0.002	0.001	0.001	0.001	0.000
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.017	0.016	0.013	0.010	0.008	0.005

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

Table 98. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Trenbolone Compound in the Pennsylvania Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90th Percentile PEC_{water} (ng/L) for the Surrogate Trenbolone Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	1.141	1.123	1.067	1.009	0.993	0.841
75% of Feedlots	0.861	0.845	0.803	0.761	0.746	0.632
50% of Feedlots	0.578	0.567	0.539	0.512	0.498	0.422
25% of Feedlots	0.295	0.290	0.274	0.258	0.251	0.215
0% of Feedlots	0.016	0.015	0.013	0.010	0.008	0.004
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.181	0.172	0.150	0.120	0.104	0.062

† The peak concentration is the 90th percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90th percentile values of the yearly maximum moving average concentration.

Appendix 8.3.5. Texas mixed-use watershed model

Table 99. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Estradiol Compound in the Texas Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90^{th} Percentile PEC_{water} (ng/L) for the Surrogate Estradiol Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	0.032	0.030	0.024	0.016	0.013	0.005
75% of Feedlots	0.031	0.029	0.023	0.016	0.013	0.005
50% of Feedlots	0.031	0.029	0.023	0.015	0.012	0.004
25% of Feedlots	0.030	0.028	0.023	0.015	0.012	0.004
0% of Feedlots	0.030	0.028	0.022	0.015	0.012	0.003
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.028	0.026	0.020	0.013	0.011	0.005

† The peak concentration is the 90^{th} percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90^{th} percentile values of the yearly maximum moving average concentration.

Table 100. Predicted Environmental Concentrations in water (PEC_{water}) for the Surrogate Trenbolone Compound in the Texas Mixed-Use Watershed Model

% of Feedlots <1000 AU that Discharge to Surface Water	90^{th} Percentile PEC_{water} (ng/L) for the Surrogate Trenbolone Compound					
	Peak †	96-Hour	21-Day	60-Day	90-Day	Annual
100% of Feedlots	0.293	0.280	0.237	0.174	0.146	0.057
75% of Feedlots	0.288	0.275	0.232	0.169	0.142	0.052
50% of Feedlots	0.282	0.268	0.226	0.164	0.136	0.047
25% of Feedlots	0.276	0.263	0.221	0.159	0.132	0.042
0% of Feedlots	0.270	0.257	0.215	0.154	0.127	0.037
Pasture Runoff Results						
100% Pasture 0% of Feedlots	0.273	0.259	0.222	0.154	0.141	0.062

† The peak concentration is the 90^{th} percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90^{th} percentile values of the yearly maximum moving average concentration.

Appendix 8.3.6. Individual contributions from combined inputs

An analysis was conducted to estimate the contribution of each exposure pathway in a watershed. The IA study region was selected for this analysis because the models predicted that IA would have the highest PEC_{water} value for the surrogate trenbolone compound. The mixed-use watershed model was run four times, each time with a single contributing source: feedlot, cropland with solid manure, cropland with collection pond water, and pasture. The PCA used for the each contributing source was kept the same as the initial mixed-use watershed modeling, namely, 0.094%, 50.96%, 5.66%, and 2.29%, respectively. However, when one source was run, the PCA for other sources were kept at zero to represent a non-contributing source. The table below summarizes trenbolone concentrations estimated from the IA study region watershed assuming there is only one contributing source in the watershed.

Table 101. Individual Contribution of Each Source to the Predicted Environmental Concentration in Water (PEC_{water}) for the Surrogate Trenbolone Compound in the Iowa Watershed

	90^{th} percentile PEC_{water} for the Surrogate Trenbolone Compound in the Iowa Watershed (ng/L)					
Contributing Source	**Peak †**	**96-Hour**	**21-Day**	**60-Day**	**90-Day**	**Annual**
Feedlot	2.447	2.409	2.318	2.181	2.097	1.746
Cropland - solid manure	0.155	0.150	0.141	0.113	0.094	0.042
Cropland - collection pond water	0.052	0.051	0.050	0.045	0.038	0.014
Pasture	0.007	0.007	0.006	0.005	0.005	0.002

† The peak concentration is the 90^{th} percentile of the yearly peak concentration for the 30-year simulation. The 96-hour, 21-day, 60-day, and 90-day values are the 90^{th} percentile values of the yearly maximum moving average concentration.

Note that these concentrations cannot be added such that the sum of the 21-day 90^{th} percentile concentrations for each source will add up to 2.417 ng/L (which is the highest surrogate trenbolone compound concentration from a scenario where 100% of all feedlots with <1000 AU in IA are directly discharging into surface waters; Table 92).

If only feedlot runoff contributed to surface water in the IA study region, the 21-day 90^{th} percentile PEC_{water} for the surrogate trenbolone compound would be 2.318 ng/L (Table 101). If only pasture runoff contributed to the water bodies of the IA study region watershed, the PEC_{water} for the surrogate trenbolone compound would be 0.006 ng/L. In addition, both sources of manured cropland (solid and pond water irrigation) resulted in a PEC_{water} of 0.191 ng/L. Therefore, concentrations from individual sources indicate that direct runoff from a feedlot is the major contributor from all sources within the watershed modeled even though the feedlot PCA is the smallest.

Appendix 9. Estimation of Feedlots in Need of Runoff Control Improvements

As discussed in Section 3.2.1, the Clean Water Act prohibits the discharge of pollutants from CAFOs into US waters without a NPDES permit. Any permit issued to a CAFO must include a CNMP that covers requirements listed under 40 CFR 122.42(e)(1) and 40 CFR 412(e)(5), including containment of wastewater runoff from the feedlot production area (i.e., no direct discharge of feedlot wastewater to surface waters). Therefore, in Section 3 of the EA, we assumed that large CAFOs with >1000 AU are collecting their wastewater runoff and will not directly discharge to surface waters.

However, small and medium AFOs[gg] with <1000 cattle are not legally required to meet the design criteria of CAFOs (Section 3.2.1) unless they are defined or designated as a medium CAFO (300-999 AU) or designated as a small CAFO (<300 AU). Some medium AFOs may be defined as a CAFO if they are found to discharge pollutants into surface waters (Section 3.2.1). In addition, some small AFOs and medium AFOs may be designated as a CAFO by local and state environmental enforcement and NPDES authorities if the AFO has the potential to impact surface waters, which would require the small and medium AFO to adhere to the criteria and BMPs that apply to a CAFO. Although it is anticipated that many, if not most, AFOs (<1000 cattle) are voluntarily employing some of these BMPs, such as a runoff controls, there is still a possibility that some AFOs will be significant polluters and are not yet designated as CAFOs; i.e., some small or medium AFOs may directly discharge wastewater to surface water. Therefore, in this Appendix, it was conservatively assumed that AFOs with <1000 AU do not have any runoff controls and directly discharge to surface water. This assumption is very conservative because it is expected that some medium AFOs are defined or designated as CAFOs and some small AFOs are likely designated.

At this time, there are no estimates available for the number or percentage of facilities that do not employ such controls (or are directly discharging to surface water). There are, however, USDA estimates of the number of AFOs that were in need of runoff control improvements in 1997 [8]. In this Appendix, using the 1997 USDA data (the most recent data available), we estimate the percentage of AFOs that may be in need of runoff control improvements. It is important to note that, although these small or medium AFOs may be in need of runoff control improvement, it does not necessarily mean that they are directly discharging to surface waters. However, in order to estimate a conservative percentage for use in the EA, we have assumed that all AFOs in need of runoff control improvements are directly discharging to surface waters.

Appendix 9.1. Estimation of the number of beef cattle in small and medium AFOs and large CAFOs

In order to determine the number of AFOs that may be directly discharging to surface waters, it is important to first determine the number and proportion of small and medium AFOs and large CAFOs in the US. Table 102 below contains the distribution of beef cattle production in the US depending on the size of the AFO or CAFO. This table was developed using data from the 2007 USDA Census of Agriculture, which is contained in Table 5 of Section 3.2.2. Based on the data presented in Table 5, the following conclusions can be made:

[gg] Note: An AFO can describe any size of animal feedlot; however, a CAFO is a specific type of AFO as defined under EPA's regulations. However, for the purpose of the discussion in this Appendix, an AFO is defined as having <1000 AU. See Section 3.2.1 for EPA's definition of an AFO.

- Farms with <35 cattle represent approximately 1.62% of the cattle population [0.47% + 0.5% + (1.29%/2)], but 66.7% of the total number of farms [34,543 + 10,574 + (11,736/2)].
- Farms with 35-350 head represent approximately 6.72% of the cattle population [(1.29%/2) + 1.63% + 2.30% + (4.29%/2)] and 25.1% of the total farms [(11,736/2) + 6,579 + 4,710 + (3,975/2)].
- Farms with 350-999 head represent approximately 7.14% of the cattle population [(4.29%/2) + 4.99%] and 5.2% of the total farms [(3,975/2) + 1,980].
- Farms with ≥1000 AU house 84.5% of all beef cattle on only 3% of the farms.

The data presented above are summarized in Table 102, and demonstrates that 85% of cattle sent to market are produced on only 3% of the farms.

Table 102. Distribution of US Beef Cattle Production by Farm Size*

Farm Size Category	Farm Size, AU	Cattle Marketed	% of Marketed	Number of Farms	% of Number of Farms
-	<35 AU	447,016	1.62%	50,975	66.7%
Small	35-349 AU	1,853,939	6.72%	19,145	25.1%
Medium	350-999 AU	1,967,925	7.13%	3,968	5.2%
Large	≥1000 AU	23,327,049	84.53%	2,299	3.0%
	Total	27,595,929	100.00%	76,387	100.0%

* Data are recalculations and derived from Table 5 and text from above.

Appendix 9.2. Magnitude of feedlot runoff issues at the time that the CAFOs regulations were proposed under the Clean Water Act

In 2003, the USDA conducted a survey based on the 1997 agricultural census to estimate the cost associated with bringing AFOs into compliance with the NPDES regulations, which included implementing a CNMP and installing improvements for manure management and runoff control [8]. The data in this survey were used to estimate the percentage of small and medium AFOs in the US that may be in need of runoff control improvements (i.e., could potential directly discharge to surface waters). This survey also contains information on manure management practices in different regions of the US along with estimates of a typical farm size and the number of cropland acres manured each year by a typical facility.

In the survey, USDA estimated the number of farms with >35 AU that would have to implement a CNMP. In the survey (page 27 of reference [8]), farms with <35 AU were considered too small to model because the diversity of their production was too great (small lots to pasture settings) and they did not produce much recoverable manure.[hh] To be consistent with the USDA approach, farms with <35 AU were excluded from the estimation of the feedlots in need of runoff control.

[hh] Based on the information in Table 102, AFOs holding <35 AU contain only 1.62% of all market beef cattle in the US, and therefore, would be expected to generate a similar percentage of total beef cattle manure in the US.

At the time of the survey (1997), there were 110,620 beef cattle operations in the US (Table 25 of reference [6]). Based on the survey, it was estimated that of those beef cattle AFOs:

- 10,159 of operations having >35 AU would need a CNMP (Table 1 of reference [8])
- 4,448 of operations having >300 AU would need a CNMP (Table A-6 of reference [8])
- 1,766 of operations having ≥1000 AU would need a CNMP (Table A-6 of reference [8])

The document did not indicate whether these feedlots were significant contributors of pollutants to surface waters, just that there was some aspect of their manure management practices that may have needed improvement to meet the requirements under the NPDES regulations.

The number of large CAFOs >1000 AU (1,766 CAFOs) needing runoff control improvements is consistent with a report from EPA where the "number of facilities that EPA expects will be designated as CAFOs by the permitting authority because they are significant contributors to water quality impairment" (pages 3-5 of reference [211]). However, the EPA estimated that only 174 operations with 300-1000 AU would be designated as CAFOs (pages 3-5 and Table 3-2 of reference [211]) compared with the 4,448 value from the USDA estimates presented above.

The following percentage factors were used by USDA to estimate the number of AFOs in need of improvements for contaminated water (page 79 of reference [8]):

> *"It was judged that the majority of fattened cattle and confined heifer operations would need contaminated water diversions. CNMP needs were assigned as follows:*
>
> - *55 percent for confined heifer and fattened cattle farms with a scrape and stack manure handling system in the South and West*
> - *40 percent for confined heifer and fattened cattle farms with a scrape and stack manure handling system in the Midwest and the Northeast*
> - *60 percent of the smaller fattened cattle operations with manure pack*
> - *50 percent of the larger fattened cattle operations with manure pack"*

Therefore, of the 10,159 beef cattle farms (with >35 AU) projected in the US that needed runoff control improvements in 1997 (see above), roughly half of them would need improvements for runoff control (10,159 / 2 = 5,080 AFOs). This estimate suggests that approximately 17% of the US beef cattle AFOs with >35 AU (5,080 AFOs / total of 29,412 AFOs with >35 AU; Table 102) are in need of runoff control improvements.

It is important to note that the needed runoff control improvements to these AFOs could include one or more of the following: the installation of a runoff collection lagoon, use of a buffer strip(s), installation of a water diversion structures, or covering the manure storage area. However, for the purposes of this EA, we conservatively assumed that these farms were in need of some type of runoff control improvement(s) and were directly discharging to surface waters.

Appendix 9.3. Farm consolidations

The number of farms in need of runoff control improvements estimated above in Appendix 9.2 was based on farm inventory data contained in the 1997 USDA Agricultural Census. Since 1997, there has been a shift in the distribution of beef cattle operations in the US, with a reduction in the number of small operations. This shift is can be seen when comparing the number of farms reported in both the 1997 and 2007 USDA Agricultural Census (the 1997 and 2007 Census data is summarized in Table 5; Section 3.2.2).

The USDA manure survey [8] indicated that the highest number of farms needing improvements were in the >35-300 AU range followed by the 300-1000 AU range. Based on the data presented in Table 5, the following conclusions can be made regarding the shift in the number of AFOs in 1997 and 2007:

- There was a reduction of 10,746 (32%) small and medium AFOs holding 20-49, 50-99 and 100-199 AU.
- The number of AFOs holding 200-999 AU remained relatively stable.
- The number of CAFOs with ≥1000 AU increased by approximately 20%.
- Overall, there was a reduction in the number of farms in the >35 AU range from 40,932 to 31,279 between 1997 and 2007, respectively, resulting in a net loss of 24% of farms with >35 AU (9,653 farms).

We would expect that the reductions in the number of small farms would likely include a disproportionate (larger) percentage of those in need of runoff control improvements; thus, reducing the estimate of 17% that was based on the 1997 USDA Census data. However, because we did not have data that specifically addressed this issue, we have not adjusted the estimate of 17%.

Appendix 9.4. Summary

Due to the lack of new information, 17% is the best estimate available at this time for the percentage of small farms in need of runoff control improvements. However, this is a national estimate and cannot be extrapolated to all regions of the country because compliance in each region is likely different. Therefore, in this risk assessment, PEC_{water} values were determined for varying percentages of AFOs with <1000 AU that directly discharge into surface waters (0, 25, 50, 75, and 100%). For the risk characterization in Section 7, the PNEC values were compared to the PEC_{water} values that were estimated conservatively assuming 50% of AFOs with <1000 head of cattle discharge directly to surface water. However, this is an extremely conservative assumption. Thus, to provide a more realistic estimate of risk, the PEC_{water} values assuming 25% of AFOs are directly discharging was used in the risk characterization as a conservative representation of the 17% national estimate calculated above.

Appendix 10. Presentation of All Surrogate Estradiol Compound RQ Values from Both the EXPRESS and Mixed-use Watershed Models

Table 103 through Table 106 summarize the individual RQ values for the surrogate estradiol compound using the PEC values for all farm-scale (runoff from cropland and pastureland) and mixed-use watershed scenarios that were developed. In order to quantitatively estimate the risks to fish reproduction-related endpoints from exposure to the surrogate estradiol compound, it was assumed that the toxicity of this compound was equal to the toxicity of either the 17α or the 17β form. Thus, to derive the RQ values for the surrogate estradiol compound, the PEC_{water} for the surrogate estradiol compound was compared to the PNEC either of 17α-estradiol (25 ng/L) or 17β-estradiol (1.4 ng/L). The maximum values from these tables were used in Table 53 and Table 54 (Section 7) to estimate the realistic worst-case RQs for the surrogate estradiol compound. Grey boxes denote scenarios with RQ values >1 (the screening level trigger value). See Section 6.3 for an explanation regarding how the PNEC values were derived and Section 7 for an explanation regarding how the RQ values were derived.

Table 103. $RQ_{\alpha 100}$ Values for Surrogate Estradiol Compound for Tilled and No-till Crop Scenarios from EPA EXPRESS Pond and Index Reservoir Models (When Assuming that the Toxicity of the Surrogate Estradiol Compound is Equivalent to the Toxicity of 17α-Estradiol)

Tilled Scenario	$RQ_{\alpha 100}$ †		No-till Scenario	$RQ_{\alpha 100}$	
	Pond	Index Reservoir		Pond	Index Reservoir
	Table 75	Table 76		Table 83	Table 84
CA1Alfalfa	0.0002	0.0006	CA corn	0.0006	0.0010
CA1Corn	0.0005	0.0008	CA cotton	0.0003	0.0003
CA1Cotton	0.0007	0.0006	IL corn	0.0005	0.0007
CA1SugarBeet	0.0001	0.0003	KS sorghum	0.0002	0.0006
ID1Potato	0.0003	0.0006	MS corn	0.0018	0.0025
IL1Alfalfa	0.0008	0.0014	MS cotton	0.0022	0.0017
IL1Corn	0.0008	0.0012	MS soybean	0.0008	0.0011
KS2Sorghum	0.0000	0.0000	NC cotton	0.0011	0.0008
ME1Potato	0.0000	0.0000	NC1 corn E	0.0005	0.0007
MI1Bean	0.0000	0.0000	NC2 corn W	0.0009	0.0014
MN1SugarBeet	0.0003	0.0006	ND corn	0.0002	0.0002
MN2Alfalfa	0.0001	0.0002	ND wheat	0.0002	0.0002
MS1Corn	0.0025	0.0034	OH corn	0.0013	0.0019
MS1Cotton	0.0021	0.0016	OR wheat	0.0000	0.0000
MS1Soybean	0.0000	0.0000	PA corn	0.0007	0.0010
NC1Alfalfa	0.0000	0.0000	TX sorghum	0.0012	0.0027
NC1Corn	0.0008	0.0013	TX wheat	0.0018	0.0016
NC1Cotton	0.0015	0.0011	**Maximum**	**0.0022**	**0.0027**
NC2Corn	0.0006	0.0009			
ND1Corn	0.0003	0.0004			
ND1Wheat	0.0003	0.0003			
OH1Corn	0.0009	0.0014			
OR1Wheat	0.0001	0.0001			
PA1Alfalfa	0.0007	0.0014			
PA1Corn	0.0005	0.0008			
TX1Alfalfa	0.0000	0.0000			
TX1Corn	0.0020	0.0030			
TX1Cotton	0.0005	0.0004			
TX1Sorghum	0.0000	0.0000			
TX2Corn	0.0005	0.0008			
TX2Cotton	0.0014	0.0011			
TX2Wheat	0.0000	0.0000			
WA1Bean	0.0000	0.0000			
WA1Potato	0.0001	0.0003			
Maximum	**0.0025**	**0.0034**			

† The RQ was calculated by dividing the PEC_{water} from the appropriate table by the PNEC for 17α-estradiol (25 ng/L); i.e., PEC_{water} of the surrogate estradiol compound/PNEC of 17α-estradiol
Grey boxes represent scenarios with RQ values >1

Table 104. $RQ_{\alpha 100}$ Values for the Surrogate Estradiol Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models (When Assuming that the toxicity of the Surrogate Estradiol Compound is Equivalent to the Toxicity of 17α-Estradiol)

Study Region	$RQ_{\alpha 100}$ †		
	100% Pasture (Table 23)	25% of AFO Feedlots Discharging (Table 29)	50% of AFO Feedlots Discharging (Table 28)
Iowa	0.0010	0.0024	0.001
Ohio	0.0010	0.0018	0.003
Pennsylvania	0.0005	0.0010	0.003
Michigan	0.0008	0.0008	0.002
Texas	0.0008	0.0009	0.004
Maximum	**0.0010**	**0.0024**	**0.001**

† The RQ was calculated by dividing the PEC_{water} from the appropriate table by the PNEC for 17α-estradiol (25 ng/L); i.e., PEC_{water} of the surrogate estradiol compound/PNEC of 17α-estradiol
Grey boxes represent scenarios with RQ values >1

Table 105. $RQ_{\beta 100}$ Values for Surrogate Estradiol Compound for Tilled and No-till Crop Scenarios from EPA EXPRESS Pond and Index Reservoir Models (When Assuming that the toxicity of the Surrogate Estradiol Compound is Equivalent to the Toxicity of 17β-Estradiol)

Tilled Scenario	$RQ_{\beta 100}$ †		No-till Scenario	$RQ_{\beta 100}$	
	Pond	Index Reservoir		Pond	Index Reservoir
	Table 75	Table 76		Table 83	Table 84
CA1Alfalfa	0.004	0.010	CA corn	0.012	0.018
CA1Corn	0.009	0.014	CA cotton	0.006	0.005
CA1Cotton	0.012	0.010	IL corn	0.009	0.013
CA1SugarBeet	0.002	0.005	KS sorghum	0.004	0.010
ID1Potato	0.005	0.010	MS corn	0.032	0.044
IL1Alfalfa	0.014	0.025	MS cotton	0.039	0.030
IL1Corn	0.015	0.022	MS soybean	0.015	0.020
KS2Sorghum	0.000	0.000	NC cotton	0.019	0.015
ME1Potato	0.000	0.000	NC1 corn E	0.009	0.013
MI1Bean	0.000	0.000	NC2 corn W	0.017	0.024
MN1SugarBeet	0.005	0.011	ND corn	0.003	0.004
MN2Alfalfa	0.002	0.004	ND wheat	0.003	0.003
MS1Corn	0.044	0.060	OH corn	0.023	0.034
MS1Cotton	0.038	0.028	OR wheat	0.001	0.001
MS1Soybean	0.000	0.000	PA corn	0.012	0.018
NC1Alfalfa	0.000	0.000	TX sorghum	0.022	0.049
NC1Corn	0.015	0.023	TX wheat	0.032	0.029
NC1Cotton	0.027	0.020	**Maximum**	**0.039**	**0.049**
NC2Corn	0.011	0.017			
ND1Corn	0.005	0.007			
ND1Wheat	0.006	0.006			
OH1Corn	0.016	0.025			
OR1Wheat	0.001	0.001			
PA1Alfalfa	0.012	0.025			
PA1Corn	0.010	0.014			
TX1Alfalfa	0.000	0.000			
TX1Corn	0.036	0.053			
TX1Cotton	0.009	0.007			
TX1Sorghum	0.000	0.000			
TX2Corn	0.009	0.013			
TX2Cotton	0.024	0.019			
TX2Wheat	0.000	0.000			
WA1Bean	0.000	0.000			
WA1Potato	0.002	0.005			
Maximum	**0.044**	**0.060**			

† The RQ was calculated by dividing the PEC_{water} from the appropriate table by the PNEC for 17β-trenbolone (1.4 ng/L); i.e., PEC_{water} of the surrogate estradiol compound/PNEC of 17β-estradiol
Grey boxes represent scenarios with RQ values >1

Table 106. $RQ_{\beta 100}$ Values for the Surrogate Estradiol Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models (When Assuming that the toxicity of the Surrogate Estradiol Compound is Equivalent to the Toxicity of 17β-Estradiol)

Study Region	$RQ_{\beta 100}$ †		
	100% Pasture (Table 23)	25% of AFO Feedlots Discharging (Table 29)	50% of AFO Feedlots Discharging (Table 28)
Iowa	0.018	0.043	0.079
Ohio	0.019	0.031	0.063
Pennsylvania	0.009	0.018	0.036
Michigan	0.015	0.014	0.026
Texas	0.014	0.016	0.016
Maximum	**0.018**	**0.043**	**0.079**

† The RQ was calculated by dividing the PEC_{water} from the appropriate table by the PNEC for 17β-trenbolone (1.4 ng/L); i.e., PEC_{water} of the surrogate estradiol compound/PNEC of 17β-estradiol
Grey boxes represent scenarios with RQ values >1

Appendix 11. Presentation of All Surrogate Trenbolone Compound RQ Values from Both the EXPRESS and Mixed-use Watershed Models

Table 107 through Table 116 summarize the individual RQ values for the surrogate trenbolone compound using the 90th percentile 21-day PEC values for all EXPRESS and mixed-use watershed scenarios that were developed. For the surrogate trenbolone compound, a somewhat different approach was used to estimate the RQ values. Because there are cattle excretion data available for trenbolone (Section 4.1.2), these data were used to proportion the PEC_{water} values for the surrogate trenbolone compound based on the relative distribution of the 17α and 17β isomers in manure. Specifically, the PEC_{water} values were multiplied by 0.20 or 0.80 to attribute a portion of the value to 17β-trenbolone and 17α-trenbolone, respectively (see Section 7 for an explanation of this distribution). The RQ values attributed to 17α-trenbolone and 17β-trenbolone (i.e., $RQ_{\alpha 80}$ or $RQ_{\beta 20}$, respectively) were calculated by dividing the specific PEC_{water} values by their respective PNEC value(s) (3.2 or 0.25-0.5 ng/L, respectively; see Table 111 through Table 114 below). In addition, to account for potential additive effects of these isomers, another set of RQ values was calculated, wherein the individual RQ values attributed to 17α-trenbolone and 17β-trenbolone were added together to estimate the final additive RQ values for the surrogate trenbolone compound (i.e., additive RQ = $RQ_{\alpha 80}$ + $RQ_{\beta 20}$; Table 115 and Table 116). In addition, for comparison purposes the RQ values assuming that 100% of the surrogate trenbolone compound is attributed to either 17α-trenbolone ($RQ_{\alpha 100}$; Table 107 and Table 108) or 17β-trenbolone ($RQ_{\beta 100}$; Table 109 and Table 110) are also presented. Grey boxes denote scenarios with RQ values >1 (the screening level trigger value). See Section 6.3 for an explanation regarding how the PNEC values were derived and Section 7 for an explanation regarding how the RQ values were derived.

Table 107. $RQ_{\alpha 100}$ Values for Surrogate Trenbolone Compound for Tilled and No-till Crop Scenarios from EPA EXPRESS Pond and Index Reservoir Models (When Attributing 100% of the PEC value to 17α-trenbolone)

Tilled Scenario	$RQ_{\alpha 100}$ †		No-till Scenario	$RQ_{\alpha 100}$ †	
	Pond	Index Reservoir		Pond	Index Reservoir
	Table 77	Table 78		Table 85	Table 86
CA1Alfalfa	0.02	0.04	CA corn	0.05	0.07
CA1Corn	0.06	0.09	CA cotton	0.03	0.02
CA1Cotton	0.07	0.05	IL corn	0.04	0.06
CA1SugarBeet	0.01	0.02	KS sorghum	0.03	0.06
ID1Potato	0.02	0.04	MS corn	0.15	0.20
IL1Alfalfa	0.06	0.09	MS cotton	0.17	0.13
IL1Corn	0.06	0.08	MS soybean	0.07	0.09
KS2Sorghum	0.03	0.07	NC cotton	0.08	0.05
ME1Potato	0.04	0.06	NC1 corn E	0.04	0.11
MI1Bean	0.01	0.02	NC2 corn W	0.08	0.06
MN1SugarBeet	0.02	0.04	ND corn	0.02	0.02
MN2Alfalfa	0.01	0.01	ND wheat	0.02	0.01
MS1Corn	0.15	0.19	OH corn	0.11	0.16
MS1Cotton	0.13	0.09	OR wheat	0.00	0.00
MS1Soybean	0.06	0.08	PA corn	0.06	0.08
NC1Alfalfa	0.03	0.05	TX sorghum	0.09	0.20
NC1Corn	0.04	0.05	TX wheat	0.15	0.13
NC1Cotton	0.10	0.07	**Maximum**	**0.17**	**0.20**
NC2Corn	0.06	0.09			
ND1Corn	0.02	0.02			
ND1Wheat	0.02	0.01			
OH1Corn	0.08	0.10			
OR1Wheat	0.01	0.01			
PA1Alfalfa	0.05	0.10			
PA1Corn	0.05	0.08			
TX1Alfalfa	0.03	0.07			
TX1Corn	0.09	0.12			
TX1Cotton	0.04	0.02			
TX1Sorghum	0.07	0.16			
TX2Corn	0.02	0.03			
TX2Cotton	0.10	0.07			
TX2Wheat	0.11	0.10			
WA1Bean	0.00	0.00			
WA1Potato	0.01	0.01			
Maximum	**0.15**	**0.19**			

† The RQ was calculated by dividing the PEC_{water} from the appropriate table by the PNEC for 17α-trenbolone (3.2 ng/L); i.e., PEC_{water} of the surrogate trenbolone compound/PNEC of 17α-trenbolone
Grey boxes represent scenarios with RQ values >1

Table 108. $RQ_{\alpha 100}$ Values for the Surrogate Trenbolone Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models (When Attributing 100% of the PEC value to 17α-trenbolone)

Study Region	$RQ_{\alpha 100}$ †		
	100% Pasture (Table 24)	**25% of AFO Feedlots Discharging (Table 29)**	**50% of AFO Feedlots Discharging (Table 28)**
Iowa	0.09	0.22	0.40
Ohio	0.09	0.14	0.29
Pennsylvania	0.05	0.09	0.17
Michigan	0.08	0.07	0.12
Texas	0.07	0.07	0.07
Maximum	**0.09**	**0.22**	**0.40**

† The RQ was calculated by dividing the PEC_{water} from the appropriate table by the PNEC for 17α-trenbolone (3.2 ng/L); i.e., PEC_{water} of the surrogate trenbolone compound/PNEC of 17α-trenbolone
Grey boxes represent scenarios with RQ values >1

Table 109. $RQ_{\beta 100}$ Values for Surrogate Trenbolone Compound for Tilled and No-till Crop Scenarios from EPA EXPRESS Pond and Index Reservoir Models (When Attributing 100% of the PEC value to 17β-trenbolone)

Tilled Scenario	$RQ_{\beta 100}$ † Pond Table 77	$RQ_{\beta 100}$ † Index Reservoir Table 78	No-till Scenario	$RQ_{\beta 100}$ † Pond Table 85	$RQ_{\beta 100}$ † Index Reservoir Table 86
CA1Alfalfa	0.13-0.25	0.28-0.57	CA corn	0.31-0.62	0.47-0.94
CA1Corn	0.37-0.73	0.55-1.10	CA cotton	0.17-0.33	0.14-0.27
CA1Cotton	0.42-0.83	0.34-0.68	IL corn	0.28-0.56	0.38-0.77
CA1SugarBeet	0.06-0.11	0.13-0.26	KS sorghum	0.18-0.36	0.39-0.78
ID1Potato	0.11-0.23	0.25-0.50	MS corn	0.96-1.92	1.27-2.54
IL1Alfalfa	0.38-0.75	0.54-1.09	MS cotton	1.10-2.21	0.81-1.62
IL1Corn	0.38-0.76	0.53-1.05	MS soybean	0.45-0.90	0.58-1.16
KS2Sorghum	0.21-0.41	0.44-0.88	NC cotton	0.49-0.98	0.33-0.65
ME1Potato	0.23-0.46	0.35-0.70	NC1 corn E	0.23-0.47	0.70-1.41
MI1Bean	0.06-0.11	0.11-0.21	NC2 corn W	0.49-0.98	0.36-0.73
MN1SugarBeet	0.10-0.20	0.22-0.45	ND corn	0.11-0.23	0.15-0.31
MN2Alfalfa	0.03-0.07	0.08-0.15	ND wheat	0.11-0.22	0.09-0.18
MS1Corn	0.96-1.91	1.24-2.48	OH corn	0.72-1.44	1.04-2.09
MS1Cotton	0.83-1.65	0.59-1.18	OR wheat	0.02-0.04	0.02-0.03
MS1Soybean	0.38-0.75	0.49-0.97	PA corn	0.36-0.71	0.52-1.04
NC1Alfalfa	0.16-0.33	0.35-0.69	TX sorghum	0.58-1.17	1.26-2.52
NC1Corn	0.23-0.46	0.33-0.66	TX wheat	0.93-1.85	0.83-1.67
NC1Cotton	0.64-1.28	0.46-0.92	**Maximum**	**1.10-2.21**	**1.27-2.54**
NC2Corn	0.36-0.73	0.57-1.14			
ND1Corn	0.10-0.20	0.14-0.29			
ND1Wheat	0.01-0.19	0.09-0.17			
OH1Corn	0.48-0.95	0.66-1.32			
OR1Wheat	0.04-0.08	0.03-0.06			
PA1Alfalfa	0.29-0.58	0.62-1.25			
PA1Corn	0.34-0.68	0.48-0.97			
TX1Alfalfa	0.17-0.33	0.43-0.87			
TX1Corn	0.56-1.11	0.79-1.58			
TX1Cotton	0.24-0.47	0.15-0.30			
TX1Sorghum	0.47-0.94	1.01-2.02			
TX2Corn	0.14-0.29	0.21-0.42			
TX2Cotton	0.61-1.23	0.45-0.89			
TX2Wheat	0.71-1.43	0.64-1.28			
WA1Bean	0.00-0.00	0.00-0.01			
WA1Potato	0.04-0.08	0.09-0.17			
Maximum	**0.96-1.91**	**1.24-2.48**			

† The RQ was calculated by dividing the PEC_{water} from the appropriate table by the PNEC for 17β-trenbolone (0.25-0.5 ng/L); i.e., PEC_{water} of the surrogate trenbolone compound/PNEC of 17β-trenbolone
Grey boxes represent scenarios with RQ values >1

Table 110. $RQ_{\beta 100}$ Values for the Surrogate Trenbolone Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models (When Attributing 100% of the PEC value to 17β-trenbolone)

Study Region	$RQ_{\beta 100}$ †		
	100% Pasture (Table 24)	**25% of AFO Feedlots Discharging (Table 29)**	**50% of AFO Feedlots Discharging (Table 28)**
Iowa	0.55-1.11	1.39-2.80	2.53-5.06
Ohio	0.59-1.18	0.93-1.86	1.84-3.68
Pennsylvania	0.30-0.60	0.55-1.10	1.08-2.16
Michigan	0.50-0.99	0.41-0.83	0.80-1.58
Texas	0.44-0.89	0.44-0.88	0.45-0.90
Maximum	**0.59-1.18**	**1.39-2.80**	**2.53-5.06**

† The RQ was calculated by dividing the PEC_{water} from the appropriate table by the PNEC for 17β-trenbolone (0.25-0.5 ng/L); i.e., PEC_{water} of the surrogate trenbolone compound/PNEC of 17β-trenbolone
Grey boxes represent scenarios with RQ values >1

Table 111. $RQ_{\alpha 80}$ Values for Surrogate Trenbolone Compound for Tilled and No-till Crop Scenarios from EPA EXPRESS Pond and Index Reservoir Models (When Attributing 80% of the PEC value to 17α-trenbolone)

Tilled Scenario	$RQ_{\alpha 80}$ †		No-till Scenario	$RQ_{\alpha 80}$ †	
	Pond	Index Reservoir		Pond	Index Reservoir
	Table 77	Table 78		Table 85	Table 86
CA1Alfalfa	0.02	0.04	CA corn	0.04	0.06
CA1Corn	0.05	0.07	CA cotton	0.02	0.02
CA1Cotton	0.05	0.04	IL corn	0.04	0.05
CA1SugarBeet	0.01	0.02	KS sorghum	0.02	0.05
ID1Potato	0.01	0.03	MS corn	0.12	0.16
IL1Alfalfa	0.05	0.07	MS cotton	0.14	0.10
IL1Corn	0.05	0.07	MS soybean	0.06	0.07
KS2Sorghum	0.03	0.06	NC cotton	0.06	0.04
ME1Potato	0.03	0.04	NC1 corn E	0.03	0.09
MI1Bean	0.01	0.01	NC2 corn W	0.06	0.05
MN1SugarBeet	0.01	0.03	ND corn	0.01	0.02
MN2Alfalfa	0.00	0.01	ND wheat	0.01	0.01
MS1Corn	0.12	0.16	OH corn	0.09	0.13
MS1Cotton	0.10	0.07	OR wheat	0.00	0.00
MS1Soybean	0.05	0.06	PA corn	0.05	0.07
NC1Alfalfa	0.02	0.04	TX sorghum	0.07	0.16
NC1Corn	0.03	0.04	TX wheat	0.12	0.10
NC1Cotton	0.08	0.06	**Maximum**	**0.14**	**0.16**
NC2Corn	0.05	0.07			
ND1Corn	0.01	0.02			
ND1Wheat	0.01	0.01			
OH1Corn	0.06	0.08			
OR1Wheat	0.01	0.00			
PA1Alfalfa	0.04	0.08			
PA1Corn	0.04	0.06			
TX1Alfalfa	0.02	0.05			
TX1Corn	0.07	0.10			
TX1Cotton	0.03	0.02			
TX1Sorghum	0.06	0.13			
TX2Corn	0.02	0.03			
TX2Cotton	0.08	0.06			
TX2Wheat	0.09	0.08			
WA1Bean	0.00	0.00			
WA1Potato	0.01	0.01			
Maximum	**0.12**	**0.16**			

† The RQ was calculated by multiplying the PEC_{water} from the appropriate table by 0.80 (i.e., 80% of surrogate trenbolone compound is attributed to 17α-trenbolone) and dividing this value by the PNEC for 17α-trenbolone (3.2 ng/L); i.e., (PEC_{water} of the surrogate trenbolone compound x 0.80)/PNEC of 17α-trenbolone

Grey boxes represent scenarios with RQ values >1

Table 112. $RQ_{\alpha 80}$ Values for the Surrogate Trenbolone Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models (When Attributing 80% of the PEC value to 17α-trenbolone)

Study Region	$RQ_{\alpha 80}$ †		
	100% Pasture (Table 24)	25% of AFO Feedlots Discharging (Table 29)	50% of AFO Feedlots Discharging (Table 28)
Iowa	0.07	0.18	0.32
Ohio	0.07	0.12	0.23
Pennsylvania	0.04	0.07	0.14
Michigan	0.06	0.05	0.10
Texas	0.06	0.06	0.06
Maximum	**0.07**	**0.18**	**0.32**

† The RQ was calculated by multiplying the PEC_{water} from the appropriate table by 0.80 (i.e., 80% of surrogate trenbolone compound is attributed to 17α-trenbolone) and dividing this value by the PNEC for 17α-trenbolone (3.2 ng/L); i.e., (PEC_{water} of the surrogate trenbolone compound x 0.80)/PNEC of 17α-trenbolone

Grey boxes represent scenarios with RQ values >1

Table 113. $RQ_{\beta 20}$ Values for Surrogate Trenbolone Compound for Tilled and No-till Crop Scenarios from EPA EXPRESS Pond and Index Reservoir Models (When Attributing 20% of the PEC value to 17β-trenbolone)

Tilled Scenario	$RQ_{\beta 20}$ † Pond Table 77	$RQ_{\beta 20}$ † Index Reservoir Table 78	No-till Scenario	$RQ_{\beta 20}$ † Pond Table 85	$RQ_{\beta 20}$ † Index Reservoir Table 86
CA1Alfalfa	0.03-0.51	0.06-0.11	CA corn	0.06-0.13	0.09-0.19
CA1Corn	0.07-0.15	0.11-0.22	CA cotton	0.03-0.07	0.03-0.05
CA1Cotton	0.08-0.17	0.07-0.14	IL corn	0.06-0.11	0.08-0.15
CA1SugarBeet	0.01-0.02	0.03-0.05	KS sorghum	0.04-0.07	0.08-0.16
ID1Potato	0.02-0.05	0.05-0.10	MS corn	0.19-0.38	0.25-0.51
IL1Alfalfa	0.08-0.15	0.11-0.22	MS cotton	0.22-0.44	0.16-0.32
IL1Corn	0.08-0.15	0.11-0.21	MS soybean	0.09-0.18	0.12-0.23
KS2Sorghum	0.04-0.08	0.09-0.18	NC cotton	0.10-0.20	0.07-0.13
ME1Potato	0.05-0.09	0.07-0.14	NC1 corn E	0.05-0.09	0.14-0.28
MI1Bean	0.01-0.02	0.02-0.04	NC2 corn W	0.10-0.20	0.07-0.15
MN1SugarBeet	0.02-0.40	0.05-0.09	ND corn	0.02-0.05	0.03-0.06
MN2Alfalfa	0.01-0.13	0.02-0.03	ND wheat	0.02-0.04	0.02-0.04
MS1Corn	0.19-0.38	0.25-0.50	OH corn	0.14-0.29	0.21-0.42
MS1Cotton	0.17-0.33	0.12-0.24	OR wheat	0.00-0.01	0.00-0.01
MS1Soybean	0.08-0.15	0.10-0.19	PA corn	0.07-0.14	0.10-0.21
NC1Alfalfa	0.03-0.01	0.07-0.14	TX sorghum	0.12-0.23	0.25-0.50
NC1Corn	0.05-0.09	0.06-0.13	TX wheat	0.19-0.37	0.17-0.33
NC1Cotton	0.13-0.26	0.09-0.18	**Maximum**	**0.22-0.44**	**0.25-0.51**
NC2Corn	0.07-0.15	0.11-0.23			
ND1Corn	0.02-0.04	0.03-0.06			
ND1Wheat	0.02-0.04	0.02-0.03			
OH1Corn	0.10-0.19	0.13-0.26			
OR1Wheat	0.01-0.16	0.01-0.01			
PA1Alfalfa	0.06-0.12	0.13-0.25			
PA1Corn	0.07-0.14	0.10-0.19			
TX1Alfalfa	0.03-0.07	0.09-0.17			
TX1Corn	0.11-0.22	0.16-0.32			
TX1Cotton	0.05-0.09	0.03-0.06			
TX1Sorghum	0.09-0.19	0.20-0.40			
TX2Corn	0.03-0.06	0.04-0.08			
TX2Cotton	0.12-0.24	0.09-0.18			
TX2Wheat	0.14-0.29	0.13-0.26			
WA1Bean	0.00-0.00	0.00-0.00			
WA1Potato	0.01-0.02	0.02-0.03			
Maximum	**0.19-0.38**	**0.25-0.50**			

† The RQ was calculated by multiplying the PEC_{water} from the appropriate table by 0.20 (i.e., 20% of surrogate trenbolone compound is attributed to 17β-trenbolone) and dividing this value by the range of PNEC values for 17β-trenbolone (0.25-0.5 ng/L); i.e., (PEC_{water} of the surrogate trenbolone compound x 0.20)/PNEC of 17β-trenbolone

Grey boxes represent scenarios with RQ values >1

Table 114. $RQ_{\beta 20}$ Values for the Surrogate Trenbolone Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models (When Attributing 20% of the PEC value to 17β-trenbolone)

Study Region	$RQ_{\beta 20}$ †		
	100% Pasture (Table 24)	**25% of AFO Feedlots Discharging (Table 29)**	**50% of AFO Feedlots Discharging (Table 28)**
Iowa	0.11-0.22	0.28-0.56	0.51-1.01
Ohio	0.12-0.24	0.19-0.37	0.37-0.74
Pennsylvania	0.06-0.12	0.11-0.22	0.22-0.43
Michigan	0.10-0.20	0.08-0.17	0.16-0.32
Texas	0.09-0.18	0.18-0.09	0.09-0.18
Maximum	**0.12-0.24**	**0.28-0.56**	**0.51-1.01**

† The RQ was calculated by multiplying the PEC_{water} from the appropriate table by 0.20 (i.e., 20% of surrogate trenbolone compound is attributed to 17β-trenbolone) and dividing this value by the range of PNEC values for 17β-trenbolone (0.25-0.5 ng/L); i.e., (PEC_{water} of the surrogate trenbolone compound x 0.20)/PNEC of 17β-trenbolone

Grey boxes represent scenarios with RQ values >1

Table 115. Additive $RQ_{\beta 20+\alpha 80}$ Values for Surrogate Trenbolone Compound for Tilled and No-till Crop Scenarios from EPA EXPRESS Pond and Index Reservoir Models (When Attributing 20% and 80% of the PEC Value to 17β- and 17α-Trenbolone, Respectively)

Tilled Scenario	Additive $RQ_{\beta 20+\alpha 80}$ †		No-till Scenario	Additive $RQ_{\beta 20+\alpha 80}$ †	
	Pond	Index Reservoir		Pond	Index Reservoir
	Table 77	Table 78		Table 85	Table 86
CA1Alfalfa	0.04-0.66	0.09-0.15	CA corn	0.10-0.16	0.15-0.25
CA1Corn	0.12-0.19	0.18-0.29	CA cotton	0.05-0.09	0.04-0.07
CA1Cotton	0.14-0.22	0.11-0.18	IL corn	0.09-0.15	0.13-0.20
CA1SugarBeet	0.02-0.29	0.04-0.07	KS sorghum	0.06-0.10	0.13-0.20
ID1Potato	0.04-0.06	0.08-0.13	MS corn	0.31-0.53	0.41-0.67
IL1Alfalfa	0.12-0.20	0.18-0.29	MS cotton	0.36-0.58	0.26-0.43
IL1Corn	0.12-0.20	0.17-0.28	MS soybean	0.15-0.24	0.19-0.31
KS2Sorghum	0.07-0.11	0.14-0.23	NC cotton	0.16-0.26	0.11-0.17
ME1Potato	0.08-0.12	0.11-0.18	NC1 corn E	0.08-0.12	0.23-0.37
MI1Bean	0.02-0.03	0.04-0.06	NC2 corn W	0.16-0.26	0.12-0.19
MN1SugarBeet	0.03-0.05	0.07-0.12	ND corn	0.04-0.06	0.05-0.08
MN2Alfalfa	0.01-0.02	0.02-0.04	ND wheat	0.04-0.06	0.03-0.05
MS1Corn	0.31-0.50	0.40-0.65	OH corn	0.23-0.38	0.34-0.55
MS1Cotton	0.27-0.43	0.19-0.31	OR wheat	0.01-0.01	0.01-0.01
MS1Soybean	0.12-0.20	0.16-0.26	PA corn	0.12-0.19	0.17-0.27
NC1Alfalfa	0.05-0.09	0.11-0.18	TX sorghum	0.19-0.31	0.41-0.66
NC1Corn	0.08-0.12	0.11-0.17	TX wheat	0.30-0.49	0.27-0.44
NC1Cotton	0.21-0.34	0.15-0.24	**Maximum**	**0.36-0.58**	**0.41-0.67**
NC2Corn	0.12-0.19	0.19-0.30			
ND1Corn	0.03-0.05	0.05-0.08			
ND1Wheat	0.03-0.05	0.03-0.05			
OH1Corn	0.16-0.25	0.21-0.35			
OR1Wheat	0.01-0.02	0.01-0.02			
PA1Alfalfa	0.09-0.15	0.20-0.33			
PA1Corn	0.11-0.18	0.16-0.25			
TX1Alfalfa	0.05-0.09	0.14-0.23			
TX1Corn	0.18-0.29	0.26-0.42			
TX1Cotton	0.08-0.12	0.05-0.08			
TX1Sorghum	0.15-0.25	0.33-0.53			
TX2Corn	0.05-0.08	0.07-0.11			
TX2Cotton	0.20-0.32	0.15-0.23			
TX2Wheat	0.23-0.38	0.21-0.34			
WA1Bean	0.00-0.00	0.00-0.00			
WA1Potato	0.01-0.02	0.03-0.05			
Maximum	**0.31-0.50**	**0.40-0.65**			

† The additive RQ was calculated by adding the RQ values from Table 111 and Table 113 ($RQ_{\alpha 80}$ + $RQ_{\beta 20}$)
Grey boxes represent scenarios with RQ values >1

Table 116. Additive $RQ_{\beta 20+\alpha 80}$ Values for the Surrogate Trenbolone Compound in the Five Selected Watersheds using the Mixed-Use Watershed Models (When Attributing 20% and 80% of the PEC Value to 17β- and 17α-Trenbolone, Respectively)

Study Region	Additive $RQ_{\beta 20+\alpha 80}$ †		
	100% Pasture (Table 24)	**25% of AFO Feedlots Discharging (Table 29)**	**50% of AFO Feedlots Discharging (Table 28)**
Iowa	0.18-0.29	0.45-0.73	0.82-1.33
Ohio	0.19-0.31	0.30-0.49	0.60-0.97
Pennsylvania	0.10-0.16	0.18-0.29	0.35-0.57
Michigan	0.16-0.26	0.14-0.22	0.26-0.42
Texas	0.14-0.23	0.14-0.23	0.15-0.24
Maximum	**0.19-0.31**	**0.45-0.73**	**0.82-1.33**

† The additive RQ was calculated by adding the RQ values from Table 112 and Table 114 ($RQ_{\alpha 80}$ + $RQ_{\beta 20}$)
Grey boxes represent scenarios with RQ values >1

Appendix 12. Field Observations of Concentrations of EB and TBA Metabolites in Fresh Manure, Manure Holding Systems, Feedlot Surfaces, Feedlot Runoff, and the Terrestrial and Aquatic Environments

As described throughout this EA, natural and synthetic estradiol and trenbolone metabolites contained in livestock waste can enter the environment through runoff and leaching from an AFO, manured cropland, and/or manure excreted directly onto pasture land. This section presents available data reported in the published literature on concentrations of estradiol and trenbolone metabolites measured in the environment, including measured concentrations in: 1) feedlot pens and soil, 2) stored manure, 3) surface runoff from feedlots, 4) concentrations in holding lagoons and ponds, and 5) surface water and groundwater concentrations. In addition, data on ED effects in fish exposed under natural conditions are also summarized. The concentrations reported in the published monitoring studies are summarized in Table 117 through Table 123 below (Appendix 12.1). The relevant studies are summarized in Appendix 12.2 and Appendix 12.3.

Appendix 12.1. Summary of field monitoring data

In feedlot material (manure + soil) collected from pens containing EB implanted cattle, 17α-estradiol is the predominant EB metabolite [212]. Similarly, in fresh dairy cattle waste from non-implanted animals, 17α-estradiol is the primary natural estrogen; however, due to transformation, the major hormone detected in wastewater farther from the manure source is estrone [213]. 17α-Estradiol and estrone are the prominent estrogens detected in dairy lagoons [10]. In aerobic waste systems, 17α-estradiol and 17β-estradiol will be transformed to estrone; however, estrone may be reduced back to 17α- and 17β-estradiol under anaerobic conditions [9]. In pits receiving manure from TBA and EB implanted cattle, the primary EB metabolite is estrone, then estriol, 17α-estradiol and 17β-estradiol [214]. As estrogens move through primary and secondary lagoons in dairy cattle operations, their concentrations are reduced [215]. Although estradiol and estrone are frequently detected in the environment, the frequency is not associated with proximity to manure piles and may be due to natural environmental production [216].

In fresh manure of TBA implanted cattle, 17α-trenbolone is the predominant isomer followed by 17β-trenbolone [13, 217]. Similarly, in pits receiving manure from TBA implanted cattle, the primary TBA metabolite is 17α-trenbolone followed by 17β-trenbolone, then trendione [214]. In anaerobic liquid manure of TBA implanted cattle, 17α-trenbolone is the predominant metabolite followed by 17β-trenbolone and trendione [218]. When these TBA metabolites are applied to soil in liquid manure, they are rapidly metabolized [218]. Although they are infrequently detected in feedlot manure and soil, the trenbolone metabolites, when detected, are degraded [13, 217]. Following natural rainfall events, 17α-trenbolone, 17β-trenbolone and trendione are rarely detected [13, 219]; however, following simulated rainfall events 17α-trenbolone and 17β-trenbolone have been detected with a higher frequency [217].

Although hormones are detected in the environment, the maximum concentrations correspond to acute exposures [206, 220, 221, 222]. 17β-Estradiol equivalent concentrations (EEQ) in bioassays of some surface water can be elevated; however, fractionation analysis of the samples has not indicated that 17β-estradiol or estrone are responsible for the estrogenic activity [223]; rather the ED activity may have been due to unidentified EDCs. When detected in surface water, the order of hormone detection

frequency was estrone > estriol > 17α-estradiol > 17β-estradiol [224]. In studies of androgenic activity observed in sites around beef CAFOs, the activity was due to natural androgens in the environment and was not attributable to the trenbolone metabolites [11, 29, 225, 226]. In an example of when BMPs are used for applying wastes to cropped fields, negligible concentrations of estrogenic compounds were detected in surface waters adjacent to the farm [227].

Studies highlighted above are reviewed in Appendix 12.2 and Appendix 12.3 below. Overall conclusions from the published field monitoring data are presented in Appendix 12.4.

Environmental concentrations of EB and TBA metabolites from the reviewed studies are summarized in the following tables:

17β-Estradiol	Table 117
17α-Estradiol	Table 118
Estrone	Table 119
Estriol	Table 120
17β-Trenbolone	Table 121
17α-Trenbolone	Table 122
Trendione	Table 123

Table 117. Concentrations of 17β-Estradiol Detected in Surface Water, Manure Lagoons, Soil and Feedlot Runoff

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
16 animals/pen; 140 mg TBA, 14 mg EB (Revalor®-H) in treated group	Solid manure	MASE/ LC-APPI-MS/MS	ND (<520 ng/kg dw) in treated animals (0% Occurrence) ND (<520 ng/kg dw) to 1300 ng/kg dw in control animals (25% Occurrence) (Table 2 of reference [13])	Bartelt-Hunt et al. [13]
	Urine soaked soil	MASE/ LC-APPI-MS/MS	ND (<520 ng/kg dw) to 18,000 ng/kg dw in treated animals (25% Occurrence) ND (<520 ng/kg dw) to 16,000 ng/kg dw in controls animals (50% Occurrence) (Table 2 of reference [13])	
Feedlot soil concentrations	Feedlot soil	MASE/ LC-APPI-MS/MS	ND (<520 ng/kg dw) to 19,000 ng/kg dw in treated animals (50% Occurrence) ND (<520 ng/kg dw) to 3,800 ng/kg dw in controls animals (50% Occurrence) (Table 2 of reference [13])	
Feedlot runoff concentration	Feedlot runoff	SPE/ LC-APPI-MS/MS	ND (<4.2 ng/L) to 1100 ng/L in treated animals (80% occurrence) Median 131 ng/L ND (<4.2 ng/L) to 1360 ng/L in control animals (77% occurrence) Median 103 ng/L (Table 1 of reference [13])	
14 steers/pen; 120 mg TBA, 24 mg EB	Fresh manure	SPE/ derivatization/ GC-MS/MS	BLQ (<500 ng/kg) (Figure 1 of reference [212])	Mansell et al. [212]
Research CAFO soil concentrations	Feedlot soil	SPE/ derivatization/ GC-MS/MS	Approx. 1000 to 2000 ng/kg Pre- and post-rainfall (% Occurrence not reported) (Figure 1 of reference [212])	
Simulated CAFO runoff concentrations	Feedlot runoff	SPE/ derivatization/ GC-MS/MS	Filtered phase, at or near LOQ, approx. 2 ng/L Suspended solids phase, approx. 5 to 15 ng/L, dependent on rainfall (Figure SI5 of Supporting Information for reference [212])	

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Dairy waste disposal system	Fresh and piled manure	Ether extraction/ derivatization/ GC-MS	ND (<667 ng/kg dw) to 153,000 ng/kg dw Decreasing with time (Table 1 of reference [213])	Zheng et al. [213]
	Dairy effluent and waste water	SPE/ derivatization/ GC-MS	Approx. 100 to 500 ng/L (Figure 2 of reference [213])	
	Lagoon water		BLQ (<4 ng/L) to Approx. 80 ng/L (Figure 2 of reference [213])	
Lagoons; Indiana (Purdue Univ.) CAFO; cattle with Revalor-S implants	Manure pits	Extraction with diethyl ether/ LC-ESI-MS/MS	Approx. 10 to 60 ng/L (from text and Figure 2 of reference [214])	Khan and Lee [214]
	Lagoon water		4.5 to 90 ng/L (Table 1 of reference [214])	
Beef cattle feedlot lagoon sample	Lagoon effluent (including suspended solids)	SPE/ derivatization/ GC-MS/MS	Approx. 100 ng/L in Dairy Lagoon ND (<20 ng/L) in Beef Feedlot Lagoon (Figure 2 of reference [215])	Hutchins et al. [215]
Milking shed effluent collection sump, or outlet of treatment pond – 18 privately owned farms	Dairy shed effluents	SPE/ derivatization/ GC-MS	1 ng/L to 310 ng/L (median 24 ng/L) (100% Occurrence – 29 of 36 samples reported) (Table 1 of reference [10])	Gadd et al. [10]
Drainage ditch waters following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	ND (<0.06 ng/L) to 20.9 ng/L (41 to 71% Occurrence) (Table S1 of reference [228])	Leet et al. [228]
Field drainage following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	BLQ (<0.21) ng/L to 20.9 ng/L (22.7% of 3512 Samples >LOQ) (Table 1 of reference [206])	Gall et al. [206]
Surface water upstream and downstream of CAFO, tile drains, and WWTP	Applied dairy Waste	Bioassay (E-screen) Confirmation: LC-MS/MS	Mean EEQ: 1032 to 1742 ng/L (Table 1 of reference [227]) ND by LC-MS/MS (Table 2 of reference [227])	Shappell et al. [227]
	Field runoff (Tile Drains)	Bioassay (E-screen) Confirmation: LC-MS/MS	Mean EEQ: 0.066 to 0.257 ng/L (Figure 3 of reference [227]) Approx. 0.040 ng/L confirmed by LC-MS/MS in 0.257 ng/L Bioassay Sample (from text of reference [227])	
	Surface water	Bioassay (E-screen)	Mean EEQ: <0.010 to 0.120 ng/L (Figure 3 of reference [227])	

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Northeastern San Joaquin Valley of Central California; rivers, canals and tile drains most likely to be impacted by agriculture; 2003-2004	Field runoff (Tile Drains)	SPE/ derivatization/ GC-MS/MS	ND (<0.1 ng/L) (0% Occurrence) (Table 2 of reference [15])	Kolodziej et al. [15]
	Surface water (Irrigation Canals)		ND (<0.1 ng/L) to 0.7 ng/L (7% Occurrence) (Table 2 of reference [15])	
	Surface water (Rivers)		ND (<0.1 ng/L) to 0.6 ng/L (9% Occurrence) (Table 2 of reference [15])	
Retention pond directly below CAFO; plus reference site	Surface water	EIA/ GC-MS	ND by GC-MS 0.084 to <3.2 ng/L by EIA (Table 3 of reference [11])	Soto et al. [11]
Sampling of 139 US streams; results for 74 samples reported	Surface water	derivatization/ GC-MS	<5 to 93 ng/L (4 (5.4%) of 74 samples >LOQ) (Table 6 of reference [229])	Barnes et al. [229]
Surface waters from prevalent land use types in California's Central Valley; 16 sites; 6 sampling times 2006-2007	Surface water	SPE/ derivatization/ GC-MS/MS	ND (<0.1 to <1 ng/L) to 0.5 ng/L (3% Occurrence) (Table S3 of Supporting Information for reference [223])	Lavado et al. [223]
Surface waters from agriculture, suburban and mixed-use areas in Chester County, PA	Surface water	SPE/ derivatization/ GC-MS	ND (<0.03 ng/L) to 5.04 ng/L (48% Occurrence) (Table 3 and Figure 2 of reference [224])	Velicu and Suri [224]
18 lakes and reservoirs in Nebraska; random stratified design based on geography	Surface water	SPE/ LC-MS/MS	BLQ (<5 ng/L) (0% of 18 samples >LOQ) (Table 1 of reference [230])	Pope et al. [230]
Surface waters from 30 sites samples in Central California where cattle grazing is predominant	Surface water	SPE/ derivatization/ GC-MS/MS	ND (<0.1 ng/L) to 1.7 ng/L (6 and 18% Occurrence; dairy and grazing rangeland) (Table 1 of reference [220])	Kolodziej and Sedlak [220]

APPI – atmospheric pressure photoionization, BLQ – below limit of quantitation, dw – dry weight, EIA – enzyme immunoassay, EEQ – estrogen activity as determined by estrogen-dependent cell line and reported as estrogen equivalents, ESI – electrospray ionization, GC – gas chromatography, LC – liquid chromatography, MASE – microwave-assisted solvent extraction, MS – mass spectrometry, MS/MS – tandem mass spectrometry, ND – not detected, POCIS – polar organic chemical integrative samplers, SPE – solid phase extraction

Table 118. Concentrations of 17α-Estradiol Detected in Surface Water, Manure Lagoons, Soil and Feedlot Runoff

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
16 animals/pen; 140 mg TBA, 14 mg EB (Revalor-H) in treated group	Solid manure	MASE/ LC-APPI-MS/MS	ND (<180 ng/kg dw) to 8,500 ng/kg dw in treated animals (25% Occurrence) ND (<180 ng/kg dw) in control animals (0% Occurrence) (Table 2 of reference [13])	Bartelt-Hunt et al. [13]
	Urine soaked soil	MASE/ LC-APPI-MS/MS	ND (<180 ng/kg dw) to 930 ng/kg dw in treated animals (50% Occurrence) ND (<180 ng/kg dw) to 930 ng/kg dw in controls animals (25% Occurrence) (Table 2 of reference [13])	
Feedlot soil concentrations	Feedlot soil	MASE/ LC-APPI-MS/MS	ND (<180 ng/kg dw) to 1,800 ng/kg dw in treated animals (50% Occurrence) ND (<180 ng/kg dw) to 1,000 ng/kg dw in controls animals (50% Occurrence) (Table 2 of reference [13])	
Feedlot runoff concentration	Feedlot runoff	SPE/ LC-APPI-MS/MS	ND (<2.7 ng/L) to 720 ng/L in treated animals (26% occurrence) Median 10.6 ng/L ND (<2.7 ng/L) to 720 ng/L in control animals (24% occurrence) Median <5 ng/L (Table 1 of reference [13])	
14 steers/pen; 120 mg TBA, 24 mg EB	Fresh manure	SPE/ derivatization/ GC-MS/MS	15,000 ng/kg dw (Figure 1 of reference [212])	Mansell et al. [212]
Research CAFO soil concentrations	Feedlot soil	SPE/ derivatization/ GC-MS/MS	Approx. 6000 to 8000 ng/kg Pre- and post-rainfall (% Occurrence not reported) (Figure 1 of reference [212])	
Simulated CAFO runoff concentrations	Feedlot runoff	SPE/ derivatization/ GC-MS/MS	Filtered phase, approx. 80 ng/L Suspended solids phase, approx. 50 to 100 ng/L, independent of rainfall (Figure SI5 of Supporting Information for reference [212])	

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Dairy waste disposal system	Fresh and piled manure	Ether extraction/ derivatization/ GC-MS	8,000 to 1.42×10^6 ng/kg dw Decreasing with time (Table 1 of reference [213])	Zheng et al. [213]
	Dairy effluent and waste water	SPE/ derivatization/ GC-MS	Approx. 375 to 2200 ng/L (Figure 2 of reference [213])	
	Lagoon water		BLQ (<4 ng/L) to Approx. 80 ng/L (Figure 2 of reference [213])	
Lagoons; Indiana (Purdue Univ.) CAFO; cattle with Revalor-S implants	Manure pits	Extraction with diethyl ether/ LC-ESI-MS/MS	Approx. 10 to 590 ng/L (from text and Figure 2 of reference [214])	Khan and Lee [214]
	Lagoon water		9 to 480 ng/L (Table 1 of reference [214])	
Beef cattle feedlot lagoon sample	Liquid manure	SPE/ derivatization/ GC-MS/MS	Approx. 200 ng/L in Dairy Lagoon Approx. 7 ng/L in Beef Feedlot Lagoon (Figure 2 of reference [215])	Hutchins et al. [215]
Milking shed effluent collection sump, or outlet of treatment pond – 18 privately owned farms	Dairy shed effluents	SPE/ derivatization/ GC-MS	110 ng/L to 11,000 ng/L (median 730 ng/L) (89.7% Occurrence – 29 of 36 samples reported) (Table 1 of reference [10])	Gadd et al. [10]
Drainage ditch waters following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	ND (<0.09 ng/L) to 26.9 ng/L (21 to 72% Occurrence) (Table S1 of reference [228])	Leet et al. [228]
Field drainage following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	BLQ (<0.29) ng/L to 51.8 ng/L (9.8% of 3512 Samples >LOQ) (Table 1 of reference [206])	Gall et al. [206]
Surface water upstream and downstream of CAFO, tile drains, and WWTP	Applied dairy Waste	Bioassay (E-screen) Confirmation: LC-MS/MS	Mean EEQ: 1032 to 1742 ng/L (Table 1 of reference [227]) 700 to 1100 ng/L by LC-MS/MS (Table 2 of reference [227])	Shappell et al. [227]
Retention pond directly below CAFO; plus reference site	Surface water	EIA/ GC-MS	ND to 0.026 by GC-MS 0.035 to <3.8 ng/L by EIA (Table 3 of reference [11])	Soto et al. [11]
Sampling of 144 US streams; results for 74 samples reported	Surface water	derivatization/ GC-MS	<5 to 74 ng/L (4.1% of 74 samples >LOQ) (Table 6 of reference [229])	Barnes et al. [229]
Surface waters from prevalent land use types in California's Central Valley; 16 sites; 6 sampling times 2006-2007	Surface water	SPE/ derivatization/ GC-MS/MS	ND (<0.1 to <1 ng/L) to 0.1 ng/L (1% Occurrence) (Table S3 of Supporting Information for reference [223])	Lavado et al. [223]
Surface waters from agriculture, suburban and mixed-use areas in Chester County, PA	Surface water	SPE/ derivatization/ GC-MS	ND (<0.03 ng/L) to 7.70 ng/L (52% Occurrence) (Table 3 and Figure 2 of reference [224])	Velicu and Suri [224]

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Surface waters from 30 sites samples in Central California where cattle grazing is predominant	Surface water	SPE/ derivatization/ GC-MS/MS	ND (<0.1 ng/L) to 25 ng/L; grazing rangeland only (31% Occurrence; grazing rangeland only) (Table 1 of reference [220])	Kolodziej and Sedlak [220]

APPI – atmospheric pressure photoionization, BLQ – below limit of quantitation, dw – dry weight, EIA – enzyme immunoassay, EEQ – estrogen activity as determined by estrogen dependent cell line and reported as estrogen equivalents, ESI – electrospray ionization, GC – gas chromatography, LC – liquid chromatography, MASE – microwave-assisted solvent extraction, MS – mass spectrometry, MS/MS – tandem mass spectrometry, ND – not detected, SPE – solid phase extraction

Table 119. Concentrations of Estrone Detected in Surface Water, Manure Lagoons, Soil and Feedlot Runoff

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
16 animals/pen; 140 mg TBA, 14 mg EB (Revalor-H) in treated group	Solid manure	MASE/ LC-APPI-MS/MS	ND (<600 ng/kg dw) to 25,600 ng/kg dw in treated animals (50% Occurrence) ND (<600 ng/kg dw) to 22,000 ng/kg dw in control animals (25% Occurrence) (Table 2 of reference [13])	Bartelt-Hunt et al. [13]
	Urine soaked soil	MASE/ LC-APPI-MS/MS	ND (<600 ng/kg dw) in treated animals (0% Occurrence) ND (<600 ng/kg dw) in controls animals (0% Occurrence) (Table 2 of reference [13])	
Feedlot soil concentrations	Feedlot soil	MASE/ LC-APPI-MS/MS	ND (<600 ng/kg dw) to 9,100 ng/kg dw in treated animals (25% Occurrence) ND (<600 ng/kg dw) to 450 ng/kg dw in controls animals (75% Occurrence) (Table 2 of reference [13])	
Feedlot runoff concentration	Feedlot runoff	SPE/ LC-APPI-MS/MS	ND (<4.9 ng/L) to 1,050 ng/L in treated animals (29% occurrence) Median 269 ng/L ND (<4.9 ng/L) to 2,600 ng/L in control animals (46% occurrence) Median 243 ng/L (Table 1 of reference [13])	
14 steers/pen; 120 mg TBA, 24 mg EB	Fresh manure	SPE/ derivatization/ GC-MS/MS	BLQ (<500 to <1000 ng/kg) (Figure 1 of reference [212])	Mansell et al. [212]
Research CAFO soil concentrations	Feedlot soil	SPE/ derivatization/ GC-MS/MS	Approx. 3000 to 5000 ng/kg Pre- and post-rainfall (% Occurrence not reported) (Figure 1 of reference [212])	
Simulated CAFO runoff concentrations	Feedlot runoff	SPE/ derivatization/ GC-MS/MS	Filtered phase, approx. 15 to 25 ng/L Suspended solids phase, approx. 10 to 20 ng/L, independent of rainfall (Figure SI5 of Supporting Information for reference [212])	

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Dairy waste disposal system	Fresh and piled manure	Ether extraction/ derivatization/ GC-MS	68,000 to 697,000 ng/kg dw Decreasing with time (Table 1 of reference [213])	Zheng et al. [213]
	Dairy effluent and waste water	SPE/ derivatization/ GC-MS	Approx. 250 to 1000 ng/L (Figure 2 of reference [213])	
	Lagoon water		BLQ (<3 ng/L) to Approx. 250 ng/L (Figure 2 of reference [213])	
Lagoons; Indiana (Purdue Univ.) CAFO; cattle with Revalor-S implants	Manure pits	Extraction with diethyl ether/ LC-ESI-MS/MS	Approx. 200 to 990 ng/L (from text and Figure 2 of reference [214])	Khan and Lee [214]
	Lagoon water		118 to 1,810 ng/L (Table 1 of reference [214])	
Beef cattle feedlot lagoon sample	Lagoon effluent (including suspended solids)	SPE/ derivatization/ GC-MS/MS	Approx. 80 ng/L in Dairy Lagoon Approx. 20 ng/L in Beef Feedlot Lagoon (Figure 2 of reference [215])	Hutchins et al. [215]
Milking shed effluent collection sump, or outlet of treatment pond – 18 privately owned farms	Dairy shed effluents	SPE/ derivatization/ GC-MS	10 ng/L to 580 ng/L (median 100 ng/L) (100% Occurrence – 29 of 36 samples reported) (Table 1 of reference [10])	Gadd et al. [10]
Drainage ditch waters following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	ND (<0.05 ng/L) to 40.0 ng/L (83 to 100% Occurrence) (Table S1 of reference [228])	Leet et al. [228]
Field drainage following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	BLQ (<0.16) ng/L to 40.0 ng/L (35.2% of 3512 Samples >LOQ) (Table 1 of reference [206])	Gall et al. [206]
Surface water upstream and downstream of CAFO, tile drains, and WWTP	Applied dairy Waste	Bioassay (E-screen) Confirmation: LC-MS/MS	Mean EEQ: 1032 to 1742 ng/L (Table 1 of reference [227]) 1000 to 3900 ng/L by LC-MS/MS (Table 2 of reference [227])	Shappell et al. [227]
Northeastern San Joaquin Valley of Central California; rivers, canals and tile drains most likely to be impacted by agriculture; 2003-2004	Field runoff (Tile Drains)	SPE/ derivatization/ GC-MS/MS	ND (<0.14 ng/L) (0% Occurrence) (Table 2 of reference [15])	Kolodziej et al. [15]
	Surface water (Irrigation Canals)		ND (<0.14 ng/L) to 17 ng/L (47% Occurrence) (Table 2 of reference [15])	
	Surface water (Rivers)		ND (<0.14 ng/L) to 0.9 ng/L (45% Occurrence) (Table 2 of reference [15])	

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Retention pond directly below CAFO; plus reference site	Surface water	EIA/ GC-MS	2.43 to 7.68 ng/L by GC-MS 0.9 to 8.3 ng/L by EIA (Table 3 of reference [11])	Soto et al. [11]
Sampling of 144 US streams; results for 74 samples reported	Surface water	derivatization/ GC-MS	<5 to 112 ng/L (6.8% of 74 samples >LOQ) (Table 6 of reference [229])	Barnes et al. [229]
Surface waters from prevalent land use types in California's Central Valley; 16 sites; 6 sampling times 2006-2007	Surface water	SPE/ derivatization/ GC-MS/MS	ND (<0.1 to <1 ng/L) to 23 ng/L (10% Occurrence) (Table S3 of Supporting Information for reference [223])	Lavado et al. [223]
Surface waters from agriculture, suburban and mixed-use areas in Chester County, PA	Surface water	SPE/ derivatization/ GC-MS	ND (<0.03 ng/L) to 2.62 ng/L (95% Occurrence) (Table 3 and Figure 2 of reference [224])	Velicu and Suri [224]
18 lakes and reservoirs in Nebraska; random stratified design based on geography	Surface water	SPE/ LC-MS/MS	BLQ (<5 ng/L) (0% of 18 samples >LOQ) (Table 1 of reference [230])	Pope et al. [230]
Surface waters from 30 sites samples in Central California where cattle grazing is predominant	Surface water	SPE/ derivatization/ GC-MS/MS	ND (<0.14 ng/L) to 38 ng/L (38 and 78% Occurrence; dairy and grazing rangeland) (Table 1 of reference [220])	Kolodziej and Sedlak [220]

APPI – atmospheric pressure photoionization, BLQ – below limit of quantitation, dw – dry weight, EIA – enzyme immunoassay, EEQ – estrogen activity as determined by estrogen dependent cell line and reported as estrogen equivalents, ESI – electrospray ionization, GC – gas chromatography, LC – liquid chromatography, MASE – microwave-assisted solvent extraction, MS – mass spectrometry, MS/MS – tandem mass spectrometry, ND – not detected, SPE – solid phase extraction

Table 120. Concentrations of Estriol Detected in Surface Water, Manure Lagoons, Soil and Feedlot Runoff

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
16 animals/pen; 140 mg TBA, 14 mg EB (Revalor-H) in treated group	Solid manure	MASE/ LC-APPI-MS/MS	ND (<1870 ng/kg dw) in treated animals (0% Occurrence) ND (<1870 ng/kg dw) in control animals (0% Occurrence) (Table 2 of reference [13])	Bartelt-Hunt et al. [13]
	Urine soaked soil	MASE/ LC-APPI-MS/MS	ND (<1870 ng/kg dw) to 4,900 ng/kg dw in treated animals (25% Occurrence) ND (<1870 ng/kg dw) to 7,000 ng/kg dw in controls animals (25% Occurrence) (Table 2 of reference [13])	
Feedlot soil concentrations	Feedlot soil	MASE/ LC-APPI-MS/MS	ND (<1870 ng/kg dw) to 4,900 ng/kg dw in treated animals (75% Occurrence) ND (<1870 ng/kg dw) to 6,400 ng/kg dw in controls animals (75% Occurrence) (Table 2 of reference [13])	
Feedlot runoff concentration	Feedlot runoff	SPE/ LC-APPI-MS/MS	ND (<7.5 ng/L) to 570 ng/L in treated animals (32% occurrence) Median 59.5 ng/L ND (<7.5 ng/L) to 390 ng/L in control animals (20% occurrence) Median <5 ng/L (Table 1 of reference [13])	
Dairy waste disposal system	Fresh and piled manure	Ether extraction/ Derivatization/ GC-MS	ND (<667 ng/kg dw) (Table 1 of reference [213])	Zheng et al. [213]
Lagoons; Indiana (Purdue Univ.) CAFO; cattle with Revalor-S implants	Manure pits	Extraction with diethyl ether/ LC-ESI-MS/MS	Approx. 10 to 2,730 ng/L (from text and Figure 2 of reference [214])	Khan and Lee [214]
	Lagoon water		1 to 960 ng/L (Table 1 of reference [214])	
Beef cattle feedlot lagoon sample	Lagoon effluent (including suspended solids)	SPE/ derivatization/ GC-MS/MS	ND (<8 ng/L) in dairy and beef feedlot lagoons (Figure 2 of reference [215])	Hutchins et al. [215]
Drainage ditch waters following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	ND (<0.21 ng/L) to 12.4 ng/L (0 to 32% Occurrence) (Table S1 of reference [228])	Leet et al. [228]
Field drainage following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	BLQ (<0.64) ng/L to 19.6 ng/L (0.8% of 3459 Samples >LOQ) (Table 1 of reference [206])	Gall et al. [206]

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Northeastern San Joaquin Valley of Central California; rivers, canals and tile drains most likely to be impacted by agriculture; 2003-2004	Field runoff (Tile Drains)	SPE/ derivatization/ GC-MS/MS	ND (<0.14 ng/L) (0% Occurrence) (Table 2 of reference [15])	Kolodziej et al. [15]
	Surface water (Irrigation Canals)		ND (<0.14 ng/L) (0% Occurrence) (Table 2 of reference [15])	
	Surface water (Rivers)		ND (<0.14 ng/L) (0% Occurrence) (Table 2 of reference [15])	
Sampling of 144 US streams; results for 74 samples reported	Surface water	derivatization/ GC-MS	<5 to 43 ng/L (19% of 74 samples >LOQ) (Table 6 of reference [229])	Barnes et al. [229]
Surface waters from prevalent land use types in California's Central Valley; 16 sites; 6 sampling times 2006-2007	Surface water	SPE/ derivatization/ GC-MS/MS	ND (<0.1 to <1 ng/L) (0% Occurrence) (Table S3 of Supporting Information for reference [223])	Lavado et al. [223]
Surface waters from agriculture, suburban and mixed-use areas in Chester County, PA	Surface water	SPE/ derivatization/ GC-MS	ND (<0.28 ng/L) to 19.7 ng/L (86% Occurrence) (Table 3 and Figure 2 of reference [224])	Velicu and Suri [224]
18 lakes and reservoirs in Nebraska; random stratified design based on geography	Surface water	SPE/ LC-MS/MS	BLQ (<5 ng/L) (0% of 18 samples >LOQ) (Table 1 of reference [230])	Pope et al. [230]

APPI – atmospheric pressure photoionization, BLQ – below limit of quantitation, dw – dry weight, EIA – enzyme immunoassay, ESI – electrospray ionization, GC – gas chromatography, LC – liquid chromatography, MASE – microwave-assisted solvent extraction, MS – mass spectrometry, MS/MS – tandem mass spectrometry, ND – not detected, SPE – solid phase extraction

Table 121. Concentrations of 17β-Trenbolone Detected in Surface Water, Manure Lagoons, Soil and Feedlot Runoff

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Solid dung collected from 12 heifers treated with 5,600 mg TBA (466.7 mg each). Dung hill sampled from top, middle, bottom, and effluent.	Solid manure	SPE/ HPLC fractionation/ EIA	19 to 4,265 ng/L (before storage) <5 to 292 ng/L (after storage) (Table 2 of reference [218])	Schiffer et al. [218]
Liquid manure in collection canal from 41 cattle treated with 3,340 mg TBA (81.5 mg each). Sampling every second week from collection canal, every 2-4 weeks from storage tank, and before spreading (5.5 month interval)	Liquid manure	SPE/ HPLC fractionation/ EIA	Approx. 35 to 185 ng/L (manure canal) Approx. 55 to 155 ng/L (storage tank) (Figure 3 and Figure 4 of reference [218])	
Soil samples following application of field with liquid manure or solid dung	Soil (w/ applied manure)	Extraction with TBME/ HPLC fractionation/ EIA	<0.4 to 8.1 ng/L (liquid manure application) <0.4 to 1.0 ng/L (solid dung application) (Table 3 of reference [218])	
16 animals/pen; 140 mg TBA, 14 mg EB (Revalor-H) in treated group	Solid manure	MASE/ LC-APPI-MS/MS	ND (<290 ng/kg dw) in treated animals (Table 2 of reference [13])	Bartelt-Hunt et al. [13]
	Urine soaked soil	MASE/ LC-APPI-MS/MS	ND (<290 ng/kg dw) in treated animals (Table 2 of reference [13])	
Feedlot soil concentrations	Feedlot soil	MASE/ LC-APPI-MS/MS	ND (<290 ng/kg dw) in treated animals (Table 2 of reference [13])	
Feedlot runoff concentration	Feedlot runoff	SPE/ LC-APPI-MS/MS	ND (<4.5 ng/L) to 270 ng/L in treated animals (2% occurrence) Median <5 ng/L (Table 1 of reference [13])	
Lagoons; Indiana (Purdue Univ.) CAFO; cattle with Revalor-S implants	Manure pits	Extraction with diethyl ether/ LC-ESI-MS/MS	Approx. 20 to 180 ng/L (from text and Figure 2 of reference [214])	Khan and Lee [214]
	Liquid manure		4 to 110 ng/L (Table 1 of reference [214])	
14 steers/pen; 120 mg TBA, 24 mg EB	Fresh manure n=8, 28 days post-implant	SPE/ derivatization/ GC-MS/MS	Mean: 3,100 ng/kg dw (from text of reference [217])	Webster et al. [217]
Research and commercial CAFO soil concentrations (n = 27)	Feedlot soil	SPE/ derivatization/ GC-MS/MS	ND (<200 ng/kg dw) to 1,300 ng/kg dw in feedlot surface soil samples (100% Occurrence in Pens A and B) (Table 1 of reference [217])	
Surface soils from commercial CAFO pens(100 mg TBA implants)	Feedlot soil	SPE/ derivatization/ GC-MS/MS	Not reported	
Simulated CAFO runoff concentrations	Feedlot runoff	SPE/ derivatization/ GC-MS/MS	Median 16 ng/L when detected (range 5 to 26 ng/L) (36.5% Occurrence) (from text of reference [217])	

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Surface soils; commercial CAFO in Nevada	Soil	derivatization/ GC-MS/MS	ND (detection limit not given) (from text of reference [219])	Parker et al. [219]
Storm water runoff sample; commercial CAFO in Iowa	Feedlot runoff	derivatization/ GC-MS/MS	31 ng/L (from text of reference [219])	
Drainage ditch waters following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	ND (<0.47 ng/L) to 28.2 ng/L (0 to 4% Occurrence) (Table S1 of reference [228])	Leet et al. [228]
Field drainage following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	BLQ (<1.6) ng/L to 162 ng/L (2.0% of 3225 Samples >LOQ) (Table 1 of reference [206])	Gall et al. [206]
Discharge drain and surface water upstream and downstream of CAFO; SW Central Ohio	Discharge Drainage	SPE/ HPLC-Fluorescence	ND (<1 to 4 ng/L) to ~20 ng/L (22.2% Occurrence) (Figure 3B of reference [29])	Durhan et al. [29]
	Discharge Drainage	GC-MS Confirmation	ND (variable; <10 to 40 ng/L) to 12 ng/L (11.1% Occurrence) (from text of reference [29])	
	Surface water	SPE/ HPLC-Fluorescence	ND (<1 to 4 ng/L) to ~7 ng/L (27.8% Occurrence) (Figure 3B of reference [29])	
6 sites total; Intermediate exposure site, retention pond, drainage canal, directly below CAFO, 3 sites along the river including a reference site; Elkhorn River, Nebraska	Surface water	EIA	<0.0004 to 0.0015 ng/L at 3 sites <0.0003 in blanks (Table 4 of reference [11])	Soto et al. [11]
18 lakes and reservoirs in Nebraska; random stratified design based on geography	Surface water	SPE/ LC-MS/MS	BLQ (<5 ng/L) (0% of 18 samples >LOQ) (Table 1 of reference [230])	Pope et al. [230]

APPI – atmospheric pressure photoionization, BLQ – below limit of quantitation, dw – dry weight, EIA – enzyme immunoassay, ESI – electrospray ionization, GC – gas chromatography, HPLC – high performance liquid chromatography, LC – liquid chromatography, MASE – microwave-assisted solvent extraction, MS – mass spectrometry, MS/MS – tandem mass spectrometry, ND – not detected, POCIS – polar organic chemical integrative samplers, SPE – solid phase extraction, TBME – tert-butyl methyl ether

Table 122. Concentrations of 17α-Trenbolone Detected in Surface Water, Manure Lagoons, Soil and Feedlot Runoff

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Solid dung collected from 12 heifers treated with 5,600 mg TBA (466.7 mg each). Dung hill sampled from top, middle, bottom, and effluent.	Solid manure	SPE/ HPLC fractionation/ EIA	227 to 75,400 ng/L (before storage) <5 to 10,100 ng/L (after storage) (Table 2 of reference [218])	Schiffer et al. [218]
Liquid manure in collection canal from 41 cattle treated with 3,340 mg TBA (81.5 mg each). Sampling every second week from collection canal, every 2-4 weeks from storage tank, and before spreading (5.5 month interval)	Liquid manure	SPE/ HPLC fractionation/ EIA	Approximately 750 to 4,250 ng/L (manure canal) Approx. 500 to 1800 ng/L (storage tank) (Figure 3 and Figure 4 of reference [218])	
Soil samples following application of field with liquid manure or solid dung	Soil (applied manure)	Extraction with TBME/ HPLC fractionation/ EIA	<0.4 to 248 ng/L (liquid manure application) <0.4 to 11 ng/L (solid dung application) (Table 3 of reference [218])	
16 animals/pen; 140 mg TBA, 14 mg EB (Revalor-H) in treated group	Solid manure	MASE/ LC-APPI-MS/MS	ND (<160 ng/kg dw) to 31,000 ng/kg dw in treated animals (25% Occurrence) ND (<160 ng/kg dw) to 5,000 ng/kg dw in untreated animals (25% Occurrence) (Table 2 of reference [13])	Bartelt-Hunt et al. [13]
	Urine soaked soil	MASE/ LC-APPI-MS/MS	ND (<160 ng/kg dw) in treated animals (0% Occurrence) (Table 2 of reference [13])	
Feedlot soil concentrations	Feedlot soil	MASE/ LC-APPI-MS/MS	ND (<160 ng/kg dw) in treated animals (0% Occurrence) (Table 2 of reference [13])	
Feedlot runoff concentration	Feedlot runoff	SPE/ LC-APPI-MS/MS	ND (<3.2 ng/L) in treated animals (0% occurrence) Median <5 ng/L (Table 1 of reference [13])	
Lagoons; Indiana (Purdue Univ.) CAFO; cattle with Revalor-S implants	Manure pits	Extraction with diethyl ether/ LC-ESI-MS/MS	Approx. 200 to 2,900 ng/L (from text and Figure 2 of reference [214])	Khan and Lee [214]
	Lagoon water		22 to 1,720 ng/L (Table 1 of reference [214])	

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
14 steers/pen; 120 mg TBA, 24 mg EB	Fresh manure n=8 28 days post-implant	SPE/ derivatization/ GC-MS/MS	Mean: 21,000 ng/kg dw (from text of reference [217])	Webster et al. [217]
Research and commercial CAFO soil concentrations (n = 27)	Feedlot soil	SPE/ derivatization/ GC-MS/MS	600 to 11,800 ng/kg dw in feedlot surface soil samples (100% Occurrence in Pens A and B) (Table 1 of reference [217])	
Surface soils from commercial CAFO pens(100 mg TBA implants)	Feedlot soil	SPE/ derivatization/ GC-MS/MS	14,000 ng/kg dw at 20 days (max) 2000 ng/kg dw at 110 days ND (<200 ng/kg dw) at 160 days (from text of reference [217])	
Simulated CAFO runoff concentrations	Feedlot runoff	SPE/ derivatization/ GC-MS/MS	Median 34 ng/L (range 1 to 390 ng/L) (100% Occurrence) (from text of reference [217])	
Surface soils; commercial CAFO in Nevada	Feedlot soil	derivatization/ GC-MS/MS	4,000 to 6,000 ng/kg dw (from text of reference [219])	Parker et al. [219]
Storm water runoff sample; commercial CAFO in Iowa	Feedlot runoff	derivatization/ GC-MS/MS	<1 ng/L (from text of reference [219])	
Drainage ditch waters following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	ND (<0.22 ng/L) to 19.1 ng/L (0 to 4% Occurrence) (Table S1 of reference [228])	Leet et al. [228]
Field drainage following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	BLQ (<0.74) ng/L to 22.7 ng/L (3.1% of 3225 Samples >LOQ) (Table 1 of reference [206])	Gall et al. [206]
Discharge drain and surface water upstream and downstream of CAFO; SW Central Ohio	Discharge Drainage	SPE/ HPLC-Fluorescence	ND (<10 ng/L) to ~120 ng/L (66.7% Occurrence) (Figure 3A of reference [29])	Durhan et al. [29]
	Discharge Drainage	GC-MS Confirmation	ND (variable; <10 to 40 ng/L) to 8 ng/L (11.1% Occurrence) (from text of reference [29])	
	Surface water	SPE/ HPLC-Fluorescence	ND (<4 ng/L) to ~50 ng/L (33.3% Occurrence) (Figure 3A of reference [29])	
6 sites total; Intermediate exposure site, retention pond, drainage canal, directly below CAFO, 3 sites along the river including a reference site; Elkhorn River, Nebraska	Surface water	EIA	0.0016 to 0.035 ng/L in 3 samples 0.0016 and 0.010 ng/L in Blanks (Table 4 of reference [11])	Soto et al. [11]

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
18 lakes and reservoirs in Nebraska; random stratified design based on geography	Surface water	SPE/ LC-MS/MS	BLQ (<5 ng/L) (0% of 18 samples >LOQ) (Table 1 of reference [230])	Pope et al. [230]

APPI – atmospheric pressure photoionization, BLQ – below limit of quantitation, dw – dry weight, EIA – enzyme immunoassay, ESI – electrospray ionization, GC – gas chromatography, HPLC – high performance liquid chromatography, LC – liquid chromatography, MASE – microwave-assisted solvent extraction, MS – mass spectrometry, MS/MS – tandem mass spectrometry, ND – not detected, POCIS – polar organic chemical integrative samplers, SPE – solid phase extraction, TBME – tert-butyl methyl ether

Table 123. Concentrations of Trendione Detected in Surface Water, Manure Lagoons, Soil and Feedlot Runoff

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Solid dung collected from 12 heifers treated with 5,600 mg TBA (466.7 mg each). Dung hill sampled from top, middle, bottom, and effluent.	Solid manure	SPE/ HPLC fractionation/ EIA	10 to 4,700 ng/L (before storage) ND (<5 ng/L) to 824 ng/L (after storage) (Table 2 of reference [218])	Schiffer et al. [218]
Liquid manure in collection canal from 41 cattle treated with 3,340 mg TBA (81.5 mg each). Sampling every second week from collection canal, every 2-4 weeks from storage tank, and before spreading (5.5 month interval)	Liquid manure	SPE/ HPLC fractionation/ EIA	Approx. 25 to 90 ng/L (manure canal) Approx. 20 to 125 ng/L (storage tank) (Figure 3 and Figure 4 of reference [218])	
Soil samples following application of field with liquid manure or solid dung	Soil (w/ applied manure)	Extraction with TBME/ HPLC fractionation/ EIA	<0.4 to 21 ng/L (liquid manure application) <0.4 to 4.1 ng/L (solid dung application) (Table 3 of reference [218])	
Lagoons; Indiana (Purdue Univ.) CAFO; cattle with Revalor-S implants	Manure pits	Extraction with diethyl ether/ LC-ESI-MS/MS	Approx. 10 to 120 ng/L (from text and Figure 2 of reference [214])	Khan and Lee [214]
	Lagoon water		6 to 150 ng/L (Table 1 of reference [214])	
14 steers/pen; 120 mg TBA, 24 mg EB	Fresh manure n=8, 28 days post-implant	SPE/ derivatization/ GC-MS/MS	ND (<200 ng/kg dw) (from text of reference [217])	Webster et al. [217]
Research and commercial CAFO soil concentrations (n = 27)	Feedlot soil	SPE/ derivatization/ GC-MS/MS	ND (<200 ng/kg dw) to 2,600 ng/kg dw In feedlot surface soil samples (100% Occurrence in Pens A and B) (Table 1 of reference [217])	
Surface soils from commercial CAFO pens(100 mg TBA implants)	Feedlot soil	SPE/ derivatization/ GC-MS/MS	Not reported	
Simulated CAFO runoff concentrations	Feedlot runoff	SPE/ derivatization/ GC-MS/MS	Median 19 ng/L when detected (range 5 to 180 ng/L) (65.1% Occurrence) (from text of reference [217])	
Surface soils; commercial CAFO in Nevada	Soil	derivatization/ GC-MS/MS	ND (detection limit not given) (from text of reference [219])	Parker et al. [219]
Storm water runoff sample; commercial CAFO in Iowa	Feedlot runoff	derivatization/ GC-MS/MS	52 ng/L (from text of reference [219])	
Drainage ditch waters following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	ND (<1.9 ng/L) to 35.4 ng/L (0 to 22% Occurrence) (Table S1 of reference [228])	Leet et al. [228]

Description of Location	Matrix	Analysis Method	Measured Concentration (source, table or figure, in publication)	Reference
Field drainage following manure application; Indiana (Purdue Univ.) CAFO; tile-drained cropland	Field runoff	SPE/ LC-ESI-MS/MS	BLQ (<6.4) ng/L to 35.3 ng/L (1.4% of 2263 Samples >LOQ) (Table 1 of reference [206])	Gall et al. [206]
6 sites total; Intermediate exposure site, retention pond, drainage canal, directly below CAFO, 3 sites along the river including a reference site; Elkhorn River, Nebraska	Surface water	EIA	0.0019 to 0.016 ng/L at 3 sites 0.0023 to 0.0088 ng/L in blanks (Table 4 of reference [11])	Soto et al. [11]
BLQ – below limit of quantitation, dw – dry weight, EIA – enzyme immunoassay, ESI – electrospray ionization, GC – gas chromatography, HPLC – high performance liquid chromatography, LC – liquid chromatography, MASE - microwave-assisted solvent extraction, MS – mass spectrometry, MS/MS – tandem mass spectrometry, ND – not detected, POCIS – polar organic chemical integrative samplers, SPE – solid phase extraction, TBME – tert-butyl methyl ether				

Appendix 12.2. Literature data on detection and degradation of estradiol and trenbolone metabolites in feedlot soil, manured soil, feedlot runoff, lagoons and manure holding ponds

Appendix 12.2.1. Concentrations of estrogens, androgens, and progesterone in manure, feedlot soils, and simulated runoff on a feedlot with steers implanted with TBA and EB [Mansell et al.]

The following study indicated that compounds with endocrine activity are naturally produced from the manure components excreted by cattle (i.e., plant material) and are not related to the steroids excreted from implants. In this study, 17α-estradiol was the predominant metabolite from EB implanted cattle.

Mansell et al. [212] assessed how rainfall events affected the release of hormones from feedlots containing steers implanted with Revalor®-S (120 mg TBA and 24 mg estradiol). Runoff and soil samples were collected after simulated rainfall on a 14-steer feedlot using different rainfall rates and aging periods. Steroid hormone concentrations were measured using SPE, derivatization, and GC-MS/MS. The highest concentration of 17α-estradiol observed in feedlot material (fresh manure) was 15,000 ng/kg followed by estrone and 17β-estradiol with maximum feedlot concentrations in the soil $\leq$5,000 ng/kg [212]. Estrone and 17β-estradiol were below the limit of quantitation in fresh manure (<500 to 1,000 ng/kg). The feedlot study also demonstrated the sum of 17α-estradiol, 17β-estradiol and estrone was relatively stable over a 14-day period, indicating minimal degradation of these combined metabolites in the feedlot environment. The concentrations of androgens (testosterone and androstenedione) and progesterone in soil declined approximately 85% after a simulated rainfall event. However, this loss could not all be accounted for by runoff, suggesting potential microbial degradation. Although testosterone was rapidly transformed in the feedlot soil, it could only account for approximately 10% of the amount of androstenedione produced (see Figure 1 of reference 212). Concentrations of 17α-estradiol in runoff after simulated rainfall were approximately 125-175 ng/L in aged and unaged plots (Figure 2 of reference 212). Estrone concentrations were approximately 25-50 ng/L and 17β-estradiol concentrations were approximately 10 ng/mL.

Appendix 12.2.2. Occurrence of trenbolone acetate metabolites in simulated confined animal feeding operation (CAFO) runoff [Webster et al.]

The following study indicated that in fresh manure of TBA implanted cattle, 17α-trenbolone (21,000 ng/kg) was the predominant isomer followed by 17β-trenbolone (3,100 ng/kg). The trenbolone metabolites were degraded in the feedlot soil. In simulated runoff trials, 17α-trenbolone was detected in all samples (1-390 ng/L) followed by trendione in 65.1% of samples (5-180 ng/L) and 17β-trenbolone in 36.5% of samples (5-26 ng/L). Percent occurrence was based on the detection limits.

Webster et al. 217 quantified TBA metabolite concentrations in beef cattle CAFO soils, manure, and runoff while identifying environmental variables governing steroid release and transport. Solid and aqueous samples were taken from a well-characterized research CAFO with simulated rainfall. Transformation of metabolites was followed in soils collected from a commercial CAFO with TBA-implanted cattle. Solid samples were solvent-extracted and analyzed by GC-MS/MS. Aqueous samples were extracted on an SPE cartridge, derivatized, and analyzed by GC-MS/MS. Although recovery was low and variable, estimates derived from this study agree with predictions of TBA metabolites in CAFO runoff.

In fresh manure collected 28 days post-implantation, 17α-trenbolone and 17β-trenbolone were present at mean concentrations of 21,000 ± 2,700 ng/kg dry weight and 3,100 ± 400 ng/kg dry weight, respectively. Trendione was not detected in the manure. Soil samples collected from pens with implanted cattle (research and commercial CAFOs) contained 17α-trenbolone at concentrations up to 11,800 ng/kg dry weight and 17β-trenbolone at concentrations up to 1300 ng/kg dry weight. TBA metabolites were also detected in surface soils of commercial CAFO pens containing implanted cattle with concentrations of 17α-trenbolone decreasing to 2000 ng/kg dry weight soil in pens at 110 days post-implantation (100 mg TBA implants). A pen containing 29 implanted cattle yielded no detectable TBA metabolites in soil at 160 days post-implantation. It was concluded that TBA metabolites were not excreted in substantial quantities and may not persist in soils. Soils at the research CAFO contained mean concentrations up to 8,200 ± 1,100 ng/kg dry weight of 17α-trenbolone and 1,200 ± 100 ng/kg dry weight of 17β-trenbolone, with higher 17α-trenbolone concentrations (approximately 20,000 ng/kg dry weight) observed in commercial CAFO soils. A first-order transformation rate of 0.028/day (25-day DT_{50}) was estimated for 17α-trenbolone in surface soils of commercial CAFOs, based on the assumption that 8% of the TBA dose is excreted as 17α-trenbolone and accounts for 95% of excreted metabolite mass.

Rainfall simulation trials in research CAFO pens were conducted using a programmable rainfall simulator design, with all trials performed after implanted cattle (120 mg TBA implant) had been in the pens for at least 14 days. Samples were collected at 5, 20, 40, and 60 days after low and high rainfall rates. Estimates of TBA metabolite concentrations in runoff samples ranged from 1-390 ng/L, with a median 17α-trenbolone concentration of 34 ng/L. Concentrations of 17α-trenbolone were detected in all samples collected and were independent of rainfall rate; however, concentrations were likely underestimated because they lacked a recovery correction. 17β-Trenbolone was detected in 23 of 63 runoff samples at concentrations ranging from 5-26 ng/L, with a median concentration of 16 ng/L when detected. Trendione was present in 41 of 63 samples with 17 of these samples below the limit of quantitation when detected. Trendione concentrations ranged from 5-180 ng/L when detected, with a median of 19 ng/L. While 17α-trenbolone concentrations declined in simulated runoff samples, there were no significant increases in trendione or 17β-trenbolone concentrations over the sample times (5-60 minutes).

Appendix 12.2.3. Concentrations of estradiol and trenbolone metabolites in cattle manure, feedlot surface soils, and natural runoff attributed to cattle administered hormone implants [Bartelt-Hunt et al.]

In the study summarized below, 17α-trenbolone was detected in fresh manure of TBA implanted cattle. As the manure aged, 17α-trenbolone was degraded. No 17α- or 17β-trenbolone was detected in feedlot soil or urine soaked soil. Following natural rainfall events, no 17α-trenbolone was detected and 17β-trenbolone was detected only 2% of the time.

Bartelt-Hunt et al. [13] evaluated the occurrence of natural and synthetic steroid hormones in feedlot runoff, manure, and soils collected from feedlot surfaces. A two-year study was conducted in which 96 beef cattle were equally divided into six pens (16 animals per pen; three replicate pens per treatment). The study consisted of a control group and a treated group administered steroid hormones via implants and feed additives. The treatment group received an implant containing 36 mg of α-zearalanol (Ralgro®) on Day 1 of the study,

followed by an implant containing 140 mg of TBA and 14 mg of estradiol (Revalor®-H) after 35 days. Treated animals also received 0.45 mg of melengestrol acetate (MGA) per day in feed beginning on Day 7. Animals were held in the pens for up to 141 days. At the conclusion of the study in 2007, soil from feedlot pens was mechanically scraped down to the clay layer and replaced with fresh soil prior to the initiation of the 2008 study. For each study period, background levels of steroid hormones were evaluated. Runoff was collected using tipping bucket samplers during each runoff event. A composite sample of each runoff event was collected manually within 5 hours and total volumes of runoff determined accordingly. Runoff samples were extracted by SPE methods and analyzed by LC-MS/MS. Feedlot surface soils and manures were extracted using microwave-assisted solvent extraction (MASE) and analyzed by LC-MS/MS.

Four rainfall events occurred in 2007, and 14 runoff events occurred in 2008. The results for this study are summarized in Table 124. This table has been consolidated to present a summary of concentrations from runoff over the two-year study period (Table 1 of [13]) and concentrations in solid samples from 2007 (Table 2 of [13]). For solid samples (feeding pen surface, urine soaked soil, and fresh manure), only selected results are provided in Table 124. Day 0 and Day 7 (2007) steroid concentrations for solids were excluded from the table because only small amounts of estrone (means as high as 25,600 $\pm$ 3,100 ng/kg dry weight) and estriol (means as high as 7,000 $\pm$ 1,200 ng/kg dry weight) were detected on these days. No other relevant hormones were detected. The concentrations in solid samples from 2008 (see supplemental information of [13]) are not included in Table 124.

Table 124. Concentration of EB and TBA Metabolites in Runoff from Cattle Feedlots (2007 and 2008) and Feeding Pen Surface Samples, Urine Soaked Surface Soil Samples, and Fresh Manure Samples in 2007

	Runoff Maximum (ng/L)					
	17β-E2	**17α-E2**	**E1**	**E3**	**17α-TB**	**17β-TB**
Treated	1100	720	1050	570	<5	270
Control	1360	720	2600	390	<5	<5
	Runoff Median (ng/L)					
Treated	131	10.6	269	59.5	<5	<5
Control	103	<5	243	<5	<5	<5
	Runoff Frequency (%)					
Treated	80%	26%	29%	32%	0%	2%
Control	77%	24%	46%	20%	0%	0%
	Feeding Pen Surface Day 46 (ng/kg dry weight)					
Treated	1,200±190	1,800±150	ND†	1,200±30	ND	ND
Control	3,800±600	1,000±140	ND	430±70	ND	ND
	Feeding Pen Surface Day 109 (ng/kg dry weight)					
Treated	19,000±4,200	190±4,500	9,100±2,600	ND	ND	ND
Control	3,500±400	150±40	80±20	ND	ND	ND
	Urine Soaked Soil Day 46 (ng/kg dry weight)					
Treated	18,000±2,200	930±520	ND	ND	ND	ND
Control	16,000±2,300	930±520	ND	ND	ND	ND
	Urine Soaked Soil Day 109 (ng/kg dry weight)					
Treated	ND	220±100	ND	ND	ND	ND
Control	4,200±600	ND	ND	ND	ND	ND
	Fresh Manure Day 46 (ng/kg dry weight)					
Treated	ND	8,500±1,600	11,000±3,100	ND	31,000±6,000	ND
Control	1.3 ±0.75	ND	22,000±2,1000	ND	5,000±2,800	ND
	Fresh Manure Day 109 (ng/kg dry weight)					
Treated	ND	ND	ND	ND	ND	ND
Control	ND	ND	ND	ND	ND	ND

† ND – not detected.
Data from Bartelt-Hunt et al. [13]

Results of the analysis of solids for 2008 were similar to 2007. In 2008, the only concentration of 17α-trenbolone above detection limits was in fresh manure on Day 47, with a mean of 55,000 ± 22,000 ng/kg dry weight compared to a mean of 31,000 ± 6,000 ng/kg on Day 46 in 2007. 17β-Trenbolone concentrations in fresh manure on Day 47 (2008) were 500 ± 300 ng/kg dry weight but not detected on Day 46 (2007). In addition, 17α-trenbolone was not detected in the feeding pen surface or urine soaked soil at any time point. Following natural feedlot runoff events, 17β-trenbolone was detected in 2% (1 of 50) of the samples with a maximum concentration of 270 ng/L [13], and 17α-trenbolone was not detected in any runoff samples (i.e., <5 ng/L).

In general, estrogen concentrations were similar in 2007 and 2008 with no observable differences between treated and control animals. For 17β-estradiol and estrone, concentrations in runoff from the control pens actually exceeded that of treated animals which strongly suggests the implants had little, if any, impact on total estrogens eliminated in the beef heifers. While the recovery for 17β-estradiol and 17α-estradiol in solid samples was 77.2% and 85.4%, respectively, and 55.6% and 28.0% in runoff samples, a relatively good comparison between treated and control animals can still be made.

Appendix 12.2.4. Trenbolone metabolite concentrations in surface soil and runoff associated with commercial CAFOs in Iowa and Nevada [Parker et al.]

The following study indicated that only 17α-trenbolone was detected in feedlot surface soil; however, in runoff, 17β-trenbolone and trendione were detected.

Parker et al. [219] described methods to quantitatively determine trace levels of trenbolone metabolites in storm water runoff from a commercial CAFO in Iowa and in surface soils from a commercial CAFO in Nevada, both of which used TBA implants in all cattle. Samples were derivatized with N-methyl-N-(trimethyl-silyl) trifluoro-acetamide (MSTFA) following SPE and clean-up methods. Soil samples were extracted with methanol. Samples were analyzed by GC-MS/MS. Analytical methods were developed utilizing a variety of representative receiving waters, including surface water from a small creek in a mixed-use agriculture-urban watershed near Reno, NV, a constructed wetland that collects and treats storm water runoff, and an irrigation canal in California that contains agricultural return water from irrigated pastureland used for cattle grazing. Overall, analyte recoveries ranged from 80-120%. Results indicated that 17β-trenbolone and trendione were detected in the CAFO storm water runoff at concentrations of 31 and 52 ng/L, respectively. In the three CAFO surface soils, only 17α-trenbolone was detected at concentrations between 4,000 and 6,000 ng/kg dry weight. Little additional information was provided because the focus of this manuscript was on the development of the analytical methods.

Appendix 12.2.5. Estrogen concentrations in fresh and aged dairy cattle manure [Zheng et al.]

The following study indicated that 17α-estradiol is the primary steroid hormone in fresh dairy waste, but estrone was the major hormone contaminant in waste water farther from the manure source due to the rapid transformation of 17α-estradiol to estrone along the dairy waste disposal system.

The concentrations of four estrogens (17β-E2, 17α-E2, E1, and E3) and two progestogens (medroxyprogesterone and progesterone) in a representative dairy waste disposal system in San Jacinto, California were determined by Zheng et al. [213]. All samples were collected from a dairy farm with approximately 2,000 dairy cows (including 1,000 milking cows). Fresh manure samples (<2 hours after deposition) were collected from six sampling locations located within barns. Aged manure samples were collected from three drying manure piles (estimated at ~2 weeks of age). Fresh dairy manure wastewater effluent samples were taken from three locations along the sewage outlet of the milking parlor after hydraulic flushing and solid waste was collected at the entrance of the outlets. Liquid samples were centrifuged to separate the liquid and solids. Solids were extracted with methanol three times and combined with the aqueous supernatant. Steroid hormones were extracted from the mixed aqueous phase by SPE, derivatized and analyzed by GC-MS/MS. For solid waste, 1 M NaOH was added to freeze-dried samples and immediately extracted with ethyl ether. The extraction procedure was carried out three times and organic phases were combined. Samples were derivatized and analyzed by GC-MS/MS. Quantification was performed by a method of standard addition to account for interference.

In dairy solid wastes, 17α-estradiol was the most abundant hormone detected (means ranged from 8,000-1,416,000 ng/kg), followed by estrone (means ranged from 68,000-697,000 ng/kg). Fresh dairy manure contained the highest concentration of total estrogens (mean 2,103,000 ng/kg) with >80% being 17α-estradiol. Manure held in dung

piles (~2 weeks old) contained the second highest total estrogen concentrations with a mean of 1,101,000 ng/kg. Concentrations of estrogens in the solids collected from dairy wastewater effluent dams (~3 months old) were much lower with means that ranged from 76,000-161,000 ng/kg. In the milking parlor effluent, concentrations of 17β-estradiol, 17α-estradiol, and estrone generally decreased with increasing distance from the source. However, the percent distribution of hormones shifted from 17β-estradiol and 17α-estradiol being the predominant hormones in fresh wastewater to E1 being the predominant hormone in wastewater collected further from the dairy parlor. Hormone concentrations in lagoon waters ranged from <4 ng/L to 80 ng/L for 17β-estradiol and 17α-estradiol, and from <3 ng/L to 250 ng/L for estrone. Concentrations in the lagoons were considerably less than drainage water. Estriol was not detected in any samples. It was concluded that 17α-estradiol was the primary steroid hormone in fresh dairy waste, but the major hormone contaminant in the environment was estrone due to the rapid transformation of 17α-estradiol to estrone along the dairy waste disposal system.

Appendix 12.2.6. Anaerobic transformation kinetics and mechanism of steroid estrogenic hormones in dairy lagoon water [Zheng et al.]

The following study indicated that 17α-estradiol and 17β-estradiol may oxidize to estrone in aqueous solutions containing dairy wastewater and that estrone may be reduced back to 17α- and 17β-estradiol under anaerobic conditions. Therefore, aeration of lagoons may aid in removal of estrogens.

In a study to investigate the transformation kinetics and mechanisms of 17β-estradiol, 17α-estradiol and estrone under anaerobic conditions, Zheng et al. [9] analyzed CAFO lagoon water spiked with stock solutions of the three hormones. CAFO lagoon water was diluted 1:1 with deionized water within an anaerobic glove bag. Solutions were purged with nitrogen gas for 30 minutes prior to use and spiked at an initial concentration of 5 mg/L for each hormone. Solutions were incubated at temperatures that ranged from 15-45°C in the dark. At regular time intervals, and within the anaerobic glove bag, aliquots of incubation solutions were withdrawn, transferred to a centrifuge tube containing equal volumes of methanol, centrifuged and filtered. Addition of methanol was used to quench hormone transformation and resulted in 95 to 100% recovery of the three estrogenic hormones. A control experiment using sterile lagoon water was also performed to determine abiotic degradation. Samples were analyzed by HPLC with a diode array detector and MS. Peak identification was performed by LC/MS. Initial biodegradation rates were determined using a pseudo-first-order kinetic model.

For all hormones, biodegradation rates increased with increasing temperature up to 35°C and decreased at 45°C. 17β-Estradiol (0.0675 h^{-1}) had the fastest degradation rate followed by 17α-estradiol (0.0172 h^{-1}) and estrone (0.00249 h^{-1}). Based on the results of the initial kinetic study, a longer-term transformation study was conducted for 52 days at 35°C at 5 mg/L. 17α-Estradiol was rapidly transformed to estrone within 2 days; however, estrone concentrations decreased over time with a concurrent formation of 17α-estradiol. 17β-Estradiol was detected after 2 days and rapidly transformed to estrone, which subsequently began to accumulate over time. Results indicated that 17α-estradiol and 17β-estradiol may oxidize to estrone in aqueous solutions containing dairy wastewater and that estrone may be reduced back to 17α- and 17β-estradiol under anaerobic conditions. Because of the potential degradation pathways, there are two consecutive reversible first-order reactions that can occur. The rate constants ranged from 0.010 day^{-1} to 1.518 day^{-1}.

Results also indicate that anaerobic degradation is rapid in the first 24 hours and slows over time. This may be of concern because 17β-estradiol and estrone have much higher estrogenic potency compared to 17α-estradiol. These compounds may persist, and possibly accumulate, in anoxic conditions (e.g., anaerobic CAFO lagoons and underlying sediments). As observed in this study by Zheng et al. [9], 77 to 85% of the initial spiked amount of hormones (17α-estradiol, 17β-estradiol, and estrone) remained after 52 days. Therefore, aeration may be very effective in eliminating these hormones because aerobic biodegradation is very rapid (e.g., estrone can be rapidly degraded to CO_2).

Appendix 12.2.7. Trenbolone metabolite concentrations and degradation in solid and liquid manure and degradation in soil [Schiffer et al.]

The following study indicated that in anaerobic liquid manure, 17α-trenbolone was the predominant metabolite followed by 17β-trenbolone and trendione. When liquid manure containing TBA metabolites was applied to soil, 17α-trenbolone, 17β-trenbolone and trendione were rapidly degraded.

Schiffer et al. [218] evaluated the residues and degradation of trenbolone metabolites in solid dung, liquid manure, and soil. There were three phases to this study that involved the determination of trenbolone metabolites in these compartments. First, 41 cattle were implanted with TBA (3,340 total mg or 81.5 mg/animal). Liquid manure was collected in a canal for two weeks then transferred to a larger storage unit under anaerobic conditions. Samples of liquid manure were taken every second week from the collection canal, every 2 or 4 weeks from the storage tank and after storage for 5.5 months before spreading onto fields. In the second phase, twelve heifers were implanted with TBA (5,600 total mg or 467 mg/animal) and solid dung was collected in a dung hill, which represented dung from Day 31 before treatment through Day 56 after treatment (estimated volume of 40 m^3 and a mass of 20 metric tons). Samples were collected from four different locations on the dung hill (top, middle, bottom, and liquid effluent). The solid dung was transferred to a sealed storage container, and samples were taken from the top, middle, and bottom after 4.5 months of storage. Finally, the third phase involved applying the solid and liquid manure to agricultural land and evaluating the degradation in soils. Soil samples were collected from three locations immediately after application and regularly each month or every second month. SPE eluates were separated by HPLC on a C18 reverse-phase column. The HPLC fractions were quantified by enzyme immunology. Procedures were validated by mass spectroscopy.

In the anaerobic liquid manure, 17α-trenbolone was the predominant metabolite followed by 17β-trenbolone and trendione. 17α-Trenbolone was 23 and 47 times more prevalent than 17β-trenbolone and trendione, respectively. The anaerobic DT_{50} in liquid manure for 17α-trenbolone and 17β-trenbolone were calculated to be 267 and 257 days, respectively. Although 17α- and 17β-trenbolone were degraded, trendione did not accumulate and remained a minor component. The overall mass balance of the principal metabolites indicated approximately 8% of the TBA released from the implants was recovered as metabolites in the liquid manure prior to storage (Table 6 of reference [218]). When soil was fertilized in the fall with fresh liquid manure, there were no detectable TBA metabolites in soil at 31 days (the first sampling point). The study was repeated in the spring with stored manure and included earlier sampling times (Table 125). On Day 1 after fertilizer was applied, the total sum of TBA metabolites was 232 ng/kg soil. The concentration declined 87% to 29 ng/kg soil after 8 days. On Day 40, no metabolites were detectable in soil (Table 125).

Table 125. Degradation of TBA Metabolites in Soil Manured with Liquid Cattle Manure in Spring

Days After Soil Fertilization (# of locations)	17α-Trenbolone, ng/kg	17β-Trenbolone, ng/kg	Trendione, ng/kg	Total Average TBA Metabolites, ng/kg
Day 1 (n=2)	248, 164	8.1, 5.1	21,18	**232.1‡**
Day 8 (n=3)	11, 8.6, 48	ND†, ND, 2.4	2.2, 1, 15	**29.4**
Day 40 (n=1)	ND	ND	ND	**ND**

† ND – not detected; Data from Schiffer et al. [218]
‡ Calculated by adding the mean of 17α-TB +17β-TB + trendione

The DT_{50} of total TBA metabolites in manured soil was ≤3 days (72 hours). This was determined by taking the slope of the natural log (LN) of the total TBA metabolite concentrations on the Y axes plotted against time (in days) on the X axis. Additional details on linear transformation to calculate a DT_{50} are found in Appendix 13.1.

Similar to liquid manure, 17α-trenbolone was the main metabolite measured in solid dung. Compared to liquid manure, the concentrations of trenbolone metabolites in solid dung were 5 to 70 times higher. The overall mass balance of the principal metabolites recovered in solid manure stored in a dung hill and storage container ranged from 3-42% of the TBA released from the implants. The measured concentrations of trenbolone metabolites are shown in Table 126. After storage, <2.9% of the dose was recovered in stored solid manure, indicating the instability of trenbolone metabolites in aerobic manure (Table 126). In effluent draining from the fresh manure, the total concentration of trenbolone metabolites was 256 ng/kg (227, 19, and 10 ng/kg for 17α-trenbolone, 17β-trenbolone, and trendione, respectively). The stored dung was then applied to cropland, and soils were collected at 26, 58, 93, 159, and 194 days after manure application. The mean concentrations of 17α-trenbolone, 17β-trenbolone and trendione in soils after 26 days were 6.7, 0.7, and 2.7 ng/kg, respectively, for a sum of trenbolone metabolites equal to 10.1 ng/kg soil (Table 3 of reference [218]). The concentrations of trenbolone metabolites were reduced further by Day 58 and were no longer detectable by Day 93.

Table 126. Residues of Trenbolone in Solid Dung of Cattle Treated with 467 mg TBA/Animal and Theoretical Percent Recoveries

Sample	17α-Trenbolone, ng/kg	17β-Trenbolone, ng/kg	Trendione, ng/kg	Sum of TBA Metabolites, ng/kg (% recovery§)
Solid dung before storage				
Fresh dung 1 m below top	13,800	1000	1,225	16,000 (9%§)
Middle of pile 2.5 m	75,400	4,265	4,700	84,400 (46%)
Old height (0.5 m)	4,726	484	405	5,620 (3.1%)
Effluent	227	19	10	256 (NA†)
Solid dung after storage and before spreading on fields				
Top of hill	<5‡, <5	11, <5	<5, <5	18 (0.01%)
Middle of hill	10,100, <5	292, <5	824, <5	5,620 (2.9%)
Bottom of hill	100, 318	60, 14	70, <5	284 (0.16%)

Data from Table 2 and Table 6 of Schiffer et al. [218]; † NA-not applicable; ‡ 5 ng/kg limit of detection.
§ % recovery: Example 16.0/181.8 = 9%, where 3,635 mg trenbolone excreted / (20 metric tons of solid dung x 1000 kg/ton) = 0.1818 mg/kg = 181.8 µg/kg starting concentration. See Table 6 of Schiffer et al. [218].

These data demonstrate the instability of trenbolone metabolites in aerobically-stored solid manure and in anaerobic liquid manure. The study also demonstrates that trenbolone and metabolites are further degraded when solid and liquid manure are applied to soil.

Appendix 12.2.8. Estrogens and synthetic androgens in manure slurry and waste lagoons from estradiol and trenbolone implanted cattle [Khan and Lee]

The following study indicated that in manure pits containing excreta from TBA and EB implanted cattle, the primary TBA metabolite was 17α-trenbolone followed by 17β-trenbolone then trendione. For estrogens, estrone was the predominant metabolite, then estriol, 17α-estradiol and 17β-estradiol.

Khan and Lee [214] quantified trenbolone and estradiol metabolites in manure collection pits and lagoons that received waste effluent from beef cattle implanted with Revalor-S (140 mg of TBA and 28 mg of estradiol). Manure pits were located directly adjacent to barns holding 91 to 110 beef cattle at the Purdue Animal Science Research and Education Center. The contents of these pits flowed into primary and secondary lagoons used for irrigation. Samples from the manure collection pits and lagoons receiving effluent were collected weekly for nine weeks following cattle implanting. Samples were extracted and analyzed by LC-ESI-MS/MS. Recoveries of 17β-trenbolone and 17β-estradiol (spiked at ~40,000 ng/L) were reported as 92 ± 7% and 100 ± 2%, respectively.

In the manure pits, the highest androgen concentrations were 2,900 ng/L for 17α-trenbolone observed two weeks after implantation. The highest 17β-trenbolone concentrations detected in the manure pits were ≤180 ng/L observed in weeks two and three, and the highest trendione concentrations were ≤120 ng/L observed in weeks two and four. The highest estrogen concentrations measured in manure pits were 990 ng/L for estrone at week four, 2730 ng/L for estriol at week six, and 590 ng/L for 17α-estradiol at week eight. Concentrations of 17β-estradiol ranged between 10 and 60 ng/L, with no clear pattern over time. In samples from the wastewater lagoons used for irrigation, concentrations ranged

from 22-1720 ng/L for 17α-trenbolone, 4-110 ng/L for 17β-trenbolone, and 6-150 ng/L for trendione. Concentrations of estrogens in lagoon waters ranged from 118-1810 ng/L for estrone, 1-960 ng/L for estriol, 9-480 ng/L for 17α-estradiol, and 4.5-90 ng/L for 17β-estradiol.

Appendix 12.2.9. Estrogen concentrations in lagoon samples from swine, poultry, and cattle CAFOs [Hutchins et al.]

The following study indicated that the total concentration of estrogens in the secondary lagoons on beef cattle feedlots was much lower than the concentrations found in the secondary lagoons of dairy cattle operations. The beef cattle secondary estrogens were also less than the estrogens in the primary lagoons of commercial swine and poultry operations.

Analysis of lagoon samples from three locations (commercial swine, poultry, and dairy and beef cattle CAFOs) for free estrogens and estrogen conjugates was performed by Hutchins et al. [215]. The authors analyzed the aqueous phases of lagoons for the presence of free estrogens (E1, 17α-E2, 17β-E2, EE2 and E3) using SPE followed by derivatization and GC-MS/MS analysis. The sediments were also analyzed following an extraction procedure using 50/50 methanol/acetone, and the extraction was repeated two additional times. To estimate the contribution of conjugated estrogens, enzyme hydrolysis using β-glucuronidase/arylsulfatase from *Helix pomatia* was performed on a second aliquot. Estrogen conjugates were directly measured from the aqueous phase using SPE and LC-MS/MS methods.

Concentrations associated with beef cattle feedlot lagoons were reported as ~20 ng/L estrone, ~7 ng/L of 17α-estradiol, and 'not detected' for 17β-estradiol and estriol (<20 ng/L and <8 ng/L, respectively) (see Figure 2 of reference [215]). The authors focused much of their discussion on the data from lagoons of commercial operations of swine, poultry and dairy cattle because these had noticeably greater concentrations when compared to beef cattle operations. For beef cattle operations, no estrogen conjugates were detected above the method detection limits, with results reported as <1.0 ng/L. The indirect analysis for estrogen conjugates indicated approximately 30% of the total estrogens from beef cattle lagoons were unidentified conjugates, and it appears these were estrone and 17α-estradiol conjugates based on the data presented.

There were many issues regarding analyses reported in the publication. The authors noted that the estrogen conjugate concentrations were extracted from complex lagoon matrices with high interference potential in the SPE and LC-MS/MS methods and should be considered in view of the blank water spike and matrix spike recoveries performed with the samples. Three estrogen conjugates (estrone-3-sulfate, 17α-estradiol-17-sulfate, and estriol-3-sulfate) had the best and most consistent recoveries; however, nine other estrogen conjugates were often low and highly variable. In addition, there was increased variability among sample locations, which the authors suggest is due to unknown additional chemical or biological differences associated with the locations.

Appendix 12.2.10. Steroid estrogens, conjugated estrogens and estrogenic activity in farm dairy shed effluents [Gadd et al.]

The following study indicated that 17α-estradiol and estrone are the most prominent estrogens detected in dairy lagoons.

Gadd et al. [10] measured the concentrations of free and conjugated estrogens in dairy farm shed effluents in New Zealand. Samples were collected from milking shed effluent collection sumps, and the outlet of treatment ponds (if present) on 18 privately owned farms with herd sizes ranging from 140-1000 cows. Samples were centrifuged, filtered, and extracted in duplicate using SPE methods followed by additional clean up steps. Steroid estrogens were analyzed by GC-MS, conjugated estrogens were analyzed by LC-MS/MS, and estrogenic activity was measured using bioassays with human breast cancer cells (MCF7-BOS). Results indicated that 17α-estradiol and estrone were the most prominent estrogens detected in the dairy shed effluents, with median concentrations of 730 ng/L and 100 ng/L, respectively (see Table 2 of reference [10]). The median 17β-estradiol concentration was 24 ng/L. EEQs measured in dairy shed effluents using bioassays ranged from approximately 10 to 650 ng/L and were highly variable (see Figure 1 of reference [10]). Conjugated estrogens contributed up to 22% of the total estrogen load from the dairy farms and the median estrogenic activity was 46 ng/L.

Appendix 12.2.11. Environmental estrogen concentrations associated with swine farms [Schuh et al.]

The following study indicated that estradiol and estrone were frequently detected in the environment and the frequency was not associated with proximity to manure piles. This high frequency may be due to natural environmental production.

Schuh et al. [216] evaluated the spatial and temporal variation of 17β-estradiol in pore water from soil cores at or near a swine facility in North Dakota. Samples were taken at four sites, 13 depths, and on five sampling dates. Sample sites were located near a manure storage pond, near lysimeter plots, near a static manure pile, and near a well installed by the state's Water Commission. Duplicate core samples were collected from each site and soil moisture and organic matter content were determined. It is important to note that estradiol was quantified in soil-water extracts (using 0.01M $CaCl_2$) and purified by SPE. Samples were analyzed by LC-MS/MS. 17β-Estradiol was detected in 37% of the samples (128 of 345 extractions) at up to 1,910 mg/L (porewater equivalents). The highest mean estradiol concentration was typically measured in soils collected near the static manure pile. Mean concentrations from each sample date from near the manure pile were in the range of 1.87-330.67 ng/L of 17β-estradiol, with a geometric mean of 81.03 ng/L. Geometric means of 17β-estradiol measured near the manure storage pond, lysimeter plot, and state well were 31.86, 16.83, and 47.97 ng/L in the water extractable fraction, respectively. However it is likely that a large portion of estradiol remained bound to the soil. The highest frequency of detection in the soils was closest to the water table, but the highest concentrations were found in the upper soil profile. It is was hypothesized that 17β-estradiol sorbed in the surface horizon where there is greater organic matter content and that estradiol persists longer near the water table as a result of anaerobic conditions. Similar to other reports, 17β-estradiol was detected in locations where manure had not been directly applied and concentrations varied across location and sampling time. 17β-Estradiol was widespread and not directly related to manure sources, climate and hydrological events.

Appendix 12.3. Literature data on concentrations of estradiol and trenbolone metabolites detected in surface waters in the US

Appendix 12.3.1. Estrogen concentrations in California inland water agriculture areas [Lavado et al.]

The following study indicated that although 17β-estradiol equivalent concentrations (EEQs) in bioassays may be elevated in surface water samples, fractionation analysis of the most active samples indicated that 17β-estradiol and estrone were not responsible for the estrogenic activity.

In a study by Lavado et al. [223], a total of 101 surface water samples were analyzed from 16 sites covering the prevalent land-use types in California's Central Valley. Samples were collected between July 2006 and April 2007 from the Sacramento-San Joaquin River system. Estrogenic activity in water samples was determined using bioassays designed to measure *in vitro* and *in vivo* production of vitellogenin mRNA by quantitative polymerase chain reaction (qPCR).

Estrogenic activity was detected in samples from six of the 16 sites. 17β-Estradiol equivalent concentrations (EEQs) in *in vitro* bioassays ranged from <0.15 ng/L to 241.8 ng/L. The sample with the highest mean *in vitro* EEQ (from the Tuolumne River, Sept. 2006) was below the detection limit (<0.15 µg/kg) as measured by the *in vivo* bioassay.

Samples with high estrogenic potential based on results from the in vitro assay were fractionated and subjected to further analyses by GC-MS/MS. Fractionation analysis of the most active samples indicated that different chemical compounds were responsible for the estrogenic activity. Further fractionation of the most active samples using HPLC showed *in vitro* and *in vivo* estrogenic activity in multiple fractions. Chemical analyses indicated only nine of 90 total samples tested were above detection limits (detection limit varied from 0.1 to 1 ng/L) for 17β-E2, 17α-E2, E1, or E3. Concentrations ranged from ND to 23 ng/L estrone (10% occurrence), ND to 0.5 ng/L of 17β-estradiol (3% occurrence), ND to 0.1 ng/L of 17α-estradiol (1% occurrence), and ND for estriol (0% occurrence) (see supplemental information Table S3 reference [223]. Testosterone was detected in two samples (0.1 and 0.6 ng/L), and androstenedione was detected in one sample (1.1 ng/L).

Appendix 12.3.2. Source of steroid hormones to surface waters from pastureland in California [Kolodziej and Sedlak]

The following study indicated that exposures to maximum measured hormone concentrations in surface waters could potentially cause reproduction effects in chronically exposed aquatic organisms. However, these concentrations were not sustained over extended periods of time and are acute exposures.

Kolodziej and Sedlak [220] measured hormone concentrations in small tributaries associated with rangeland grazing cattle in California. Sampling locations were representative of small headwater creeks found in many watersheds where cattle grazing is the predominant land use. Many of these tributaries are dry during much of the year and likely are not significant areas of fish reproduction. Collected water samples were filtered, extracted using SPE discs, and analyzed by GC-MS/MS. Maximum concentrations of 17α-estradiol, 17β-estradiol, estrone and testosterone measured in the rivers were 25, 1.7, 38, and 4.3 ng/L, respectively. All steroid analytes were detected in at least one of the

88 water samples, with estrone being detected most frequently. Estrone was detected in 78% of the samples at concentrations up to 38 ng/L. 17α-Estradiol was present in 31% of water samples at concentrations up to 25 ng/L. 7β-Estradiol was present in 18% of water samples at concentrations up to 1.7 ng/L. Only 2 of the 88 samples were detected at concentrations above 1 ng/L. Testosterone was also measured in 11% of water samples and did not exceed 2.3 ng/L. Although maximum measured hormone concentrations could potentially cause reproduction effects in chronically exposed aquatic organisms, these concentrations were not sustained over extended periods of time [221], and therefore effects on fish are not expected. The maximum steroid concentrations occurred during the wet season, while during the dry season, concentrations were usually <0.3 ng/L (LOQ) [221]. Locations that frequently exhibited elevated hormone concentrations were those that had low stream flows, suggesting less in-stream dilution. These high concentrations were comparable to (or higher than) concentrations observed in surface waters near dairy operations and in WWTP effluent. Therefore, although there were not enough monitoring data at the time of this work, the potential for persistent elevated steroid concentrations may be a concern in some locations.

Appendix 12.3.3. Sediment and watershed ED analyses in agricultural intensive areas of Nebraska

An examination of the density of feedlots in the Elkhorn River area (northeast Nebraska) indicates this area has a very high density of feedlot cattle and is an appropriate area to look for potential effects of CAFOs on the aquatic environment (Figure 4). The following four studies were conducted in this region.

The anti-estrogenic activity of sediments from agriculturally intense watersheds [Sellin Jeffries et al.]

The following study demonstrated that sediments can cause defeminization of female fathead minnows. However, the authors could not definitively demonstrate a causal relationship between the presence of 17β-trenbolone in the sediments and endocrine disrupting effects on fathead minnows.

A study conducted by Sellin Jeffries et al. [225] examined the potential for sediments and water in watersheds with intensive beef cattle production to cause androgenic effects in female fathead minnows. Samples were collected from Bow Creek (sample sites at East Bow Creek and the confluence of Bow Creek and the Missouri River) and Elkhorn River watersheds. The water and sediment were analyzed for 46 pesticides using GC-MS and 20 steroid hormones using LC-MS/MS. The potential for androgenic activity was measured by hepatic VTG and estrogen receptor alpha (ERα) mRNA expression in minnows. The study detected 17β-trenbolone in the sediment of the Elkhorn and Confluence Rivers indicating the presence of synthetic hormones in surface waters from use in beef cattle. No pesticides were detected.

Fathead minnows were also exposed in laboratory aquaria for 7 days to sediment and/or water collected from East Bow Creek, the Confluence River, and Elkhorn River. The natural hormones epitestosterone, testosterone, 17α-estradiol, estrone, progesterone and 4-androstenedione were observed at various times during aquarium incubations. Hepatic VTG and estrogen receptor alpha (ERα) mRNA expression were also measured. Females exposed to sediments from the Confluence River experienced defeminization with a significant reduction in ERα expression. However, chemical analyses were not conducted

for this test to determine steroid hormone concentrations in sediments. Therefore, the defeminization of fish cannot be definitively linked to the presence of steroid hormones. When the study was repeated the following year, 17β-trenbolone was detected in the sediments but exposed females did not show evidence of defeminization. Given this, the authors could not definitively demonstrate a causal relationship between the presence of 17β-trenbolone in the sediments and endocrine disrupting effects on fathead minnows.

Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in Eastern Nebraska [Soto et al.]

In the following study, androgenic activity was observed in sites around beef CAFOs and at a reference site. The authors concluded that the activity was due to natural androgens in the environment and were not necessarily attributable to the trenbolone metabolites.

In 1999, 2000, and 2001, Soto et al. [11] analyzed water samples from six sites on the Elkhorn River in Nebraska for feedlot effluent contaminants as a potential source of androgens. The Elkhorn River is a 4000 sq. mile watershed with high density livestock operations along the river and approximately 1.2 million head of cattle maintained in the area. Water samples were collected from a feedlot retention pond, a drainage canal 0.5 km downstream from the feedlot, a stream with intermediate livestock impact, and three sites along the Elkhorn River, including sites near CAFOs and reference sites with no apparent feedlot activity in the surrounding areas. The reference site was the furthest location (80 km) from the nearest feedlot. Water samples were extracted using dichloromethane and analyzed by GC-MS. However, the 2001 water samples were extracted via immunoaffinity columns, compounds were separated by HPLC, and estrogen and trenbolone fractions were analyzed by enzyme immunoassay (EIA). Unfortunately, it was reported that there was a low signal-to-noise ratio when analyzing estrogens and androgens by GC and EIA which was problematic. The only measurable analyte by EIA and GC-MS in these three tributaries was estrone (E1) with concentrations ranging from <0.21 to 2.16 ng/L, depending on the detection method.

In the same study, Soto et al. [11] also conducted bioassays using androgen- and estrogen-target human breast cancer cells (MCF7-BOS or MCF7-AR1 cells). Estrogenic and androgenic activities of water sample extracts were measured in the range of 0.76 to 1.15 picomolar (pM) and 2.45 to 4.58 pM, respectively. This corresponds to estradiol equivalent concentrations ranging from 0.21-0.31 ng/L and trenbolone equivalent concentrations ranging from 0.66-1.24 ng/L. Estrogenic and androgenic activities measured in these bioassays were higher in the retention pond, drainage canal, and stream drains (all within close proximity to the cattle feedlot), with estrogenic activity ranging from 0.65-2.23 pM, or estradiol equivalent concentrations ranging from 0.18-0.61 ng/L. While androgen levels were considerably lower at the furthest site from feedlots when compared to levels in the retention pond, estrogen levels were comparable with that of the stream site with intermediate livestock impact. Androgenic activity in bioassays ranged from 3.83-9.62 pM, which is equivalent to trenbolone concentrations from 1.04-2.60 ng/L. The highest concentration of androgens was detected in samples from a cattle effluent holding pond directly below the feedlot. In water samples collected in 1999 and 2000 from the retention pond and reference site, estrogenic and androgenic activities were similar (i.e., within 2- to 3-fold between years). Estrone concentrations determined by enzyme immunoassay (EIA) and GC-MS were slightly higher in samples collected in September 2000 when compared to June 1999. In samples collected in July 2001 from the retention pond, a stream with intermediate impact, and reference site, the concentrations of

trenbolone metabolites as measured by EIA ranged from <0.0003 to 0.035 ng/L with the highest being 17α-trenbolone. It was concluded that marginal levels of 17α- and 17β-trenbolone contributed to 0.1 to 1.1% of the total androgenic activity measured in the bioassays and that the majority of androgenic activity may be attributed to naturally-occurring androgens. The highest levels of estrogens measured were 8.30 ng/L estrone, <3.80 ng/L of 17α-estradiol, and <3.2 ng/L of 17β-estradiol, the latter being limited by the detection limits of the analytical methods. Although androgenic activity was observed, the authors concluded it was due to natural androgens in the environment and was not necessarily attributable to the trenbolone metabolites.

Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow [Orlando et al.]

In the following study, fathead minnows exposed to cattle feedlot effluents exhibited altered reproduction biology and androgenic responses. Regardless of the presence of feedlot effluents, there were viable populations of the minnows in the streams below the outfall of feedlots or fields with dispersed cattle.

Orlando et al. [226] examined whether endocrine activity could be detected in natural stream/river systems below feedlots by studying the reproductive endocrinology and secondary sex characteristics of the fathead minnow (FHM), *Pimephales promelas*, living upstream and downstream of Nebraskan cattle feedlots. The sites of research were: 1) a stream directly below the effluent outflow of a high density cattle feedlot, the “contaminated” site, and 2) a stream that received runoff from fields where cattle were more widely dispersed, the “intermediate” exposure site. Both sites drain into the Elkhorn River and have several commercial feedlots that release effluents into retaining ponds which then drain into the river. Additionally, “reference” sites were those that were upriver from the feedlots and the streams of which flowed into the river but with no apparent feedlot activity in the surrounding areas.

Morphological measurements of FHMs collected from each site were obtained. Hepatic tissue and gonads were removed to determine their mass, and then the gonads were transferred to an explant culture where the *in vitro* gonadal synthesis of sex steroid hormones in male and female FHMs was examined. The *in vitro* production of estradiol and testosterone in female FHMs and testosterone in male FHMs were measured via radioimmunoassay on extracts of the culture media. Bioassays for hormonal activity (androgenic and estrogenic) were performed with water collected from the contaminated, intermediate and reference sites. Also, water was obtained from a retaining pond located immediately at the base of a feedlot whose outflow is the headwaters for the contaminated site. These water samples were analyzed for *in vitro* androgenic and estrogenic activity. Additionally, water samples collected from the retaining pond at the base of the feedlot was assayed to determine if feedlot effluent displayed androgen receptor agonist activity, i.e., induction of human androgen receptor-dependent gene expression in CV-1 cells from a monkey kidney line. Hormones were not quantified in this study.

All statistical tests for significance were at an alpha level of 0.05. No significant differences were noted in length and mass, as well as ovary or liver mass, among female FHMs from the contaminated, intermediate, and reference sites. Interocular distance (IOD) was significantly different, with females from the contaminated and intermediate sites having smaller distances than females from the reference site. Head widths were not different. No significant differences were noted in length or body mass among male fish collected at the

three sites. In males, there was a significant difference in testicular but not hepatic mass. As was the case for females, the IOD was significantly different with males from the contaminated and intermediate sites having reduced distances compared with males from the reference site. No significant difference in ovarian estradiol synthesis was seen among the three sites, and mean ovarian synthesis of testosterone was not different among sites. A significant difference was apparent when the data from the females were evaluated as a ratio of estrogen to androgen. The females from the contaminated and intermediate sites had a decreased estrogen:androgen ratio, based on a decrease in estradiol synthesis and an increase in testosterone synthesis. No overt characteristics were seen in either male or female fish that suggested environmental exposure to estrogens. In the *in vitro* assay however, potent androgenic responses to feedlot effluent were detected. The authors did not identify the agents in the feedlot effluent that produced androgenic responses in the FHM.

Regardless of the presence of feedlot effluents, there were still viable populations of FHM at all of the sites that were studied.

Geographic trends in contamination of Nebraska's surface waters as indexed by sex steroids of common carp [Pope et al.]

The following study indicated that there is not a geographic trend in the occurrence of ED compounds in the lakes of Nebraska.

The results of Soto et al. [11] were also supported by a survey conducted by Pope et al. [230]. Water and fish tissue (gonads and liver) and blood (n=20 fish per site) samples were collected from 18 lakes and reservoirs in Nebraska. Sites were randomly selected based on the type of water body and geographic location within the state. Water samples were analyzed for: 4-androstenedione, androsterone, 17α-trenbolone, 17β-trenbolone, α-zearalenol, β-zearalenol, estriol, estrone, ethinylestradiol, melengestrol acetate, progesterone, 17β-estradiol and testosterone. Samples were analyzed using on-line solid phase extraction (SPE) LC-MS/MS. Plasma estradiol and testosterone levels in carp were determined by radioimmunoassay, and hepatic VTG mRNA expression was determined by real-time PCR for five male carp from each field site. No steroid hormones were measured above the limit of detection (5 ng/L) with the exception of one sample at 6 ng/L of androsterone. Significantly higher plasma estradiol/testosterone ratios were detected in male carp collected from Lake Zorinski when compared to any other site ($p<0.0001$). In addition, male carp from Lake Ericson had significantly higher ratios when compared to nine of the other sites. There were no significant differences in hepatic VTG mRNA expression of male carp from any of the field sites (statistical significance was set at $\alpha = 0.05$). The authors concluded that, despite their *a priori* expectations, they did not find a geographic trend in the occurrence of EDCs.

Appendix 12.3.4. Estrogens and androgens in surface waters surrounding a dairy CAFO in North Dakota [Shappell et al.]

The following study demonstrated that when farmers use BMPs for applying wastes from a large dairy farm, there were negligible estrogenic compounds in surface waters adjacent to the farm.

Shappell et al. [227] evaluated estrogen loading from dairy waste application and estrogenic activity of surface waters surrounding a large dairy CAFO in North Dakota that employed BMPs for land application of dairy waste. Application rates were compliant with BMPs for

nutrient loading according to the USDA Natural Resource Conservation Service, and each field received only a single application. Surface waters upstream and downstream of the CAFO and manured fields receiving dairy waste were sampled in April, June, and July during the period of wastewater land application. Tile-drain samples were also tested from two locations, as were two sites upstream and downstream of a nearby WWTP. Samples were extracted using SPE and evaluated for estrogenic activity in bioassays using human breast cancer cells (MCF7-BOS). Surface waters with the highest 17β-estradiol equivalents were confirmed by LC-MS/MS. Although there was evidence of estrogens in samples taken from tile-drains, estrogenic activity of all sites was below 1 ng/L. Mean 17β-estradiol equivalent concentrations were not different along the creek within and across sampling dates ($p>0.20$), whether upstream or downstream of the WWTP or CAFO. Mean 17β-estradiol equivalents in the creek samples ranged from 0.044-0.120 ng/L and decreased to <0.010 ng/L in July. The authors concluded that the use of BMPs for applying wastes from a large dairy farm resulted in negligible estrogenic contributions to surface waters adjacent to the farm.

Appendix 12.3.5. Androgenic activity and concentrations of trenbolone metabolites in runoff and rivers in Ohio [Durhan et al.]

The following study indicated that 17α- and 17β-trenbolone can be detected in the discharge drain of a cattle facility. However, the authors were unable to conclude that 17α- and 17β-trenbolone were responsible for androgenic activity in the river.

In 2002 and 2003, Durhan et al. [29] collected samples on nine different occasions from three locations on and adjacent to a CAFO in southwest Ohio: 1) 572 meters upstream from all drainage from the facility, 2) a discharge drain that collects runoff from the two sets of buildings housing cattle, and 3) 381 meters downstream from the discharge drain. The feedlot held 9800 head of cattle and consisted of eight cattle buildings on 96 ha. The facility was said to have used Revalor-S implants (Intervet, Inc.), which contains 120 mg of TBA and 24 mg of 17β-estradiol in six pellets. To determine the occurrence of 17α- and 17β-trenbolone in the drainage discharge and in the stream adjacent to the CAFO, samples were processed by filtration, followed by SPE, and then analyzed by HPLC with fluorescence detection (LOD = 4 ng/L). A subset of samples were further analyzed by GC-MS (LOD was variable and ranged from <10 to 100 ng/L) to validate the HPLC analysis. In addition, the *in vitro* androgenic activity was analyzed using CV-1 cells that had been transiently co-transfected with human androgen receptor and reporter gene constructs. Many of the CV-1 assays were performed only once due to logistical constraints. However, samples collected on four of the sampling occasions (February 2002, March 2002, October 2002, and March 2003) were assayed three separate times, and these data were used to estimate a mean and for statistical analyses.

The *in vitro* bioassays demonstrated that there was no significant androgenic activity ($p>0.05$) in samples collected upstream and downstream from discharge drains. However, the mean androgenic activity in samples collected from a discharge drain (samples from four of the nine collection times), was significantly above media controls and comparable to the positive control, dihydrotestosterone (DHT). It is important to note that although the mean androgenic activity of the discharge drain samples was significantly above media controls, the androgenic activity from the discharge drain samples was significantly elevated in only half of the nine samples collected, i.e., half of the samples were not significantly elevated compared to the media controls. The authors also reported high variability in the CV-1 cell bioassay with a relatively flat dose response relationship. The assay achieved statistical

significance with ≥5.4 ng/L of 17β-trenbolone and ≥13.6 ng/L of 17α-trenbolone. The authors noted that because of the variations in efficiency of transfection of the receptor/reporter gene construct between experiments, the CV-1 assay is considered a semi-quantitative measure of androgenic activity.

Although there was no androgenic activity demonstrated in the bioassays of samples collected upstream and downstream of the CAFO drainage, 17α- and 17β-trenbolone were detected in four and three, respectively, of the nine samples at varying concentrations (Figure 3 of Durhan et al. [29]). However both 17α- and 17β-trenbolone were detected more often in the discharge drain samples than in the stream samples, and 17α-trenbolone was detected more frequently and at higher concentrations than 17β-trenbolone. 17α-Trenbolone was detected in six of the nine samples from the discharge drain with concentrations ranging from <10 to 120 ng/L, whereas 17β-trenbolone was only detected in two of the nine discharge drain samples at 10 and 20 ng/L. GC-MS analysis was also used to confirm concentrations of the trenbolone metabolites though the detection limits varied greatly (10 to 100 ng/L) and, according to the authors, did not allow a sufficient number of confirmatory analyses to validate the HPLC results.

Interpretation of the data presented in this publication is very difficult due to the analytical variability. Although Durhan et al. [29] were able to detect 17α- and 17β-trenbolone in the discharge drain of a cattle facility, they were unable to conclude that 17α- and 17β-trenbolone were responsible for the androgenic activity observed in the study. The authors suggested that additional work is needed to determine the influence of natural steroid hormones, such as testosterone, on the androgenic activity in the water associated with these sites.

Appendix 12.3.6. Hormone monitoring at the Purdue University Agricultural Experiment Station in Indiana

The following two studies were conducted at the Purdue University Agricultural Experiment Station in Indiana.

Hormone discharges from a Midwest tile-drained agroecosystem receiving animal wastes [Gall et al.]

In the following study, estrone was the estrogen detected most frequently. Androgen concentrations were highest in fall and winter. Concentrations of TBA metabolites when detected ranged from 1.6-162 ng/L (Table 121 through Table 123). Testosterone and androstenedione were the most frequently detected androgens, with synthetic androgens detected in <15% of samples. These data demonstrate that natural and synthetic hormones can move (presumably through soil micropores) to the ditches around tile-drained agricultural fields.

Gall et al. [206] monitored the presence of natural and synthetic hormones from tile-drained fields at the Purdue University Agricultural Experiment Station in Indiana. Waste from beef cattle, dairy cattle, poultry, and swine facilities were applied to agricultural fields. Application methods involved solids broadcasting, pivot irrigation, or subsurface injection, depending on the manure source (*viz.*, solid waste, lagoon, liquid slurry storage units). The beef cattle were implanted with Revalor-S containing 28 mg 17β-estradiol and 140 mg of TBA. Both solids and liquids from collection lagoons were applied to agricultural fields. Analyses were conducted for 17α- and 17β-estradiol, estrone, estriol, testosterone, androstenedione, 17α-

and 17β-trenbolone and trendione at seven sampling stations over a 15-month period. Hormones were extracted from water samples by SPE and analyzed by HPLC-ESI-MS/MS. Measured estrogen concentrations (17α-estradiol, 17β-estradiol, estrone, and estriol) were highest in spring and summer and ranged from 1.3-51.8 ng/L (Table 117 through Table 120). Estrone was detected most frequently, probably because estrone is more stable than other estrogens. Androgen concentrations were highest in fall and winter [209]. Concentrations of TBA metabolites ranged from 1.6-162 ng/L (Table 121 through Table 123). Testosterone and androstenedione were the most frequently detected androgens, with synthetic androgens detected in <15% of samples. It was noted that hormones were subject to sorption and microbial degradation prior to extracting the water samples in the laboratory. The authors indicated that there was the potential for substantial degradation of some hormones within the first 24 hours after water collection, and therefore hormone concentrations from subsurface tile drain and ditch network samples from this study are likely underestimated.

In general, these data demonstrated that natural and synthetic hormones can move (presumably through soil micropores) to the ditches around tile-drained agricultural fields. It also demonstrated that the appearance of these hormones is transient. It can be concluded that the primary source of estrogen is not directly attributable to hormone implants in beef cattle at this location because estrogen data from this study were confounded by applications of multiple sources of waste (dairy cattle, swine, and poultry).

Assessing impacts of land-applied manure from concentrated animal feeding operations on fish populations [Leet et al.]

The following study indicates that the maximum (acute) measured concentrations of androgens and estrogens in surface water can be relatively high. However, average concentrations of 17α-estradiol, estrone, and trendione were 1.13, 1.46, and 2.84 ng/L, respectively. Average concentrations of 17β-estradiol, estriol, 17α-trenbolone, and 17β-trenbolone were all <0.57 ng/L.

To evaluate the impact of land-applied CAFO manure on fish populations, Leet et al. [222, 228] evaluated water samples from one reference site (stream) and two sites near CAFOs (i.e., ditches) that received tile-drain discharge and runoff from adjacent agricultural fields. One ditch received subsurface tile-drain discharge from fields applied with solid manure and lagoon effluent from beef and dairy CAFOs. The other ditch received runoff from fields that were subsurface injected with swine manure and irrigated with swine and poultry waste lagoon effluent. There were only seven water samples collected from the reference site in comparison to approximately 250 samples collected from each CAFO-impacted site. Water samples were passed through SPE columns and analyzed by HPLC-ESI-MS/MS. Fish abundance, species richness, and reproduction characteristics of creek chub (*Semotilus atromaculatus*) were also evaluated. In addition, *in situ* exposures of fathead minnow embryos (6 week exposure) and caged adults (7-day exposure) were conducted. However, because of confounding factors such as the presence of pesticides, nutrients, and the potential lack of spawning habitats, results of the fish studies are not discussed in this EA.

High concentrations of hormones were detected in samples collected during storm events that followed land applications of CAFO manure. Hormones were detected in over 80% of ditchwater samples, with estrone detected most frequently and estriol detected the least. Natural androgens were detected more frequently than synthetic ones, with testosterone having the highest concentration. Maximum and average measured concentrations of each

hormone were reported, and total 17β-estradiol equivalents (EEQ) and 17β-trenbolone equivalents (TEQ) were calculated from maximum estrogen and androgen concentrations. EEQ means ranged from 7.74-32.95 ng/L in ditch water and was 0.59 ng/L at the reference site. TEQs were in the range of 4.4-34.28 ng/L in ditch water. At the reference site, the TEQ value was 0.01 ng/L. While the maximum measured concentrations were considerably higher, the average concentrations at each site were typically <1 ng/L. Average concentrations of 17α-estradiol, estrone, and trendione were 1.13, 1.46, and 2.84 ng/L, respectively. However, average concentrations of 17β-estradiol, estriol, 17α-trenbolone, and 17β-trenbolone were all <0.57 ng/L.

Appendix 12.3.7. Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment [Kolodziej et al.]

In the following study, it was demonstrated that that fish excrete comparable amounts of steroids as livestock when excretion rates are normalized to animal mass. In surface water monitoring, all maximal concentrations of androgens and estrogens were <1 ng/L, with the exception of testosterone at 1.9 ng/L.

Kolodziej et al. [15] measured androgen, estrogen and progestin concentrations in water samples from multiple sources including groundwater monitoring wells, dairy waste lagoons, surface waters impacted by agricultural operations (upstream and downstream of dairy farms and irrigation canal discharge points), three state fish hatcheries (influent water and effluent at end of raceway), a fish ladder, and a spawning site on a river. Steroid hormones were extracted from water samples using SPE followed by derivatization and analyzed by GC-MS/MS. Recovery of the matrix spikes ranged from 56-85% and correlated with the recovery of the surrogate standard, mesterolone. In lagoon water, steroid hormone concentrations ranged from <LOD to 650 ng/L and varied considerably between sampling dates.

Steroid hormones were detected in seven of the 26 shallow groundwater samples, but concentrations were considerably lower than in the lagoon samples (less than approximately 16 ng/L based on a visual estimation from Figure 1B in reference [15]). There was no consistency with respect to the specific hormone detected in the groundwater by location or sampling time. In the six tile-drain groundwater samples, no steroid hormones were detected above the LOD. Hormones were detected sporadically in river and irrigation canal samples taken from six locations in three rivers and from nine locations in six irrigation canals, but there was no correlation between concentration and location. The maximum concentrations of estrone detected in river and irrigation canal samples were 0.9 and 17 ng/L, respectively. The maximum 17β-estradiol concentrations in the river and irrigation canal samples were 0.6 and 0.7 ng/L, respectively. The maximum concentrations of testosterone from the river and irrigation canal samples were 0.6 and 1.9 ng/L, respectively. Estriol was not detected in any groundwater or river samples (LOQ = 0.4 ng/L). Interestingly, all tile-drain pump discharge samples had no measureable levels of 17β-estradiol, estrone, or estriol. The low level of estrogens in the field samples and tile-drain effluent indicate steroid hormones are likely strongly adsorbed and/or degraded as wastewater infiltrates to the soil prior to reaching groundwater.

In fish hatchery effluent, testosterone, androstenedione, and estrone were detected at concentrations ranging from 0.1-0.8 ng/L. It was estimated that fish excrete comparable amounts of steroids as livestock when excretion rates are normalized to animal mass.

Although concentrations in hatchery effluent are low, fish populations could potentially be a notable source of hormones into the aquatic environment.

Appendix 12.3.8. Steroid hormones in surface water of agricultural, suburban, and mixed-use areas in Pennsylvania [Velicu and Suri]

The following study indicated that the order of hormone detection frequency in surface water was estrone > estriol > 17α-estradiol > 17β-estradiol and was similar for the three land use areas sampled.

Velicu and Suri [224] collected surface water samples in the fall from 21 locations in agricultural, suburban and mixed-use areas in Chester County, PA and analyzed the samples for 11 natural and synthetic steroid hormones. Water samples were filtered prior to passing though SPE columns, derivatized, and analyzed by GC-MS. Detection limits for 17β-estradiol and 17α-estradiol were 0.03 ng/L, and detection limits for estrone and estriol were 0.3 ng/L. Estrone had the highest detection frequency of >90%, with concentrations ranging from 0.66-2.62 ng/L. Estrone was detected in all but one site. The second most frequently detected estrogen was estriol (>80%), with concentrations ranging from 0.33-19.7 ng/L. 17α-Estradiol ranged from 0.04-7.70 ng/L and 17β-estradiol ranged from 0.09-5.04 ng/L. The order of detection frequency was determined to be estrone > estriol > 17α-estradiol > 17β-estradiol and was somewhat similar for the three land use areas sampled (i.e., agricultural, suburban and mixed-use areas).

Appendix 12.3.9. Estrogen concentrations detected in US surface waters during the USGS surveillance program from 1999-2000 [Barnes et al.]

The following study indicated that estrogens were detected in relatively few stream samples. 17β-Estradiol was detected in 5.4% of samples and estriol was the most frequently detected estrogen, with approximately 19% of samples above the LOD.

As part of the first nationwide surveillance program conducted by the USGS in 1999-2000 [229], samples were collected from 139 streams in 30 states and analyzed for 95 different organic wastewater contaminants, including 17β-E2, 17α-E2, E1, and E3. However, not all water samples were tested for the presence of estrogens. Sites more susceptible to contamination due to the proximity to urban areas or livestock production were selected. Samples were processed using continuous liquid-liquid extraction (CLLE) with methylene chloride, derivatized, and analyzed by GC-MS/MS. Eighty-two of the 95 compounds tested (86%) were detected in the water samples, and a total of 80% of the stream samples contained at least one contaminant. The high frequency of contaminant detection may be a result of selecting sites that were susceptible to contamination. In general, steroid hormone concentrations were below the limit of detection (5 ng/L). Out of 74 water samples sampled for steroid hormones:

- 17α-Estradiol was detected in 3 samples (23 to 74 ng/L)
- 17β-Estradiol was detected in 4 samples (9 to 93 ng/L)
- Estrone was detected in 5 samples (8 to 112 ng/L)
- Estriol was detected in 14 samples (6 to 43 ng/L)

Despite the high frequency of detection of many contaminants, estrogens were detected in relatively few stream samples. 17β-Estradiol was detected in 5.4% of samples. Estriol was the most frequently detected estrogen, with approximately 19% of samples above the LOD.

Appendix 12.4. Conclusions of field monitoring

The following conclusions are supported by studies discussed in Appendix 12.1. The concentrations of EB and TBA metabolites reported in the studies reviewed for field monitoring are presented in Table 117 through Table 123.

- 17α isomers of estradiol and trenbolone are found in higher concentrations in manure (both fresh and aged) than the 17β isomers and estrone or trendione.
- Some degradation of both EB and TBA metabolites may occur in manure storage systems, including in the feedlot manure pack, dung piles, storage systems, and in retention lagoons/ponds.
- EB and TBA metabolites in manure applied to soil are degraded.
- Detection of EB and TBA metabolites in surface waters is infrequent; specifically, the 17α and 17β isomers of estradiol and trenbolone are detected infrequently in surface water, and when detected, concentrations are typically low.
- Monitoring studies have not definitively linked estradiol and trenbolone metabolites, or the fish ED effects observed at a site, to CAFOs or the use of EB and TBA implants in beef cattle. Studies indicate that observed ED effects on fish is a complex issue that is likely due to multiple contributing sources (i.e., WWTP, fish hatcheries, wildlife) and several different compounds with estrogenic and androgenic activity.

Appendix 13. Executive Summaries of Zoetis-owned Confidential Information and Studies

Table 127. List of Confidential Information and Studies and Appendices Where the Public Executive Summary is Located

Information / Study	Reference
Appendix 13.1. e-Mail correspondence on half-life of exemestane in aerobic and anaerobic OECD 308 water-sediment systems. CONFIDENTIAL.	[231]
Appendix 13.2. Concentration of [^{14}C] and 17β-estradiol in edible tissues of cattle following implantation of ^{14}C-trenbolone acetate and estradiol benzoate. Doc. # RS-95921 ATv 5940. Syntex Research. 23 January 1992 CONFIDENTIAL.	[232]
Appendix 13.3. Characterization of the 14C-residues present in the liver, bile and feces of cattle following implantation of 14C-trenbolone acetate and estradiol benzoate. Doc. # RS-95921 ATv 6724. Syntex Research. 27 February 1992 (amended August 1994) CONFIDENTIAL.	[233]
Appendix 13.4. Depletion of trenbolone acetate and estradiol benzoate from Synovex Plus Long-Acting Implants in cattle. Experiment 0738-B-US-I-98; Final Report GASR 04-41.00. CONFIDENTIAL.	[234]
Appendix 13.5. Preliminary aqueous photolysis of 17α-trenbolone. Syntex Doc# RS-95921 CH 0209 CONFIDENTIAL	[235]
Appendix 13.6. 17α-hydroxy trenbolone: acute toxicity to the water flea, *Daphnia magna*, under static conditions. 16 November 1994. Syntex Doc# RS-95921 CH 0285. CONFIDENTIAL.	[236]
Appendix 13.7. 5[^{14}C]17α-Trenbolone – determining the adsorption/desorption coefficient (K_{OC}) in 5 soils following OECD Guideline 106. 27 March 2012. Pfizer Study Number 1A72N-60-11-772. CONFIDENTIAL	[237]
Appendix 13.8. [^{14}C]-Trendione – determining the adsorption/desorption coefficient (K_{OC}) in 5 soils following OECD Guideline 106. 27 March 2012. Pfizer Study Number 1A72N-60-11-773. CONFIDENTIAL	[238]
Appendix 13.9. Transformation and mineralization of 17α-[^{14}C]-estradiol in two aerobic aquatic sediment systems following OECD Guideline 308. Pfizer Study Number 1A72N-60-11-768. 06 June 2012. CONFIDENTIAL	[239]
Appendix 13.10. Transformation and mineralization of 17α-[^{14}C]-estradiol in two anaerobic aquatic sediment systems following OECD Guideline 308. Pfizer Study Number 1A72N-60-11-769. 06 June 2012. CONFIDENTIAL	[240]
Appendix 13.11. Transformation and mineralization of [^{14}C]-17α-trenbolone in two aerobic aquatic sediment systems following OECD Guideline 308. Pfizer Study Number 1A72N-60-11-770. 18 June 2012. CONFIDENTIAL	[241]
Appendix 13.12. 17α-[^{14}C]-Estradiol - short-term reproduction assay with fathead minnow (*Pimephales promelas*) following OECD Guideline 229. Pfizer Study Number 1A73N-60-11-785. 07 June 2012. CONFIDENTIAL	[242]
Appendix 13.13. [^{14}C]-17α-Trenbolone - short-term reproduction assay with fathead minnow (*Pimephales promelas*) following OECD Guideline 229. Pfizer Study Number A5Y3N-US-12-001. 08 June 2012. CONFIDENTIAL	[243]
Appendix 13.14. [^{14}C]-17α-Trenbolone - short-term reproduction assay with medaka (O*ryzias latipes*) following OECD Guideline 229. Pfizer Study Number 1A73N-60-11-786. 06 June 2012. CONFIDENTIAL	[244]

Appendix 13.1. DT_{50} of exemestane in aerobic/anaerobic water sediment systems

The DT_{50} values of exemestane in aerobic and anaerobic water-sediment are shown in Table 128. These data are from a study conducted for a publication, but the anaerobic DT_{50} was not reported in the publication [83]. These results were obtained from a personal communication with the author of the publication [231]. To calculate the DT_{50}, the concentration data were transformed by taking the natural log (LN), and plotting the LN (concentration) on Y axis and day on the X axis. The slope of the line (k = first-order decay rate constant) was determined and the DT_{50} calculated using the following equation:

DT_{50} = LN(2)/k. For example, DT_{50} for aerobic Choptank River = LN(2)/0.037 = 18.7 days

The values for k are shown in the kinetic plots of the degradation data in Figure 27 and Figure 28.

The methodology for LN transforming first-order degradation data to determine a DT_{50} and DT_{90} is detailed in a publication by Scherr et al. [30].

Table 128. Aerobic and Anaerobic DT_{50} of Exemestane in Water/Sediment

Site	DT_{50} (Days)	
	Aerobic	Anaerobic
Choptank River	18.7	33
Turkey Creek	26.7	36.5

Figure 27. Aerobic Transformation of Exemestane in Water/Sediment

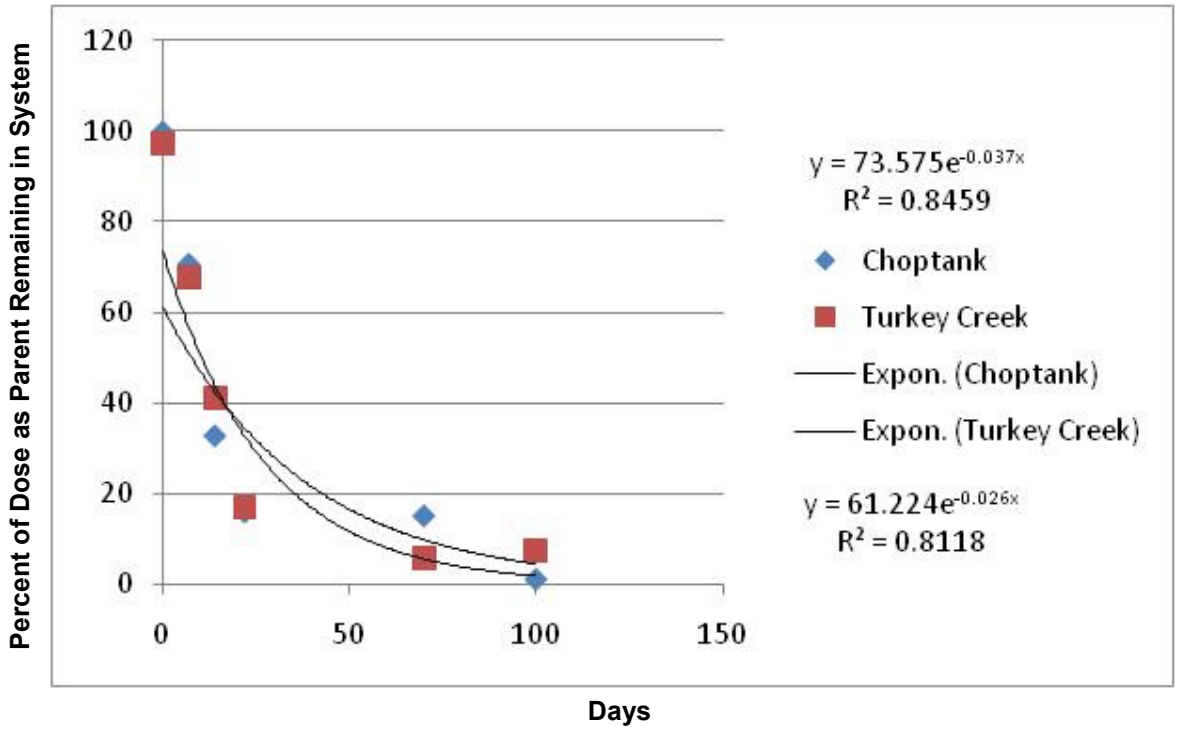
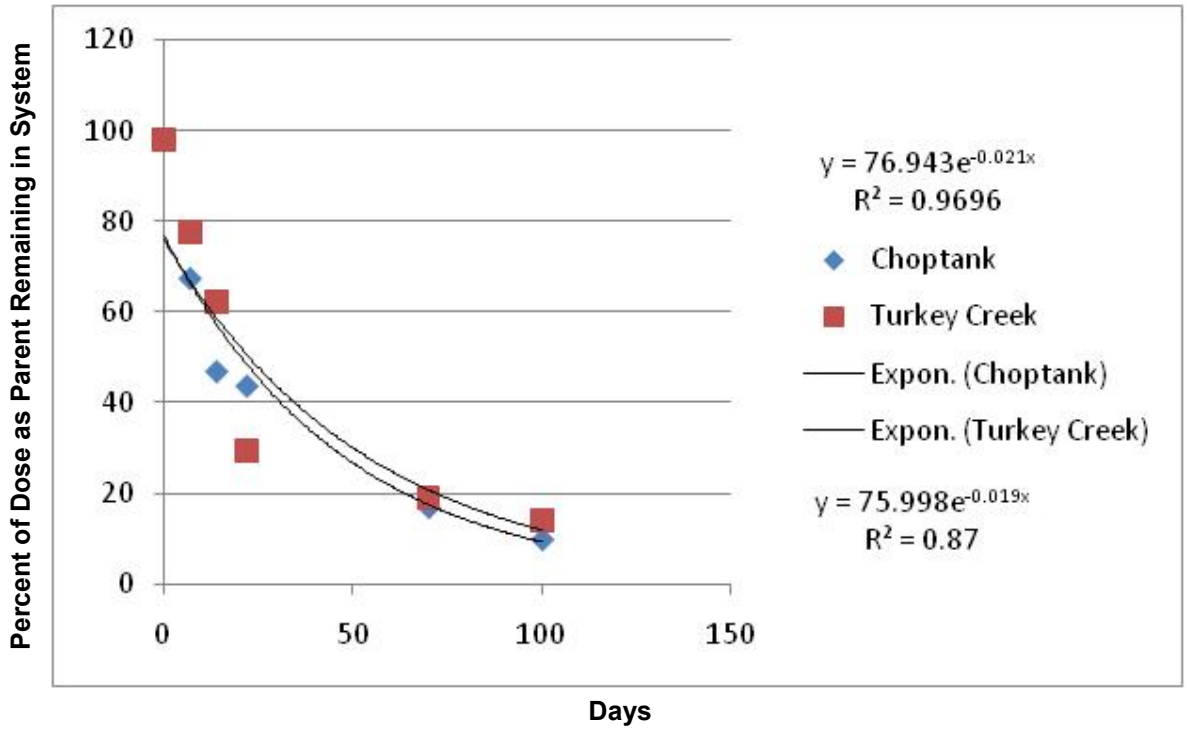


Figure 28. Anaerobic Transformation of Exemestane in Water/Sediment



Appendix 13.2. Concentrations of ^{14}C-trenbolone in edible tissues of implanted cattle

Title: Concentration of [^{14}C] and 17β-estradiol in edible tissues of cattle following implantation of ^{14}C-trenbolone acetate and estradiol benzoate. 23 Jan 1992. [232]

Syntex Document Number: Doc. # RS-95921 ATv 5940

GLP: No

Purpose: The study was designed to investigate the metabolic disposition of trenbolone acetate in cattle, and to provide data for the establishment of the withdrawal period for the TBA and EB combined product.

General Design: The purpose of this study was 1) to determine the concentrations of the total radioactivity of TBA related resides in edible tissues and bile, and the concentration of 17β-estradiol in fat and muscle collected from the control animals and from the treated cattle slaughtered at 15 and 30 days after implantation of ^{14}C-TBA and EB, 2) to characterize the ^{14}C-residues present in liver, bile, and feces, and 3) to obtain the excretion profile for the administered ^{14}C-TBA.

a. Test Animals:
   24 calves, each weighing between 211-327 kg, were divided into three groups, each with four steers and four heifers.

b. Dosage and Route of Administration:
   Each animal in Groups 1 and 2 received one implant containing 300 mg of ^{14}C-TBA and 42 mg of EB in the middle 1/3 of the posterior pinna of the right ear on Study Day 0. Group 3 was a control group.

c. Testing:
   Animals in Groups 1 and 2 were euthanized on Study Day 30 and 15, respectively. Control animals were euthanized on Study Day 29. Samples of fat, liver, kidney, muscle, bile, and blood were collected at various times throughout the study period and at the end. Urine and feces produced on Study Days 1, 2, 3, 4, 5, 8, 11, 15, and 30 were collected from animals in Group 1. The concentration of radiolabeled trenbolone was measured via liquid scintillation counting (LSC).

Results: There was no evidence of a significant difference between the mean concentrations of ^{14}C-residues in tissues collected at 15 and 30 days after implantation. Furthermore, there was no evidence of significant differences in residue concentrations between the steers and heifers.

Results showed that concentrations of 17β-estradiol in fat and muscle collected from animals euthanized at 15 days post-implantation were similar to those in the corresponding tissues from animals euthanized at 30 days. The average concentrations of 17β-estradiol in fat were 83.5 and 81.8 pg/g at 15 and 30 days respectively, and in muscle were 17.9 and 19.0 pg/g at 15 and 30 days, respectively.

Samples of bile collected from animals in Groups 1 and 2 contained high levels of ^{14}C-residues, indicating that bile is one of the major routes of excretion in cattle. Amounts of ^{14}C-residues excreted in urine collected on the first two days after implantation were significantly higher than those collected on other days. The total amounts of trenbolone excreted in urine and feces on most collection days were consistent in individual animals and between collection times. On average, approximately 0.21% and 1.05% of the dose were excreted in the urine and feces, respectively, on a daily basis. The mean excretion rate of total radioactivity in urine was 16.42%, with the major route of excretion in feces at 83.58%.

Appendix 13.3. ^{14}C-TBA residue characterization in urine and feces of cattle

Title: Characterization of the ^{14}C-residues present in the liver, bile, and feces of cattle following implantation of ^{14}C-trenbolone acetate and estradiol benzoate. 27 Feb 1992 Amended Aug 1994. [233]

Syntex study Number: RS-95921 ATv 6724

GLP: No

Purpose: This study was conducted to determine the concentrations of the total radioactivity of TBA-related residues in edible tissues, bile, plasma, urine and feces from cattle following implantation with ^{14}C-TBA and EB.

General Design: The study design was described in Appendix 13.2 and the samples came from that study. Samples were prepared and analyzed by HPLC analysis to characterize the ^{14}C-residues from liver, bile, and feces from treated and untreated animals.

Results: On average, 48%, 68%, and 50% of the radioactivity present in the liver, bile, and feces, respectively, were extracted into organic solvent. HPLC analysis of the radioactivity extracted from the liver showed the presence of three main drug-related components, which were shown by enzymatic reactions to be either glucuronide or sulfate conjugates. The deconjugated materials were examined by mass spectrometric (MS) techniques. One of the components was identified as the glucuronide conjugate of 17α-trenbolone. The other two components were not identified. 17α-Trenbolone glucuronide was also the major component in bile. A minor unidentified component was also present in some of the bile samples. 17α-Trenbolone, rather than its glucuronide, was the major metabolite present in feces which accounted for, on average, 47.2% of the total radioactivity recovered via HPLC. 17β-Trenbolone was present in small amounts (mean of 1.68%). The data showed no evidence for the presence of TBA. A minor metabolite comprising 6.2% of the radioactivity with retention time of 16 minutes may correspond to 17α-trenbolone glucuronide which has been shown to be a major metabolite in liver and bile. In addition, there did not appear to be a difference in the metabolism of TBA between steers and heifers.

Appendix 13.4. Explant study for Synovex ONE (a.k.a. SYNOVEX Plus Long-Acting[ii]) and SYNOVEX Plus

Title: Depletion of trenbolone acetate and estradiol benzoate from SYNOVEX Plus Long-Acting Implants in Cattle (Report No. GASR 04-41.00). 13 December 1999. [234]

GLP: Yes

Purpose: The purpose of this study was to determine the *in vivo* depletion of TBA and EB in coated (~15% w/w) Synovex ONE-F implants (which are referred to as SYNOVEX Plus Long-Acting implants in the report, but will be marketed as Synovex ONE). Depletion from the commercial SYNOVEX® Plus™ implants was also determined for comparison purposes.

General Design: A 200-day study to evaluate the depletion of TBA and EB from coated SYNOVEX Plus Long-Acting implants and commercial SYNOVEX® Plus™ implants in cattle was conducted.

a. Test Animals: 30 Hereford or Hereford crossbred steers, which weighed approximately 200-350 kg BW. The cattle were blocked into six weight blocks of five animals each based on pre-treatment BW (Day -1) and randomly assigned to an explant day (40, 81, 120, 160, or 200) within each block.
b. Dosage Form: All the animals received a coated SYNOVEX Plus Long-Acting implant in one ear and a commercial SYNOVEX® Plus™ implant in the other ear.
c. Animal Husbandry: On Day 0, each implant formulation was subcutaneously inserted into the middle 1/3 of either the right or left ear (ear location was randomly assigned). All implanted cattle were maintained together (on pasture or fed alfalfa/grass hay, haylage, or corn silage when pasture was insufficient) and each animals diet was supplemented daily in the morning with approximately 1 kg of a 16% crude protein concentrate. The feeding rate of the concentrate was adjusted to 2 kg/animal/day as the trial progressed in order to maintain an average daily gain of at least 0.5 kg/head/day.

[ii] The initial proposed trade name for Synovex ONE-F and –G was Synovex Long-Acting (LA)-F and –G. therefore, in several cited technical reports, ONE-F and ONE-G were referred to as LA-F and LA-G, respectively. The term LA refers to long-acting; however, because this product is considered an extended release product the final agreed upon names are Synovex ONE-F and ONE-G.

Results: The overall health of the animals was good throughout the study. Ears of all animals were examined and palpated on Day 40. No implants were lost and implant site reactions were minimal. A few animals received concurrent treatment for pinkeye, but the treatments were not expected to affect the outcome of the study. Body weights of each animal were determined on Day -1 and again just prior to the specific sacrifice day for each animal (i.e., Day 40, 81, 120, 160, and 200). The average weight gains ranged from 0.812-1.220 kg/day.

Implants were removed from groups consisting of six steers on Days 40, 81, 130, 160, and 200 and analyzed for TBA and EB content. Simple regression analyses of the depleted amounts of TBA versus days and EB versus days were performed separately for the SYNOVEX Plus Long-Acting and SYNOVEX Plus implants and the slopes were estimated. The depletion rate (or slope) of TBA from the SYNOVEX Plus Long-Acting implant was 0.9466 mg/day which was significantly less than the depletion rate of 1.703 mg/day from the commercial SYNOVEX Plus implant (one-sided Z-test at $\alpha \leq 0.05$). EB depletion was also significantly lower (0.1049 mg/day vs. 0.1980 mg/day) from the coated SYNOVEX Plus Long-Acting implant than from the commercial SYNOVEX Plus implant. Using the values in Table 129 below, calculation of the ratio of TBA slopes for the two implant products yielded a value of 0.55, whereas calculation of the comparable slope ratio for EB yielded a value of 0.53.

Table 129. Daily Release Rate of TBA and EB from Synovex ONE and SYNOVEX Plus Implants

Active Ingredient	Implant Depletion Slopes		ONE-F/Plus Ratio
	Synovex ONE-F	SYNOVEX Plus	
TBA	-0.9466 mg/day	-1.7073 mg/day	0.5544
EB	-0.1049 mg/day	-0.1980 mg/day	0.5298
TBA/EB ratio	9.02	8.62	1.047

Appendix 13.5. Aqueous photolysis of 17α-trenbolone

Title: Preliminary aqueous photolysis of 17α-trenbolone [235]. 17 January 1995.

Syntex Study Number: RS-95921 CH 0290

GLP: No. This study followed OECD 316 guidance but was classified as preliminary because it was not conducted under GLP.

The purpose of this study was to evaluate the extent of photolysis during a one-day exposure to sunlight under winter conditions. The work was conducted in three parts on three separate days, with each part corresponding to an exposure at a designated pH. For photolysis at a pH of 5, a 1.78×10^{-5} M 17α-trenbolone solution was prepared in a 0.1 M ammonium phosphate buffer. For photolysis at pH 7, a 2.89×10^{-5} M 17α-trenbolone solution was prepared in 0.1 M sodium phosphate buffer. For photolysis at pH 9, a 4.59×10^{-5} M solution of 17α-trenbolone solution was prepared in 0.1 M sodium borate buffer.

In each phase of the study, both foil wrapped and unwrapped quartz tubes containing a TBA solution were exposed to sunlight. At 30 to 45 minute intervals, one set of tubes consisting of two exposed and one wrapped tube were sampled for analysis by HPLC. As the control tubes indicated negligible non-photolytic reactions, the photolytic rate constant was calculated from the best fit of the natural logarithm of C_0/C_t versus exposure time, and the DT_{50} calculated from the rate constant. C_0 = initial time-0 concentration and C_t = concentration at time (t).

17α-Trenbolone was found to undergo extensive photolysis in a one-day period, with half-lives at pH 5, 7, and 9 of 12.6, 3.1, and 3.3 hours, respectively. The longer half-life at pH 5 may be attributed to foggy, cloudy weather conditions.

A *p*-nitroanisole/pyridine ultraviolet (UV) sunlight actinometer was used on the same day as the study at pH 7 to adjust calculations for UV irradiance fluctuations during the exposure period. The actinometer samples were exposed and sampled in the same manner as the 17α-trenbolone samples. The corresponding summer, fall, and winter photolysis DT_{50} values were 0.169, 0.355, and 0.608 days, respectively.

Appendix 13.6. 17α-Trenbolone acute toxicity to *Daphnia magna*

Title: 17-α-hydroxy trenbolone: acute toxicity to the water flea, *Daphnia magna*, under static conditions. 16 Nov 1994. [236].

Syntex Study Number: RS-95921 CH 0285.

GLP: Not specified

Daphnids were exposed to 17α-trenbolone for 48 hours under static conditions. Nominal concentrations of 17α-trenbolone were 0, 0.10, 1.00, 10.0, and 100 mg/L. Measured concentrations were <0.015, 0.0181, 0.182, 1.75, and 17.9 mg/L of 17α-trenbolone, respectively. The 48-hour LC_{50} was 0.34 mg/L of 17α-trenbolone, based on measured concentrations. The 95% confidence interval ranged from 0.16-0.70 mg/L of 17α-trenbolone. The NOEC was <0.0181 mg/L of 17α-trenbolone based on the significant mortality observed at this and higher test concentrations (10% mortality was observed at the lowest measured concentration of 0.0181 mg/L).

Appendix 13.7. K_{OC} of 17α-trenbolone in five soils following OECD Guideline 106

Title: 5[^{14}C]-17α-Trenbolone – Determining the adsorption/desorption coefficient (K_{OC}) in 5 soils following OECD Guideline 106. 27 March 2012. [237].

Pfizer Study Number: 1A72N-60-11-772

GLP: Yes

General Design: The adsorption and desorption isotherm experiments for 5[^{14}C]-17α-trenbolone were conducted using the batch equilibrium method recommended in OECD Guideline 106 [50]. Adsorption and desorption of 5[^{14}C]-17α-trenbolone was determined, in duplicate, in five soils at five nominal concentrations of 0.010, 0.050, 0.100, 0.503 and 1.02 mg/L. The properties of the test soils are listed in Table 130. The experiments were conducted at an optimal soil-to-solution ratio (i.e., 1:5) at equilibrium (i.e., four hours for adsorption and 24 hours for desorption determined during an adsorption kinetics study). 17α-Trenbolone was found to be stable during the study period with >95% recovery after four hours. All samples were analyzed via LSC to determine the radioactivity in the aqueous and soil samples at each time point. The stability of 17α-trenbolone was determined via HPLC.

Results: The results of the study are shown in Table 131. The mean percent adsorption of 5[^{14}C]-17α-trenbolone was 84.0% to Don Uglem (DU) soil, 66.2% to Mutchler Sandy Loam (MSL) soil, 60.7% to Montana (MT) soil, 70.3% to Ostlie East (OE) soil, and 52.1% to Roger Myron (RM) soil. The mean percent desorption of 5[^{14}C]-17α-trenbolone was 4.50% from the DU soil, 7.75% from the MSL soil, 13.0% from the MT soil, 5.49% from the OE soil and 16.0% from the RM soil.

The K^F_{OC} value using the Freundlich adsorption isotherm was 517 mL/g for DU soil, 409 mL/g for MSL soil, 608 mL/g for MT soil, 277 mL/g for OE soil and 490 mL/g for RM soil. The corresponding log K_{OC} values would be 2.71 for DU soil, 2.61 for MSL soil, 2.78 for MT soil, 2.44 for OE soil, and 2.69 for RM soil (Table 131). The coefficient of determination (r^2) and the Freundlich adsorption and desorption parameters (1/n) were both generally near 1.0. These data demonstrate the appropriateness of the log-transformed Freundlich equation to estimate Kd and K_{OC}.

The mean percent adsorption and desorption of 5[^{14}C]-17α-trenbolone for all of the soils was 66.7% and 9.35%, respectively. The mean K^F_{OC} and desorption coefficient (K^F_{des}) value for the five soils was 460 mL/g and 22.1 mL/g, respectively.

Table 130. Soil Properties

Soil ID USDA Textural Class	Don Uglem (DU) Clay Loam	Mutchler Sandy Loam (MSL) Sandy Clay Loam	Montana (MT) Clay	Ostlie East (OE) Sandy Clay Loam	Roger Myron (RM) Sandy Loam
% Sand	44	64	34	58	82
% Silt	23	11	21	11	3
% Clay	33	25	45	31	15
Bulk Density (g/cc)	1.01	1.06	1.17	1.04	1.20
Available Nutrients (ppm):					
Calcium	2160	2260	3210	4160	1070
Magnesium	515	483	814	404	187
Sodium	11	16	13	40	8
Potassium	453	172	419	201	185
Hydrogen	30	22	29	18	35
Olsen Phosphorus	62	43	9	10	68
Total Nitrogen (%)	0.367	0.179	0.115	0.337	0.098
Soluble Salts (mmhos/cm)	0.31	0.69	0.62	1.09	0.32
% Organic Matter	6.5	3.0	1.5	5.2	1.5
% Organic Carbon	3.8	1.8	0.9	3.1	0.9
pH (1:1 soil/water ratio)	5.2	6.3	7.9	7.5	5.2
Cation Exchange Capacity					
(meq/100 g)	19.3	18.1	26.8	26.7	10.9
Capacity at 1/3 bar	29.2	20.6	24.4	26.4	10.6
Capacity at 15 bar	22.5	16.2	18.8	18.8	7.3

Table 131. 17α-Trenbolone Freundlich Isotherm Results, Mean Percent Adsorption and Desorption

Soil Type	Mean % Adsorption	K^F ads (mL/g)	K^Foc (mL/g)	Mean % Desorption	K^Fdes (mL/g)
DU Soil	84.0	19.8	517	4.50	49.7
MSL Soil	66.2	7.22	409	7.75	13.7
MT Soil	60.7	5.36	608	13.0	24.1
OE Soil	70.3	8.49	277	5.49	14.5
RM Soil	52.1	4.32	490	16.0	8.53
Mean	**66.7**	**9.04**	**460**	**9.35**	**22.1**

Appendix 13.8. K_{OC} of trendione in five soils following OECD Guideline 106

Title: [^{14}C]Trendione – Determining the adsorption/desorption coefficient (K_{OC}) in 5 soils following OECD Guideline 106. 27 March 2012. [238].

Pfizer Study Number: 1A72N-60-11-773

GLP: Yes

General Design: The adsorption and desorption isotherm experiment for 5[^{14}C]-trendione was conducted using the batch equilibrium method recommended in OECD Guideline 106 [50]. Adsorption and desorption of 5[^{14}C]-trendione was determined, in duplicate, in five soils at five nominal concentrations of 0.00989, 0.0500, 0.101, 0.495, and 0.992 mg/L. The properties of the test soils are listed in Table 130 above. The experiment was conducted at an optimal soil-to-solution ratio (i.e., 1:5 for soils) at equilibrium (i.e., 48 hours for adsorption and desorption determined during an adsorption kinetics study). The soil properties are shown in Table 130 of Appendix 13.7. Trendione was found to be stable during the study period with >95% recovery after 4 hours. All samples were analyzed via LSC to determine the radioactivity in the aqueous and soil samples at each time point. The stability of trendione was determined via HPLC.

Results: The results of the study are shown in Table 132. The mean percent adsorption of [^{14}C]trendione was 86.6% to DU soil, 81.6% to MSL soil, 65.4% to MT soil, 84.6% to OE soil and 65.9% to RM soil. The mean percent desorption of [^{14}C]-trendione was 4.19% from DU soil, 6.89% from MSL soil, 7.41% from MT soil, 4.87% from OE soil and 9.85% from RM soil.

The K^F_{OC} value (using the Freundlich adsorption isotherm) was 1226 mL/g for DU soil, 1403 mL/g for MSL soil, 2604 mL/g for MT soil, 1508 mL/g for OE soil and 2281 mL/g for RM soil. The corresponding log K_{OC} values would be 3.09 for DU soil, 3.15 for MSL soil, 3.42 for MT soil, 3.18 for OE soil, and 3.36 for RM soil (Table 132). The coefficient of determination (r^2) and the Freundlich adsorption and desorption parameters (1/n) were both generally near 1.0. These data demonstrate the appropriateness of the log-transformed Freundlich equation to estimate Kd and K_{OC}.

The mean percent adsorption and desorption of [^{14}C]-trendione for all of the soils was 76.8% and 6.64%, respectively. The mean K^F_{OC} and K^Fdes value for the five soils was 1804 mL/g and 43.8 mL/g, respectively

Table 132. Trendione Freundlich Isotherm Results, Mean Percent Adsorption and Desorption

Soil Type	Mean % Adsorption	K^Fads (mL/g)	K^F_{OC} (mL/g)	Mean % Desorption	K^Fdes (mL/g)
DU Soil	86.6	46.9	1226	4.19	72.7
MSL Soil	81.6	24.8	1403	6.89	33.5
MT Soil	65.4	23.0	2604	7.41	29.6
OE Soil	84.6	46.1	1508	4.87	57.2
RM Soil	65.9	20.1	2281	9.85	26.1
Mean	**76.8**	**32.2**	**1804**	**6.64**	**43.8**

Appendix 13.9. Aerobic 17α-estradiol transformation in water/sediment systems following OECD Guideline 308

Title: Transformation and mineralization of 17α-[^{14}C] estradiol in two aerobic aquatic sediment systems following OECD Guideline 308. [239]

Date: 06 June 2012

Pfizer Study Number: 1A72N-60-11-768

CRO Study Number: 2438.6667

GLP: Yes

Test Conditions: The rate of aerobic transformation of 17α-[^{14}C]-estradiol was studied at a nominal concentration of 1.0 mg/L and a temperature of 20 ± 2°C for 120 days in two aerobic sediments varying in pH, textural characteristics, organic matter content and microbial content. Sediments from Taunton River and Weweantic River contained 3.8% and 1.2% OC, respectively. Water-sediment samples from each test system were assayed at 0, 1, 3, 7, 14, 29, 43, 56, 77 and 120 days after dosing.

The untreated flooded sediment samples were equilibrated at test conditions under aerobic conditions for at least one week. Following equilibration, the water layers of each of the systems were treated with 17α-[^{14}C]-estradiol to achieve a nominal estradiol concentration of 1.0 mg/L in the water layer. The aerobic incubation of treated test systems was performed continuously by bubbling hydrated air through the water layer for 120 days. Potassium hydroxide (KOH) and ethylene glycol traps were used in the flow-through aerobic test systems to collect $^{14}CO_2$ and any volatile components that evolved during the study.

Analysis: At each sampling interval, the water-sediment samples from each test system were separated into water and sediment fractions. The water phase was analyzed for total radioactivity by LSC and for distribution of radioactivity by HPLC/RAM. The sediment phase was extracted once with acetonitrile (150 mL) and once with acetonitrile: purified reagent water (80:20, v:v, 150 mL). At each sampling day, with the exception of Day 0, the sediment was extracted one additional time each with acetonitrile: purified reagent water: hydrochloric acid (80:20:0.1, v:v:v, 150 mL) for a total of three extractions. On Day 120, a fourth extraction[jj] was included for the Weweantic River sediment samples only, using acetonitrile: purified reagent water: hydrochloric acid (80:20:0.1, v:v:v, 150 mL). The sediment extracts were assayed for total radioactivity by LSC and by HPLC/RAM to quantify 17α-[^{14}C]-estradiol and biodegradation products in the sediment phase. Following extraction, the sediment-bound residues were quantified by combustion analysis and residues in the traps used to capture volatile organic materials were quantified by LSC.

[jj] The fourth extraction for the Day 120 Weweantic River sediment sample was added as a confirmatory step to ensure that all the 'extractable' 14C-activity was recovered and characterized as such. Outcome of the 3rd extraction showed the Weweantic River sediment extraction recovering about 2X as much as the Taunton River (~6-7% vs. 2.3% respectively). As a precaution, the additional extraction was performed on the Weweantic River sample to ensure no extractable residues remained. The 4th extraction only recovered an additional 3% of the dosed activity. This amount was added to the total activity recovered as 'extractable' for the Weweantic River sample and essentially confirmed that only three extractions were needed.

Results/Conclusions: 17α-[^{14}C]-Estradiol was transformed and degraded under the aerobic conditions of this study. Three known metabolites, estriol, 17β-estradiol and estrone, were observed at retention times of approximately 15, 21, and 25 minutes throughout the study in both the water and sediment phases of both the Taunton River and Weweantic River test systems, but estriol did not exceed 2% of the applied radioactivity (AR). In the water phase, estriol, 17β-estradiol and estrone reached maximum average[kk] percentages (average of two replicates) of 0.8%, 1.4%, and 2.1% of the AR, respectively. In the sediment phase, they were detected at higher percentages of 1.8%, 5.8%, and 16.7%, respectively. This indicates that the transformation products were principally in the sediment phase. Additional minor areas of radioactivity were observed in some of the chromatograms. In all cases, these individual peaks represented <10% AR, and were not considered further.

Ultimate biodegradation was observed in the aerobic test systems and the cumulative amount of evolved $^{14}CO_2$ was 27.5% AR for the Taunton River aerobic test system and 11.4% AR for the Weweantic aerobic test system at Day 120. Volatile organics were ≤0.01% AR in the Taunton River and the Weweantic River aerobic test systems.

Aqueous Dissipation DT_{50}: 17α-[^{14}C]-Estradiol

The aqueous DT_{50} for 17α-[^{14}C]-estradiol was estimated to be 9.8 days and 12 days for Taunton River and Weweantic River, respectively. The aqueous dissipation rate is based on the disappearance of 17α-[^{14}C]-estradiol from the water layer due to biotransformation and sorption to sediment. The half-life was estimated using a conservative approach by simple linear regression assuming linear kinetics based on a one-compartment model.

Total System Transformation DT_{50}: 17α-[^{14}C]-Estradiol and Total Drug

The total system transformation DT_{50} values for 17α-estradiol and total drug (17α-estradiol + 17β-estradiol + estrone) were estimated by fitting a single compartment model (using simple linear or single first-order (SFO) non-linear regression). Selection of the best model was made using a chi-square (χ^2) goodness-of-fit statistic. The total system DT_{50} was based on the depletion of 17α-estradiol (or total drug) from the total water-sediment system as determined by the amount of 17α-estradiol (or total drug) found in the water and sediment extractables. The kinetic results for the best-fitting model, the non-linear SFO, are summarized in Table 133 below.

The values for the total system transformation DT_{50} values were used in subsequent EXPRESS and watershed modeling runs. The most conservative value (longest half-life) from the two water-sediment systems was used in the modeling.

[kk] The 'maximum average' value is the maximum reported value (from both sediments) of all of the averaged values determined (average of 2 replicates) at each of the time points for each of the transformation products.

Table 133. Aerobic Dissipation Half-Life (DT_{50}) of 17α-Estradiol and 17α-Estradiol Plus Transformation Products (total drug) in the Total System

Test system	Sample	DT_{50} (days)	k (day^{-1})
Taunton River	Total system – 17α-Estradiol	8.5	0.0816
	Total system – Total drug*	31.1	0.0223
Weweantic River	Total system – 17α-Estradiol	16.3	0.0425
	Total system – Total drug*	25.5	0.0272
	Mean – 17α-Estradiol	12.4	
	Mean – Total drug	28.2	

* Total drug = sum (α-estradiol + β-estradiol + estrone)

Total system transformation half-life: transformation products 17β-estradiol and estrone

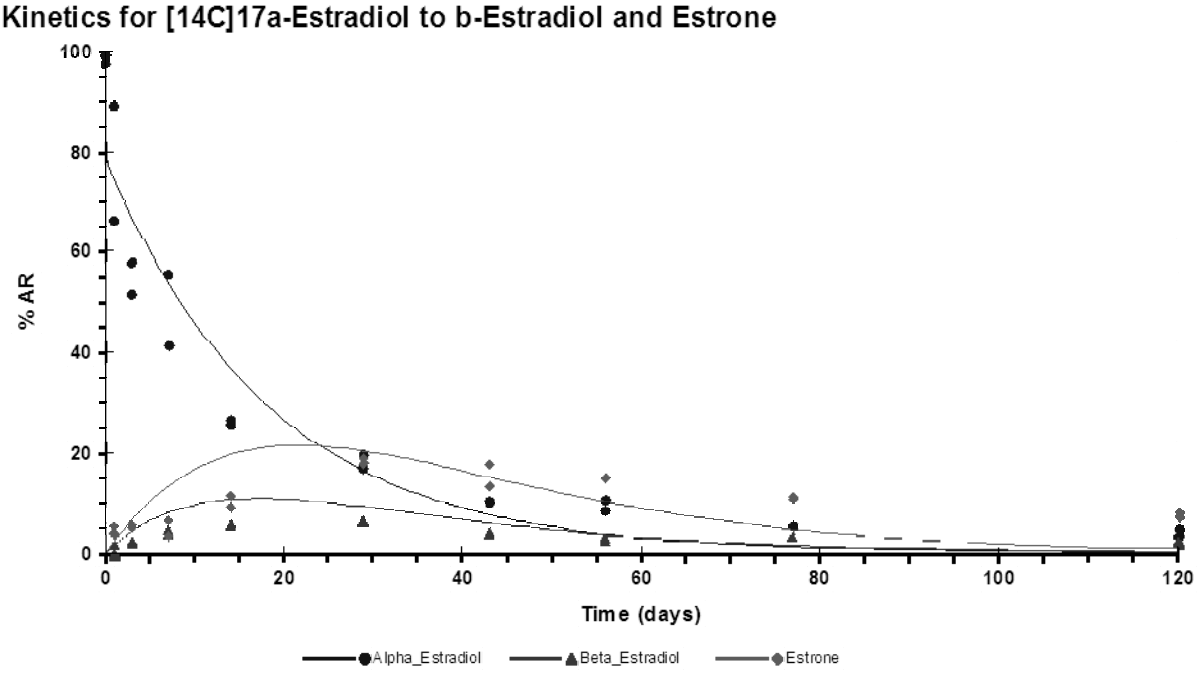
The total system transformation DT_{50} values for the metabolites 17β-estradiol and estrone were estimated using a kinetic model that describes the transformation of 17α-estradiol to two subsequent metabolites. All three metabolites were modeled using single first-order kinetics (SFO). The total system transformation DT_{50} was based on the depletion of each metabolite from the total water-sediment system as determined by the amount of metabolite found in the water and sediment extractables. The results of these analyses are presented in Table 134 below.

The data confirm that the two transformation products 17β-estradiol and estrone have similar transformation DT_{50} values when compared to that of 17α-estradiol. The transformation products did not persist.

Table 134. Aerobic Half-Life (DT_{50}) of Transformation Products in Total System

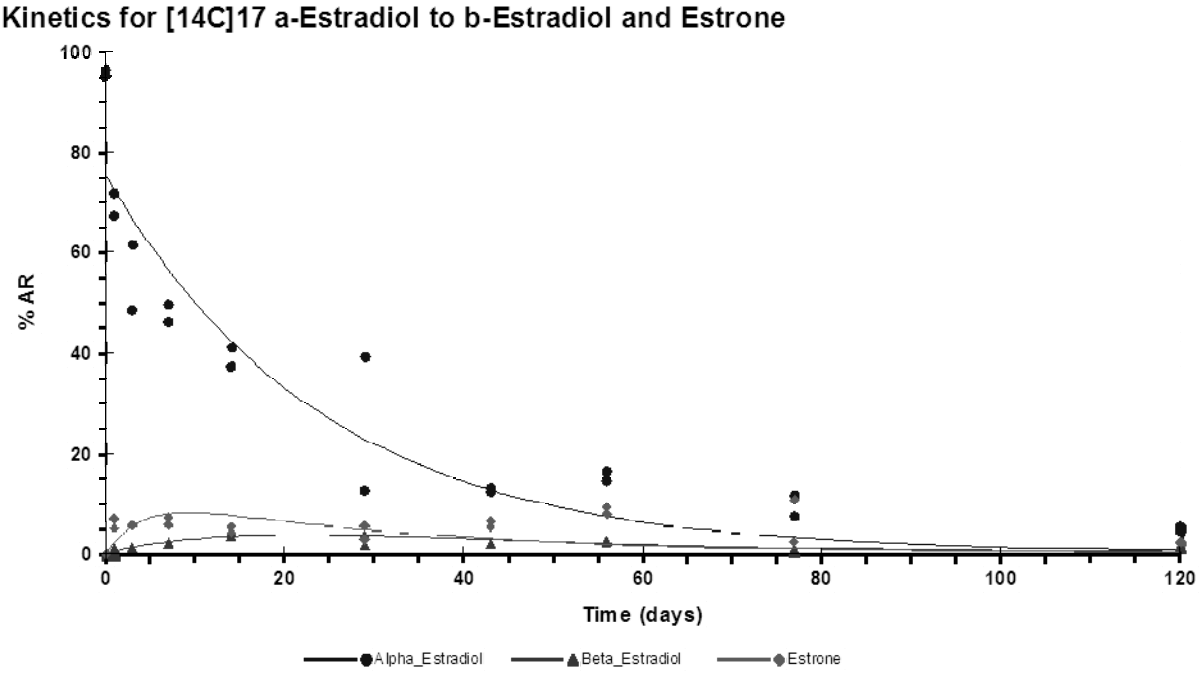
Test System	Endpoint	17β-Estradiol	Estrone
Taunton River	DT_{50} (days)	11.0	10.2
Weweantic River	DT_{50} (days)	13.6	2.9
	Mean – DT_{50} (days)	12.3	6.6

Figure 29. Kinetics of Aerobic 17α-Estradiol Transformation in Taunton River Water/Sediment (Total System)



Circle: 17α-Estradiol, Triangle: 17β-Estradiol, Diamond: Estrone
% AR = percent applied radioactivity

Figure 30. Kinetics of Aerobic 17α-Estradiol Transformation in Weweantic River Water/Sediment (Total System)



Circle: 17α-Estradiol, Triangle: 17β-Estradiol, Diamond: Estrone
% AR = percent applied radioactivity

Appendix 13.10. Anaerobic 17α-estradiol transformation in water/sediment systems following OECD Guideline 308

Title: Transformation and mineralization of 17α-[14C] estradiol in two anaerobic aquatic sediment systems following OECD Guideline 308. [240]

Date: 06 June 2012

Pfizer Study Number: 1A72N-60-11-769

CRO Study Number: 2438.6666

GLP: Yes

Test Conditions: The rate of anaerobic transformation of 17α-[^{14}C]-estradiol was studied at a nominal concentration of 1.0 mg/L and a temperature of 20 ± 2°C for 203 days in two anaerobic sediments varying in pH, textural characteristics, organic matter content and microbial content. The OC content in the Taunton River and Weweantic River sediments were 3.6% and 0.82%, respectively. Water-sediment samples from each test system were assayed at 0, 7, 14, 30, 60, 100, 151 and 203 days after dosing.

The untreated flooded sediment samples were equilibrated under anaerobic conditions for at least one week. Following equilibration, 17α-[^{14}C]-estradiol was added to the water layers of each of the systems to achieve a nominal concentration of 1.0 mg/L. The test systems were sparged with nitrogen gas until anaerobic conditions in the water and sediment were achieved (<-100 mV redox potential). All anaerobic test vessels were continuously aerated with hydrated nitrogen over the 203-day incubation period. KOH and ethylene glycol traps were used in the flow through anaerobic test systems to collect $^{14}CO_2$ and any volatile components that evolved during the study.

Analysis: At each sampling time point, the water-sediment samples from each test system were separated into water and sediment fractions. The water phase was analyzed for total radioactivity by LSC and for distribution of radioactivity by HPLC/RAM. The sediment phase was extracted once with acetonitrile (150 mL) and once with acetonitrile:purified reagent water (80:20, v:v, 150 mL). At each sampling time point, with the exception of Day 0, the sediment was extracted one additional time each with acetonitrile:purified reagent water:hydrochloric acid (80:20:0.1, v:v:v, 150 mL) for a total of three extractions. The sediment extracts were radioassayed for total radioactivity by LSC and by HPLC/RAM to quantify 17α-[^{14}C]-estradiol and biodegradation products in the sediment phase. Following extraction, the sediment-bound residues were quantified by combustion analysis and residues in the traps used to capture volatile organic materials were quantified by LSC.

Results/Conclusions: 17α-[^{14}C]-Estradiol was transformed and degraded under the anaerobic conditions of this study. Three known transformation products, estriol, 17β-estradiol and estrone were observed at retention times of approximately 15, 21, and 25 minutes, respectively, throughout the study in both the water and sediment phases for the Taunton River and Weweantic River test systems. In the water phases, estriol, 17β-estradiol and estrone reached maximum average[ll] percentages of 0.70, 1.39, and

[ll] The 'maximum average' value is the maximum reported value (from both sediments) of all of the averaged values determined (average of 2 replicates) at each of the time points for each of the transformation products.

3.12% of the AR, respectively. In the sediment phases, estriol was not detected and 17β-estradiol and estrone reached maximum average percentages of 11.67% and 18.41%, respectively. This indicates that the transformation products were principally in the sediment phase. Additional minor quantities of radioactivity were observed in some of the chromatograms. In all cases, these individual peaks represented <10% of the AR, and were not considered further.

Ultimate biodegradation was observed in the anaerobic test systems and the cumulative amount of evolved $^{14}CO_2$ was 32.66% AR for the Taunton River anaerobic test system and 63.48% AR for the Weweantic River anaerobic test system at Day 203. These data suggest a major degradation pathway for estradiol in the environment, under anaerobic conditions, is the microbial cleavage of the steroid ring (position of ^{14}C-label) and its subsequent mineralization. Volatile organics were ≤0.02% AR in the Taunton River and the Weweantic River anaerobic test systems.

Aqueous Dissipation DT_{50}: 17α-Estradiol

The aqueous DT_{50} values for 17α-estradiol were estimated to be 17.3 and 16.0 days for Taunton and Weweantic Rivers, respectively. The aqueous dissipation rate is based on the disappearance of 17α-estradiol from the water layer due to biotransformation and sorption to sediment. The DT_{50} was estimated using a conservative approach using simple linear regression assuming linear kinetics based on a one-compartment model

Total System Transformation DT_{50}: 17α-Estradiol and Total Drug

The total system transformation DT_{50} values for 17α-estradiol and total drug (α-estradiol + β-estradiol + estrone) were estimated by fitting a single compartment model (using simple linear or SFO non-linear regression). Selection of the best model was made using a chi-square (χ^2) goodness-of-fit statistic. The total system DT_{50} was based on the depletion of 17α-estradiol (or total drug) from the total water-sediment system as determined by the amount of 17α-estradiol (or total drug) found in the water and sediment extractables. The kinetic results for the best-fitting model, the non-linear SFO, are summarized in Table 135 below.

The values for the total system transformation DT_{50} were used in subsequent EXPRESS and watershed modeling runs. The most conservative values (longest half-life) from the two water-sediment systems were used in the modeling.

Table 135. Anaerobic Dissipation Half-Life (DT_{50}) of 17α-Estradiol and 17α-Estradiol plus Transformation Products (total drug) in the Total System

Test system	Sample	DT_{50} (days)	k (day^{-1})
Taunton River	Total system – 17α-Estradiol	60.6	0.011
	Total system –Total drug*	107.8	0.0064
Weweantic River	Total system – 17α-Estradiol	62.8	0.0110
	Total system – Total drug*	103.5	0.0070
	Mean – 17α-Estradiol	61.7	
	Mean – Total drug	105.7	

* Total Drug = sum (α-estradiol + β-estradiol + estrone)

Total system transformation DT_{50}: transformation products β-estradiol and estrone

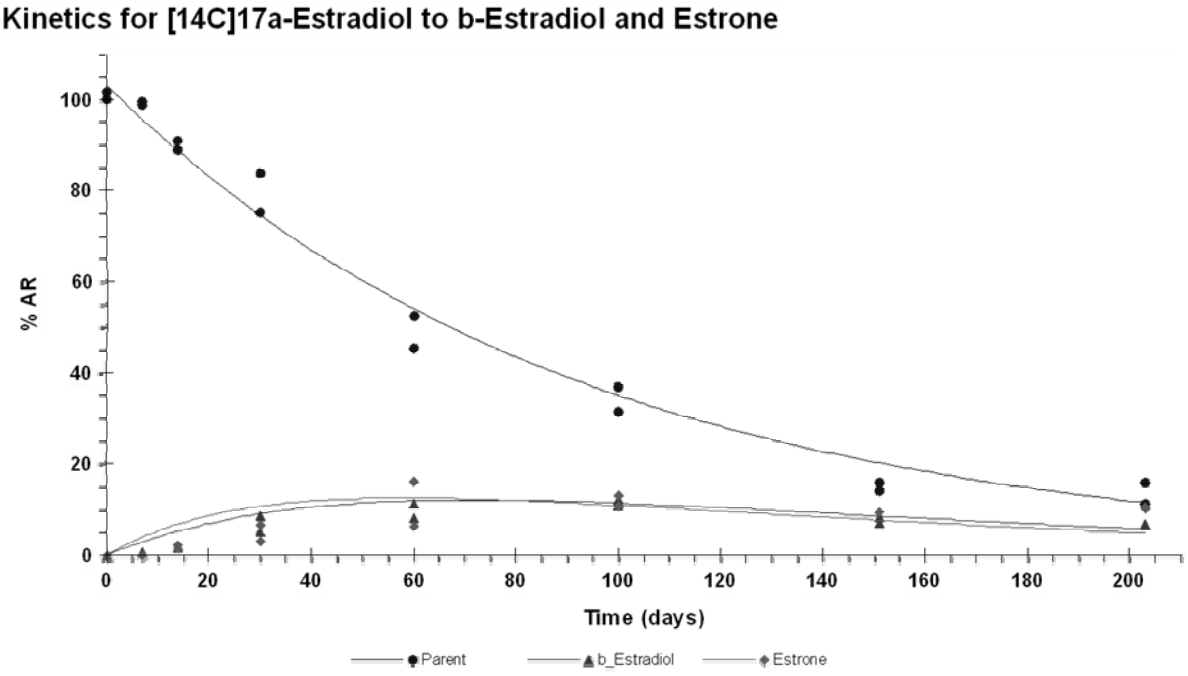
The total system transformation DT_{50} values for the metabolites 17β-estradiol and estrone were estimated using a kinetic model that describes the transformation of 17α-estradiol to two subsequent metabolites. All three metabolites were modeled using single first-order kinetics (SFO). The total system transformation DT_{50} was based on the depletion of each metabolite from the total water-sediment system as determined by the amount of metabolite found in the water and sediment extractables. The results of these analyses are presented in Table 136 below.

The data confirm that the two transformation products 17β-estradiol and estrone generally have shorter half-lives when compared to that of 17α-estradiol. The transformation products did not persist.

Table 136. Anaerobic Dissipation Half-Life (DT_{50}) of Transformation Products in the Total System

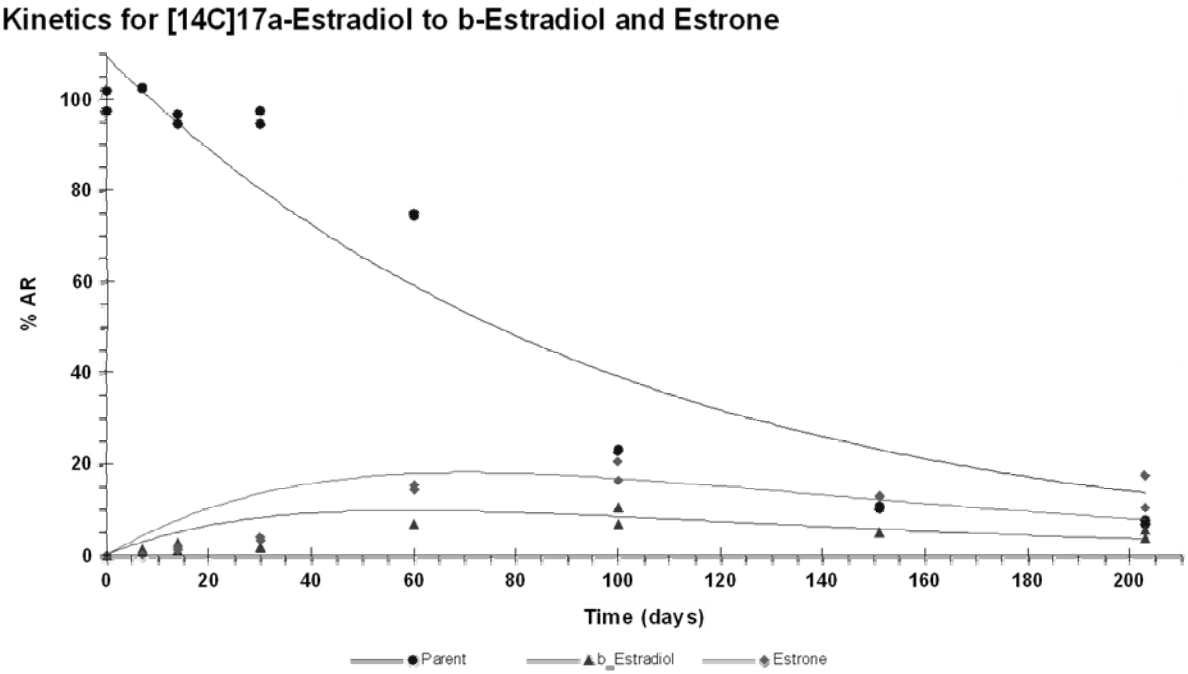
Anaerobic Half-Life of Transformation Products In Total System			
Test System	**Endpoint**	**17β-Estradiol**	**Estrone**
Taunton River	DT_{50} (days)	39.5	15.7
Weweantic River	DT_{50} (days)	26.7	22.1
	Mean – DT_{50} (days)	33.1	18.9

Figure 31. Kinetics of Anaerobic 17α-Estradiol Transformation in Taunton River Water/Sediment (Total System)



Circle: 17α-Estradiol, Triangle: 17β-Estradiol, Diamond: Estrone
% AR = percent applied radioactivity

Figure 32. Kinetics of Anaerobic 17α-Estradiol Transformation in Weweantic River Water/Sediment (Total System)



Circle: 17α-Estradiol, Triangle: 17β-Estradiol, Diamond: Estrone
% AR = percent applied radioactivity

Appendix 13.11. Aerobic 17α-trenbolone transformation in water/sediment systems following OECD Guideline 308

Title: Transformation and mineralization of [^{14}C]-17α-trenbolone in two aerobic aquatic sediment systems following OECD Guideline 308 [241]

Date: 18 June 2012

Pfizer Study Number: 1A72N-60-11-770

CRO Study Number: 2438.6669

GLP: Yes

Test Conditions: The rate of aerobic transformation of [^{14}C]-17α-trenbolone was studied at a nominal concentration of 1.0 mg/L and a temperature of 20 ± 2°C in two aerobic sediments varying in pH, textural characteristics, organic matter content and microbial content. The OC content in the Taunton River and Weweantic River sediments were 3.8% and 1.2%, respectively. Water-sediment samples were assayed from the Taunton River at 0, 1, 3, 7, 14, 28, 56, 77, and 119 days after dosing and from the Weweantic River at 0, 1, 3, 7, 14, 28, 56, 100, and 150 days after dosing.

The untreated flooded sediment samples were equilibrated at test conditions under aerobic conditions for at least one week. Following equilibration, [^{14}C]-17α-trenbolone was added to the water layers of each of the systems to achieve a nominal concentration of 1.0 mg/L in the water layer. Aerobic conditions in the test systems was maintained by continuously bubbling hydrated air through the water layers for 119 and 150 days for the Taunton River and Weweantic River test systems, respectively. KOH and ethylene glycol traps were used in the flow through aerobic test systems to collect $^{14}CO_2$ and any volatile components that evolved during the study.

Analysis: At each sampling interval, the water-sediment samples from each test system were separated into water and sediment fractions. The water phase was analyzed for total radioactivity by LSC and for distribution of radioactivity by HPLC/RAM. The sediment phase was extracted once with acetonitrile (150 mL) and once with acetonitrile:purified reagent water (80:20, v:v, 150 mL). At each sampling day, with the exception of Day 0, the sediment was extracted one additional time each with acetonitrile:purified reagent water:hydrochloric acid (80:20:0.1, v:v:v, 150 mL) for a total of three extractions. On Day 100 and 150, a fourth extraction was included for the Weweantic River sediment samples, using acetonitrile:purified reagent water:hydrochloric acid (80:20:0.1, v:v:v, 150 mL). The sediment extracts were assayed for total radioactivity by LSC and by HPLC/RAM to quantify [^{14}C]-17α-trenbolone and biodegradation products in the sediment phase. Following extraction, the sediment-bound residues were quantified by combustion analysis and volatile organic traps were quantified by LSC.

Results/Conclusions: [^{14}C]-17α-Trenbolone was transformed and degraded under the aerobic conditions of this study. Two known transformation products, 17β-trenbolone and trendione, were observed at respective retention times of approximately 19.5 and 25.9 minutes throughout the study in both the water and sediment phases of both the Taunton River and Weweantic River test systems. In the water phase, 17β-trenbolone and trendione reached maximum average[mm] percentages of 1.89% and 4.16% of the AR, respectively. In the sediment phase, they were detected at percentages of 5.00% and 11.68%, respectively. This indicates that the transformation products were principally in the sediment phase. Additional minor areas of radioactivity were observed in some of the chromatograms. However, in all cases, these individual peaks represented <10% of the AR and were not considered further.

Ultimate biodegradation was observed in the aerobic test systems and the cumulative amount of evolved $^{14}CO_2$ was 4.43% AR for the Taunton River aerobic test system and 11.09% AR for the Weweantic River aerobic test system at Day 119 and Day 150, respectively. Volatile organics were $\leq$0.02% AR in the Taunton River and the Weweantic River aerobic test system.

Aqueous dissipation DT_{50}: 17α-trenbolone

The aqueous DT_{50} for 17α-trenbolone was estimated to be 5.4 and 21.0 days for Taunton River and Weweantic River, respectively. The aqueous dissipation rate is based on the disappearance of 17α-trenbolone from the water layer due to biotransformation and sorption to sediment. The DT_{50} was estimated using a conservative approach by simple linear regression assuming linear kinetics based on a one-compartment model.

Total system transformation DT_{50}: 17α-trenbolone and total drug

The total system transformation DT_{50} for 17α-trenbolone and total drug (17α-trenbolone + 17β-trenbolone + trendione) was estimated by fitting a single compartment model (using simple linear or SFO non-linear regression). Selection of the best-fit model was made using a chi-square (χ^2) goodness-of-fit statistic. The total system DT_{50} was based on the depletion of 17α-trenbolone (or total drug) from the total water-sediment system as determined by the amount of 17α-trenbolone (or total drug) found in the water and sediment extractables. The kinetic results for the best model are summarized in Table 137 below.

The values for the total system transformation DT_{50} were used in subsequent EXPRESS and watershed modeling runs. The most conservative value (longest half-life) from the two water-sediment systems was used in the modeling.

[mm] The 'maximum average' value is the maximum reported value (from both sediments) of all of the averaged values determined (average of 2 replicates) at each of the time points for each of the transformation products.

Table 137. Aerobic Dissipation Half-Life (DT_{50}) of 17α-Trenbolone and 17α-Trenbolone plus Transformation Products (total drug) in the Total System

Test system	Sample	DT_{50} (days)	k (day^{-1})
Taunton River	Total system – 17α-Trenbolone	21.2	0.0327
	Total system – Total drug*	34.7	0.0200
Weweantic River	Total system – 17α-Trenbolone	46.5	0.0149
	Total system – Total drug*	53.3	0.0130
Mean – 17α-Trenbolone		33.9	
Mean – Total drug		44.0	

* Total Drug = sum (17α-trenbolone + 17β-trenbolone + trendione)

Total system transformation DT_{50}: transformation products 17β-estradiol and estrone

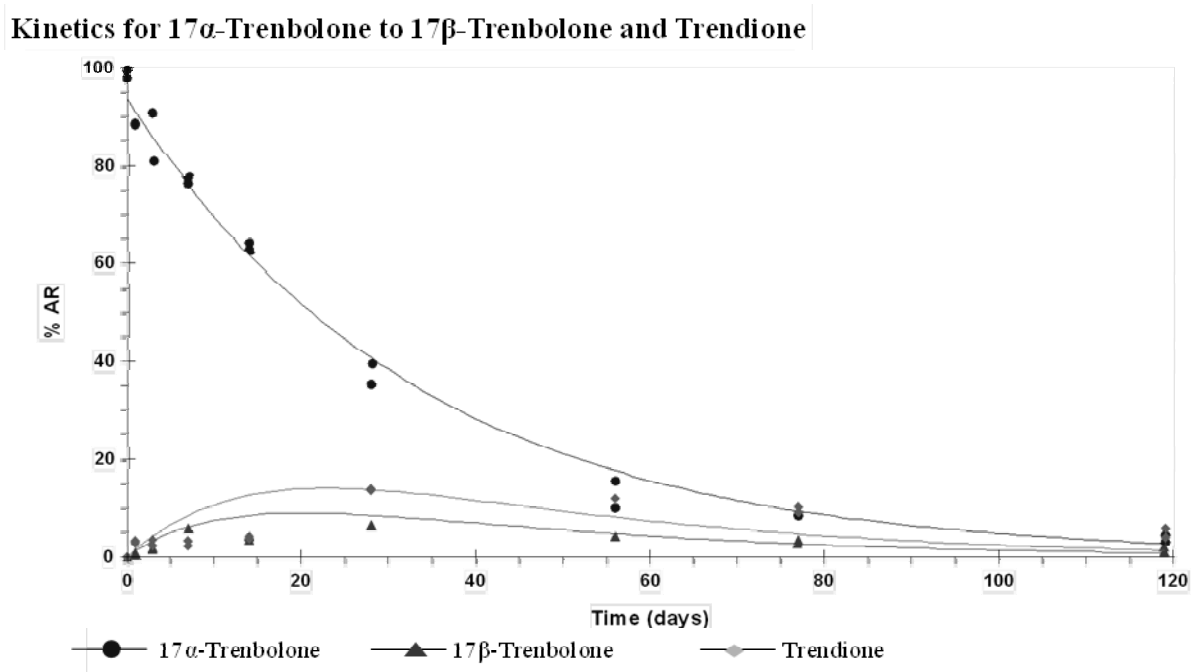
The total system transformation DT_{50} values for the transformation products, 17β-trenbolone and trendione, were estimated from a model that depicts the transformation of 17α-trenbolone to two subsequent transformation products. All three metabolites were modeled using single first-order kinetics (SFO). The total system DT_{50} was based on the depletion of each transformation product from the total water-sediment system as determined by the amount of transformation product found in the water and sediment extractables. The results of these runs are presented in Table 138 below.

The data confirm that the two transformation products 17β-trenbolone and trendione have shorter transformation half-lives when compared to that of 17α-trenbolone. The transformation products did not persist.

Table 138. Aerobic Dissipation Half-Life (DT_{50}) of Transformation Products in the Total System

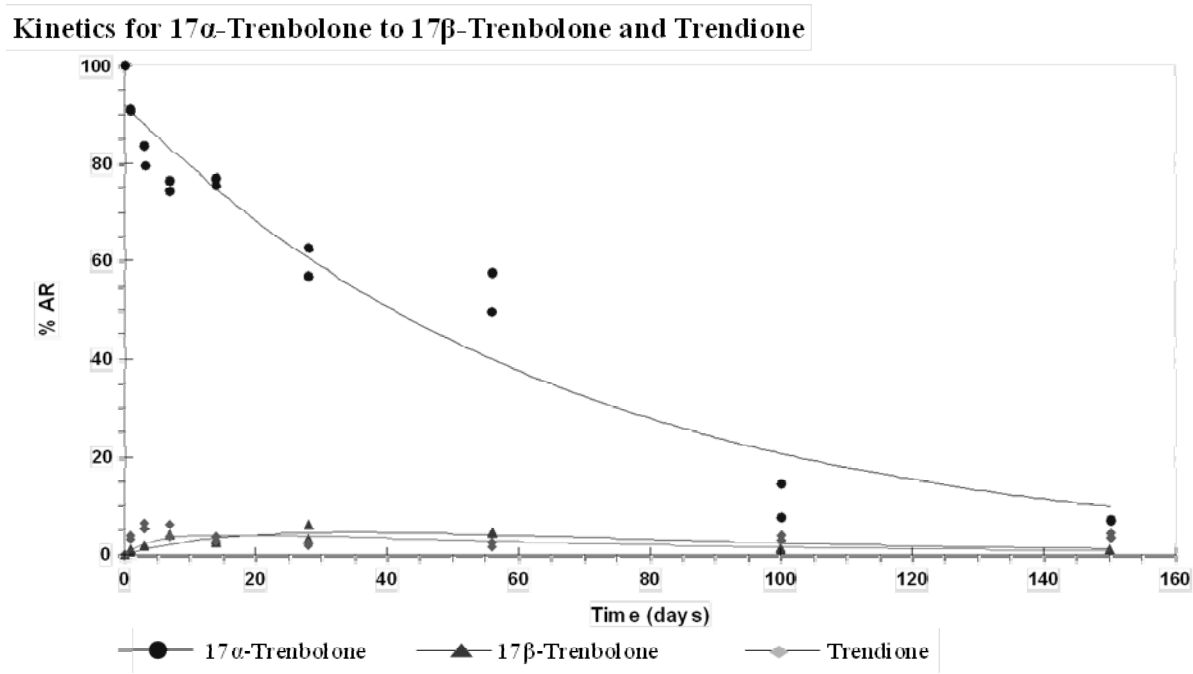
Test System	Endpoint	17β-Trenbolone	Trendione
Taunton River	DT_{50} (days)	9.1	6.6
Weweantic River	DT_{50} (days)	14.0	2.8
	Mean – DT_{50} (days)	11.6	4.7

Figure 33. Kinetics of Aerobic 17α-Trenbolone Transformation in Taunton River Water/Sediment (Total System)



Circle: 17α-Trenbolone, Triangle: 17β-Trenbolone, Diamond: Trendione
% AR = percent applied radioactivity

Figure 34. Kinetics of Aerobic 17α-Trenbolone Transformation in Weweantic River Water/Sediment (Total System)



Circle: 17α-Trenbolone, Triangle: 17β-Trenbolone, Diamond: Trendione
% AR = percent applied radioactivity

Appendix 13.12. 17α-estradiol effects on fish reproduction in fathead minnow following OECD Guideline 229

Title: 17α-[^{14}C]-estradiol - short-term reproduction assay with fathead minnow (*Pimephales promelas*) following OECD Guideline 229. [242]

Date: 07 June 2012

Pfizer Study Number: 1A73N-60-11-785

CRO Study Number: 2438.6684

GLP: Yes

Introduction: The effects of 17α-estradiol on fish reproduction endpoints were evaluated in a short-term reproduction assay with fathead minnows (*Pimephales promelas*) following OECD Guideline 229 [124] using 17α-[^{14}C]-estradiol. The study was conducted in compliance with GLP at nominal exposure concentrations of 2.7, 8.5, 27, 83, and 260 ng/L of 17α-estradiol. Statistical evaluation of the following endpoints was determined based on mean measured concentrations of 2.5, 7.2, 25, 80, and 250 ng/L of 17α-estradiol:

- Adult survival
- Fecundity (number of eggs/female/day)
- Gonadosomatic index (GSI)
- Nuptial tubercle score
- Blood plasma vitellogenin (VTG) concentration
- Fertilization rate
- Hatching success
- Percent normal fry at hatch
- Percent normal fry following yolk sac absorption

The fecundity endpoint, associated with adverse reproduction effects, was used to determine the NOEC, LOEC, and MATC values for the study.

The fathead minnows (*P. promelas*) used during this study were obtained from a laboratory supply of reproductively mature animals in spawning condition. The test animals originated from brood stock maintained at the testing facility for more than 30 years, which originated from the U.S. EPA's Environmental Research Laboratory, Duluth, Minnesota. The fish used for this exposure were approximately 16 to 18 weeks old at the start of the pre-exposure period and were approximately 18 to 20 weeks old at exposure initiation. Prior to initiation of exposure, the reproductive activity of the fish was assessed during a 14-day pre-exposure period. Prior to exposure initiation and throughout the exposure period, the fish were fed a measured amount of live brine shrimp (*Artemia salina*) twice daily. Following the 14-day pre-exposure phase, the 28 spawning groups with the greatest number of eggs/female/day were added to the flow through exposure system using a random block design. The exposure system was designed to provide five concentrations of the 17α-[^{14}C]-estradiol, a

dilution water control and a solvent control to four replicate aquaria. Each replicate tank contained four female and two male fish. The exposure was maintained for a period of 21 days, during which, survival and the appearance of the fish, behavior, secondary sex characteristics and fecundity were assessed daily. Water samples for each treatment were collected weekly and analyzed for 17α-estradiol using LSC, and the composition of [^{14}C] residues in the highest concentration was confirmed using HPLC/RAM.

As defined by the OECD Guideline 229, the following acceptance criteria were achieved in this study:

- Survival of ≥90% in the controls over the duration of the 21-day spawning period.
- Dissolved oxygen concentration was maintained at a minimum of 60% of air saturation throughout the exposure period.
- Water temperature was maintained within 1.5°C between test tanks at any one time during the exposure period and maintained within ±2°C of the 25°C temperature.
- Evidence was obtained to demonstrate that the concentrations of 17α-estradiol in solution had been satisfactorily maintained within ±20% of the mean measured values included in the final report.
- Greater than 95% fertility of eggs from the control animals during the exposure.

Fish actively spawned in all replicates prior to initiating chemical exposure and in control replicates during the test.

Results

Survival

Survival of male and female fish was assessed. Following 21 days of exposure to 17α-estradiol, mean percent survival among male fish in the control, solvent control and all treatment levels was 100%. Mean percent survival among female fish in both the control and solvent controls was 94%. Mean percent survival among female fish exposed to 2.5, 7.2, 25, 80, and 250 ng/L of 17α-estradiol was 88, 100, 94, 100, and 100%, respectively. No significant difference ($p>0.05$) was observed in survival among the treatment levels tested.

Egg Production, Viability and Hatchability

The mean number of eggs per female per reproductive day for the control and solvent control was 18.7 and 16.7, respectively. The mean number of eggs per female per reproductive day for the 2.5, 7.2, 25, 80, and 250 ng/L of 17α-estradiol treatment levels was 17.2, 20.9, 16.1, 19.7 and 19.6, respectively. No significant difference ($p>0.05$) was observed in the mean number of eggs per female per day for any of the treatment levels tested when compared to the pooled controls (17.7 eggs/female/day).

The percentage of viable eggs in both the control and solvent control averaged 97%. The percentage of viable eggs in the 2.5, 7.2, 25, 80 and 250 ng/L of 17α-estradiol treatment levels averaged 98, 97, 97, 98 and 98%, respectively. No significant difference ($p>0.05$) was observed in the percent viable eggs for any of the treatment levels tested when compared to the pooled control (i.e., 97%).

Embryo hatching success in the control and solvent control averaged 95 and 97%, respectively. Mean embryo hatching success in the 2.5, 7.2, 25, 80 and 250 ng/L of 17α-estradiol treatment levels was 87, 95, 91, 90 and 91%, respectively. No significant difference ($p>0.05$) was observed in mean embryo hatching success for any of the treatment levels tested compared to the pooled control (i.e., 96%).

F1 Generation Development

The mean percent normal larvae at hatch in both the control and solvent control were 100%. The mean percent normal larvae at hatch in the 2.5, 7.2, 25, 80 and 250 ng/L of 17α-estradiol treatment levels was 100, 100, 100, 99 and 100%, respectively. No significant difference ($p>0.05$) was observed in mean percent normal larvae at hatch for any of the treatment levels tested compared to the pooled control (i.e., 100%).

The mean percent normal larvae following yolk sac absorption in both the control and solvent control was 100%. The mean percent normal larvae following yolk sac absorption in the 2.5, 7.2, 25, 80 and 250 ng/L of 17α-estradiol treatment levels was 100, 100, 100, 99, and 100%, respectively. No significant difference ($p>0.05$) was observed in mean percent normal larvae following yolk sac absorption for any of the treatment levels tested compared to the pooled control (i.e., 100%).

Tubercle Scoring

The median male tubercle score in the control and solvent control was 38 and 37, respectively. Mean tubercle scores in the 2.5, 7.2, 25, 80 and 250 ng/L of 17α-estradiol treatment levels were 39, 36, 39, 39 and 36, respectively. No significant difference ($p>0.05$) was observed in mean tubercle scores for any of the treatment levels tested compared to the pooled control (i.e., 38).

The females in this exposure did not develop nuptial tubercles as a result of exposure. The tubercle score for all females in all treatment levels tested and the control was 0.

GSI

The mean male GSI in both the control and solvent control was 1.4%. The mean male GSI among fish exposed to the 2.5, 7.2, 25, 80, and 250 ng/L of 17α-estradiol treatment levels was 1.5, 1.4, 1.3, 1.6, and 1.5%, respectively. No significant difference ($p>0.05$) was observed in mean male GSI among fish exposed to any of the treatment levels tested compared to the pooled control (i.e., 1.4%).

The mean female GSI in the control and solvent control was 13 and 12%, respectively. The mean female GSI in the 2.5, 7.2, 25, 80, and 250 ng/L of 17α-estradiol treatment levels was 11, 13, 12, 13, and 12%, respectively. No significant difference ($p>0.05$) was observed in mean female GSI for any of the treatment levels tested compared to the pooled control (i.e., 12%).

VTG

The mean male VTG concentration in the control and solvent control was 888 and 260 ng/mL, respectively. The mean male VTG concentration in the 2.5, 7.2, 25, 80, and 250 ng/L of 17α-estradiol treatment levels was 195, 46, 404, 529, and 8.9 x 10^5 ng/mL, respectively. A significant difference in mean male VTG concentration in the 250 ng/L of 17α-estradiol treatment level was observed when compared to the control (i.e., 888 ng/mL; p = 0.0466) and solvent control (i.e., 260 ng/mL; p = 0.0342). However, a significant difference (p=0.1454) was not observed in mean male VTG when compared to the pooled control. The difference between these procedures is likely due to the increased variation when pooling the control data. In order to provide a conservative effect concentration for VTG, comparison with the control was reported.

The mean female VTG concentration in the control and solvent control was 1.8 x 10^6 ng/mL and 1.6 x 10^6 ng/mL, respectively. The mean female VTG concentration in the 2.5, 7.2, 25, 80, and 250 ng/L of 17α-estradiol treatment levels was 1.4 x 10^6, 1.4 x 10^6, 1.5 x 10^6, 1.3 x 10^6, and 1.7 x 10^6 ng/mL, respectively. No significant difference (p>0.05) was observed in mean female VTG concentration among fish exposed to any of the treatment levels tested compared to the pooled control (i.e., 1.7 x 10^6 ng/mL).

The NOEC and LOEC values for each of the endpoints evaluated are presented in Table 139 below.

Table 139. Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) from 21-Day Exposure of Fathead Minnow (*Pimephales promelas*) to 17α-[^{14}C]-Estradiol

Endpoints	NOEC (ng/L)	LOEC (ng/L)
F0 Generation		
Survival	250	>250
Fecundity	250	>250
Secondary Sex Characteristics (nuptial tubercle score)-Females	250	>250
Secondary Sex Characteristics (nuptial tubercle score)-Males	250	>250
Gonadosomatic Index (GSI)-Females	250	>250
Gonadosomatic Index (GSI)-Males	250	>250
Vitellogenin-Females	250	>250
Vitellogenin-Males	80	250
F1 Generation		
Hatching Success	250	>250
Fertilization Success	250	>250
% Normal Larvae at Hatch	250	>250
% Normal Larvae Following Yolk Sac Absorption	250	>250

Conclusion

Analysis of the exposure solutions of 17α-estradiol resulted in mean measured concentrations of 2.5, 7.2, 25, 80 and 250 ng/L (84.7 to 96.4% of nominal). The NOEC, LOEC and MATC values selected for the short-term effects of 17α-estradiol on fathead minnow reproduction are 250 ng/L, >250 ng/L and $\geq$250 ng/L, respectively.

Figure 35. Fecundity (Number of Eggs Per Female Per Reproductive Day) During the 21-Day Exposure of Fathead Minnow (*Pimephales promelas*) to 17α-[^{14}C]-Estradiol (Error Bar = SD)

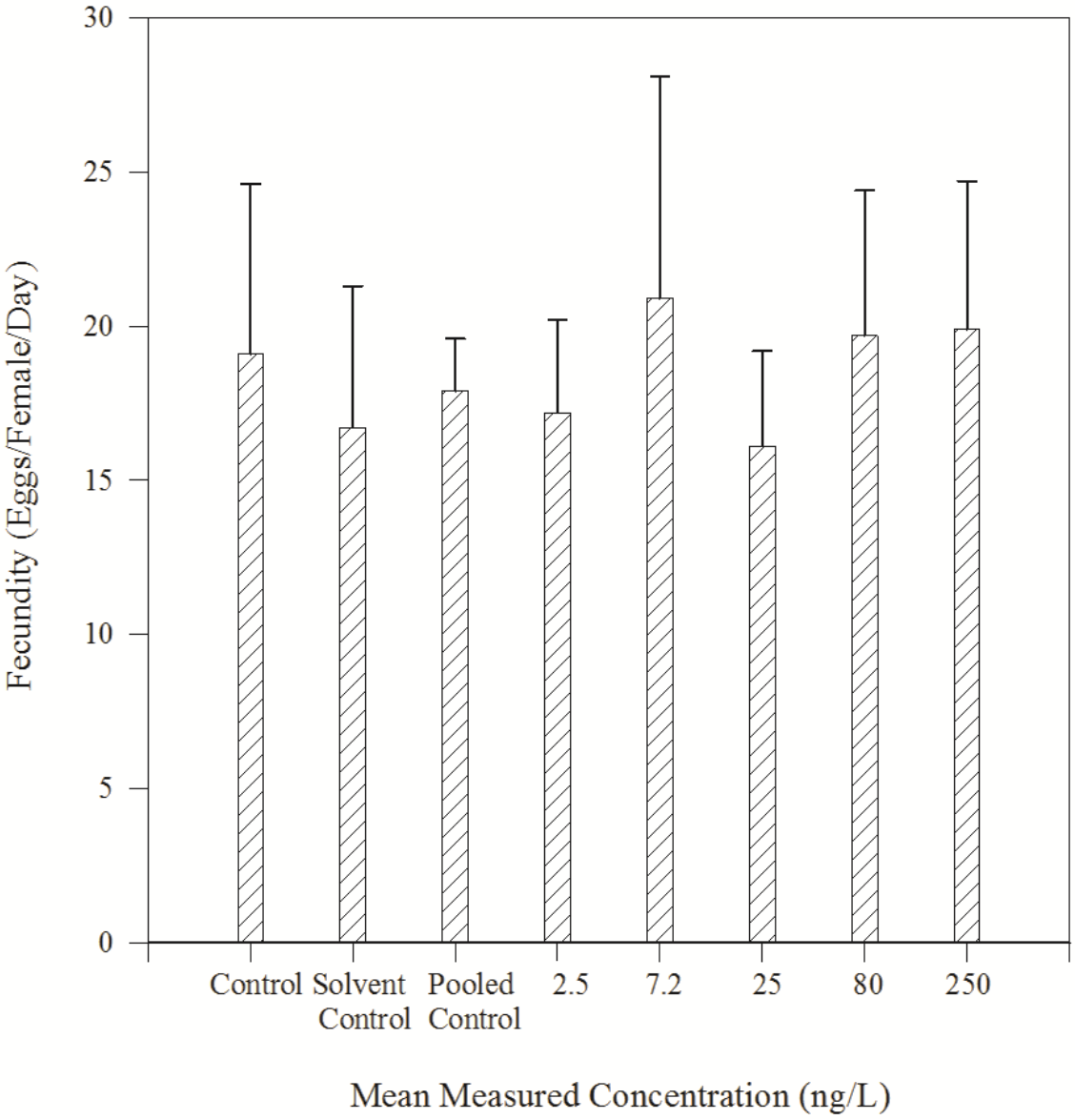
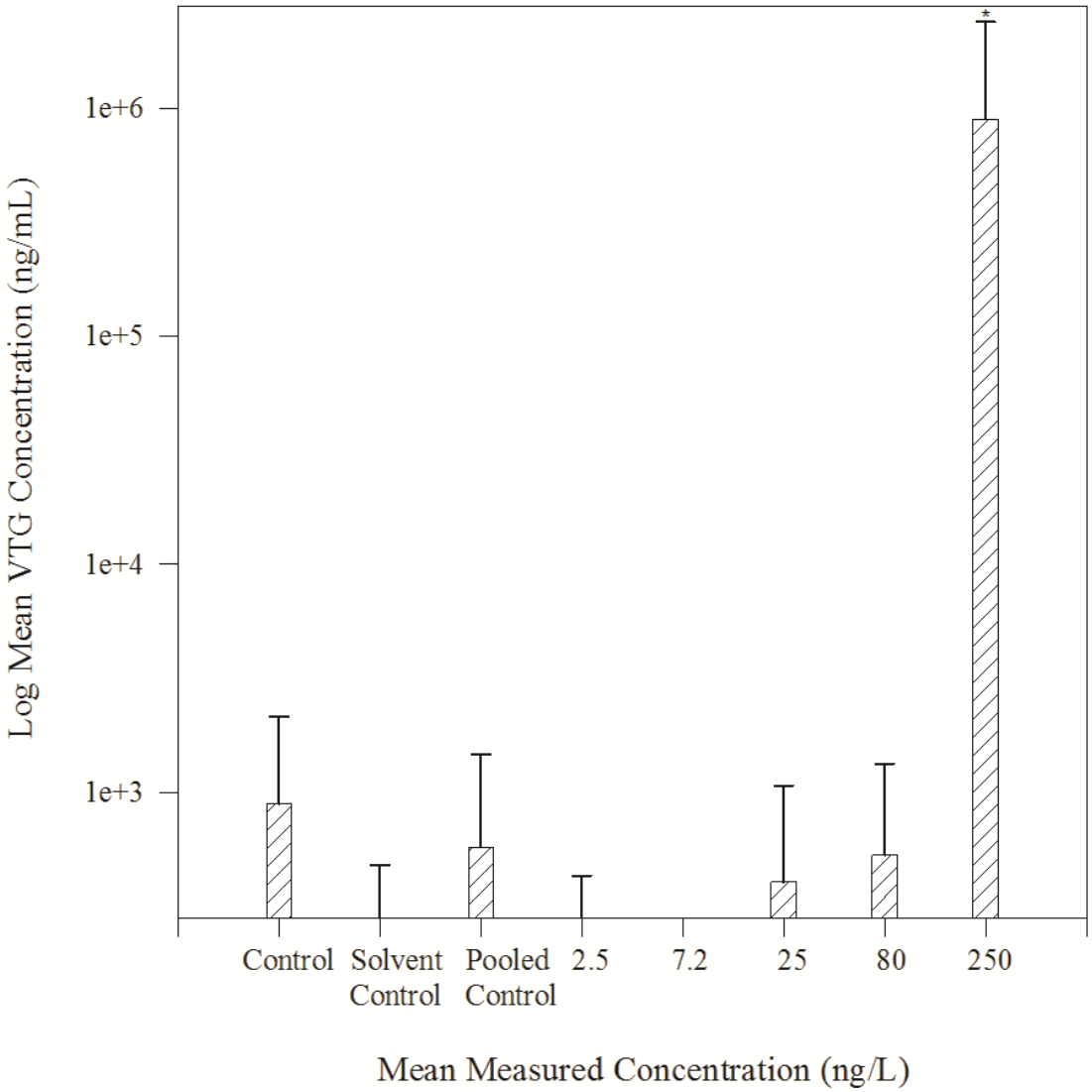


Figure 36. Male Vitellogenin Concentration During the 21-Day Exposure of Fathead Minnow (*Pimephales promelas*) to 17α-[^{14}C]-Estradiol (Error Bar = SD)



*Significantly different compared to the control,
based on Jonckheere-Terpstra Step-Down Test (p = 0.0466).

Appendix 13.13. 17α-trenbolone effects on fish reproduction in fathead minnow following OECD Guideline 229

Title: 17α-[^{14}C]-trenbolone - short-term reproduction assay with fathead minnow (*Pimephales promelas*) following OECD Guideline 229. [243]

Date: 08 June 2012

Pfizer Study Number: A5Y3N-US-12-001

CRO Study Number: 2438.6689

GLP: Yes

Introduction: The effects of 17α-trenbolone on fish reproduction endpoints were evaluated in a short-term reproduction assay with fathead minnow (*P. promelas*) following OECD Guideline 229 [124] using [^{14}C]-17α-trenbolone. The study was conducted in compliance with GLP at nominal exposure concentrations of 1.3, 3.9, 12, 38, and 120 ng/L of 17α-trenbolone. Statistical evaluation of the endpoints was determined based on mean measured concentrations of 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone.

The experimental design, observed endpoints, study and analytical methods, and OECD 229 Guideline acceptance criteria were the same as those outlined in Appendix 13.12.

Results

Survival

Following 21 days of exposure, mean percent survival among male fish in both the control solvent control was 100%. Mean percent survival in the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 100, 100, 88, 100, and 88%, respectively. No significant difference ($p>0.05$) was observed in percent survival among male fish in any of the treatment levels tested compared to the pooled control (i.e., 100%).

Mean percent survival among female fish in the control and solvent control was 94 and 88%, respectively. Mean percent survival among female fish exposed to the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 100, 88, 88, 100, and 100%, respectively. No significant difference ($p>0.05$) was observed in percent survival among female fish in any of the treatment levels tested compared to the pooled control (i.e., 91%).

Egg Production, Viability and Hatchability

The mean number of eggs per female per reproductive day for the control and solvent control was 10.3 and 15.2, respectively. The mean number of eggs per female per reproductive day for the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 8.2, 11.9, 12.9, 13.0, and 5.54, respectively. A significant difference ($p=0.0166$) was observed in mean number of eggs per female per day for the 120 ng/L of 17α-trenbolone treatment level compared to the pooled control (i.e., 12.7 eggs/female/day).

The percentage of viable eggs in both the control and solvent control averaged 98%. The percentage of viable eggs in the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels averaged 99, 99, 99, 99, and 97%, respectively. No significant difference ($p>0.05$) was observed in percentage of viable eggs for any of the treatment levels tested compared to the pooled control (i.e., 98%).

Embryo hatching success in both the control and solvent control averaged 91%. Mean embryo hatching success in the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 89, 90, 95, 92, and 96%, respectively. No significant difference ($p>0.05$) was observed in mean embryo hatching success for any of the treatment levels tested compared to the pooled control (i.e., 91%).

F1 Generation Development

The mean percent normal larvae at hatch in both the control and solvent control was 100%. The mean percent normal larvae at hatch in the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 99, 100, 99, 100, and 100%, respectively. No significant difference ($p>0.05$) was observed in mean percent normal larvae at hatch for any of the treatment levels tested compared to the pooled control (i.e., 100%).

The mean percent normal larvae following yolk sac absorption in both the control and solvent control was 100%. The mean percent normal larvae following yolk sac absorption in the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 99, 100, 100, 100 and 100%, respectively. No significant difference ($p>0.05$) was observed in mean percent normal larvae following yolk sac absorption for any of the treatment levels tested compared to the pooled control (i.e., 100%).

Tubercle Scoring

The median tubercle score in the control and solvent control was 33 and 34, respectively. Median tubercle scores in the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels were 31, 33, 36, 33, and 30, respectively. No significant difference ($p>0.05$) was observed in median tubercle scores for any of the treatment levels tested compared to the pooled control (i.e., 33).

The females did not develop nuptial tubercles as a result of exposure. The tubercle score for all females in all treatment levels tested and the controls was 0.

GSI

The mean male GSI in both the control and solvent control was 1.2%. The mean male GSI among fish exposed to the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 1.0, 1.6, 1.1, 1.3, and 1.4%, respectively. No significant difference ($p>0.05$) was observed in mean male GSI among fish exposed to any of the treatment levels tested compared to the pooled control (i.e., 1.2%).

The mean female GSI in both the control and solvent control was 12%. The mean female GSI in the 1.2, 3.5, 11, 35, and 120 of ng/L of 17α-trenbolone treatment levels was 14, 12, 13, 13, and 15%, respectively. A significant difference ($p=0.0296$) was observed in mean female GSI for the 120 ng/L treatment level compared to the pooled control (i.e., 12%).

VTG

The mean male VTG concentration in the control and solvent control was 348 and 176 ng/mL, respectively. The mean male VTG concentration in the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 643, 262, 360, 420, and 997 ng/mL, respectively. No significant difference ($p>0.05$) for male VTG concentration was observed in any of the treatment levels tested compared to the pooled control (i.e., 262 ng/mL).

The mean female VTG concentration in the control and solvent control was 2.2×10^6 ng/mL and 3.9×10^6 ng/mL, respectively. The mean female VTG concentration in the 1.2, 3.5, 11, 35, and 120 ng/L of 17α-trenbolone treatment levels was 2.2×10^6, 2.6×10^6, 1.6×10^6, 3.3×10^6, and 1.9×10^6 ng/mL, respectively. No significant difference ($p>0.05$) was observed in mean female VTG concentration among fish exposed to any of the treatment levels tested compared to the pooled control (i.e., 3.0×10^6 ng/mL).

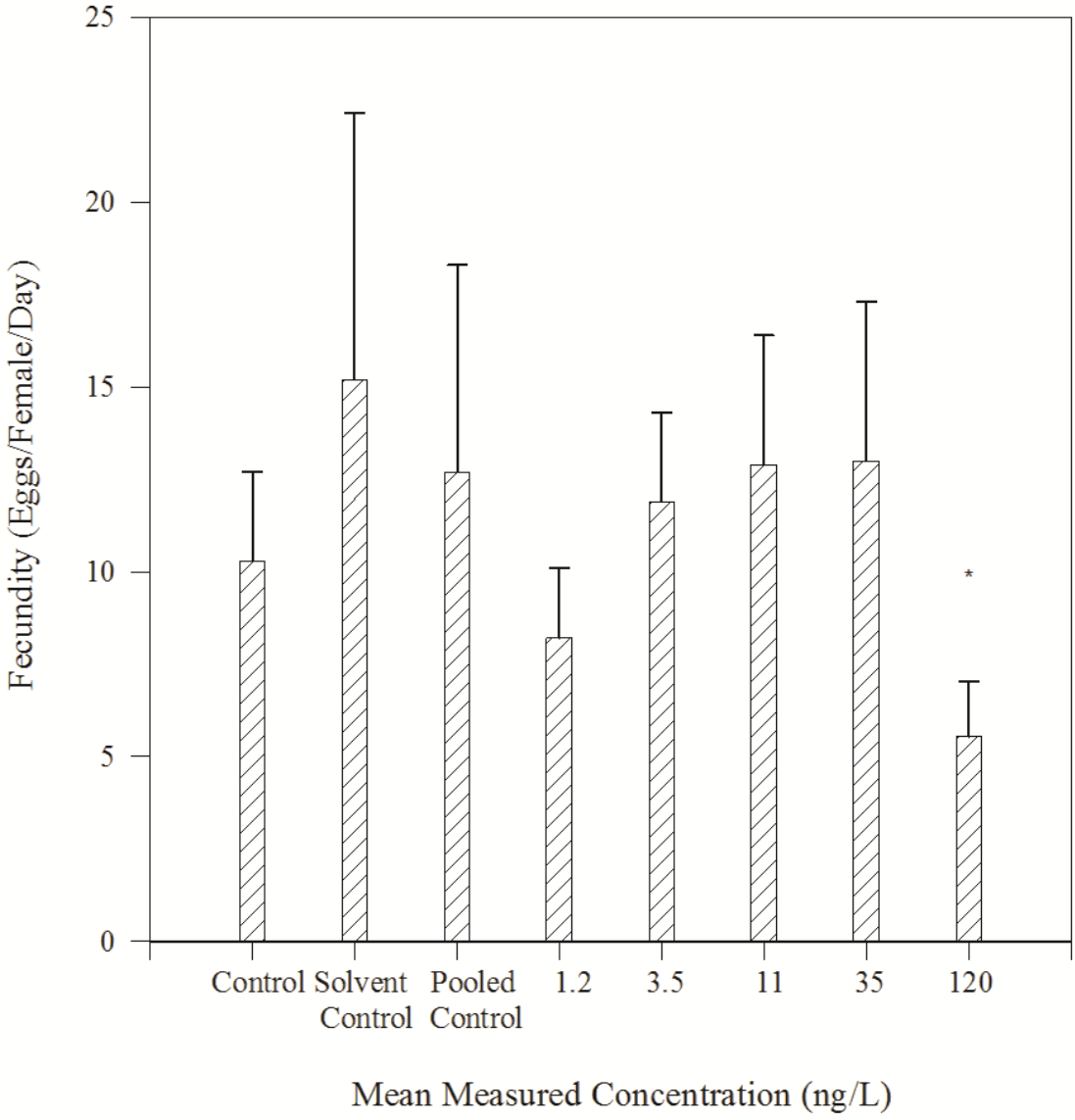
The NOEC and LOEC values for each of the endpoints evaluated are presented in Table 118 below.

Table 140. Lowest Observed Effects Concentration (LOEC) and No Observed Effect Concentration (NOEC) from 21-Day Exposure of Fathead Minnow (*Pimephales promelas*) to 17α-[^{14}C]-Trenbolone

Endpoints	NOEC (ng/L)	LOEC (ng/L)
F0 Generation		
Survival	120	>120
Fecundity	35	120
Secondary Sex Characteristics (nuptial tubercle score)-Females	120	>120
Secondary Sex Characteristics (nuptial tubercle score)-Males	120	>120
Gonadosomatic Index (GSI)-Females	35	120
Gonadosomatic Index (GSI)-Males	120	>120
Vitellogenin-Females	120	>120
Vitellogenin-Males	120	>120
F1 Generation		
Hatching Success	120	>120
Fertilization Success	120	>120
% Normal Larvae at Hatch	120	>120
% Normal Larvae Following Yolk Sac Absorption	120	>120

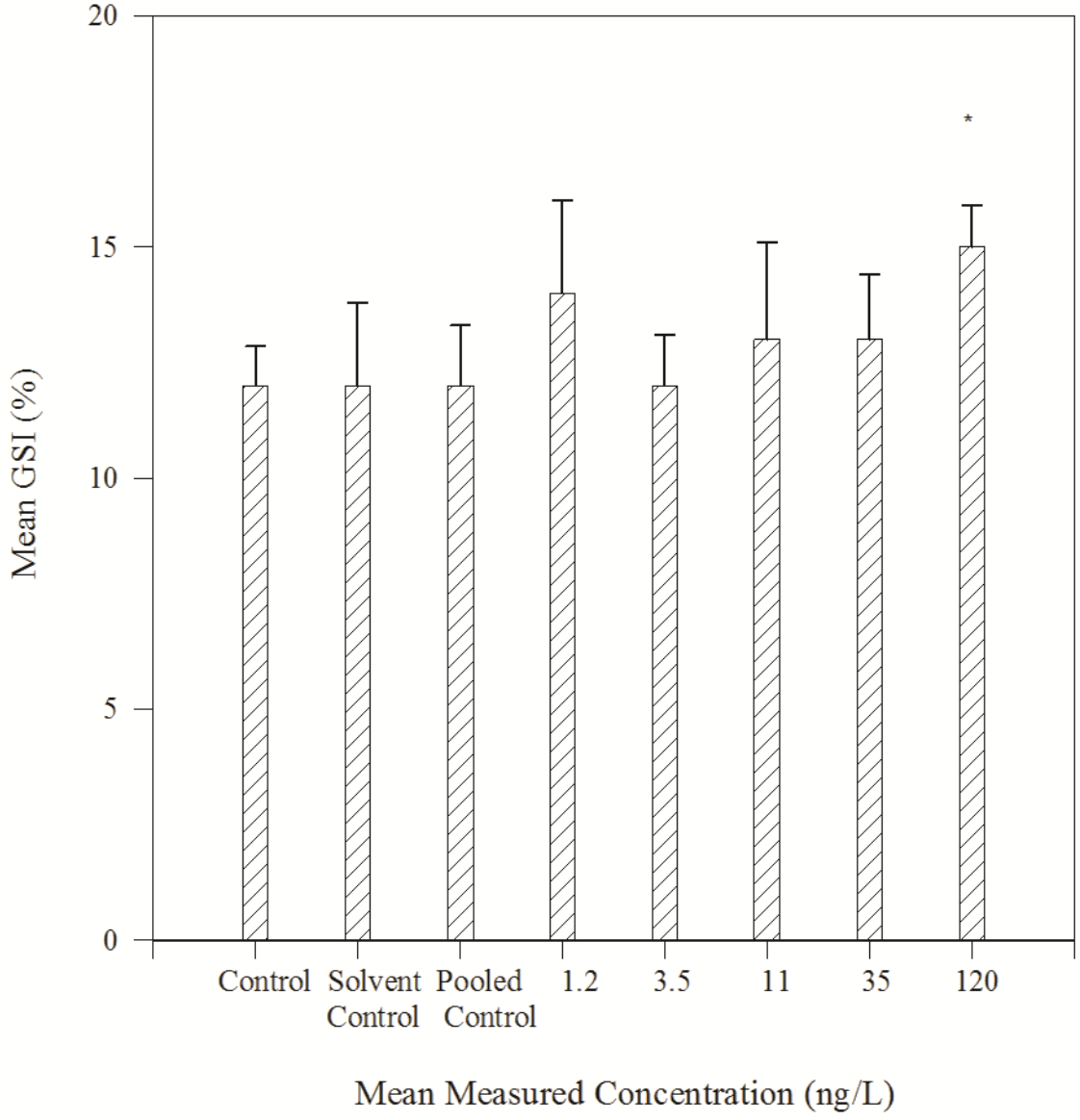
Conclusions: Analysis of the exposure solutions of 17α-trenbolone provided mean measured concentrations of 1.2, 3.5, 11, 35, and 120 ng/L (88-100% of nominal). The NOEC, LOEC, and MATC values determined for the short-term reproduction effects of 17α-trenbolone on fathead minnow are 35 ng/L, 120 ng/L, and 65 ng/L, respectively.

Figure 37. Fecundity (Number of Eggs per Female per Reproductive Day) During the 21-Day Exposure of Fathead Minnow (*Pimephales promelas*) to [^{14}C]-17α-Trenbolone (Error Bar = SD)



*Significantly different compared to the pooled control, based on Dunnett's Multiple Comparison Test (p = 0.0166).

Figure 38. Female GSI During the 21-Day Exposure of Fathead Minnow (*Pimephales promelas*) to [^{14}C]-17α-Trenbolone (Error Bar = SD)



*Significantly different compared to the pooled control, based on Dunnett's Multiple Comparison Test (p = 0.0296).

Appendix 13.14. 17α-trenbolone effects on fish reproduction in medaka following OECD Guideline 229

Title: 17α-[^{14}C]-trenbolone - short-term reproduction assay with medaka (*Oryzias latipes*) following OECD Guideline 229 [244].

Date: 06 June 2012

Pfizer Study Number: 1A73N-60-11-786

CRO Study Number: 2438.6687

GLP: Yes

Introduction: The effects of 17α-trenbolone on fish reproduction were evaluated in a short-term reproduction assay with medaka (*Oryzias latipes*) following OECD Guideline 229 [124] using [^{14}C]-17α-trenbolone. The study was conducted in compliance with GLP at nominal exposure concentrations of 1.3, 3.9, 12, 38 and 120 ng/L. Statistical evaluation of the following endpoints were determine based on mean measured concentrations of 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone:

- Adult survival
- Fecundity (number of eggs/female/day)
- Gonadosomatic index (GSI)
- Secondary sex characteristics (papillary processes)
- Hepatic vitellogenin (VTG) concentration
- Fertilization rate
- Hatching success
- Percent normal fry at hatch
- Percent normal fry following yolk sac absorption

The medaka (*Oryzias latipes*) used during this study were originally obtained from Aquatic Research Organisms, a commercial supplier in Hampton, New Hampshire. The fish were purchased as embryos and were reared to sexual maturity at the testing facility. The animals used for this exposure were approximately 15 weeks old at the start of the pre-exposure period and were approximately 17 weeks old at exposure initiation. Prior to initiation of exposure, the reproductive activity of the fish were assessed during a 14-day pre-exposure period.

Following the 14-day pre-exposure phase, the 14 spawning groups with the greatest number of eggs/female/day were added to the flow-through exposure system using a randomized block assignment to the treatment groups. Each replicate tank contained five female and five male fish, for a total of 10 males and 10 females per treatment. For this study, one of the test vessels (38 ng/L nominal, replicate B) was improperly loaded with nine females and one male and was excluded from all data pertaining to fecundity, GSI, survival, fertilization success, and vitellogenin concentration. The adult phase of the exposure was maintained

for 21 days, during which, survival and the appearance of the fish, behavior, and fecundity were assessed daily. The exposure was continued for an additional eight days to collect F1 embryo data. Water samples for each treatment were collected weekly and analyzed for 17α-trenbolone via LSC, and the composition of [^{14}C] residues in the highest concentration were confirmed via HPLC/RAM.

- As defined by the OECD Guideline 229 and the study protocol, the following acceptance criteria were achieved in this study:
- Survival of $\geq$90% in the controls over the duration of the 21-day spawning period.
- Dissolved oxygen concentration was maintained at a minimum of 60% of air saturation throughout the exposure period.
- Water temperature was maintained within 1.5°C between test tanks at any one time during the exposure period and within $\pm$2°C of the 25°C temperature.
- Evidence was obtained to demonstrate that the concentrations of 17α-trenbolone in solution have been satisfactorily maintained within $\pm$20% of the mean measured values included in the final report.
- Fish actively spawned in all replicates prior to initiating chemical exposure and in control replicates during the test.

Results

Survival

Following 21 days of exposure, mean percent survival among female and male fish in the control, solvent control and all treatment levels tested was 100%. No significant difference ($p>0.05$) was observed in percent survival among fish in any of the treatment levels tested compared to the pooled control (i.e., 100%).

Egg Production, Viability and Hatchability

The number of eggs per female per reproductive day for the control and solvent control averaged 23.7 and 25.1, respectively. The number of eggs per female per reproductive day for the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels averaged 24.6, 27.8, 23.9, 25.3, and 25.9, respectively. No significant difference ($p>0.05$) was observed in the number of eggs per female per day for any of the treatment levels tested compared to the pooled control (i.e., 24.4 eggs/female/day).

The percentage of viable eggs in the control and solvent control averaged 94 and 92%, respectively. The percentage of viable eggs in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels averaged 94, 92, 91, 94, and 90%, respectively. No significant difference ($p>0.05$) was observed in percentage of viable eggs for any of the treatment levels tested compared to the pooled control (i.e., 93%).

Embryo hatching success in both the control and solvent control averaged 97%. Embryo hatching success in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels averaged 91, 92, 95, 89, and 85%, respectively. A significant difference was observed in embryo hatching success for the 33 ng/L ($p=0.0162$) and 110 ng/L ($p=0.0014$) treatment levels compared to the pooled control (i.e., 97%).

F1 Generation Development

The percent normal larvae at hatch in both the control and solvent control averaged 100%. The percent normal larvae at hatch in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels averaged 100, 100, 99, 100, and 98%, respectively. A significant difference ($p=0.0100$) in percent normal larvae at hatch was observed for the 110 ng/L of 17α-trenbolone treatment level compared to the pooled control (i.e., 100%). While the variability observed at the 110 ng/L concentration is higher than that observed at lower treatment levels or controls, the response observed for this endpoint does not appear to be biologically relevant. Similar variability was observed at the 10 ng/L treatment level and these abnormalities were not observed following yolk sac absorption.

The percent normal larvae following yolk sac absorption in both the control and solvent control averaged 100%. The percent normal larvae following yolk sac absorption in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels averaged 100, 100, 99, 100, and 99%, respectively. No significant difference ($p>0.05$) was observed in percent normal larvae following yolk sac absorption for any of the treatment levels tested compared to the pooled control (i.e., 100%).

GSI

The mean male GSI in the control and solvent control was 1.4 and 1.0%, respectively. The mean male GSI among fish exposed to the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels was 1.1, 1.2, 1.1, 1.1, and 1.1%, respectively. No significant difference ($p>0.05$) was observed in mean male GSI among fish exposed to any of the treatment levels tested compared to the pooled control (i.e., 1.2%).

The mean female GSI in the control and solvent control was 12 and 12%, respectively. The mean female GSI in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels was 11, 11, 12, 11, and 11%, respectively. No significant difference ($p>0.05$) was observed in mean female GSI for any of the treatment levels tested compared to the pooled control (i.e., 12%).

Papillary Process Scoring

The mean papillary process scores for males in the control and solvent control was 107 and 105, respectively. The mean papillary process scores for males in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels were 113, 111, 106, 105, and 114, respectively. No significant difference ($p>0.05$) was observed in mean papillary process scores for any of the treatment levels tested compared to the pooled control (i.e., 106).

The mean papillary process score for females in both the control and solvent control was 0. The mean papillary process scores for females in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels were 0, 0, 0, 0, and 3.3, respectively. A significant difference ($p=0.0142$) was observed in mean papillary process scores for the 110 ng/L treatment level compared to the pooled control (i.e., 0).

VTG

The mean male VTG concentration in the control and solvent control was 849 and 1770 ng/mL, respectively. The mean male VTG concentration in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels treatment levels was 3188, 1339, 6642, 419, and 18458 ng/mL, respectively. No significant difference ($p>0.05$) was observed in mean male VTG concentration among fish exposed to any of the treatment levels tested compared to the pooled control (i.e., 1310 ng/mL).

The mean female VTG concentration in the control and solvent control was 3.4×10^5 ng/mL and 3.5×10^5 ng/mL, respectively. The mean female VTG concentration in the 1.1, 4.2, 10, 33, and 110 ng/L of 17α-trenbolone treatment levels treatment levels was 4.0×10^5, 2.7×10^5, 2.2×10^5, 2.5×10^5, and 2.2×10^5 ng/mL, respectively. A significant difference was observed in female VTG concentration in the 10 ng/L ($p=0.0366$), 33 ng/L ($p=0.0204$), and 110 ng/L ($p=0.0026$) treatment levels compared to the pooled control (i.e., 3.5×10^5 ng/mL).

The NOEC and LOEC values for each of the endpoints evaluated are presented in Table 141 below.

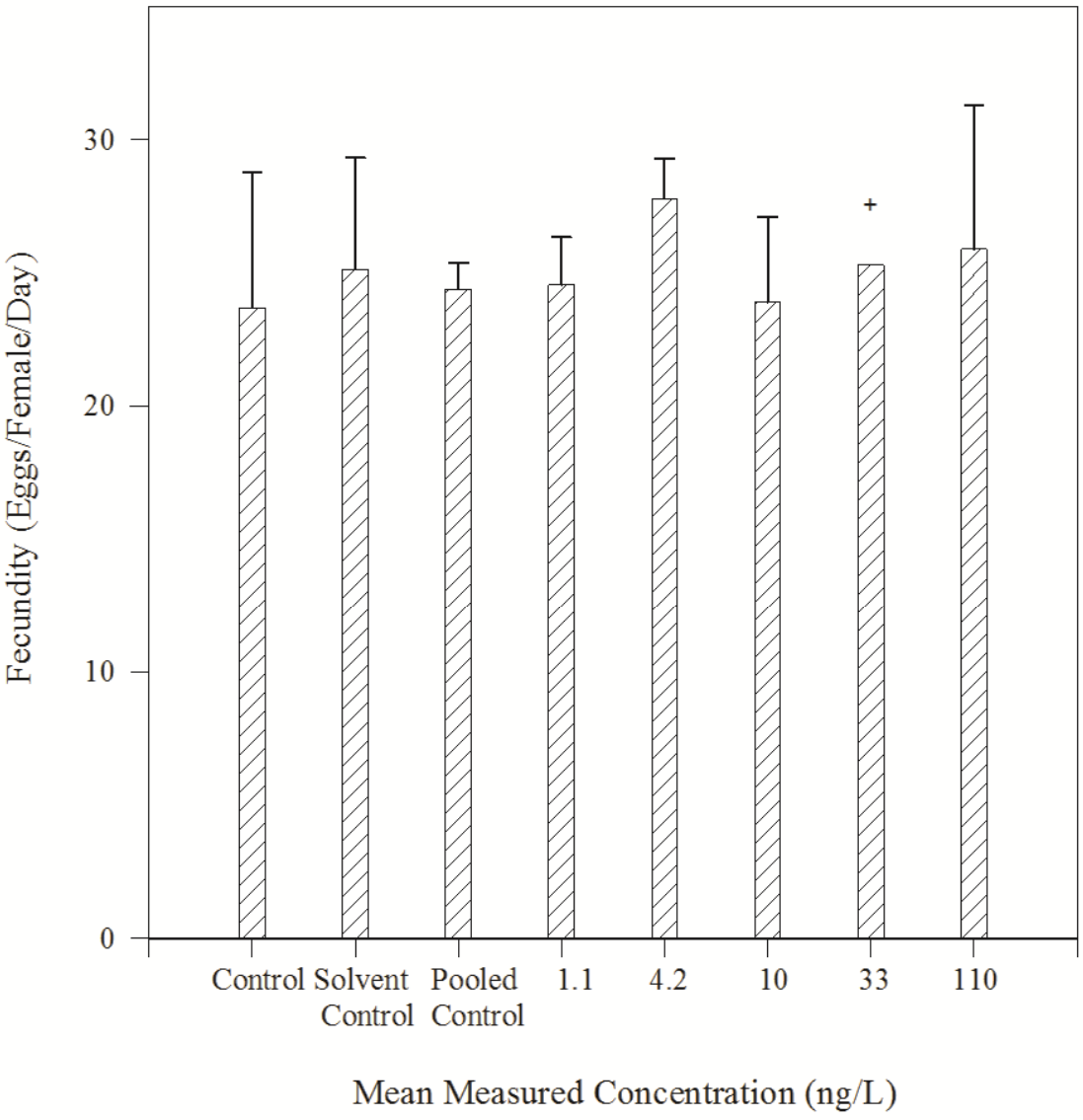
Table 141. Lowest Observed Effects Concentration (LOEC) and No Observed Effect Concentration (NOEC) from 21-Day Exposure of Medaka (*Oryzias latipes*) to 17α-[^{14}C]-Trenbolone

Endpoints	NOEC (ng/L)	LOEC (ng/L)
F0 Generation		
Survival	110	>110
Fecundity	110	>110
Secondary Sex Characteristics (papillary processes)-Females	33	110
Secondary Sex Characteristics (papillary processes)-Males	110	>110
Gonadosomatic Index (GSI)-Females	110	>110
Gonadosomatic Index (GSI)-Males	110	>110
Vitellogenin-Females	4.2	10
Vitellogenin-Males	110	>110
F1 Generation		
Hatching Success	10	33
Fertilization Success	110	>110
% Normal Larvae at Hatch	33	110
% Normal Larvae Following Yolk Sac Absorption	110	>110

Conclusions

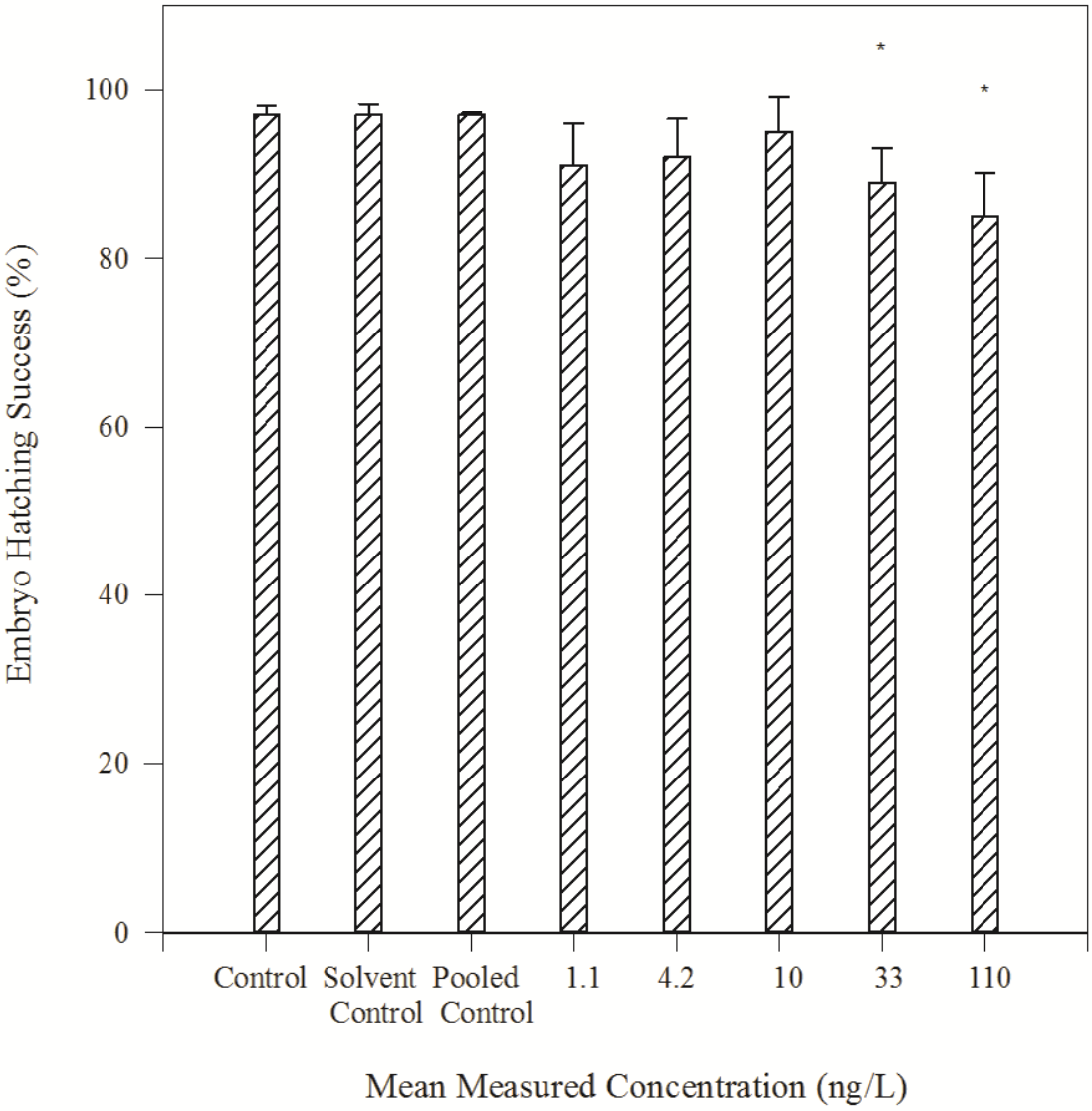
Analysis of the exposure solutions of 17α-trenbolone provided mean measured concentrations of 1.1, 4.2, 10, 33 and 110 ng/L (86-110% of nominal). The NOEC, LOEC and MATC values determined for the short-term reproduction effects of 17α-trenbolone on medaka were 110 ng/L, >110 ng/L, and >110 ng/L, respectively.

Figure 39. Fecundity (Number of Eggs per Female Per Reproductive Day) During the 21-Day Exposure of Medaka (*Oryzias latipes*) to [^{14}C]-17α-Trenbolone (Error Bar = SD)



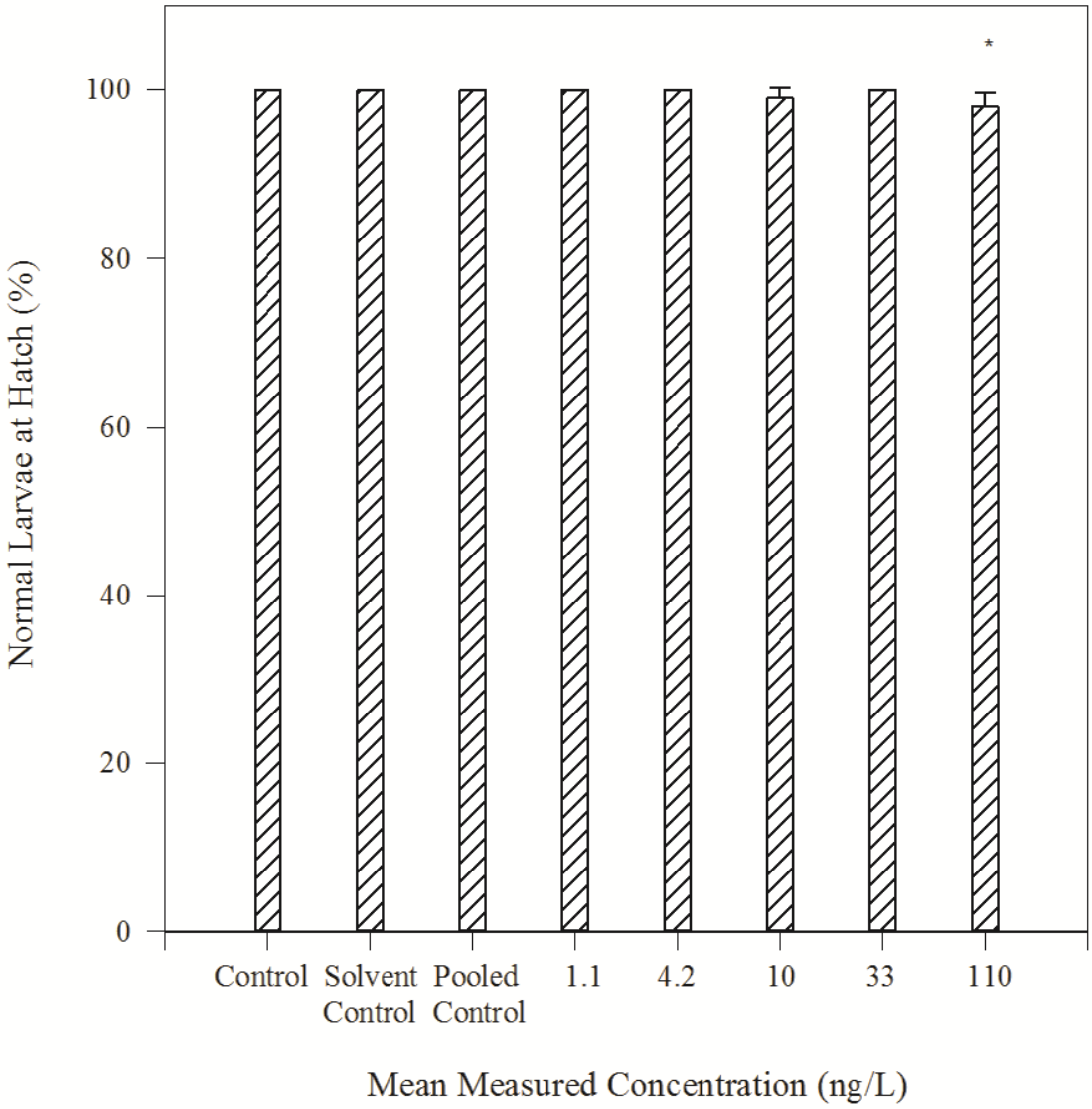
[+]Data presented for this treatment level are for replicate A only. Data for replicate B of this treatment level were excluded from statistical analysis due to an inappropriate sex ratio (1 male, 9 females).

Figure 40. Embryo Hatching Success During the 21-Day Exposure of Medaka (*Oryzias latipes*) to [^{14}C]-17α-Trenbolone (Error Bar = SD)



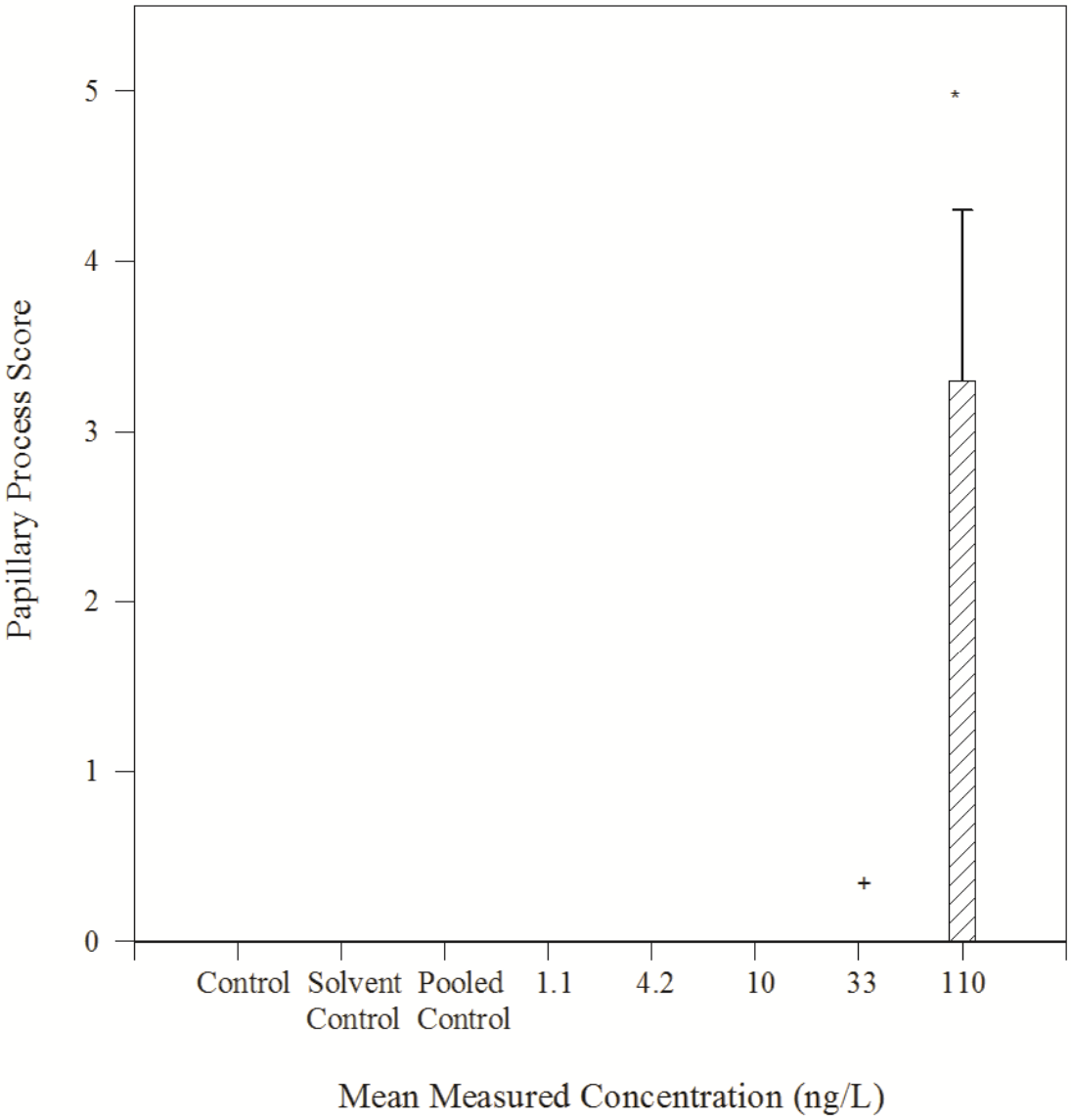
*Significantly different compared to the pooled control, based on Jonckheere-Terpstra's Step-Down Test (p = 0.0162 and 0.0014, respectively, for the 33 and 110 ng/L treatment levels).

Figure 41. Percent Normal Larvae at Hatch During the 21-Day Exposure of Medaka (*Oryzias latipes*) to [^{14}C]-17α-Trenbolone (Error Bar = SD)



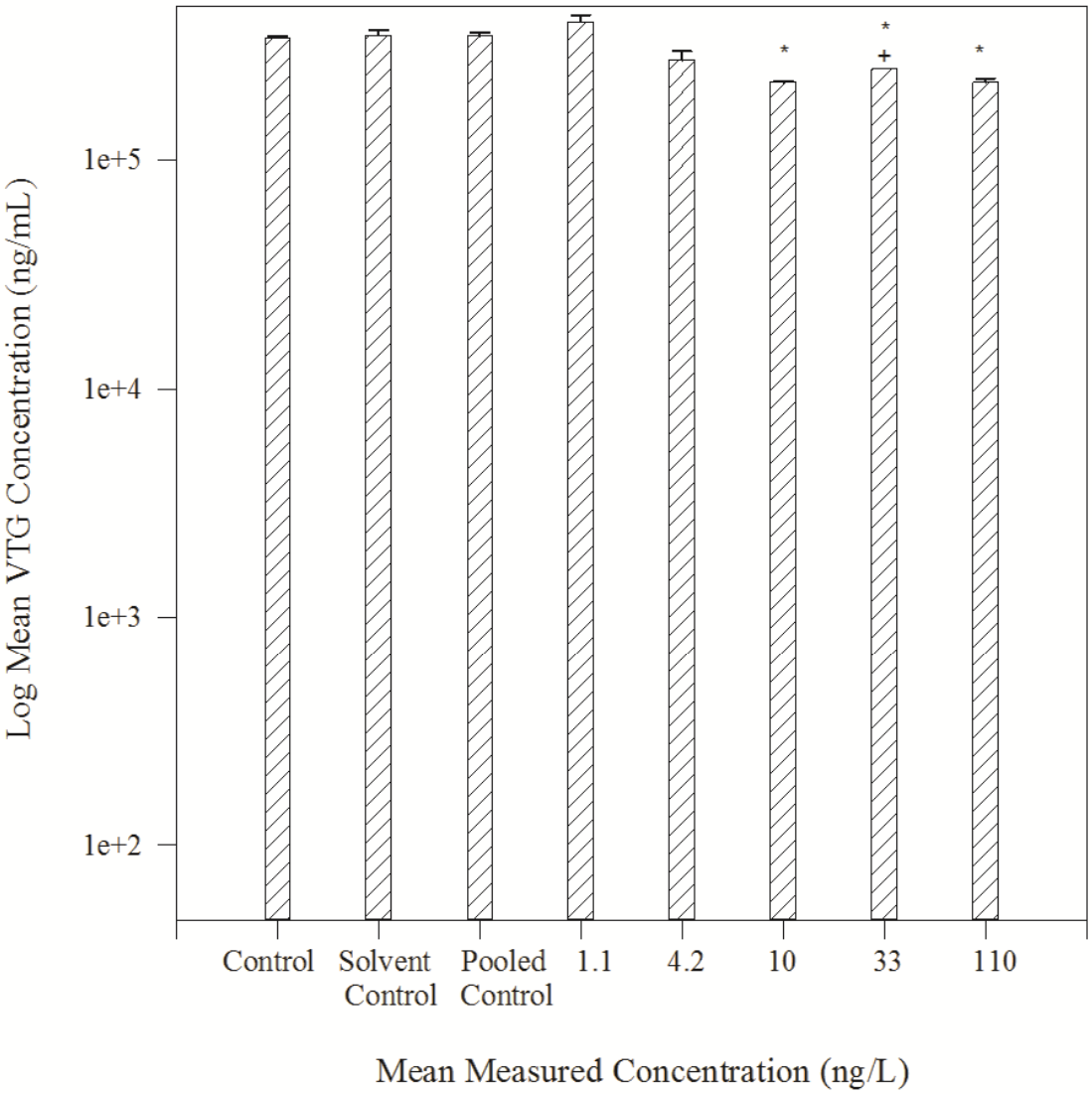
*Significantly different compared to the pooled control, based on Jonckheere-Terpstra's Step-Down Test (p = 0.0100). Response not considered to be biologically relevant, as abnormalities observed at hatch were not observed following yolk sac absorption.

Figure 42. Female Secondary Sex Characteristics (Papillary Processes) during the 21-Day Exposure of Medaka (*Oryzias latipes*) to [^{14}C]-17α-Trenbolone (Error Bar = SD)



[+]Data presented for this treatment level are for replicate A only. Data for replicate B of this treatment level were excluded from statistical analysis due to an inappropriate sex ratio (1 male, 9 females).
*Significantly different compared to the pooled control, based on Jonckheere-Terpstra Step-Down Test (p = 0.0142).

Figure 43. Female Vitellogenin Concentration During the 21-day Exposure of Medaka (*Oryzias latipes*) to [^{14}C]-17α-Trenbolone (Error Bar = SD)



*Significantly different compared to the pooled control, based on Jonckheere-Terpstra Step-Down Test (p = 0.0366, 0.0204 and 0.0026, respectively, for the 10, 33 and 110 ng/L treatment levels).
+Data presented for this treatment level are for replicate A only. Data for replicate B of this treatment level were excluded from statistical anlaysis due to an inappropriate sex ratio (1 male, 9 females).

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236. 17α-hydroxy trenbolone: acute toxicity to the water flea, *Daphnia magna*, under static conditions. 16 November 1994. Syntex Doc# RS-95921 CH 0285. (See Appendix 13.6 for Study summary).

237. 5[^{14}C]-17α-trenbolone – determining the adsorption/desorption coefficient (Koc) in 5 soils following OECD Guideline 106. 27 March 2012. Pfizer Study Number 1A72N-60-11-772. (See Appendix 13.7 for Study summary).

238. [^{14}C]-trendione – determining the adsorption/desorption coefficient (Koc) in 5 soils following OECD Guideline 106. Pfizer study number 1A72N-60-11-773. 27 March 2012. (See Appendix 13.8 for Study summary).

239. Transformation and mineralization of 17α-[^{14}C]-estradiol in two aerobic aquatic sediment systems following OECD Guideline 308. Pfizer study number 1A72N-60-11-768. 06 June 2012. (See Appendix 13.9 for Study summary).

240. Transformation and mineralization of 17α-[^{14}C]-estradiol in two anaerobic aquatic sediment systems following OECD Guideline 308. Pfizer study number 1A72N-60-11-769. 06 June 2012. (See Appendix 13.10 for Study summary).

241. Transformation and mineralization of [^{14}C]-17α-trenbolone in two aerobic aquatic sediment systems following OECD Guideline 308. Pfizer study number 1A72N-60-11-770. 18 June 2012. (See Appendix 13.11 for Study summary).

242. 17α-[^{14}C]-estradiol - short-term reproduction assay with fathead minnow (*Pimephales promelas*) following OECD Guideline 229. Pfizer study number 1A73N-60-11-785. 07 June 2012. (See Appendix 13.12 for Study summary).

243. [^{14}C]-17α-trenbolone - short-term reproduction assay with fathead minnow (*Pimephales promelas*) following OECD Guideline 229. Pfizer study number A5Y3N-US-12-001. 08 June 2012. (See Appendix 13.13 for Study summary).

244. [^{14}C]-17α-trenbolone - short-term reproduction assay with medaka (*Oryzias latipes*) following OECD Guideline 229. Pfizer study number 1A73N-60-11-786. 06 June 2012. (See Appendix 13.14 for study summary).

Attachment 1. Waterborne Environmental Study Report

TITLE

EXPOSURE ASSESSMENT OF TRENBOLONE AND ESTRADIOL IN SURFACE WATER ASSOCIATED WITH THE USE OF SYNOVEX-ONE AS A GROWTH ENHANCER IN BEEF CATTLE

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May 3, 2013

PERFORMING LABORATORY

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PROJECT ID

Waterborne Study Number: 734.01

Zoetis Study Number: A7X0R-US-12-003

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1. Summary

An aquatic exposure assessment was conducted for trenbolone and estradiol associated with the use of Synovex-One in beef cattle. Trenbolone and estradiol are released into the environment through manure. Three primary sources of manure in a watershed—feedlot, manured croplands, and pasture—were modeled in each study region of Iowa, Pennsylvania, Ohio, Michigan, and Texas to estimate concentrations of trenbolone and estradiol in surface water.

The assessment utilized a modeling framework established by the U.S. Environmental Protection Agency (USEPA) for pesticide registration. USEPA's approved regulatory models PRZM and EXAMS were used for modeling chemical runoff and erosion from cropland. The European version of PRZM (winPRZM) was modified to simulate application, runoff, and erosion on a feedlot and pasture.

Regions that may be most vulnerable to trenbolone and estradiol exposure in surface water were identified by conducting a national geospatial analysis that combined normal annual precipitation with areas of high beef cattle production and density of feedlots. This analysis resulted in five study regions in the states of Iowa, Pennsylvania, Ohio, Michigan, and Texas.

Among the five selected regions the Iowa study region was predicted to have the highest environmental concentrations of trenbolone and estradiol. Among all sources of manure, feedlots were predicted to contribute the highest amounts of trenbolone and estradiol. The 21-day 90^{th} percentile concentrations of trenbolone were 2.417, 1.067, 1.831, 0.774, and 0.237 ng/L for Iowa, Pennsylvania, Ohio, Michigan, and Texas, respectively. The 21-day 90^{th} percentile concentrations of estradiol were 0.216, 0.101, 0.176, 0.072, and 0.024 ng/L for Iowa, Pennsylvania, Ohio, Michigan, and Texas, respectively.

2. Introduction

Synovex-One is administered to beef cattle as an ear implant which contains the compounds trenbolone acetate and estradiol benzoate. Active metabolites of trenbolone and estradiol are released into the environment through manure excreted by cattle implanted with Synovex-One. The manure containing these active metabolites is a potential source of environmental risk. The concentration of trenbolone and estradiol in the surface water was estimated from the three main sources of manure that have been indentified (feedlot cattle, as fertilizer on cropland and pasture cattle). Trenbolone and estradiol can end up in surface water through runoff and erosion processes occurring from these sources.

The fate and transport of trenbolone and estradiol is governed by the complex interaction of factors including their mode of entry into the environment, physio-chemical properties that govern mobility and persistence, the hydrologic response of soils, weather conditions, hydrology of the application sites and management practices of the farms and feedlot.

To assess the exposure potential of trenbolone and estradiol in the environment, five regions were selected by conducting a national geospatial analysis and were modeled using the USEPA exposure assessment approach. The two main objectives of this study are the following:

1. Identify areas with a high potential for runoff of trenbolone and estradiol into surface water
2. Conduct fate and transport modeling of trenbolone and estradiol for these selected areas using PRZM, winPRZM and EXAMS models following USEPA Tier-2 modeling approach

Sections 3 and 4 discuss the first objective and section 5 discusses the second objective in detail.

3. Selection of Study Regions with a High Exposure Potential of Trenbolone and Estradiol into Surface Water Using National Geospatial Analysis

A national geospatial analysis was conducted to identify regions of high potential vulnerability of trenbolone and estradiol runoff or erosion into surface water by spatially overlaying areas of high beef cattle production, feedlots, and normal annual precipitation. Five study regions (one within each of the states of Iowa, Pennsylvania, Ohio, Michigan, and Texas) were identified for further analysis and modeling in subsequent phases. The importance of the national analysis is that it places the results of the subsequent modeling into a national perspective, and promotes confidence that the subsequent modeling represents a realistic “intense use” case that can be applied to any region across the U.S. The five regions have been identified for detailed characterization and model setup. These regions were selected based on the density and existence of multiple feedlot size categories, manure acres treated, and pastured cattle.

The following sections are designed to justify the selection of the study regions using maps and tables of county-level, Census of Agriculture tabular statistics on beef feedlot density, density of acres of pasture/cropland treated with manure, and pastured cattle density. Cattle statistics were collected from the NASS 2007 Census of Agriculture [USDA-NASS, 2009] and from the NASS 2007 survey data available via the QuickStats website [USDA-NASS, 2011].

3.1. Selection of Counties with a High Density of Beef Feedlots

Although it is not legal for any size feedlot to allow direct surface runoff of manure into waterways, in many states, animal feeding operations less than 1,000 head (AFOs) do not require the same level of permit as lots over 1,000 head (CAFOs). While smaller feedlots have fewer head of cattle, surface runoff may be more likely because large lots are required to have more rigorous runoff prevention controls. Therefore, identifying the spatial distribution of both small and large feedlots across the U.S. was important. The Census of Agriculture feedlot

statistics were used to distinguish small and large feedlots across the U.S. These data are organized into categories of less than and greater than 500 head of feedlot cattle.

A series of maps were made to visualize the density of beef cattle in feedlots in counties across the U.S. Figure 1 presents the ranking of feedlot cattle normalized by county area (i.e., total cattle on feed per county acre). The map highlights the fact that while feedlot cattle are dispersed across a large portion of the U.S., feedlot cattle density is the greatest in areas of Iowa, Nebraska, Kansas, and several other states in the more arid regions of the U.S. Only a few counties east of the Mississippi River in Illinois, Ohio, Michigan and Pennsylvania are above the 90th percentile for total feedlot density. Figure 2 indicates that beef cattle density on feedlots with greater than 500 head tends to be the highest for counties in the more arid areas of eastern Colorado, northern Texas, southwest Kansas, Nebraska, and northwestern Iowa. These larger feedlots are used as an indicator of permitted feedlots (CAFOs) having greater than 1000 head. Figure 3 shows that smaller feedlots, which are used as indicators of non-permitted feedlots (AFOs), tend to be more concentrated in eastern Minnesota and Nebraska, Wisconsin, Iowa, and locations eastward.

3.2. Selection of Counties with a High Density of Cropland with Manure Applied

The application of animal manure to cropland is an important practice for managing animal waste and in return is a valuable source of nutrients for crop production as well as for maintaining soil structure. The rate of application of manure to cropland is a function of the nutrient requirements for the crop and the availability of nutrients in the manure. Water bodies adjacent to cropland with manure applied have the potential for exposure to nutrients and drugs found in manure, particularly after rainfall events.

County level census statistics on lands which had manure applied in 2007 are mapped for the U.S. in Figure 4. In this map, lands treated with manure may be from a combination of cattle, swine, and poultry because the Census of Agriculture data do not distinguish between manure types. What's important to recognize from this map is that many of the counties with the highest densities of manure application also have the highest feedlot cattle densities. The majority of counties in the 90th percentile of manure application density are in Iowa, Minnesota, and Wisconsin. Many of the same counties are in the upper percentiles for feedlot cattle density as well. Furthermore, two counties in Texas are in the upper 90th percentile for both manure application and large feedlots (lots over 500 head). Lastly, several counties across Pennsylvania, Michigan, and Ohio rank high (over 90th percentile) in terms of manure application density and small feedlot density (fewer than 500 head).

3.3. Selection of Counties with a High Density of Pasture Cattle

The number of pastured beef cattle was estimated using a combination of Census of Agriculture county and state statistics. Pastured cattle are not a category of the cattle identified in the

Census of Agriculture and was, therefore, obtained on a county level by subtraction (total cattle minus all other categories of cattle). The subtraction method is discussed in further detail in Section 4.5.

Figure 5: Density of Pastured Beef Cattle (Estimated from the 2007 Census of Agriculture) presents the percent rank of pasture cattle densities for counties in the U.S. The map indicates that a large portion of the counties with a high density of pasture cattle are west of the Mississippi River, primarily in Oklahoma, Nebraska, Texas and Kansas. However, a number of counties across the U.S. have a high density of pasture cattle, including counties also having a high density of cropland manured and feedlots. Many counties in Iowa, Pennsylvania, Ohio, Michigan, and Texas rank relatively high in all beef cattle categories.

3.4. Final Selection of High Exposure Potential Study Regions

Based on an overlay of information on feedlots, pastured cattle, crop and pasture lands manured, and rainfall, five study regions representing a variety of beef cattle characteristics, climatic conditions, and geographic regions were selected for further analysis and environmental fate modeling. These study regions are located in Iowa, Pennsylvania, Ohio, Michigan, and Texas. Note that the four of five study regions are represented by a single county, the Iowa study region, on the other hand, includes two adjacent counties. The placement of the study regions in the national distribution for each of the variables examined (feedlots, pastured cattle, crop and pasture lands manured, and rainfall) is presented in Table 1. The five selected study regions rank high in terms of the examined metrics. Figure 1 through Figure 8 highlight the location of the five study regions across the U.S. in relation to beef cattle and rainfall metrics. National rainfall mapping was accomplished using the Parameter-elevation Regression Independent Slopes Model (PRISM) data from Oregon State University, a spatially gridded (1 kilometer) average monthly and annual precipitation dataset for the period 1971–2000 [ORSU-PRISM, 2002]. Figure 9 maps the study regions examined, with the counties of interest outlined in red.

Table 1 shows that the counties contain a large density of cattle on feedlots in general and on both large and small size lots, and are in the 97th percentile of counties in the nation for acres treated with manure. All counties are in the 95th percentile in the national distribution for feedlot cattle density (less than and greater than 500 head combined). From a national perspective, all sites are in moderate to low rainfall regions; however, if the geographic extent was constrained to moderate and high beef cattle productions regions, the sites would rank much higher in terms of rainfall. The counties are representative of near worst-case scenarios in terms of runoff potential for trenbolone and estradiol based upon density of beef cattle production and relative rainfall.

Lyon and Sioux counties are located in the northwest portion of Iowa, are adjacent to each other, and are processed as a single study region for reasons made clear below. These counties rank very high (above 95th percentile) in terms of feedlot characteristics (both for less than and greater than 500 head) as well as for crop and pasture lands manured. Pasture cattle

rank above the 90th percentile. This study region has the second lowest rainfall totals of the regions examined.

Castro County, Texas, has some of the largest feedlots in the U.S. over 500 head, as indicated by its ranking above the 99th percentile. Only 0.65% of the beef cattle in Castro are on feedlots with less than 500 head of cattle this represents approximately 22,000 head. Castro County is in an arid region, representing some of the lowest rainfall totals in the U.S. Ranking for pasture cattle density and crop and pasture lands manured are high.

Huron County, Michigan, and Mercer County, Ohio, rank similarly in terms of feedlot statistics. These counties are dominated by smaller feedlots, but large feedlots are also present. Rainfall totals are similar between the two study regions with Mercer County having slightly more rainfall than Huron County. Pasture cattle density is lower for these two counties than the other study regions examined.

Lancaster County, Pennsylvania, was selected because of the density of small feedlots (99.6th percentile for lots under 500 head) in a region with the highest precipitation from a cattle production perspective. A significant amount of pastured cattle and crop and pasture lands manured are also present in the county.

4. Selection of a High Exposure Potential Watershed within each Study Region

4.1. Watershed Level Data Requirements

The study regions selected represent too large an area with too much diversity in waterways, feedlots, cropland and pasture to be modeled in its entirety. Therefore, each study region required further refinement. The U.S. Geological Survey (USGS) defines a watershed as a geographic area of land, water and biota within the confines of a drainage divide. Watersheds in the U.S. have been delineated by the U.S. Geological Survey using a national standard hierarchical system. The smallest unit of categorization of watershed that the USGS uses is the sub-watershed (12-digit Hydrologic Unit Code (HUC)) [USDA-NRCS-NCGC, 2006]. It is this unit that is most appropriate for modeling and will be referred to hereafter as a watershed.

The Pesticide Root Zone Model (PRZM) used for watershed modeling estimates the mass of chemical loaded to a body of water from a single input source that represents the entire watershed area. Therefore, to estimate mass loadings from different sources (feedlots, cropland and pasture), PRZM model was run separately for each source. Since entire watershed land area represents the loading source for a PRZM run, the USEPA uses percent cropped area (PCA) factors to scale the PRZM estimated mass loadings to be representative of the modeled watershed area that is actually contributing to the chemical concentrations coming from cropland, pasture or feedlot. Therefore, to estimate PCA factors, the following are required for each watershed modeled:

1. Percent of total watershed area that is feedlot
2. Percent of total watershed area with crop/pasture-lands applied with solid manure and/or water from collection ponds
3. Percent of total watershed area that is pasture

For ease of discussion these percent areas are referred to as watershed densities in the following sections.

4.2. Selection of a High Exposure Potential Watershed in the Lyon/Sioux, Iowa, and Castro, Texas Study Regions

A watershed selection process was conducted in the Lyon/Sioux, Iowa, and Castro, Texas study regions to identify the 90th percentile watershed in terms of exposure potential of trenbolone and estradiol to surface waters for subsequent watershed modeling. The rationale for selecting the 90th percentile for modeling is based on guidance from the USEPA for applying the Pesticide Root Zone Model (PRZM) to model the fate and transport of pesticide to surface waters [SAP,1998]. The Iowa and Texas study regions represent 98th percentile or greater in terms of beef cattle density at the county-level and now within these regions the 90th percentile watershed is being selected for modeling. Data are not available for the other study regions (Michigan, Ohio, Pennsylvania) to provide a ranking of watersheds; therefore, an alternative method will be used and is described in subsequent sections.

In order to select a 90th percentile watershed from the distribution of all watersheds in the Lyon/Sioux, Iowa, and Castro, Texas study regions, an *exposure potential of trenbolone* to surface waters (Exposure Index) was computed and ranked. The Exposure Index was computed for the 42 HUC-12 watersheds in the Iowa study area and for the 35 watersheds covering Castro, Texas.

To calculate the watershed-level Exposure Index for trenbolone, a weighted, annual loading of trenbolone per watershed (g/ac) was computed for every watershed in the two regions. Annual loadings were based upon weighted sum total loadings of trenbolone via

1. beef cattle excretion onto feedlots <1,000 AU,
2. crop and pasture lands with beef cattle manure applied, and
3. beef cattle excretion onto pasture lands.

Preliminary watershed modeling results showed that trenbolone contributed significantly more to watershed loadings than estradiol; therefore, only trenbolone was used in the equation to compute the Exposure Index. While the preliminary PRZM results are not provided with this report, final PRZM results support the assumption that trenbolone contributions are most relevant. As indicated in Section5.5.1, the 21-day 90th percentile predicted concentration for trenbolone is over 11 times greater than that of estradiol. Including estradiol loadings in the index calculation would not have affected the relative ranking of the watersheds, nor the 90th percentile watershed ultimately selected.

An initial understanding of the degree to which each of the three sources makes to the overall trenbolone loadings was critical for deciding what makes a watershed more vulnerable than another. These findings indicated that watersheds with a high density of feedlots under 1,000 head were the most vulnerable, followed by manured crop/pasture lands, and lastly by stocked pasture lands. Section 5.5.1.1 supports this by presenting preliminary PRZM modeling which indicated the individual contribution from feedlots to the cumulative total watershed concentration was nearly 95%, followed by manured crop/pasture lands (less than 10% of total), and lastly by pasture lands stocked with cattle (less than 1%).

The following equation was used to calculate the watershed-level Exposure Index for trenbolone:

$$\begin{aligned}\text{Weighted Total Loading (g)} &= \left(\text{feedlot area (ac)} \times \text{trenbolone loading}\left(\frac{\frac{\text{g}}{\text{ac}}}{\text{day}}\right) \times 365\ \text{(days)} \times 100\right) \\ &+ \left(\text{manure applied crop or pasture area (ac)} \right. \\ &\left. \times \text{trenbolone loading}\left(\frac{\frac{\text{g}}{\text{ac}}}{\text{application}}\right) \times 2\ \text{applications} \times 10\right) \\ &+ \left(\text{pasture area (ac)} \times \text{trenbolone loading}\left(\frac{\frac{\text{g}}{\text{ac}}}{\text{day}}\right) \times 212\ \text{(days on pasture)} \times 1\right)\end{aligned}$$

Formula Notes:

- Trenbolone loadings:
  - Feedlot = 20.56 g/ac/day or 50.8 g/ha/day [Table 17 and Zoetis, 2012].
  - Manured cropland/pasture = 0.561 g/ac/application or 0.2601 g/ha/application [Table 17 and Zoetis, 2012].
  - Pasture = 0.0014 g/ac/day or 0.00342 g/ha/day [Table 17 and Zoetis, 2012].
- The acres of feedlots, pasture/cropland manured, and pasture acres are all derived from the GIS data and the methodology is discussed in subsequent sections.

Note the factors of 100, 10, and 1 are applied to the area (acres) of feedlot, manured crop/pasture lands, and pasture lands with cattle, respectively, to weight the contribution that each source represents. As discussed in the paragraph above, and further supported in Section 5.5.1.1, preliminary PRZM modeling results which indicated feedlots contribute the greatest amount of trenbolone to overall watershed loadings and are therefore weighted most heavily (indicated by a factor of 100), followed by manured crop/pasture lands (factor of 10), and lastly pasture lands stocked with cattle (factor of 1). However, while feedlots contribute the greatest trenbolone loads on a grams per acre basis, they represent a much smaller area of the

watershed than do manured crop/pasture lands or pasture lands with cattle. Therefore, a weighting factor of 100 is used to ensure watersheds with greatest density of feedlots rank very high. Similar logic is applied to crop or pasture lands with manure applied.

Upon calculating the Exposure Index for every watershed in the Iowa and Texas study regions, the indexes were ranked and the 90th percentile watershed was selected. The summary statistics of the beef density metrics at the 100th, 90th, 75th, 50th, and 10th percentiles (where percent rank is based on the Exposure Index) in the Lyon/Sioux Counties in the Iowa study region are presented in Table 2, and Table 3 for Castro County, Texas. The density statistics for the 90th percentile watershed in these tables were used in subsequent modeling.

Figure 10 presents the watershed modeled in Iowa and Figure 11 maps the watershed modeled in Texas. These maps highlight the location of the regional location of the watershed and regional land cover characteristics. For the Texas figure, only CAFOs are mapped; AFOs are not present in the Texas database (refer to Appendix 1) and cannot be displayed in the map.

4.3. Computation of Density of Feedlots in the Selected Watersheds for Lyon/Sioux, Iowa, and Castro, Texas Study Regions

This section of the report discusses the approach to calculating the feedlot density (i.e., percent of the watershed land area that is feedlot less than 1,000 head).

Feedlot density refers to the percent of the watershed area comprised of feedlots fewer than 1,000 head of cattle. For each watershed, the maximum permitted head of cattle on lots under 1,000 head were tabulated from the state GIS databases. To estimate the acreage of feedlots in the watershed, a stocking density of 15m^2 per animal unit was used [Zoetis, 2012]. The density of feedlots less than 1,000 head was calculated for each watershed by multiplying the cattle counts in the GIS database by 15m^2 and normalizing by watershed area. Thus for the Iowa watersheds:

$$\text{watershed land area that is feedlots of} < 1000 \text{ head (acres)} = (\text{cattle on feedlots} < 1000 \text{ hd}) \times \left(15 \ {}^{\text{m}^2}/_{\text{hd}}\right) \times \left({}^{1 \text{ acre}}/_{4046.86 \text{ m}^2}\right)$$

For the selected watershed the number of permitted cattle on feedlots of < 1,000 head was 5,373 resulting in 19.915 acres of watershed land area that is feedlot of <1,000 head.

For Lyon and Sioux counties in Iowa, the feedlot metrics (both AFO and CAFOs) are calculated directly from the Iowa DNR AFO database [Iowa DNR, 2005]. However, for Castro County, Texas, it was necessary to estimate the fraction of beef cattle on AFOs (lots under 1,000 head) in Castro since the TCEQ CAFO database only tabulates CAFOs (lots over 1,000 head) [TCEQ, 2007]. To do so, the finest resolution data on feedlot sizes available in the 2007 Census of Agriculture were used. County-level Census statistics indicate that for Castro County, 0.7% of beef are on lots fewer than 500 head and state level figures indicate that 0.5% of beef are on lots between 500-999 head. Taken together, these statistics indicate that a small proportion of

the total beef, approximately 1.178% (0.654% + 0.524%), are on AFOs in Castro County. The equation below and Table 4 presents the approach taken to estimating the fraction of beef on lots under 1,000 head in Castro County.

$$\begin{aligned}\text{cattle on feedlots} < 1000\ \text{hd} &= (341694\ \text{total cattle on all feedlots}) \\ &\times (0.00654\ \text{proportion of cattle on feedlots of} \\ &< 500\ \text{hd} + 0.00524\ \text{proportion of cattle on feedlots of}\ 500 - 999\ \text{hd})\end{aligned}$$

This results in 4,025 head of cattle on feedlots of <1000 head for Castro county (Table 5), however this value needs to be divided amongst the 35 watersheds of Castro county. At the watershed level the total numbers of "permitted" cattle represent cattle on CAFOs. The county-level estimate of beef on AFOs is proportionally-weighted to the watersheds using the CAFO beef counts from the TCEQ CAFO GIS database, resulting in watersheds with the largest number of CAFO beef to have a higher potential for cattle on AFOs. For the each watershed:

$$\text{Cattle on feedlots} < 1000\ \textit{head in watershed} = \left(\frac{\text{CAFO cattle in watershed (hd)}}{\text{total cattle CAFO in county (hd)}}\right) \cdot (4028\ \text{AFO cattle in cou}\textit{nty})$$

For the selected watershed, the cattle on AFOs (lots <1000 head) were estimated to be 247 head (Table 6).

It is important to note that the permitted beef CAFO (>1,000 head) counts from the TCEQ CAFO database largely disagreed with the Census of Agriculture. The total permitted beef count in the TCEQ CAFO database showed 1,393,490 in Castro, whereas the Census of Agriculture reported a total of 341,694 beef (of any size, not just >1,000 AU). To estimate the number of cattle in each watershed, it is necessary to account for the mismatch between beef that is "permitted" versus beef that actually passes through the county (per Census of Agriculture) because only permitted cattle numbers are available at the watershed level. CAFO beef were scaled according to the ratio of permitted beef in the watershed to permitted in the study region. For example, the watershed modeled in Castro (HUC 120500050303) had 85,500 permitted beef which represents 6.14% of the total permitted beef in the study area (85,500/1,393,490 = 6.14%), so the modeled watershed received 6.14% of the Census of Agriculture total beef (6.14% x 341,694 = 20,965 beef on CAFOs in the modeled watershed in Castro, TX).

Table 5 provides the total count and total area of all watersheds, along with feedlot beef counts, for all five study regions. Table 6 provides similar information but for the specific watershed ultimately modeled in each study region.

The summary statistics of the beef density metrics at the 100th, 90th, 75th, 50th, and 10th percentiles (percent rank based on the Exposure Index results; refer to Section 4.2) in the Iowa study region are presented in Table 2, and Table 3 for Castro County, Texas. As previously mentioned, due to a lack of feedlot related GIS data, an alternative approach was required for Michigan, Ohio, and Pennsylvania, to estimate the feedlot density.

4.4. Computation of Density of Crop and Pasture Lands with Manure Applied in the Lyon/Sioux, Iowa, and Castro, Texas Study Regions

This section discusses the approach to calculating the density of crop/pasture - lands within the watershed. This density will represent the percent of watershed area with crop/pasture - lands to which solid manure and water from collection ponds is applied.

The density of manured acres refers to the percentage of cropland in the watershed treated with liquid and solid manure applications. The calculation assumes that cattle on any lot size contribute manure, so all feedlot cattle from the state GIS databases are tabulated for the watersheds (i.e., both AFOs and CAFOs contribute). To estimate the acreage of cropland potentially treated with manure, regional manure application rates to reach the phosphorus requirements of corn for silage or grain were multiplied by the number of feedlot cattle in the watershed. The application rate for Pennsylvania was based on the P_2O_5 requirement of corn for silage. The four other site applications were based on the P_2O_5 requirement of corn for grain harvested [Zoetis, 2012]. The last column of Table 7, "Acres Required per Feedlot Animal Unit AU," provides the values used for each study region. The following equations provide the derivation of the manured land area:

$$\mathrm{P_2O_5 requirement\ kg/acre} = \text{ yield } \left(\frac{\mathrm{bu}}{\mathrm{acre}}\right) \times \left(\mathrm{P_2O_5 \text{ removed by crop } \left(\frac{kg}{bu}\right)}\right),$$

$$\text{Total annual } \mathrm{P_2O_5} \text{ present in cattle manure within watershed(kg)} = \left(\mathrm{P_2O_5 \text{ produced}} \left(\frac{\mathrm{kg}}{\mathrm{hd \times day}}\right)\right)(365\ days) \times (\text{feedlot cattle within watershed, hd}),$$

$$\mathrm{P_2O_5 \text{ produced}} \left(\frac{\mathrm{kg}}{\mathrm{hd \times day}}\right) = 0.0747 \text{ [Zoetis 2012]},$$

$$\text{Acres manured within watershed} = \frac{\text{Total annual } \mathrm{P_2O_5} \text{ present in cattle manured within watershed}}{\mathrm{P_2O_5} \text{ requirement/acre}},$$

For the selected watershed in Iowa this results in 11,983 manured acres of the 21,128 total watershed acres (56.62%) as shown in Table 8.

It is assumed that the manure generated by feedlot cattle is applied to the cropland within the same watershed as the feedlot, except when the amount of manure (based on P_2O_5 requirements) exceeds the amount of available cropland. Only in the Castro County, Texas study region were there watersheds in which the manure produced in the watershed exceeded the available cropland. For these watersheds, the maximum crop area (all cropped agriculture land use types) from the NASS CDL (remotely sensed geospatial data) [USDA-NASS-RDD-GIB-SARS, 2011] was used. For Castro, this results in 100% of the cropland (i.e., [acres of NASS CDL cropland / watershed acre] *100) in the watershed being treated with manure.

The percent-rankings in Table 2 (Iowa study region), and Table 3 (Texas study region) are based upon the computed Exposure Index (refer to Section 4.2 for details on how that is

computed) and provide the watershed-level manured acres densities for the modeled watershed.

Table 8 presents the distribution of all possible watershed-level feedlot and manured acres densities in the Iowa and Texas study regions (where the ranking is based on actual watershed-level feedlot and manured acres densities that existed in all the watersheds for each study region, not based on computed Exposure Index). The purpose of Table 8 is to present the full range of possible feedlot and manured acres densities that existed in all the watersheds in the two study regions. At the bottom of Table 8, the densities for the modeled watershed are provided in order to place their values into the context of the full range of possible values. As discussed in Section 4.2, due to a lack of feedlot related GIS data, an alternative approach was required in Michigan, Ohio, and Pennsylvania, to estimate the crop/pasture lands manured density and are therefore not presented in this table.

4.5. Computation of Density of Lands Used for Pasturing or Grazing of Cattle in All Five Study Regions

This section of the report discusses the approach to calculating the density of pasturelands within the watershed. For all five study regions (not just Iowa and Texas), pasture density relates to percent of the watershed comprised of lands potentially used for pasturing or grazing cattle. To estimate the pasture density at the watershed level in the five study regions, the grass and pasture/hay land use types from the NASS CDL (remotely sensed geospatial data) were used to locate lands in the watershed with the potential for pasturing and grazing of beef cattle in combination with the Census of Agriculture figures on pasturing acres.

While the NASS CDL dataset contains refined spatial locations of agriculture, for grassy land cover types it is more appropriate to combine the NASS CDL dataset with the county-level Census of Agricultural data when estimating actual pasture land acreage. This is because the areas of grassy land cover types in the NASS CDL are often misrepresented from an acreage perspective and can be improved by incorporating official Census of Agriculture pasture land area acreages. For each of the watersheds in the study regions, the calculated percent of the watershed in pasture reflects the actual acreage of lands used for pasturing and grazing from the Census of Agriculture distributed over the locations of grass and pasture/hay land use types from the NASS CDL. The distribution of Census of Agriculture county-level acreage to the watershed is performed based on the relative proportion of NASS CDL based grass/pasture/hay acreage that the watershed represents compared with all grass/pasture/hay acreage across all watersheds. For instance, in Iowa, the NASS CDL data estimates that the modeled watershed (HUC 101702032002) contains 1.5% of the total grass/pasture/hay acreage in the study region; therefore, it receives 1.5% of the county-level pasture land area acreages as estimated by the Census of Agriculture. The following formula shows the derivation of pasture acres and density for the modeled Iowa watershed:

$$\text{pastured acres in watershed} = \left(\text{Lyon County pasture acres } (9{,}071) + \text{Sioux county pasture acres } (23{,}280)\right) \times 0.015 = 485 \text{ acres},$$

$$\text{density of pasture in watershed} = \frac{485 \text{ acres of pasture}}{21{,}128 \text{ acres in watershed}} = 2.29\%,$$

The approach uses the best source of pastured land area (the Census of Agriculture) and estimates its location within the watershed using the location information from the NASS CDL remote sensing spatial data.

Table 9 presents the distribution of all possible watershed-level pasture cattle densities in all five study regions. The purpose of Table 9 is to present the full range of possible pasture cattle densities that existed in all the watersheds in the five study regions. At the bottom of Table 9, the densities for the modeled watershed are provided in order to place their values into the context of the full range of possible values.

As a verification of the pasture calculations, the number of pastured cattle can be used to back-calculate the pastured acres. At the county level, lands used for pasturing or grazing of beef cattle were estimated using a combination of NASS county and state statistics. Because a specific pastured beef cattle category is not present in the Census of Agriculture at the county level it was required to estimate the pastured cattle by subtracting all the classes of cattle from the total cattle (Table 10). By assuming a fixed cattle density per pasture acre (3.15 AU/acre) the watershed pasture acres were used to determine the capacity of the pastures (Table 11). These values were then compared to the county level (subtraction results) to look for agreement (see bottom of Table 11). In all but one case the cattle estimates using the watershed method exceeded the county estimates and in all but one case the watershed estimates were within ±30% of county estimates.

As discussed in Section 4.2, due to a lack of feedlot related GIS data, an alternative approach was required in Michigan, Ohio, and Pennsylvania, to estimate the two feedlot derived densities (i.e., feedlot density and crop/pasture lands manured density). However, grass/pasture/hay spatial data (i.e., NASS CDL) is available in Michigan, Ohio, and Pennsylvania to calculate actual pasture densities and therefore it was used. As indicated in Table 9, the 90th percentile pasture density was conservatively used in Michigan, Ohio, and Pennsylvania.

4.6. Estimation of Density of Feedlots and Density of Crop/pasture-lands Manured in the Pennsylvania, Michigan, and Ohio Study Regions

For the study regions in Pennsylvania, Michigan, and Ohio, state and county regulatory agencies do not prepare a publically available database of AFO locations and characteristics, but according to the Census of Agriculture, there are a substantial number of feedlots of all sizes in these states. What is available is the total number of feedlot cattle within each of the

study regions and feedlots >1,000 head (CAFOs) within each of the study regions. It is certain that these regions have characteristics more similar to the Iowa study region than the Texas study region. Therefore, Iowa data was used to scale the study region data down to watershed level data within the Michigan, Pennsylvania, and Ohio study regions. In the Iowa study region of all beef cattle on feedlots of <1,000 head 4.5% of them are in the modeled Iowa watershed, this same ratio was used to estimate the number of beef cattle on AFOs in the modeled watersheds in the Michigan, Pennsylvania, and Ohio study regions. Similarly, in the Iowa study region of all beef cattle on feedlots of >1,000 head 6.2% of them are in the 90th percentile Iowa modeled watershed, so 6.2% of the total feedlot cattle on CAFOs in the Michigan, Pennsylvania, and Ohio study region were estimated to be within the modeled watershed. The following equations demonstrate the process:

$$\%\ \text{of cattle on AFOs in IA study region that are in the modeled watershed} = \frac{5{,}373}{119{,}885} = 4.5\%$$

$$\%\ \text{of cattle on CAFOs in IA study region that are in the modeled watershed} = \frac{10{,}410}{168{,}521} = 6.2\%$$

$$\text{Estimated number of cattle on AFO in the MI, OH or PA modeled watershed} = (\text{number of cattle on AFO in study region}) \times 0.045$$

$$\text{Estimated number of cattle on CAFO in the MI, OH or PA modeled watershed} = (\text{number of cattle on CAFO in study region}) \times 0.062$$

In a similar way the size of the theoretical modeled watersheds in Michigan, Ohio and Pennsylvania can be estimated:

$$\%\ \text{of total land area of the study region that the IA watershed represents} = \frac{21{,}128}{995{,}598} = 2.1\%$$

$$\text{Estimated size of modeled watershed in MI, OH or PA (acres)} = (\text{total land area of study region}) \times 0.021$$

Finally density of AFO feedlots can be determined:

$$\text{watershed land area that is feedlots of} < 1{,}000\ \text{head (acres)} = (\text{Estimated number of cattle on AFO in the MI, OH or PA modeled watershed}) \times \left(15\ {}^{\mathrm{m}^2}/_{\mathrm{hd}}\right) \times \left({}^{1\ \text{acre}}/_{4046.86\ \mathrm{m}^2}\right)$$

$$\text{density of AFO feedlots with modeled watershed (\%)} = 100 \times \frac{\text{watershed land area that is feedlots of} < 1{,}000\ \text{head (acres)}}{\text{total watershed land area}}$$

For density of crop/pasture lands manured, the equations provided in Section 4.3 apply using the appropriate crop and yields for in each study region (Michigan, Ohio, Pennsylvania; Table 7) and the estimated feedlot cattle numbers and size of the watershed given above.

Table 12 presents the estimated feedlot densities, crop/pasture lands manured densities, and watershed areas for these three regions. The table is organized into three parts. The upper section highlights pertinent statistics from the 90th percentile Iowa watershed that was referenced in order to estimate beef metrics in Pennsylvania, Michigan, and Ohio. The middle section tabulates cattle counts at the county and watershed levels in Pennsylvania, Michigan, and Ohio. County-level counts are from the 2007 Census of Agriculture and watershed cattle figures are calculated using the method described above. The lower portion of the table presents the final beef cattle densities estimated for the modeled watersheds in Pennsylvania, Michigan, and Ohio. Note that, as discussed in Section 4.5 pasture related GIS data is available in Michigan, Ohio, and Pennsylvania to calculate actual pasture densities and therefore no data scaling from the Iowa watershed was required. As indicated in Table 9, the 90th percentile pasture density was used in Michigan, Ohio, and Pennsylvania.

4.7. Final Beef Cattle Counts and Densities for the Modeled Watersheds in All Five Study Regions

Table 13 presents the final beef cattle counts and cattle densities calculated for each modeled watershed (ranking based on the computed Exposure Index (Iowa and Texas); refer to Section 4.2 for details on calculations) in each of the five study regions. The table highlights the number of cattle on feedlots less than 1,000 head and greater than 1,000, along with counts of pastured cattle. Watershed density factors (i.e., “PCA factors”) for each of the parameters (feedlot acres, pastureland acreage, and manured acres) which are used in the subsequent modeling are also presented.

Section 5 and beyond discuss the exposure modeling and incorporation of the watershed density factors.

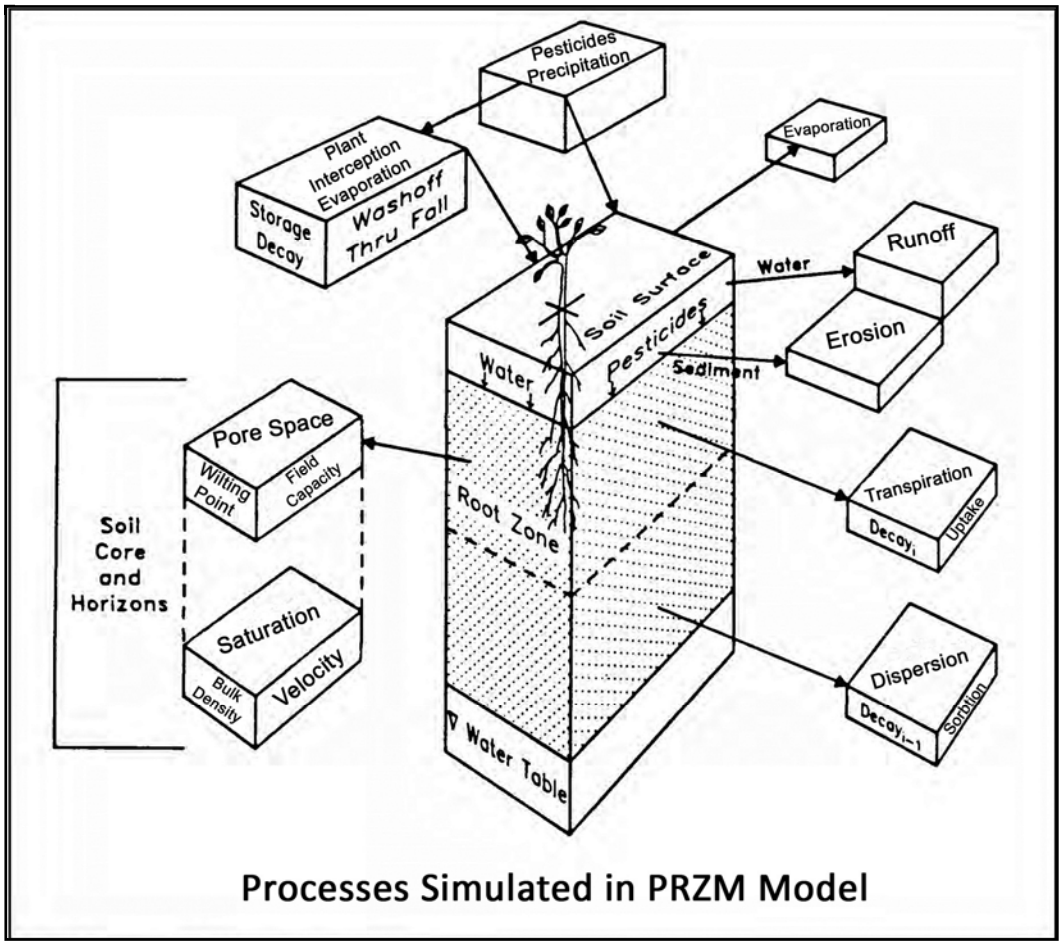
5. Modeling Trenbolone and Estradiol in Surface Water as a Mixed-Use Watershed Using USEPA’s Exposure Assessment Approach

5.1. Model Selection

The fate of trenbolone and estradiol in the environment associated with the use of Synovex-One administered to beef cattle as an ear implant is governed by the complex interaction of factors that include their mode of entry into the environment, chemical properties that effect mobility and persistence, the chemistry of the surrounding environment, the hydrologic response of soils and drainage features to weather conditions, and management practices of the farm and feedlot. The Pesticide Root Zone Model (PRZM) version 3.12 and the Exposure Analysis Modeling System (EXAMS) version 2.98 models were selected to simulate the fate of trenbolone and estradiol in a watershed because of their ability to simulate relevant dissipation pathways, the acceptance of these models for pesticide registration by the U.S. Environmental Protection Agency’s (USEPA) Office of Pesticide Programs (OPP) and the relative ease in

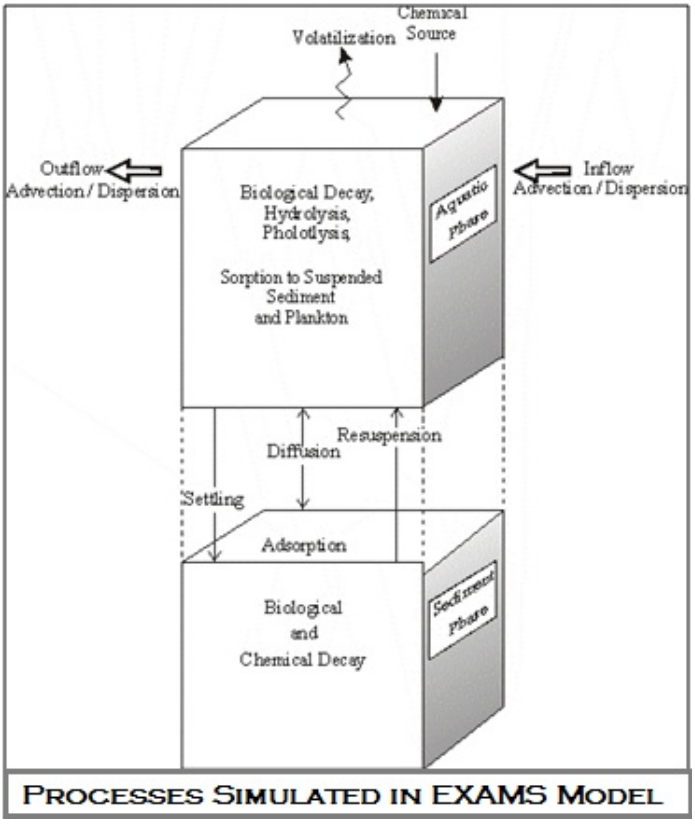
adopting USEPA's procedures for Synovex-One risk assessment. The USEPA has developed 128 standard scenarios for pesticide risk assessment that represent a combination of climatic conditions, crop management practices, and soil properties across the U.S.

PRZM is a one-dimensional compartmental model that simulates chemical movement in an agricultural field. PRZM is run on a daily time step with 30-year historic weather data. Runoff and erosion of soil are calculated based on the Soil Conservation Service curve number technique and the Universal Soil Loss Equation, respectively [Carousel et al., 2005]. Dissolved, adsorbed, and vapor-phase concentrations of the chemical in the soil are estimated by simultaneously considering processes such as uptake by plants, surface runoff, erosion, decay, volatilization, foliar washoff, advection, dispersion, and retardation. Various processes simulated in the PRZM model are shown in figure below.



Source: Carousel et al., (2005)

Daily edge-of-field loadings of the chemical dissolved in runoff and sorbed to sediment as predicted by PRZM (i.e., the PRZM output) are input into a waterbody simulated by EXAMS. EXAMS simulates the fate and transport of the chemical in a receiving waterbody with processes including volatilization, sorption, hydrolysis, biodegradation, and photolysis as shown in the figure below.



USEPA has two standard receiving waterbodies modeled in EXAMS: a farm pond and an index reservoir. The farm pond is a 1-ha by 2-m deep waterbody modeled with a 10-ha watershed simulated by PRZM and is used for ecological assessments of a chemical. The index reservoir is a 640-m long x 82-m wide x 2-m deep waterbody modeled with a 172.8-ha watershed simulated by PRZM and is used by the USEPA for drinking water assessments of a chemical. EXAMS is a steady state model and both water bodies have constant volume. The farm pond is modeled as static with no outflow and the index reservoir is configured with flow.

EXAMS outputs the annual pesticide exposure concentration in the water column from a 30-year simulation that are expressed as instantaneous maximum (peak), maximum 96-hour average, maximum 21-day average, maximum 60-day average, maximum 90-day average and annual average. The 90th percentile concentrations are used for exposure assessments by USEPA. Appendix 2 describes the calculation of 90th percentile concentrations.

Three main sources of manure – cropland, feedlot, and pasture, were used in the modeling to assess the fate and transport of trenbolone and estradiol using PRZM and EXAMS. The index reservoir waterbody was selected over the farm pond because it represents a watershed environment that can be configured to represent various proportions of feedlot, pasture and cropland in a watershed.

Since the PRZM v 3.12 model was developed to simulate the fate and transport of a chemical applied in an agricultural field, it was used for modeling fate and transport of trenbolone and estradiol applied on cropland in the form of manure applied to the fields.

To simulate surface losses of a veterinary drug from a feedlot and pasture, enhancements were made to winPRZM model version 4.51 [FOCUS, 2001]. The winPRZM model is a version of PRZM model used in European Union (EU) for pesticide risk assessment and has a capability to be modified if needed. Modifications to the winPRZM code were made such that the following functions could be simulated:

1. To model the feedlot environment, a constant concentration of the chemical was maintained in the top 10 cm of the feedlot manure pack. No applications of the chemical are made. The amount of chemical or veterinary drug lost from the feedlot through runoff and erosion is replenished the next day to maintain constant concentration. Degradation of the compounds in feedlot manure is not included in this model.
2. To model cattle grazing on pasture, daily loadings or applications of the compound excreted in the form of manure can be modeled with user-specified start and stop dates.

Several other models were considered to evaluate fate of trenbolone and estradiol in surface water, but were found to be less suitable for various reasons. APEX and SWAT are water quality models developed by the U.S. Department of Agriculture to simulate the fate and transport of nutrients and pesticides in watershed systems. Both models can simulate fate and transport of nutrients from a farm, feedlot, or pasture and include components like buffer strips, manure management, and grazing. However, these models cannot model the fate and transport of continuous daily loading of veterinary drug applied in a feedlot or pasture.

As a co-author of the PRZM and winPRZM models, it was possible for Waterborne Environmental, Inc., to modify the winPRZM model for feedlot and manure practices in a relatively straightforward way compared to modifying APEX or SWAT to model the fate and transport of veterinary drugs in feedlots and pasture.

The VETCALC model was developed for estimating fate of veterinary drugs in European settings. The model contains 12 scenarios representing a range of agricultural and manure management practices, soils and pedoclimatic conditions across Europe. Since this model is developed for EU conditions, it cannot be used for the U.S. without modifying/adapting it to U.S conditions. Additionally, VETCALC is not accepted by regulatory authorities in the EU.

RIVWQ and WASP are two other receiving waterbody models that simulate fate and persistence of chemicals in an aquatic ecosystem. RIVWQ is a steady state model that predicts water quality in a flowing waterbody, when linked with PRZM. WASP is an unsteady state flow model that simulates chemical behavior in a receiving waterbody when linked with models like PRZM to receive loadings. RIVWQ and WASP may be used as higher tier risk assessments in this case. RIVWQ and WASP are candidate models for watershed modeling with PRZM if the detail and sophistication is warranted. Table 14 shows a model selection matrix that rates the model on the basis of complexity of inputs required and processes simulated.

In summary, the PRZM-EXAMS modeling structure was used to simulate the environmental fate and transport of veterinary drugs, based on its ability to simulate important governing processes and an existing framework and scenarios for regulatory assessments in the U.S. Modifications

were made to winPRZM model to allow the daily loadings of veterinary drug to pasture and constant concentration in feedlots.

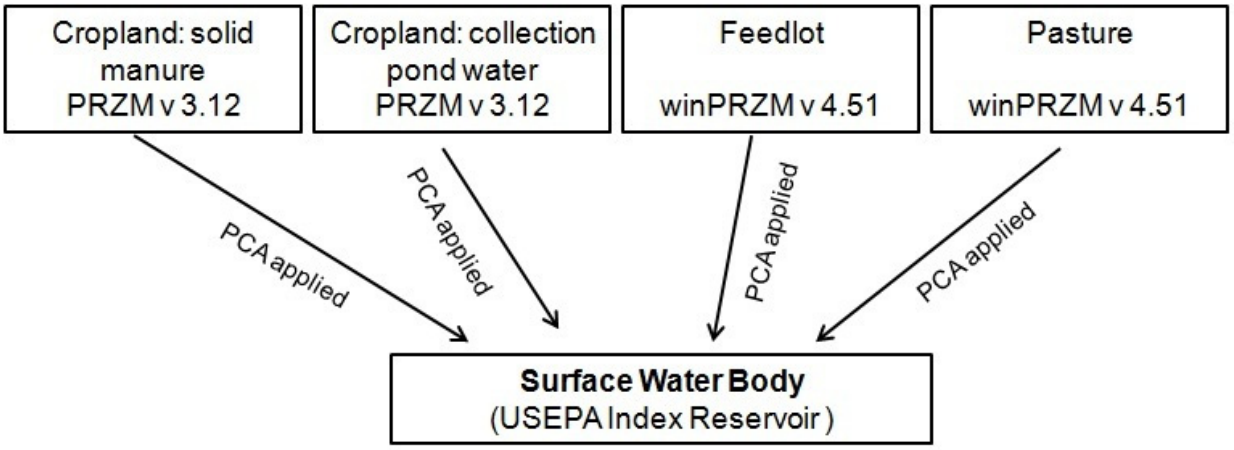
5.2. Modeling Synovex-One Metabolites Trenbolone and Estradiol using USEPA's Exposure Assessment Approach

Synovex-One is a product containing trenbolone acetate and estradiol benzoate and is administered to beef cattle as an ear implant. Active metabolites of trenbolone and estradiol are excreted in feces of implanted cattle. The manure containing these active metabolites is a potential source of environmental risk. The three main sources through which the trenbolone and estradiol can be released in to the environment via manure are feedlots, pasture and croplands:

1) Feedlots: Trenbolone and estradiol contained in the manure excreted by beef cattle on feedlots may end up in a receiving waterbody through surface runoff and erosion from the less than 1000 animal unit (AU) feedlots [Zoetis, 2012] that do not have adequate runoff control in place and discharge runoff to nearby surface water bodies.
2) Croplands:
   a) Manure from the feedlots is collected and stored for application to the fields to be used as fertilizer. The manure can be applied as broadcast or tilled into the land. Runoff and erosion from fields applied with manure is a source for trenbolone and estradiol to enter a receiving waterbody.
   b) Feedlots with more than 1000 AU (CAFO) are required to contain the runoff from the feedlots utilizing runoff control structures such as holding ponds or lagoons. Runoff collected in these holding ponds or lagoons is mixed with irrigation water and applied to croplands. Runoff and erosion from such croplands is also a source for trenbolone and estradiol to enter a receiving waterbody.
3) Pasture: Animals grazing on the pasture excrete manure on the surface of the pasture land. Runoff and erosion from surface of the pasture is also a source for trenbolone and estradiol to enter a receiving waterbody.

The USEPA's exposure assessment approach for modeling a watershed for human health assessment (using the index reservoir waterbody) was followed to model trenbolone and estradiol in selected counties for five regions: Iowa, Michigan, Texas, Ohio and Pennsylvania (Section 3.4). The mixed-use watershed was modeled consisting of feedlot, cropland (applied with solid manure and water from collection ponds) and pasture as potential sources of trenbolone and estradiol. Percent cropped area (PCA) factors (also referred to as watershed density factors in Section 4.7) are used by the USEPA to scale the final PRZM estimated concentrations to be representative of the area in the watershed that is actually contributing to the chemical concentrations in runoff and erosion. In this case, PCA factors were used to scale the watershed area that contributed to concentrations coming from cropland, pasture and feedlot. Figure 12 shows a schematic of modeling using USEPA's exposure assessment approach.

Four sources of manure containing trenbolone and estradiol were modeled with individual PRZM runs. Modeling for cropland, for both solid manure and collection pond water, was conducted using PRZM v 3.12 and modeling for feedlot and pasture were conducted using winPRZM v 4.51. The PCA factors (or watershed density factors) for each source were applied to the daily edge-of-field loadings generated by each PRZM run. After the PCA factors were applied, the daily edge-of-field loadings from four sources were added, to create an input to the USEPA index reservoir waterbody (modeled by EXAMS) as shown in the schematic below.



The development of PCA factors (or watershed density factors) is discussed in detail in Section 4 of this report. The mixed-use watershed was modeled for 30 years and the 90th percentile concentrations (calculations described in Appendix 2) of trenbolone and estradiol in the water column were used for the exposure assessment.

5.3. PRZM and EXAM Inputs

The required inputs used in PRZM and EXAMS models were selected based on conservative assumptions. The summary of the input selection process is described in this section. A detailed list of inputs used in each region's PRZM input file is listed in Appendix 3 to Appendix 7. The modeling parameters used for physical chemical properties, degradation rates in soil and water-sediment, nutrient requirements of individual crops and the application rates of trenbolone and estradiol to pasture, manured crops, and feedlots were all calculated and presented in detail in the Environmental Assessment (EA) [Zoetis, 2012] for Synovex-One.

5.3.1. Physical and Chemical Properties of Estradiol and Trenbolone Metabolites

Table 15 lists the physical and chemical properties trenbolone and estradiol used in PRZM and EXAM runs. More detail about the sources of the data can be found in Zoetis [2012].

5.3.2. Application Method and Timing

Cropland applied with solid manure: For modeling solid manure applications, solid manure was applied two times a year - in spring before planting and in fall after harvest of the corn. In the real world, farmers would not apply manure on a day of rainfall. But it is not possible to select a different application day (with zero rainfall) each year for 30 years in PRZM model. Therefore, application timing for this modeling study was selected with a conservative but not worst-case approach and within the limitations of the model. Based on visual inspection of 30-year weather data, a day was picked with the lowest amount of rainfall over 30 years and no large storms occurring on that day in any of the 30 years.

To determine the most appropriate application date, emergence and harvest dates for each site were taken from PRZM crop scenarios. Planting date was calculated as 10 days before emergence date. A 10-day period was selected ending 5 days before planting date and beginning 5 days after the harvest date for selecting application date for spring and fall, respectively. Using the PRZM weather files a pivot table (Microsoft Excel ®) was generated for the selected 10 day period consisting of daily rainfall for each day for the 30 years. Total rainfall over 30 years for each day in that period was also calculated. By visual inspection of the rainfall data in the 10-day period, application date was selected using the following criteria: Among the days with the lowest total rainfall, a day with no storm greater than 2.5 cm occurring in any year on that day or the following day was selected as the application day. If the criteria was not met within the 10-day selected period, additional 5 days were considered. For example, in case of Iowa spring application, planting date is May 15 and a period of 10 days was selected from May 1 to May 10, which is ending 5 days before the planting date. May 4 was selected to be most appropriate application day following the criteria mentioned above.

The application dates selected for each site are used for all 30 years and are presented in Table 16.

Solid manure was modeled as tilled-in at an incorporation depth of 15 cm in Iowa, Michigan, and Texas regions. In Pennsylvania and Ohio counties, no-till corn is a common agricultural practice; therefore, Pennsylvania and Ohio regions were modeled as no-till [Zoetis, 2012]. To model no-till cropping scenarios for Pennsylvania and Ohio, manure was applied as broadcast (on surface) at an incorporation depth of 5 cm. Incorporation depth of 5 cm is for surface application is assumed to reflect the inherent surface roughness that exists to varying degrees in all soils. Trenbolone and Estradiol in manure was assumed to be uniformly incorporated for the depth of application (or incorporation depth) which is represented in PRZM by using Chemical Application Method (CAM) of 4 for both till and no-till applications.

Cropland applied with collection pond water: Runoff stored in collection ponds or lagoons is usually applied along with irrigation water to the fields. It was assumed that collected pond water was applied four times a year during the growing season spaced out at equal intervals. The application dates selected for each site are presented in Table 16.

Since collection pond water is applied with irrigation water, it is applied on the surface of the soil. Therefore, incorporation depth of 5 cm was used with uniform incorporation (CAM = 4).

Feedlot: It was assumed that a constant concentration of the trenbolone and estradiol is present in the top 10 cm of feedlot "soil" which is assumed to be manure. Therefore, no applications were modeled for feedlot. A uniform incorporation (CAM=4) was assumed for top 10 cm depth. The constant concentration of 50.8 g/ha for trenbolone was input in the model and 6.57 g/ha for estradiol [Zoetis, 2012 and Table 17]. This concentration was held constant for all days in 30 years.

Pasture: It is assumed that the drug is applied daily (i.e., the drug is released daily on to the pasture during the time period the cows are grazing on the pasture). According to ISU-IBC [2007], the average date for cows to start grazing on pasture in Iowa is April 1st. Therefore, it was assumed that cows start grazing on pasture from April 1st for Iowa, Ohio, Pennsylvania, and Michigan regions. In northern panhandle of Texas, cattle start grazing on pasture around March/early April time period (Personal Communication with Mike Moseley, Zeotis [May, 2012]). Therefore, it was assumed that cows start grazing on pasture from March 1st for the Texas region.

Excretion end times from grazing cattle were estimated based on the number of days it would take to completely exhaust all of trenbolone and estradiol from the Synovex-One implant: 211 days for trenbolone and 270 days for estradiol [Zoetis, 2012]. Therefore, grazing ends for trenbolone and estradiol on October 28th and December 26th for Iowa, Ohio, Pennsylvania and Michigan, respectively. For Texas, grazing ends for trenbolone on September 27th and estradiol on November 26th.

Pasture was modeled as uniformly incorporated (CAM = 4) applications on the surface at a 5 cm incorporation depth.

5.3.3. Application Rates

Details of estimation of yearly application rates for cropland, feedlot and pasture are given in [Zoetis, 2012]. Application rates for loading of trenbolone and estradiol used in four PRZM runs for all sites are listed in Table 17. The application rates used in modeling are presented in g/ha for each application event.

Cropland: The manure application rates for cropland were estimated based on daily release rate of trenbolone and estradiol and P_2O_5 requirement of corn silage for Pennsylvania and P_2O_5 requirement of corn for grain harvested for other four regions. Details for total (yearly) application rate for cropland are presented in Table 7 and [Zoetis, 2012). Application rates for solid manure and collection pond water were calculated by splitting the yearly manured cropland application rates as 90% and 10%, respectively [Zoetis, 2012].

Based on application timing discussed in Section 5.3.2, yearly solid manure application rate is divided into two to estimate the application rate for spring and fall applications. Therefore, the

application rate for each application event for solid manure will be half of the yearly application rate to the cropland. Yearly collection pond water application rate is split into four equal parts to estimate the application rate for each application event.

Pasture: Pasture application rates are estimated based on daily release rate of trenbolone and estradiol and cattle stocking density for pasture of 3.15 head/acre [Zoetis, 2012]. The application rate for pasture is presented as a daily application rate, and it is applied daily for the number of days (Section 5.3.2) it takes for trenbolone or estradiol to be completely released from the Synovex-One implant.

Feedlot: Application rate for feedlots is same as the constant concentration assumed for the feedlot PRZM run. The constant concentration in the feedlot manure pack was estimated based on daily release rate of trenbolone and estradiol from Synovex-One and stocking density of 270 cattle per acre and number of days between manure collection [Zoetis, 2012].

5.3.4. Weather Parameters

The USEPA daily weather data for 30 years was used for PRZM and EXAM simulations. Weather stations closest to the selected counties in each region were selected. The following weather stations (weather file name included in parenthesis) were selected: Iowa – Sioux City, SD (w14944.dvf); Pennsylvania – Harrisburg, Pennsylvania (w14751.dvf); Ohio – Dayton, Ohio (w93815.dvf); Michigan – Flint, Michigan (w14826.dvf) and Texas – Amarillo, Texas (w23047.dvf).

Daily weather data included precipitation, pan evaporation, mean temperature, wind speed at 10 m, solar radiation, FAO short grass ETo (Reference evapotranspiration for grass calculated using Food and Agriculture Organization (FAO) Irrigation and Drainage paper 56 method), daylight station pressure, daylight relative humidity, daylight opaque sky cover, daylight temperature, daylight broadband aerosol, daylight prevailing wind speed at 10 m and daylight prevailing wind direction [Carousel et al., 2005].

The average annual rainfall for the selected weather stations is 60.67 cm, 102.88 cm, 93.17 cm, 76.97 cm and 49.70 cm for Iowa, Pennsylvania, Ohio, Michigan and Texas, respectively.

5.3.5. Crop Parameters

Some of the key PRZM crop related input parameters are interception storage, rooting depth, aerial coverage and canopy height, emergence, maturation and harvest dates.

Cropland: Corn was modeled for each region. For the Iowa, Pennsylvania, Ohio and Texas regions, standard USEPA PRZM corn scenarios were used [USEPA, 2011].

USEPA has not developed a corn scenario for Michigan. Therefore, a corn scenario for the Michigan region was created using interception storage, rooting depth, aerial coverage and canopy height parameters that were used in the standard corn scenarios. Emergence,

maturation, and harvest dates for corn in Michigan were taken from USDA Agricultural Handbook [USDA, 2000].

Pasture: The USEPA standard PRZM "RangeBSS" (rangeland) scenario was used for modeling pasture for Texas region [USEPA, 2011]. The crop parameters for this scenario were applied to the creation of Iowa, Michigan, Ohio and Pennsylvania pasture scenarios. Emergence, maturation and harvest dates for simulating pasture in four northern regions were assumed to be April 1, April 15, and November 15, respectively. These dates were selected to correspond with the start of grazing dates (Section 5.3.2) in these regions.

Note that emergence dates for pasture represent when grass is likely to become green after overwintering. Grass is assumed to be dormant from harvest to emergence of the following year.

Feedlot: Crop was not modeled for feedlots.

5.3.6. Soil Parameters

Important PRZM soil input parameters include depth of soil core, each soil layer (or horizon) thickness, bulk density, organic carbon content, field capacity, and wilting point for each horizon. These parameters vary for different soil types.

Cropland: For the Iowa, Pennsylvania, Ohio, and Texas regions, standard PRZM corn scenarios were used and soil parameters were kept the same as those used in the standard scenarios. For the Michigan region, soil parameters were taken from USEPA's standard scenario for Michigan beans [USEPA, 2011], because the soil type used in that scenario was a benchmark soil for Huron County, Michigan, which is the selected county of the region for simulations in this study.

The soil types and slopes used for each region were Iowa – Fayette silty clay loam and 6% slope; Pennsylvania – Hagerstown Silt Loam and 6% slope; Ohio – Cardington Silt Loam and 6% slope; Michigan – Toledo silt clay and 1% slope; and Texas – Axtell Sandy Loam and 6% slope.

Pasture: Standard PRZM rangeland scenario was used and the soil parameters were kept the same as those used in the scenario for Texas region. Soil type used in the Texas rangeland scenario is Brackett-Rock outcrop-Comfort complex with a 4% land slope. For the Iowa, Michigan, Ohio, and Pennsylvania regions, pasture scenarios were created using the same soil parameters and slopes as in their respective corn scenarios.

Feedlot: Soil profile for a feedlot was represented in a different manner than for a cropland. The top 10 cm layer of feedlot soil profile was assumed to be manure and next 10 cm was assumed to be "interface layer" which is a mixture of manure and soil. The top 20 cm of feedlot was collectively simulated as "manure pack." Bulk density, organic carbon content, field capacity, wilting point for each 10 cm horizon was taken from Cole et al. [2009], Kissinger et al. [2007],

and Meilke et al. [1974]. The top 20 cm feedlot soil profile was assumed to be the same for all regions.

Soil horizon layers deeper than 20 cm for all regions were assumed to be the same as soil used in their respective corn scenario because at this depth soil is not sensitive to runoff or erosion Slope of the feedlot was assumed to be 4% for all regions [FASS, 2010].

5.3.7. Runoff Parameters

Runoff in the PRZM model is based on the Soil Conservation Service curve number technique. Curve numbers range from 1 to 100 and are based on soil type, hydrologic condition of the soil and type of crop. Soil types range from A (sandy soils) to D (clayey soils), where runoff potential increases from A to D type. A higher curve number will result in more runoff. PRZM requires three curve numbers for fallow, cropping and residue conditions. All curve numbers were selected for antecedent moisture condition (AMC) II [Carousel et al., 2005], which represents average initial soil moisture content. The model adjusts the AMC II curve numbers during a simulation as a function of soil moisture.

Cropland: For the Iowa, Pennsylvania, Ohio, and Texas regions, standard PRZM corn scenarios were used along with the recommended curve numbers. The following curve numbers (for fallow, cropping and residue) are used in the standard scenarios and selected based on following soil types:

- Iowa – 86 79 86; B soil type, poor condition
- Pennsylvania – 89 83 89; C soil type, good condition
- Ohio – 91 87 91; C soil type, poor condition
- Texas – 92 89 92; D soil type, poor condition

For the Michigan region, soil type and condition were taken from the Michigan beans standard PRZM scenario. For Michigan, D soil type and poor condition were used to estimate curve numbers for corn crop – 94 91 94 [Carousel et al., 2005].

In Pennsylvania and Ohio counties, no-till corn is a common agricultural practice; therefore, Pennsylvania and Ohio regions were modeled as no-till [Zoetis, 2012]. For no-till conditions, curve numbers for tilled conditions are reduced by 10%. See Section 5.3.9 for more details. Therefore, the following curve numbers were used in Pennsylvania and Ohio corn scenarios for modeling no-till cropping conditions: Pennsylvania – 80 75 80 and Ohio – 82 78 82.

Pasture: The standard PRZM range scenario was used and curve numbers were kept the same as those used in the scenario for Texas region. The curve numbers used were: 87 83 86 based on C soil type.

For Iowa, Michigan, Ohio, and Pennsylvania, curve numbers were selected based on soil types and conditions used in cropland scenarios and pasture crop. The following curve numbers were

used for pasture scenarios: Iowa – 82 79 82; Pennsylvania – 85 79 85; Ohio – 89 86 89; and Michigan – 93 89 93.

Feedlot: A highly conservative curve number of 95 for feedlot for fallow, residue and cropping conditions was selected for all regions [Williams et al., 2006].

5.3.8. Erosion Parameters

Erosion is calculated in PRZM based on Modified Universal Soil Equation for Small Systems (MUSS). Sensitive erosion parameters include USLE K (soil erodibility factor), USLE LS (topographic factor), USLE P (practice factor), USLE C (cover management factor) and Manning's N. Details about these parameters can be found in Carousel et al. [2005].

Cropland: For the Iowa, Pennsylvania, Ohio, and Texas regions, standard PRZM corn scenarios were used with the recommended erosion parameters. For the Michigan region, USLE K, USLE LS, and USLE P were taken from the Michigan beans USEPA standard PRZM scenario. USLE C factors and Manning's N were taken from USDA Agricultural Handbook [USDA, 2000] for corn crop in Michigan based on conventional tillage.

In Pennsylvania and Ohio counties, no-till corn is a common agricultural practice; therefore, Pennsylvania and Ohio regions were modeled as no-till [Zoetis, 2012]. See Section 5.3.9 for modifications made for no-till.

Pasture: Standard PRZM range scenario was used and all erosion parameters were kept the same as those in the scenario for the Texas region.

For the Iowa, Michigan, Ohio, and Pennsylvania pasture scenarios, USLE K, USLE LS, and USLE P parameters were taken from their respective corn scenarios. USLE C factors and Manning's N for these regions were taken from USDA Agricultural Handbook [USDA, 2000] for pasture crop in the Pennsylvania and Michigan regions.

Feedlot: Erosion from a feedlot was modeled using manure erosion equation adapted from the APEX model [Williams et al., 2006]. The APEX model uses a modified MUST equation that simulates erosion from a manure covered surface.

$$YMNU = ME * (Q * qp)^{0.5} * PE * SL * RSDM^{M} * \exp(-CAGPM * AGPM))$$

where:

- YMNU is the manure erosion (ton ha^{-1}).
- ME is the manure erosion equation coefficient. Larger values increase manure erosion. Ranges from 0.1 to 0.5. Conservative = 0.25.
- Q is the runoff volume (mm).
- qp is the peak runoff rate (mm h^{-1}).
- PE is the erosion control practice factor. From Table 5.5 and 5.6 of PRZM or APEX, table based on 4% slope.

- SL is the slope length and steepness factor – used slope of 4% in feedlots.
- RSDM is the manure on the soil surface (ton/ha). Estimated = 12 ton/ha. Based on daily manure production of animal for 300 kg animal (18.2 kg) [NRCS, 2012] and stocking density of feedlot ($15m^2$/AU).
- M modifies the equation based on weight of manure on soil surface. Ranges from 0.1 to 1.0. Conservative = 0.5.
- AGPM is the standing live and dead plant material. It is negligible for feedlot surface because there is no plant growth. It is simulated daily.
- CAGPM is a coefficient for AGPM. It modifies the erosion estimate based on above ground plant material. Plant material live or dead reduces manure erosion. Values vary between 0.1-1.5. Conservative = 0.15.

Conservative estimates are based on personal communication with Jimmy Williams, author of the APEX model.

USLE C factors and Manning's N for feedlot were set to represent fallow/no residue conditions [Carousel et al., 2005].

5.3.9. No-till Parameters

For regions where no-till is a common agricultural practice, no-till was modeled for cropland scenarios. For modeling no-till, runoff curve numbers and erosion parameters (USLE C factors and Manning's N) were changed to represent no-till conditions.

Under no-till conditions, runoff and erosion is reduced compared to tilled conditions due to presence of crop residue on the surface. To simulate reduced runoff and erosion, curve numbers for tilled conditions are reduced by 10% [Campbell, 2006] and USLE C factors and Manning's N were changed to represent no-till conditions. Erosion parameters for no-till conditions were taken from USDA Agricultural Handbook [USDA, 2000].

5.4. Percent Crop Area (PCA) Factors

PCA factors also referred to as watershed density factors were applied to the PRZM outputs before the chemical loading enters the waterbody. The PCA factors used for each region are listed in Table 18. The development of PCA factors is discussed in detail in Section 4.

5.5. Mixed-Use Watershed Modeling Results

5.5.1. Worst-Case Results

The predicted environmental concentrations of trenbolone and estradiol in watersheds of five selected regions resulting following the use of Synovex-One in beef cattle are summarized below. These concentrations were estimated from combined contributions (based on PCA

factors) from feedlots, pasture and manured cropland discharging to surface water in the watershed assuming 100% of feedlots with <1000 AU do not control runoff and thus in that sense these are worst-case concentrations.

		90th percentile concentrations in water (ng/L)					
Region	**Compound**	**Peak**	**96-hr**	**21-day**	**60-day**	**90-day**	**Yearly**
Iowa	Trenbolone	2.550	2.510	2.417	2.265	2.143	1.769
	Estradiol	0.235	0.228	0.216	0.195	0.182	0.139
Pennsylvania	Trenbolone	1.141	1.123	1.067	1.009	0.993	0.841
	Estradiol	0.110	0.106	0.101	0.097	0.093	0.073
Ohio	Trenbolone	1.943	1.901	1.831	1.763	1.696	1.418
	Estradiol	0.183	0.181	0.176	0.160	0.155	0.120
Michigan	Trenbolone	0.825	0.813	0.774	0.731	0.722	0.595
	Estradiol	0.080	0.078	0.072	0.068	0.065	0.051
Texas	Trenbolone	0.293	0.280	0.237	0.174	0.146	0.057
	Estradiol	0.032	0.030	0.024	0.016	0.013	0.005

Highest 21-day 90th percentile concentration of trenbolone and estradiol were predicted for the Iowa study region. The 21-day 90th percentile predicted concentrations of trenbolone were 2.417, 1.067, 1.831, 0.774 and 0.237 ng/L for Iowa, Pennsylvania, Ohio, Michigan and Texas, respectively. The 21-day 90th percentile predicted concentrations of estradiol were 0.216, 0.101, 0.176, 0.072, and 0.024 ng/L for Iowa, Pennsylvania, Ohio, Michigan and Texas, respectively.

5.5.1.1. Individual Contributions

Feedlot is the major contributing source of manure containing trenbolone and estradiol, as compared to manured cropland and pasture. Feedlots are highly prone to surface losses through runoff and erosion because of bare (uncropped) feedlot surface covered with loose un-compacted manure pack containing high amount of trenbolone and estradiol (50.8 g/ha) compared to cropland and pasture (Table 17),

An analysis was done to estimate the contribution of each manure source in a watershed. The Iowa study region and trenbolone were selected for this analysis as highest worst-case concentrations were predicted for this case. The mixed-use watershed model was run four times, each time with a single contributing source – feedlot, cropland with solid manure, cropland with collection pond water and pasture and the PCA used for the each contributing source was 0.094%, 50.96%, 5.66% and 2.29%, respectively (Table 18). A PCA factor of zero was used for other sources to represent a non-contributing source. The table below summarizes the trenbolone 90th percentile concentrations estimated from the Iowa study region watershed assuming there is only one contributing source in the watershed.

	90th percentile concentrations in water (ng/L)					
Contributing Source	**Peak**	**96-hr**	**21-day**	**60-day**	**90-day**	**Yearly**
Feedlot	2.447	2.409	2.318	2.181	2.097	1.746
Cropland-solid manure	0.155	0.150	0.141	0.113	0.094	0.042
Cropland- collection pond water	0.052	0.051	0.050	0.045	0.038	0.014
Pasture	0.007	0.007	0.006	0.005	0.005	0.002

Note that these concentrations will not sum to exactly the worst-case 90th percentile concentration provided in Section 5.5.1 when all sources are contributing.

If feedlot was the only contributor to water in the Iowa study region, the predicted trenbolone 21-day 90th percentile concentration would be 2.318 ng/L, which is almost 95% of worst-case concentration (2.417 ng/L). If only pasture was contributing to the waterbodies of the Iowa study region watershed, trenbolone 21-day 90th percentile concentration would be 0.006 ng/L, which is less than 1% of the worst-case concentration. Both sources of manured cropland contribute less than 10% of the total concentration. Therefore, concentrations from individual sources indicate that feedlot is the major contributor among other sources even though feedlot PCA is the smallest.

5.5.1.2. Discussion

Model parameters that are the most sensitive for PRZM simulations are parameters associated with application (rate, number, and intervals), runoff and erosion. The driving factors for runoff and erosion occurring on cropland, feedlot, and pasture are rainfall, soil type (i.e., curve number, organic carbon), and erosion (c-factors, slope). Another sensitive parameter within the watershed is the PCA factor (watershed density factor) of each of the contributing manure sources (cropland, feedlot, and pasture).

The effect of soil type, curve number, erosion parameters, slope, and application rate can be seen from concentrations generated from feedlot, pasture, and cropland (Section 5.5.1.1) The effect of number of <1000 head beef cattle, pasture land, and cropland manured areas (described by PCA factors) can be seen in the concentrations predicted for each region (Section5.5.1).

In the case of feedlots, high application rate (50.8 g/ha) with high runoff/erosion potential (bare surface, high OC ~38% and high curve number of 95) compared to cropland and pasture, resulted in feedlots being a dominant contributing source of mass load to the water body (Section 5.5.1.1). Therefore, regions with the highest PCA factor for feedlots resulted in the highest concentrations. Iowa with a PCA of 0.094% had the highest concentrations followed by Ohio, Pennsylvania, Michigan, and Texas. Texas had the lowest concentrations and the lowest PCA factor for feedlots (0.003%).

The highest cropland application rate (0.74 g/ha) was used in Pennsylvania but since Pennsylvania had the lowest PCA factor for cropland (9.41%), the application rate did not cause high concentrations.

An example of the influence of weather can be seen by comparing the 21-day 90th percentile concentrations. Texas resulted in the overall lowest 21-day 90th percentile concentration among the other regions because the weather station had the lowest average annual rainfall compare to the other regions (Section5.3.4).

5.5.2. Use of Best Management Practices

The worst-case concentrations shown above used the PCA factor (or watershed density factors) for feedlots that includes all the possible <1000 AU feedlots in a watershed contributing to the surface water through runoff and erosion. This is an extremely conservative assumption because in reality, not all < 1000 AU feedlots discharge to nearby surface water bodies. Therefore, an analysis was done to estimate concentrations if only 75%, 50%, 25% and 0% of the feedlot PCA factor were contributing to the waterbody. The model was run by changing the feedlot PCA factor to 75%, 50%, 25% and 0% of the feedlot PCA factor for each region. The 90th percentile concentrations predicted for all regions with varying feedlots PCAs are presented in Table 19 to Table 28. The 90th percentile concentrations were plotted for each time period (peak, 96 hr, 21-day, 60 day, 90 day, and annual) and best fit line was plotted and a linear regression equation was estimated (using Microsoft Excel) for each time period (Table 29). Figure 13 to Figure 17 show the graphical representation of 21-day 90th percentile concentrations with varying feedlot PCAs and the associated regressions equations estimated for trenbolone and estradiol for the five regions. Regression equations are provided so that concentrations can be predicted for any level of feedlot discharge.

Decreasing feedlot PCA factor decreases 21-day 90th percentile concentrations more rapidly for all sites except Texas as demonstrated by considerably lower slope of Texas regression equations. The Texas region had a relatively high PCA factor for cropland (53.6%) compared to a feedlot PCA factor of 0.003%. In Texas, cropland slope, soil type, and curve number are more sensitive parameters since cropland is the major contributor of mass loadings to the water body. Following the procedure described in Section 5.5.1.1, it was estimated that both sources of cropland (collection pond water and solid manure) contributed more than 85% of the mass loading for Texas's worst case 21-day 90th percentile trenbolone concentrations, whereas the feedlot contributed about 16%. Since feedlot is not a major contributor for the Texas worst-case concentrations, the effect of varying the feedlot PCA factor for the Texas region results in a much flatter line (Figure 17) as compared to other regions.

If best management practices are followed by all the feedlot owners in the watershed and there is no direct discharge from any feedlot to a nearby waterbody, the predicted trenbolone and estradiol concentrations in watersheds of five selected regions are summarized below.

		90th percentile concentrations in water (ng/L)					
Region	**Compound**	**Peak**	**96-hr**	**21-day**	**60-day**	**90-day**	**Yearly**
Iowa	Trenbolone	0.164	0.159	0.150	0.120	0.102	0.048
	Estradiol	0.017	0.017	0.015	0.012	0.009	0.003
Pennsylvania	Trenbolone	0.016	0.015	0.013	0.010	0.008	0.004
	Estradiol	0.002	0.002	0.001	0.001	0.001	0.0003
Ohio	Trenbolone	0.069	0.067	0.058	0.042	0.032	0.014
	Estradiol	0.007	0.007	0.006	0.004	0.003	0.001
Michigan	Trenbolone	0.030	0.029	0.026	0.021	0.016	0.011
	Estradiol	0.003	0.003	0.002	0.002	0.001	0.001
Texas	Trenbolone	0.270	0.257	0.215	0.154	0.127	0.037
	Estradiol	0.030	0.028	0.022	0.015	0.012	0.003

These concentrations are resulting from runoff and erosion from only pasture and manure croplands in the watershed. Because feedlot is the major contributor among other modeled manure sources, eliminating feedlot as contributing source resulted in 21-day trenbolone and estradiol concentrations for Iowa study region dropping down to 0.150 and 0.015 ng/L, respectively, as compared to the 2.417 and 0.216 ng/L worst-case concentrations.

Realistically speaking, not all feedlot owners with <1000 head of cattle will follow best management practices and control runoff from feedlots. Therefore, it was estimated by Zoetis [2012] that about 85% of feedlot owners will follow best management practices in a watershed. Using the linear regression equations presented in Table 29, concentrations for each region were calculated if 85% of the feedlot owners in a watershed would practice best management practices to control runoff from feedlots and presented below.

		90th percentile (ng/L)					
Region	**Compound**	**Peak**	**96-hr**	**21-day**	**60-day**	**90-day**	**Yearly**
Iowa	Trenbolone	0.507	0.499	0.481	0.443	0.413	0.308
	Estradiol	0.048	0.046	0.042	0.037	0.034	0.024
Pennsylvania	Trenbolone	0.184	0.180	0.169	0.251	0.155	0.130
	Estradiol	0.018	0.016	0.016	0.016	0.014	0.011
Ohio	Trenbolone	0.330	0.322	0.305	0.290	0.273	0.223
	Estradiol	0.032	0.031	0.029	0.027	0.025	0.019
Michigan	Trenbolone	0.145	0.143	0.135	0.122	0.119	0.097
	Estradiol	0.014	0.014	0.013	0.012	0.010	0.008
Texas	Trenbolone	0.273	0.260	0.218	0.157	0.130	0.040
	Estradiol	0.030	0.028	0.022	0.015	0.012	0.004

A realistic scenario where 85% of the feedlot owners in a watershed control runoff from feedlots would result in a 21-day trenbolone and estradiol concentrations of 0.481 ng/L and 0.042 ng/L

as compared to worst-case concentration of 2.417 and 0.216 ng/L, respectively for Iowa study region.

It may be noted that 90th percentile concentrations estimated by using regressions equations may sometimes not exactly match the 90th percentile concentrations calculated by running EXAMS with varying PCAs. Even though linear relationship exists between concentrations at the varying feedlot PCAs (as demonstrated by R^2 close to 1.0 for regression lines estimated for each time period), minor differences in concentrations can be attributed to the procedures of calculating running averages for different time periods and calculating 90th percentiles in the model.

5.6. Additional Analysis Requested by the Sponsor

In addition to the mixed-use watershed models presented in the EA [Zoetis, 2012], the sponsor also requested three additional analysis discussed below.

5.6.1. Worst-Case Pasture

Based on sponsor's request, an analysis was conducted to calculate concentrations assuming the entire watershed consisted of pasture land for estimating worst-case concentrations resulting from pasture. Table 30 shows the concentrations for all regions assuming the entire watershed was covered with pasture cattle. Highest 21-day 90th percentile concentrations, for trenbolone (0.295 ng/L) and estradiol (0.026 ng/L) were predicted for the Ohio region. The Michigan region was modeled with the highest pasture runoff curve numbers (93,89,93) and 1% slope, it resulted in lower concentrations than the Ohio region which was modeled with slightly lower curve numbers (89,86,89) but a 6% slope.

5.6.2. No-till Cropping Scenarios

The sponsor also requested to develop additional 17 no-till crop scenarios from USEPA standard crop scenarios following the procedure described in Section 5.3.9. No-till crop scenarios were developed for corn, wheat, cotton, soybean and sorghum, which are the principle crops grown under no-till. Table 31 records the principal crops grown in each study region according to the 2007 Census of Agriculture. The runs were made using both farm pond and index reservoir waterbody scenarios. Additional details about the no-till scenarios are provided in Zoetis [2012] and the results for trenbolone and estradiol concentrations for 17 no-till scenarios are presented in Table 32 to Table 35.

5.6.3. Leaching from Unpaved Feedlots

The sponsor also requested to estimate leaching potential from an unpaved feedlot [Zoetis, 2012]. The Koc of the chemical, organic carbon content of the soil and curve number are the primary factors that affect runoff and leaching potential. Both trenbolone and estradiol have relatively high Koc values (in range of 1000). The top 10 cm soil layer for feedlot was simulated

to have high organic carbon content (38%) [Cole et al., 2009]. That, combined with a high curve number of 95 used for feedlots, makes a feedlot surface relatively impervious, thereby minimizing leaching potential.

The PRZM model also estimates leaching below the soil core. The soil core depth for the five regions that were modeled ranged from 100 to 150 cm. The yearly “pesticide leached below core depth” values in the PRZM model for feedlot for trenbolone were estimated to be zero for all five regions, signifying that leaching below a feedlot is negligible.

6. Conclusions

An aquatic exposure assessment was conducted for trenbolone and estradiol associated with the use of Synovex-One on beef cattle. The assessment utilized a modeling framework established by the U.S. Environmental Protection Agency (USEPA) for pesticide registration. The scenarios developed by USEPA were modified to address trenbolone and estradiol release from manure using feedlot, pasture, and cropland as sources. USEPA’s approved regulatory models PRZM and EXAMS were used for modeling chemical runoff and erosion from cropland. The winPRZM model, the European version of PRZM was modified to simulate application, runoff, and erosion from feedlot and pasture surfaces.

Regions that may be most vulnerable to trenbolone and estradiol exposure in surface water were identified to be modeled by conducting a national geospatial analysis that combined normal annual precipitation with areas of high beef cattle production and density of feedlots. Five study regions (one within each of the states of Iowa, Texas, Pennsylvania, Michigan, and Ohio) were identified for analysis. The importance of the national analysis is that it places the modeling results into a national context and promotes confidence that the results represent a realistic “intense use” case that can be applied to any region across the U.S.

Per unit area, feedlots were found to be the most significant contributor followed by cropland and pasture. The highest concentrations in surface water were predicted for Iowa study region followed by Ohio, Pennsylvania, Michigan and Texas.

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Tables

Table 1: Relative Ranking of Study Regions in the National Distribution of Beef Cattle and Rainfall Characteristics

State	County	Feedlot Density Rank (n=1,380)	>500 Head Feedlot Density Rank (n=290)	<500 Head Feedlot Density Rank (n=1,345)	Acres Manure Density Rank (n=2,887)	Pasture Cattle Density Rank (n=3,019)	Annual Rainfall Rank (n=3,110)	March Rainfall Rank (n=3,110)	October Rainfall Rank (n=3,110)
IA	Lyon	98.9%	96.8%	99.7%	99.6%	92.5%	23.0%	22.6%	20.2%
IA	Sioux	99.5%	98.4%	99.8%	99.9%	97.5%	23.3%	24.0%	20.8%
MI	Huron	95.5%	89.6%	98.1%	97.9%	75.3%	29.7%	31.6%	33.1%
OH	Mercer	96.0%	88.4%	99.4%	99.8%	88.4%	39.8%	40.5%	29.5%
PA	Lancaster	94.9%	72.8%	99.6%	100.0%	94.9%	56.1%	52.9%	68.4%
TX	Castro	99.9%	99.6%	74.1%	95.4%	99.7%	11.1%	5.1%	15.0%

Beef related statistics in this table from 2007 Census of Agriculture County Statistics. Rainfall information from PRZM Weather data.
“n “ in each table heading refers to number of counties in U.S. with >0 for the category. For example, there are 1,380 counties with beef feedlots in the US per the ’07 Census of Agriculture.

Table 2: Beef Densities for the Watersheds Ranked by their Exposure Index in order to Select a 90th percentile Watershed in the Lyon & Sioux Counties Study Region

Percent Rank^ (n=42 watersheds)	Feedlot (<1,000 head) Density (% of watershed area) /% Rank	Manured Acres Density (%) /% Rank	Pasture Density (%) / % Rank	Watershed Area (acres) / % Rank
100th Percentile	0.121/ 100%	67.74/ 93%	2.75/ 46%	13,019/ 81%
90th Percentile* (HUC 101702032002)	**0.094†/ 90%**	**56.62†/ 88%**	**2.29†/ 29%**	**21,128/ 68%**
75th Percentile	0.074/ 76%	15.12/ 54%	4.39/ 85%	10,977/93%
50th Percentile	0.036/ 51%	7.58/ 24%	1.52/ 9%	12,202/ 83%
10th Percentile	.007/ 10%	1.49/ 2%	1.72/ 17%	10,163/ 98%

^Percent rank based upon the computed Exposure Index (refer to Section 4.2 for details on how it is computed).

*Actual watershed selected for modeling

†Densities (PCAs) used for watershed modeling. In the watershed selected to model in Iowa, the feedlot density <1,000 head was a principle driver for the selection since its contribution to potential surface water pollution was greater than crop land or pasture land. Also note that the percentiles are not 90% in manured acres (88%) and pasture (29%) because the ranking is based on the Exposure Index discussed in Section 4.2. The feedlot size was based on the actual cattle AFOs locations within the watershed from the Iowa DNR database, in combination with a feedlot stocking density for cattle on lots <1,000 AU of 15m^2 per animal unit. The manured acreage was based on the actual cattle numbers from AFOs and CAFOs in the Iowa DNR database within the watershed, in combination with a crop application rate for manure (Section 4.4 discussed this). The pasture land within the watershed (from the NASS CDL remote sensing data) was adjusted by '07 Census of Agriculture acreage (refer to Section 4.5) and multiplied by the stocking density of pasture cattle (3.15 AU per acre) to estimate pasture cattle counts.

Table 3: Beef Densities for the Watersheds Ranked by their Exposure Index in order to Select a 90th percentile Watershed in the Castro, Texas Study

Percent Rank^ (n=35 watersheds)	Feedlot (<1,000 head) Density (% of watershed area) /% Rank	Manured Acres Density (%) /% Rank	Pasture Density (%) /% Rank	Watershed Area (acres) /% Rank
100th Percentile	0.008/ 100%	50.01/ 62%	5.04/ 64%	33,079/ 27%
90th Percentile* (HUC 120500050303)	**0.003†/ 91%**	**53.59†/ 94%**	**4.36†/ 55%**	**27,073/ 65%**
75th Percentile	0.002/ 76%	34.0/ 77%	1.7/ 0%	27,208/ 62%
50th Percentile	0.00003/ 50%	4.46/ 50%	2.20/ 5%	38,084/ 15%
10th Percentile	0.00/ 0%	0.00/ 0%	4.35/ 52%	32,353/ 32%

^Percent rank based upon the computed Exposure Index (refer to Section 4.2 for details on how it is computed).
*Actual watershed selected for modeling.
†Densities (PCAs) used for watershed modeling. In the watershed selected to model in Texas, the feedlot density <1,000 head and acres manured (because of the large number of beef on CAFOs) were the principle driver for the selection since their contribution to potential surface water pollution was greater than pastureland cattle. Also note that the percentiles are not 90% in manured acres (94%) and pasture (55%) because of the method of ranking is based on the Exposure Index discussed in Section 4.2. The feedlot area was based on the Texas DEQ GIS CAFO database in combination with an estimation of cattle on AFOs as discussed in Section 4.3 and a feedlot stocking density for cattle on lots <1,000 head of $15m^2$ per animal unit. The manured acreage was based on the Texas DEQ CAFO database within the watershed in combination with '07 Census of Agriculture data and a crop application rate for manure (Section 4.4 discusses this). The pasture land within the watershed (from the NASS CDL remote sensing data) was adjusted by '07 Census of Agriculture acreage (refer to Section 4.5) and multiplied by the stocking density of pasture cattle (3.15 AU per acre) to estimate pasture cattle counts.

Table 4: Estimate of Beef Cattle on Feedlots Less Than 1,000 Head in Castro, Texas

Castro County	% of Total Cattle on Feed	Source	Resulting Feedlot Cattle
Total Cattle on Feed	100	Census of Agriculture, County Stats	341,694
Cattle in lots <500 head	0.654	Census of Agriculture, County Stats	2,232
Cattle in lots 500-999 head	0.524	Census of Agriculture, State Stats	1,789
Total <1000 head	1.178	State and County level combined	4,025

Castro county feedlots <1,000 head was estimated from county data of 0.654% in lots <500 AU + state level estimate of 0.524% in lots 500-999 AU as presented Section 4.3. Briefly, this calculation was performed since county level data was only available for <500 head farms (’07 Census of Agriculture) but State level data is available for 500- 999 head farms. The majority of beef cattle are in the Texas Pan Handle region making the extrapolation of a state estimate practical to the Castro Study Region.

Table 5: Area (Acres) and Feedlot Beef Cattle Counts for All Watersheds in the Five Study Regions Examined

Study Region	Total Area (Acres) & Count of All Watersheds (USGS HUC12) in Study Region^	Total Cattle on Feedlots in All Watersheds in Study Region	Total Cattle on Feedlots <1,000 head in All Watersheds in Study Region	Total Cattle on Feedlots >1,000 head in All Watersheds in Study Region	Source of Study Region Cattle Counts
Lyons & Sioux County, Iowa	995,598 (n = 42 watersheds)	288,406	119,885	168,521	Iowa DNR AFO/ CAFO GIS Database†
Castro, Texas	742,331 (n=35 watersheds)	341,694	4,025	341,694	Texas CEQ CAFO GIS Database in combination with '07 Cens of Ag State & County Stats††
Mercer, Ohio	317,468 (n=18 watersheds)	28,448	27,509	939	'07 Cens of Ag County & State Stats*
Huron, Michigan	504,592 (n= 27 watersheds)	45,367	20,869	24,498	'07 Cens of Ag County & State Stats*
Lancaster, Pennsylvania	753,360 (n=33 watersheds)	43,349	40,661	2,688	'07 Cens of Ag County & State Stats*

This table provides feedlot cattle totals in all the watersheds in each study region, along with the watershed counts and acreages.
^The total area of all watersheds (above) differs from the areas of the counties in Table 10 because watersheds cross county lines.
†For Iowa, CAFO and AFO beef counts represent "permitted beef" from the GIS database.
††For TX, beef counts for CAFOs represent "permitted beef" from GIS database adjusted to match the Census of Agriculture figures (refer to Section 4.3 for the approach). Note that it was conservatively assumed that 100% of the beef in Castro are on lots >1,000 head when calculating CAFO beef counts; therefore both "Total Cattle of Feedlots" and "Total Cattle on Feedlots >1,000" are the same at 341,694 (Table 5 above). Beef counts on AFOs were estimated from state and county data as described in Section 4.3 and Table 4. The estimation indicated that approximately 1.178% of beef are on AFOs (<1,000 head) in Castro (1.178% * 341,694 = 4,025).
*For Ohio, Michigan, and Pennsylvania, the number of beef on lots <1,000 head was calculated from state and county-level Census of Agriculture stats. Refer to the row labeled "% of Cattle on feedlots <1,000 head "in Table 10 for details on how that was determined (for example, 93.8% of beef are lots <1,000 AU (Table 10), so 0.938 * 43,349 = 40,661).

Table 6: Area (Acres) and Feedlot Beef Cattle Counts for the Modeled Watersheds in the Five Study Regions

Study Region	Watershed ID (HUC 12)	Watershed Area (Acre)	Cattle on Feedlots <1,000 head in Watershed	Cattle on Feedlots >1,000 head in Watershed	Source of Watershed Cattle Counts
Lyons & Sioux County, Iowa	101702032002	21,128	5,373	10,410	Iowa DNR AFO GIS Database†
Castro, Texas	120500050303	27,073	247	20,965	Texas CEQ CAFO GIS Database in combination with '07 Cens of Ag State & County Stats††
Mercer, Ohio	Watershed Area Estimated ^	6,737	1,233	167 round up to 1,000 AU~	Feedlot Cattle Estimated ^
Huron, Michigan	Watershed Area Estimated ^	10,708	935	1,513	Feedlot Cattle Estimated ^
Lancaster, Pennsylvania	Watershed Area Estimated ^	16,051	1,822	142 round up to 1,000 AU~	Feedlot Cattle Estimated ^

This table provides feedlot cattle counts for the modeled watersheds in each study region, along with the watershed identifier (USGS HUC ID) for the watershed examined.
†For Iowa, CAFO and AFO beef counts represent “permitted beef” from the GIS database.
††For TX, beef counts for CAFOs represent “permitted beef” from GIS database adjusted to match the Census of Agriculture figures (refer to Section 4.3 for the approach). Beef counts on AFOs at the county-level were proportionally weighted to the watershed based on the density of CAFOs in each watershed. This process is described in detail in Section 4.3.
^In Ohio, Michigan, and Pennsylvania, the watershed modeled was theoretical with the feedlot beef counts and the watershed area estimated using the approach discussed in Section 4.6.
~During the estimation process applied in Ohio, Michigan, and Pennsylvania, the county level data that was extrapolated (Section 4.6) to the watershed level resulted in not enough cattle in lots >1,000 head. Therefore it was assumed one CAFO was located in the watershed and the value of 167 or 142 head was rounded up to 1,000 AU to calculate the density since a >1,000 head lot cannot contain less than 1,000 head.

Table 7: Calculation of Acres of Land Required Per Animal for each Study Region and Crop

State	Crop (yield)	Expected yield (bu/acre)	P_2O_5 Removal by Crop (lb/bu)	Acres Required Per Feedlot Animal Unit AU
Iowa	Corn Grain (bu)	203	0.39	0.758 §
Pennsylvania	Corn Silage (bu)	178	0.63	0.535
Ohio	Corn Grain (bu)	175	0.39	0.879
Michigan	Corn Grain (bu)	175	0.39	0.879
Texas	Corn Grain (bu)	225	0.39	0.684

§ An example of the acreage requirement from the yearly production of manure from one AU is given here (365 d x 0.0747 kg P_2O_5 produced/AU/d) / (203 bu/acre x 0.39 lb P_2O_5/bu x kg/2.2046 lb) = 0.758 acres/AU. See section 4.4 for details.

Table 8: Distribution of Watershed Feedlot Cattle Densities and Manured Acres Densities in Relation to the Modeled Watersheds for Iowa and Texas Study Areas

	Iowa Watershed (n=42 watersheds)		Texas Watershed (n=35 watersheds)	
Percent Rank^	**Feedlot (<1,000 head) Density (%)**	**Manured Acres Density (%)**	**Feedlot (<1,000 head) Density (%)**	**Manured Acres Density (%)**
100th Percentile	0.12	84.0	0.008	65.24
90th Percentile	0.09	61.39	0.003	52.22
75th Percentile	0.07	24.61	0.002	33.99
50th Percentile	0.036	14.79	0.0003	4.46
10th Percentile	.007	4.94	0.0	0.0
Modeled Watershed Density	0.094%†	56.52%†	0.003%‡	53.59%‡

^Percent rank is based on actual feedlot and manured acres densities that existed in all the watersheds for each study region. At the bottom of the table, the densities for the modeled watershed are provided in order to place their values into the context of the full range of possible values.
Modeled 90th percentile watershed PCA data from † Table 2 for Iowa and ‡ Table 3 for Texas. These data indicate that both the feedlot density and manured acres were close to the 90th percentile distribution of the study area.

Table 9: Distribution of Pasture Densities in Relation to the Modeled Watersheds in All Five Study Regions

Percent Rank^	Iowa Study Area Pasture Density (%) (n=42 watersheds)	Texas Study Area Pasture Density (%) (n=35 watersheds)	Pennsylvania Study Area Pasture Density (%) (n=33 watersheds)	Ohio Study Area Pasture Density (%) (n=18 watersheds)	Michigan Study Area Pasture Density (%) (n=27 watersheds)
100th Percentile	10.82	11.92	3.54	3.85	4.6
90th Percentile	6.07	9.75	2.67	2.93	4.05
75th Percentile	3.49	7.20	2.28	2.74	3.56
50th Percentile	3.04	4.29	1.84	2.24	2.24
10th Percentile	1.52	2.38	1.03	1.14	0.63
Modeled Watershed PCA	2.29% †	4.36% ‡	2.67% ‡‡	2.93% ‡‡	4.05 ‡‡

^Percent rank is based on actual pasture densities that existed in all the watersheds for each study region. At the bottom of the table, the densities for the modeled watershed are provided in order to place their values into the context of the full range of possible values.
Modeled 90th percentile watershed PCA data from † Table 2 for Iowa and ‡ Table 3 for Texas. ‡‡ For Michigan, Pennsylvania and Ohio, the actual 90th percentile pasture density was used in the modeling (refer to Section 4.5 for more information on pasture).

Table 10: Calculation of Pasture Cattle and Cattle in Lots <1,000 AU from Census of Agriculture Data

	Michigan Huron County	Ohio Mercer County	Pennsylvania Lancaster County	Texas Castro County	Iowa Lyon	Iowa Sioux
County Area (acres)	317,161	303,801	629,314	582,814	376,926	492,369
Total Cattle	105,734	79,058	270,577	530,890	143,014	328,317
Beef cows	1,321	2,091	6,289	11,458	12,030	14,754
Estimate of Beef Replacement Heifers	404‡ (30.6%)	439 (21.0%)	1,679 (26.7%)	1,696 (14.8%)	1,792 (14.9%)	2,198 (14.9%)
Milk Cows	27,237	21,515	109,653	28,702	10,941	21,706
Estimate of Dairy replacement heifers	11,249‡ (41.3%)	8,240 (38.3%)	53,839 (49.1%)	12,830 (44.7%)	6,247 (57.1%)	12,394 (57.1%)
Cattle on feed (feedlot cattle)	45,367	28,448	43,349	341,694	83,710	219,534
% of Cattle on feedlots <1,000 head	46% §	96.7% §	93.8% §	1.18% §§	34.0%¶	34.0%¶
Pasture cattle (subtraction)†	20,156	18,325	55,768	134,510	28,294	57,731

‡Beef and dairy replacement heifer data is not available on a county level. Therefore the state level data for beef cows and beef replacement heifers was used to determine the percent of replacement heifers in relation to cows and the state wide data extrapolated to the county level. For example in Michigan there are 33,000 beef replacement heifers and 108,000 beef cows (30.6%) according to the 2007 Census of Agriculture. This factor was then used to estimate county level beef replacement heifers from the number of beef cows (1,321 beef cows X 30.6% = 404 replacement heifers). This same procedure was used for milk cows and dairy replacement heifers.

†By subtraction (total cattle – (cows + cattle on feed + replacement heifers)). The estimate of pasture cattle also includes bulls and stags therefore this estimate is conservative for the number of pasture cattle. Also not all pasture cattle will be treated with Synovex-One since it is only for older cattle not first years.

§The percent of beef cattle in feedlots <1,000 head was estimated from data from individual counties' websites giving the number of cattle in the county in CAFOs ([MI CAFOs, 2011], [OH CAFOs, 2011], [PA CAFOs, 2010]).

§§Castro county feedlots <1,000 head was estimated from '07 Census of Agriculture county data of 0.654% in lots <500 head + state level estimate of 0.524% in lots 500-999 head (discussed in Section 4.3).

¶Values for feedlots <1,000 head of the actual watershed modeled in Lyon/Sioux Counties were obtained from Iowa DNR AFO GIS database [Iowa DNR, 2005].

Table 11: County-level Pasture Acreage and Cattle Numbers used when Calculating Watershed-level Pasture Acreage and Cattle Numbers

	Michigan Huron County	Ohio Mercer County	Pennsylvania Lancaster County	Texas Castro County	Iowa Lyon	Iowa Sioux
Pasturing Acreage from Census of Agriculture‡	12,685*	6,785*	14,468*	48,810*	9,071*	23,280*
Stocking Density (head/acre)	3.15	3.15	3.15	3.15	3.15	3.15
Estimated Pasture cattle†(Acres*Density)	39,958	21,372	45,574	153,751	28,573	73,332
County Pasture Cattle Estimate ††	20,156	18,325	55,768	134,510	28,294	57,731
Difference Between Pasture Cattle calculated via "Acres*Density" and "County Pasture Cattle Estimate"‡‡	98% greater than county estimate	16% greater than county estimate	18% less than county estimate	14% greater than county estimate	1% greater than county estimate	27% greater than county estimate

‡These are the figures that the NASS CDL (Remotely Sensed GIS data) Pasture/Hay acres were scaled to in order to account for mis-representation at the watershed-level. Refer to Section 4.6 for an explanation of why this was performed. After applying the scaling, the Pasture/Hay acreage will sum up to these figures for all the watersheds in a study area (e.g., the total acreage for the 27 watersheds in Michigan will sum up to 12,685.

* County-level figure from 2007 Census of Agriculture, "Crop Land used only for pasture or grazing (acres)" for Michigan, Ohio, and Sioux, Iowa. For Pennsylvania, Texas, and Lyon, Iowa, this is a county-level figure from 2007 Census of Agriculture, "Pasture Land All Types (Acres)".

†Pasture cattle numbers determined by pasture acreage. Each acre was multiplied by 3.15 cattle per acre to estimate the number of pasture cattle (e.g., For Michigan, 12,685*3.15=39,958).

††This is the county-level estimate of cattle from Table 10 (row labeled "Pasture cattle (subtraction)"and the value used to validate the watershed-level numbers.

‡‡This indicates that for all study regions, with the exception of Pennsylvania, the scaling approach applied to account for mis-representation, conservatively over-estimates the pasture cattle when compared with the county-level figure used as validation. For Pennsylvania, the scaling approach under-represented beef by 18%; however, the pasture cattle figure used as validation (55,768) includes bulls and stags therefore this estimate is conservative for the number of pasture cattle. Also not all pasture cattle will be treated with Synovex-One since it is only for older cattle not first years.

Table 12: Calculations for Estimating Feedlot Beef Cattle Statistics in the Modeled Watersheds in the Michigan, Pennsylvania, and Ohio Study Regions by Extrapolating Iowa Feedlot Beef Cattle Density Characteristics

Iowa study region feedlot beef cattle populations statistics applied to estimate feedlot beef cattle in the modeled watersheds in Pennsylvania, Ohio, and Michigan					
Iowa HUC12 Watershed ID	Total Area of All Watersheds in Study Region (acres)	Modeled Watershed Area (acres) / Study Area (acres) = % of Total Study Area		Cattle on Feedlots <1,000 in Watershed / Cattle on Feedlots <1,000 head in Study Area	Cattle on Feedlots > 1,000 in Watershed / Cattle on Feedlots > 1,000 head in Study Area
101702032002 †	995,598	21,128 / 995,598 = 2.1%		5,373/ 119,885 = 4.5%	10,410/ 168,521 = 6.2%
Extrapolation of Iowa high density watershed beef cattle characteristics to estimate feedlot beef cattle modeled watersheds in Pennsylvania, Ohio, and Michigan					
Study Region	Feedlot Cattle in County	No. Cattle on Feedlots <1,000 in County	No. Cattle on Feedlots >1,000 in County	No. Cattle on Feedlots <1,000 in Estimated Watershed	No. Cattle on Feedlots >1,000 in Estimated Watershed
Pennsylvania†	43,349	40,661	2,688	4.5% * 40,661 = 1,822	6.2% * 2,288 = 142 round to 1,000 #
Ohio†	28,448	27,509	939	4.5% * 27,509 = 1,233	6.2% * 2,688 = 167 round to 1,000 #
Michigan†	45,367	20,869	24,498	4.5% * 20,869 = 935	6.2% * 24,498 = 1,513
Final beef cattle densities (i.e., PCAs) estimated and watershed area for the estimated for modeled watersheds in Pennsylvania, Ohio, and Michigan					
Study Region	Total Area of All Watersheds in Study Region (acres)	Area of Estimated Watershed (acres)	Estimated Feedlot Density (%)	Estimated Cropland Manured Density (%)	Actual 90th percentile Pasture Density (%)
Pennsylvania	753,360†	2.1% * 753,360 = 16,051	0.040% ##	9.41% **	2.67% ‡‡
Ohio	317,468†	2.1% *317,458 = 6,737	0.068% ##	29.13% **	2.93% ‡‡
Michigan	504,592†	2.1% * 504,592 = 10,708	0.032% ##	20.10% **	4.05% ‡‡

†Watershed and study region area (acres) and cattle population numbers are found in Table 5 and Table 6, respectively.

#Extrapolation of county level data to watershed level, resulted in not enough cattle in lots >1,000 head. Therefore it was assumed one CAFO was located in the watershed and the value of 142 AU to was rounded up to 1,000 AU to calculate the PCA since a >1,000 head lot cannot contain less than 1,000 head.

##Feedlot Density example calculation: 1,822 AU *15m^2 per AU = 27,330 m^2 * .000247 m^2 per acre = 6.75 feedlot acres / 16,051 watershed acres = 0.04%

**Cropland manured example calculation: (1,822 AU + 1000 AU) X 0.535 acres per AU (Table 11) = 1,510 acres / 16,061=9.41%

‡‡Actual 90th percentile pasture densities from Table 9.

Table 13: Final Beef Cattle Counts and Densities for the Modeled Watersheds

State Counties	Michigan Huron County	Ohio Mercer County	Pennsylvania Lancaster County	Texas Castro County	Iowa Lyon + Sioux Counties
Watershed Area (acres)	10,708	6,737	16,051	27,073	21,128
# Beef Cattle in <1,000 head feedlots	935^	1,233	1,822	247	5,373
# Beef Cattle in >1,000 head feedlots	1,513#	167 round up to 1,000 AU	142 round up to 1,000 AU	20,965	10,410
# Beef Pasture Cattle	1,366*	622	1,350	3,720	1,525
	Watershed Density Factors (PCAs) for Modeled Watersheds				
Feedlot <1,000 head	0.032%	0.068%	0.04%	0.003%	0.094%
Cropland Manured	20.10%	29.13%	9.41%	53.59%	56.62%
Beef Pasture Land	4.05%	2.93%	2.67%	4.36%	2.29%

^Example calculation of beef cattle on AFOs from feedlot density: 10,708 acres X 0.0324% = 3.467 acres of feedlots / 15m2 per AU (or 0.00371 acres per AU) = 935 beef on feedlots.
#Example calculation of beef cattle on CAFOs from cropland manured density: 10,708 acres X 20.10% = 2,152.3 acres manured / 0.879 acres per AU (P_2O_5 rate from Table 11) = 2,449 total beef – 935 AFO beef = 1,513 beef on CAFOs.
*Example calculation of pasture cattle from pasture land density: 10,708 acres X 4.05% = 433.7 acres of pasture land X 3.15 AU/acre = 1,366 beef on pasture.

Table 14: Model Selection Matrix

Approach	Developer	Type	Output	Input Parameters	Complexity	Comments
GENEEC/ FIRST	USEPA-OPP	meta-model	pesticide	application rates, chemical properties	low	Meta-model of PRZM-EXAMS
PRZM-EXAMS	USEPA -OPP	mechanistic	pesticide	crop, location, application rates/dates, chemical properties		Single PRZM run into single EXAMS compartment. PE5 and EXPRESS modeling platforms.
APEX	USDA-ARS	mechanistic	water, sediment, nutrient, pesticide	pesticide rates, chemical properties, soil, cropping, and weather parameters		Developed to extend EPIC to small watersheds. Continuous simulation model to evaluate land management strategies in whole farm/small watershed systems.
winPRZM-EXAMS	Waterborne Environmental, Inc.	mechanistic	water, sediment, chemical	chemical rates, chemical properties, soil, cropping, two-compartment waterbody, and weather parameters		PRZM (Version 4.51) adapted for pasture and feedlot loadings into EXAMS waterbody.
winPRZM-RIVWQ	Waterborne Environmental, Inc.	mechanistic	water, sediment, chemical	chemical rates, chemical properties, soil, cropping, channel geometry, and weather parameters		Multiple PRZM runs provide loadings into tributary model. PRZM Version 4.51 adapted for pasture and feedlot loadings.
SWAT	USDA-ARS	mechanistic	water, sediment, nutrient, pesticide	pesticide properties, chemical properties, soil, cropping, and weather parameters		Appropriate for small or large watersheds. Subbasins are homogenous HRUs. GIS GUI interface.
PRZM-WASP5	USEPA -ORD	mechanistic	water, sediment, chemical	pesticide properties, chemical properties, soil, cropping, weather parameters, channel properties	high	Unsteady flow model. WASP is a merge of DYNHYD and EXAMS. Duplicated input entry. Prone to instability. Would not recommend for upland watersheds.

Table 15: Physical and Chemical Properties of Estradiol and Trenbolone Metabolites

Parameter	Trenbolone Metabolites	Estradiol Metabolites
Molecular Weight	270.4	272.4
Vapor Pressure (Torr)	7.5E-10	7.5E-11
Henry's Constant (atm-m^3/mole)*	7.41E-13	6.9E-12
Aqueous Solubility (mg/L)	360	3.9
Hydrolysis	Stable (assumed zero)	Stable(assumed zero)
Soil Koc	912	1259
Soil DT_{50} (days)	3.0	3.1
Anaerobic water sediment DT_{50} (days)	191.0	107.8
Aerobic water sediment DT_{50} (days)	53.3	31.1

* Henry's constant (atm-m^3/mole) = (vapor pressure (torr) / 760) / (solubility (mg/L) /Mol. Weight)

Table 16: Application Timing Selected for Cropland Applications

Study Region	Cropland- solid manure	Cropland-collection pond water
Iowa	4-May and 26-Oct	30-May, 30-June, 30-July and 30-Aug
Pennsylvania	15-Mar and 11-Oct	30-Apr, 30-May, 30-June and 30-July
Ohio	15-Apr and 11-Nov	30-May, 30-June, 30-July and 30-Aug
Michigan	26-Apr and 29-Oct	30-May, 30-June, 30-July and 30-Aug
Texas	24-Feb and 24-Sep	30-Mar, 30-Apr, 30-May and 30-June

Table 17: Application Rates used in PRZM Simulations in g/ha

	Trenbolone				Estradiol			
Study Region	**Cropland-solid manure**	**Cropland-collection pond water**	**Feedlot**	**Pasture**	**Cropland-solid manure**	**Cropland-collection pond water**	**Feed-lot**	**Pasture**
Iowa	0.3468	0.1734	50.8	0.00342	0.0454	0.0227	6.57	0.00044
Pennsylvania	0.4912	0.2456	50.8	0.00342	0.0642	0.0321	6.57	0.00044
Ohio	0.2990	0.1495	50.8	0.00342	0.0391	0.0195	6.57	0.00044
Michigan	0.2990	0.1495	50.8	0.00342	0.0391	0.0195	6.57	0.00044
Texas	0.3844	0.1922	50.8	0.00342	0.0503	0.0251	6.57	0.00044

Note: The yearly application rates in mg/acre were derived and presented in the EA for Synovex [Zoetis, 2012]. The application rates listed in this table are single application rates in g/ha as used in the modeling. Total yearly application rate of solid manure applied to crop land, is split into half representing - one half of the watershed is manured in spring and the other half in fall. Collection pond water applied to cropland is split into four representing four applications made throughout the cropping season. For example: In case of Iowa, the trenbolone application rate to cropland is 0.28069 g/ac. (0.28069 g/ac X 2.471 ha/ac X ½ watershed manured = 0.3468 g/ha. More details can be found in Section 5.3.3.

Table 18: PCA Factors or Watershed Density Factors Used for Each Region (%)

Study Region	Feedlot	Pasture	Cropland	Cropland-solid manure (split 90% of total cropland)	Cropland-pond water (split 10% of total cropland)
Iowa	0.094	2.29	56.62	50.96	5.66
Pennsylvania	0.040	2.67	9.41	8.47	0.94
Ohio	0.068	2.93	29.13	26.22	2.91
Michigan	0.032	4.05	20.10	18.09	2.01
Texas	0.003	4.36	53.59	48.23	5.36

Table 19: Trenbolone Concentrations for Iowa Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	**App rate (g/ha)**	**# of App**	**CAM**	**DEPI (cm)**	**Application date**	**PCA (%)**	**Peak**	**96 Hr**	**21 Day**	**60 Day**	**90 Day**	**Yearly**
Cropland-solid	0.3468	2	4	15	4-May and 26-Oct	50.96	2.550	2.510	2.417	2.265	2.143	1.769
Cropland-pond water	0.1734	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	50.8	NA	4	10	NA	0.094*						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.29						
Cropland-solid	0.3468	2	4	15	4-May and 26-Oct	50.96	1.951	1.920	1.854	1.731	1.645	1.345
Cropland-pond water	0.1734	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	50.8	NA	4	10	NA	0.071†						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.29						
Cropland-solid	0.3468	2	4	15	4-May and 26-Oct	50.96	1.330	1.309	1.264	1.182	1.123	0.905
Cropland-pond water	0.1734	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	50.8	NA	4	10	NA	0.047‡						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.29						
Cropland-solid	0.3468	2	4	15	4-May and 26-Oct	50.96	0.737	0.724	0.699	0.666	0.623	0.484
Cropland-pond water	0.1734	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	50.8	NA	4	10	NA	0.024§						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.29						
Cropland-solid	0.3468	2	4	15	4-May and 26-Oct	50.96	0.164	0.159	0.150	0.120	0.102	0.048
Cropland-pond water	0.1734	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	50.8	NA	4	10	NA	0**						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.29						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 20: Estradiol Concentrations for Iowa Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	**App rate (g/ha)**	**# of App**	**CAM**	**DEPI (cm)**	**Application date**	**PCA (%)**	**Peak**	**96 Hr**	**21 Day**	**60 Day**	**90 Day**	**Yearly**
Cropland-solid	0.0454	2	4	15	4-May and 26-Oct	50.96	0.235	0.228	0.216	0.195	0.182	0.139
Cropland-pond water	0.0227	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	6.57	NA	4	10	NA	0.094*						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.29						
Cropland-solid	0.0454	2	4	15	4-May and 26-Oct	50.96	0.180	0.174	0.165	0.148	0.139	0.105
Cropland-pond water	0.0227	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	6.57	NA	4	10	NA	0.071†						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.29						
Cropland-solid	0.0454	2	4	15	4-May and 26-Oct	50.96	0.122	0.119	0.111	0.099	0.094	0.070
Cropland-pond water	0.0227	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	6.57	NA	4	10	NA	0.047‡						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.29						
Cropland-solid	0.0454	2	4	15	4-May and 26-Oct	50.96	0.067	0.065	0.060	0.054	0.050	0.037
Cropland-pond water	0.0227	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	6.57	NA	4	10	NA	0.024§						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.29						
Cropland-solid	0.0454	2	4	15	4-May and 26-Oct	50.96	0.017	0.017	0.015	0.012	0.009	0.003
Cropland-pond water	0.0227	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	5.66						
Feedlot	6.57	NA	4	10	NA	0**						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.29						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 21: Trenbolone Concentrations for Pennsylvania Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	App rate (g/ha)	# of App	CAM	DEPI (cm)	Application date	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
cropland-solid	0.4912	2	4	5	15-Mar and 11-Oct	8.47	1.141	1.123	1.067	1.009	0.993	0.841
cropland-pond water	0.2456	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
feedlot	50.8	NA	4	10	NA	0.04*						
pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.67						
cropland-solid	0.4912	2	4	5	15-Mar and 11-Oct	8.47	0.861	0.845	0.803	0.761	0.746	0.632
cropland-pond water	0.2456	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
feedlot	50.8	NA	4	10	NA	0.03†						
pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.67						
cropland-solid	0.4912	2	4	5	15-Mar and 11-Oct	8.47	0.578	0.567	0.539	0.512	0.498	0.422
cropland-pond water	0.2456	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
feedlot	50.8	NA	4	10	NA	0.02‡						
pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.67						
cropland-solid	0.4912	2	4	5	15-Mar and 11-Oct	8.47	0.295	0.290	0.274	0.258	0.251	0.215
cropland-pond water	0.2456	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
feedlot	50.8	NA	4	10	NA	0.01§						
pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.67						
cropland-solid	0.4912	2	4	5	15-Mar and 11-Oct	8.47	0.016	0.015	0.013	0.010	0.008	0.004
cropland-pond water	0.2456	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
feedlot	50.8	NA	4	10	NA	0**						
pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.67						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 22: Estradiol Concentrations for Pennsylvania Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	App rate (g/ha)	# of App	CAM	DEPI (cm)	Application date	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
Cropland-solid	0.0642	2	4	5	15-Mar and 11-Oct	8.47	0.110	0.106	0.101	0.097	0.093	0.073
Cropland-pond water	0.0321	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
Feedlot	6.57	NA	4	10	NA	0.04*						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.67						
Cropland-solid	0.0642	2	4	5	15-Mar and 11-Oct	8.47	0.082	0.080	0.076	0.073	0.070	0.055
Cropland-pond water	0.0321	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
Feedlot	6.57	NA	4	10	NA	0.03†						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.67						
Cropland-solid	0.0642	2	4	5	15-Mar and 11-Oct	8.47	0.055	0.054	0.051	0.048	0.047	0.037
Cropland-pond water	0.0321	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
Feedlot	6.57	NA	4	10	NA	0.02‡						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.67						
Cropland-solid	0.0642	2	4	5	15-Mar and 11-Oct	8.47	0.028	0.027	0.025	0.024	0.023	0.019
Cropland-pond water	0.0321	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
Feedlot	6.57	NA	4	10	NA	0.01§						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.67						
Cropland-solid	0.0642	2	4	5	15-Mar and 11-Oct	8.47	0.002	0.002	0.001	0.001	0.001	0.000
Cropland-pond water	0.0321	4	4	5	30-Apr, 30-May, 30-Jun, 30-Jul	0.94						
Feedlot	6.57	NA	4	10	NA	0**						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.67						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 23: Trenbolone Concentrations for Ohio Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	App rate (g/ha)	# of App	CAM	DEPI (cm)	Application date	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
Cropland-solid	0.299	2	4	5	15-Apr and 11-Nov	26.22	1.943	1.901	1.831	1.763	1.696	1.418
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	50.8	NA	4	10	NA	0.068*						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.93						
Cropland-solid	0.299	2	4	5	15-Apr and 11-Nov	26.22	1.461	1.429	1.375	1.325	1.275	1.066
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	50.8	NA	4	10	NA	0.051†						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.93						
Cropland-solid	0.299	2	4	5	15-Apr and 11-Nov	26.22	0.979	0.958	0.920	0.888	0.852	0.713
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	50.8	NA	4	10	NA	0.034‡						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.93						
Cropland-solid	0.299	2	4	5	15-Apr and 11-Nov	26.22	0.498	0.487	0.464	0.450	0.430	0.361
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	50.8	NA	4	10	NA	0.017§						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.93						
Cropland-solid	0.299	2	4	5	15-Apr and 11-Nov	26.22	0.069	0.067	0.058	0.042	0.032	0.014
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	50.8	NA	4	10	NA	0**						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	2.93						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 24: Estradiol Concentrations for Ohio Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	App rate (g/ha)	# of App	CAM	DEPI (cm)	Application date	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
Cropland-solid	0.0391	2	4	5	15-Apr and 11-Nov	26.22	0.183	0.181	0.176	0.160	0.155	0.120
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	6.57	NA	4	10	NA	0.068*						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.93						
Cropland-solid	0.0391	2	4	5	15-Apr and 11-Nov	26.22	0.138	0.136	0.132	0.120	0.116	0.090
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	6.57	NA	4	10	NA	0.051†						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.93						
Cropland-solid	0.0391	2	4	5	15-Apr and 11-Nov	26.22	0.092	0.091	0.088	0.080	0.078	0.060
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	6.57	NA	4	10	NA	0.034‡						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.93						
Cropland-solid	0.0391	2	4	5	15-Apr and 11-Nov	26.22	0.047	0.046	0.044	0.041	0.040	0.031
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	6.57	NA	4	10	NA	0.017§						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.93						
Cropland-solid	0.0391	2	4	5	15-Apr and 11-Nov	26.22	0.007	0.007	0.006	0.004	0.003	0.001
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.91						
Feedlot	6.57	NA	4	10	NA	0**						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	2.93						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 25: Trenbolone Concentrations for Michigan Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	**App rate (g/ha)**	**# of App**	**CAM**	**DEPI (cm)**	**Application date**	**PCA (%)**	**Peak**	**96 Hr**	**21 Day**	**60 Day**	**90 Day**	**Yearly**
Cropland-solid	0.299	2	4	15	26-Apr and 29-Oct	18.09	0.825	0.813	0.774	0.731	0.722	0.595
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	50.8	NA	4	10	NA	0.032*						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	4.05						
Cropland-solid	0.299	2	4	15	26-Apr and 29-Oct	18.09	0.622	0.613	0.585	0.550	0.543	0.448
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	50.8	NA	4	10	NA	0.024†						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	4.05						
Cropland-solid	0.299	2	4	15	26-Apr and 29-Oct	18.09	0.421	0.414	0.396	0.368	0.364	0.301
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	50.8	NA	4	10	NA	0.016‡						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	4.05						
Cropland-solid	0.299	2	4	15	26-Apr and 29-Oct	18.09	0.221	0.217	0.207	0.189	0.185	0.154
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	50.8	NA	4	10	NA	0.008§						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	4.05						
Cropland-solid	0.299	2	4	15	26-Apr and 29-Oct	18.09	0.030	0.029	0.026	0.021	0.016	0.011
Cropland-pond water	0.1495	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	50.8	NA	4	10	NA	0**						
Pasture	0.00342	211	4	5	1-Apr to 28-Oct	4.05						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 26: Estradiol Concentrations for Michigan Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	App rate (g/ha)	# of App	CAM	DEPI (cm)	Application date	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
Cropland-solid	0.0391	2	4	15	26-Apr and 29-Oct	18.09	0.080	0.078	0.072	0.068	0.065	0.051
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	6.57	NA	4	10	NA	0.032*						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	4.05						
Cropland-solid	0.0391	2	4	15	26-Apr and 29-Oct	18.09	0.060	0.059	0.054	0.051	0.049	0.039
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	6.57	NA	4	10	NA	0.024†						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	4.05						
Cropland-solid	0.0391	2	4	15	26-Apr and 29-Oct	18.09	0.040	0.040	0.037	0.034	0.033	0.026
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	6.57	NA	4	10	NA	0.016‡						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	4.05						
Cropland-solid	0.0391	2	4	15	26-Apr and 29-Oct	18.09	0.021	0.021	0.019	0.017	0.017	0.013
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	6.57	NA	4	10	NA	0.008§						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	4.05						
Cropland-solid	0.0391	2	4	15	26-Apr and 29-Oct	18.09	0.003	0.003	0.002	0.002	0.001	0.001
Cropland-pond water	0.0195	4	4	5	30-May, 30-Jun, 30-Jul, 30-Aug	2.01						
Feedlot	6.57	NA	4	10	NA	0**						
Pasture	0.0004	270	4	5	1-Apr to 26-Dec	4.05						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 27: Trenbolone Concentrations for Texas Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	App rate (g/ha)	# of App	CAM	DEPI (cm)	Application date	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
Cropland-solid	0.3844	2	4	15	24-Feb and 24-Sep	48.23	0.293	0.280	0.237	0.174	0.146	0.057
Cropland-pond water	0.1922	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36						
Feedlot	50.8	NA	4	10	NA	0.003*						
Pasture	0.00342	211	4	5	1-Mar to 27-Sep	4.36						
Cropland-solid	0.3844	2	4	15	24-Feb and 24-Sep	48.23	0.288	0.275	0.232	0.169	0.142	0.052
Cropland-pond water	0.1922	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36						
Feedlot	50.8	NA	4	10	NA	0.0023†						
Pasture	0.00342	211	4	5	1-Mar to 27-Sep	4.36						
Cropland-solid	0.3844	2	4	15	24-Feb and 24-Sep	48.23	0.282	0.268	0.226	0.164	0.136	0.047
Cropland-pond water	0.1922	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36						
Feedlot	50.8	NA	4	10	NA	0.0015‡						
Pasture	0.00342	211	4	5	1-Mar to 27-Sep	4.36						
Cropland-solid	0.3844	2	4	15	24-Feb and 24-Sep	48.23	0.276	0.263	0.221	0.159	0.132	0.042
Cropland-pond water	0.1922	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36						
Feedlot	50.8	NA	4	10	NA	0.0008§						
Pasture	0.00342	211	4	5	1-Mar to 27-Sep	4.36						
Cropland-solid	0.3844	2	4	15	24-Feb and 24-Sep	48.23	0.270	0.257	0.215	0.154	0.127	0.037
Cropland-pond water	0.1922	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36						
Feedlot	50.8	NA	4	10	NA	0**						
Pasture	0.00342	211	4	5	1-Mar to 27-Sep	4.36						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 28: Estradiol Concentrations for Texas Region for all Sources and Varying Feedlot PCAs

							90th percentile concentrations (ng/L)					
Land use	App rate (g/ha)	# of App	CAM	DEPI (cm)	Application date	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
Cropland-solid	0.0503	2	4	15	24-Feb and 24-Sep	48.23						
Cropland-pond water	0.0251	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36	0.032	0.030	0.024	0.016	0.013	0.005
Feedlot	6.57	NA	4	10	NA	0.003*						
Pasture	0.0004	270	4	5	1-Mar to 26-Nov	4.36						
Cropland-solid	0.0503	2	4	15	24-Feb and 24-Sep	48.23						
Cropland-pond water	0.0251	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36	0.031	0.029	0.023	0.016	0.013	0.005
Feedlot	6.57	NA	4	10	NA	0.0023†						
Pasture	0.0004	270	4	5	1-Mar to 26-Nov	4.36						
Cropland-solid	0.0503	2	4	15	24-Feb and 24-Sep	48.23						
Cropland-pond water	0.0251	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36	0.031	0.029	0.023	0.015	0.012	0.004
Feedlot	6.57	NA	4	10	NA	0.0015‡						
Pasture	0.0004	270	4	5	1-Mar to 26-Nov	4.36						
Cropland-solid	0.0503	2	4	15	24-Feb and 24-Sep	48.23						
Cropland-pond water	0.0251	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36	0.030	0.028	0.023	0.015	0.012	0.004
Feedlot	6.57	NA	4	10	NA	0.0008§						
Pasture	0.0004	270	4	5	1-Mar to 26-Nov	4.36						
Cropland-solid	0.0503	2	4	15	24-Feb and 24-Sep	48.23						
Cropland-pond water	0.0251	4	4	5	30-Mar, 30-Apr, 30-May, 30-Jun	5.36	0.030	0.028	0.022	0.015	0.012	0.003
Feedlot	6.57	NA	4	10	NA	0**						
Pasture	0.0004	270	4	5	1-Mar to 26-Nov	4.36						

* 100% of feedlot PCA; † 75% of feedlot PCA; ‡ 50% of feedlot PCA; § 25% of feedlot PCA; ** 0% of feedlot PCA

Table 29: Linear Regression Equations Estimated for each Time Period by Plotting 90th percentile Concentrations from Varying Feedlot PCAs

Region	Drug	Peak	96 hr	21 Day	60 Day	90 Day	Yearly
IA	Trenbolone	y = 0.0239x + 0.1489	y = 0.0236x + 0.1447	y = 0.0228x + 0.1388	y = 0.0214x + 0.1215	y = 0.0204x + 0.1066	y = 0.0172x + 0.0496
	Estradiol	y = 0.0022x + 0.0149	y = 0.0021x + 0.0144	y = 0.002x + 0.0117	y = 0.0018x + 0.0096	y = 0.0017x + 0.0081	y = 0.0014x + 0.0031
PA	Trenbolone	y = 0.0113x + 0.0149	y = 0.0111x + 0.0138	y = 0.0105x + 0.0118	y = 0.01x + 0.0101	y = 0.0099x + 0.0067	y = 0.0084x + 0.0044
	Estradiol	y = 0.0011x + 0.0015	y = 0.001x + 0.0014	y = 0.001x + 0.0008	y = 0.001x + 0.0007	y = 0.0009x + 0.0006	y = 0.0007x + 0.0004
OH	Trenbolone	y = 0.0188x + 0.0479	y = 0.0184x + 0.0464	y = 0.0178x + 0.0382	y = 0.0173x + 0.03	y = 0.0167x + 0.0226	y = 0.0141x + 0.0119
	Estradiol	y = 0.0018x + 0.0049	y = 0.0018x + 0.0044	y = 0.0017x + 0.0036	y = 0.0016x + 0.0027	y = 0.0015x + 0.0022	y = 0.0012x + 0.0009
MI	Trenbolone	y = 0.008x + 0.0252	y = 0.0079x + 0.0242	y = 0.0075x + 0.0225	y = 0.0071x + 0.0153	y = 0.0071x + 0.012	y = 0.0058x + 0.0097
	Estradiol	y = 0.0008x + 0.0023	y = 0.0008x + 0.0022	y = 0.0007x + 0.002	y = 0.0007x + 0.0014	y = 0.0006x + 0.0011	y = 0.0005x + 0.0008
TX	Trenbolone	y = 0.0002x + 0.2702	y = 0.0002x + 0.257	y = 0.0002x + 0.215	y = 0.0002x + 0.1541	y = 0.0002x + 0.1272	y = 0.0002x + 0.037
	Estradiol	y = 2E-05x + 0.0298	y = 2E-05x + 0.0278	y = 2E-05x + 0.022	y = 2E-05x + 0.0146	y = 2E-05x + 0.0116	y = 2E-05x + 0.0033

Note: Graphical plots of 90th percentile concentrations for each time period were plotted and linear line was fitted through the data to estimate linear regression equation in Microsoft Excel. Plots for 21-day 90th percentile concentrations are presented from Figure 13 to Figure 17. Plots for other time periods are not presented in the report for the sake of brevity.

Table 30: Trenbolone and Estradiol Concentrations Assuming 100% Watershed is Pasture

							90th percentile concentrations (ng/L)					
Chemical	**Application rate (g/ha)**	**# of Applications**	**CAM**	**DEPI (cm)**	**Application dates**	**PCA (%)**	**Peak**	**96 hr**	**21 Day**	**60 Day**	**90 Day**	**Yearly**
Iowa												
Trenbolone	0.00342	211	4	5	1-Apr to 28-Oct	100	0.324	0.314	0.277	0.228	0.203	0.107
Estradiol	0.00044	270	4	5	1-Apr to 26-Dec	100	0.032	0.030	0.025	0.019	0.016	0.007
Pennsylvania												
Trenbolone	0.00342	211	4	5	1-Apr to 28-Oct	100	0.181	0.172	0.150	0.120	0.104	0.062
Estradiol	0.00044	270	4	5	1-Apr to 26-Dec	100	0.017	0.016	0.013	0.010	0.008	0.005
Ohio												
Trenbolone	0.00342	211	4	5	1-Apr to 28-Oct	100	0.343	0.331	0.295	0.231	0.208	0.132
Estradiol	0.00044	270	4	5	1-Apr to 26-Dec	100	0.032	0.031	0.026	0.019	0.018	0.011
Michigan												
Trenbolone	0.00342	211	4	5	1-Apr to 28-Oct	100	0.293	0.282	0.248	0.207	0.186	0.106
Estradiol	0.00044	270	4	5	1-Apr to 26-Dec	100	0.026	0.025	0.021	0.016	0.014	0.008
Texas												
Trenbolone	0.00342	211	4	5	1-Mar to 27-Sep	100	0.273	0.259	0.222	0.154	0.141	0.062
Estradiol	0.00044	270	4	5	1-Mar to 26-Nov	100	0.028	0.026	0.020	0.013	0.011	0.005

Table 31: Principal Crops Grown in Each Study Region per 2007 USDA, Census of Agriculture

Crop	Acres	% of Total
Sioux and Lyon Counties, Iowa		
Corn for grain	395,255	55.50%
Soybeans for beans	265,180	37.20%
Soybeans for beans	31,818	4.50%
Forage - land used for all hay and all haylage, grass silage, and greenchop	17,432	2.40%
Oats for grain	1,747	0.20%
Wheat for grain, all	1,187	0.20%
All Other Crops	56	0.00%
Total Acres	712,675	
Mercer County, Ohio		
Corn for grain	101,078	39.00%
Soybeans for beans	100,178	38.60%
Wheat for grain, all	24,072	9.30%
Forage - land used for all hay and all haylage, grass silage, and greenchop	16,990	6.60%
Corn for silage or greenchop	15,891	6.10%
All other crops	1,120	0.40%
Total Acres	259,329	
Huron County, Michigan		
Corn for grain	99,757	26.90%
Dry edible beans, excluding limas	72,896	19.70%
Wheat for grain, all	58,801	15.90%
Sugarbeets for sugar	52,740	14.20%
Soybeans for beans	31,935	8.60%
Corn for silage or greenchop	25,664	6.90%
Forage - land used for all hay and all haylage, grass silage, and greenchop	25,240	6.80%
All other crops	3,667	1.00%
Total Acres	370,700	

Table 31: Principal Crops Grown in Each Study Region per 2007 USDA, Census of Agriculture (continued)

Crop	Acres	% of Total
Lancaster County, Pennsylvania		
Corn for grain	101,981	32.00%
Forage - land used for all hay and all haylage, grass silage, and greenchop	84,366	26.50%
Corn for silage or greenchop	68,238	21.40%
Soybeans for beans	30,673	9.60%
Wheat for grain, all	11,763	3.70%
Barley for grain	7,543	2.40%
Vegetables harvested for sale	6,139	1.90%
Tobacco	6,095	1.90%
All other crops	1,900	0.60%
Total Acres	318,698	
Castro County, Texas		
Corn for grain	98,414	32.80%
Wheat for grain, all	84,940	28.30%
Forage - land used for all hay and all haylage, grass silage, and greenchop	34,467	11.50%
Cotton, all	30,484	10.20%
Sorghum for grain	24,212	8.10%
Corn for silage or greenchop	16,688	5.60%
Sorghum for silage or greenchop	10,699	3.60%
Total Acres	299,904	

Table 32: Trenbolone Concentrations for 17 No-Till Crop Scenarios Modeled with Pond EXAMS Scenario

							90th percentile concentrations (ng/L)					
Scenario	App rate (kg/ha)	# of App	App timing	CAM	DEPI (cm)	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
CA corn	0.0011	1	21 days before planting	4	5	100	0.190	0.182	0.156	0.120	0.112	0.065
CA cotton	0.0009	1	7 days after harvest	4	5	100	0.098	0.094	0.083	0.052	0.047	0.026
IL corn	0.0007	1	28 days before planting	4	5	100	0.165	0.158	0.139	0.105	0.090	0.040
KS sorghum	0.0003	1	7 days before planting	4	5	100	0.112	0.107	0.091	0.066	0.054	0.021
MS corn	0.0006	1	14 days before planting	4	5	100	0.598	0.571	0.479	0.349	0.288	0.111
MS cotton	0.0006	1	1 days before planting	4	5	100	0.699	0.668	0.552	0.384	0.312	0.114
MS soybean	0.0004	1	21 days before planting	4	5	100	0.266	0.255	0.224	0.177	0.148	0.055
NC corn E	0.0004	1	28 days before planting	4	5	100	0.142	0.136	0.117	0.088	0.075	0.033
NC corn W	0.0008	1	14 days before planting	4	5	100	0.300	0.287	0.247	0.186	0.157	0.067
NC cotton	0.0007	1	1 days before planting	4	5	100	0.298	0.286	0.244	0.183	0.154	0.062
ND corn	0.0005	1	21 days after harvest	4	5	100	0.058	0.058	0.056	0.053	0.052	0.020
ND wheat	0.0004	1	28 days after harvest	4	5	100	0.056	0.056	0.054	0.052	0.050	0.021
OH corn	0.0007	1	28 days after harvest	4	5	100	0.417	0.401	0.359	0.215	0.201	0.111
OR wheat	0.0007	1	7 days before planting	4	5	100	0.011	0.011	0.009	0.007	0.006	0.004
PA corn	0.001	1	14 days before planting	4	5	100	0.210	0.201	0.178	0.134	0.114	0.047
TX sorghum	0.0003	1	7 days before planting	4	5	100	0.371	0.351	0.291	0.201	0.161	0.057
TX wheat	0.0004	1	21 days before planting	4	5	100	0.590	0.556	0.463	0.334	0.282	0.098

Table 33: Trenbolone Concentrations for 17 No-Till Crop Scenarios Modeled with Index Reservoir EXAMS Scenario

							90th percentile concentrations (ng/L)					
Scenario	App rate (kg/ha)	# of App	App timing	CAM	DEPI (cm)	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
CA corn	0.0011	1	21 days before planting	4	5	61	0.277	0.267	0.235	0.182	0.153	0.057
CA cotton	0.0009	1	7 days after harvest	4	5	33	0.077	0.075	0.067	0.042	0.030	0.013
IL corn	0.0007	1	28 days before planting	4	5	61	0.233	0.224	0.192	0.139	0.112	0.038
KS sorghum	0.0003	1	7 days before planting	4	5	91	0.239	0.229	0.194	0.139	0.110	0.036
MS corn	0.0006	1	14 days before planting	4	5	61	0.860	0.810	0.634	0.398	0.300	0.086
MS cotton	0.0006	1	1 days before planting	4	5	33	0.544	0.513	0.405	0.251	0.188	0.054
MS soybean	0.0004	1	21 days before planting	4	5	57	0.351	0.333	0.290	0.207	0.161	0.048
NC corn E	0.0004	1	28 days before planting	4	5	61	0.199	0.191	0.163	0.117	0.095	0.031
NC corn W	0.0008	1	14 days before planting	4	5	61	0.436	0.416	0.352	0.247	0.197	0.064
NC cotton	0.0007	1	1 days before planting	4	5	33	0.230	0.220	0.182	0.126	0.100	0.034
ND corn	0.0005	1	21 days after harvest	4	5	61	0.081	0.080	0.076	0.070	0.066	0.026
ND wheat	0.0004	1	28 days after harvest	4	5	38	0.049	0.047	0.044	0.040	0.038	0.016
OH corn	0.0007	1	28 days after harvest	4	5	61	0.603	0.582	0.522	0.300	0.222	0.099
OR wheat	0.0007	1	7 days before planting	4	5	38	0.010	0.009	0.008	0.006	0.005	0.002
PA corn	0.001	1	14 days before planting	4	5	61	0.303	0.291	0.259	0.190	0.155	0.053
TX sorghum	0.0003	1	7 days before planting	4	5	91	0.810	0.766	0.630	0.415	0.319	0.096
TX wheat	0.0004	1	21 days before planting	4	5	38	0.537	0.505	0.416	0.285	0.228	0.059

Table 34: Estradiol Concentrations for 17 No-Till Crop Scenarios Modeled with Pond EXAMS Scenario

							90th percentile concentrations (ng/L)					
Scenario	App rate (kg/ha)	# of App	App timing	CAM	DEPI (cm)	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
CA corn	0.00014	1	21 days before planting	4	5	100	0.0214	0.0201	0.0161	0.0111	0.0090	0.0032
CA cotton	0.00011	1	7 days after harvest	4	5	100	0.0098	0.0092	0.0077	0.0046	0.0031	0.0013
IL corn	0.00009	1	28 days before planting	4	5	100	0.0157	0.0148	0.0119	0.0081	0.0064	0.0023
KS sorghum	0.00003	1	7 days before planting	4	5	100	0.0084	0.0079	0.0062	0.0041	0.0032	0.0011
MS corn	0.00008	1	14 days before planting	4	5	100	0.0621	0.0580	0.0448	0.0289	0.0225	0.0071
MS cotton	0.00008	1	1 days before planting	4	5	100	0.0763	0.0713	0.0546	0.0334	0.0255	0.0079
MS soybean	0.00005	1	21 days before planting	4	5	100	0.0259	0.0243	0.0207	0.0148	0.0117	0.0037
NC corn E	0.00006	1	28 days before planting	4	5	100	0.0165	0.0155	0.0123	0.0082	0.0065	0.0022
NC corn W	0.00011	1	14 days before planting	4	5	100	0.0309	0.0290	0.0233	0.0157	0.0126	0.0044
NC cotton	0.00009	1	1 days before planting	4	5	100	0.0367	0.0346	0.0271	0.0179	0.0142	0.0050
ND corn	0.00007	1	21 days after harvest	4	5	100	0.0053	0.0050	0.0041	0.0038	0.0036	0.0014
ND wheat	0.00006	1	28 days after harvest	4	5	100	0.0059	0.0056	0.0044	0.0039	0.0037	0.0015
OH corn	0.00009	1	28 days after harvest	4	5	100	0.0404	0.0382	0.0322	0.0180	0.0142	0.0068
OR wheat	0.00009	1	7 days before planting	4	5	100	0.0012	0.0011	0.0009	0.0006	0.0005	0.0002
PA corn	0.00013	1	14 days before planting	4	5	100	0.0211	0.0199	0.0164	0.0111	0.0089	0.0031
TX sorghum	0.00004	1	7 days before planting	4	5	100	0.0442	0.0407	0.0307	0.0185	0.0139	0.0042
TX wheat	0.00005	1	21 days before planting	4	5	100	0.0635	0.0580	0.0445	0.0282	0.0226	0.0060

Table 35: Estradiol Concentrations for 17 No-Till Crop Scenarios Modeled with Index Reservoir EXAMS Scenario

							90th percentile concentrations (ng/L)					
Scenario	App rate (kg/ha)	# of App	App timing	CAM	DEPI (cm)	PCA (%)	Peak	96 Hr	21 Day	60 Day	90 Day	Yearly
CA corn	0.00014	1	21 days before planting	4	5	61	0.0314	0.0299	0.0250	0.0177	0.0142	0.0046
CA cotton	0.00011	1	7 days after harvest	4	5	33	0.0078	0.0074	0.0064	0.0039	0.0026	0.0009
IL corn	0.00009	1	28 days before planting	4	5	61	0.0229	0.0218	0.0178	0.0119	0.0092	0.0027
KS sorghum	0.00003	1	7 days before planting	4	5	91	0.0184	0.0173	0.0139	0.0090	0.0068	0.0021
MS corn	0.00008	1	14 days before planting	4	5	61	0.0892	0.0830	0.0617	0.0355	0.0259	0.0071
MS cotton	0.00008	1	1 days before planting	4	5	33	0.0600	0.0557	0.0413	0.0231	0.0168	0.0046
MS soybean	0.00005	1	21 days before planting	4	5	57	0.0349	0.0326	0.0276	0.0183	0.0137	0.0038
NC corn E	0.00006	1	28 days before planting	4	5	61	0.0240	0.0228	0.0184	0.0121	0.0093	0.0028
NC corn W	0.00011	1	14 days before planting	4	5	61	0.0451	0.0425	0.0342	0.0221	0.0169	0.0050
NC cotton	0.00009	1	1 days before planting	4	5	33	0.0284	0.0267	0.0206	0.0128	0.0098	0.0030
ND corn	0.00007	1	21 days after harvest	4	5	61	0.0077	0.0074	0.0061	0.0050	0.0047	0.0021
ND wheat	0.00006	1	28 days after harvest	4	5	38	0.0053	0.0051	0.0042	0.0031	0.0029	0.0014
OH corn	0.00009	1	28 days after harvest	4	5	61	0.0590	0.0563	0.0478	0.0273	0.0182	0.0081
OR wheat	0.00009	1	7 days before planting	4	5	38	0.0010	0.0010	0.0008	0.0006	0.0005	0.0001
PA corn	0.00013	1	14 days before planting	4	5	61	0.0307	0.0292	0.0247	0.0165	0.0128	0.0039
TX sorghum	0.00004	1	7 days before planting	4	5	91	0.0965	0.0892	0.0681	0.0398	0.0291	0.0081
TX wheat	0.00005	1	21 days before planting	4	5	38	0.0580	0.0532	0.0410	0.0252	0.0193	0.0049

Figures

Figure 1: Density of Beef Cattle Feedlots (2007 Census of Agriculture)

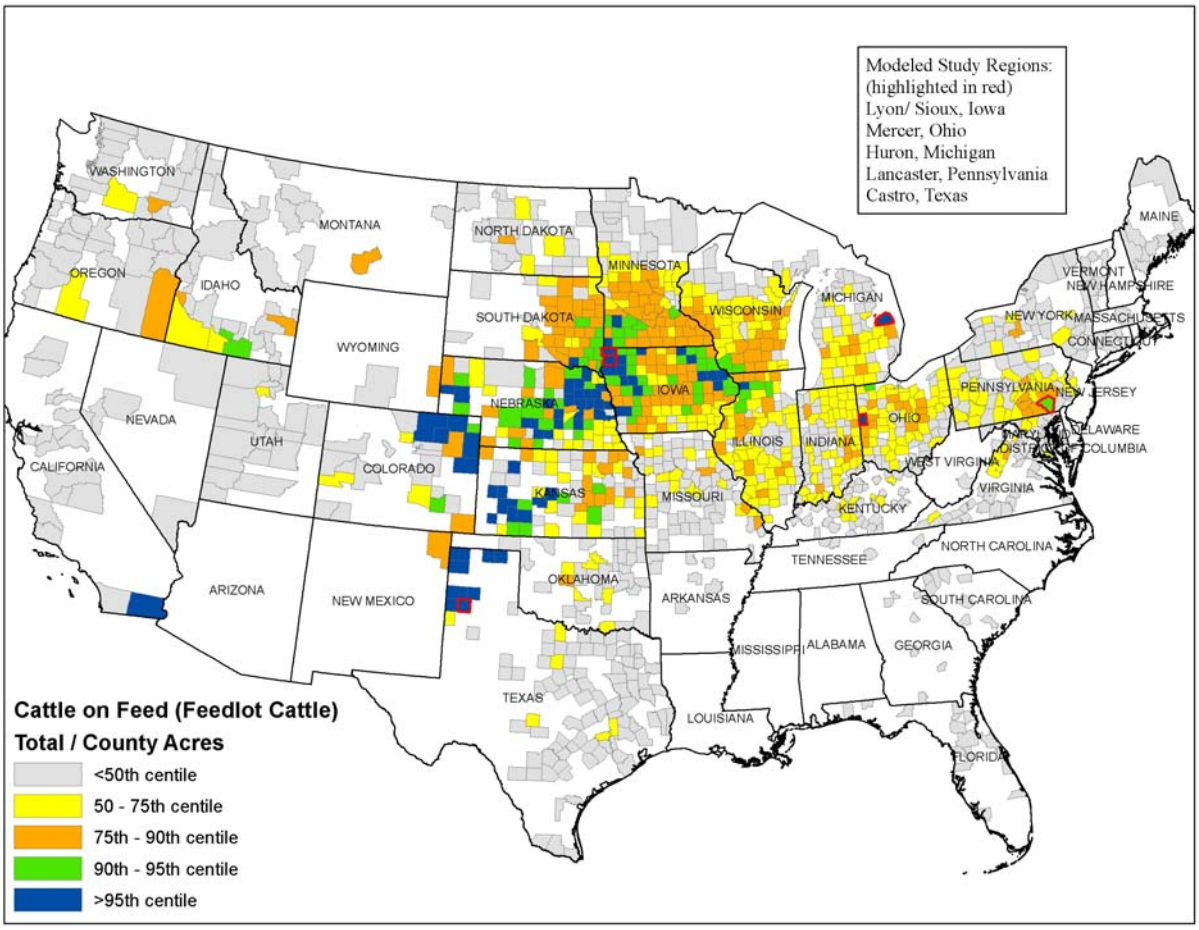


Figure 2: Density of Beef Cattle Feedlots with Greater Than 500 Head (2007 Census of Agriculture)

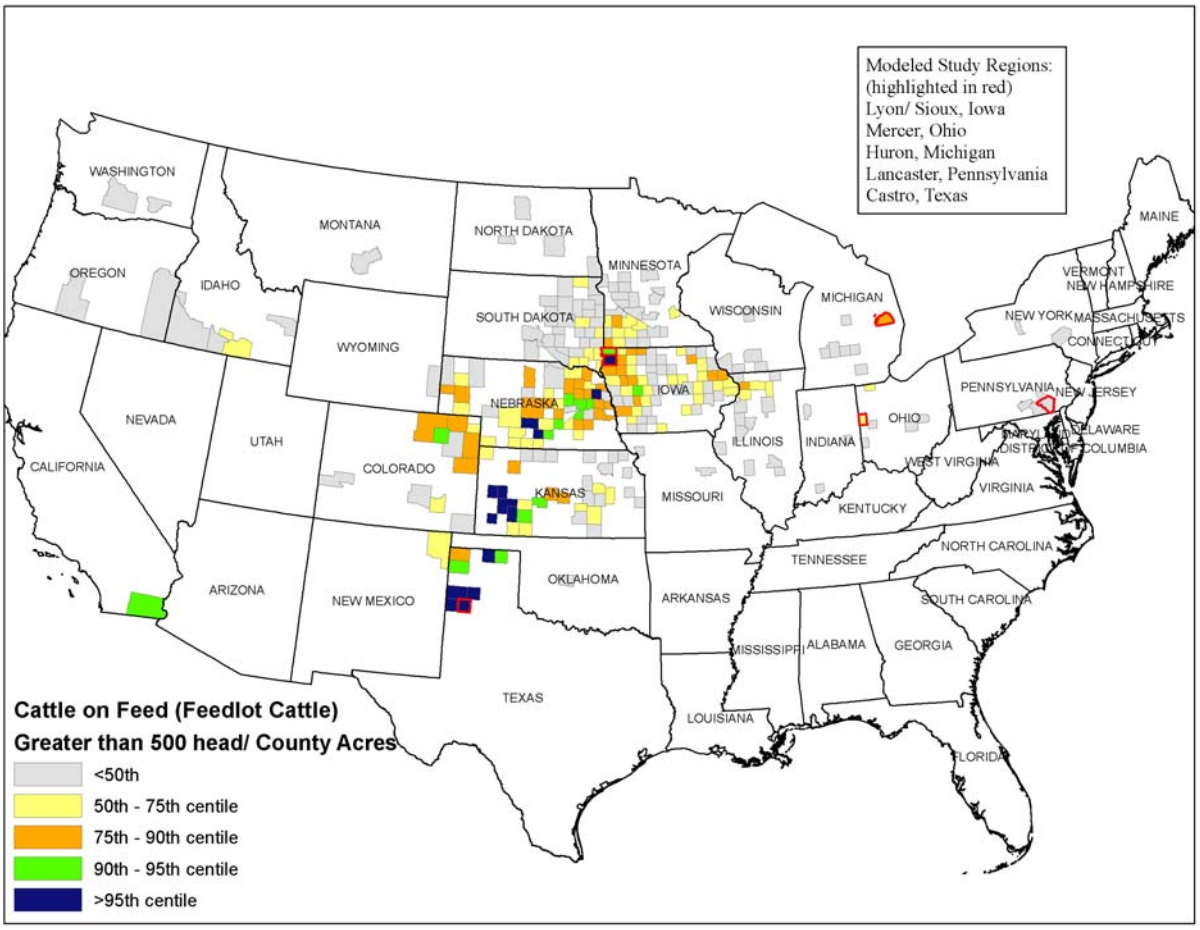


Figure 3: Density of Beef Cattle Feedlots with Less Than 500 Head (2007 Census of Agriculture)

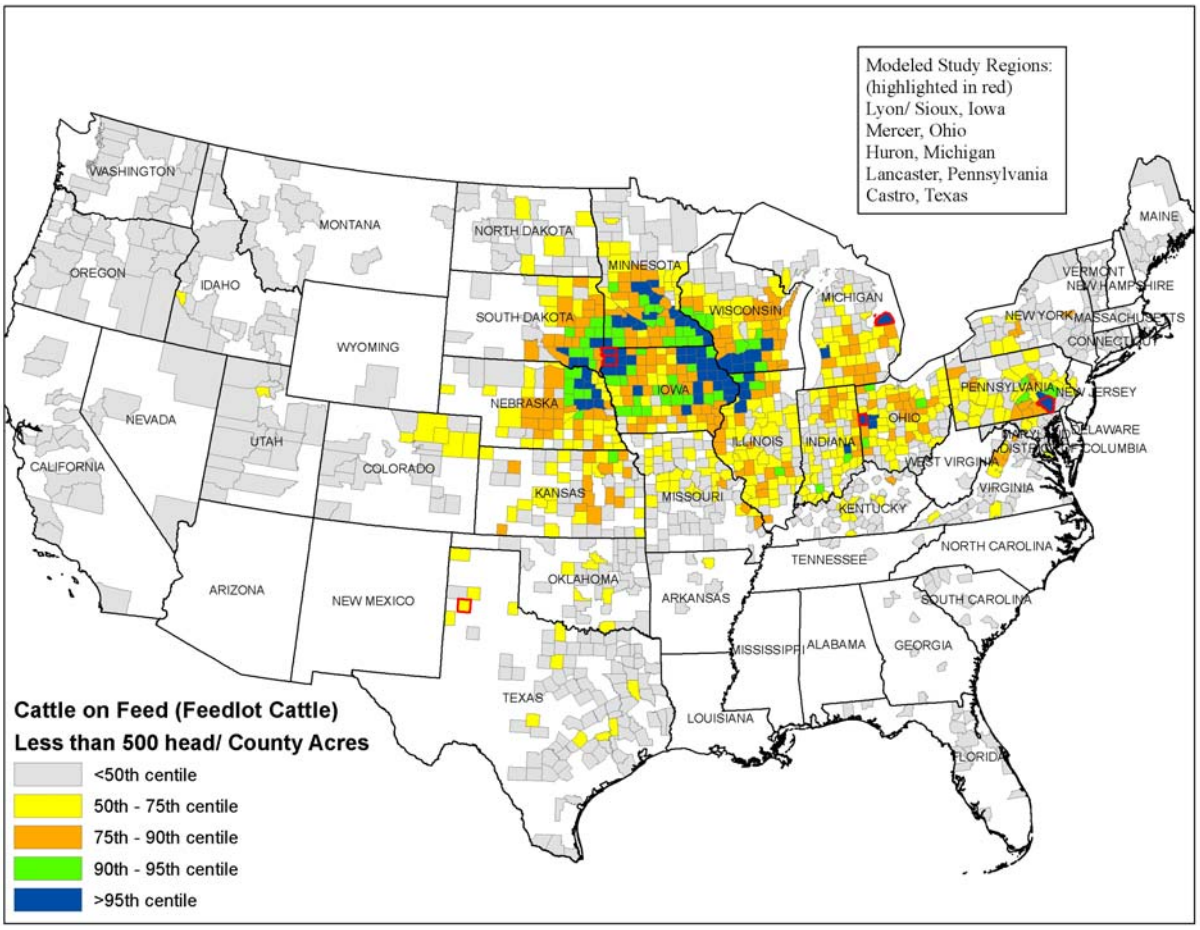


Figure 4: Density of Acres of Manure Applied from All Animal Types (2007 Census of Agriculture)

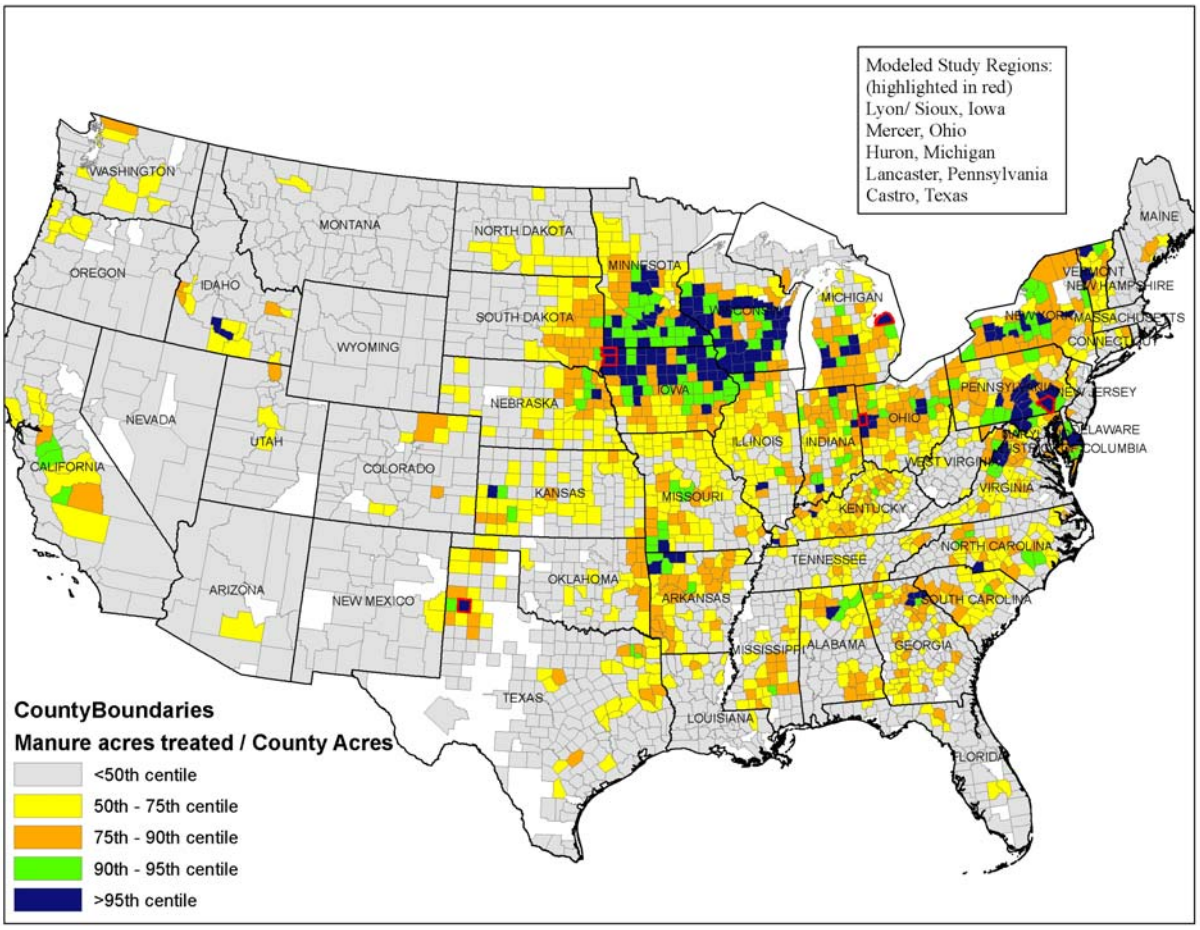


Figure 5: Density of Pastured Beef Cattle (Estimated from the 2007 Census of Agriculture)

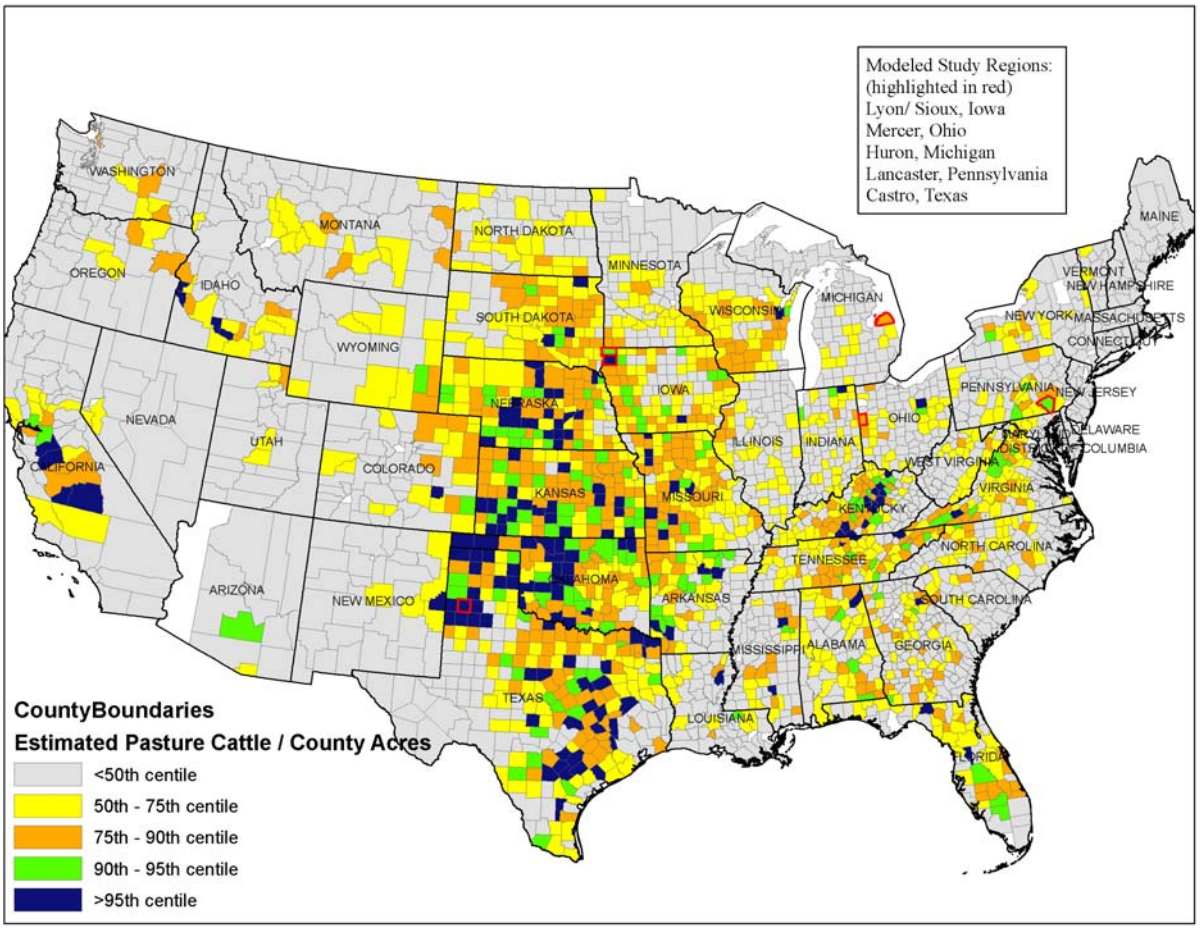


Figure 6: Long Term (1971-2000) Average Total Annual Rainfall (inches)

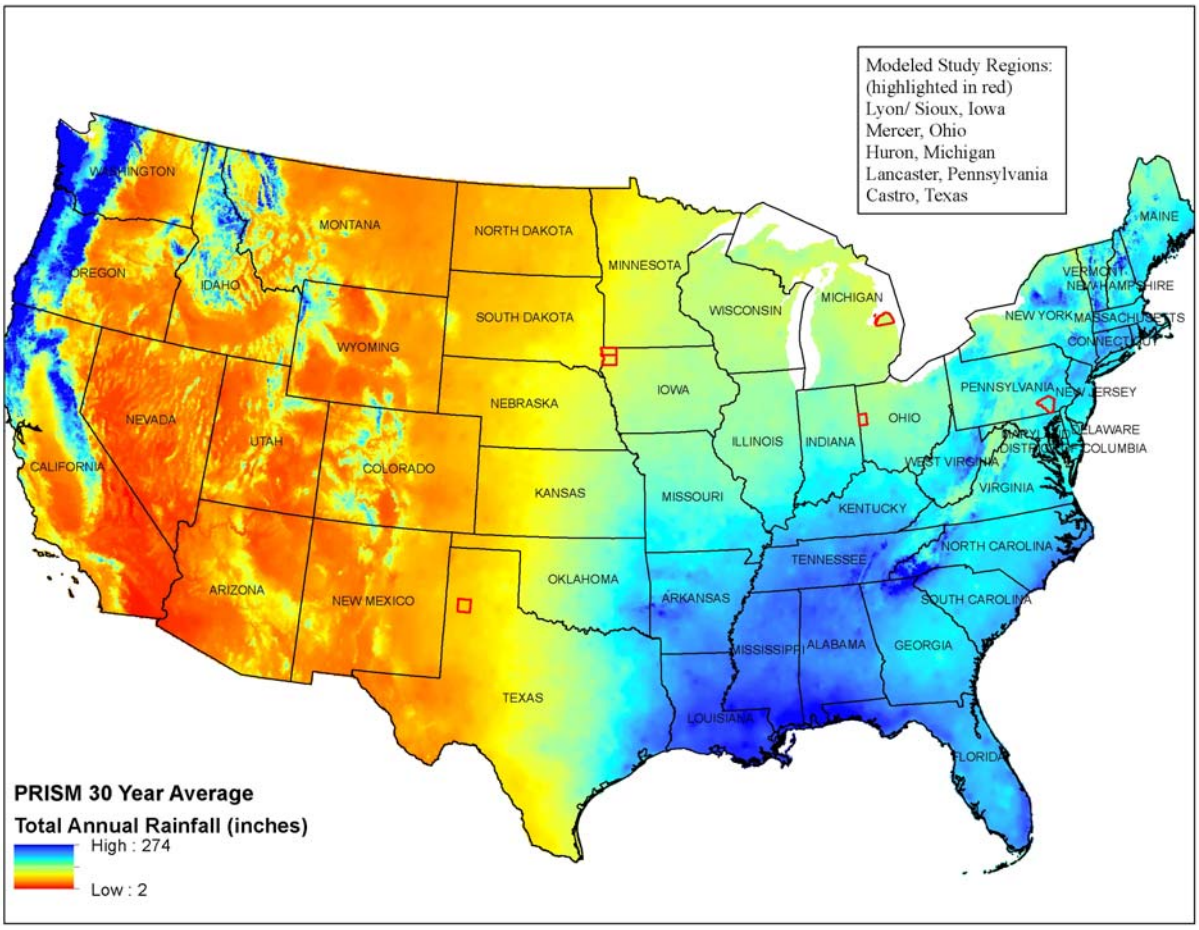


Figure 7: Long Term (1971-2000) Average Total October Rainfall (inches)

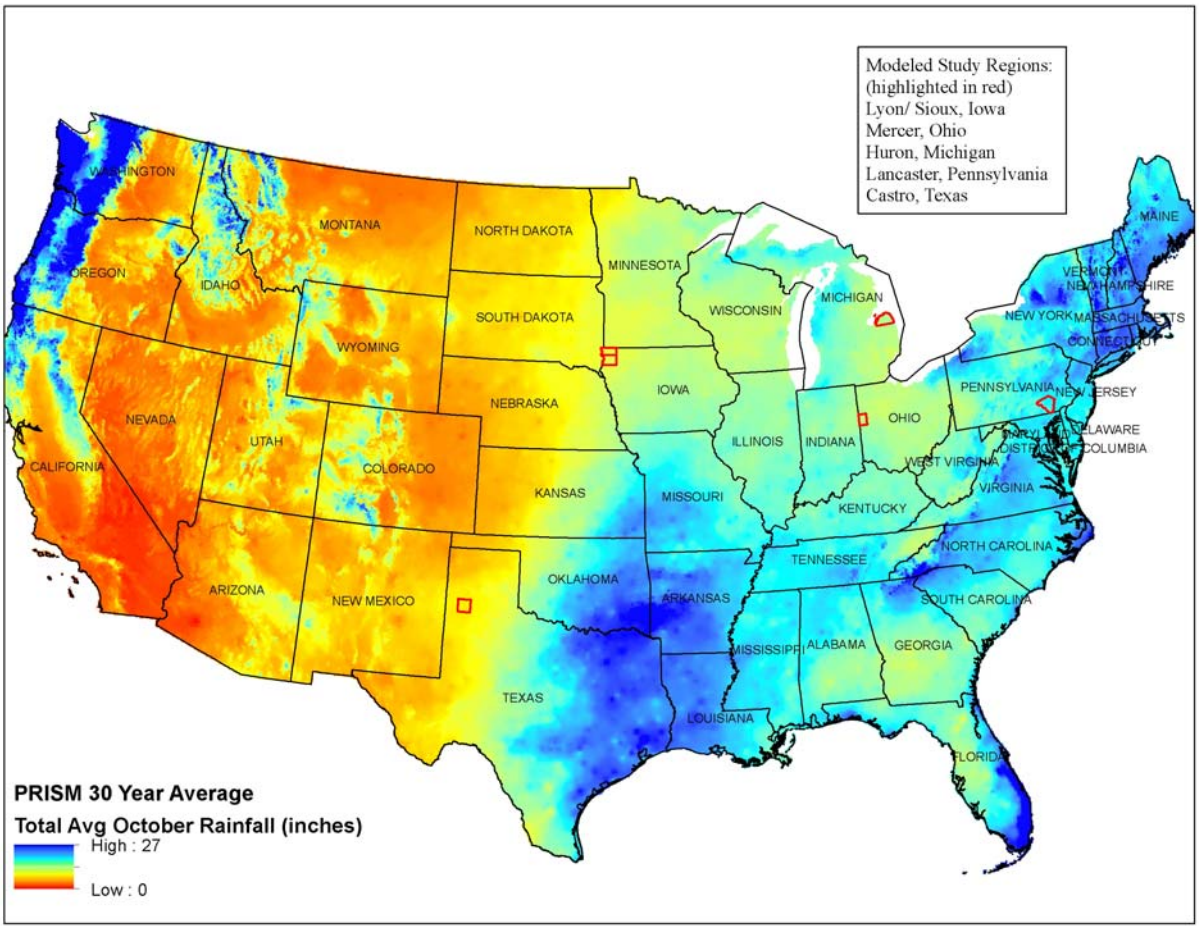


Figure 8: Long Term (1971-2000) Average Total March Rainfall (inches)

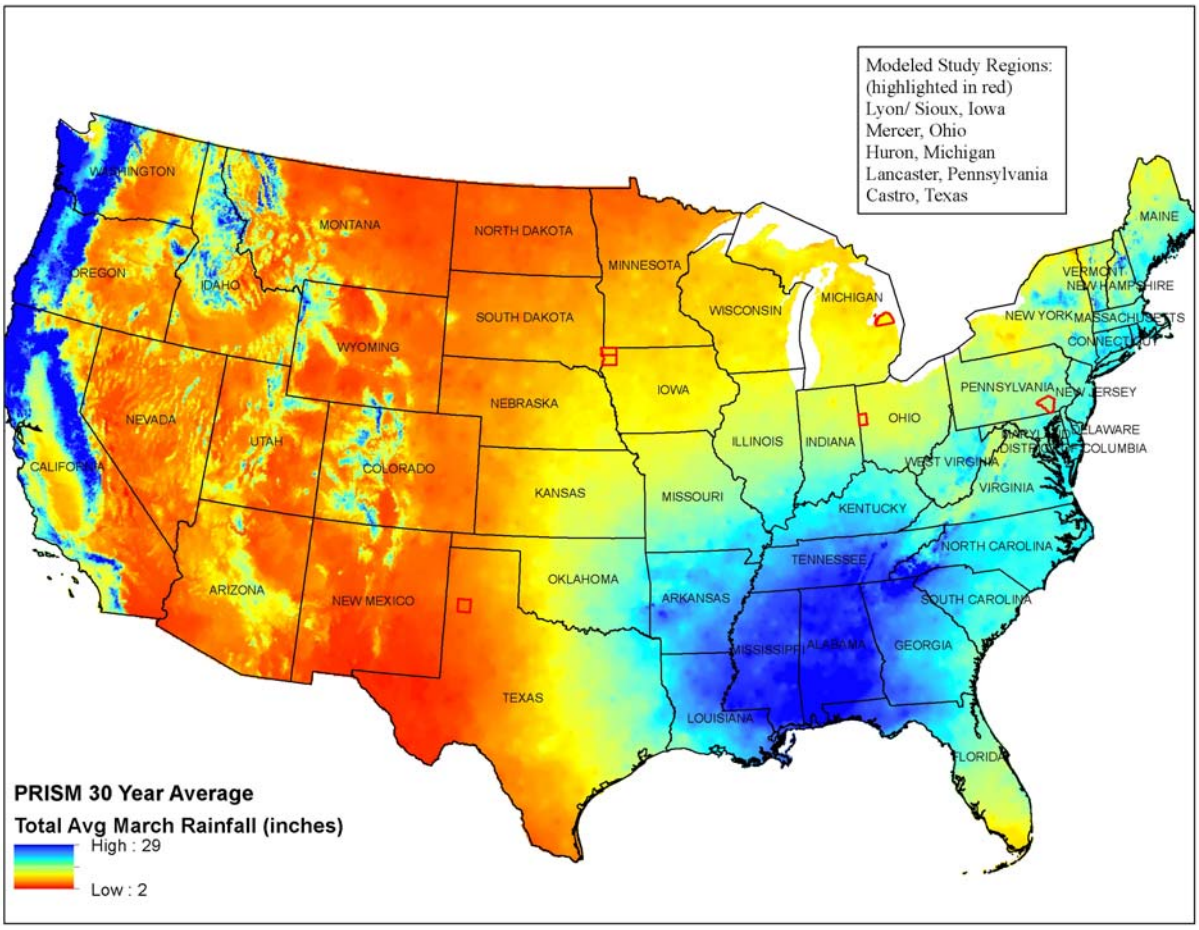


Figure 9: Five Study Regions Examined

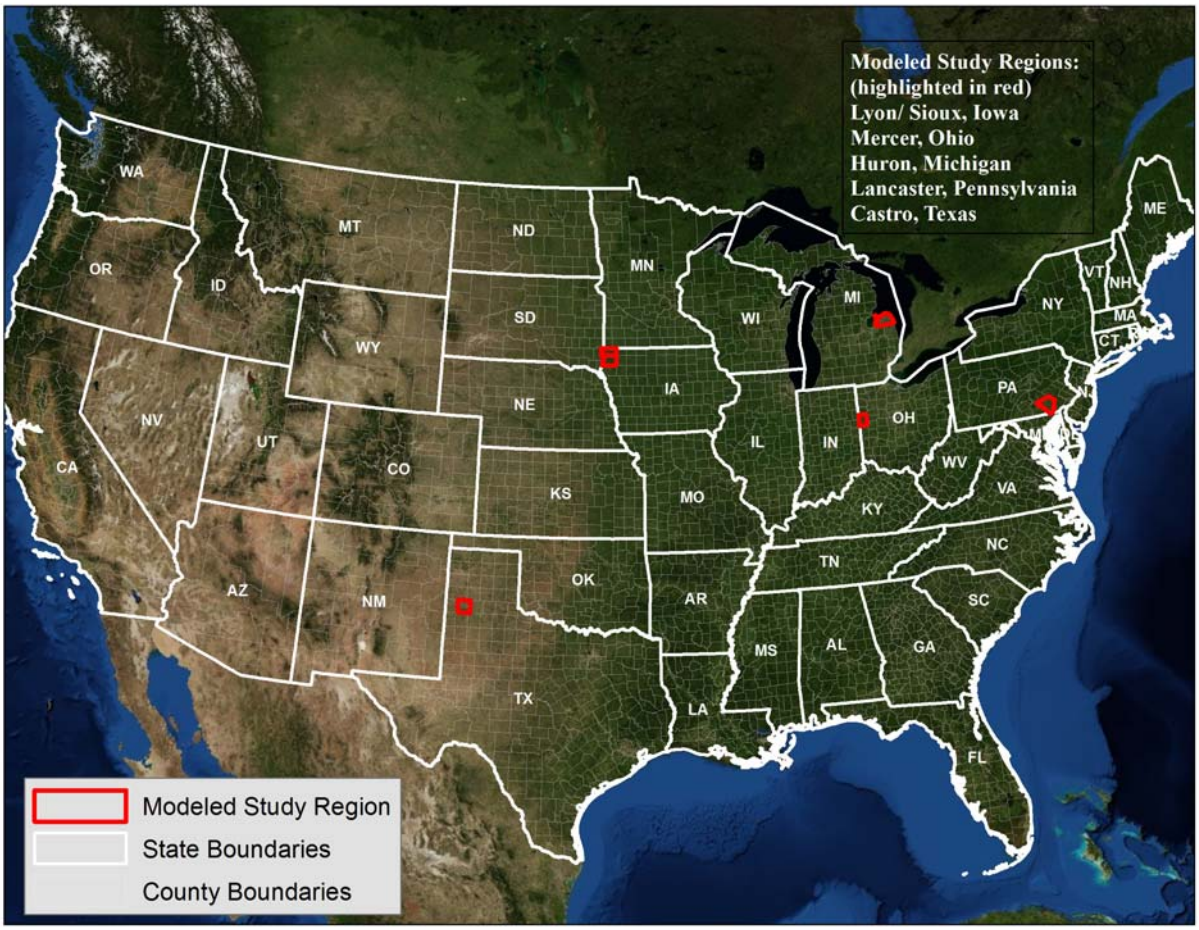


Figure 10: Location and Land Cover Characteristics of the Modeled Watershed in Iowa

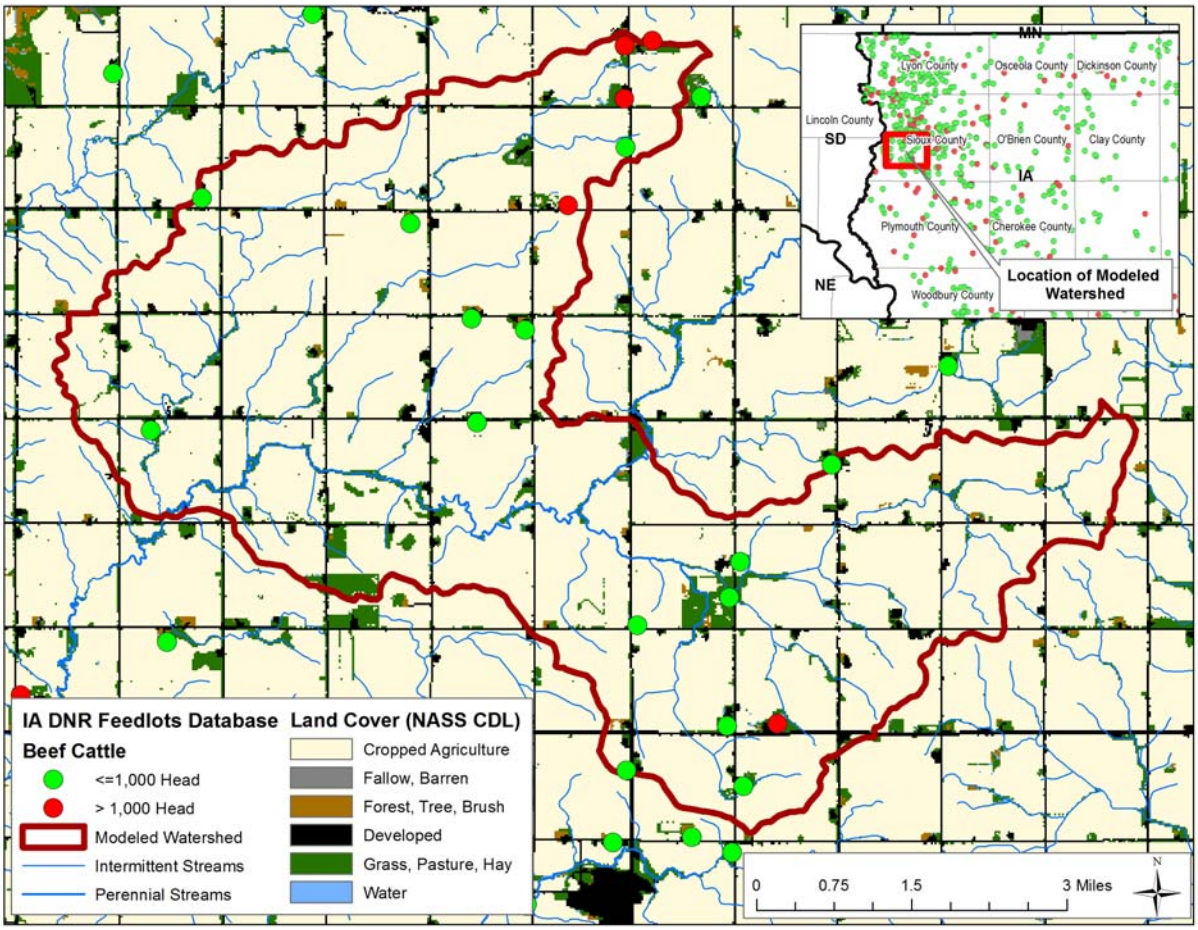


Figure 11: Location and Land Cover Characteristics of the Modeled Watershed in Texas

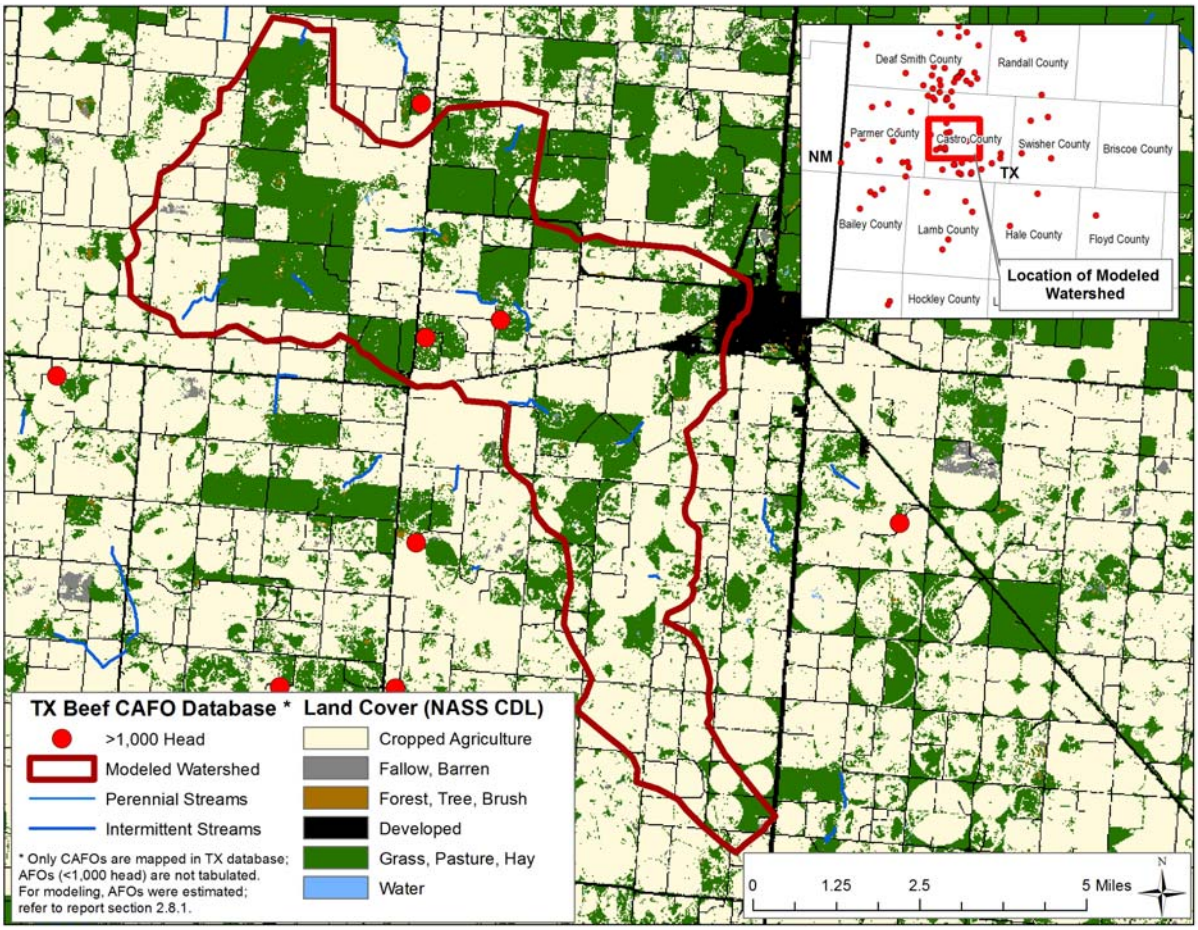


Figure 12: Schematic of Modeling Using USEPATier-2 Approach

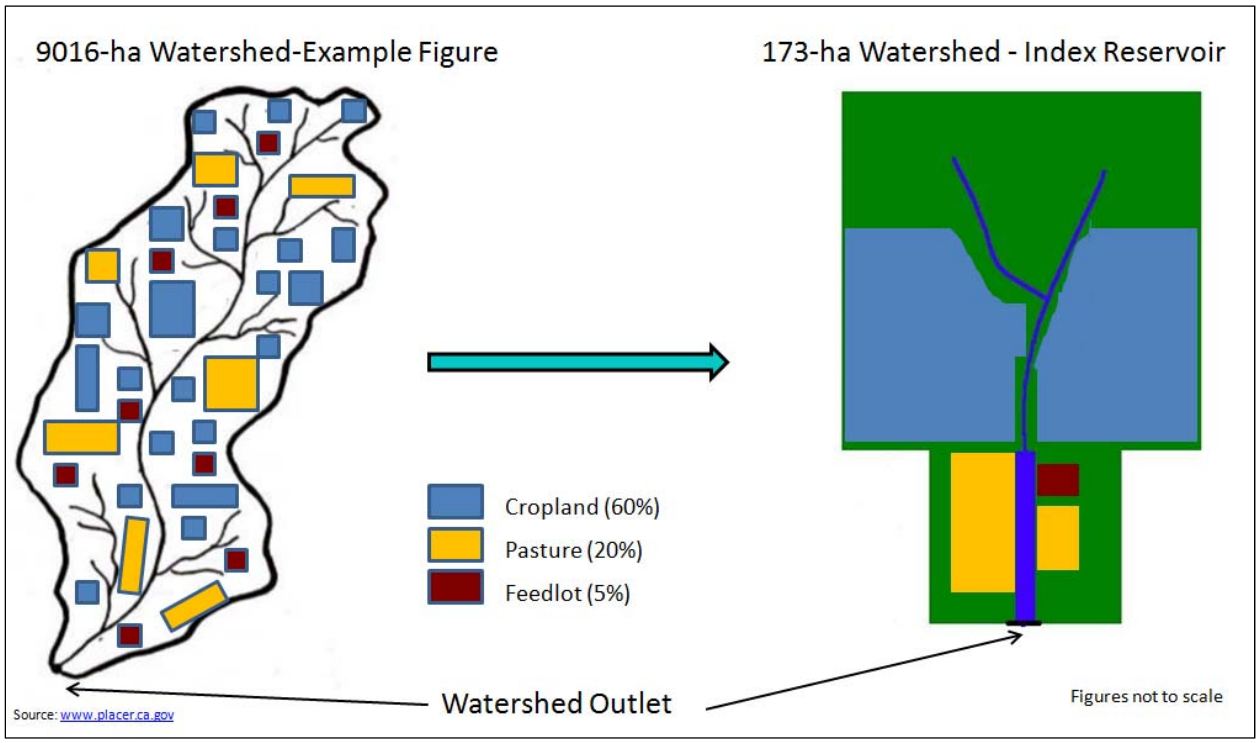


Figure 13: Effect of Varying <1000 AU Feedlot PCA Factor from 0 to 100% for Iowa Region

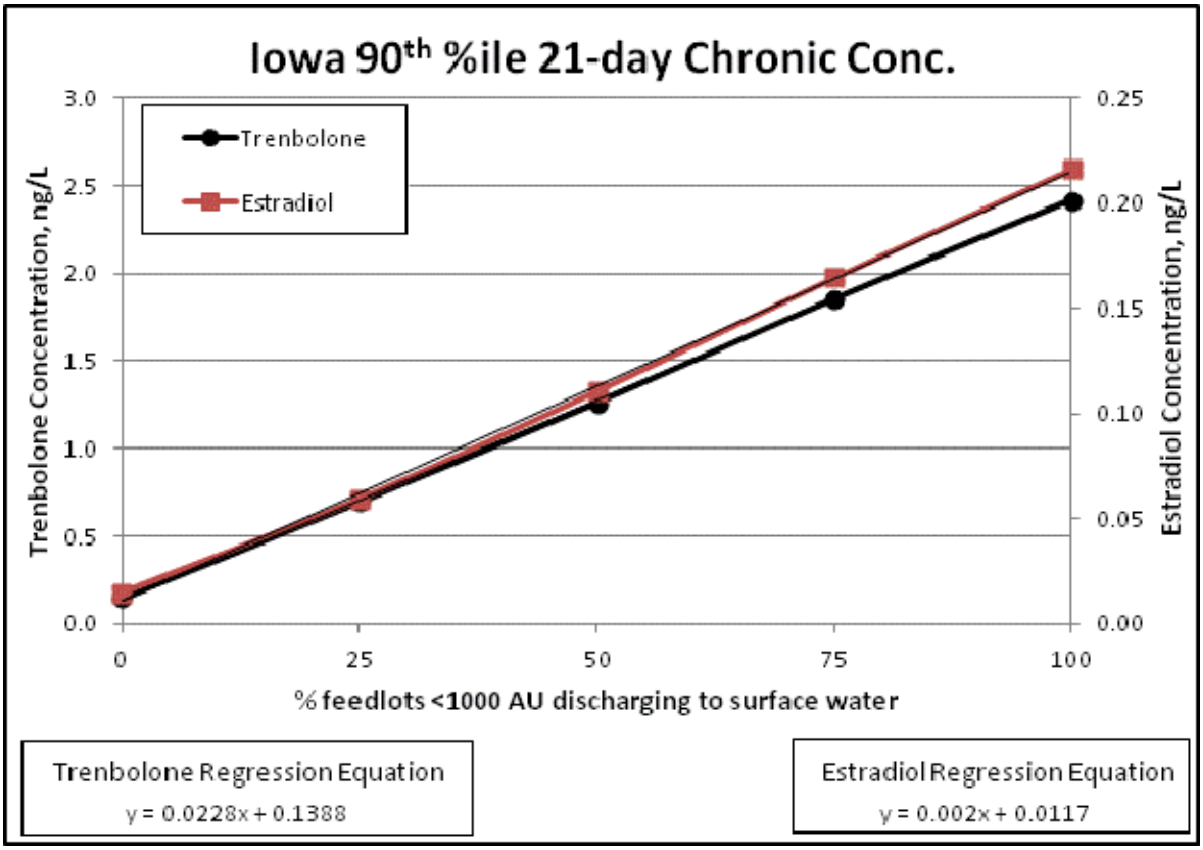


Figure 14: Effect of Varying <1000 AU Feedlot PCA Factor from 0 to 100% for Pennsylvania Region

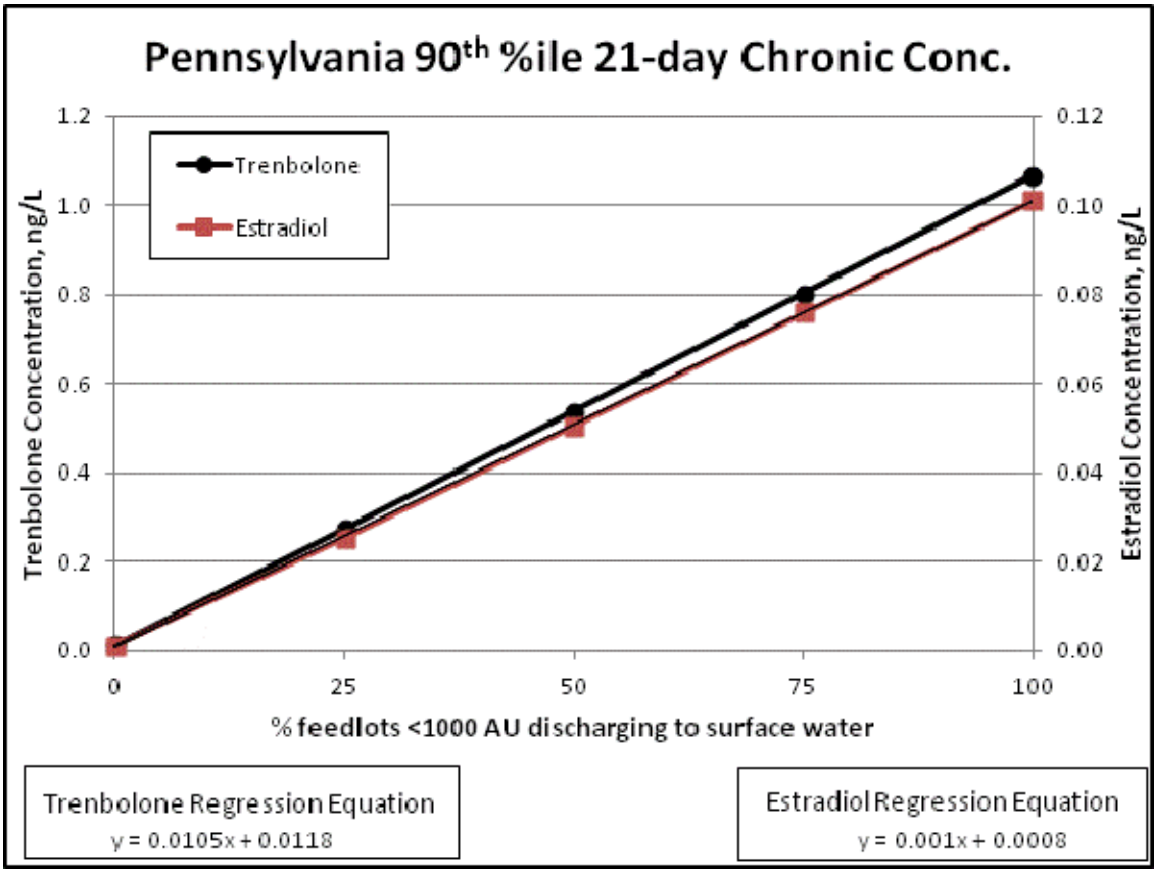


Figure 15: Effect of Varying <1000 AU Feedlot PCA Factor from 0 to 100% for Ohio Region

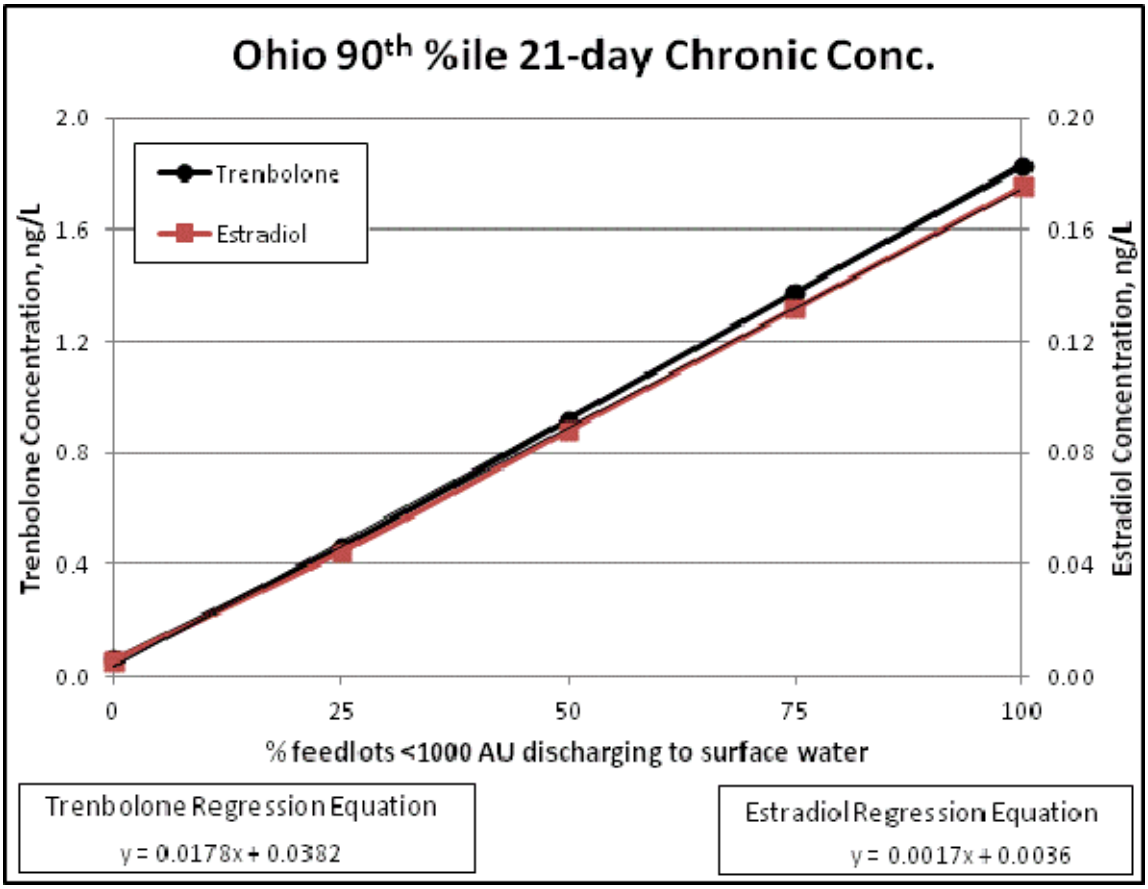


Figure 16: Effect of Varying <1000 AU Feedlot PCA Factor from 0 to 100% for Michigan Region

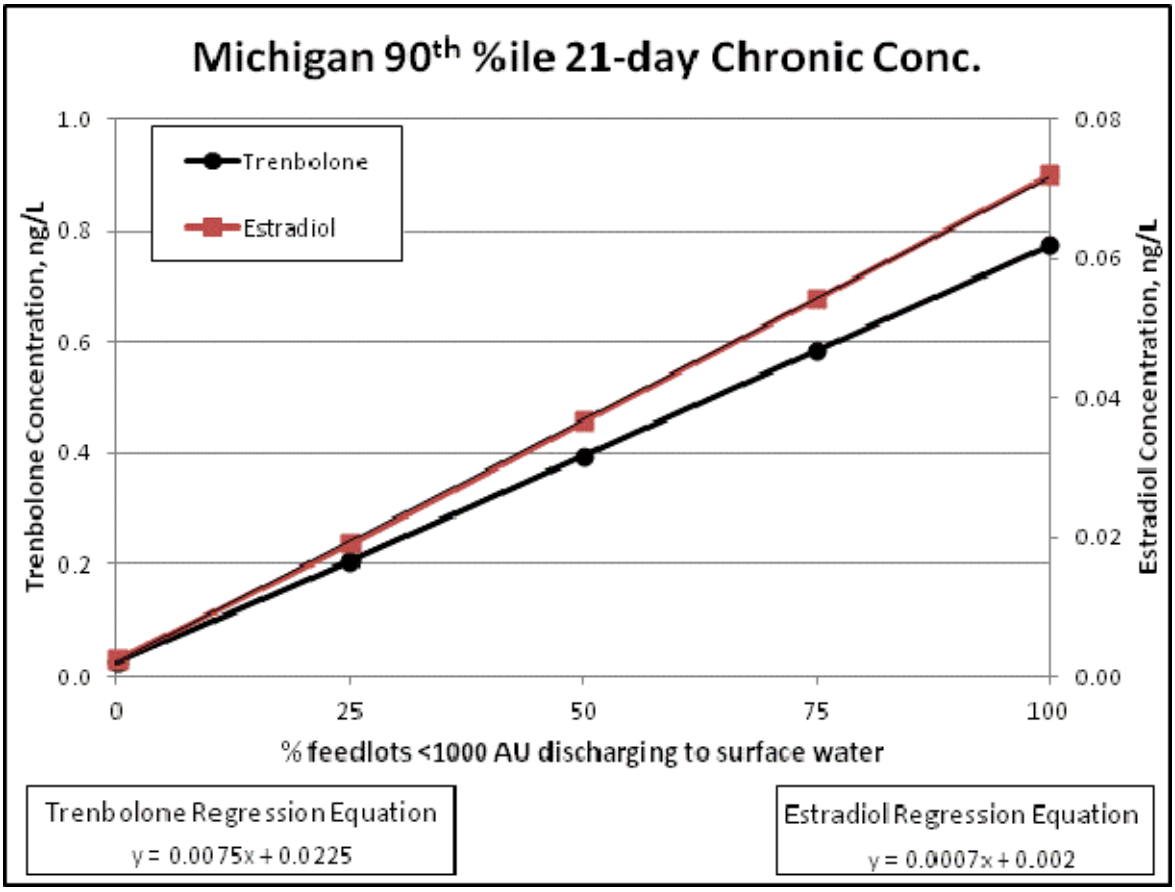
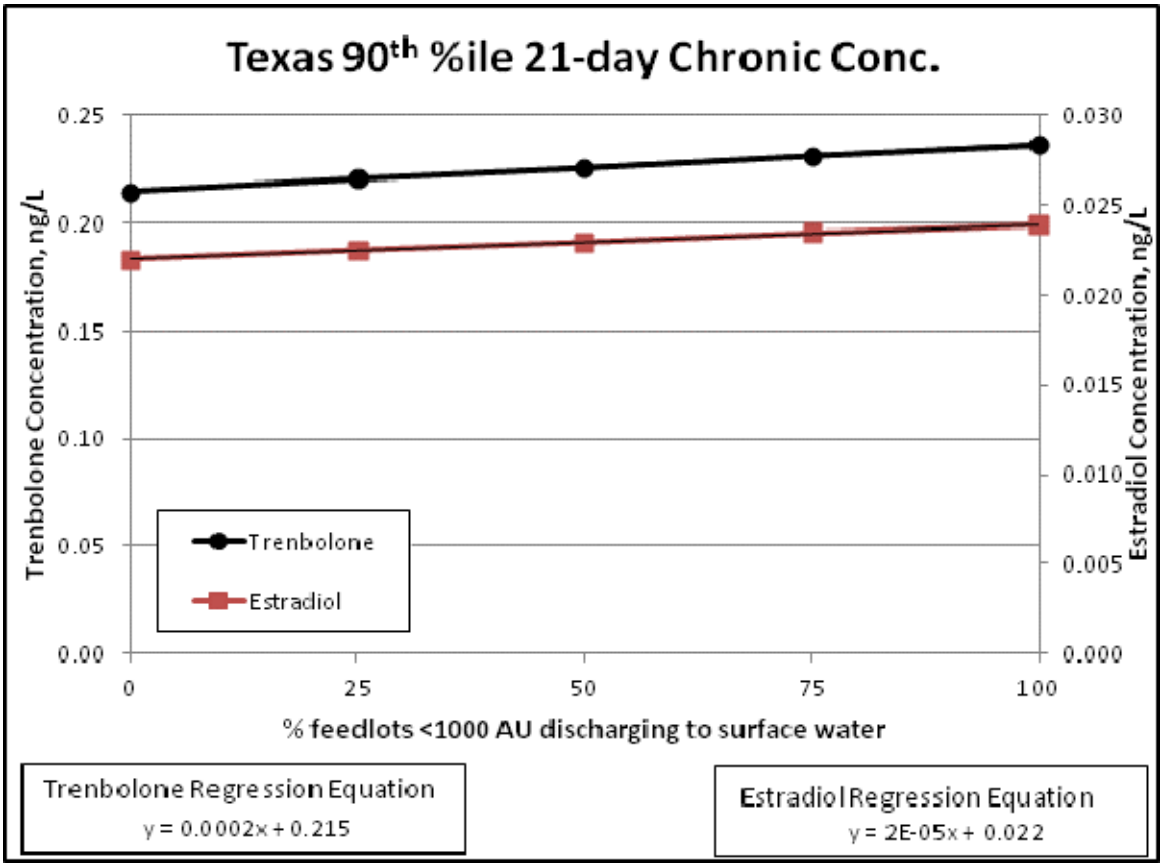


Figure 17: Effect of Varying <1000 AU Feedlot PCA Factor from 0 to 100% for Texas Region



Appendix 1 Data Sources

This section documents the data used to perform a national geospatial analysis to identify regions of high potential vulnerability to runoff and erosion of trenbolone and estradiol into surface water and to characterize watersheds within the selected regions according to the density of beef feedlots, cropland treated with beef manure, and pastured beef cattle.

Census of Agriculture Data for County-level Analysis

County- and state-level cattle statistics were collected from the NASS 2007 Census of Agriculture [USDA-NASS, 2009] and from the NASS 2007 survey data available via the QuickStats website [USDA-NASS, 2011]. NASS statistics were used to identify counties in the U.S. with high beef cattle densities and, therefore, a high potential for trenbolone and estradiol use. This data is also used to assist in the calculation of watershed metrics for the selected study regions. For instance, NASS statistics were used to locate counties in the upper 5th percentile for feedlot densities (having less than 500 head). This is a Census of Agriculture category that most similarly represents Animal Feeding Operations (AFOs) which are not required to attain a permit through the National Pollutant Discharge Elimination System (NPDES) permit program of the Clean Water Act, and therefore have a greater potential for exposure of trenbolone and estradiol to surface waters than larger feedlots. Survey data from the QuickStats website were used to supplement the Census of Agriculture data because they included additional cattle categories that were used to estimate beef and dairy replacement heifers, along with other useful metrics.

Animal Feeding Operations Databases for Watershed-level Analysis

Public geospatial data with the highest resolution were used when available to further characterize, in detail, the study regions ultimately chosen for modeling. For both the Iowa and Texas study regions, the location and characteristics of permitted Animal Feeding Operations are publically available as digital, geographically referenced data.

The Iowa Department of Natural Resources Animal Feeding Operations Database records the geographic coordinates of each feedlot and categorizes them into two classes: Animal Feeding Operations (AFOs) of less than 1,000 head and Confined Animal Feeding Operations (CAFOs) of over 1,000 head [Iowa DNR, 2005]. Note that the database stores the maximum head of beef cattle permitted, not the actual number of cattle that passed through the lot in any given year. To verify the utility of the Iowa DNR feedlot database, the beef cattle counts were cross-checked against the 2007 Census of Agriculture statistics, which verified that the Iowa DNR state data captured 78% of the total beef cattle in the Census of Agriculture. The disparity is a function of the purpose and method for conducting the two surveys and the temporal differences between the two databases. For the two counties that were ultimately modeled, Lyon and Sioux counties (ranking first and third in the state for total beef cattle production using the 2007 Census of Agriculture), the Iowa DNR database had good agreement with the Census of Agriculture with only a 3.1% difference in the count of beef cattle. These statistics indicate that while the Iowa DNR does not entirely represent all feedlots in the state, it does well represent feedlots in the

counties which were modeled. The beef cattle totals from the Census of Agriculture and Iowa DNR database are charted (Figure A. 1) for the top 50 counties in total beef cattle production. Figure A. 2 maps the feedlot locations in the Iowa DNR database to visualize the spatial distribution across the state. Figure A. 3 maps selected DNR feedlots in Sioux and Lyon counties in the context of surface water and watershed boundaries. Figure A. 4 highlights the positional accuracy of the beef farm database locations in relation to aerial photography database. The figure shows that for the examples selected, the beef farms overlay well with real world farms.

The Texas Commission on Environmental Quality (TCEQ) database includes the maximum permitted beef cattle counts and locational information for Confined Animal Feeding Operations (CAFOs) of greater than 1,000 head [TCEQ, 2007]. Animal Feeding Operations (AFOs) of less than 1,000 head are not tabulated by the Texas state regulatory agency and are therefore not included in the TCEQ CAFO database. In order to estimate the proportion of beef AFOs in Castro County, the study region modeled, the finest resolution data on feedlot sizes available in the 2007 Census of Agriculture were used. County-level census statistics indicate that for Castro County, 0.7% of beef are on lots of fewer than 500 head, and state-level figures indicate that 0.5% of beef are on lots of between 500-999 head [USDA-NASS, 2009]. Taken together, these statistics indicate that a very small proportion (less than 2%) of the total beef cattle are on AFOs of less than 1,000 head in Castro County. This procedure is discussed further within Section 4.2

Watershed Boundaries

For the study regions examined in detail, surface water and drainage areas were identified using the medium-resolution National Hydrography Dataset Plus [NHDPlus; USEPA, USGS, 2005] in combination with USDA-NRCS-developed 12 digit Hydrologic Cataloging Unit (HUC-12) subwatershed boundaries [USDA-NRCS-NCGC, 2006]. There are 87,500 HUC12 subwatersheds across the conterminous U.S., with an average size of 35.6 mi^2 and ranging between less than 1 and 1,704 mi^2. This subwatershed dataset was selected because the size range represents a more reasonable analysis area than HUC8 watersheds (average of 1,481 mi^2) or NHDPlus catchments (average of 1.3 mi^2). Only the subwatersheds and their corresponding drainage networks entirely contained in the study region (i.e., county boundaries) were examined, since feedlot locations and characteristics may not be known for adjacent counties or states. For instance, the study region examined in Iowa is adjacent to Nebraska, where feedlot locational data are not publically available. Therefore, any subwatersheds extending into Nebraska were not examined.

Land Use/ Land Cover

Land use/land cover metrics were calculated for each of the study regions using the NASS Cropland Data Layer (CDL). The CDL consists of digital data layers with specific agriculture information, including pasturelands and croplands, which are suitable for use in a Geographic Information System (GIS). NASS CDL are aggregated to multiple standardized categories for display purposes with the emphasis being agricultural land cover. The CDL uses satellite

imagery to locate and identify crops over an entire state, making it a “census by satellite,” and is ground verified by local Farm Service Agency offices. The CDL program also provides NASS with state- and county-level estimates of major commodities multiple times during the growing season, helping improve agency estimates. For example, NASS uses CDL-derived data to generate winter wheat estimates for their June 30th Crop Acreage Report and corn and soybean estimates for their August Crop Production Report [USDA-NASS-RDD-GIB-SARS, 2011].

Precipitation Data for County-level Analysis

Long term average rainfall characteristics such as annual and monthly totals were mapped for the U.S. counties having a co-occurrence of high beef cattle densities and high rainfall totals. These counties were identified as potential study regions, since rainfall is a primary driver in the movement of trenbolone and estradiol into surface water. National rainfall mapping was accomplished using the Parameter-elevation Regression Independent Slopes Model (PRISM) data from Oregon State University, a spatially gridded (1 kilometer) average monthly and annual precipitation dataset for the period 1971–2000 [ORSU-PRISM, 2002]. PRISM uses point data and a digital elevation model (DEM) to generate estimates of climate parameters. The PRISM modeling system of the Spatial Climate Analysis Service at Oregon State University is directed and sponsored by the NRCS National Water and Climate Center in Portland, Oregon. Figure 6 through Figure 8 map the rainfall characteristics for the US.

APPENDIX 1 FIGURES

Figure A. 1: Count of Beef Cattle per Iowa DNR AFO Database and 2007 Census of Agriculture Database for the Top 50 Feedlot Counties in Iowa

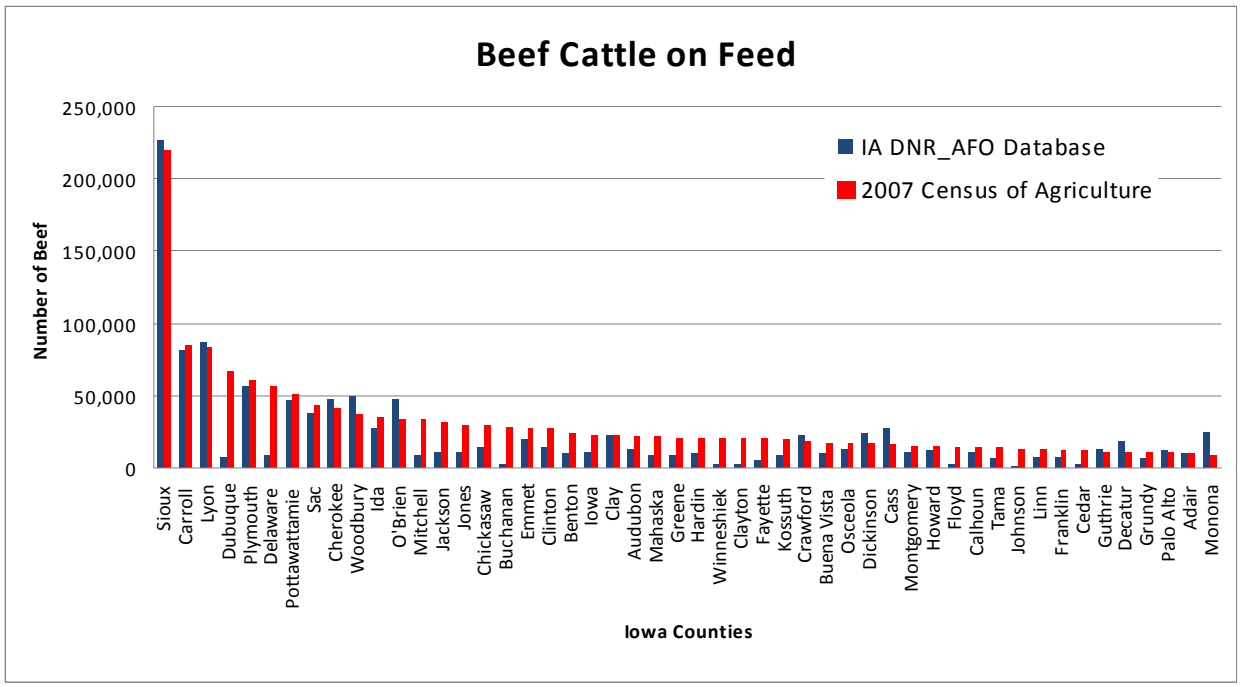


Figure A. 2: Iowa DNR Animal Feeding Operations Database

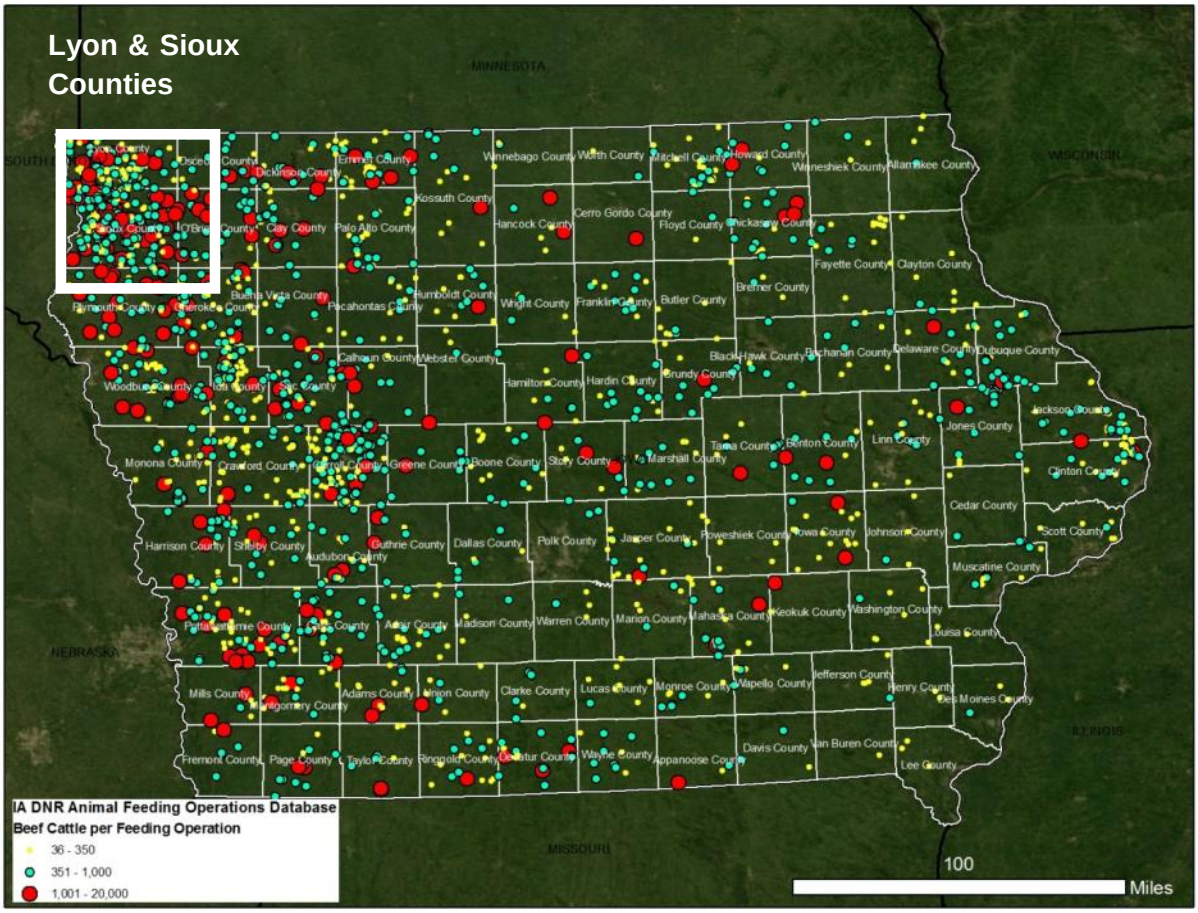


Figure A. 3: Iowa DNR Animal Feeding Operations Database with NHD Surface Water & HUC12 Watersheds

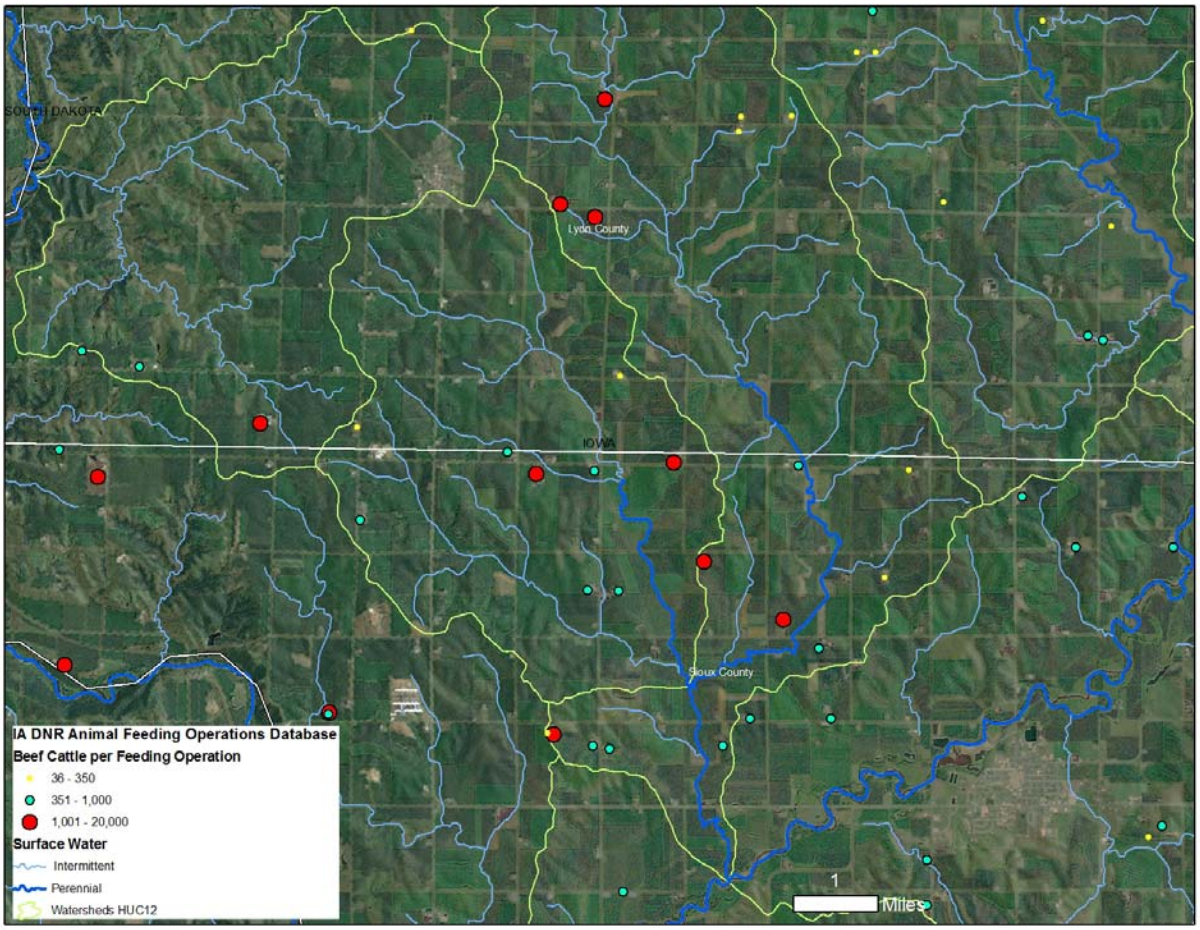
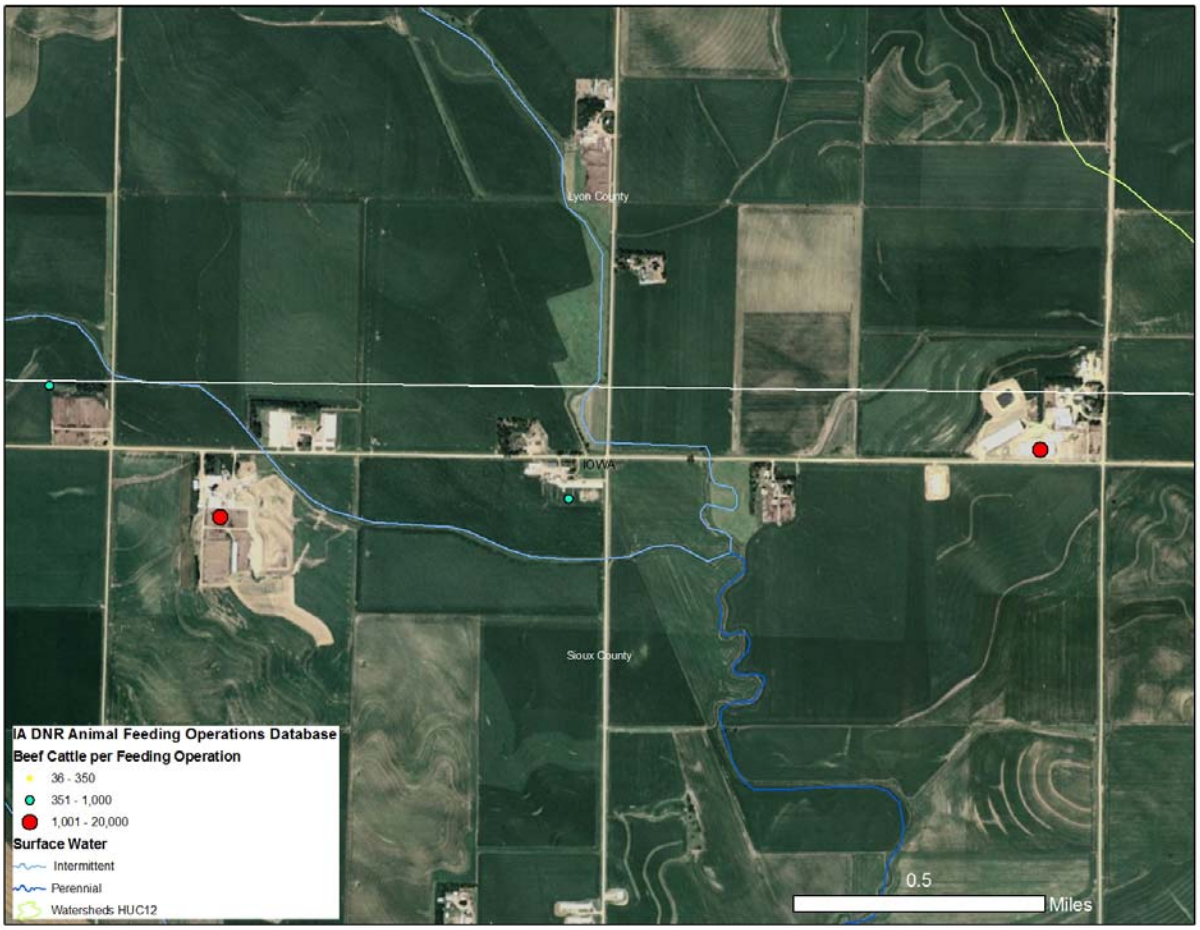


Figure A. 4: Iowa DNR Animal Feeding Operations Database with Air Photography



Appendix 2 Calculations of Temporal Probability of Occurrence

The simulation models incorporated into USEPA's framework (i.e., PRZM and EXAMS) produce output on a daily time step. EXAMS summarizes the daily values into an annual series for different exposure durations (instantaneous peak, 96 hr, 21-day, 60 day, 90 day, and annual). Where instantaneous peak represents the predicted daily concentration, the 96-hr, 21, 60, and 90-day are moving averages of the duration specified and annual is the average across the year. For example, to calculate the maximum 21-day series, for each year of simulation, the average concentration is calculated for days 1 to 21, 2 to 22, 3 to 23, ...345 to 365. For each statistic and each year of the simulation the maximum value is chosen as the annual maximum series. This results in 30 values of instantaneous peaks, 96-hr moving averages, etc. As recommended by the USEPA the upper 10^{th} percentile (i.e., 90^{th} percentile) from these annual maximum series will be used for risk assessment. The 21-day moving average data is the primary endpoint.

To determine the 90^{th} percentile values, a probability analysis is performed on the annual maximum series of predicted concentrations for a given exposure duration. The Weibull plotting position (Haan, 1977) is used to calculate the upper 10^{th} percentile concentrations. The Weibull plotting position allows concentrations to be expressed in a temporal probability context (i.e., frequency of occurrence). For example concentrations of a 10^{th} percentile are estimated to occur on average once in a 10-year period.

Annual maximum series of predicted concentrations are ranked in descending order from 1 to 30 (corresponding to 30 years of simulation). For the annual values (n = 30), the highest value (ranked from high to low) has a rank of 1 and the lowest value has a rank of 30. The equation for the Weibull plotting position is shown below:

$$\text{Weibull plotting position} = \left(\frac{Rank}{n+1}\right)*100$$

The upper 10^{th} percentile or 90^{th} percentile Weibull plotting position is then determined by interpolation as shown below:

$$90^{th}percentile = (1-0.9)\cdot x_4 + 0.9\cdot x_3$$

Where x_3 and x_4 are the 3^{rd} and 4^{th} rank values in the series, respectively.

Appendix 3 PRZM Inputs Specific to Iowa Modeling

Parameter	Pasture	Feedlot	Crop	Rationale/source
Pan Factor (PFAC)	0.76	0.76	0.76	Used to estimate daily evapotranspiration. Figure 5.1 PRZM manual
Snowmelt factor (SFAC)	0.36	0.36	0.36	Depends upon forest cover. Figure 5.1 PRZM manual
Pan factor flag	0	0	0	Signifies PAN data read
Min depth of evaporation (ANETD)	17.5	17.5	17.5	Depends upon geographical location. Figure 5.2 PRZM manual
Surface condition of initial crop (ISCOND)	3	1	1	1= fallow, 2= cropping, 3= residue
Erosion Flag	4	5	4	4- MUSS (PRZM manual) and 5- modified MUST equation
USLE K or RSDM	0.37	12 t/ha	0.37	USLE K - Depends upon textural class and OM; Table 5.3 PRZM manual. RDSM is manure of surface in t/ha
USLE LS	3.73	1	3.73	Depends upon % slope and slope length. Table 5.5 PRZM manual
USLE P	1	1	0.8	Depends upon slope and land practice used. Table 5.6 PRZM manual
Area of field - ha (AFIELD)	172.8	172.8	172.8	Standard area used for index reservoir
AGPM	NA	0	NA	AGPM is standing living and dead plant material for erosion equation
Location of NCRS 24 hyetograph	3	3	3	Depends upon geographical location. Figure 5.12 PRZM manual.
Land slope % (SLP)	6	4	6	Standard scenario land slope for cropland and pasture.; 4% slope is recommended (FASS Guide)
Hydraulic length	600	600	600	Standard value for index reservoir
No of crops (NDC)	1	1	1	PRZM requires to simulate at least one crop
Max interception storage -cm (CINTCP)	0.2	0	0.25	Depends upon crop canopy
Max Rooting depth cm (AMXDR)	43	0	90	Depends upon crop. able 5.9 PRZM manual
Max areal coverage % (COVMAX)	97	0	100	Depends upon crop canopy
Curve Number (CN)	82 79 82	95 95 95	86 79 86	CN for fallow, cropping and residue. Table 5.16 PRZM manual
Max canopy height at maturation -cm (HTMAX)	122	0	300	Depends upon crop , Table 5.16 PRZM manual

Parameter	Pasture	Feedlot	Crop	Rationale/source
Day and Month for USLEC and N	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	2505 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 2010 0111 1611 0112 1612 0101 1601 0102 1602 0103 1603 0104 1604 0105 1605	Pasture = USDA Agriculture Handbook Feedlot = For Fallow Conditions – PRZM Manual Crop = USEPA Standard Scenario
USLE C factor	0.001 for all year	1 for all year	.307 .263 .141 .089 .086 .092 .097 .102 .104 .104 .104 .016 .017 .017 .017 .017 .230 .228 .227 .226 .227 .233 .245 .267 .267 .307	Depends upon crop, rotation and management of land. Table 5.7 PRZM manual
Manning's N	0.11 for all year	0.05 for all year	0.014 for all year	Roughness coefficient for overland flow. Table 5.46 PRZM manual
Date of emergence	1-Apr	1-Jan	25-May	Pasture is simulated as all year crop. No crop is simulated for feedlot. And standard Iowa corn cropping dates are used for cropland
Date of maturation	15-Apr	2-Jan	24-Jul	
Date of harvest	15-Nov	31-Dec	19-Oct	
Total number of applications	211 for T and 270 for E	NA	60 for solid and 120 for pond water	Total number of applications in one year
Farm flag	6	4	0	4- feedlot and 6- pasture; Flag used in modified PRZM
Application date	1-Apr	1-Jan	Refer Table 16	USEPA Standard Scenario
Application stop date	28 Oct for T and 26 Dec for E	NA	NA	Only used for modified PRZM
WINDAY	0	0	0	USEPA Standard Scenario
Chemical Application Method (CAM)	4	4	4	4- Soil applied -uniform with depth
Depth of incorporation - cm (DEPI)	5	10	5,15	5 cm for surface application (pond water and pasture), 10 cm for feedlot and 15 cm for solid manure application
Application rate-kg/ha	Refer Table 17	Refer Table 17	Refer Table 17	
Application efficiency	1	1	1	USEPA Standard Scenario
Spray drift	0	0	0	USEPA Standard Scenario
Filtration Parameter (FILTRA)	0	0	0	USEPA Standard Scenario

Parameter	Pasture	Feedlot	Crop	Rationale/source
IPSCND	1	1	1	USEPA Standard Scenario
Plant uptake factor (UPTKF)	0	0	0	USEPA Standard Scenario
Total depth of soil core	152	152	152	USEPA Standard Scenario
Bulk density (BD FLAG)	0	0	0	USEPA Standard Scenario
Field capacity and Wilting point (TH FLAG)	0	0	0	USEPA Standard Scenario
KD FLAG	1	1	1	USEPA Standard Scenario
Drainage flag (HSWZT)	0	0	0	USEPA Standard Scenario
MOC flag	0	0	0	USEPA Standard Scenario
Irrigation flag (IRFLAG)	0	0	0	USEPA Standard Scenario
Soil temp flag (ITFLAG)	0	0	0	USEPA Standard Scenario
Thermal conductivity (ID FLAG)	0	0	0	USEPA Standard Scenario
Biodegradation (BIO FLAG)	0	0	0	USEPA Standard Scenario
Diffusion Coefficient (cm2/day) (DAIR)	0	0	0	USEPA Standard Scenario
Henry's Law constant - dimensionless	0	0	0	USEPA Standard Scenario
Enthalpy of vaporization (ENPY)	0	0	0	USEPA Standard Scenario
PCMC	4	4	4	USEPA Standard Scenario
SOL(KOC)	912 for T and 1259 for E	912 for T and 1259 for E	912 for T and 1259 for E	Adsorption coefficient (Koc)
Horizon number	1	1	1	Number of the soil layer in soil profile
Horizon thickness-cm	10	10	10	For pasture and cropland - standard soil parameters from Iowa scenario are used. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
Bulk density	1.4	0.85	1.4	
Field Capacity (FC)	0.31	0.45	0.31	
Wilting Point (WP)	0.12	0.14	0.12	
Organic Carbon (OC)	0.93	38	0.93	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	2	2	2	Number of the soil layer in soil profile

Parameter	Pasture	Feedlot	Crop	Rationale/source
Horizon thickness-cm	5	10	5	For pasture and cropland - standard soil parameters from Iowa scenario are used. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
Bulk density	1.4	1.25	1.4	
FC	0.31	0.321	0.31	
WP	0.12	0.202	0.12	
OC	0.93	31	0.93	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	3	3	3	Number of the soil layer in soil profile
Horizon thickness-cm	125	120	125	For pasture, cropland and feedlot - standard soil parameters from Iowa scenario are used.
Bulk density	1.37	1.37	1.37	
FC	0.279	0.279	0.279	
WP	0.079	0.079	0.079	
OC	0.14	0.14	0.14	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	4	4	4	Number of the soil layer in soil profile
Horizon thickness-cm	12	12	12	For pasture, cropland and feedlot - standard soil parameters from Iowa scenario are used.
Bulk density	1.48	1.48	1.48	
FC	0.309	0.309	0.309	
WP	0.119	0.119	0.119	
OC	0.14	0.14	0.14	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)

Appendix 4 PRZM Inputs Specific to Pennsylvania Modeling

Parameter	Pasture	Feedlot	Crop	Rationale/source
Pan Factor (PFAC)	0.76	0.76	0.76	Used to estimate daily evapotranspiration. Figure 5.1 PRZM manual
Snowmelt factor (SFAC)	0.36	0.36	0.36	Depends upon forest cover. Figure 5.1 PRZM manual
Pan factor flag	0	0	0	Signifies PAN data read
Min depth of evaporation (ANETD)	17.5	17.5	17.5	Depends upon geographical location. Figure 5.2 PRZM manual
Surface condition of initial crop (ISCOND)	3	1	3	1= fallow, 2= cropping, 3= residue
Erosion Flag	4	5	4	4- MUSS (PRZM manual) and 5- modified MUST equation
USLE K or RSDM	0.32	12 t/ha	0.32	USLE K - Depends upon textural class and OM; Table 5.3 PRZM manual. RDSM is manure of surface in t/ha
USLE LS	1.34	1	1.34	Depends upon % slope and slope length. Table 5.5 PRZM manual
USLE P	1	1	0.5	Depends upon slope and land practice used. Table 5.6 PRZM manual
Area of field - ha (AFIELD)	172.8	172.8	172.8	Standard area used for index reservoir
AGPM	NA	0	NA	AGPM is standing living and dead plant material for erosion equation
Location of NCRS 24 hyetograph	3	3	3	Depends upon geographical location. Figure 5.12 PRZM manual.
Land slope % (SLP)	6	4	6	Standard scenario land slope for cropland and pasture. 4% slope for feedlot (FASS guide)
Hydraulic length	600	600	600	Standard value for index reservoir
No of crops (NDC)	1	1	1	PRZM requires to simulate at least one crop
Max interception storage -cm (CINTCP)	0.2	0	0.25	Depends upon crop canopy
Max Rooting depth cm (AMXDR)	43	0	90	Depends upon crop. Table 5.9 PRZM manual
Max areal coverage % (COVMAX)	97	0	100	Depends upon crop canopy
Curve Number (CN)	85 79 85	95 95 95	80 75 80	CN for fallow, cropping and residue. Table 5.16 PRZM manual
Max canopy height at maturation -cm (HTMAX)	122	0	300	Depends upon crop. Table 5.16 PRZM manual

Parameter	Pasture	Feedlot	Crop	Rationale/source
Day and Month for USLEC and N	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 2010 0111 1611 0112 1612 0101 1601 0102 1602 0103 1603 0104	Pasture = USDA Agriculture Handbook Feedlot = For Fallow Conditions – PRZM Manual Crop = USEPA Standard Scenario
USLE C factor	0.001 for all year	1 for all year	076 .116 .134 .120 .084 .059 .052 .058 .062 .066 .081 .113 .127 .012 .013 .014 .014 .015 .042 .043 .044 .045 .048 .054 .063	Depends upon crop, rotation and management of land. Table 5.7 PRZM manual
Manning's N	0.11 for all year	0.05 for all year	0.023 for all year	Roughness coefficient for overland flow. Table 5.46 PRZM manual
Date of emergence	1-Apr	1-Jan	16-Apr	Pasture is simulated as all year crop. No crop is simulated for feedlot. And standard Pennsylvania corn cropping dates are used for cropland
Date of maturation	15-Apr	2-Jan	4-Jul	
Date of harvest	15-Nov	31-Dec	1-Oct	
Total number of applications	211 for T and 270 for E	NA	60 for solid and 120 for pond water	Total number of applications in one year
Farm flag	6	4	0	4- feedlot and 6- pasture; Flag used in modified PRZM
Application date	1-Apr	1-Jan	Refer Table 16	USEPA Standard Scenario
Application stop date	28 Oct for T and 26 Dec for E	NA	NA	Only used for modified PRZM
WINDAY	0	0	0	USEPA Standard Scenario
Chemical Application Method (CAM)	4	4	4	4- Soil applied -uniform with depth
Depth of incorporation - cm (DEPI)	5	10	5	5 cm for surface application (pond water, solid manure (no-till) and pasture) and 10 cm for feedlot
Application rate- kg/ha	Refer Table 17	Refer Table 17	Refer Table 17	
Application efficiency	1	1	1	USEPA Standard Scenario
Spray drift	0	0	0	USEPA Standard Scenario
Filtration Parameter (FILTRA)	0	0	0	USEPA Standard Scenario
IPSCND	1	1	1	USEPA Standard Scenario
Plant uptake factor (UPTKF)	0	0	0	USEPA Standard Scenario

Parameter	Pasture	Feedlot	Crop	Rationale/source
Total depth of soil core	100	100	100	USEPA Standard Scenario
Bulk density (BD FLAG)	0	0	0	USEPA Standard Scenario
Field capacity and Wilting point (TH FLAG)	0	0	0	USEPA Standard Scenario
KD FLAG	1	1	1	USEPA Standard Scenario
Drainage flag (HSWZT)	0	0	0	USEPA Standard Scenario
MOC flag	0	0	0	USEPA Standard Scenario
Irrigation flag (IRFLAG)	0	0	0	USEPA Standard Scenario
Soil temp flag (ITFLAG)	0	0	0	USEPA Standard Scenario
Thermal conductivity (ID FLAG)	0	0	0	USEPA Standard Scenario
Biodegradation (BIO FLAG)	0	0	0	USEPA Standard Scenario
Diffusion Coefficient (cm2/day) (DAIR)	0	0	0	USEPA Standard Scenario
Henry's Law constant - dimensionless	0	0	0	USEPA Standard Scenario
Enthalpy of vaporization (ENPY)	0	0	0	USEPA Standard Scenario
PCMC	4	4	4	USEPA Standard Scenario
SOL(KOC)	912 for T and 1259 for E	912 for T and 1259 for E	912 for T and 1259 for E	Adsorption coefficient (Koc)
Horizon number	1	1	1	Number of the soil layer in soil profile
Horizon thickness-cm	10	10	10	For pasture and cropland - standard soil parameters from Pennsylvania scenario are used. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
Bulk density	1.6	0.85	1.6	
Field Capacity (FC)	0.282	0.45	0.282	
Wilting Point (WP)	0.122	0.14	0.122	
Organic Carbon (OC)	2.9	38	2.9	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	2	2	2	Number of the soil layer in soil profile
Horizon thickness-cm	40	10	40	For pasture and cropland - standard soil parameters from Pennsylvania scenario are used. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
Bulk density	1.7	1.25	1.7	
FC	0.242	0.321	0.242	
WP	0.142	0.202	0.142	
OC	0.174	31	0.174	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	3	3	3	Number of the soil layer in soil profile
Horizon thickness-cm	50	30	50	For pasture and feedlot - standard soil parameters from Pennsylvania scenario are used. For Feedlot, standard Pennsylvania soil
Bulk density	1.8	1.7	1.8	
FC	0.245	0.242	0.245	

Parameter	Pasture	Feedlot	Crop	Rationale/source
WP	0.145	0.142	0.145	Parameters from second horizon were used
OC	0.116	0.174	0.116	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	NA	4	NA	Number of the soil layer in soil profile
Horizon thickness-cm	NA	50	NA	For Feedlot, standard Pennsylvania soil parameters from third horizon were used
Bulk density	NA	1.8	NA	
FC	NA	0.245	NA	
WP	NA	0.145	NA	
OC	NA	0.116	NA	
DWRATE , DSRATE	NA	0	NA	Degradation rate (LN(2)/half life)

Appendix 5 PRZM Inputs Specific to Ohio Modeling

Parameter	Pasture	Feedlot	Crop	Rationale/source
Pan Factor (PFAC)	0.77	0.77	0.77	Used to estimate daily evapotranspiration. Figure 5.1 PRZM manual
Snowmelt factor (SFAC)	0.36	0.36	0.36	Depends upon forest cover. Figure 5.1 PRZM manual
Pan factor flag	0	0	0	Signifies PAN data read
Min depth of evaporation (ANETD)	17.5	17.5	17.5	Depends upon geographical location. Figure 5.2 PRZM manual
Surface condition of initial crop (ISCOND)	3	1	3	1= fallow, 2= cropping, 3= residue
Erosion Flag	4	5	4	4- MUSS (PRZM manual) and 5- modified MUST equation
USLE K or RSDM	0.37	12 t/ha	0.37	USLE K - Depends upon textural class and OM; Table 5.3 PRZM manual. RDSM is manure of surface in t/ha
USLE LS	1.34	1	1.34	Depends upon % slope and slope length. Table 5.5 PRZM manual
USLE P	1	1	1	Depends upon slope and land practice used. Table 5.6 PRZM manual
Area of field - ha (AFIELD)	172.8	172.8	172.8	Standard area used for index reservoir
AGPM	NA	0	NA	AGPM is standing living and dead plant material for erosion equation
Location of NCRS 24 hyetograph	3	3	3	Depends upon geographical location. Figure 5.12 PRZM manual.
Land slope % (SLP)	6	4	6	Standard scenario land slope for cropland and pasture. 4% slope for feedlot (FASS guide)
Hydraulic length	600	600	600	Standard value for index reservoir
No of crops (NDC)	1	1	1	PRZM requires to simulate at least one crop
Max interception storage -cm (CINTCP)	0.2	0	0.25	Depends upon crop canopy
Max Rooting depth cm (AMXDR)	43	0	90	Depends upon crop. Table 5.9 PRZM manual
Max areal coverage % (COVMAX)	97	0	100	Depends upon crop canopy
Curve Number (CN)	89 86 89	95 95 95	82 78 82	CN for fallow, cropping and residue. Table 5.16 PRZM manual
Max canopy height at maturation -cm (HTMAX)	122	0	300	Depends upon crop. Table 5.16 PRZM manual

Parameter	Pasture	Feedlot	Crop	Rationale/source
Day and Month for USLEC and N	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 2010 0111 1611 0112 1612 0101 1601 0102 1602 0103 1603 0104 1604	Pasture = USDA Agriculture Handbook Feedlot = For Fallow Conditions – PRZM Manual Crop = USEPA Standard Scenario
USLE C factor	0.001 for all year	1 for all year	.070 .083 .074 .043 .029 .030 .034 .038 .042 .046 .065 .075 .008 .008 .009 .009 .009 .023 .023 .024 .024 .025 .028 .034 .042	Depends upon crop, rotation and management of land. Table 5.7 PRZM manual
Manning's N	0.11 for all year	0.05 for all year	0.014 for all year	Roughness coefficient for overland flow. Table 5.46 PRZM manual
Date of emergence	1-Apr	1-Jan	1-May	Pasture is simulated as all year crop. No crop is simulated for feedlot. And standard Ohio corn cropping dates are used for cropland
Date of maturation	15-Apr	2-Jan	26-Sep	
Date of harvest	15-Nov	31-Dec	25-Oct	
Total number of applications	211 for T and 270 for E	NA	60 for solid and 120 for pond water	Total number of applications in one year
Farm flag	6	4	0	4- feedlot and 6- pasture; Flag used in modified PRZM
Application date	1-Apr	1-Jan	Refer Table 16	USEPA Standard Scenario
Application stop date	28 Oct for T and 26 Dec for E	NA	NA	Only used for modified PRZM
WINDAY	0	0	0	USEPA Standard Scenario
Chemical Application Method (CAM)	4	4	4	4- Soil applied -uniform with depth
Depth of incorporation - cm (DEPI)	5	10	5	5 cm for surface application (pond water, solid manure (no-till) and pasture) and 10 cm for feedlot
Application rate- kg/ha	Refer Table 17	Refer Table 17	Refer Table 17	
Application efficiency	1	1	1	USEPA Standard Scenario
Spray drift	0	0	0	USEPA Standard Scenario
Filtration Parameter (FILTRA)	0	0	0	USEPA Standard Scenario

Parameter	Pasture	Feedlot	Crop	Rationale/source
IPSCND	1	1	1	USEPA Standard Scenario
Plant uptake factor (UPTKF)	0	0	0	USEPA Standard Scenario
Total depth of soil core	100	100	100	USEPA Standard Scenario
Bulk density (BD FLAG)	0	0	0	USEPA Standard Scenario
Field capacity and Wilting point (TH FLAG)	0	0	0	USEPA Standard Scenario
KD FLAG	1	1	1	USEPA Standard Scenario
Drainage flag (HSWZT)	0	0	0	USEPA Standard Scenario
MOC flag	0	0	0	USEPA Standard Scenario
Irrigation flag (IRFLAG)	0	0	0	USEPA Standard Scenario
Soil temp flag (ITFLAG)	0	0	0	USEPA Standard Scenario
Thermal conductivity (ID FLAG)	0	0	0	USEPA Standard Scenario
Biodegradation (BIO FLAG)	0	0	0	USEPA Standard Scenario
Diffusion Coefficient (cm2/day) (DAIR)	0	0	0	USEPA Standard Scenario
Henry's Law constant - dimensionless	0	0	0	USEPA Standard Scenario
Enthalpy of vaporization (ENPY)	0	0	0	USEPA Standard Scenario
PCMC	4	4	4	USEPA Standard Scenario
SOL(KOC)	912 for T and 1259 for E	912 for T and 1259 for E	912 for T and 1259 for E	Adsorption coefficient (Koc)
Horizon number	1	1	1	Number of the soil layer in soil profile
Horizon thickness-cm	10	10	10	For pasture and cropland - standard soil parameters from Ohio scenario are used. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
Bulk density	1.6	0.85	1.6	
Field Capacity (FC)	0.294	0.45	0.294	
Wilting Point (WP)	0.086	0.14	0.086	
Organic Carbon (OC)	1.16	38	1.16	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)

Parameter	Pasture	Feedlot	Crop	Rationale/source
Horizon number	2	2	2	Number of the soil layer in soil profile
Horizon thickness-cm	12	10	12	For pasture and cropland - standard soil parameters from Ohio scenario are used. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
Bulk density	1.6	1.25	1.6	
FC	0.294	0.321	0.294	
WP	0.086	0.202	0.086	
OC	1.16	31	1.16	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	3	3	3	Number of the soil layer in soil profile
Horizon thickness-cm	78	2	78	For pasture and feedlot - standard soil parameters from Ohio scenario are used. For Feedlot, standard Ohio soil parameters from second horizon were used
Bulk density	1.65	1.6	1.65	
FC	0.147	0.294	0.147	
WP	0.087	0.086	0.087	
OC	0.174	1.16	0.174	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	NA	4	NA	Number of the soil layer in soil profile
Horizon thickness-cm	NA	78	NA	For Feedlot, standard Ohio soil parameters from third horizon were used
Bulk density	NA	1.65	NA	
FC	NA	0.147	NA	
WP	NA	0.087	NA	
OC	NA	0.174	NA	
DWRATE , DSRATE	NA	0	NA	Degradation rate (LN(2)/half life)

Appendix 6 PRZM Inputs Specific to Michigan Modeling

Parameter	Pasture	Feedlot	Crop	Rationale/source
Pan Factor (PFAC)	0.76	0.76	0.76	Used to estimate daily evapotranspiration. Figure 5.1 PRZM manual
Snowmelt factor (SFAC)	0.36	0.36	0.36	Depends upon forest cover. Figure 5.1 PRZM manual
Pan factor flag	0	0	0	Signifies PAN data read
Min depth of evaporation (ANETD)	12.5	12.5	12.5	Depends upon geographical location. Figure 5.2 PRZM manual
Surface condition of initial crop (ISCOND)	3	1	3	1= fallow, 2= cropping, 3= residue
Erosion Flag	4	5	4	4- MUSS (PRZM manual) and 5- modified MUST equation
USLE K or RSDM	0.2	12 t/ha	0.2	USLE K - Depends upon textural class and OM; Table 5.3 PRZM manual. RDSM is manure of surface in t/ha
USLE LS	0.2	1	0.2	Depends upon % slope and slope length. Table 5.5 PRZM manual
USLE P	1	1	1	Depends upon slope and land practice used. Table 5.6 PRZM manual
Area of field - ha (AFIELD)	172.8	172.8	172.8	Standard area used for index reservoir
AGPM	NA	0	NA	AGPM is standing living and dead plant material for erosion equation
Location of NCRS 24 hyetograph	3	3	3	Depends upon geographical location. Figure 5.12 PRZM manual.
Land slope % (SLP)	1	4	1	Standard scenario land slope for cropland and pasture. 4% slope for feedlot (FASS guide)
Hydraulic length	600	600	600	Standard value for index reservoir
No of crops (NDC)	1	1	1	PRZM requires to simulate at least one crop
Max interception storage -cm (CINTCP)	0.2	0	0.25	Depends upon crop canopy
Max Rooting depth cm (AMXDR)	43	0	90	Depends upon crop. Table 5.9 PRZM manual
Max areal coverage % (COVMAX)	97	0	100	Depends upon crop canopy
Curve Number (CN)	93 89 93	95 95 95	94 91 94	CN for fallow, cropping and residue. Table 5.16 PRZM manual
Max canopy height at maturation -cm (HTMAX)	122	0	300	Depends upon crop. Table 5.16 PRZM manual

Parameter	Pasture	Feedlot	Crop	Rationale/source
Day and Month for USLEC and N	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	1505 1605 0106 1506 1606 0107 1607 0108 1608 0109 1609 0110 1610 2010 2510 0111 1611 0112 1612 0101 1601 0102 1602 0103 1603 0104 1604 2004 0105	Pasture and Crop = USDA Agricultural Handbook Pasture = For Fallow Conditions – PRZM Manual
USLE C factor	0.001 for all year	1 for all year	.669 .665 .650 .622 .514 .369 .265 .239 .243 .282 .367 .394 .394 .038 .101 .113 .128 .138 .146 .342 .348 .353 .358 .365 .382 .406 .422 .579 .610	Depends upon crop, rotation and management of land. Table 5.7 PRZM manual
Manning's N	0.11 for all year	0.05 for all year	0.014 for all year	Roughness coefficient for overland flow. Table 5.46 PRZM manual
Date of emergence	1-Apr	1-Jan	15-May	Pasture is simulated as all year crop. No crop is simulated for feedlot. And Michigan corn cropping dates taken from USDA Agricultural Handbook
Date of maturation	15-Apr	2-Jan	15-Aug	
Date of harvest	15-Nov	31-Dec	20-Oct	
Total number of applications	211 for T and 270 for E	NA	60 for solid and 120 for pond water	Total number of applications in one year
Farm flag	6	4	0	4- feedlot and 6- pasture; Flag used in modified PRZM
Application date	1-Apr	1-Jan	Refer Table 16	UEPA Standard Scenario
Application stop date	28 Oct for T and 26 Dec for E	NA	NA	Only used for modified PRZM
WINDAY	0	0	0	USEPA Standard Scenario
Chemical Application Method (CAM)	4	4	4	4- Soil applied -uniform with depth
Depth of incorporation - cm (DEPI)	5	10	5,15	5 cm for surface application (pond water and pasture), 10 cm for feedlot and 15 cm for solid manure application
Application rate- kg/ha	Refer Table 17	Refer Table 17	Refer Table 17	
Application efficiency	1	1	1	USEPA Standard Scenario
Spray drift	0	0	0	USEPA Standard Scenario
Filtration Parameter (FILTRA)	0	0	0	USEPA Standard Scenario
IPSCND	1	1	1	USEPA Standard Scenario

Parameter	Pasture	Feedlot	Crop	Rationale/source
Plant uptake factor (UPTKF)	0	0	0	USEPA Standard Scenario
Total depth of soil core	100	100	100	USEPA Standard Scenario
Bulk density (BD FLAG)	0	0	0	USEPA Standard Scenario
Field capacity and Wilting point (TH FLAG)	0	0	0	USEPA Standard Scenario
KD FLAG	1	1	1	USEPA Standard Scenario
Drainage flag (HSWZT)	0	0	0	USEPA Standard Scenario
MOC flag	0	0	0	USEPA Standard Scenario
Irrigation flag (IRFLAG)	0	0	0	USEPA Standard Scenario
Soil temp flag (ITFLAG)	0	0	0	USEPA Standard Scenario
Thermal conductivity (ID FLAG)	0	0	0	USEPA Standard Scenario
Biodegradation (BIO FLAG)	0	0	0	USEPA Standard Scenario
Diffusion Coefficient (cm2/day) (DAIR)	0	0	0	USEPA Standard Scenario
Henry's Law constant - dimensionless	0	0	0	USEPA Standard Scenario
Enthalpy of vaporization (ENPY)	0	0	0	USEPA Standard Scenario
PCMC	4	4	4	USEPA Standard Scenario
SOL(KOC)	912 for T and 1259 for E	912 for T and 1259 for E	912 for T and 1259 for E	Adsorption coefficient (Koc)
Horizon number	1	1	1	Number of the soil layer in soil profile
Horizon thickness-cm	10	10	10	For pasture and cropland - standard soil parameters from Michigan scenario are used. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
Bulk density	1.1	0.85	1.1	
Field Capacity (FC)	0.377	0.45	0.377	
Wilting Point (WP)	0.257	0.14	0.257	
Organic Carbon (OC)	3.48	38	3.48	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	2	2	2	Number of the soil layer in soil profile
Horizon thickness-cm	12	10	12	For pasture and cropland -

Parameter	Pasture	Feedlot	Crop	Rationale/source
Bulk density	1.1	1.25	1.1	standard soil parameters from Michigan scenario are used. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
FC	0.377	0.321	0.377	
WP	0.257	0.202	0.257	
OC	3.48	31	3.48	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	3	3	3	Number of the soil layer in soil profile
Horizon thickness-cm	78	2	78	For pasture and feedlot - standard soil parameters from Michigan scenario are used. For Feedlot, standard Michigan soil parameters from second horizon were used
Bulk density	1.8	1.1	1.8	
FC	0.314	0.377	0.314	
WP	0.254	0.257	0.254	
OC	0.29	3.48	0.29	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	NA	4	NA	Number of the soil layer in soil profile
Horizon thickness-cm	NA	78	NA	For Feedlot, standard Michigan soil parameters from third horizon were used
Bulk density	NA	1.8	NA	
FC	NA	0.314	NA	
WP	NA	0.254	NA	
OC	NA	0.29	NA	
DWRATE , DSRATE	NA	0	NA	Degradation rate (LN(2)/half life)

Appendix 7 PRZM Inputs Specific to Texas Modeling

Parameter	Pasture	Feedlot	Crop	Rationale/source
Pan Factor (PFAC)	0.69	0.71	0.71	Used to estimate daily evapotranspiration. Figure 5.1 PRZM manual
Snowmelt factor (SFAC)	0.36	0.36	0.36	Depends upon forest cover. Figure 5.1 PRZM manual
Pan factor flag	0	0	0	Signifies PAN data read
Min depth of evaporation (ANETD)	25	25	25	Depends upon geographical location. Figure 5.2 PRZM manual
Surface condition of initial crop (ISCOND)	3	1	3	1= fallow, 2= cropping, 3= residue
Erosion Flag	4	5	4	4- MUSS (PRZM manual) and 5- modified MUST equation
USLE K or RSDM	0.37	12 t/ha	0.31	USLE K - Depends upon textural class and OM; Table 5.3 PRZM manual. RDSM is manure of surface in t/ha
USLE LS	0.69	1	1.34	Depends upon % slope and slope length. Table 5.5 PRZM manual
USLE P	1	1	1	Depends upon slope and land practice used. Table 5.6 PRZM manual
Area of field - ha (AFIELD)	172.8	172.8	172.8	Standard area used for index reservoir
AGPM	NA	0	NA	AGPM is standing living and dead plant material for erosion equation
Location of NCRS 24 hyetograph	3	3	3	Depends upon geographical location. Figure 5.12 PRZM manual.
Land slope % (SLP)	4	4	6	Standard scenario land slope for cropland and pasture. 4% slope for feedlot (FASS guide)
Hydraulic length	600	600	600	Standard value for index reservoir
No of crops (NDC)	1	1	1	PRZM requires to simulate at least one crop
Max interception storage -cm (CINTCP)	0.2	0	0.25	Depends upon crop canopy
Max Rooting depth cm (AMXDR)	43	0	90	Depends upon crop. Table 5.9 PRZM manual
Max areal coverage % (COVMAX)	97	0	100	Depends upon crop canopy
Curve Number (CN)	87 83 86	95 95 95	92 89 92	CN for fallow, cropping and residue. Table 5.16 PRZM manual
Max canopy height at maturation -cm (HTMAX)	122	0	300	Depends upon crop. Table 5.16 PRZM manual

Parameter	Pasture	Feedlot	Crop	Rationale/source
Day and Month for USLEC and N	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612	1603 0104 1004 1504 1604 0105 1605 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 0111 1611 0112 1612 0101 1601 0102 1602 0103	Pasture and Crop = USEPA Standard Scenario Feedlot = For Fallow Conditions – PRZM Manual
USLE C factor	0.001 for all year	1 for all year	.356 .489 .540 .562 .561 .549 .440 .318 .241 .219 .221 .222 .225 .242 .294 .211 .222 .196 .165 .150 .132 .285 .298 .312 .327 .340	Depends upon crop, rotation and management of land. Table 5.7 PRZM manual
Manning's N	0.11 for all year	0.05 for all year	0.014 for all year	Roughness coefficient for overland flow. Table 5.46 PRZM manual
Date of emergence	1-Mar	1-Jan	16-Mar	For Pasture, Texas range standard scenario cropping dates are used. No crop is simulated for feedlot. And standard Texas corn cropping dates are used for cropland
Date of maturation	15-Jun	2-Jan	25-Jul	
Date of harvest	15-Nov	31-Dec	10-Sep	
Total number of applications	211 for T and 270 for E	NA	60 for solid and 120 for pond water	Total number of applications in one year
Farm flag	6	4	0	4- feedlot and 6- pasture; Flag used in modified PRZM
Application date	1-Mar	1-Jan	Refer Table 16	USEPA Standard Scenario Standard Scenario
Application stop date	28 Oct for T and 26 Dec for E	NA	NA	Only used for modified PRZM
WINDAY	0	0	0	USEPA Standard Scenario
Chemical Application Method (CAM)	4	4	4	4- Soil applied -uniform with depth
Depth of incorporation - cm (DEPI)	5	10	5,15	5 cm for surface application (pond water and pasture), 10 cm for feedlot and 15 cm for solid manure application
Application rate- kg/ha	Refer Table 17	Refer Table 17	Refer Table 17	
Application efficiency	1	1	1	USEPA Standard Scenario
Spray drift	0	0	0	USEPA Standard Scenario

Parameter	Pasture	Feedlot	Crop	Rationale/source
Filtration Parameter (FILTRA)	0	0	0	USEPA Standard Scenario
IPSCND	1	1	1	USEPA Standard Scenario
Plant uptake factor (UPTKF)	0	0	0	USEPA Standard Scenario
Total depth of soil core	43	100	100	USEPA Standard Scenario
Bulk density (BD FLAG)	0	0	0	USEPA Standard Scenario
Field capacity and Wilting point (TH FLAG)	0	0	0	USEPA Standard Scenario
KD FLAG	1	1	1	USEPA Standard Scenario
Drainage flag (HSWZT)	0	0	0	USEPA Standard Scenario
MOC flag	0	0	0	USEPA Standard Scenario
Irrigation flag (IRFLAG)	0	0	0	USEPA Standard Scenario
Soil temp flag (ITFLAG)	0	0	0	USEPA Standard Scenario
Thermal conductivity (ID FLAG)	0	0	0	USEPA Standard Scenario
Biodegradation (BIO FLAG)	0	0	0	USEPA Standard Scenario
Diffusion Coefficient (cm2/day) (DAIR)	0	0	0	USEPA Standard Scenario
Henry's Law constant - dimensionless	0	0	0	USEPA Standard Scenario
Enthalpy of vaporization (ENPY)	0	0	0	USEPA Standard Scenario
PCMC	4	4	4	USEPA Standard Scenario
SOL(KOC)	912 for T and 1259 for E	912 for T and 1259 for E	912 for T and 1259 for E	Adsorption coefficient (Koc)
Horizon number	1	1	1	Number of the soil layer in soil profile
Horizon thickness-cm	10	10	10	For pasture and cropland - standard soil parameters from Texas corn and Texas range scenario, respectively. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
Bulk density	1.4	0.85	1.6	
Field Capacity (FC)	0.28	0.45	0.174	
Wilting Point (WP)	0.164	0.14	0.064	
Organic Carbon (OC)	1.16	38	0.58	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	2	2	2	Number of the soil layer in soil profile
Horizon thickness-cm	5	10	10	For pasture and cropland - standard soil parameters from

Parameter	Pasture	Feedlot	Crop	Rationale/source
Bulk density	1.4	1.25	1.6	Texas corn and Texas range scenario, respectively. For feedlot - Meilke et. al., 1974 and Cole et. al., 2009
FC	0.28	0.321	0.174	
WP	0.164	0.202	0.064	
OC	1.16	31	0.58	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)
Horizon number	3	3	3	Number of the soil layer in soil profile
Horizon thickness-cm	28	80	80	For pasture and cropland - standard soil parameters from Texas corn and Texas range scenario, respectively. For feedlot - standard soil parameters from Texas corn scenario
Bulk density	1.4	1.7	1.7	
FC	0.251	0.235	0.235	
WP	0.142	0.165	0.165	
OC	0.73	0.29	0.29	
DWRATE , DSRATE	0.231 for T and 0.223 for E	0	0.231 for T and 0.223 for E	Degradation rate (LN(2)/half life)