Veterinary Medicine Research & Development
Kalamazoo, Michigan 49007
United States

Environmental Assessment for Re-Implant Use of Synovex® Choice, Synovex® Plus, and Synovex® ONE Ear Implant Products in Feedlot Beef Steers and Heifers

Active Ingredients: Trenbolone Acetate, Estradiol Benzoate

Date: 21 April 2022

LIST OF ABBREVIATIONS AND ACRONYMS

ac	Acre
AF	Assessment factor
AFO	Animal feeding operation
AOP	Adverse outcome pathway
AR	Androgen receptor
ARTA	Androgen receptor transactivation assay
AU	An animal unit equals one beef steer or heifer on pasture or feedlot.
BMP	Best management practice associated with manure management
CAFO	Concentrated animal feeding operation
CAS	Registry numbers assigned by the Chemical Abstracts Service
CNMP	Comprehensive nutrient management plan
CVM	FDA's Center for Veterinary Medicine
DC	Deconjugated, i.e., enzymatically treated during analysis to deconjugate conjugated steroids
DT_{50}	Time to dissipate or degrade to one-half of the initial concentration. The term half-life is used interchangeably with DT_{50} in this document.
dw	Dry weight
E1	Estrone
E2	Estradiol
17α-E2	17α-Estradiol
17β-E2	17β-Estradiol
E3	Estriol
EA	Environmental assessment
EB	Estradiol benzoate
EDC	Endocrine disrupting compound(s)
EPA	US Environmental Protection Agency
EXAMS	US EPA's Exposure Analysis Modeling System
GC-MS/MS	Gas chromatography-tandem mass spectrometry/mass spectrometry
GLP	Good Laboratory Practices
ha	Hectare (2.471 acre/ha)
HPLC	High Performance Liquid Chromatography
HUC	Hydrologic Unit Code (HUC) 12-digit Watershed Boundary Dataset
IUPAC	International Union for Pure and Applied Chemistry
JECFA	Joint Expert Committee for Food Additives of Codex Alimentarius
K_d	Soil distribution coefficient
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of detection
LOQ	Lower limit of quantitation
LSC	Liquid scintillation counting or liquid scintillation counter
MW	Molecular weight
NA	Not applicable, not available, or not analyzed
NADA	New Animal Drug Application
NASS	USDA National Agricultural Statistics Service
nc	Not calculated
nd	Not determined or not detected
ND	Not deconjugated, i.e., not enzymatically treated during analysis to deconjugate conjugated steroids

NEPA	National Environmental Policy Act
nm	Not measured
NMP	Nutrient management plan for manure
NOEC	No Observed Effect Concentration
NPDES	EPA National Pollutant Discharge Elimination System
NR	Not reported
ONE-F	Synovex® ONE Feedlot
ONE-G	Synovex® ONE Grower, formerly Synovex® ONE Grass
P	Phosphorus
Pa	Pascal
PCA	Percent cropped area
PEC or PEC_{water}	Predicted environmental concentration (in water). PEC and PEC_{water} are used interchangeably in this EA.
PNEC	Predicted no effect concentration
PRZM	US EPA's pesticide root zone model
RQ	Risk quotient
SDS	Safety data sheet (material safety data sheet, MSDS)
SETAC	Society of environmental toxicology and chemistry (professional organization)
TB	Trenbolone
17α-TB	17α-Trenbolone
17β-TB	17β-Trenbolone.
TBA	Trenbolone acetate
TDO	Trendione
US or USA	United States of America
USDA	United States Department of Agriculture
USGS	United States Geological Survey

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	Exposure to a single chemical by multiple pathways and routes of exposure (e.g., runoff from CAFO, pasture, and cropland).

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, 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 exogenous compound that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones responsible for maintenance of homeostasis, reproduction, development, or behavior
Mixed-use watershed	In this EA, a mixed-use watershed is a watershed receiving estradiol and trenbolone metabolites from multiple exposure pathways, which include runoff from manured croplands, pasture cattle, AFOs with <1000 beef cattle, and application of runoff water collection from a lagoon to cropped fields from CAFOs with ≥1000 beef cattle.
NOEC	The highest concentration of the toxicant tested that has no significant observable effect on the organism(s) exposed to it
PCA	The percentages of land area in a watershed allocated to different livestock uses such as feedlot, pasture, and cropland for cattle
RQ	The predicted environmental concentration (PEC) for a substance divided by the predicted no effect concentration (PNEC)
Surrogate estradiol compound	A single estradiol-like compound with the physical-chemical and environmental fate properties that conservatively are a composite of representative metabolites of estradiol benzoate (17β-estradiol, 17α-estradiol, and estrone).
Surrogate trenbolone compound	A single trenbolone-like compound with the physical-chemical and environmental fate properties that conservatively are a composite of representative metabolites of trenbolone acetate (17β-trenbolone, 17α-trenbolone, and trendione).

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EXECUTIVE SUMMARY

A unified environmental assessment (EA) was prepared to support re-implant use of Synovex® Choice, Plus, ONE Feedlot, and ONE Grower ear implant products in beef steers and heifers fed in confinement. An exposure assessment was conducted in which predicted environmental concentrations (PECs) of estradiol benzoate (EB) and trenbolone acetate (TBA) metabolites were calculated for an intense-use watershed in Iowa. The Iowa watershed had the highest exposure in the 2014 EA for Synovex ONE and represents an intensive use, reasonable worst-case scenario for the United States.

PECs were determined for a “surrogate estradiol compound” and a “surrogate trenbolone compound” which are estradiol-like and trenbolone-like compounds with physical-chemical and environmental fate properties that conservatively are a composite of representative metabolites of EB and TBA. PECs for several re-implant scenarios using different combinations of Synovex implants were modeled and the combined contributions from feedlots, pasture, and manured cropland discharging to surface water were determined. PECs used for risk assessment were the 90th percentiles of the annual maximum 21-day concentrations from simulations spanning a 30-year period.

Risk characterization was based upon the risk quotient (RQ) method in which RQ equals the PEC divided by the PNEC (predicted no effect concentration) for a sensitive species. An RQ value in the range of 1 or less indicates that significant environmental effects are highly unlikely at the predicted level of exposure. Daily RQs for all implantation scenarios also were calculated to identify any time periods in the 30-year period when the daily RQ exceeded 1.

RQs based upon upper 90th percentile PECs were <1 for both estradiol and trenbolone in all single implant and re-implant scenarios. Daily RQs for estradiol were <1 in all scenarios. Daily RQs for trenbolone were <1 in all trenbolone scenarios except the two re-implant scenarios with the highest 90th percentile PECs. Over the 30-year simulation period, each of two scenarios had a single short-duration event with a maximum daily RQ close to 1.

Based on all available information and the RQ values determined in this EA, we conclude that no significant environmental impacts are expected from re-implant use of Synovex Choice, Plus, ONE Feedlot, or ONE Grower in beef steers and heifers. There are many conservative assumptions and mitigating factors used throughout this assessment that further reduce the risk associated with this proposed use of Synovex products.

1. PURPOSE AND NEED

In accordance with the Code of Federal Regulations 21 CFR 25.15(a), all applications or petitions requesting agency action require the submission of an environmental assessment (EA) or a claim of categorical exclusion to evaluate whether the approval of the product will cause significant impacts to the environment. This EA is provided to support supplemental New Animal Drug Applications (NADAs) for re-implant regimens of Synovex® Choice, Plus, ONE products containing trenbolone acetate (TBA) and estradiol benzoate (EB).

Synovex Choice, Plus, ONE Feedlot (ONE-F), and ONE Grower (ONE-G) are approved products for use in beef cattle fed in confinement (feedlots). However, increased use of a drug may occur if the drug will be administered at higher dosage levels, for longer duration, and/or for different indications than previously in effect (21 CFR 25.5(b)(4)). Accordingly, the

FDA has determined that an EA is required for re-implant use of Synovex products containing EB and TBA.

2. DESCRIPTION OF PROPOSED ACTION

The proposed action is for supplemental approvals for Synovex Choice (NADA 141-043), Synovex Plus (NADA 141-043), and Synovex ONE Feedlot (NADA 141-348) for steers and heifers fed in confinement for slaughter in which Synovex Choice is applied as the first implant and Synovex Choice, ONE Feedlot, or Plus is administered 60 to 120 days later. A single unifying EA has been written to address these re-implant uses as well as other uses that could be considered in the future. See Section 5.2.

3. RISK ASSESSMENT APPROACH

A comprehensive environmental risk assessment document (EA) was published in 2014 to support the approval of Synovex ONE [1]. The EA for Synovex ONE is referred to as the "2014 EA" throughout this document. The approach is described briefly below. Refer to the 2014 EA for descriptions of assumptions, approaches, and methods.

First, physical-chemical properties and degradation rates of EB and TBA metabolites in manure, soil, water, and sediment were determined to estimate the fate and movement of these compounds in soil and water. Parameters were established for a "surrogate estradiol compound" and "surrogate trenbolone compound". These are estradiol-like and trenbolone-like compounds with physical-chemical and environmental fate properties that conservatively are a composite of representative metabolites of EB and TBA.

Second, PECs were determined for the surrogate estradiol and surrogate trenbolone compounds for five regions of the USA. These regions were selected as regions with high potential vulnerability to surface waters from runoff or erosion of trenbolone and estradiol residues from manure from implanted animals. Each PEC used for risk assessment was the 90^{th} percentile of the annual maximum 21-day moving average concentrations over a 30-year simulation period.

Third, fish were determined to be a highly sensitive sentinel species for understanding the impact of endocrine disruption in the aquatic environment. Zoetis-owned data and data from the literature regarding the effects of 17α-estradiol, 17β-estradiol, 17α-trenbolone, and 17β-trenbolone on fish reproduction were used to establish no observed effect concentrations (NOECs). The NOEC for each compound was divided by an assessment factor (AF) to calculate a predicted no effect concentration (PNEC).

Lastly, assessment of risk associated with Synovex ONE for the five modeled regions was based upon the RQ values for the surrogate estradiol and surrogate trenbolone compounds.

The models employed and the parameters chosen provided conservative estimates. This resulted in an EA with a high margin of safety and an overestimation of environmental exposure. It was demonstrated in the 2014 EA that metabolites of EB and TBA excreted in manure from cattle treated with Synovex ONE did not pose a significant environmental risk to terrestrial or aquatic environments.

A similar approach was used in this EA to assess the environmental safety of re-implant uses of Synovex products. An aquatic exposure assessment was conducted for TBA and EB for re-implant uses of Synovex ear implant products in feedlot cattle. Diagrams summarizing the risk assessment approach are provided in Appendix 1 and Appendix 2 for EB and TBA, respectively.

The risk assessment consisted of:

- An exposure assessment in which predicted environmental concentrations (PEC_{water}, PECs) were determined for metabolites of estradiol benzoate (EB) and trenbolone acetate (TBA) for representative re-implant combinations of Synovex products. A new buildup mixed-use watershed model was developed to estimate PECs from cattle receiving more than one implant. Because there are many possible re-implant combinations and regimens, uses with the highest predicted exposures were evaluated in this EA.
- An effects assessment in which predicted no effect concentrations (PNEC) were derived in the 2014 EA for metabolites of trenbolone (17α-trenbolone and 17β-trenbolone) and metabolites of estradiol (17α-estradiol and 17β-estradiol), and
- Risk characterization for EB and TBA metabolites for each of the modeled scenarios. Risk characterization was based upon the risk quotient (RQ) method in which RQ equals the ratio of PEC to PNEC. An RQ value in the range of 1 or less indicates that significant environmental effects are highly unlikely.

Except for revisions required for the buildup model and inclusion of new data available since the 2014 EA was published, the many conservative assumptions in the 2014 EA were retained in this EA with four minor differences. First, the Sioux/Lyon watershed in Iowa was selected as a conservative ‘intensive use’ case that can be applied to any region of the United States. Second, for greatest conservatism, RQs were calculated for the surrogate estradiol compound based upon the PNEC of 17β-estradiol (the most potent metabolite). Third, RQ values for the surrogate trenbolone compound were calculated as the sum of the RQs for 17β-trenbolone and 17α-trenbolone with 5% of the TBA metabolite residues assigned to 17β-trenbolone and 95% to 17α-trenbolone based on new data. Fourth, in addition to RQ values calculated based on the 90th percentile annual maxima, daily RQ values were calculated to identify any events over the 30-year modeling period with RQ values >1.

4. ACTIVE SUBSTANCES IN SYNOVEX CHOICE, PLUS, AND ONE

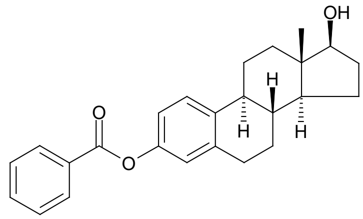
Estradiol benzoate (EB) - The IUPAC (International Union for Pure and Applied Chemistry) name for estradiol benzoate (CAS 50-50-0) is: [(8R,9S,13S,14S,17S)-17-hydroxy-13-methyl-6,7,8,9,11,12,14,15,16, 17-decahydrocyclo-penta[a]-phenanthren3-yl] benzoate. Molecular weight = 376.5 g/mol.

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. Molecular weight = 312.4 g/mol.

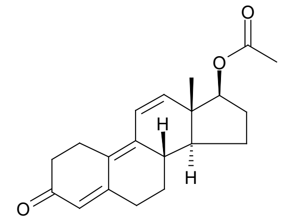
Chemical structures of EB and TBA are shown in Figure 1.

Figure 1. Structures of Estradiol Benzoate (EB) and Trenbolone Acetate (TBA)

EB



TBA



5. MARKETED PRODUCTS

5.1. Formulation and Dose

Synovex products are implanted by subcutaneous injection in the ear of cattle to provide sustained release of trenbolone acetate (TBA) and estradiol benzoate (EB) *in vivo*. Amounts of TBA and EB in Synovex Choice, Plus, ONE Feedlot (ONE-F), and ONE Grower (ONE-G) are shown in Table 1.

Table 1. Amounts of TBA and EB in Synovex Choice, Plus, and ONE Products

Product	Number of Pellets	Coating	EB (mg)	TBA (mg)
Choice	4	None	14	100
Plus	8	None	28	200
ONE Feedlot (ONE-F)	8	Coated	28	200
ONE Grower (ONE-G)	6	Coated	21	150

Synovex Choice, Plus, ONE-F, and ONE-G contain individual pellets containing 3.5 mg estradiol benzoate (EB) and 25 mg trenbolone acetate (TBA). Choice and Plus contain 4 and 8 uncoated pellets and ONE-G and ONE-F contain 6 and 8 pellets. Pellet composition is the same for all products, however pellets for ONE-F and ONE-G are coated with a porous polymer film. Pellet ingredients are described in Table 2. The ingredients of the film coating are described in Table 3.

Table 2. Synovex Choice, Plus, and ONE Pellet Ingredients

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

Table 3. 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	---

The benzoate and acetate groups of EB and TBA, respectively, are rapidly cleaved to form estradiol and trenbolone and related metabolites in cattle. To determine potential environmental effects of EB and TBA metabolites excreted by cattle, the total base activities of estradiol and trenbolone in Synovex Choice, Plus, ONE-F, and ONE-G were calculated using the molecular weight (MW) conversion factors below. The corresponding base activities of estradiol and trenbolone in each implant product are provided in Table 4.

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%

Table 4. Base Activity of Total Estradiol and Trenbolone Dose in Synovex Products

Implant Product	Total Dose of TBA (mg)	Trenbolone-Equivalent Dose (mg)	Total Dose of EB (mg)	Estradiol-Equivalent Dose (mg)
Choice	100	86.55	14	10.13
Plus	200	173.1	28	20.26
ONE Grower	150	129.8	21	15.19
ONE Feedlot	200	173.1	28	20.26

Synovex products are manufactured in strict accordance with procedures approved under NADAs 141-043 and 141-348. Pellets are placed in molded low density polyethylene (LDPE) resin cartridges containing 10 channels that are intended for use with an implant gun. Each of the ten channels in a cartridge contain 4, 6, or 8 pellets. Ten cartridges are placed on a plastic tray and sealed in a laminated foil pouch. At time of use, a cartridge is placed in an implant gun, and all of the individual pellets in a single channel of the cartridge are injected into the ear of an animal.

5.2. Label Indications and Frequency of Use

Synovex Choice, Plus, and ONE products are currently approved for single implant use, i.e., an animal in a production class may only be implanted once. Supplemental approvals are sought for NADAs 141-043 and 141-348 for re-implant use of Synovex Choice, Plus, and ONE-F in beef steers and heifers fed in confinement for slaughter in which Choice is applied as the first implant and a second implant of Choice, ONE Feedlot, or Plus is administered 60 to 120 days later. A single unifying EA has been written to support those claims along with other re-implant uses that could be considered in the future. Accordingly, the following combinations and use regimens have been evaluated in this EA:

- Choice or Plus as lead implant and a second implant of Choice, Plus, ONE-F, or ONE-G is administered 60 to 120 days later,
- ONE-F or ONE-G as lead implant and a second implant of Choice, Plus, ONE-F, or ONE-G is administered 140 to 200 days later,
- Choice or Plus as lead implant, a second implant of Choice or Plus is administered 60 to 120 later, and a third implant of Plus or a lower dose implant is administered 60 to 120 days after the second implant, and
- Choice or Plus as lead implant, a second implant of ONE-F or ONE-G is administered 60 to 120 days later, and a third implant of Plus or a lower dose implant is administered 140 to 200 days after the second implant.

5.3. Disposal of Synovex Choice, Plus, and ONE Implants

Safety Data Sheet (SDS): No change to the SDS for Synovex Choice and Plus (NADA 141-043) or the SDS for Synovex ONE (NADA 141-348). *"Avoid release to the environment. Do not discharge into drains, water courses or onto the ground. Considering the relevant known environmental and human health hazards of the material, review and implement appropriate technical and procedural wastewater and waste disposal measures to prevent occupational exposure and environmental release. It is recommended that waste minimization be practiced. The best available technology should be utilized to prevent environmental releases. This may include destructive techniques for waste and wastewater. Dispose of contents/container in accordance with local/regional/national/international regulations."*

Label: No change to labels for Synovex Choice and Plus (NADA 141-043) or Synovex ONE Feedlot or ONE Grower (NADA 141-348). "*Synovex ear implant waste materials should be disposed of according to prescribed Federal, State, and Local guidelines."*

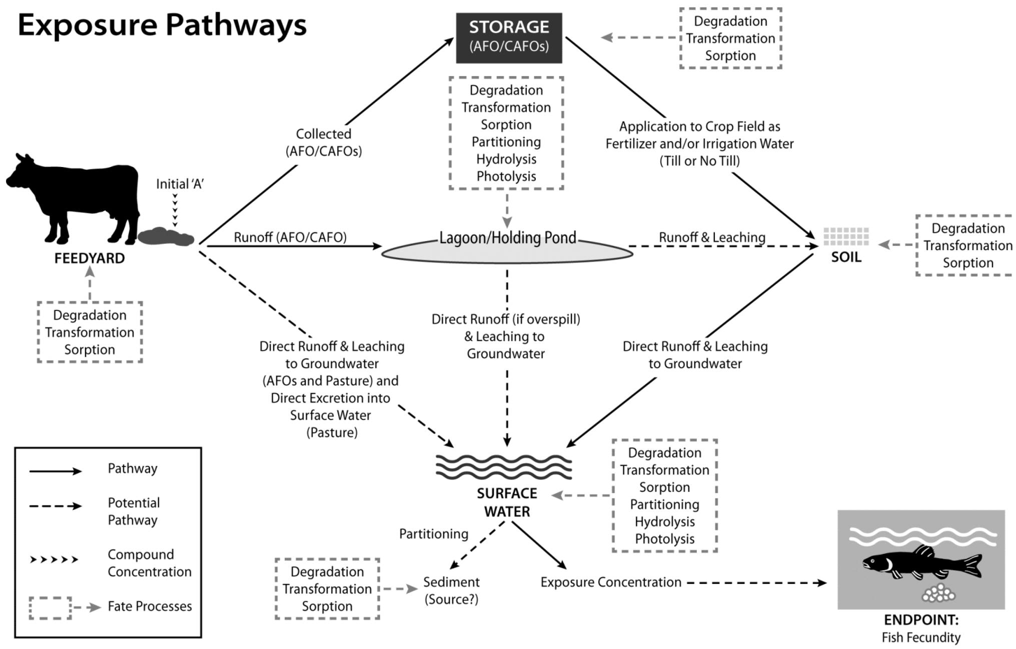
6. ECOSYSTEMS AT RISK FROM BEEF CATTLE PRODUCTION

Synovex Choice, Plus, and ONE may be administered to beef cattle housed in an animal feeding operation (AFO). Trenbolone, estradiol, and their metabolites are excreted in manure from treated cattle in AFOs. Manure is stored for various periods of time and later applied to cropland as fertilizer. In addition to feedlot uses, beef cattle are also raised on pasture and may be administered Synovex ONE-G.

Ecosystems at risk from the use of Synovex products and the potential for aggregate exposure from multiple sources of EB and TBA metabolites released in the environment were identified in the 2014 EA. Beef cattle production is primarily located inland so that EB and TBA metabolites from the use of Synovex implants could be introduced into the environment through direct runoff from AFOs, application of manure or wastewater to cropland as fertilizer, or deposition of manure on pastureland. Ecosystems potentially at risk are therefore primarily freshwater watersheds.

Potential sources (exposure pathways) were described in the 2014 EA. For each source and pathway, an evaluation was conducted for the potential for EB and TBA metabolite residues to migrate to surface water via surface runoff, erosion, and/or leaching to groundwater or via movement of groundwater through the subsurface to surface waters. Exposure pathways found to be potential contributors are described in Section 3.4.1 of the 2014 EA. See Figure 2 below. Sources and pathways that were determined to be negligible contributors to surface water were eliminated from further evaluation. Accordingly, models selected for both the 2014 EA and this EA were surface water runoff/erosion models.

Figure 2. Pathways for Components in Manure to Reach Surface and Groundwater



In studies evaluating the effects of EDCs (endocrine disrupting compounds) on non-target species, fish and amphibians were identified as the most sensitive. As noted in the 2014 EA, there are no data indicating that terrestrial organisms such as earthworms and plants are as sensitive to exposures of steroid hormones. This EA continues to focus on the potential for EB and TBA metabolites to migrate to and potentially impact freshwater aquatic environments from feedlot, cropland, and pasture sources in a watershed.

7. AREAS OF BEEF CATTLE PRODUCTION

7.1. Inventory of Beef Cattle in Confinement

To support environmental modeling, data for the population and distribution of pasture and feedlot cattle were evaluated. Spatial and temporal data were provided in Sections 3.1 and 3.2 of the 2014 EA. Section 3.2.2 of the 2014 EA summarized the inventory of beef cattle in feedlots based on the 2007 United States Department of Agriculture (USDA) Census of Agriculture. At that time, most cattle (85%) were raised on large capacity farms with ≥1000 animal units (AU) that comprised 3% of total farms.

The most recent census is the 2017 USDA Census of Agriculture [2]. Data for the number of farms as a function of size, cattle inventory, and percentages of inventory are provided in Table 5. Data for the number of farms in 1997 and 2007 are shown for comparison.

Table 5. Total USA Cattle on Feed by Farm Size - 2017 Census of Agriculture

Farms with # Animals (mean of range)		# Farms 2017[a]	Number of Cattle Marketed 2017[a]	% Marketed 2017	# Farms 2007[b]	# Farms 1997[c]
1-19	(10)	5,549	76,410	0.31	45,117	69,688
20-49	(35)	8,266	254,516	1.03	11,736	19,295
50-99	(75)	5,086	345,414	1.40	6,579	9,052
100-199	(150)	3,977	541,678	2.20	4,710	5,424
200-499	(350)	3,792	1,148,594	4.66	3,975	3,867
500-999	(750)	1,801	1,255,909	5.09	1,980	1,397
1000-2499	(1750)	907	1,317,593	5.35	1,264	939
2500-4999	(3750)	324	1,103,824	4.48	366	318
5000 or more	(>5000)	571	18,606,059	75.48	669	640
Total		**30,273**	**24,649,997**	**100**	**76,396**	**110,620**

[a]2017 Census of Agriculture [2]. Table 13. Cattle and Calves – Sales: 2017 and 2012. Cattle on feed.
[b]2007 Census of Agriculture [3]. Table 13. Cattle and Calves – Sales: 2007 and 2002. Cattle on feed.
[c]1997 Census of Agriculture [4]. Table 25. Cattle and Calves – Sales: 1997 and 1992. Cattle fattened on grain and concentrate for slaughter.

According to the 2017 survey, most cattle (85%) are raised on large capacity farms with ≥1000 AU that comprise 6% of total farms. From 1997 to 2017, there were consistent declines in the total number of farms and the number of small operations with <500 AU (animal units) on a nationwide level. From 2007 (data used in the 2014 EA) through 2017, there has been a 62% reduction in the number of small and medium AFOs holding <1000 AU and a 22% reduction in the number of large CAFOs with ≥1000 AU. There also has been a general trend of declining numbers of cattle marketed over time (data not shown).

7.2. AFOs Assumed to Discharge Directly to Surface Waters

In the 2014 EA, it was estimated that 17% of small and medium AFOs on a nationwide basis may be in need of runoff control improvements and thus could potentially directly discharge to surface waters. This value was rounded to 25% (Appendix 9 of the 2014 EA). Note that this value did not include adjustments for increased voluntary compliance or enforcement of the Clean Water Act and the NPDES (EPA National Pollutant Discharge Elimination System) program and is therefore conservative.

Most of the use of ear implants occurs in large farms with ≥1000 AU (CAFOs) that are regulated under the Clean Water Act. CAFOs are less likely to be polluters of surface water because these operations are required to minimize potential pollution of surface water through use of runoff containment systems and to apply manure to agricultural fields in accordance with Comprehensive Nutrient Management Plans (CNMPs) under Best Management Practices (BMPs).

With the reduction of small and medium farms from 1997 to 2017 described in Section 7.1 and continuing improvements in manure management practices in the United States, the percentage of farms in need of runoff control improvements is significantly lower than the estimate of 17% based upon the 1997 data. For the purposes of this EA, a value of 25% is retained as a conservative estimate for the percentage of small and medium AFOs that could potentially directly discharge feedlot runoff to surface waters in a local watershed.

7.3. Watershed Selection for Modeling

National statistics, while informative, do not directly apply to potential use of ear implants in watersheds with high beef cattle densities. The mixed-use watershed models utilized in the 2014 EA and this EA are based upon the cattle population within individual watersheds.

Watersheds used in the 2014 EA were selected by conducting a national geospatial analysis to identify regions with high potential vulnerability of estradiol and trenbolone compounds in surface waters due to runoff or erosion. The analysis considered areas with high beef cattle density, high feedlot density, and annual precipitation. Watersheds were selected in Iowa, Texas, Michigan, Pennsylvania, and Ohio. See Appendix 3.1.

In environmental modeling conducted for the 2014 EA, the Sioux/Lyon county region of Iowa consistently produced the highest PECs. This region is a HUC 12 level watershed, which is local sub-watershed in USGS/EPA EnviroAtlas. Based on the USDA survey of agriculture data from 2002, 2007, 2012, and 2017 (Appendix 3.2), the Sioux/Lyon watershed continues to rank in the upper 98th percentile or greater in terms of beef cattle density and the upper 99th percentile or greater for smaller operations (<500 head of cattle).

To confirm that the Sioux/Lyon watershed continued to produce the highest predicted environmental exposures among the five watersheds, modeling simulations were conducted with the buildup model for the five study regions using a representative re-implant scenario. The results (Appendix 3.3) confirmed that the Iowa watershed produced the highest PECs and is thus a reasonable worst-case scenario for the United States. Accordingly, all modeling simulations in this EA were performed using the Iowa watershed. PCA values (percent cropped area, percentages of a watershed allocated to different land uses) were updated based on the 2017 cattle census data. See Table 25 of Appendix 3.3.

8. ENVIRONMENTAL FATE OF EB AND TBA METABOLITES

The environmental degradation pathways of EB and TBA metabolites are similar regardless of whether residues enter the terrestrial or aquatic environment. Upon entering the environment in cattle manure, EB and TBA metabolites may be present in manure piles, remain in a feedlot or pasture, enter a manure storage lagoon, or be applied to agricultural soil. The metabolites can potentially bind to manure or soil and they can be transformed and degraded in the environment. Some metabolites may enter the aquatic environment where additional binding, transformation, and degradation can occur.

In the 2014 EA and this EA, "surrogate estradiol compound" describes an estradiol-like compound with physical-chemical and environmental fate properties that conservatively is a composite of representative metabolites of EB: 17β-estradiol, 17α-estradiol, and estrone (E1). Similarly, "surrogate trenbolone compound" describes a trenbolone-like compound with physical-chemical and environmental fate properties that conservatively is a composite of representative metabolites of TBA: 17β-trenbolone, 17α-trenbolone, and trendione.

Physical-chemical property data and environmental fate data are important input parameters in environmental models that were used to estimate the partitioning, persistence, and mobility of compounds in different environmental compartments. Environmental fate properties used in the 2014 EA were based upon data from Zoetis-owned studies and literature data available at the time. The approach and methods used to establish physical-chemical and environmental fate parameters are described in Section 4.2 of the 2014 EA.

A comprehensive literature search was conducted for the period from 2014 to present to determine if environmental fate properties should be updated for the current EA. No articles were found that justify a revision to the environmental fate properties. Environmental fate properties used for the current EA are summarized in Table 6.

Table 6. Environmental Fate Properties of Trenbolone and Estradiol 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 K_{OC}	912	1259
Soil DT50 (days)	3.0	3.1
Anaerobic water sediment DT50 (days)	191.0	107.8
Aerobic water sediment DT50 (days)	53.3	31.1

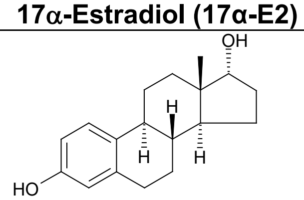
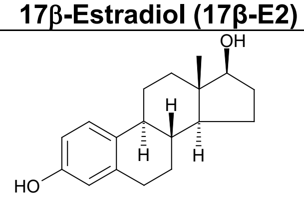
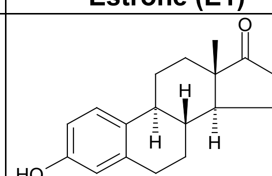
* Henry's constant (atm-m^3/mole) = (vapor pressure (torr) / 760) / (solubility (mg/L) /Molecular Weight)

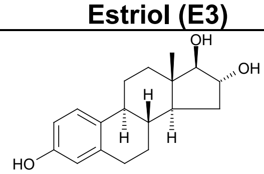
9. METABOLISM OF ESTRADIOL BENZOATE IN CATTLE

The metabolism of EB in cattle was described in Section 4.1.1 of the 2014 EA. Upon solubilization and absorption of EB from the ear, EB is hydrolyzed to 17β-estradiol (17β-E2) in cattle. 17β-E2 is likely further metabolized to 17α-estradiol (17α-E2), which is the principal metabolite identified in cattle excreta. 17β-E2, estrone (E1), and other related compounds have been identified as minor metabolites in cattle manure.

17β-E2 is recognized as the primary compound responsible for the growth performance enhancement effect observed in cattle, whereas 17α-E2 is the principal metabolite identified in cattle manure. Chemical structures are shown in Table 7.

Table 7. Metabolites of Estradiol Benzoate

Name	17α-Estradiol (17α-E2)	17β-Estradiol (17β-E2)	Estrone (E1)
Structure			
CAS Number	57-91-0	50-28-2	53-16-7
Molecular Weight	272.37	272.37	270.37

Name	Estriol (E3)
Structure	
CAS Number	50-27-1
Molecular Weight	288.38

It should be noted that the “principal metabolite identified in excreta” is not necessarily the same as the “principal metabolite excreted from cattle” because additional transformation processes may occur after residues in cattle manure enter the environment. Field monitoring data summarized in the 2014 EA indicate that 17α-E2 and E1 with a small amount of 17β-E2 are the primary metabolites found in cattle waste on a feedlot, whether excreted in this form by cattle or transformed after excretion.

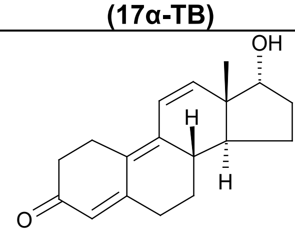
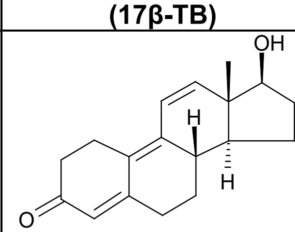
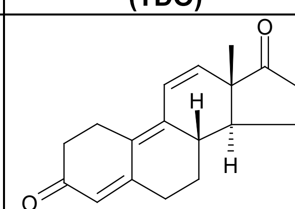
10. METABOLISM OF TRENBOLONE ACETATE (TBA) IN CATTLE

10.1. Background

Metabolism of TBA in cattle was described in Section 4.1.2 of the 2014 EA. Data available at that time demonstrated that TBA is hydrolyzed to 17β-trenbolone (17β-TB) after absorption from the ear and then extensively metabolized in cattle to 17α-trenbolone (17α-TB) along with trendione (TDO) and other metabolites. Small amounts of 17α-TB or TDO can be transformed to 17β-TB by interconversion processes in the environment. Ultimately, all metabolites undergo further degradation to less potent compounds (ultimately to CO_2) and do not accumulate in the environment.

17β-TB is recognized as the primary compound responsible for growth performance enhancement effects observed in cattle, whereas 17α-TB is the principal metabolite in cattle excreta. Chemical structures of 17α-TB, 17β-TB, and TDO are shown in Table 8.

Table 8. Metabolites of Trenbolone Acetate

Name	17α-Trenbolone (17α-TB)	17β-Trenbolone (17β-TB)	Trendione (TDO)
Structure			
CAS Number	80657-17-6	10161-33-8	4642-95-9
Molecular Weight	270.37	270.37	268.36

A key study in the 2014 EA was a Zoetis-owned ^{14}C cattle metabolism and excretion study conducted by Syntex in beef steers and heifers implanted with target doses of 300 mg ^{14}C-TBA and nonlabeled EB (Syntex study). Total radioactivity excreted in urine and feces was determined for the first 30 days after implantation. Metabolite profiling was performed for feces samples by HPLC (high performance liquid chromatography) with radioactivity detection. Urine samples were not profiled.

Metabolism and excretion of TBA was similar in steers and heifers. An average of 83.6% of the total radioactivity was excreted in feces and 16.4% in urine. Approximately 50% of the radioactive residues in feces were not extractable. It was acknowledged that non-extractable residues may not be bioavailable or readily mobile in the environment.

Free 17α-TB (nonconjugated) was the primary metabolite in feces and accounted for an average of 47.2% of total radioactivity. The 17α-TB glucuronide conjugate comprised an average of 6.2% of the extractable residues. Total 17α-TB (sum of free and glucuronide forms) in feces was 53.4%. An average of 1.68% of the extractable radioactivity in feces was 17β-TB. Four additional metabolites together comprised 32.6% of the total radioactivity and were each ≤10% of the extractable residues. TBA and TDO were not detected. See Section 4.1.2 and Appendices 13.2 and 13.3 of the 2014 EA for additional information.

Data from this study and the literature were used in the 2014 EA to allocate the percentages of the PEC for the surrogate trenbolone metabolite in risk quotient calculations. Due to lack of data for the composition and activity of trenbolone metabolites in urine at the time of the 2014 EA, all radioactive residues in urine were considered to be biologically active and conservatively assigned as 17β-TB. See Section 7 of the 2014 EA.

Since the time of the 2014 EA, additional data are available regarding the nature of trenbolone metabolites excreted by cattle in urine and feces (manure). Data regarding the composition of metabolites in cattle excreta are presented in Section 10.2. Data regarding the transformation of metabolites in excreta are presented in Section 10.3. Data regarding the activity of metabolites are presented in Section 10.4. Conclusions from all data and allocation of metabolite residues to activity bins are summarized in Sections 10.5 and 10.6.

10.2. Composition of Trenbolone Metabolites in Cattle Excreta

10.2.1. Metabolite Composition of Cattle Excreta – Zoetis ^{14}C Study

A radiolabel study was conducted by Zoetis in a steer and heifer implanted with [^{14}C]-TBA and non-labeled EB to supplement the data from the Syntex study. There were two primary objectives: (1) to collect total radioactive residue and metabolite profiling data for urine, feces, and manure for up to 70 days post-implantation, and (2) to develop more sensitive analytical methods with improved chromatographic separation to better characterize the composition of trenbolone-related metabolites in cattle excreta.

10.2.1.1. In-Life Phase and Excretion of Total Radioactive Residues

A radiolabel excretion study (Zoetis study A432R-GB-16-417 [6] (Appendix 4.1)) was conducted in a steer and heifer implanted with 200 mg [^{14}C]-TBA and 28 mg non-labeled EB. Urine, feces, and manure were collected periodically through 70 or 71 days after implantation. Total radioactive residue (TRR) concentrations were determined in urine by liquid scintillation counting (LSC) and by combustion-LSC for feces and manure. The excretion rate of total radioactivity was similar for the two animals and peaked between Days 3 and 14. The average daily excretion rate was 0.775% of dose/day (1.5 mg/day) with an average of 28% of the radioactivity excreted in urine and 72% in feces.

10.2.1.2. Metabolite Profiling Phase

Metabolite profiles for urine, feces, and manure samples collected in the Zoetis ^{14}C cattle excretion study were determined in Zoetis study A432R-US-17-536 [7] by reverse-phase HPLC-fraction collection-TopCount analysis. Method development was conducted in Zoetis study A432R-US-17-535 [8]. Additional information is provided in Appendix 4.2 and Appendix 4.3.

Feces: Metabolite profiles in feces were similar for the two animals and over time. The average extractability of ^{14}C residues from feces was 60%. Percentages of 17α-TB, 17β-TB, TDO, and the sum of all other metabolites in the extractable fraction of feces were 32%, 0.8%, 1.4%, and 66% for the steer and 34%, 1.2%, 1.6%, and 64% for the heifer. Five metabolites (unidentified) were quantified besides 17α-TB, 17β-TB, and TDO. Mean percentages of metabolites F5 and F4 were 7.2% and 5.9% for the steer and 8.9% and

6.6% for the heifer, respectively. Each of the other three metabolites were present at <1% to 5% of the total extractable radioactive residues. There were many additional low-level metabolites that together made up 44% and 41% of the extractable residues in steer and heifer feces, respectively.

Due to use of more sensitive instrumentation in the Zoetis ^{14}C study, many low-level metabolites quantified in the Zoetis study were not detected or not chromatographically separated in the Syntex study. As a result, peak area percentages reported for 17α-TB and other metabolites in the Syntex study are generally higher than those observed in the Zoetis study. After accounting for that factor, metabolite profiles were similar across studies.

Observations from the Zoetis ^{14}C study for feces were: (1) 17α-TB was the primary metabolite, (2) most 17α-TB was present in the free (unconjugated) form, (3) metabolites were observed with similar relative abundances and elution order as those observed in the Syntex study, (4) most of the radioactive residue (ca. 65%) was comprised of metabolites other than 17α-TB, 17β-TB, and TDO, and (5) 17β-TB and TDO each comprised <2% of total radioactive residues, on average.

Urine: Because urine was not profiled in the Syntex study, the Zoetis study provides useful information about the composition of TBA metabolites in urine. Metabolite profiles of 24-hour collections of urine (bulk urine) were similar for the steer and heifer and over time. Mean percentages of 17α-TB, 17β-TB, TDO, and the sum of all other radioactive metabolites were 12%, 1.2%, 1.4%, and 85% of total radioactive residues for the steer and 17%, 1.3%, 0.98%, and 81% for the heifer, respectively. Urinary metabolite U7 (unidentified, similar retention time as fecal metabolite F5) comprised an average of 9.5% of the radioactive residues in steer urine and 7.2% in heifer urine. Several other metabolites comprised <1% to 5% each, and many additional low-level metabolites together comprised 54% to 56% of the total radioactive residues. Metabolite profiles of clean catch urine samples after enzymatic deconjugation closely resembled metabolite profiles of 24-hour urine collections.

Observations from the Zoetis ^{14}C study were: (1) clean catch urine contained mostly conjugated metabolites, whereas bulk urine contained mostly nonconjugated metabolites that had extensively self-deconjugated during the 24-hour collection period, (2) 17α-TB and U7 were the primary metabolites in urine, (3) most of the total radioactive residues in urine (81 to 85%) are metabolites other than 17α-TB, 17β-TB, and TDO, and (4) 17β-TB and TDO each comprised <2% of total radioactive residues, on average.

Manure: Extractability of ^{14}C residues and metabolite profiles for manure samples collected in the first 3 to 5 weeks after dosing were similar to feces. Mean percentages of 17α-TB, 17β-TB, TDO, and the sum of all other radioactive metabolites were 33%, 1.0%, 1.8%, and 64%. Residue extractability declined and metabolite profiles changed after 3 to 5 weeks of manure accumulation, indicating that residues had degraded in aged manure under environmental conditions.

Additional data: TBA metabolite profiles also were determined in samples prepared for companion study A430R-US-18-617 [9]. See Section 10.4 and Appendix 4.5. Composite samples and selected individual samples of urine and feces were prepared using excreta collected in study A432R-GB-16-417. Samples were analyzed with and without enzymatic deconjugation.

Mean percentages of 17α-TB, 17β-TB, TDO, and the sum of all other radioactive metabolites in composite urine were 9.3%, 1.2%, 1.2%, and 88%, respectively. Mean percentages in deconjugated samples were 11%, 1.8%, 1.3%, and 87%, respectively. Concentration percentages were similar after deconjugation treatment because conjugated metabolites in bulk urine had extensively self-deconjugated during collection.

Mean percentages of 17α-TB, 17β-TB, TDO, and the sum of all other metabolites in composite feces were 32%, 1.5%, 2.1%, and 65%, respectively, of total extractable radioactive residues. Mean percentages for deconjugated samples were 32%, 2.4%, 1.9%, and 64%, respectively. Concentration percentages were similar after deconjugation treatment because feces contains mostly nonconjugated metabolites due to deconjugation by microflora in the gastrointestinal tract of cattle.

10.2.2. Metabolite Composition of Cattle Excreta – Literature Data

Additional data were published since the time of the 2014 EA regarding the concentrations of trenbolone metabolites in cattle excreta. Because metabolism of TBA in cattle is similar regardless of the implant product administered, data regarding trenbolone metabolism are not limited to studies using Zoetis ear implant products. Data acquired in studies using other ear implant products also are relevant for this EA.

10.2.2.1. Urine and Feces

In Biancotto et al. [10] (Appendix 5.1), urine was collected from 16 beef steers implanted with 200 mg TBA and 40 mg estradiol. Maximum concentrations of 17α-TB and 17β-TB were observed by Day 7 and declined by Day 63. Concentrations increased on Day 68 due to the second phase of release. The average ratio of 17α-TB:17β-TB in urine was 85:15.

In Blackwell et al., 2014 [11] (Appendix 5.2), 8 cattle were implanted with 200 mg TBA and 40 mg estradiol. 17α-TB was the primary metabolite and was excreted mostly in the nonconjugated form in feces and mostly in the conjugated form in urine. 17α-TB, 17β-TB, and TDO in urine accounted for 86.6%, 11.7%, and 1.7%, respectively, as the concentration sum of the three analytes. 17α-TB, 17β-TB, and TDO in the extractable residues in feces accounted for 95.3%, 4.0%, and 0.7%, respectively, as the concentration sum.

In Blackwell et al., 2015 [12] (Appendix 5.3), percentages of 17α-TB, 17β-TB, and TDO as their concentration sum in freshly collected pooled urine from 8 cattle implanted with 200 mg TBA and 40 mg estradiol were 85.1%, 12.4%, and 2.5%, respectively. Percentages as the concentration sum in freshly collected pooled feces were 99.3%, not detected, and 0.7%, respectively.

In Challis et al. [13] (Appendix 5.4), the average concentrations of 17α-TB and 17β-TB were 41±30 ng/g and 3±2 ng/g in fresh feces samples collected from 240 cattle treated with TBA-containing implants in two cattle cycles. TBA and TDO were below the detection limit in all samples. Averaged over 2 years, percentages of 17α-TB, 17β-TB, and TDO as their concentration sum were 93 to 95%, 5 to 7%, and 0% (not detected), respectively. Results from contemporaneous samples collected from commercial feedlots were similar.

10.2.2.2. Manure

Cattle manure is mostly composed of feces, but it also contains residues excreted in urine. Common forms of cattle manure are solid and liquid manure. Solid manure produced in a feedlot contains plant material used as litter/bedding and some surface soil that has absorbed feces and urine excreted by animals. Liquid manure is produced in more intensive livestock rearing facilities, typically without litter/bedding material. Liquid manure also contains residues excreted by cattle in urine and feces.

A difficulty in assessing manure data across studies is that manure may not be collected as a fresh sample or it may be amassed over time as a mixture of fresh and aged manure. To establish the trenbolone metabolite composition of cattle manure, information in this section focuses on studies with samples collected within 1 to 2 weeks of production by cattle. Data from studies not meeting this criterion were omitted for brevity. See the 2014 EA for data presented previously.

In Study I of Schiffer et al. [14] (Appendix 5.5), 17α-TB in liquid cattle manure was 22 times higher than 17β-TB and 49 times higher than TDO. Calculated concentration percentages of 17α-TB, 17β-TB, and TDO were 93.8%, 4.3%, and 1.9% as their concentration sum.

In Bartelt-Hunt et al. [15] (Appendix 5.6), 17α-TB was 100 times greater than 17β-TB in cattle manure samples collected 7 days after implantation in each of two years.

In Khan and Lee [16] (Appendix 5.7), the concentration of 17α-TB in cattle manure was 16 times higher than 17β-TB and 24 times higher than TDO. Concentration percentages were 91% for 17α-TB, 5.7% for 17β-TB, and 3.8% for TDO as their concentration sum. Percentages in irrigation water were similar to manure.

In a study by Jones et al. [17] (Appendix 5.8), most results for 17β-TB and TDO in cattle manure were below the detection limit, however 17α-TB was the predominant metabolite.

In Challis et al. [13] (Appendix 5.4), concentrations of 17α-TB and 17β-TB in pen floor samples (manure with urine and straw bedding) from TBA-implanted animals were lower than freshly collected feces from the same animals. TDO was not detected. Average percentages of 17α-TB, 17β-TB, TDO in manure were similar to feces.

10.2.2.3. Literature Reviews

In a 2016 SETAC Pellston Workshop, trenbolone was selected as a representative androgen agonist for a case study based on the quality and quantity of information [18]. The group concluded that: (1) the principal route of trenbolone entering the environment was through leaching of metabolites from cattle manure, (2) trenbolone is excreted from cattle primarily as 17α-TB, (3) 17β-TB, TDO, and other metabolites are minor, (4) presence of 17α-TB and 17β-TB in run-off from cattle feedlots is infrequent and occurs at ng/L levels when present, (5) 17α-TB is the most relevant metabolite in the aquatic environment due to its higher input concentrations, persistence, and mobility, and (6) interconversion of trenbolone metabolites may occur in some fish species and in the environment under some conditions. The group concluded that most surveillance studies report measured environmental concentrations of 17α-TB, when present, that are an order of magnitude higher than 17β-TB and TDO and thus support these findings.

A review by Ankley et al. [19] reported the same conclusions regarding the principal route, composition, and fate of trenbolone metabolites in the environment. The authors concluded that 17β-TB concentrations in solution in the environment will be significantly lower than 17α-TB based on: (1) low initial concentrations of 17β-TB excreted by cattle, (2) lower mobility/greater affinity of 17β-TB when interacting with solids in the environment, and (3) faster environmental degradation of 17β-TB than 17α-TB.

10.2.3. Cross-Study Comparisons

In non-label studies reported in the literature, 17α-TB, 17β-TB, and TDO concentrations often are expressed as percentages of the three analytes relative to each other. This approach can be used for all studies that quantify concentrations of these analytes. This information can be used in a weight of evidence approach to compare data across studies and establish the composition of trenbolone metabolites excreted by cattle. Data for several studies are summarized in that fashion in Table 29, Table 30 and Table 31 of Appendix 6.

A flaw in this approach is that these calculated percentages are based upon only three metabolites. This ignores the contributions of most of the other TBA metabolites actually present in excreta which were shown to be 86.5% on average for urine, 65% for feces, and 64% for manure in the Zoetis ^{14}C studies. Consequently, percentages of the three analytes as their concentration sum grossly overestimate actual percentages of these metabolites in excreta.

Using the results from the Zoetis ^{14}C study as a bridge, we can estimate the true concentration percentages that would have been observed in nonlabel studies if investigators could quantitate all (total) trenbolone-related metabolites in excreta and not just two or three analytes. This approach is described in Appendix 6. Concentration percentages of 17α-TB, 17β-TB, and TDO expressed relative to all (total) TBA metabolite residues are summarized in Table 29, Table 30, and Table 31 of Appendix 6 for urine, feces, and manure, respectively.

As shown in these tables, results are highly consistent in studies conducted by several researchers using different implant products, study designs, experimental procedures, and analytical methods. This information provides weight of evidence for the composition of trenbolone metabolites in excreta. Conclusions are summarized below.

Urine: Four studies were conducted by three investigators with fresh urine collected from 34 animals. Metabolites were excreted primarily as conjugated compounds and are rapidly deconjugated under environmental conditions or after brief contact with feces. Based upon results from Zoetis studies A432R-US-17-536 and A430R-US-18-617 [7,9], Biancotto et al. [10], and Blackwell et al. [11,12], measured or calculated percentages of 17α-TB, 17β-TB, and TDO vs. total TBA metabolites ranged from 9.3% to 14.5%, 1.2 to 2.0%, and 0.23% to 1.3%. Data were consistent among all studies and demonstrated that percentages of 17α-TB, 17β-TB, and TDO determined in the Zoetis studies are supported by the weight of evidence. Average percentages of 17α-TB, 17β-TB, and TDO based upon the data from Zoetis studies A432R-US-17-536 and A430R-US-18-617 were 11%, 1.4%, and 1.2%, respectively.

Feces: Five studies were conducted by four investigators with fresh feces collected from 266 animals. Metabolites were excreted primarily as nonconjugated compounds. Based upon results from the Syntex study [1], Zoetis studies A432R-US-17-536 and A430R-US-18-617 [7,9], Blackwell et al. [11,12], and Challis et al. [13], measured or calculated percentages of 17α-TB, 17β-TB, and TDO ranged from 31.5% to 53.4%, not detected to 2.4%, and not detected to 2.1% vs. total TBA metabolites. Data were consistent among all studies and demonstrated that percentages of 17α-TB, 17β-TB, and TDO determined in the Zoetis studies are supported by the weight of evidence. Average percentages of 17α-TB, 17β-TB, and TDO based upon the data from Zoetis studies A432R-US-17-536 and A430R-US-18-617 were 32%, 1.6%, and 1.8%, respectively.

Manure: Five studies were conducted by five investigators with fresh manure collected from 489 total animals. Metabolites were present primarily as nonconjugated compounds. Metabolite percentages in manure were similar to feces. Based upon results from Zoetis study A432R-US-17-536 [7], Schiffer et al. [14], Bartelt-Hunt et al. [15], Khan and Lee [16], and Challis et al. [13], measured or calculated percentages of 17α-TB, 17β-TB, and TDO ranged from 32.6% to 35.6%, 0.4% to 2.2%, and not detected to 1.8% vs. total TBA metabolites. Data were consistent among all studies and demonstrated that the percentages of 17α-TB, 17β-TB, and TDO determined in Zoetis study A432R-US-17-536 of 33%, 1.0%, and 1.8%, respectively, are supported by the weight of evidence.

10.3. Post-Excretion Transformation of Trenbolone Metabolites

Data from the Zoetis-owned studies and the literature were presented in Section 10.2 for studies focusing on the relative abundances of trenbolone metabolites in freshly excreted, freshly collected samples. However, 17α-TB, 17β-TB, and TDO can transform in the environment after excretion by cattle. This aspect was considered in the 2014 EA.

In a review by Lange et al. [20], the authors described work by van der Merwe in which trenbolone degraded in urine under direct sunlight and by Vogt in which 17α-TB in feces degraded to non-detectable levels by 7 days when stored at room temperature. The authors noted work by Schiffer et al. [14] regarding the instability of 17α-TB, 17β-TB, and TDO in liquid manure and dung. The authors also described results from soil column experiments in which 17β-TB was converted to 17α-TB and TDO and the potential for interconversion of 17α-TB, 17β-TB, and TDO in the environment.

In Study II of Schiffer et al. [14] (Appendix 5.5), manure from implanted animals was collected from the top, middle, bottom, and effluent areas of a dung pile. Absolute concentrations of 17α-TB, 17β-TB, and TDO in samples from different locations in the pile were highly variable, however relative percentages of the metabolites were similar among samples. Analyte concentrations in samples after 4.5 months of storage were significantly lower in most samples, thus indicating extensive degradation of analytes during manure storage.

In Jones et al. [17] (Appendix 5.8), transformation rates of 17α-TB in manure were determined. Half-lives were 4.1, 2.7, and 1.6 days for samples stored in the dark at 1, 19, or 33°C, respectively. In sunlit samples with an average air temperature of 33°C, 17α-TB decreased by 2-fold in the first 24 hours and then stabilized.

In Blackwell et al., 2015 [12] (Appendix 5.3), feces, urine, and simulated manure (mixture of urine and feces) from implanted cattle were stored in the dark at 21°C. Dissipation half-lives of 17α-TB were 9.5, 5.1, and 8.7 days for urine, feces, and simulated manure, respectively. 17α-TB conjugates in urine were rapidly converted to free steroids (half-life: 1.0 days). Small transient increases in 17β-TB and TDO were attributed to interconversion of 17α-TB, 17β-TB, and TDO in samples.

In Webster et al. [21] (Appendix 5.9), concentration percentages in manure accumulating over 28 days under field conditions from implanted cattle were consistent with data from other nonlabel studies. There were no significant changes in the relative proportions of 17α-TB, 17β-TB, and TDO in manure accumulating in a pen or feedlot over time.

In Challis et al. [13] (Appendix 5.4), concentrations of 17α-TB and 17β-TB from animals treated with TBA and ractopamine dissipated quickly following the final implant, especially in summer months (June and July). At the end of the feeding phase (post-trial, 2017-2018), concentrations of 17α-TB had declined to ≤1 ng/g after 10 days and below the limit of detection after 22 days. 17β-TB also dissipated and was below the limit of detection in all samples collected after May in the 2017-2018 and 2018-2019 seasons.

In Khan et al. [22] (Appendix 5.10), soil microcosms were fortified with 17α-TB, 17β-TB, or TDO and incubated under aerobic conditions at 22°C. Concentrations of the metabolites declined according to pseudo first-order kinetics, with faster degradation of 17α- and 17β-TB than TDO. 17α-TB, 17β-TB, and TDO formed small amounts of each other.

In Cole et al. [23] (Appendix 5.11), microcosms prepared using inocula from water sources were fortified with 17α-TB, 17β-TB, or TDO. Half-lives were 0.9, 1.3, and 2.2 days for 17β-TB, TDO, and 17α-TB, respectively, and were temperature dependent. The analytes declined to non-detectable levels by 15 days. These data demonstrate that the three metabolites interconvert among each other to various extents, but the predominant transformation pathway is for 17α-TB, 17β-TB, and TDO to be metabolized to other metabolites that cannot revert to 17α-TB, 17β-TB, and TDO.

These studies demonstrate that interconversion of 17α-TB, 17β-TB, and TDO is complex. First, 17α-TB, 17β-TB, and TDO dynamically interconvert among each other. Second, transformation of 17α-TB, 17β-TB, and TDO to other metabolites is extensive and limits the extent to which these compounds can interconvert among each other. Third, as described in Section 10.2.3, 17α-TB makes up approximately one-third of the total trenbolone-related residues in cattle manure and percentages of 17β-TB and TDO are both are very low (<2%). This limits the extent that interconversion can occur in the environment because precursor amounts are initially low and rapidly decline over time.

10.4. Activity of Trenbolone Metabolites

10.4.1. Background

In vivo toxicology studies in fish demonstrated that 17β-TB is approximately 10x more potent than 17α-TB (Section 11). As supporting information that this relationship is similar in other vertebrates (mammals), the JECFA (Joint Expert Committee for Food Additives) concluded that 17α-TB had one tenth of the activity of 17β-TB based upon oral studies in pig and rat [24,25].

Data are more limited for TDO. *In vitro* data demonstrate that TDO has similar or lower androgenic activity than 17α-TB [26,27]. *In vivo* data in Japanese medaka by Forsgren et al. [28] confirm that TDO has detectable endocrine activity. With interconversion of 17α-TB and 17β-TB observed in some fish species [29] through TDO as an intermediate, animals dosed independently with 17α-TB or 17β-TB in toxicology studies presumably are exposed to all three compounds to some extent by autoexposure.

Regarding other TBA metabolites excreted by cattle, the Syntex and Zoetis ^{14}C studies demonstrated that most of the TBA metabolites in cattle excreta are compounds other than 17α-TB, 17β-TB, and TDO. Because the androgenic activities of unidentified metabolites are not known, Zoetis adapted and validated the androgen receptor transactivation assay (ARTA) reported by Blake et al. [27] and applied this assay to characterize the androgenic activity of TBA metabolite residues in excreta collected in the Zoetis ^{14}C cattle study.

The following topics are discussed in this section: (1) the adverse outcome pathway approach which justifies the use of *in vitro* assays to assess the relative potency of TBA metabolites, (2) *in vitro* assay data reported in the literature for 17α-TB, 17β-TB, and TDO, (3) validation and implementation of a reporter assay (ARTA) to provide androgenic activity data for this EA, (4) literature data regarding the activity of TBA metabolites in cattle excreta, and (5) conclusions for environmental assessment based upon weight of evidence.

10.4.2. Use of *In Vitro* Assays to Predict the Activity of EDCs

10.4.2.1. Adverse Outcome Pathway (AOP)

In reviews by Ankley and others [30-33], an adverse outcome pathway (AOP) approach has been widely accepted to evaluate androgen receptor (AR)-mediated effects in vertebrates. Adverse outcome pathways depict causal linkages between a molecular initiating event and adverse outcome(s). In the case of trenbolone and its metabolites, the molecular initiating event (AR binding) is definitively linked to an adverse outcome (reproductive effects in fish).

In the 2016 SETAC Pellston Workshop on *Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Substances* [18], a weight of evidence evaluation was conducted on the effects of trenbolone. The group concluded that: (1) due to conservation of mechanism across vertebrates, mammalian AR and mammalian cell lines are effective tools for predicting effects in fish, and (2) *in vitro* assays focusing on androgen agonism as the molecular initiating event are appropriate for determining the activity of TBA metabolites.

10.4.2.2. *In Vitro* Androgen Receptor Data

A review of published *in vitro* data for trenbolone by the SETAC work group [18] indicated that the rank order potency of the trenbolone metabolites was 17β-TB > 17α-TB ≥ TDO. When compared to natural substances, the potency of 17β-TB was similar to dihydrotestosterone and greater than testosterone. The potencies of 17α-TB and TDO were lower than 17β-TB and testosterone but greater than androstenedione, androsterone, and other lower-potency androgens. If a relative potency of 1.0 is assigned to 17β-TB, the relative potency of 17α-TB would be approximately 0.1, which aligns with the greater androgenic activity of 17β-TB in reproduction studies in fish and mammals. Published data are sparse for TDO but indicate that its potency is probably more similar to 17α-TB than 17β-TB.

10.4.2.3. Assay Selection and Validation

For an assay to be used to assess the activity of TBA metabolites in cattle excreta: (1) the assay must be able to differentiate among strong and weak AR agonists and non-active compounds, (2) 17β-TB must be shown to be significantly more potent than 17α-TB with clear discrimination between dose-response curves, and (3) additive activity must be demonstrated. The androgen receptor transcriptional activation assay (ARTA) described by Blake et al. [27] met all criteria and was selected to evaluate the relative androgenic activity of trenbolone metabolites in the Zoetis studies.

ARTA utilizes a human breast cancer cell line (MDA-kb2) containing a functional human androgen receptor that is stably transfected with a luciferase reporter gene introduced by plasmid insertion in the MMTV-LTR (mouse mammary tumor virus-long terminal repeat) enhancer region. ARTA was successfully transferred to Zoetis laboratories, and assay performance was evaluated in Zoetis study A436R-US-18-616 [34] (Appendix 4.4). Results from assay validation experiments confirmed that ARTA was suitable for characterizing the androgenic activity of excreta from implanted cattle.

10.4.2.4. Androgenic Activity of Trenbolone Metabolites – Zoetis Data

ARTA was used in Zoetis study A430R-US-18-617 [9] (Appendix 4.5) to characterize the androgenic activity of trenbolone metabolites in excreta collected in the Zoetis ^{14}C study (Section 10.2.1.1, Appendix 4.1). Urine and feces samples were prepared with and without enzymatic deconjugation and then analyzed by: (1) LSC to measure total radioactive residues, (2) HPLC-TopCount to establish metabolite profiles, and (3) ARTA to determine relative androgenicity. Metabolite profiling results for urine and feces samples prepared for ARTA analysis were summarized in Section 10.2.1.2.

Low androgenic activity was detected in 24-hour urine collections and clean catch urine from treated cattle. Activity did not increase after deconjugation treatment. The combined response of all TBA metabolites in urine was significantly lower than the 17α-TB and 17β-TB positive controls, indicating that all other metabolites present in urine besides 17α-TB, 17β-TB, and TDO did not contribute significant androgenic activity.

Interpreting the androgenic activity of TBA metabolites in feces was complicated by significant androgenic activity of endogenous substances naturally present in cattle feces. Based upon qualitative comparisons of dose response curves of control feces from nontreated cattle and control feces fortified with 17β-TB and 17α-TB, the combined TBA metabolites in feces had significantly lower activity than 17β-TB but similar activity as 17α-TB and endogenous substances. Activity did not increase after deconjugation treatment. Because there are many common TBA metabolites present in urine and feces and metabolites in urine had relatively low activity, this suggests that TBA metabolites in feces other than 17β-TB, 17α-TB, and TDO also have lower androgenic activity.

Because metabolite U7/F5 was a significant metabolite in both urine and feces, U7/F5 was isolated and evaluated in ARTA in Zoetis study A636Z-US-19-691 [35] (Appendix 4.6). Androgenic response for the U7/F5 isolate was not detected at concentrations that produced robust responses for 17β-TB and 17α-TB.

In conclusion, data from these studies demonstrate that the combined TBA metabolites in cattle urine had lower additive activity than 17α-TB, 17β-TB, and TDO and that the combined TBA metabolites in cattle feces had similar activity to endogenous matter and 17α-TB alone but lower activity than 17β-TB. Results indicate that most trenbolone-related metabolites have significantly lower activity individually than 17α-TB and 17β-TB.

10.4.2.5. Androgenic Activity of TBA Metabolites – Literature Data

Several studies were reported in the literature in which *in vitro* assays or *in vivo* bioassays were used to assess androgenic activity in animal excreta or environmental samples. Results for selected studies are described below. Refer to Appendix 12 of the 2014 EA for information about studies described previously.

In Michalsen et al. [36], metabolites of 17β-TB, 17α-TB, and TDO were produced by incubating each compound individually in rat and bovine liver microsomes. All three compounds produced a complex mixture of monohydroxy and higher order hydroxylation products. Some interconversion was observed among 17β-TB, 17α-TB, and TDO. AR binding affinity was markedly reduced in biotransformation samples, which indicated that metabolites of 17β-TB, 17α-TB, and TDO were less potent than their precursor compounds.

In Durhan et al. [37], androgenic activity of cattle feedlot runoff was determined in samples collected upstream, at the discharge drain, and downstream. Activity in downstream samples was similar to upstream samples. Not all drain samples had detectable activity.

In Lorenzen et al. [38], municipal biosolids and dairy cow, beef cattle, swine, and chicken excreta were analyzed for androgenic activity using a reporter assay. Concentrations were reported as 'hormone equivalents' relative to testosterone. In beef cattle, no activity was detected in fecal pats collected 118 and 119 days after implanting heifers with Synovex Plus or from steers at 12 and 32 days after implantation with Component TE-S. Concentrations of 20 and 13 ng/g were observed in fecal pats from heifers at 22 and 49 days after implanting with Component TE-H which decreased to <5 ng/g or was not detected at later timepoints. For comparison, manure from non-lactating pregnant cows had the highest concentrations of all samples (>1500 ng/g), stored dairy cow manure had levels of 300 ng/g, and no androgenic activity was detected in manure from early gestation cows.

In Sellin et al. [39], a fathead minnow bioassay was used to assess the impact of exposure to urine and fecal slurries from nonimplanted and TBA/estradiol-implanted steers. No effects were observed in male or female fish exposed to urine from untreated or implanted cattle. No effects were observed in female fish exposed to fecal slurries from untreated or implanted cattle. Increased vitellogenin expression was observed in male fish exposed to fecal slurry from implanted cattle which was attributed to estrogenic and not androgenic activity.

In Cavallin et al. [40], surface water was collected from six basins near farming operations. Hormone concentrations were measured chemically. Androgenic activity was determined using a reporter assay. *In vivo* activity in samples was determined by static exposure of fathead minnows with the following measurements: hepatic expression of vitellogenin, plasma vitellogenin, and *ex vivo* testosterone and estradiol. All samples exhibited detectable androgenic activity due to endogenous substances (dairy and poultry) or endogenous and exogenous substances (beef cattle). Only the NY dairy sample (no implant use) had androgenic activity above the significant response level of the reporter assay.

10.5. Trenbolone Residue Allocation for Risk Quotient Calculations

Due to the potency differences between 17β-TB and 17α–TB, a binning approach is useful to classify TBA metabolites into three categories based upon relative activity.

1. 17β-TB: metabolites(s) with potencies > 17α–TB but ≤ 17β-TB
2. 17α-TB: metabolite(s) with potencies ≤ 17α-TB
3. Non-active/low activity: metabolite(s) with non-detectable activity or significantly lower potencies than 17α-TB

With sufficient evidence, metabolites in the third category could be subtracted from the total residues present in excreta due to lack of activity. To be conservative in this EA, no metabolites were assigned to the third category.

In allocating the PEC in risk quotient calculations for trenbolone described in Section 13 of this EA, two main sources of metabolites are considered: those directly excreted by cattle and those potentially produced by biotransformation in the environment after excretion. Both sources were considered when assigning metabolite percentages in the 2014 EA.

Regarding the composition of TBA metabolites excreted by cattle, it should be acknowledged that the percentages of 17α-TB, 17β-TB, and TDO used in this EA were based on analysis of fresh excreta. As noted, absolute concentrations of 17α-TB, 17β-TB, and TDO decrease over time after excretion by cattle. Therefore, percentages of 17α-TB, 17β-TB, and TDO relative to total TBA metabolite residues in excreta also decrease over time. To be conservative in this EA, the composition of TBA metabolites in excreta was based upon fresh excreta.

Regarding post-excretion degradation and biotransformation processes, laboratory and mechanistic studies described in Section 10.3 demonstrate that a small amounts of 17α-TB, 17β-TB, and TDO may be produced from each other under certain conditions. The data indicate that interconversion among these three compounds is complex and that reactions are dynamic and short-lived. The predominant pathway is for 17α-TB, 17β-TB, TDO, and other metabolites to be converted to less potent compounds (ultimately to CO_2) that do not convert back to 17α-TB, 17β-TB, TDO and do not accumulate in the environment.

10.5.1. 17α-Trenbolone

As described in Section 10.2.3, concentration percentages of 17α-TB were 11% in urine, 32% in feces, and 33% in manure. Because manure is the matrix most relevant to feedlot runoff, the percentage assigned to 17α-TB for the purposes of this EA is 33%. In the 2014 EA, it was assumed that up to 3% of 17α-TB excreted by cattle may be converted to 17β-TB in the environment. This equates to approximately 1% (3% of 33%). Therefore, the value assigned to 17α-TB is 32% (33% - 1%).

10.5.2. 17β-Trenbolone

As described in Section 10.2.3, concentration percentages of 17β-TB were 1.4% in urine, 1.6% in feces, and 1.0% in manure. The percentage assigned to 17β-TB for the purposes of this EA will be the highest percentage: 1.6%. This is a conservative assignment because this value is greater than the average percentage in manure (1.0%) and because approximately half of the residues in feces and manure are non-extractable and probably not available in the environment.

It is also conservative to apply an interconversion factor for post-excretion production of 17β-TB in the environment. As described in Section 10.5.1, 17β-TB potentially formed from 17α-TB is 1%. Adding both sources (1.6% + 1%) produces a value of 2.6% for 17β-TB.

10.5.3. Trendione

As described in Section 10.2.3, concentration percentages of TDO were 1.2% in urine, 1.8% in feces, and 1.8% in manure. For the purposes of this EA, a value of 1.8% is assigned to TDO based on the higher percentages of TDO in feces and manure. This is conservative because half of the residues in manure are non-extractable and probably not available in the environment.

10.5.4. All Other Metabolites (All Else)

Urine, feces, and manure contain many metabolites that together comprise most of the TBA metabolite mass. The ARTA studies conducted by Zoetis and the data reported in the literature indicate that the androgenic activity of TBA metabolites in excreta is mostly due to presence of 17α-TB, 17β-TB, and TDO. Recognizing that all other metabolites together have lower activity, these residues have been treated as a single common activity pool for simplification. The percentage of total residues is determined by subtracting the contributions of 17α-TB (32%), 17β-TB (2.6%), and TDO (1.8%) from 100% to produce a value of 64%.

10.5.5. Metabolite Percentages for RQ Calculations

The percentages below were used in RQ calculations for trenbolone in Section 13 based upon a weight of evidence approach, erring on the side of conservatism.

17β-TB: 5%. This pool includes the amounts of 17β-TB (2.6%) and TDO (1.8%) excreted directly by animals along with those potentially produced post-excretion in the environment. The sum (4.4%) is rounded up to 5%. To be conservative in the EA, all TDO residues were assigned to this activity pool due to uncertainty about the *in vivo* activity of TDO and address potential interconversion of TDO to 17β-TB.

17α-TB: 95%. This activity pool includes 17α-TB and all other TBA metabolites not assigned to the 17β-TB activity pool. This approach is highly conservative because the combined androgenic activity of residues in urine and feces was significantly less than 17α-TB in cattle urine and less than or equal to 17α-TB in cattle feces.

10.6. Conclusions Regarding Metabolites of Trenbolone Acetate

A key study in the 2014 EA was a ^{14}C cattle metabolism and excretion study conducted by Syntex. Additional ^{14}C cattle excretion and activity studies were conducted by Zoetis to supplement the data from the Syntex study. In addition to the Zoetis and Syntex studies, data from literature studies and published interdisciplinary reviews support the findings of the Syntex and Zoetis studies. Data from all studies demonstrate that:

- The principal route of trenbolone entering the environment is through leaching of metabolites from cattle manure.
- TBA is hydrolyzed to 17β-trenbolone (17β-TB) after absorption in cattle which is then extensively metabolized to other metabolites.
- Most TBA metabolites are excreted in feces as free (non-conjugated) metabolites and as conjugated metabolites in urine. Conjugated metabolites rapidly self-deconjugate after excretion by cattle.
- TBA metabolites are excreted from cattle primarily as 17α-TB, which is the primary metabolite in feces and a major metabolite in urine. 17α-TB is the most relevant metabolite in the aquatic environment due to its higher input concentrations, persistence, and mobility.
- 17β-TB and TDO are low-level metabolites that each comprise <2% of total radioactive residues in urine, feces, and manure, on average.
- Most of the total radioactive residues in manure are comprised of metabolites other than 17α-TB, 17β-TB, and TDO. A major metabolite in urine was metabolite U7 which had the same retention time as fecal metabolite F5.
- In general, metabolites of TBA undergo further degradation to less potent compounds (ultimately to CO_2) that do not accumulate in the environment.

- Small amounts of 17α-TB, 17β-TB, and TDO can be formed from each other by interconversion processes in the environment but the prevailing biotransformation process is to form other metabolites that cannot revert to 17α-TB, 17β-TB, and TDO.

The above information was used to assign percentages of TBA metabolite residues for use in RQ calculations: 5% to 17β-TB and 95% to 17α-TB. There are several conservative and precautionary assumptions in these assignments. First, percentages were based upon results from fresh excreta, whereas metabolite concentrations have been shown to decrease over time in aged excreta. Second, all residues attributed to TDO were assigned to 17β-TB to address potential formation of 17β-TB from TDO and uncertainty regarding the *in vivo* activity of TDO. Third, 95% of TBA metabolites were assigned to the 17α-TB pool. This pool contains 17α-TB present in excreta along with >85% of the residues in urine and >60% in feces which have lower androgenic activity than 17α-TB. Lastly, it was assumed that non-extractable residues were 100% bioavailable even though half the residues in feces and manure are non-extractable.

11. EFFECTS ASSESSMENT

An updated literature search was conducted as part of the preparation of this EA to determine if new effects data were available for trenbolone, estradiol and their metabolites. The recent literature search did not identify any new effects data that were more sensitive than those used in the 2014 EA. Therefore, the effects data used in the 2014 EA to evaluate the potential for environmental impacts from the proposed use are considered appropriate and conservative for the current assessment.

Due to the use pattern of hormone implants and the potential for multiple EDC sources in a watershed, there is the potential for chronic exposure in the environment. Ear implants may be present in most cattle in an AFO for up to 365 days a year. The sustained-release characteristics of these products may result in metabolites of TBA and EB entering the environment on a continuous or semi-continuous basis.

Based on their 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. As described in the 2014 EA, fish were identified as the most sensitive sentinel taxonomic group of aquatic organisms to evaluate the effects of exposure to estradiol and trenbolone. Population-relevant endpoints from Zoetis-owned studies and studies in the scientific literature were used to establish no observed effect concentration (NOEC) values for 17α-E2, 17β-E2, 17α-TB, and 17β-TB.

The NOEC value for each compound was divided by an assessment factor (AF), or uncertainty factor, to calculate a predicted no effect concentration (PNEC). PNEC values for each compound are summarized in Table 9. See Section 6 of the 2014 EA for additional information regarding studies and methodology used to derive NOEC and PNEC values.

Table 9. 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 (17α-E2)	250	10	25
17β-estradiol (17β-E2)	2.86	2[a]	1.4
17α-trenbolone (17α-TB)	32	10	3.2
17β-trenbolone (17β-TB)	2.5-5.0	10	0.25-0.5

[a] An AF (assessment factor) of 2 was selected for 17β-E2 based upon results of 5 chronic fish reproduction studies in 4 species, Four of which were >100 days duration. One evaluated effects over multiple generations.

12. EXPOSURE ASSESSMENT METHODOLOGY

12.1. General Approach

A comprehensive environmental risk assessment (EA) was published in 2014 to assess the environmental safety of Synovex ONE [1]. A high level overview of the methodologies used in the 2014 EA was described in Section 3. A similar risk assessment approach was used in this EA to evaluate the environmental safety of re-implant uses of Synovex products. Most of the conservative assumptions used in the 2014 EA were retained.

Previous modeling for the 2014 EA was conducted for cattle administered a single Synovex ONE Feedlot implant in which TBA and EB were released at a constant rate over time. A new mixed-use watershed model (buildup model) was required to estimate PECs for trenbolone and estradiol metabolites in cattle receiving more than one implant per cattle production cycle. As in the 2014 EA, PECs include aggregate environmental inputs from runoff from feedlot, pasture, and cropland but in cattle treated with more than one implant.

Many conservative assumptions were made in the 2014 assessment. Modifications required for the new model or to incorporate new information in this EA are designated in bold below.

- Risk assessment focused on areas of the United States with a high potential for trenbolone and estradiol exposure to aquatic organisms. **The Sioux/Lyon watershed in Iowa was selected as a conservative intensive use case applicable to any region of the United States.**
- **A modified mixed-use watershed model (buildup model) was developed to derive PEC_{water} values for trenbolone and estradiol metabolites from feedlot cattle receiving more than one implant.**
- Feedlots were stocked every day of the 30-year modeling period with a high stocking density (270 head/acre).**100% of cattle were implanted at all times with at least one implant.** Cattle were immediately restocked between production cycles.
- All pasture animals were implanted with Synovex ONE-G with a high pasture stocking density (3.15 head/acre).
- **Each simulation was conducted assuming sequential uninterrupted production cycles over 30 consecutive years using the same re-implant regimen. For comparison, single implant scenarios for Choice, Plus, ONE-F, and ONE-G were modeled assuming all animals were implanted with a single implant and that animals were present continuously in a feedlot for 30 years.**
- There was no decrease in excreted daily masses of TBA and EB metabolite residues due to metabolism in cattle. In other words, the daily rate of TBA and EB released by implants and absorbed by cattle equals the daily rate of TBA and EB metabolite residues excreted by cattle. **100% of TBA metabolite residues were assumed to be excreted as active metabolites vs. 71.5% in the 2014 EA.**
- **In all simulations, it was assumed that manure was not removed from the feedlot until the end of each cattle production cycle.**
- The surrogate trenbolone and surrogate estradiol metabolite concept was utilized for TBA and EB metabolites. Conservative environmental fate parameters established in the 2014 EA were used in this EA.

- Except for limited interconversion of 17α-TB to 17β-TB in the environment after excretion by cattle, it was assumed that no degradation of TBA or EB metabolites occurred in manure.
- The cropland application rate was calculated from the daily amounts of trenbolone or estradiol metabolite residues released by cattle, the phosphorus application rate for corn grain, and the daily phosphorus excretion rate in beef cattle manure.
- All manure generated in feedlots was applied to croplands up to the total acreage of cropland in the watershed, 90% as solid manure and 10% as liquid manure. **It was assumed that 100% of operations used no till practices for solid manure application to cropland (5 cm depth) and a 5 cm depth for liquid applications**.
- Solid manure was applied two times per year in the spring and fall before and after the corn crop cycle. Liquid manure was applied four times per year with irrigation water during the crop cycle.
- The curve number for feedlots used in NRCS curve number method was 95, which reflects high runoff potential. Runoff curve numbers (CN) were used in the PRZM model to determine the amount of rainfall that becomes runoff. The CN reflects both the soil properties and land cover of an area.
- 25% of Animal Feeding Operations with <1,000 head (AFOs) were assumed to be in need of runoff control improvements and thus potentially directly discharge to surface waters as a reasonable worst-case for a local watershed.
- Modeling addressed the combined (aggregate) TBA and EB metabolite runoff from feedlots, pasture cattle, and cropland treated with manure assuming that all cattle in feedlots and pastureland in the watershed received implants. **Percentages assigned to these land uses in the Sioux/Lyon watershed were updated based on current data.**
- PECs were based on the 90th percentile of the annual maximum 21-day concentrations determined for a 30-year modeling period. **Daily PECs were calculated over the modeling period** as additional information.
- **Percentages of TBA metabolites assigned to 17α-TB and 17β-TB for RQ calculations were 95% and 5%, respectively, based on new data**.
- For estradiol, separate risk quotient calculations were performed in the 2014 EA assuming that either 100% 17α-E2 or 100% 17β-E2 comprised all EB metabolite residues. **For additional conservatism in this EA, risk quotient calculations were performed for estradiol assuming that all residues were 17β-E2.**

Modeling simulations were performed for implantation scenarios with different numbers of implants (one, two, and three implants per cycle), re-implant timing, and grow out periods (total residence time in the feedlot, days on feed). In all simulations, it was assumed that manure was not removed from the feedlot until the end of each cattle production cycle and that not all manure was removed after the previous cycle concluded.

Because there are many possible re-implant combinations and use regimens, representative combinations with the highest predicted exposures were selected. The regimen (implant timing and grow out period) with the highest predicted environmental exposure was selected for each of these combinations of implants.

12.2. Model Technology

For the 2014 EA, USEPA was using PRZM version 3.12 [41] to simulate chemical mass balance in the terrestrial environment and Exposure Analysis Modeling System (EXAMS) version 2.98 [42] to simulate chemical mass balance in the aquatic environment. Modifications were made to PRZM to simulate daily amounts of estradiol and trenbolone residues in certain months of the year from pasture and daily constant concentrations of estradiol and trenbolone from feedlots. The manure erosion equation from the Agricultural Policy / Environmental eXtender model (APEX) model [43] also was incorporated into the model. See Section 5 of the 2014 EA.

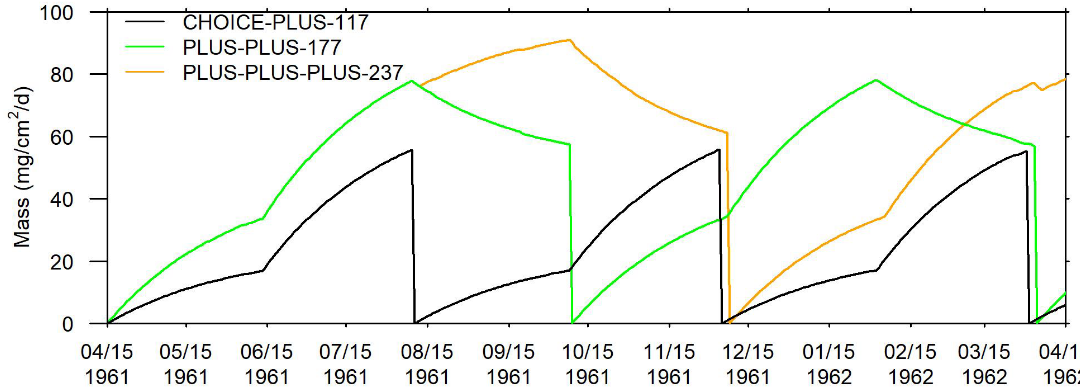
The European version of PRZM, version 4.73 (winPRZM), developed under the European Commission's FOCUS DG SANTE (the Forum for the co-ordination of pesticide fate models and their use) [44,45] was the source model for the modifications used in the 2014 EA due to familiarity and ownership of the code by Waterborne Environmental Inc. To support the modeling conducted for this EA, the cropland scenario in winPRZM was tested against PRZM-3.12 prior to and after the addition of the modifications and was found to produce similar results.

Additional modifications were required for this EA in order for winPRZM to model trenbolone and estradiol releases when different implants, singly or together, release TBA and EB metabolites over time. This updated version of winPRZM (SynovexPRZM) accommodates different schedules and dose rates of animal treatment and the accumulation of manure containing trenbolone and estradiol residues in a feedlot over time.

Using a daily time step, the model calculates the buildup of feedlot manure over time. Each day, masses of surrogate trenbolone or surrogate estradiol compound from one or more actively releasing implants are added to the residues remaining from the previous time step. Total drug at any point in time is assumed to be uniformly distributed in the feedlot and uniformly mixed in the manure pile by cattle movement. Mixing occurs in the active layer (upper 10 cm) of manure. Once the manure depth exceeds 10 cm, residues below 10 cm are buried and unavailable for mixing as was assumed in the 2014 EA. Each day, a depth of manure equal to the daily addition is buried and the next daily addition is mixed with the active manure pile.

For illustration, the mass of trenbolone in the top 10 cm of manure layer is shown in Figure 3 for three scenarios: (1) Choice-Plus-117, (2) Plus-Plus-177, and (3) Plus-Plus-Plus-237. See Appendix 7 for a description of these implantation scenarios. The image depicts three full cycles for Choice-Plus-117, two cycles for Plus-Plus-177, and one and a half cycles for Plus-Plus-Plus-237. Upon completing each cattle production cycle (grow out period), manure is removed from the feedlot, new animals enter and are immediately implanted, and the accumulation cycle begins anew. The same process occurs for EB metabolite mass in the manure layer, however the shape of the profiles and timings are different due to the different rates and durations of release of EB from Synovex implant products.

Figure 3. Mass of Trenbolone Residues in the Top 10 cm Manure Layer in a Feedlot



As of the date of this EA, USEPA has replaced PRZM-3.12 and EXAMS with PRZM5 [46] and the Variable Volume Water Model, VVWM [47]. Simulations for this EA were conducted with SynovexPRZM and VVWM. PECs for the Iowa cropland scenario generated by SynovexPRZM were compared to those with PRZM5 and found to be similar.

12.3. Watershed Confirmation

As noted in Section 7.3, the Sioux/Lyon watershed in Iowa was selected as a conservative intensive use case that can be applied to any region of the United States. This watershed was chosen because it: (1) was in the 98th percentile or greater for beef cattle density at the county level based on the 2017 USDA Census of Agriculture, (2) was in the 99th percentile or greater since 2002 for operations with greater risk (<500 head), and (3) consistently produced the highest PECs among the five modeled regions in the 2014 EA. All modeling simulations in this EA were performed using Sioux/Lyon watershed with updated PCA values of 0.108% for feedlots, 88.7% for cropland, and 4.12% for pastureland. See Table 25 of Appendix 3.3.

12.4. Environmental Fate Properties

A comprehensive literature review was conducted from 2014 to present that focused on the environmental fate, metabolism, and toxicology of trenbolone and estradiol and their metabolites. This review indicated that the surrogate metabolite concept and the environmental fate data used in the 2014 EA continue to be valid. Environmental fate properties used in this EA are summarized in Table 6 in Section 8.

12.5. Amounts of EB and TBA Residues Released Per Day in Manure

For mass balance, the average daily rates of EB and TBA absorbed by cattle from ear implants and the total amounts of TBA and EB excreted as their metabolites in manure are equal. As noted in Section 5.1, benzoate and acetate groups of EB and TBA, are rapidly cleaved to form estradiol and trenbolone in cattle. To determine potential environmental effects of surrogate estradiol and surrogate trenbolone compounds excreted by cattle, daily amounts of estradiol and trenbolone excreted from implanted animals were calculated by applying a molecular weight conversion factor of 0.7235 to convert EB to estradiol (E2) and 0.8655 to convert TBA to trenbolone (TB).

Values for the average daily amounts of EB and TBA released from Synovex Plus and Synovex ONE-F were established in Appendix 13.4 of the 2014 EA. The excretion rates of

EB and TBA from Choice (4 uncoated pellets) are one half of Plus (8 uncoated pellets). The excretion rates of EB and TBA from ONE-G (6 coated pellets) are three quarters of ONE-F (8 coated pellets). Daily amounts of EB and TBA excreted from cattle as estradiol and trenbolone equivalents are summarized in Table 10 and Table 11.

Table 10. Rates and Durations of Estradiol Release for Synovex Implants

Implant	Total Dose (mg EB)	Estimated Duration of Release (days)[C]	Average Daily Release of EB (mg/d)[B]	Average Daily Release of Estradiol (mg/d)[A]
ONE-F	28	267	0.1049	0.07590
ONE-G	21	Same as ONE-F	0.0787[D]	0.05692
Plus	28	141	0.1980	0.1433
Choice	14	Same as Plus	0.0990[E]	0.07163

[A] Molecular weight conversion from EB to E2. Ratio = 0.7235.
[B] Average daily release values for EB are from Appendix 13.4 of the 2014 EA. Assumed constant average daily release of EB from implant(s) over the entire duration in the feedlot.
[C] Estimated duration of release = total EB in implant (28 mg for Plus and ONE-F, 21 mg for ONE-G, and 14 mg for Choice) ÷ average daily release rate of EB.
[D] Coated pellets: ONE-F = 28 mg vs. ONE-G = 21 mg. Multiplied rate for ONE-F by 0.75 for ONE-G.
[E] Uncoated pellets: Plus = 28 mg. Choice = 14 mg. Multiplied rate for Plus by 0.5 for Choice

Table 11. Rates and Durations of Trenbolone Release for Synovex Implants

Implant	Total Dose (mg TBA)	Estimated Duration of Release (days)[C]	Average Daily Release of TBA (mg/d)[B]	Average Daily Release of Trenbolone (mg/d)[A]
ONE Feedlot (ONE-F)	200	211	0.9466	0.8193
ONE Grower (ONE-G)	150	Same as ONE-F	0.7100[D]	0.6145
Plus	200	117	1.7073	1.4777
Choice	100	Same as Plus	0.8537[E]	0.7388

[A] Molecular weight conversion from TBA to TB. Ratio = 0.8655.
[B] Average daily release values for TBA are from Appendix 13.4 of the 2014 EA. Assumed constant average daily release of TBA from implant(s) over the entire duration in the feedlot.
[C] Estimated duration of release = total mg TBA in implant (200 mg for Plus and ONE-F, 150 mg for ONE-G, and 100 mg for Choice) ÷ average daily release rate of TBA.
[D] Coated pellets: ONE-F = 200 mg. ONE-G = 150 mg. Multiplied rate for ONE-F by 0.75 for ONE-G.
[E] Uncoated pellets: Plus = 200 mg. Choice = 100 mg. Multiplied rate for Plus by 0.5 for Choice

Daily excretion rates of estradiol and trenbolone residues in manure from individual implants can be expressed as grams per hectare as shown in Table 12. An example calculation is shown in Appendix 8 for Synovex Plus.

Table 12. Daily Excretion Rates in Feedlot Manure for Individual Implants

Implant	Daily Trenbolone Excretion Rate (g/ha)	Daily Estradiol Excretion Rate (g/ha)
ONE-F	0.5466	0.0506
ONE-G	0.4100	0.0380
Plus	0.9859	0.0956
Choice	0.4929	0.0478

12.6. Implantation Scenarios

To meet the objectives of producers for specific groups of cattle, implant selections and regimens vary considerably. Because there are many possible re-implant schemes that can be used, representative scenarios were selected for different combinations of lead and subsequent implants, re-implant timing, grow-out periods (days on feed), number of production cycles per year, etc. The scenarios modeled in this EA for trenbolone and for estradiol are described in Appendix 7. For each re-implant combination, the regimen or regimens predicted to produce the highest environmental exposure for each combination of Synovex products were identified and used in modeling.

In all modeled scenarios, it was assumed that: (1) animals were restocked immediately after completing the previous cattle production cycle, (2) all manure produced by animals in each production cycle remained in the feedlot and thus was available for runoff, (3) 70% of manure was removed at the end of the grow-out period before restocking with fresh animals, and (4) no till practices were used for manure application to cropland.

These assumptions are conservative. First, animals may not be restocked immediately or stocked only seasonally. Second, it is common practice to remove or mound manure in the lot periodically which lowers the amount of manure present and the surface area of manure available for potential runoff. And third, different tillage practices are used to incorporate manure into cropland, some of which reduce the amount of manure residue in runoff.

12.7. Model Input Parameters and Simulation Procedures

Individual simulations of SynovexPRZM were run for each of the three sources of trenbolone and estradiol residues: feedlot, pasture, and manured cropland. Each simulation was run for 30 consecutive years using historical weather data for the region. The time series outputs for trenbolone and estradiol in runoff were then multiplied by the proportions of feedlot, pasture, and manured cropland in the watershed (PCA factors, Section 12.3) to calculate the total mass of residues of the surrogate estradiol compound and the surrogate trenbolone compound entering the reservoir on each day of the 30-year simulation. The resulting buildups of trenbolone and estradiol in the reservoir were simulated with VVWM.

Next, consistent with the approach of the 2014 EA, a series of 21-day concentrations were calculated as rolling average values over the 30-year simulation. The maximum 21-day concentration of the surrogate estradiol compound and the surrogate trenbolone compound for each year were ranked and the 90th percentile concentration for each was used as the PEC value for risk assessment. The 90th percentile of the annual maximum series corresponds to a 10-year return period.

The inputs used in SynovexPRZM and VVWM models were selected based on conservative assumptions. Table 6 lists the physical-chemical and environmental fate properties of trenbolone and estradiol used in SynovexPRZM and VVWM runs. Weather, crop, soil, runoff, and erosion parameters were the same as those used in the 2014 EA. Specific input parameters are described in Sections 12.7.1 to 12.7.6. Alterations to SynovexPRZM are provided in Appendix 9.

12.7.1. Manure Depth

The uniform depth of manure generated each day on feedlot was estimated as 0.18 cm. The derivation of this value is provided in Appendix 10.

12.7.2. Application Method and Timing

Cropland scenarios used in this EA were the same as the 2014 EA. In modeling, all manure generated on feedlots is applied to croplands up to the total acreage of cropland in the watershed. 90% of the manure is applied as solid manure and 10% as liquid manure. Solid manure is applied two times a year, in the spring and fall, before and after corn crop cycle. Liquid manure is applied four times a year with irrigation water during the crop cycle. Planting and application scenarios for each year of the 30-year simulation are described in Table 13 below.

Table 13. Annual Cropland Scenarios Used for Environmental Modeling

Date	Action
5/4	manure application – solid
5/25	crop emergence
5/30	manure application – liquid
6/30	manure application – liquid
7/24	crop maturation
7/30	manure application – liquid
8/30	manure application – liquid
10/19	crop harvest
10/26	manure application – solid

12.7.3. Application Rates

In animal production areas, manure application rates are influenced by phosphorus buildup, which limits the amount of manure that can be applied to fields. Manure application rates were calculated using a ratio between the phosphorus (P_2O_5) present in manure and the amounts of TBA and EB metabolite residues released from one or more implants during a feedlot cycle. In the 2014 EA and this EA, it was assumed that all soils had a starting phosphorus content of zero, thus assuring that the maximum amount is applied to cropland.

Manure contains the combined proportional amounts of trenbolone and estradiol residues contributed by each implant. In scenarios in which only a portion of the TBA and EB in an implant is released (i.e., animals are removed from feedlots before implants have fully released), only the fraction released was used to calculate the amount in the applied manure. Application rates of trenbolone and estradiol residues for each scenario and land use are presented in Table 34 and Table 35 of Appendix 11 with an example calculation.

No changes were made to pasture simulations from the 2014 EA because re-implantation is not an approved use in pasture cattle. For implanted animals in pasture, daily excretion occurs from April 1st to October 28th every year.

12.7.4. Cropland Tillage Parameters

A summary of tillage practices for Iowa and for Lyon and Sioux counties are summarized in Table 14 below. Data were obtained from USDA NASS [2].

Table 14. Tillage Practices in Iowa (Statewide) and Sioux and Lyon Counties, 2017

	Iowa	Lyon	Sioux
Total Cropland (acres)	26,545,960	318,213	453,455
No Till (acres)	8,196,199 (31%)	66,480 (21%)	89,870 (20%)
Reduced Tillage (acres)	10,132,599 (38%)	146,956 (46%)	211,087 (47%)
Conventional Tillage (acres)	5,018,129 (19%)	93,967 (30%)	132,188 (29%)

To assess the effect of different tillage practices upon runoff of trenbolone and estradiol residues, a sensitivity analysis was conducted comparing conventional tillage versus no till practices. The different inputs used for the tillage options are shown in Table 15. These values were obtained from the PRZM3 manual [41]. The results from the sensitivity analysis are shown in Appendix 12 with Choice-Plus as an example scenario. No till application to cropland produced the highest PECs for trenbolone and estradiol residues.

Table 15. Inputs Used for Tillage Options in Environmental Modeling

	No Till	Conventional Tillage
Curve Number Change	-10%	0%
RUSLE C Factors	No Till	Conventional Till
Solid Application Depth (cm)	5	15
Liquid Application Depth (cm)	5	5

Although conventional and reduced tillage practices together currently account for 57% of cropped acres in Iowa statewide and 76% in Sioux and Lyon counties, other regions of the United States may use no till practices to a greater extent and percentages may change in any region. Therefore, no till cropland application was used in this EA to be conservative.

12.7.5. Feedlot Scraping Efficiency

Scraping efficiency, the amount of total manure produced in a cattle feedlot that is removed before the next cattle cycle, was an assumed value. In practice, it may not be possible to remove all manure from a feedlot after each production cycle. There may be some areas where manure is completely scraped to the hard pack and other areas with greater or lesser depth due to unevenness of the feedlot surface. We are not aware of specific data to justify scraping efficiency, and this parameter could vary depending upon the physical details of feedlots as well as animal and manure management practices.

To address the uncertainty of this parameter, a sensitivity analysis was conducted by examining output produced using values of 70% and 90% for the Choice-Plus no till scenario. In this scenario, 30% or 10% of the total manure produced in the previous cattle production cycle remained in the feedlot at the start of the next cycle. As shown by results in Appendix 12, 90th percentile PECs did not change significantly. To be conservative, except for the sensitivity analysis described above, a scraping efficiency of 70% was used for the modeling conducted for this EA.

12.7.6. Source Contributions

Time series loadings predicted by SynovexPRZM for feedlots, cropland, and pasture were scaled to reflect the fraction of the watershed from each source using PCA factors of 0.108%, 88.7%, and 4.12%, respectively (see Table 25 in Appendix 3.3). The remaining area of the watershed (7%) was assumed to be cropland not treated with manure. In modeling the runoff from feedlots, it was assumed that all CAFOs were in compliance with the Clean Water Act and that 25% of small and medium AFOs could potentially directly discharge to surface water in a local watershed (see Section 7.2).

13. RISK CHARACTERIZATION

Risk characterization in this EA was based upon the risk quotient (RQ) method in which RQ equals the PEC divided by the PNEC for a sensitive species. An RQ value in the range of 1 or less indicates that significant environmental effects are unlikely. Due to the many conservative assumptions used when deriving the PEC and PNEC values, RQs calculated in this EA over-represent the potential risk for environmental impact due to use of Synovex implants when used singly or in combination in feedlot beef cattle.

As noted in Section 12, PECs for the surrogate estradiol and surrogate trenbolone compounds were estimated for the Sioux/Lyon watershed for several representative implantation uses of Synovex implants. The regimen that produced the highest predicted environmental exposure was modeled as a worst-case use for each use (modeled scenario). If it was unclear whether a shorter or longer grow-out period in the feedlot produced higher exposures, both regimens were modeled. The regimen with the highest RQ was then used to represent that implant use.

RQs for the surrogate estradiol and trenbolone compounds were calculated for single implant and re-implant scenarios using the 90th percentile 21-day PEC values calculated as described in Section 12 and the PNEC values described in Section 11. Daily RQ values for the surrogate estradiol and trenbolone compounds were calculated and plotted versus time to identify any occasions when RQ values exceeded 1 over a 30-year period.

13.1. Risk Quotients (RQs) for the Surrogate Estradiol Compound

To calculate RQs for estradiol in the 2014 EA, it was assumed that the toxicity of the surrogate estradiol compound was equal to 17α-estradiol (17α-E2) because 17α-E2 was the primary metabolite in manure. RQs based upon 17β-E2 also were calculated in the 2014 EA for comparison. For simplification and to be most conservative in this EA, RQ values based upon the PNEC for 17β-E2 were used because these RQ values are always greater than those based upon 17α-E2. The RQ value for each implantation scenario was calculated using the equation below.

$$Estradiol\ RQ = \frac{PEC}{PNEC_{17\beta-E2}}$$

Results for RQs based upon the 90th percentile maximum annual series PECs are shown in Table 16 for representative scenarios modeled for this EA. Refer to Appendix 7 for a description of each implant combination and regimen.

Table 16. Risk Quotients for the Surrogate Estradiol Compound

Implantation Scenario	21d PEC (ng/L)	RQ	Event Count (# events with Daily RQ ≥ 1)
Choice	0.05	0.03	0
Plus	0.09	0.07	0
ONE-F	0.05	0.04	0
ONE-G	0.04	0.03	0
Choice-Plus-141	0.09	0.07	0
Choice-Plus-201	0.09	0.07	0
Choice-Choice	0.07	0.05	0
Choice-ONE-F	0.07	0.05	0
Plus-Plus-141	0.14	0.10	0
Plus-Plus-201	0.13	0.09	0
Plus-Choice	0.12	0.08	0
Plus-ONE-F	0.12	0.08	0
ONE-F-Plus-267	0.09	0.06	0
ONE-F-Plus-281	0.09	0.06	0
ONE-F-Choice	0.07	0.05	0
ONE-F-ONE-F	0.07	0.05	0
ONE-G-Plus-267	0.08	0.06	0
ONE-G-Plus-281	0.07	0.05	0
ONE-G-Choice	0.06	0.04	0
ONE-G-ONE-F	0.06	0.04	0
Choice-Plus-Plus-201	0.12	0.09	0
Choice-Plus-Plus-261	0.08	0.06	0
Choice-Choice-Plus-201	0.09	0.07	0
Choice-Choice-Plus-261	0.07	0.05	0
Choice-ONE-F-Plus-327	0.10	0.07	0
Choice-ONE-F-Plus-341	0.10	0.07	0
Plus-Plus-Plus-201	0.16	0.11	0
Plus-Plus-Plus-261	0.10	0.07	0

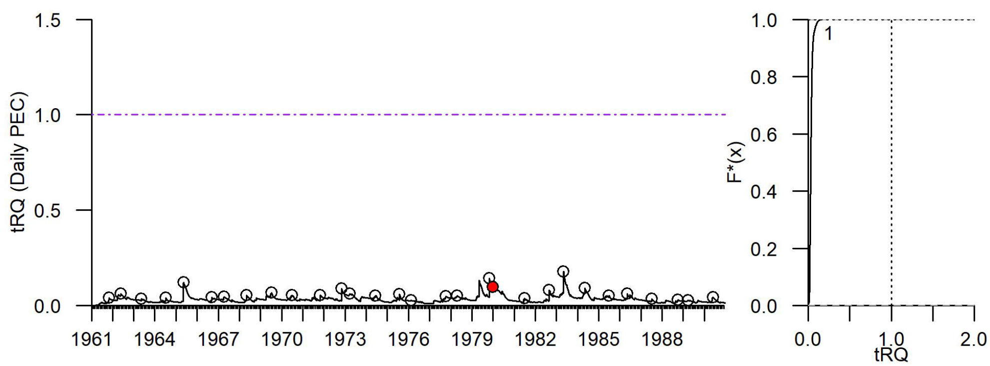
As shown in Table 16, RQs were <1 for all single implant and re-implant uses. The maximum RQ was 0.11 for Plus-Plus-Plus-201, which was a Synovex Plus implant followed by second and third Plus implants 60 and 120 days later and a 201 day grow-out period in the feedlot. All RQs are highly conservative because it was assumed that 100% of the estradiol residues excreted in cattle manure are comprised of 17β-E2 or substances with similarly high estrogenic activity. As noted in the 2014 EA, field monitoring data clearly indicate that 17α-estradiol, estrone, and other less potent compounds are the primary metabolites in cattle waste.

To further characterize risk, daily RQs for the surrogate estradiol compound for each implantation scenario were calculated over the 30-year modeling period and depicted graphically. The calculated daily RQ values overestimate risk because a PEC from a daily (acute) exposure is compared to a PNEC based upon chronic exposure. A PNEC for 17β-E2 has not been established for acute exposure.

With this caveat, daily RQ values were calculated using the chronic PNEC value for 17β-E2 to identify any times during the 30-year modeling period with greater risk of exposure to estradiol-related metabolites. Example output is shown in Figure 4 for Plus-Plus-Plus-201, the scenario with the highest predicted environmental exposure. Daily RQ values over the

30-year modeling period for the Plus-Plus-Plus scenario are shown in the left panel of the figure. The annual maximum values are depicted by black circles and the 90th percentile annual maximum is depicted by the red circle. The right panel is a cumulative distribution function of all daily values for the 30-year period. The fraction of values less than or equal to 1 is indicated by a number in the top left of the right panel. As shown by the data in Table 16 and Figure 4, there were no occasions (events) when the daily RQ exceeded 1.

Figure 4. Daily Estradiol RQs for the Plus-Plus-Plus-201 Scenario



13.2. Risk Quotients (RQs) for Surrogate Trenbolone Compound

A modified approach was used to determine RQ values for the surrogate trenbolone compound because reliable data are available regarding the composition of trenbolone-related residues excreted by cattle. These data were used to proportion the PEC value for each implantation scenario between the 17α and 17β isomers of trenbolone.

As described in Sections 10.5 and 10.6 of this EA, 5% of the PEC value for the surrogate trenbolone compound was assigned to 17β-TB, the most potent trenbolone metabolite. The remainder was conservatively assigned to 17α-TB, also a potent metabolite. RQs for the surrogate trenbolone compound were calculated as the sum of RQs for 17α-TB and 17β-TB. The PNECs used in this calculation were 3.2 ng/L for 17α-TB and 0.25 ng/L for 17β-TB.

$$Trenbolone\ RQ = RQ_{17\alpha-TB} + RQ_{17\beta-TB} = \frac{PEC \times 0.95}{PNEC_{17\alpha-TB}} + \frac{PEC \times 0.05}{PNEC_{17\beta-TB}}$$

Results for RQs based upon 90th percentile 21-day moving average maximum annual series PECs are summarized in Table 17. Refer to Appendix 7 for a description of each re-implant combination and regimen.

Table 17. Risk Quotients for the Surrogate Trenbolone Compound

Application Scenario	21d PEC (ng/L)	RQ	Event Count (# events with Daily RQ ≥ 1)
Choice	0.48	0.24	0
Plus	0.95	0.47	0
ONE-F	0.55	0.27	0
ONE-G	0.41	0.21	0
Choice-Plus-117	0.88	0.44	0
Choice-Plus-177	0.91	0.45	0
Choice-Choice	0.68	0.34	0
Choice-ONE-F	0.71	0.35	0
Plus-Plus-117	1.36	0.68	1
Plus-Plus-177	1.26	0.63	0
Plus-Choice	1.16	0.58	0
Plus-ONE-F	1.18	0.59	0
ONE-F-Plus-211	0.85	0.42	0
ONE-F-Plus-257	0.88	0.44	0
ONE-F-Choice	0.70	0.35	0
ONE-F-ONE-F	0.72	0.36	0
ONE-G-Plus-211	0.72	0.36	0
ONE-G-Plus-257	0.76	0.38	0
ONE-G-Choice	0.57	0.28	0
ONE-G-ONE-F	0.58	0.29	0
Choice-Plus-Plus-177	1.17	0.58	0
Choice-Plus-Plus-237	1.08	0.54	0
Choice-Choice-Plus-177	0.89	0.44	0
Choice-Choice-Plus-237	0.88	0.44	0
Choice-ONE-F-Plus-271	0.93	0.46	0
Choice-ONE-F-Plus-317	0.91	0.45	0
Plus-Plus-Plus-177	1.51	0.75	1
Plus-Plus-Plus-237	1.37	0.68	1

RQs were <1 for all single implant and re-implant scenarios. The maximum RQ was 0.75 for the Plus-Plus-Plus-177 scenario, which was an initial Plus implant followed by a second and third Plus implant 60 and 120 days later and a grow-out period of 177 days in the feedlot. As shown in Table 17, RQ values for the two Choice-Plus scenarios with different grow-out periods (Choice-Plus-117 and Choice-Plus-177) were similar. For the Plus-Plus and Plus-Plus-Plus scenarios, two regimens were modeled by varying grow-out period in the feedlot. Plus-Plus-117 had a greater RQ value than Plus-Plus-177. Plus-Plus-Plus-177 had a greater RQ value than Plus-Plus-Plus-237, respectively. As noted, the higher RQ value was used to characterize the risk for those re-implant uses.

To demonstrate the impact of re-implantation, results for re-implant uses were compared with single implant uses. RQ values for all two-implant combinations with Choice as lead implant were greater than a single Choice implant but less than or similar to a single Plus implant. For two-implant scenarios, Plus-Plus-117 produced the greatest RQ (0.68), which was 1.4x greater than a single Plus implant. Plus-Plus also had greater a RQ value than three-implant combinations with Choice as the lead implant.

To further characterize risk, RQs for the surrogate trenbolone compound for each scenario were calculated for each day of the 30-year modeling period and depicted graphically. As noted in Section 13.1 for estradiol, the calculated daily RQ values overestimate risk because a PEC from a daily (acute) exposure is compared to a PNEC based upon chronic exposure. PNECs for 17α-TB and 17β-TB have not been established for acute exposure.

With this caveat, daily RQ plots were created for all implant simulations. For brevity, output is provided only for the two combination uses with occasions (events) when daily RQ values exceeded 1. These were Plus-Plus-117 (Figure 5) and Plus-Plus-Plus-177 (Figure 6). As shown in Table 17, all other single and multiple implant uses had no daily RQ excursions.

Figure 5. Daily Trenbolone RQs for the Plus-Plus-117 Scenario

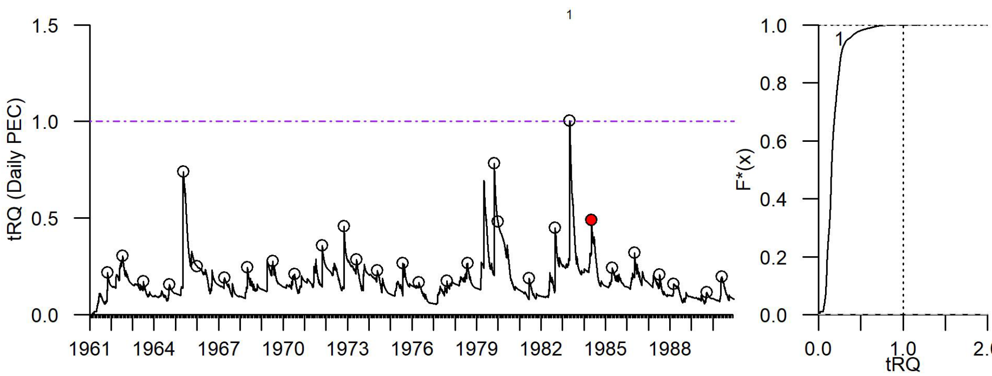
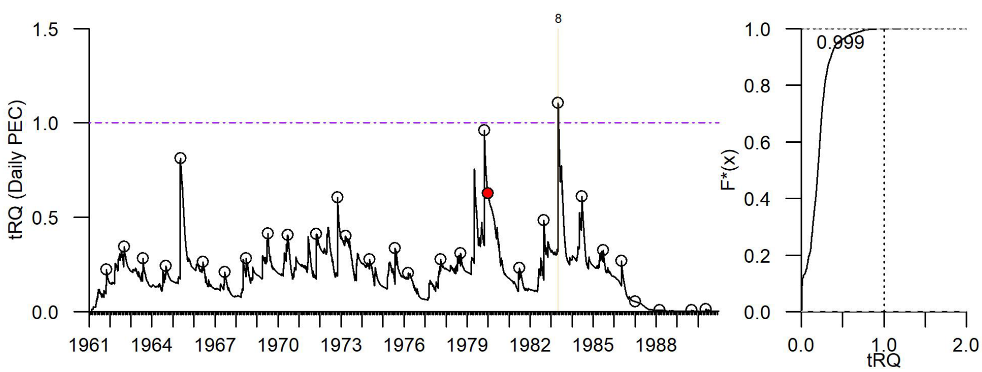


Figure 6. Daily Trenbolone RQs for the Plus-Plus-Plus-177 Scenario



To further characterize these results, the beginning date, peak RQ, arithmetic RQ, and duration of each event are summarized in Table 18. The arithmetic average RQ over the duration of the event is a better estimate of the RQ for the excursion event than the maximum daily RQ, because it better matches the timescale in which the PNEC was determined (21 days).

For the Plus-Plus-117 scenario, the single event lasted 1 day with a daily RQ of 1.00. For the Plus-Plus-Plus-177 scenario, the event lasted 8 days, the average RQ was 1.05, and the fraction of all values less than or equal to 1 was 0.999. Both single events occurred in May 1983 during a time of excessive rainfall towards the end of a cattle cycle. The peak also occurred the day before removing accumulated manure from the feedlot.

Table 18. Implantation Scenarios for Trenbolone with Events with RQ ≥1.0

Scenario	Event	Begin Date	Peak RQ	Arith. Avg. RQ	Duration (days)
Plus-Plus-117	1	5/6/1983	1.00	1.00	1
Plus-Plus-Plus-177	1	5/6/1983	1.11	1.05	8

In both of these re-implant scenarios, the duration of exposure was short relative to the duration of the toxicity studies used to assign PNEC values. There was a lack of repeated exposure exceeding the PNEC threshold and thus a long recovery period between seasons/years. Additionally, fish respond rapidly upon introduction and removal of trenbolone (rapid uptake and depuration) [48] so that exposures would be transient after short events. Therefore, there is low risk of population-level impacts from single or successive exposures.

13.3. Risk Characterization Conclusions

This EA was written as a unifying EA to address all possible combinations of single and re-implant uses of Synovex Choice, Plus, ONE Feedlot, and ONE Grower. Because it was impractical to model all possible combinations and regimens, representative implantation scenarios were selected with different lead and follow-on implants using regimens that produced the greatest potential for estradiol and trenbolone exposure.

Combinations of two implants that were modeled for this EA are designated by a check mark (√) in Table 19. Combinations with lead or follow-on implants that were not modeled but have lower release rates (doses) of EB and TBA and thus lower RQs are signified by a letter corresponding to the higher-exposure combination(s) that support its use. RQs also were determined for four representative three-implant combinations with high-exposure regimens and grow-out periods from 6 to >10 months on feed: Choice-Choice-Plus, Choice-ONE-F-Plus, Choice-Plus-Plus, and Plus-Plus-Plus.

Table 19. Single and Re-implant Uses of Synovex Products Evaluated in this EA

Lead Implant	Second Implant				
	None (single implant)	Choice	ONE Feedlot	Plus	ONE Grower
Choice	√	√	√	√	√
ONE Feedlot	√	√	√	√	√
Plus	√	√	√	√	√
ONE Grower	√	A	B	C	B

[A] Addressed by higher-dose (higher exposure) ONE Feedlot-Choice combination
[B] Addressed by higher-dose (higher exposure) ONE Feedlot-ONE Feedlot combination
[C] Addressed by higher-dose (higher exposure) ONE Feedlot-Plus combination

Considering all available information and the RQ values determined for this EA, no significant environmental impacts are expected from re-implant use of Synovex Choice, Plus, ONE Feedlot, or ONE Grower in beef steers and heifers. As described in Section 15.2, there are many conservative assumptions and mitigating factors that could not be quantified in this risk assessment that further reduce the risk associated with this proposed use.

14. STEROID HORMONES IN ENVIRONMENT

Endocrine disrupting compounds (EDCs) are exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior [49]. EDCs can enter terrestrial and aquatic environments from several sources and can affect the endocrine function of organisms. An explanation of how the endocrine system functions, how EDCs can disrupt endocrine function, and examples of endocrine disruption in wildlife are provided in the 2012 report from the United Nations Environment Programme and the World Health Organization (WHO), *State of the Science of Endocrine Disrupting Chemicals – 2012* [50].

As noted in Section 8 of the 2014 EA, steroid hormones are known EDCs. Natural and anthropogenic steroid hormones can be introduced into terrestrial and aquatic environments from domestic animals and humans by various routes. EDCs such as biocides, plasticizers, and pharmaceuticals entering the environment can interfere with natural hormone actions by exerting direct action on hormone receptors and receptor function or by controlling hormone delivery to the receptor [50]. Use of Synovex Choice, Plus, and ONE products containing EB and TBA in beef steers and heifers could be one potential source of estrogens and androgens entering the environment.

As noted in the 2014 EA, research has focused on the potential exposure and effects of natural and synthetic steroids in the environment as a potential cause of endocrine disruption observed in wildlife, including fish. Establishing a direct causal link between the observed endocrine disruption and specific sources is difficult due to the complex chemical (e.g., mixtures of EDCs) and physical (e.g., fate and distribution of compounds) interactions that may occur in the environment.

If EB and TBA metabolites from implanted animals were to enter the aquatic environment in high enough concentrations, exist for a sufficient duration, or add significant mass to EDCs already present in the environment, this potentially could cause endocrine disrupting effects in fish and amphibians in waterways located near AFOs. The 2014 EA focused on the potential for cumulative exposure of steroid hormones in the aquatic environment and included a comparison of the contributions of steroids entering the environment from use of Synovex ONE vs. other sources.

In the 2014 EA, it was determined that the contribution of estrogens and androgens entering the aquatic environment from use of Synovex ONE was 1% or less relative to the overall load of estrogens and androgens already present from human and livestock sources. The 90th percentile PEC estimates for estradiol and trenbolone calculated in this EA using the buildup model and additional data now available (Table 16 and Table 17) are similar in magnitude to the PECs calculated for the 2014 EA using the previous model, assumptions, and data in effect at that time (Tables 28 and 29 of the 2014 EA). Therefore, re-implant use of Synovex products is expected to have very little impact to the overall load of endocrine disruptors already present in the environment.

New information has been published in the literature for natural and anthropogenic sources of EDCs since the time of the 2014 EA, however the same conclusions from the cumulative exposure assessment in the 2014 EA are still relevant. For brevity, this topic is not addressed further. The reader is referred to Section 8 of the 2014 EA for more information.

15. SOURCES OF UNCERTAINTY AND CONSERVATIVE ASSUMPTIONS

In conducting a risk assessment of this nature and in designing models to predict environmental concentrations, some level of uncertainty is inevitable. Numerous conservative assumptions were made throughout this EA to account for uncertainty or lack of data in some cases. Uncertainties and conservative assumptions used in this EA are summarized below. Most of these points are the same as those described in the 2014 EA except instances in which new assumptions were required for the buildup model used in this EA or because new data were available.

15.1. Sources of Uncertainty

Potential sources of uncertainty in the modeling and assessment include:

- Incomplete information on the effects of estradiol and trenbolone metabolites on fish survival, development, and reproduction over several generations.
- Environmental modeling programs have not been formally validated, however they have been used widely by the USEPA for pesticide risk assessment for many years.
- Large CAFOs (>1000 AU) that may be 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 antibiotics, which may be present in manure of implanted cattle, on the degradation rate of estradiol and trenbolone in soil is not well characterized.
- Incomplete information on the effects of mixtures of estradiol and trenbolone metabolites on fish reproductive endpoints.
- Insufficient information to determine cumulative exposures of steroid hormones and other EDCs in the aquatic environment.

15.2. Conservative Assumptions

A partial list of conservative quantitative and qualitative assumptions used in this EA is provided below.

Exposure Assessment

- PEC estimates used to calculate RQs are the 90th percentiles of maximum annual 21-day moving averages determined from 30-year simulations. As stated in the 2014 EA, these values are considered to be conservative estimates of aquatic exposures.
- It was assumed that 100% of cattle were implanted, that cattle were present every day in the feedlot at the maximum stocking density, there was no vacancy in the feedlot, and cattle were immediately restocked between production cycles.

- Each modeling run for an implant scenario was conducted assuming non-interrupted cattle production cycles using the same combination of implants and re-implantation regimen for 30 consecutive years.
- It was assumed that 100% of the market share for growth-promoting implants used in these feedlots is attributed to Synovex products.
- Each modeling run for an implantation scenario was conducted using the regimen (re-implant time and grow-out period) that produced the greatest environmental exposure of trenbolone- and estradiol-related metabolites.
- It was assumed that 100% of pasture cattle are treated with Synovex ONE Grower with 100% market share. This assumption is an overestimate because only stocker pasture cattle may be treated. As described in the 2014 EA, only half of pasture cattle are stockers.
- It was assumed that 100% of the trenbolone metabolite residues excreted by cattle had activity in this EA vs. 71.5% in the 2014 EA.
- Although estradiol and trenbolone degrade in feedlot soil, stored manure, and runoff collection lagoons, no degradation of estradiol or trenbolone metabolites to less potent substances was assumed.
- All environmental fate parameters for the surrogate estradiol and surrogate trenbolone compounds were conservative. See Section 4.2 of the 2014 EA.
- Several studies published in the literature demonstrate that both trenbolone and estradiol are photosensitive and degrade rapidly when exposed to sunlight (Section 4.2 of the 2014 EA). It was assumed that photodegradation is not a significant dissipation route in the environment.
- In the 2014 EA, the EPA crop scenarios were developed to represent sites where specific crops are grown that may be vulnerable to surface runoff. These scenarios were designed to have conservative properties that would result in greater contaminant runoff and leaching. These included soil types prone to erosion, runoff, and steep slopes. See Section 5 of the 2014 EA for information on the EPA models.
- No till parameters (5 cm tillage depth) were used for solid manure application in cropland SynovexPRZM runs because this practice results in higher runoff potential for trenbolone and estradiol-related residues vs. conventional or reduced tillage.
- It was assumed that 25% of AFOs with <1000 AU may potentially directly discharge feedlot runoff to surface waters in a local watershed without considering facilities that retain runoff water (drainage basin, lagoon or pond, storage tank, etc.). As noted in Section 7.2, the 25% value is highly conservative and does not include adjustments for increased voluntary compliance or enforcement of the Clean Water Act or the NPDES program.
- Overall masses of EB and TBA metabolites entering a watershed are overestimated. It was assumed that 100% of the EB and TBA metabolites excreted on a feedlot are present in runoff from the feedlot surface and that the same 100% are also present (following a holding period) in the manure applied to cropland. In reality, <100% of the EB and TBA metabolites in manure would be applied to cropland.

- In manured fields in animal production areas, phosphorus can build up and limit the amount of manure that can be applied to cropland. In the 2014 EA and this EA, it was assumed that all soils had a phosphorus content of zero, thus assuring that the maximum amount of manure is applied to cropland.
- In the 2014 EA and this EA, manure is applied on the same day each year over 30-year period. This date was conservatively selected based upon a sensitivity analysis conducted for the 2014 EA (Appendix 7.1.1 of the 2014 EA).
- In the 2014 EA and this EA, typical best management practices (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 modeling, even though many of these mitigation practices commonly are used by farmers and result in lower environmental exposure to local water bodies.

Effects Assessment

- There were multiple chronic studies ($\geq$ 21 day exposures, some multi-generational) used to derive the PNEC values for the 17α and 17β isomers of estradiol and trenbolone. See Section 6 of the 2014 EA.
- To derive PNECs for 17α-E2, 17α-TB, and 17β-TB, an assessment factor of 10 was applied to account for uncertainties in laboratory studies. An AF of 2 was used to derive the PNEC for 17β-E2, even though a large data set (21 NOEC values from 19 studies in 8 fish species) was available. See Section 6.3 of the 2014 EA.

Risk Characterization

- Based upon information in the literature, the primary metabolite present in beef cattle manure is 17α-E2 with only minor amounts of 17β-E2. The 17α and 17β isomers are further degraded in the environment to less potent substances. It was conservatively assumed in this EA that 100% of the estradiol-related residues had activity equivalent to 17β-E2, the most potent metabolite.
- Available information indicates that TBA is extensively metabolized in cattle to many less-potent metabolites and that very small amounts (<2%) of 17β-TB and TDO are excreted by cattle. A conservative estimate of 5% was made for the fraction of TBA metabolite residues in manure attributed to 17β-TB (most potent TBA metabolite). This estimate included percentages of 17β-TB and TDO excreted by cattle in manure and an additional amount potentially formed by interconversion of 17α-TB and TDO to 17β-TB after excretion by cattle.
- For additional conservatism, all trenbolone-related residues not assigned to 17β-TB were assigned to 17α-TB (95%) even though the combined activity of all TBA metabolite residues was less than 17α-TB.
- Approximately half of the trenbolone metabolite residues in feces and manure were non-extractable. Similarly, not all estradiol metabolite residues in excreta may be extractable. It was assumed in this EA that 100% of the residues in excreta are bioavailable even though all or some of the non-extractable residues may not be mobile or bioavailable in the environment.

16. CONCLUSIONS

A unified environmental assessment was prepared to support re-implantation use of Synovex ear implant products (Choice, Plus, ONE Feedlot, and ONE Grower) in beef steers and heifers fed in confinement. An exposure assessment was conducted in which predicted environmental concentrations (PECs) of estradiol benzoate and trenbolone acetate metabolites were calculated for a hypothetical realistic intense-use watershed. Based on all available information and the RQ values determined in this EA, no significant environmental impacts are expected from single use or re-implant use of Synovex Choice, Plus, ONE Feedlot, or ONE Grower in beef steers and heifers.

17. ALTERNATIVES TO THE PROPOSED ACTIONS

The only alternative to the proposed action is the “no action” alternative, which would be the failure to approve re-implant uses of SYNOVEX Choice, Plus, and ONE. Based on the analysis in this environmental assessment, Zoetis does not believe that significant environmental impacts will occur from this action. Therefore, the “no action” alternative was eliminated from consideration.

18. PERSONS AND AGENCIES CONSULTED

This EA was prepared with input and assistance from members of the Environmental Safety Team in the Office of New Animal Drug Evaluation in FDA’s Center for Veterinary Medicine.

19. ACKNOWLEDGMENTS

Waterborne Environmental, Inc. developed the environmental exposure models used in the 2014 EA for Synovex ONE and this EA. Team members who contributed to this project were: W. Martin Williams, Colleen A. Roy, Ishadeep Khanijo*, J. Mark Cheplick, Amy Ritter, Joshua Amos*, Nikki Maples-Reynolds, and Nicholas S. Green*.

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* Previously associated with Waterborne Environmental, Inc.

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21. APPROVAL

Dated: XXXXXXXXXXXXXXXX Signed: See appended electronic signature page

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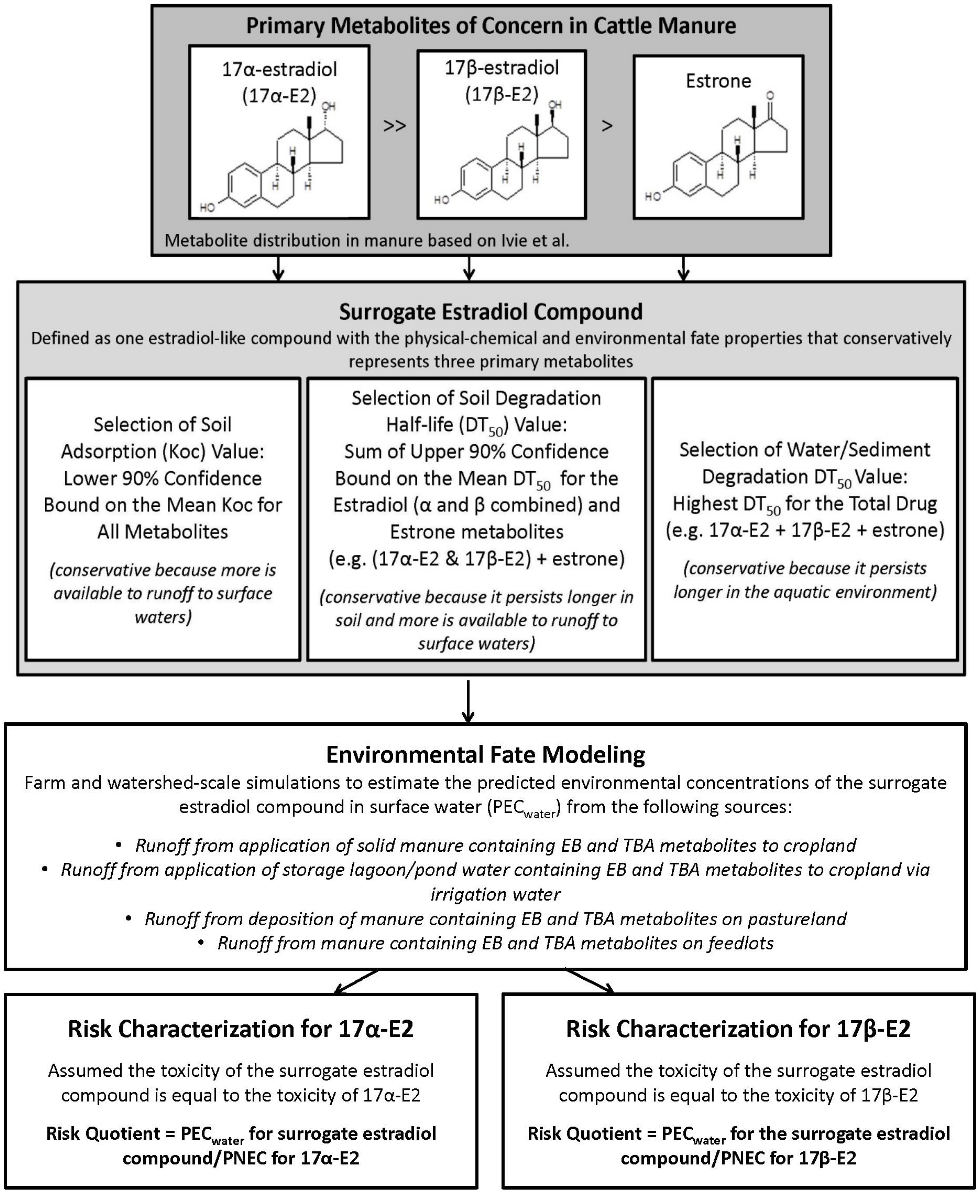
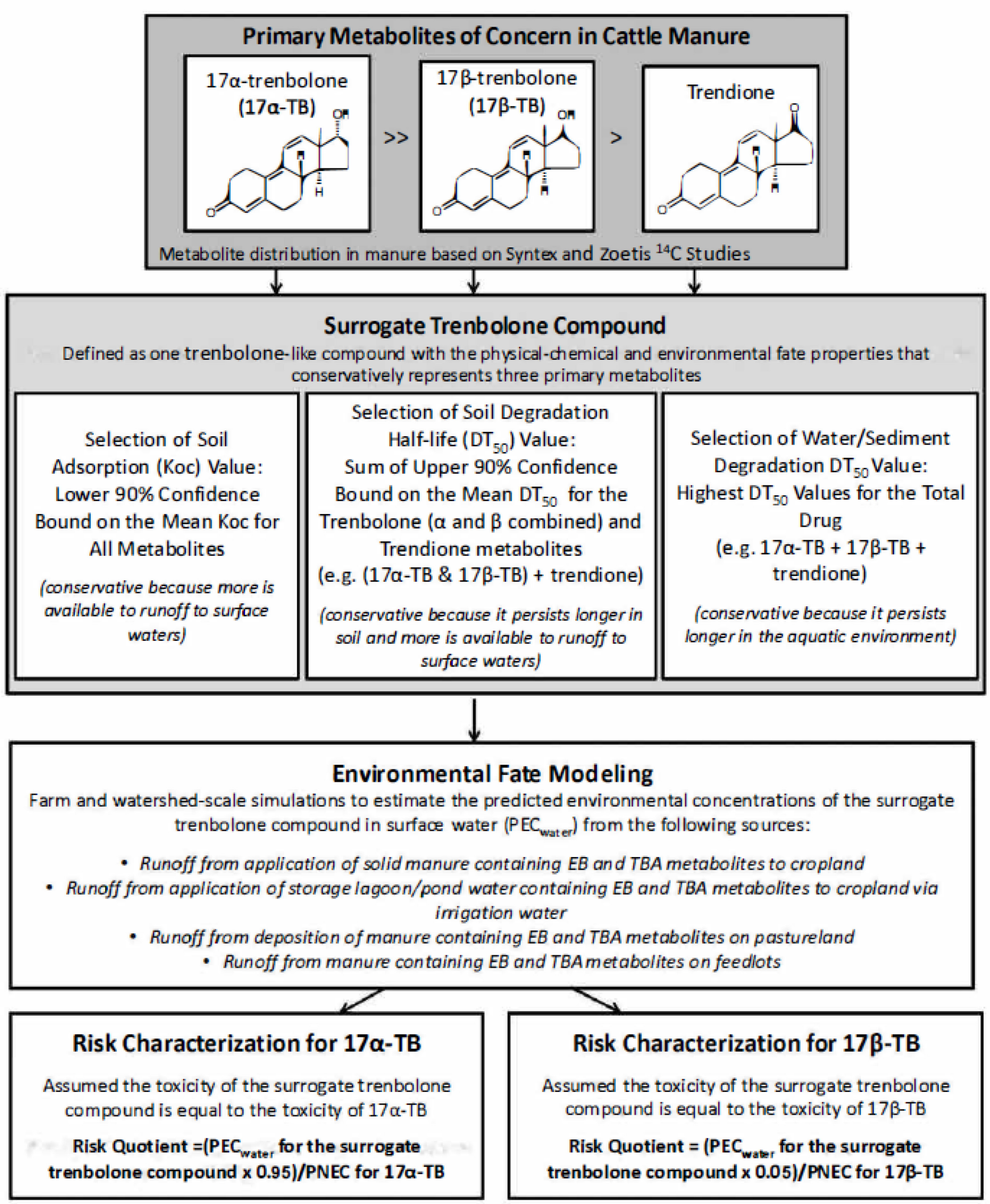
Appendix 1. Conceptual Model for the Surrogate Estradiol Compound



Ivie et al., 1986 [51]

Two sets of risk quotients were calculated for estradiol metabolites in the 2014 EA, the first based upon the PNEC of 17α-E2 (principal metabolite) and the second based upon the PNEC for 17β-E2 (most potent metabolite). Because higher risk quotients are always produced when calculated using the PNEC for 17β-E2, risk quotients in this EA were calculated based upon 17β-E2 to conservatively address the risk for all EB metabolites.

Appendix 2. Conceptual Model for the Surrogate Trendione Compound



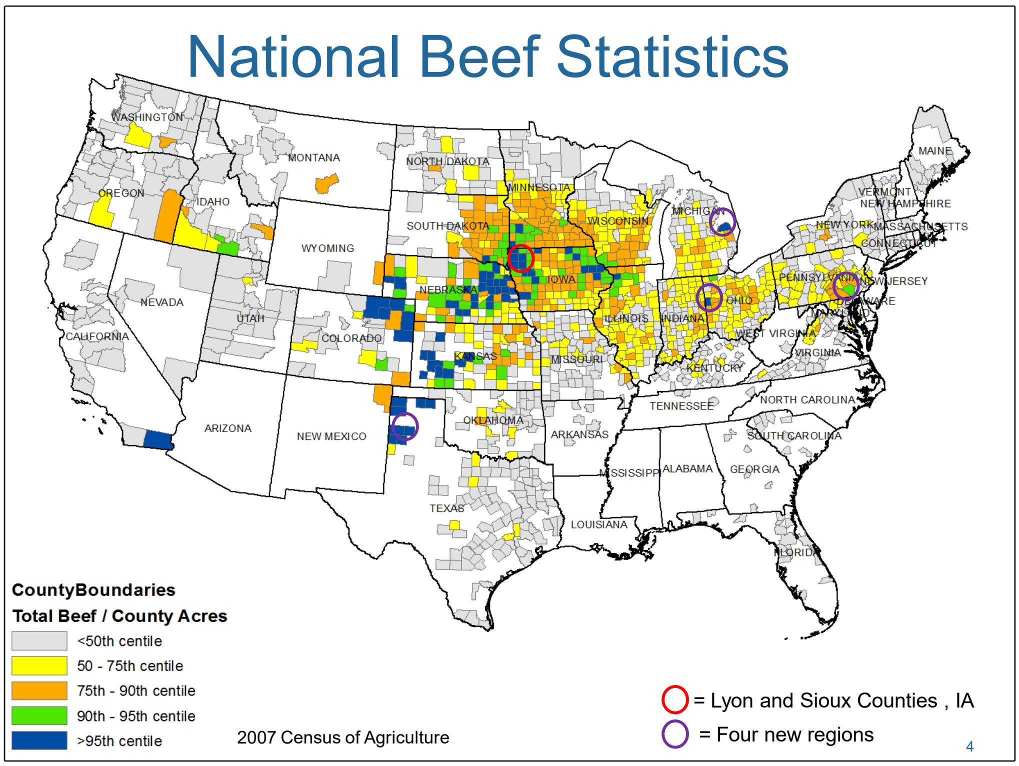
Appendix 3. Watershed Selection for Environmental Modeling

Appendix 3.1. National geospatial analysis

The model watersheds used for the 2014 Synovex ONE EA were selected by conducting a national geospatial analysis to identify regions of high potential vulnerability to estradiol and trenbolone compounds from runoff or erosion into surface waters. The analysis considered areas with high beef cattle density, high density of feedlots, and normal annual precipitation.

Five regions were selected for the 2014 EA based upon cattle and feedlot statistics data from the 2007 USDA Census of Agriculture. The regions selected for the 2014 EA were Huron County, MI; Mercer County, OH; Lancaster County, PA; Castro County, TX; Sioux and Lyon Counties, IA. These regions are circled in Figure 7 below.

Figure 7. Beef Feedlot Cattle Density by County in the Continental United States



Appendix 3.2. Rankings of study regions in the 2014 EA

The relative rankings of each of the five study regions used in the 2014 EA for the period from 2002 through 2017 are summarized in Table 20 below. These data indicate that the five study regions, and particularly the Sioux/Lyon region, remain highly relevant for the purposes of this EA.

Table 20. Relative Ranking of Study Regions Over Time (2002 to 2017)

2002 Census (for historical reference)						
State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Manure Density Rank	Pasture Cattle Density Rank
		(n=1,953)	(n=700)	(n=1,772)	(n=2,795)	(n=3,019)
Iowa	Lyon	98.7%	90.4%	99.7%	98.9%	83.1%
Iowa	Sioux	99.6%	97.1%	100.0%	99.9%	89.7%
Michigan	Huron	96.9%	91.8%	99.2%	96.3%	68.6%
Ohio	Mercer	92.9%	0.0%	97.2%	99.6%	68.8%
Pennsylvania	Lancaster	96.0%	78.1%	99.9%	100.0%	95.1%
Texas	Castro	99.8%	99.1%	69.3%	90.4%	99.3%

2007 Census						
State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Manure Density Rank	Pasture Cattle Density Rank
		(n=1,380)	(n=290)	(n=1,345)	(n=2,887)	(n=3,019)
Iowa	Lyon	98.9%	96.8%	99.7%	99.6%	92.5%
Iowa	Sioux	99.5%	98.4%	99.8%	99.9%	97.5%
Michigan	Huron	95.5%	89.6%	98.1%	97.9%	75.3%
Ohio	Mercer	96.0%	88.4%	99.4%	99.8%	88.4%
Pennsylvania	Lancaster	94.9%	72.8%	99.6%	100.0%	94.9%
Texas	Castro	99.9%	99.6%	74.1%	95.4%	99.7%

2012 Census						
State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Manure Density Rank	Pasture Cattle Density Rank
		(n=1,203)	(n=602)	(n=836)	(n=2,852)	(n=3,019)
Iowa	Lyon	98.9%	95.5%	99.5%	99.9%	92.6%
Iowa	Sioux	99.8%	98.8%	99.6%	100.0%	97.1%
Michigan	Huron	97.0%	94.3%	98.2%	98.5%	85.6%
Ohio	Mercer	94.5%	85.1%	94.8%	99.7%	78.4%
Pennsylvania	Lancaster	94.4%	82.8%	99.8%	100.0%	95.7%
Texas	Castro	99.6%	99.3%	0.0%	91.3%	98.4%

2017 Census						
State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Manure Density Rank	Pasture Cattle Density Rank*
		(n=1,104)	(n=645)	(n=826)	(n=2,884)	(n=3,019)
Iowa	Lyon	99.0%	98.4%	99.2%	99.9%	92.6%
Iowa	Sioux	99.6%	99.6%	99.6%	99.9%	97.1%
Michigan	Huron	94.9%	96.5%	98.8%	98.7%	85.6%
Ohio	Mercer	95.1%	93.5%	97.9%	99.5%	78.4%
Pennsylvania	Lancaster	91.1%	90.9%	99.3%	99.8%	95.7%
Texas	Castro	99.3%	99.7%	0.0%	94.7%	98.4%

* Not updated. Based on 2012 Census, as pasture cattle density rank has no material impact on RQs

Appendix 3.3. Watershed selection for this EA

In the environmental modeling conducted for the 2014 EA, the Sioux/Lyon county region of Iowa consistently produced the highest PECs. This region is a HUC 12 level watershed, which is classified as a local sub-watershed in USGS/EPA EnviroAtlas. This Iowa watershed is in the upper 98th percentile or greater in terms of beef cattle density and the upper 99th percentile or greater for smaller operations (<500 head of cattle).

To confirm that the Iowa watershed continued to produce the highest predicted exposures, a sensitivity analysis for direct runoff of feedlot manure prior to scraping was conducted during the development of the buildup model. The objective was to verify that the Iowa watershed was the highest exposure scenario. An example scenario (Choice-Plus-60/180) was utilized in which Choice is implanted on Day 0, Plus is implanted on Day 60, and the grow-out period in the feedlot is 180 days. This scenario was run for trenbolone for the five regions used in the 2014 EA (Huron County, MI; Mercer County, OH; Lancaster County, PA; Castro County, TX; Sioux/Lyon Counties, IA) using each region's weather file and PCAs (percent cropped areas, the percentages of different land uses in a watershed). Results are shown in Table 21.

Table 21. Percent Crop Area (PCA) Values from the 2014 Synovex ONE EA

PCA values from 2014 EA	IA	MI	OH	PA	TX
Feedlot	0.094%	0.032%	0.068%	0.04%	0.003%
Applied Cropland	56.62%	20.10%	29.13%	9.41%	53.59%
Pasture	2.29%	4.05%	2.93%	2.67%	4.36%

The USEPA weather stations used in the assessment were: IA-Sioux City, SD (w14944.dvf), Pennsylvania-Harrisburg, PA (w14751.dvf), Ohio-Dayton, OH (w93815.dvf), Michigan-Flint, MI (w14826.dvf), and Texas-Amarillo, TX (w23047.dvf).

Initially, feedlot trenbolone flux values were evaluated. To determine trenbolone loadings originating solely from feedlots, AFOs were the primary contributors of TBA metabolite residues in manure. All other areas of the watershed were assigned as cropland. Results shown in Table 22 demonstrate that IA and OH had higher 21-day PEC values than PA, MI, and TX.

Table 22. 21-Day PECs for Trenbolone Based Upon Feedlot Flux Values

Region	21-day PEC Trenbolone (ng/L)
IA	0.286
MI	0.124
OH	0.283
PA	0.153
TX	0.007

As a next step, simulations were repeated to include the TB flux from all three land types. The results shown in Table 23 demonstrate that the Sioux/Lyon, Iowa 21-day PEC value was significantly higher than the other regions and thus remains the most conservative. The comparison was based on PCAs from the 2014 EA due to the intensive labor involved in updating watershed-specific PCAs for all land uses.

Table 23. 21-Day PECs for Trenbolone Including All Land Types

Region	21-day PEC Trenbolone (ng/L)
IA	0.541
MI	0.160
OH	0.328
PA	0.158
TX	0.189

With the Sioux/Lyon watershed identified as the watershed with highest environmental exposures, the 2017 Census of Agriculture [2] was used to compare cattle on feed for Sioux/Lyon counties versus the other regions. There was an increase in total cattle on feed in Sioux/Lyon counties compared to the other states. See Table 24 below. Therefore, the Sioux/Lyon watershed stands out as an even more worst-case scenario using the most current data.

Table 24. 2017 Census of Agriculture Data for Five Regions of Interest

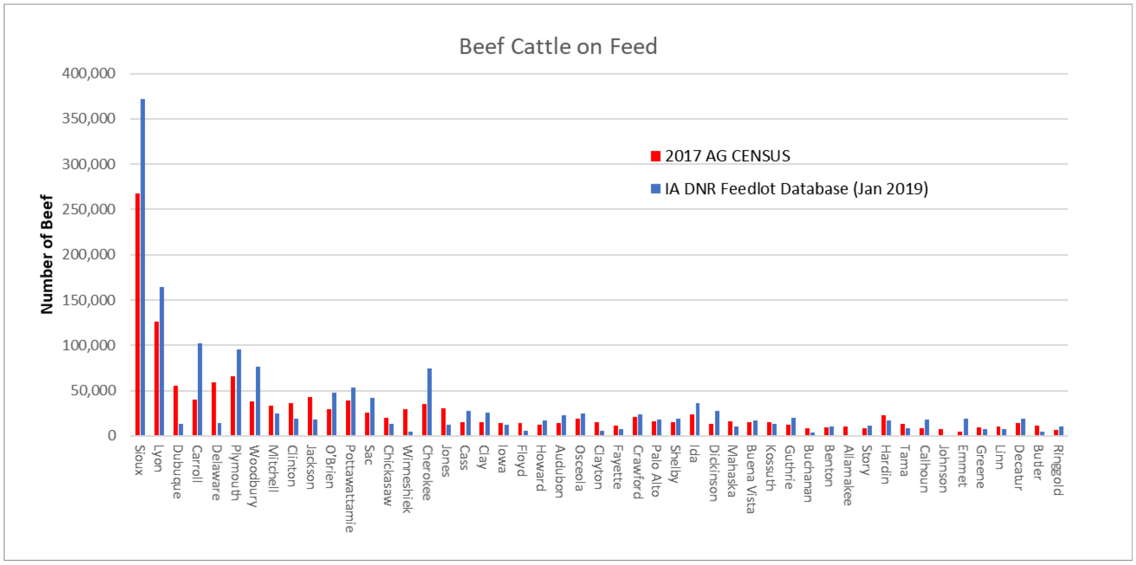
	MI	OH	PA	TX	IA
	Huron County	**Mercer County**	**Lancaster County**	**Castro County**	**Sioux/Lyon Counties**
Acres	317,161	303,801	629,314	582,814	869,295
Cattle on feed 2007	45,367	28,448	43,349	341,694	303,244
Cattle on feed 2017	50,691	28,977	29,786	272,913*	394,153
% change 2007-2017	11.7%	1.9%	-31.3%	NA*	30.0%

Cattle on feed (beef feedlot cattle) is the sum of cattle of feed >500 head and cattle on feed <500 head from the 2007 and 2017 Census of Agriculture.

*2,231 cattle on feed <500 head reported in Castro County, TX, in 2007 Census of Agriculture. All subsequent years not disclosed for confidentiality due to limited number of farms.

Specifically for the Iowa watershed, the most recent Department of Natural Resources (DNR) Feedlot location database [52] was evaluated to identify any changes in watershed-level feedlot cattle densities. Comparisons of feedlot counts in the DNR database with the 2017 USDA Census of Agriculture (Figure 8 below) indicate that the DNR database better reflects actual numbers of beef cattle passing through a feedlot facility because the database represents “permitted cattle”. For beef cattle on pasture, the most recent USDA NASS Cropland Data Layer [5] was used to re-calculate pasturelands in which pasture cattle may be stocked.

Figure 8. Comparison of Feedlot Beef Cattle Counts from Iowa DNR Feedlot Database and 2017 Census of Agriculture



Summary information for the Sioux/Lyon watershed is shown in Table 25 below. These data indicate that there are higher numbers of AFO, CAFO, and pasture beef cattle and higher percentages of AFO feedlots, manured cropland, and pastureland in the Iowa watershed in 2017 than 2007. The higher PCA values for those land uses have been used for all modeling conducted for this EA.

Table 25. Comparison of the Sioux/Lyon Watershed by Year

Assessment	AFO Beef Head	CAFO Beef Head	Pasture Cattle Beef Head	% AFO Feedlot	% Cropland Manured	% Pastureland
2014 EA (2007 USDA data)	5,373	10,410	1,525	0.094%	56.6%	2.29%
Current EA (2017 USDA data)	6,130	17,800	2,743	0.108%	88.7%	4.12%

Appendix 4. Zoetis-Owned Confidential Information Used in this EA

Table 26. List of Appendices Where the Public Executive Summary is Located

Information / Study	Reference
Appendix 4.1. Total radioactive residues of ^{14}C-trenbolone acetate (TBA) in excreta from beef cattle following the implantation of SYNOVEX PLUS implants containing ^{14}C-trenbolone acetate pellets into the ear. Charles River Report 287640 (Zoetis study A432R-GB-16-417), 02 June 2021. **CONFIDENTIAL**	[6]
Appendix 4.2. Development of methods for analysis of ^{14}C-trenbolone acetate metabolite residues in cattle excreta from Study A432R-GB-16-417. Zoetis Report A432R-US-17-535, 20 December 2019. **CONFIDENTIAL**	[8]
Appendix 4.3. Profiling ^{14}C-trenbolone acetate metabolite residues in cattle excreta from study A432R-GB-16-417. Zoetis Report A432R-US-17-536, 20 December 2019. **CONFIDENTIAL**	[7]
Appendix 4.4. Qualification of an androgen receptor transactivation assay (ARTA) to characterize the relative bioactivity of trenbolone metabolites in cattle excreta. Zoetis Report A436R-US-18-616, 26 December 2019. **CONFIDENTIAL**	[34]
Appendix 4.5. Characterization of the relative bioactivity of trenbolone metabolites in cattle excreta using an androgen receptor transactivation assay (ARTA), Zoetis Report A430R-US-18-617, 01 May 2020. **CONFIDENTIAL**	[9]
Appendix 4.6. Isolation and purification of trenbolone metabolite U7 from cattle urine and characterization of its androgenic activity. Zoetis Report A636Z-US-19-691, 22 August 2019. **CONFIDENTIAL**	[35]

Appendix 4.1. Total radioactive residues of ^{14}C- TBA in cattle excreta

Title: Total radioactive residues of ^{14}C-trenbolone acetate (TBA) in excreta from beef cattle following the implantation of SYNOVEX PLUS implants containing ^{14}C-trenbolone acetate pellets into the ear; Amendment 03

Study number: Charles River report 287640, Zoetis reference number A432R-GB-16-417

GLP: No

Purpose: To collect urine, feces, and manure samples from beef cattle administered ear implants containing [^{14}C]-TBA and estradiol benzoate (EB) for total radioactive residue determination, metabolite profiling, and androgenic activity characterization

Test facility: Charles River Laboratories, Tranent, UK

Study design: On Day 1 (time 00:00), two cattle were implanted with 200 mg [^{14}C]-TBA and 28 mg nonlabeled EB. Samples of bulk urine and feces were collected from metabolism cages at 24-hour intervals: at pre-treatment (controls) and at 1, 3, 7, 14, 21, 28, 42, 56, and 70 days after implantation. Clean catch urine samples were collected as grab samples on the same collection days. Manure samples were collected weekly as composite samples from multiple sites in the pen. Animals were euthanized on Day 71, and bile, urine, and feces were collected at necropsy.

Animals: 2 commercial beef cattle, 1 heifer and 1 steer weighing 264 and 266 kg three days before dosing. Animals were housed in metabolism cages during sampling times and were otherwise housed in separate pens with bedding.

Dosage and route of administration: Each implant contained 8 pellets with identical composition and dose as the commercial Synovex Plus formulation. Total nominal dose was 200 mg [^{14}C]-TBA and 28 mg EB. Animals received a single subcutaneous dose in the middle-third of the ear with an implant gun.

Analytical methods: Individual urine, bile, and composite manure samples were divided into 2 subsamples each by the test facility. Feces samples were homogenized with water before dividing into 2 subsamples. TRR concentrations in urine and bile were determined by liquid scintillation counting (LSC). TRR in feces homogenates and manure were determined by combustion-LSC.

Results: The TRR excretion rate (total ^{14}C residues in urine, feces, and cage washes over time) was similar for the two animals and peaked between Days 3 and 14. The average daily excretion rate over the study was 0.775% of dose/day (1.5 mg/day). The mean fraction of TRR excreted in urine was 28% (steer: 33%, heifer 24%). The mean fraction of TRR excreted in feces was 72% (steer: 67%, heifer: 76%). Total TBA excreted over the study was estimated as 50 to 55% of the administered dose.

Appendix 4.2. Method development for metabolite analysis of excreta samples

Title: Development of methods for analysis of ^{14}C-trenbolone acetate metabolite residues in cattle excreta from Study A432R-GB-16-417

Study number: Zoetis reference number A432R-US-17-535

GLP: No

Purpose: To establish methods to profile trenbolone metabolites in excreta samples from Zoetis study A432R-GB-16-417

Test facility: Zoetis VMRD (Veterinary Medicine Research & Development), Kalamazoo, MI, USA

Study design: A gradient reverse-phase high performance liquid chromatography (HPLC) method was developed. Sample preparation procedures were developed for urine to de-salt and concentrate samples by solid phase extraction. Extraction conditions for feces were developed. Sample preparation procedures were established to concentrate fecal extracts for analysis. Samples from Zoetis study A432R-GB-16-417 (Appendix 4.1) were used in this study.

Analytical methods: Prepared excreta samples were analyzed by liquid scintillation counting (LSC) to determine extraction efficiency (feces) and sample preparation recoveries. Selected prepared samples were analyzed by HPLC-TopCount: reverse-phase HPLC, collection of chromatographic fractions in LumaPlate 96-well plates, detection of ^{14}C using a TopCount plate reader, and chromatogram reconstruction using ARC software. Metabolite profiles for [^{14}C]-TBA-fortified control samples and representative excreta samples with incurred residues were examined with and without enzymatic deconjugation with *Helix pomatia* extract.

Results: The HPLC method separated many TBA metabolites across a wide polarity range. Except for clean catch urine that contained mostly conjugated metabolites, metabolites in bulk urine collected from metabolism cages had extensively self-deconjugated naturally over the 24-hour collection period. Residues in feces were mostly unconjugated metabolites.

Prepared (concentrated) urine samples had improved detection and quantitation of low-level residues vs. non-concentrated samples. Sequential extraction of feces produced extraction efficiencies of approximately 60% vs. total ^{14}C residues. ^{14}C residues in post-extracted feces were shown to be bound/non-extractable following sequential extraction with other solvents and after sequential acid and alkaline digestion. Acceptable chromatographic separation, peak shape, and recovery were produced in HPLC-TopCount analyses of urine and feces.

Appendix 4.3. Metabolite profiling of ^{14}C-TBA metabolites in excreta

Title: Profiling ^{14}C-trenbolone acetate metabolite residues in cattle excreta from study A432R-GB-16-417

Study number: Zoetis reference number: A432R-US-17-536

GLP: No

Purpose: To determine the metabolite profiles of trenbolone-related metabolite residues in cattle excreta collected from study A432R-GB-16-417

Test facility: Zoetis VMRD, Kalamazoo, MI, USA

Study design: Methods of analysis were established in Zoetis study A432R-US-17-535 (Appendix 4.2). Samples from Zoetis study A432R-GB-16-417 (Appendix 4.1) were used in this study.

Analytical methods: Urine samples were prepared by solid phase extraction (Oasis HLB). Feces and manure were extracted with methanol and concentrated for analysis. Prepared samples were analyzed by LSC and by HPLC-TopCount: gradient reverse-phase HPLC, collection of chromatographic fractions in LumaPlate 96-well plates, detection of ^{14}C using a TopCount plate reader, and chromatogram reconstruction using ARC software.

Results: Metabolite profiles for bulk urine were similar for the steer and heifer and over time. Mean abundances of 17α-TB, 17β-TB, and TDO vs. total ^{14}C residues were 12%, 1.2%, and 1.4% for the steer and 17%, 1.3%, and 0.98% for the heifer, respectively. Mean abundance of metabolite U7/F5 in bulk urine was 9.5% for the steer and 7.2% for the heifer. Several metabolites were present at mean abundances of <1% to 5% of total ^{14}C residues. Numerous low-level metabolites together comprised a mean of 54% and 56% of total residues for the steer and heifer, respectively. Most clean catch urine samples contained primarily conjugated metabolites, with percentages varying among the samples.

Metabolite profiles for feces were similar for the steer and heifer and over time. Mean extractability of ^{14}C residues was 60%. Mean abundances of 17α-TB, 17β-TB, and TDO vs. total ^{14}C residues were 32%, 0.8%, and 1.4% for the steer and 34%, 1.2%, and 1.6% for the heifer, respectively. Mean abundances of metabolites U7/F5 and F4 were 7.2% and 5.9% for the steer and 8.9% and 6.6% for the heifer, respectively. Several metabolites each were present at <1 to 5% of total residues. Many low-level metabolites together comprised 44% and 41% of the remaining residues for the steer and heifer, respectively.

Metabolite profiles of manure collected in the first 3 to 5 weeks post-treatment resembled feces profiles. Mean abundances of 17α-TB, 17β-TB, and TDO vs. total ^{14}C residues were 33%, 1.0%, and 1.8%, respectively. Residue extractability decreased and profiles differed significantly in samples collected at later time periods that contained a mixture of fresh and aged manure, indicating that metabolites had degraded under ambient conditions.

Appendix 4.4. Development and validation of ARTA

Title: Qualification of an androgen receptor transactivation assay (ARTA) to characterize the relative bioactivity of trenbolone metabolites in cattle excreta

Study number: Zoetis reference number: A436R-US-18-616

GLP: No

Purpose: Development and validation of an androgen receptor transactivation assay (ARTA) to determine the relative bioactivity of trenbolone metabolites in cattle excreta

Test facility: Zoetis VMRD, Kalamazoo, MI, USA

Study design: ARTA was evaluated in three types of samples: pure compounds in solution, excreta from nontreated animals analyzed with and without trenbolone reference standards, and representative excreta samples from implanted cattle. Samples from Zoetis study A432R-GB-16-417 (Appendix 4.1) were used in this study. The following validation experiments were conducted:

1. Determination of EC_{50} values for representative test substances and verification that activity of 17β-TB > 17α-TB ≥ TDO,
2. Confirmation that results were not affected by ^{14}C present in excreta samples,
3. Confirmation that ARTA produced directionally correct dose response for mixtures (additivity)
4. Receptor specificity experiments with glucocorticoids and progestogens,
5. Evaluation of cytotoxicity due to endogenous substances and determination of the minimum required dilution,
6. Evaluation of background androgenic response due to endogenous substances, and
7. Evaluation of excreta from nontreated animals fortified with 17α-TB, 17β-TB, and TDO and excreta samples from implanted and nontreated animals.

Analytical methods: Samples were analyzed using the MDA-kb2 cell line. MDA-kb2 cells contain the human androgen receptor (AR) and a stably transfected luciferase reporter gene introduced by plasmid insertion in the MMTV-LTR (mouse mammary tumor virus-long terminal repeat) enhancer region.

Androgenic activity was determined using the Promega Steady-Glo® Luciferase Assay System. The relative luminescence in each well of a plate was quantified. Cytotoxicity was determined using the Promega Celltiter-Glo® Luminescent Cell Viability Assay. Cell viability was determined by the presence of ATP which must be present in metabolically active cells. An 80% relative response threshold was used to identify sample concentrations at which ARTA responses were potentially affected by cytotoxicity.

Results: The assay validation data demonstrated that ARTA met all assay requirements to analyze urine and feces samples from implanted cattle.

Appendix 4.5. Relative activity of TBA metabolites in cattle excreta

Title: Characterization of the relative bioactivity of trenbolone metabolites in cattle excreta using an androgen receptor transactivation assay (ARTA)

Study number: Zoetis reference number: A430R-US-18-617

GLP: No

Purpose: To characterize the relative androgenic activity of trenbolone metabolites in urine and feces of implanted cattle relative to trenbolone metabolite reference standards

Test facility: Zoetis VMRD, Kalamazoo, MI, USA

Study design: Composite urine and feces from implanted cattle; control urine and feces; feces collected on Days 3, 14, and 42; and clean catch urine collected on Days 7, 42, and 70 were prepared with and without enzymatic deconjugation with *Helix pomatia*. Each prepared sample was analyzed by LSC to measure total radioactive residues, HPLC-TopCount to determine the metabolite composition, and ARTA to determine relative androgenicity. Samples from Zoetis study A432R-GB-16-417 (Appendix 4.1) were used in this study.

Analytical methods: Samples were serially diluted to produce a range of exposure concentrations to establish a dose response curve for each sample. Samples in the resulting dilution series were diluted with cell culture medium by a minimum required dilution of 1/100 for urine and 1/200 for feces. Each sample was analyzed in two matched 96-well plates: the first to evaluate relative androgenic activity and the second to monitor for cytotoxicity. Each plate contained the dilution series for the sample (9 concentrations), negative control (no cells), positive control (17β-TB, 1 μM), and solvent control, 8 replicates of each. Six replicates were analyzed without nilutamide and two replicates were analyzed with nilutamide to confirm androgenic activity, if present. Samples were analyzed by ARTA as described in Appendix 4.4.

Results: Mean percentages of 17α-TB, 17β-TB, TDO, and the sum of all other radioactive metabolites in composite urine were 9.3%, 1.2%, 1.2%, and 88% of the total ^{14}C residues, respectively. Mean percentages in deconjugated urine samples were 11%, 1.8%, 1.3%, and 87%, respectively. Mean percentages of 17α-TB, 17β-TB, TDO, and the sum of all other metabolites in composite feces were 32%, 1.5%, 2.1%, and 65% of total extractable radioactive residues. Mean percentages in deconjugated feces samples were 32%, 2.4%, 1.9%, and 64%. Concentration percentages were similar after deconjugation treatment because conjugated metabolites in bulk urine had extensively self-deconjugated during collection and because feces was deconjugated by microflora in the gastrointestinal tract of cattle before excretion.

Low androgenic activity was detected in bulk urine (24-hour collections) and clean catch urine from implanted cattle. The combined androgenic response of all trenbolone-related metabolites in urine was significantly lower than the 17α-TB and 17β-TB positive controls. Activity did not increase after deconjugation treatment for either bulk or clean catch urine.

Interpreting the activity of TBA metabolites in feces was complicated by androgenic activity from endogenous substances that was similar in magnitude to 17α-TB. Qualitative comparisons of dose response curves for samples from treated animals were made versus control feces from untreated animals and control feces fortified with 17β-TB and 17α-TB. The combined metabolites in feces from implanted animals had significantly lower activity than 17β-TB but similar activity to endogenous substances and 17α-TB. Activity in feces did not increase after deconjugation treatment. Common metabolites present in both urine and feces based on chromatographic retention time had insignificant activity when present in urine which suggests that most TBA metabolites are less active than 17α-TB.

Appendix 4.6. Androgenic activity of TBA metabolite U7/F5

Title: Isolation and purification of trenbolone metabolite U7 from cattle urine and characterization of its androgenic activity

Study number: Zoetis reference number: A636Z-US-19-691

GLP: No

Purpose: To isolate and purify TBA metabolite U7/F5 and characterize its androgenic activity in ARTA

Test facility: Zoetis VMRD, Kalamazoo, MI, USA

Study design: Composite urine was prepared using specimens from the steer in Zoetis study A432R-GB-16-417 (Appendix 4.1).

Analytical methods: The sample was concentrated by solid-phase extraction and fractionated by gradient reverse-phase HPLC. Fractions from serial HPLC injections were collected at 0.15-minute intervals into 96-well plates. Contents of wells corresponding to U7/F5 were combined for all injections, evaporated to dryness, and reconstituted. The isolate was further purified in a second round of gradient reverse-phase HPLC. The trenbolone-equivalent concentration of purified U7/F5 isolate was 0.18 μM with no detectable ^{14}C impurities. The isolate was analyzed by ARTA as described in Appendix 4.4.

Results: Purified metabolite U7/F5 was analyzed by ARTA vs. 17β-TB and 17α-TB positive controls. Androgenic response for metabolite U7/F5 was not detected in ARTA, indicating that metabolite U7/F5 has much lower androgenic activity than both 17β-TB and 17α-TB.

Appendix 5. Executive Summaries of Literature Studies Used in this EA

Table 27. List of Appendices Where the Public Executive Summary is Located

Information / Study	Reference
Appendix 5.1. Biancotto et al. Urinary concentration of steroids in bulls under anabolic treatment by Revalor-XS® implant. J Analyt Meth Chem 2016; Article ID 8013175:1-16.	[10]
Appendix 5.2. Blackwell et al. (2014). Characterization of trenbolone acetate and estradiol metabolite excretion profiles in implanted steers. *Environ Toxicol Chem* 2014; 33(12):2850-2858.	[11]
Appendix 5.3. Blackwell et al. (2015). Transformation kinetics of trenbolone acetate metabolites and estrogens in urine and feces of implanted steers. *Chemosphere* 2015; 138:901-907.	[12]
Appendix 5.4. Challis et al. Ractopamine and other growth-promoting compounds in beef cattle operations: fate and transport in feedlot pens and adjacent environments. *Environ Sci Technol*. 2021; 55(3), 1730-1739.	[13]
Appendix 5.5. Schiffer et al. The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. Environ Health Perspect 2001; 109(11):1145-1151.	[14]
Appendix 5.6. Bartelt-Hunt SL et al. Effect of growth promotants on the occurrence of endogenous and synthetic steroid hormones on feedlot soils and in runoff from beef cattle feeding operations. *Environ Sci Technol* 2012; 46:1352-1360.	[15]
Appendix 5.7. Khan and Lee. Estrogens and synthetic androgens in manure slurry from trenbolone acetate/estradiol implanted cattle and in waste-receiving lagoons used for irrigation. *Chemosphere* 2012; 89:1443-1449.	[16]
Appendix 5.8. Jones et al. Mass balance approaches to characterizing the leaching potential of trenbolone acetate metabolites in agro-ecosystems. *Envir Sci Technol* 2014; 48:3715-3723.	[17]
Appendix 5.9. Webster JP, Kover SC, Bryson RJ, Harter T, Mansell DS, Sedlak, DL, Kolodziej EP. Occurrence of trenbolone acetate metabolites in simulated confined animal feeding operation (CAFO) runoff. *Environ Sci Technol* 2012;46:2803-2810.	[21]
Appendix 5.10. Khan B, Lee LS, Sassman SA. Degradation of synthetic androgens 17α- and 17β-trenbolone in agricultural soils. *Environ Sci Technol* 2008;42:3570-3574.	[22]
Appendix 5.11. Cole EA, McBride SA, Kimbrough KC, Lee J, Marchand EA, Cwiertny DM, Kolodziej EP. Rates and product identification for trenbolone acetate metabolite biotransformation under aerobic conditions. *Envir Toxicol Chem* 2015;34:1472-1484.	[23]

Appendix 5.1. Biancotto et al., 2016

Title: Urinary concentration of steroids in bulls under anabolic treatment by Revalor-XS® implant

Citation: Biancotto G, Stella R, Barrucci F, Lega F, Angeletti R. *J Analyt Meth Chem* 2016; Article ID 8013175:1-16.

Study design: Thirty two (32) bulls were divided into two groups of 16 each: a group implanted with Revalor XS containing 200 mg TBA and 40 mg estradiol and a nontreated control group. Urine was collected from pre-dose through 68 days on treatment.

Analytical methods: 17α-TB and 17β-TB were measured by LC-MS/MS after enzymatic deconjugation with *Helix pomatia*. Decision limits (CCα) for 17α-TB and 17β-TB were 0.23 and 0.17 ng/mL, respectively. Detection capabilities (CCβ) were 0.39 and 0.29 ng/mL, respectively. TDO was not measured.

Results: Average concentrations of 17α-TB and 17β-TB in samples collected through 68 days of implantation are reported in Table 4 of the article. Maximum concentrations of 17α-TB (2.06 ng/mL) and 17β-TB (0.49 ng/mL) were observed at Day 7 and declined to 0.8 to 0.9 ng/mL for 17α-TB and to 0.14 ng/mL for 17β-TB by Day 63. Concentrations of both analytes increased on Day 68 due to the second phase of API release from Revalor XS.

Average concentrations of 17β-TB reported in italics in Table 4 of Biancotto et al. were below the detection capability limit (CCβ). Only the average concentrations on Days 7 and 68 were greater than CCβ. Despite this limitation, the relationship between 17α- and 17β-TB can be estimated. For Days 7 and 68, the concentration ratios of 17α-TB:17β-TB were 81:19 and 85:15, respectively. The mean ratio was 85:15 based on data for all time points.

Appendix 5.2. Blackwell et al., 2014

Title: Characterization of trenbolone acetate and estradiol metabolite excretion profiles in implanted steers

Citation: Blackwell BR, Brown TR, Broadway PR, Buser MD, Brooks JC, Johnson BJ, Cobb GP, Smith PN. *Environ Toxicol Chem* 2014;33(12):2850-2858.

Study design: 8 cattle were implanted with Revalor XS (200 mg TBA and 40 mg estradiol). Serum and urine and feces grab samples were collected through 112 days after implantation.

Analytical methods: Concentrations of 17α-TB, 17β-TB, and TDO were determined by LC-MS/MS. Urine and feces samples were analyzed before and after *Helix pomatia* treatment to determine the relative abundances of free and conjugated steroids.

Results: The primary trenbolone metabolite in serum was 17β-TB. The average peak concentration of 450 pg/mL was observed on Day 1 and declined to 180 pg/mL by Day 112. The increase in the serum concentration of 17β-TB on Days 56 to 70 was consistent with the biphasic release pattern of Revalor XS implants. 17α-TB and TDO were present at lower concentrations in serum and detected infrequently (LOD = 5 pg/mL).

The primary metabolite in excreta was 17α-TB. 17α-TB concentrations in urine peaked by Day 7. 17α-TB conjugates comprised 92% of total 17α-TB. 17α-TB concentrations peaked by Day 7 in feces and were mostly nonconjugated residues. For 17α-TB excreted in urine and feces, 84% was excreted in feces and 8.1% was conjugated, mostly in urine.

17α-TB, 17β-TB, and TDO in urine accounted for 86.6%, 11.7%, and 1.7%, respectively, relative to the concentration sum of the three analytes. 17α-TB, 17β-TB, and TDO in feces accounted for 95.3%, 4.0%, and 0.7% relative to their concentration sum.

Appendix 5.3. Blackwell et al., 2015

Title: Transformation kinetics of trenbolone acetate metabolites and estrogens in urine and feces of implanted steers

Citation: Blackwell BR, Johnson BJ, Buser MD, Cobb GP, Smith PN. *Chemosphere* 2015; 138:901-907.

Study design: Urine and feces grab samples were collected from eight cattle on Days 1 through 7 after implantation. Aliquots of pooled feces, pooled urine, and simulated manure (1:1 mixture of feces and urine) were stored loosely covered in the dark at 21°C.

Analytical methods: Samples were analyzed through 112 days of incubation. Concentrations of 17α-TB, 17β-TB, and TDO were determined by LC-MS/MS. Samples were analyzed with and without enzymatic hydrolysis with *Helix pomatia* to determine concentrations of free and total metabolites.

Results: Percentages of 17α-TB, 17β-TB, and TDO versus their concentration sum were 85.1%, 12.4%, and 2.5%, respectively, in urine and 99.3%, not detected, and 0.7%, respectively, in feces. Conjugates of 17α-TB rapidly converted to free steroids during storage (half-life: 1.0 days). 17α-TB in urine (free + conjugates) increased in the first 3 days to 145% which was attributed to natural deconjugation of some conjugates that had not been cleaved completely in day-0 samples by *Helix pomatia*.

Conjugates in simulated manure were considerably lower than calculated concentrations based upon levels present in urine and feces alone, presumably due to deconjugation by β-glucuronidases and other enzymes present in feces. Because urine in a feedlot mixes with fresh feces and interacts with soil or pen surfaces, metabolite conjugates in urine in a feedlot setting deconjugate faster than urine maintained separately.

Metabolite transformation of 17α-TB in the dark at 21°C was determined by measuring the decline of 17α-TB (free + conjugates) over time. Decline of 17α-TB fit a pseudo-first order decay model. Dissipation half-lives were 9.5, 5.1, and 8.7 days for urine, feces, and simulated manure, respectively. Feces and simulated manure samples were visibly desiccated by Days 28 and 84 which may have affected substrate availability or microbial activity and decreased transformation rate.

To determine the potential for secondary metabolite formation, the production and loss of 17β-TB and TDO from 17α-TB were measured over time. Abundances were reported as mol% vs. the initial concentration of 17α-TB. 17β-TB in urine increased from 12% to 14% of the initial 17α-TB concentration at the first timepoint and declined to below the limit of detection within 14 days. 17β-TB in feces increased to 4% and rapidly declined to below the detection limit by 14 days. TDO increased in the first 7 days from 2.5% to a maximum of 3.9% in urine and from 0.7 mol% to a maximum of 1% in feces and then declined to less than 1% in urine and feces by Day 21. Transient increases in 17β-TB and TDO were attributed to conversion of 17α-TB to 17β-TB and TDO.

Appendix 5.4. Challis et al. 2021

Title: Ractopamine and other growth-promoting compounds in beef cattle operations: fate and transport in feedlot pens and adjacent environments

Citation: Challis JK, Sura S, Cantin J, Curtis AW, Shade KM, McAllister TA, Jones PD, Giesy JP, Larney FJ. *Environ Sci Technol* 2021;55(3):1730-1739.

Study Design: Cattle (groups of 10 animals per pen, four pens per treatment) were fed for 259 days in 2017-2018 and 273 days in 2018-2019. Six treatments were administered per group: (1) control heifers (no treatment), (2) TBA + estradiol-implanted heifers, (3) heifers fed continuously with melengestrol acetate (MGA), (4) control steers (no treatment), (5) TBA-implanted steers, and (6) steers implanted with TBA and fed ractopamine hydrochloride the last 42 days prior to slaughter (TBA+RAC). Animals receiving TBA implants received doses of 100 mg, 100 mg, and 200 mg approximately 80 days apart. With 40 animals per treatment and 3 TBA treatments, 240 beef cattle were treated with TBA in the 2 year study.

Twenty samples of fresh feces were sampled per pen on 3 consecutive days, 2 weeks after each of the three implants were administered. Twenty samples of pen floor material (mix of manure with urine and straw bedding) were collected from each pen once monthly. Each set of 20 feces and floor pen samples collected within a pen were composited to make a single feces and single floor pen sample per pen and sampling time. TBA, 17α-TB, 17β-TB, and TDO were quantified by liquid chromatography-tandem mass spectroscopy with limits of detection ranging from 0.05 to 0.8 ng/mL depending upon analyte and matrix.

Extensive sampling from floor pens also was also conducted in the 2017-2018 trial in TBA+RAC-treated animals after 200 days and again 10 and 22 days post-trial after animals had been removed. Manure samples were collected from four commercial feedlots at 5-6 sampling points over a 2 year period (2016-2018) and analyzed using the same methods.

Simulated runoff experiments were also conducted. Because the focus of this EA is on data regarding the concentrations of TBA metabolites in cattle excreta, these data are not discussed further in this EA.

Study Results: The predominant metabolite in feces and pen floor samples was 17α-TB. Fresh feces samples had higher concentrations of 17α-TB (average: 41±30 ng/g) and 17β-TB (average 3±2 ng/g) than pen floor samples. TBA and TDO were below the detection limit in all samples. Averaged over 2 years, the 17α-TB:17β-TB:TDO ratio in fresh feces and pen floor samples ranged from 93:7:0 to 95:5:0. Contemporaneous results from samples collected from commercial feedlots were similar to those measured in this feeding study.

Regarding fate and dissipation of TBA metabolite residues, concentrations of 17α-TB and 17β-TB from TBA+RAC treated animals dissipated quickly following the final implant, especially in summer months (June and July). At the end of the 2017-2018 trial, concentrations of 17α-TB had declined to ≤1 ng/g after 10 days and below the limit of detection after 22 days. Similarly, concentrations of 17β-trenbolone dissipated over time and were below the limit of detection for all samples collected after May in both the 2017-2018 and 2018-2019 trials.

Appendix 5.5. Schiffer et al., 2001

Title: The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies

Citation: Schiffer B, Daxenberger A, Meyer K, Meyer HHD. *Environ Health Perspect* 2001; 109(11):1145-1151.

Study design: In Study I of Schiffer et al., 41 cattle were implanted with TBA. Liquid manure was collected into a collection canal and pumped into a cylindrical storage tank. Samples of liquid manure were collected every 2 weeks. Manure in the storage tank was sampled every 2 or 4 weeks and again before spreading on fields. Liquid manure in the canal was not mixed before sampling, whereas material in the storage pit was stirred prior to sampling.

In Study II, 12 cattle were implanted with TBA. Stables housing the animals were cleaned periodically and manure was moved to a dung pile. The pile contained the combined excreta accumulated over 87 days (31 days before through 56 days after implantation). At the end of the collection period, samples were collected from the top, middle, bottom, and effluent areas of the pile. The pile was then transferred to a storage area and samples were collected 4.5 months later.

Analytical methods: 17α-TB, 17β-TB, and TDO were quantitated in samples by reverse-phase (C_{18}) HPLC with fraction collection. Fractions containing analytes were quantified by enzyme immunoassay. Concentrations of 17β-TB and TDO were determined by relative cross-reactivity vs. 17α-TB. A recovery factor was applied to correct for analyte loss during sample preparation. Detection limits were 4 pg/g for liquid manure and 5 pg/g for solid dung. To confirm the results, analytes in two samples were quantitated by GC-MS as their heptafluorobutyryl derivatives. Presence of 17α-TB and 17β-TB were confirmed by GC-MS. TDO was not confirmed due to assay interference.

Results: For Study I, analyte concentrations in liquid manure were highly variable due to lack of mixing prior to sampling. Absolute concentrations of trenbolone metabolites varied from sample to sample and were <1000 to 4000 pg/g for 17α-TB, 80 to 180 pg/g for 17β-TB, and 20 to 80 pg/g for TDO (approximate values from Figure 3 of Schiffer et al.). Relative concentrations of 17α-TB in liquid manure were consistently 22x higher than 17β-TB and 49x higher than TDO. Using these numeric relationships, mean concentration percentages were calculated to be 93.8%, 4.3%, and 1.9% for 17α-TB, 17β-TB, and TDO as their concentration sum.

For Study II, data in Table 2 of Schiffer et al. were used to estimate the proportional relationships of the three analytes in dung accumulated over 87 days under simulated field conditions. Results are presented in Table 28. Absolute analyte concentrations in samples from different locations in a pile were highly variable due to non-uniformity/lack of mixing of residues throughout the pile, migration of residues from the surface to deeper in the pile due to precipitation, difference in water content among samples, and differential degradation of residues within the pile. Absolute concentrations of trenbolone metabolites were highest in middle of the dung pile and varied as a function of location. Percentages of metabolites relative to their concentration sum were consistent in the pile and effluent zone.

Analyte concentrations in dung samples after 4.5 months of storage were significantly lower and below the detection limit in several samples, thus indicating extensive degradation of analytes during storage. Concentrations in the center of the pile were higher than the top or bottom and had similar analyte percentages as samples collected before storage. Concentration percentages in samples from the bottom of the pile were highly variable due to extensive degradation.

Table 28. Relative Percentages of TBA Metabolites in Manure (Schiffer et al.)

Sample	17α-TB (pg/g)	17β-TB (pg/g)	TDO (pg/g)	Sum total (pg/g)	17α-TB (%)	17β-TB (%)	TDO (%)	17α-TB vs. 17β-TB	17α-TB vs. TDO
Study I: Liquid manure (collected biweekly)									
Liquid manure	<1000-4000	80-180	20-80	Not determined	93.8*	4.3*	1.9*	22x	49x
Study II: Solid dung before storage (collected for 87 days, 31 days before through 56 days on treatment)									
Fresh (~ 1 m from top)	13820	1000	1,225	16045	86.1	6.2	7.6	13.8x	11.3x
Medium (height 2.5 m)	75400	4265	4700	84365	89.4	5.1	5.6	17.7x	16.0x
Old (0.5 m from bottom)	4726	484	405	5615	84.2	8.6	7.2	9.8x	11.7x
Effluent zone	227	19	10	256	88.7	7.4	3.9	11.9x	22.7x
Mean, all zones					87.1	6.8	6.1	13.3x	15.4x
Study II: Solid dung before spreading on fields (additional 4.5 months storage), results for 2 samples per location									
Top of hill	nd, nd	11, nd	nd, nd	nc	nc	nc	nc	nc	nc
Middle of hill	10100, nd	292, nd	824, nd	11216, nc	90.0, nc	2.6, nc	7.3, nc	34.6x, nc	12.3x, nc
Bottom of hill	100, 318	60, 14	70, nd	230, 332	43.5, 95.8	26.1, 4.2	30.4, 0	1.7x, 22.7x	1.4x, >100x

* Calculated using the reported relationship that concentrations of 17α-trenbolone were 22x higher than 17β-trenbolone and 49x higher than trendione on average. nd = not detected. nc = not calculated

Appendix 5.6. Bartelt-Hunt et al., 2012

Title: Effect of growth promotants on the occurrence of endogenous and synthetic steroid hormones on feedlot soils and in runoff from beef cattle feeding operations

Citation: Bartelt-Hunt SL, Snow DD, Kranz WL, Mader TL, Shapiro CA, van Donk SJ, Shelton DP, Tarkalson DD, Zhang TC. *Environ Sci Technol* 2012; 46:1352-1360.

Study design: A two-year study was conducted in a simulated confined beef cattle production facility in 2007 and 2008. Each year, 96 heifers were divided into a treated group and a control group of 48 animals each. Treated animals received a Ralgro implant (36 mg of α-zearalanol) on Day 1, a Revalor H implant (140 mg TBA and 14 mg estradiol) 35 days later, and 0.45 mg melengestrol acetate per day in feed on Day 7 through the end of each annual trial. Animals were held in pens for 112 days in 2007 and for 141 days in 2008. Soil and manure were mechanically scraped to the clay layer and fresh soil was added before initiating the second phase in 2008.

Analytical methods: Manure, surface soil, and urine-soaked soil were sampled on Days 7, 46, and 109 in 2007 and on Days 7, 47, and 138 in 2008. Feedlot runoff was collected from each pen following significant rain events. 17α-TB and 17β-TB and other analytes were measured in runoff, manure, and soil by LC-MS/MS. TDO was not measured.

Results: In samples collected on Day 7 of each year, the 17α-TB concentration in manure was approximately 100x higher than 17β-TB (99:1). 17α-TB concentrations in accumulated manure were 31 ng/g-dw (dry weight) on Day 46 in 2007 and 55 ng/g-dw on Day 47 in 2008, which were the only timepoints with detectable concentrations. 17β-TB was not detected at Day 46 in 2007, was detected at 0.5 ng/g-dw on Day 47 in 2008, and was not detected at later timepoints.

Feedlot runoff was collected from each pen following significant rain events. 17β-TB was detected (270 ng/L) in one runoff event over the two-year period, whereas 17α-TB was not detected in that event. Neither analyte was detected in urine-soaked soil samples, thus confirming that other metabolism/degradation pathways degraded trenbolone metabolites excreted from cattle. Given the large predominance of 17α-TB in manure produced by cattle and non-detectable levels of 17α-TB and 17β-TB at the feedlot surface, the reported level of 17β-TB in that runoff event was inconsistent with the other findings from this study and with the collective data from several studies by other investigators. This conclusion was substantiated by independent reviewers [18,19].

Appendix 5.7. Khan and Lee, 2012

Title: Estrogens and synthetic androgens in manure slurry from trenbolone acetate/estradiol implanted cattle and in waste-receiving lagoons used for irrigation

Citation: Khan B and Lee LS. *Chemosphere* 2012; 89:1443-1449.

Study design: 201 cattle were implanted with Revalor-S (140 mg TBA and 28 mg estradiol) and housed at the Purdue Animal Science Research and Education Center. Animals were housed in two barns: 110 animals in the west barn and 91 animals in the east barn. Each barn had slatted floors with manure and urine collection pits on the right and left side of the barn. The collection pits were flushed weekly into a two-stage unaerated lagoon system with the first stage (Lagoon I) collecting solids and the second stage (Lagoon II) collecting effluent used to flush manure pits and irrigate agricultural fields.

Analytical methods: Triplicate samples of manure slurry from collection pits of the west barn housing 110 animals were collected weekly for nine weeks, and triplicate samples of Lagoon II flushing water were collected weekly. Concentrations of 17α-TB, 17β-TB, TDO, and other analytes were measured by LC-MS/MS.

Results: Peak concentrations of 17α-TB and 17β-TB in manure collected from the west barn occurred at 2 weeks post-dose and at 2 and 4 weeks for TDO. 17α-TB was the most abundant metabolite. The maximum concentration of 17α-TB (2.90 µg/L) was 16x higher than 17β-TB (≤0.18 µg/L) and 24x higher than TDO (≤0.12 µg/L). Based on these numeric relationships, concentration percentages were calculated to be 90.6% for 17α-TB, 5.7% for 17β-TB, and 3.8% for TDO as their concentration sum.

Irrigation water was also analyzed. Maximum concentrations of trenbolone metabolites in irrigation water occurred 4 to 5 weeks post-dose. Relative abundances of the three metabolites in irrigation water were similar to manure. 17α-TB was the most abundant metabolite in the first nine weeks, with average concentrations of 0.29-1.53 µg/L. Concentrations of 17β-TB (0.02-0.1 µg/L) and TDO (0.01-0.11 µg/L) were approximately 15x lower than 17α-TB.

Appendix 5.8. Jones et al., 2014

Title: Mass balance approaches to characterizing the leaching potential of trenbolone acetate metabolites in agro-ecosystems

Citation: Jones GD, Benchetler PV, Tate KW, Kolodziej EP. *Envir Sci Technol* 2014; 48:3715-3723.

Study design: Three steers were implanted with Revalor G (40 mg TBA and 8 mg estradiol). Manure samples were collected through 113 days after implantation. Metabolite transformation rates were determined in fresh manure by measuring the concentration of 17α-TB daily in samples stored in the dark or direct sunlight.

Analytical methods: Concentrations of 17α-TB, 17β-TB, TDO were determined by GC-MS/MS after derivatization with MSTFA-I_2 (methyl-N-(trimethylsilyl)trifluoro-acetate-iodine). The authors reported good analytical recoveries of 17α-TB and 17β-TB, but low recoveries of TDO (33%).

Results: 17α-TB concentrations in manure peaked at 64 ng/g-dw (dry weight) at 24 hours after implantation and decreased to 10 ng/g-dw by 113 days. 17β-TB was detected through 7 days (1.7 to 3.8 ng/g-dw) and only sporadically thereafter. TDO was detected only twice in the first 4 days at 1.3 and 2.5 ng/g-dw. Due to the low TBA dose, most results for 17β-TB and TDO were below the detection limit.

17α-TB depletion in manure followed first-order kinetics. Depletion half-lives were 4.1, 2.7, and 1.6 days for samples stored in the dark at 1, 19, or 33°C, respectively. In sunlit samples (33°C average air temperature), the 17α-TB concentration decreased approximately 2-fold from 53 ng/g-dw to 24 ng/g-dw in the first 24 hours and then stabilized presumably due to desiccation of samples under arid conditions.

The authors also conducted controlled leaching and mass transfer experiments. Results are outside the scope of this review, however the authors estimated that 9.3% of implant mass was excreted in manure as 17α-TB and <1% as 17β-TB and TDO. The authors also estimated that most (>97%) of the total excreted mass of 17α-TB transforms to other uncharacterized metabolites.

Appendix 5.9. Webster et al., 2011

Title: Occurrence of trenbolone acetate metabolites in simulated confined animal feeding operation (CAFO) runoff

Citation: Webster JP, Kover SC, Bryson RJ, Harter T, Mansell DS, Sedlak, DL, Kolodziej EP. *Environ Sci Technol* 2012;46:2803-2810.

Study design: Simulated rainfall experiments were conducted at a research CAFO using feces collected from fourteen steers receiving an implant containing 120 mg TBA and 24 mg estradiol. Manure was collected from eight animals at 28 days. Spatially composited samples of manure and soil (top 5 cm) collected from additional animals. Note: for the purposes of this EA, manure samples in this study were classified as aged manure because manure was accumulated over 4 weeks under field conditions.

Analytical methods: Concentrations of 17α-TB, 17β-TB, and TDO were determined by GC-MS/MS after derivatization with MSTFA-I_2 (methyl-N-(trimethylsilyl)trifluoro-acetate-iodine).

Results: Concentrations of 17α-TB and 17β-TB were 21 and 3.1 ng/g-dw, respectively. Trendione was not detected (nd). Concentration percentages were 87%, 13%, and nd for 17α-trenbolone, 17β-trenbolone, and trendione as their concentration sum.

17α-TB was the predominant metabolite in spatially composited samples of manure and soil (top 5 cm). Ratios of 17α-TB and 17β-TB were similar to manure. TDO was not detected (nd). The reduced metabolite concentrations in these samples relative to fresh manure reflects extensive biotransformation (degradation) of TBA metabolites on CAFO surfaces.

Appendix 5.10. Khan et al. 2008

Title: Degradation of synthetic androgens 17α- and 17β-trenbolone in agricultural soils

Citation: Khan B, Lee LS, Sassman SA. *Environ Sci Technol* 2008;42:3570-3574.

Study design: Soil microcosms were prepared using three soils. One soil also was augmented with manure at a level equivalent to 20 tons/acre. Samples were fortified with 17α-TB or 17β-TB at concentrations of 0.05, 0.1, 1, 7, and 10 mg/kg and with TDO at concentrations of 0.04, 3, and 3.5 mg/kg. These samples were incubated under aerobic conditions at 22 ± 2°C.

Analytical methods: Metabolite concentrations were measured by reverse-phase HPLC with ultraviolet or mass spectrometric detection.

Results: The concentration of each metabolite declined according to pseudo first-order kinetics, with faster degradation rates observed for 17α-TB and 17β-TB than TDO. Both 17α-TB and 17β-TB formed TDO in small amounts. Approximately 1.5% of TDO was converted to 17β-TB regardless of fortification concentration. No direct conversion of 17β-TB to 17α-TB was observed, however a small amount (<1%) of 17α-TB was converted to 17β-TB, presumably through TDO as an intermediate. The degradation rate of 17α-TB was not affected by addition of manure to soil but increased for TDO. Production of 17β-TB from 17α-TB in soil was similar (1.9%) in a study conducted by Zoetis (see Section 4.2.7 of the Synovex One EA [1]).

Appendix 5.11. Cole et al. 2015

Title: Rates and product identification for trenbolone acetate metabolite biotransformation under aerobic conditions

Citation: Cole EA, McBride SA, Kimbrough KC, Lee J, Marchand EA, Cwiertny DM, Kolodziej EP. *Envir Toxicol Chem* 2015;34:1472-1484.

Study design: Microcosms were prepared using inocula from water sources and individually fortified at concentrations of 1400 ng/L of 17α-TB, 17β-TB, or TDO.

Analytical methods: Analytes were measured by GC-MS/MS to determine transformation rates.

Results: Half-lives were 0.9, 1.3, and 2.2 days for 17β-TB, TDO, and 17α-TB, respectively, and were temperature dependent. In 17α-TB-fortified samples, 17α-TB rapidly declined to form mostly other metabolites along with low amounts of 17β-TB and TDO. Of the TDO and 17β-TB produced, both rapidly declined over time. In 17β-TB-fortified samples, 17β-TB rapidly declined to mostly other metabolites along with TDO and low amounts of 17α-TB. In TDO-fortified samples, TDO rapidly declined in the first day to form primarily other metabolites along with low amounts of 17α-TB and 17β-TB. All analytes decreased to non-detectable levels by 15 days.

Appendix 6. Cross-Study Comparisons of TBA Metabolites in Excreta

In non-label studies reported in the literature, 17α-TB, 17β-TB, and TDO concentrations often are expressed as percentages of the three analytes relative to each other. This approach can be used for both radiolabel and nonlabeled studies.

Because 17α-TB and 17β-TB have the same molecular weight and because the molecular weight of TDO differs by only 2 Daltons, analyte concentration percentages can be calculated as shown below. Calculations performed by substituting metabolite percentages in place of concentrations produce the same numeric results.

%17α-TB = Conc 17α-TB / [Conc 17α-TB + Conc 17β-TB + Conc TDO] x 100%

%17β-TB = Conc 17β-TB / [Conc 17α-TB + Conc 17β-TB + Conc TDO] x 100%

%TDO = Conc TDO / [Conc 17α-TB + Conc 17β-TB + Conc TDO] x 100%

Accordingly, concentration percentages of 17α-TB, 17β-TB, and TDO for all studies with reliable data were calculated to express the proportional relationship of the three trenbolone metabolites to each other. This information is valuable for a weight of evidence approach to compare data across studies and establish the actual composition of trenbolone metabolites in excreta. Results for urine, feces, and manure from nonlabel and radiolabel studies are summarized in Table 29, Table 30, and Table 31 of this Appendix. In each table, a shorthand notation is used for Zoetis studies in which the first, third, and last 3 characters denote the study number (e.g, A3536 = A432R-US-17-536).

The calculated percentages of 17α-TB, 17β-TB, and TDO express the numeric relationship of the three known active trenbolone metabolites to each other. However, these values grossly overestimate the actual percentages of the three metabolites in excreta because they do not account for most of the total trenbolone metabolite residues actually present in excreta: 86.5% on average for urine, 65% for feces, and 64% for manure.

Using the results from the Zoetis ^{14}C study as a bridge, we can estimate the concentration percentages in nonlabel studies that would have been observed if all investigators had the analytical capability to quantitate all (total) trenbolone-related metabolites and not just two or three analytes. This is accomplished by multiplying the concentration percentages determined for the three analytes by the following factors: 0.135 for urine, 0.35 for feces, and 0.36 for manure (the combined percentage of all other metabolites observed in each of these sample types in the Zoetis ^{14}C studies).

Results for urine, feces, and manure in all studies are summarized in the rightmost columns of Table 29, Table 30, and Table 31 of this Appendix. The resulting percentages (measured values for radiolabel studies, calculated values for nonlabel studies) are more accurate estimates for the actual concentration percentages of 17α-TB, 17β-TB, and TDO in samples relative to total (all) trenbolone-related metabolites in excreta.

Table 29. Percentages of Trenbolone Metabolites in Cattle Urine

Data Source	No. of Animals	Type of Study and Method of Analysis	Proportions of 17α-TB, 17β-TB, TDO to Each Other			Measured (m) or Calculated (c) Percentages of Metabolites vs. Total Trenbolone Metabolite Residues in a Sample			
			17α-TB	17β-TB	TDO	17α-TB	17β-TB	TDO	All Else
Syntex (2014 EA) [1]	8	^{14}C, HPLC-Radiometric	nm	nm	nm	nm	nm	nm	nm
Zoetis A3536 (ND) [7] Appendix 4.3	2	^{14}C, HPLC-Radiometric	84.3	7.9	7.8	14.5m	1.2m	1.2m	83.1m
Zoetis A3617 (ND) [9] Appendix 4.5			79.8	10.3	9.9	9.3m	1.2m	1.2m	88.4m
Zoetis A3617 (DC) [9] Appendix 4.5			77.4	13.3	9.3	10.5m	1.8m	1.3m	86.5m
Biancotto (DC) 10] Appendix 5.1	16	Nonlabel, LC-MS/MS	85	15	nm	11.5c	2.0c	nm	86.5c
Blackwell (DC) [11] Appendix 5.2	8	Nonlabel, LC-MS/MS	86.6	11.7	1.7	11.7c	1.6c	0.23c	86.5c
Blackwell (DC) [12] Appendix 5.3	8	Nonlabel, LC-MS/MS	85.1	12.4	2.5	11.5c	1.7c	0.33c	86.5c

nm = not measured. nd = not detected. nc = not calculated. ND = Non-deconjugated, analyzed without *Helix pomatia* enzymatic treatment. DC = Deconjugated, analyzed with *Helix pomatia* enzymatic treatment.

Calculated percentages in the right 4 columns include the contributions of other metabolite residues besides 17α-TB, 17β-TB, and TDO. These values were calculated by assigning 86.5% to 'all other metabolites' based on the ^{14}C data and multiplying percentages of 17α-TB, 17β-TB, and TDO expressed as their concentration sum by 0.135 (100% - 86.5% = 13.5%).

Table 30. Percentages of Trenbolone Metabolites in Cattle Feces

Data Source	No. of Animals	Type of Study and Method of Analysis	Proportions of 17α-TB, 17β-TB, TDO to Each Other			Measured (m) or Calculated (c) Percentages of Metabolites vs. Total Trenbolone Metabolite Residues in a Sample			
			17α-TB	17β-TB	TDO	17α-TB	17β-TB	TDO	All Else
Syntex (2014 EA) [1]	8	^{14}C, HPLC-Radiometric	96.8c	3.2c	nd	53.4m	1.8m	nd	44.8c
Zoetis A3536 (ND) [7] Appendix 4.3	2	^{14}C, HPLC-Radiometric	92.8	2.9	4.4	32.7m	1.0m	1.5m	64.8m
Zoetis A3617 (ND) [9] Appendix 4.5			90.0	4.2	5.8	31.8m	1.5m	2.1m	64.8m
Zoetis A3617 (ND) [9] Appendix 4.5]			88.2	6.6	5.2	31.5m	2.4m	1.9m	64.4m
Blackwell (DC) [11] Appendix 5.2	8	Nonlabel, LC-MS/MS	95.3	4.0	0.7	33.3c	1.4c	0.25c	65c
Blackwell (DC) [12] Appendix 5.3	8	Nonlabel, LC-MS/MS	99.3	nd	0.7	34.8c	nd (0)	0.23c	65c
Challis et al. [13] Appendix 5.4	240	Nonlabel, LC-MS/MS	94 (avg)	6 (avg)	nd	32.9c	2.1c	nd (0)	65c

Calculated by assigning 65% to 'all other metabolite residues' based on the ^{14}C data and multiplying percentages of 17α-TB, 17β-TB, and TDO expressed as their concentration sum by 0.35 (100% - 65% = 35%). See Table 29 for other footnotes.

Table 31. Percentages of Trenbolone Metabolites in Cattle Manure

Data Source	No. of Animals	Type of Study and Method of Analysis	Proportions of 17α-TB, 17β-TB, TDO vs. Each Other			Measured (m) or Calculated (c) Percentages of Metabolites vs. Total Trenbolone Metabolite Residues in a Sample			
			17α-TB	17β-TB	TDO	17α-TB	17β-TB	TDO	All Else
Zoetis A3536 (ND) [7] Appendix 4.3	2	^{14}C, HPLC-Radiometric	92.0	2.9	5.1	33.1m	1.0m	1.8m	64.1m
Schiffer [14], Phase I Appendix 5.5	41	Nonlabel, HPLC-fraction collection, enzyme immunoassay	93.8*	4.3*	1.9*	33.8c	1.5c	0.7c	64c
Bartelt-Hunt [15] Appendix 5.6	96	Nonlabel, LC-MS/MS	99	1	nm	35.6c	0.4c	nm	64c
Khan and Lee [16] Appendix 5.7	110 (west barn)	Nonlabel, LC-MS/MS	90.6*	5.7*	3.8*	32.6c	2.1c	1.4c	64c
Challis et al. [13], floor pen, Appendix 5.4	240	Nonlabel, LC-MS/MS	94 (avg)	6 (avg)	nd	33.8c	2.2c	nd (0)	64c
Schiffer [14], Phase II Appendix 5.5	12	Nonlabel, HPLC-fraction collection, enzyme immunoassay	87.1	6.8	6.1	31.4c**	2.4c**	2.2c,**	64**
Schiffer [14], Phase II 'stored' Appendix 5.5		As above, pile was moved and sampled 4.5 months later	90.0	2.6	7.3	32.4c**	0.9c**	2.6c,**	64**
Webster [21] Appendix 5.9	8	Nonlabel, GC-MS/MS	87	13	nd	31.3c**	4.7c**	nd (0)	64**

Calculated by assigning 64% to 'all other metabolite residues' based on the ^{14}C data and multiplying percentages of 17α-TB, 17β-TB, and TDO expressed as their concentration sum by 0.36 (100% - 64% = 36%). See Table 29 for other footnotes.
* Calculated from the reported proportional relationships in Schiffer et al. Khan and Lee.
** Values should be interpreted with caution for aged manure samples amassed over time. These values are used for illustration purposes.

Appendix 7. Implantation Scenarios Modeled for this EA

Table 32. Implant Combinations and Regimens for Estradiol

Implant Scenario	Implant 1 (on day 0)	Implant 2 (Implant day)	Implant 3 (Implant day)	Days Per Production Cycle	Production Cycles Per Year
Choice	Choice	--	--	141	2.59
Plus	Plus	--	--	141	2.59
ONE Feedlot (ONE-F)	ONE-F	--	--	267	1.37
ONE Grower (ONE-G)	ONE-G	--	--	267	1.37
Choice-Plus-141	Choice	Plus (60)	--	141	2.59
Choice-Plus-201	Choice	Plus (60)	--	201	1.82
Choice-Choice	Choice	Choice (60)	--	141	2.59
Choice-ONE-F	Choice	ONE-F (60)	--	141	2.59
Plus-Plus-267	Plus	Plus (60)	--	141	2.59
Plus-Plus-281	Plus	Plus (60)	--	201	1.82
Plus-Choice	Plus	Choice (60)	--	141	2.59
Plus-ONE-F	Plus	ONE-F	--	141	2.59
ONE-F-Plus-267	ONE-F	Plus (140)	--	267	1.37
ONE-F-Plus-281	ONE-F	Plus (140)	--	281	1.30
ONE-F-Choice	ONE-F	Choice (140)	--	267	1.37
ONE-F-ONE-F	ONE-F	ONE-F (140)	--	267	1.37
ONE-G-Plus-267	ONE-G	Plus (140)	--	267	1.37
ONE-G- Plus-281	ONE-G	Plus (140)	--	281	1.30
ONE-G-Choice	ONE-G	Choice (140)	--	267	1.37
ONE-G-ONE-F	ONE-G	ONE-F (140)	--	267	1.37
Choice-Plus-Plus-201	Choice	Plus (60)	Plus (120)	201	1.82
Choice-Plus-Plus-261	Choice	Plus (60)	Plus (120)	261	1.40
Choice-Choice-Plus-201	Choice	Choice (60)	Plus (120)	201	1.82
Choice-Choice-Plus-261	Choice	Choice (60)	Plus (120)	261	1.40
Choice-ONE-F-Plus-327	Choice	ONE-F (60)	Plus (200)	327	1.12
Choice-ONE-F-Plus-341	Choice	ONE-F (60)	Plus (200)	341	1.07
Plus-Plus-Plus-201	Plus	Plus (60)	Plus (120)	201	1.82
Plus-Plus-Plus-261	Plus	Plus (60)	Plus (120)	261	1.40

Table 33. Implant Combinations and Regimens for Trenbolone

Implant Scenario	Implant 1 (on day 0)	Implant 2 (Implant day)	Implant 3 (Implant day)	Days Per Production Cycle	Production Cycles Per Year
Choice	Choice	--	--	117	3.12
Plus	Plus	--	--	117	3.12
ONE Feedlot (ONE- F)	ONE-F	--	--	211	1.73
ONE Grower (ONE-G)	ONE-G	--	--	211	1.73
Choice-Plus-117	Choice	Plus (60)	--	117	3.12
Choice-Plus-177	Choice	Plus (60)	--	177	2.06
Choice-Choice	Choice	Choice (60)	--	117	3.12
Choice-ONE-F	Choice	ONE-F (60)	--	117	3.12
Plus-Plus-117	Plus	Plus (60)	--	117	3.12
Plus-Plus-177	Plus	Plus (60)	--	177	2.06
Plus-Choice	Plus	Choice (60)	--	117	3.12
Plus-ONE-F	Plus	ONE-F	--	117	3.12
ONE-F-Plus-211	ONE-F	Plus (140)	--	211	1.73
ONE-F-Plus-257	ONE-F	Plus (140)	--	257	1.42
ONE-F-Choice	ONE-F	Choice (140)	--	211	1.73
ONE-F-ONE-F	ONE-F	ONE-F (140)	--	211	1.73
ONE-G-Plus-211	ONE-G	Plus (140)	--	211	1.73
ONE-G-Plus-257	ONE-G	Plus (140)	--	257	1.42
ONE-G-Choice	ONE-G	Choice (140)	--	211	1.73
ONE-G-ONE-F	ONE-G	ONE-F (140)	--	211	1.73
Choice-Plus -Plus-177	Choice	Plus (60)	Plus (120)	177	2.06
Choice-Plus-Plus-237	Choice	Plus (60)	Plus (120)	237	1.54
Choice-Choice-Plus-177	Choice	Choice (60)	Plus (120)	177	2.06
Choice-Choice-Plus-237	Choice	Choice (60)	Plus (120)	237	1.54
Choice-ONE-F-Plus-271	Choice	ONE-F (60)	Plus (200)	271	1.35
Choice-ONE-F-Plus-317	Choice	ONE-F (60)	Plus (200)	317	1.15
Plus-Plus-Plus-177	Plus	Plus (60)	Plus (120)	177	2.06
Plus-Plus-Plus-237	Plus	Plus (60)	Plus (120)	237	1.54

Appendix 8. Example Calculation for Daily Excretion Rate

Daily excretion rates of estradiol and trenbolone residues in manure from release of TB and TBA from individual Synovex implants can be expressed as grams per hectare. Daily release rates for trenbolone and estradiol residues from individual Synovex implants are calculated by substituting the daily excretion rate of estradiol or trenbolone from Table 10 or Table 11 into this calculation.

An example calculation for the daily trenbolone release rate from Synovex Plus is shown below.

Daily excretion rate per animal unit (from Table 10 or Table 11)	=	$1.4777 \frac{mg}{head}$
Daily excretion rate per area	=	$1.4777 \frac{mg}{head} \times 270 \frac{head}{ac}$
	=	$398.979 \frac{mg}{ac}$
	=	$398.979 \frac{mg}{ac} \times 0.001 \frac{g}{mg} \times 2.47105 \frac{ac}{ha}$
	=	$0.9859 \frac{g}{ha}$

Appendix 9. Alterations to SynovexPRZM

Appendix 9.1. Alterations to PRZM for Feedlot and Pasture

Feedlot Model:

The winPRZM model was modified to simulate the release of veterinary medicines in manure excreted from livestock in animal feeding operations (AFOs) and confined animal feeding operations (CAFO). The model can accommodate different schedules and dose rates of animal treatment, accumulation of manure and pharmaceutical residues in the feedlot over time, and the removal of manure from feedlots from periodic scraping. User inputs include the date, dose, and payout period in which residues are released from the animal; the amount of manure generated each day on a feedlot based on stocking density and fresh manure bulk density); and the amount of medicine excreted in manure each day (or application rate), which depends on implant release rate and stocking density, implant interval and scraping schedule.

Using a daily time step, the model calculates the buildup of manure based on daily rate of excretion. Pharmaceutical mass in the daily addition of manure is mixed with pre-existing residue remaining from the previous time steps. Residues are uniformly mixed in the manure pile to account for the mixing that occurs from cattle movement in the feedlot.

The amount of residue available for runoff is function of depth is based on the runoff extraction model in PRZM and winPRZM (Figure 1 of Appendix 9.1). That is, a greater fraction of the residue in the surface of the manure pile will be extracted during a runoff event than those deeper in the manure pile. Mixing occurs in the active (upper 10 cm) of the manure pile. Once the manure pile exceeds 10 cm, residues below 10 cm are buried and not available for mixing. After that point in time, a depth of manure equal to the daily addition is buried and the daily addition of vet med mass is mixed to the active manure pile.

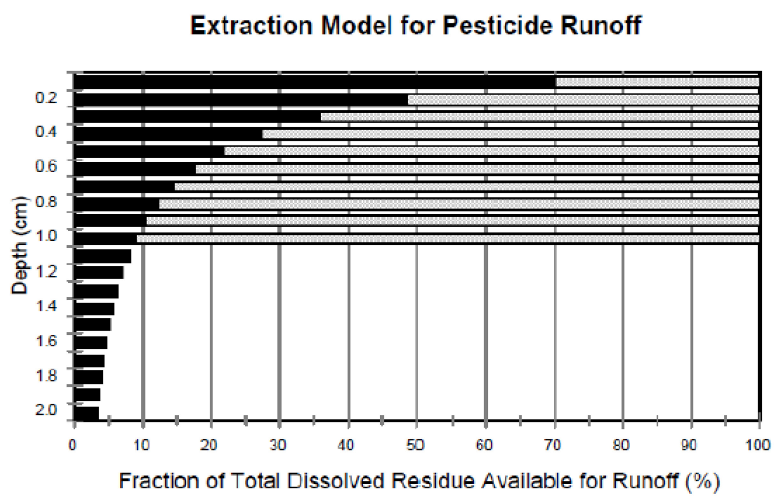


Figure 1. **Residue availability in runoff as a function of manure (source: Suárez, 2006)**

Pasture Model:

Changes were made to the application routine for SynovexPRZM to simulate application of chemical on pasture surface for a user-specified period that animals are out to pasture. To incorporate such a scenario, daily applications of chemical will occur on pasture surface between a user supplied start and stop date in the input file.

For both the Feedlot and Pasture models, the amount of pharmaceutical loss in eroded manure is predicted is based on the U.S. Department of Agriculture's APEX model [43].

Cropland Model:

Cropland scenarios did not require code modifications. The user specifies the dates and rates during the year that manure is applied to cropland.

Appendix 9.2. Alterations for Synovex PRZM Source Code

Changes from PRZM including those applied in the 2014 EA and this EA are marked in red text. All changes were made to construct the buildup model that addresses overlapping release of TBA and EB from more than one Synovex implant product.

Changes to RDPRZM Subroutine in RSINP2.FOR

```
RECORD 13
1010 FORMAT(10I8)
 READ(MESAGE,1010,END=910,ERR=920) NAPS,NCHEM,FRMFLG,DK2FLG,MANCPT

 Multiple FRMFLG options added:
 FRMFLG=5, Feedlot-Constant soil concentration
 FRMFLG=6, Pasture
 FRMFLG=7, Obsolete, Superceded by FRMFLG 8
 FRMFLG=8, Feedlot-Manure mixing zone

 MANCPT: Manure mixing zone entered
  as number of manure (soil) compartments FRMFLG=8)

 Note: NAPS should be set to 1 for FRMFLG=5

           DEPI transforms when FRMFLG=8 to signify the depth
added/subtracted per day of manure

RECORD 18.2 IF((FRMFLG.EQ.5).OR.(FRMFLG.EQ.6).OR.
  (FRMFLG.EQ.7).OR.(FRMFLG.EQ.8))THEN
 1010 FORMAT(10I8)
 READ(MESAGE,1010,END=910,ERR=920) NCLND

 NCLND: Number of scraping events (total for sn)

RECORD 18.5 IF((FRMFLG.EQ.5).OR.(FRMFLG.EQ.6).OR.
  (FRMFLG.EQ.7).OR.(FRMFLG.EQ.8))THEN
 READ(MESAGE,'(2X,3I2,I8,F8.0)',END=910,ERR=92
 1 CPD,CPM,CLNYR(I),CLNCMP(I),CLNPCT(I) (re 1 to NCLND)

 CPD: Clean Day
 CPM: Clean Month
 CLNYR: Clean Year
 CLNCMP: Number of manure compartments to remoLG 5,7,8)
 CLNPCT: Percent removal of mass from compartmFLG 5,7,8)
```

RSPRZ1.FOR

```
 SUBROUTINE PRZM
 I   (RSTFG, NUMFIL, MCARLO, SEPTON, NITRON,
 I   MODID, RSDAT, REDAT, LPRZRS,
 I   LPRZOT, LPRZIN, LWDMS,
 I   LMETEO, LSPTIC, LNITAD, LIRRG1,LHRMET,
 I   LTMSRS, SRNFG, BASEND, IPRZM, ITSAFT, NLDLT)
C
C + + + PURPOSE + + +
C called by EXESUP to execute PRZM
C Modification date: 2/18/92 JAM
```

```
C
cwinter
c 2
 USE WINTERACTER
 TYPE(WIN_MESSAGE) MESSAGE
C
cwinter
c 1
 include 'resource.inc'
 INCLUDE 'CDAYS.INC'
C
C + + + DUMMY ARGUMENTS + + +
 INTEGER SRNFG,BASEND,RSTFG,NUMFIL,IPRZM,ITSAFT,NLDLT,
 1  KLIN,DEPICNT
 INTEGER RSDAT(3),REDAT(3),LPRZRS,LPRZOT,LIRRG1,LHRMET,
 1  LPRZIN,LMETEO,LSPTIC,LNITAD,LTMSRS,LWDMS,K1
 LOGICAL MCARLO,SEPTON,NITRON,APPLY
 CHARACTER*3 MODID(NUMFIL)
 REAL CURVN,DDLN
 INTEGER*4 RODPTH
C
C + + + ARGUMENT DEFINITIONS + + +
C RSTFG - restart starting flag
C NUMFIL - max. number of open files
C MCARLO - flag for Monte Carlo on
C SEPTON - septic effluent on flag
C NITRON - nitrogen modeling on flag
C MODID - model id (pest,conc,water)
C RSDAT - restart starting date
C REDAT - restart ending date
C LPRZRS - unit number for przm restart file
C LPRZOT - unit number for przm output file
C LPRZIN - unit number for przm input file
C LMETEO - unit number for meteorlogical file
C LSPTIC - unit number for septic effluent file
C LNITAD - unit number for nitrogen atmospheric deposition
C LTMSRS - unit number for time series file
C LWDMS - unit number for WDM file
C SRNFG - starting run flag
C BASEND - base node for PRZM
C IPRZM - current przm zone
C ITSAFT - current time step
C NLDLT - maximum days in a time step (31)
C
C + + + PARAMETERS + + +
C
 INCLUDE 'PPARM.INC'
 INCLUDE 'PMXPDT.INC'
 INCLUDE 'PMXNSZ.INC'
 INCLUDE 'PMXZON.INC'
C
C + + + COMMON BLOCKS + + +
C
 INCLUDE 'CMET.INC'
 INCLUDE 'CMISC.INC'
 INCLUDE 'CVMISC.INC'
 INCLUDE 'CPRZST.INC'
```

```
 INCLUDE 'CHYDR.INC'
 INCLUDE 'CPEST.INC'
 INCLUDE 'CCROP.INC'
 INCLUDE 'CIRGT.INC'
 INCLUDE 'CECHOT.INC'
 INCLUDE 'CPTAP.INC'
 INCLUDE 'CFILEX.INC'
 INCLUDE 'CBIO.INC'
 INCLUDE 'EXAM.INC'
 INCLUDE 'CNITR.INC'
C
C + + + LOCAL VARIABLES + + +
C
 INTEGER J,I,LDAY,FDAY,JP1,MNTHP1,EYRFG,
 1  K, NMCDAY,LPAD,elpsed,ITYPE,CLNPAD,P,QQ
 INTEGER FLPS,FLCN
 REAL  ATEMP(2),PWIND(2),R0,pctot(3),s2tot(3),
 1  OLDKH(NCMPTS),ZCH,URH,ZRH,TOTCR
 REAL*8 DKBIO(3,NCMPTS),PP2
 CHARACTER*4 YEAR,MNTH,DAY,CONC
 INTEGER ILDLT,IERROR,isim1
 LOGICAL MCTFLG,IRDAY,FATAL
 CHARACTER*80 MESAGE
C
C + + + INTRINSICS + + +
C
 INTRINSIC MOD
C
C + + + EXTERNALS + + +
C
 EXTERNAL SUBIN,RSTGET,RSTGT1,KHCORR,ACTION,GETMET,PLGROW
 EXTERNAL IRRIG,HYDROL,EVPOTR,HYDR1,HYDR2,EROSN,SLTEMP,FARM
 EXTERNAL PZSCRN,PESTAP,PLPEST,CANOPY,BIODEG,SLPST0,SLPST1
 EXTERNAL MOC,MASBAL,OUTCNC,OUTRPT,OUTPST,OUTHYD,OUTTSR
 EXTERNAL MCPRZ,RSTPUT,RSTPT1,SUBOUT,PRZEXM,ERRCHK
 EXTERNAL SEPTIN,NITR,NITRAP,NITBAL,OUTCNI,OUTNIT,ZIPR
C
C + + + DATA INITIALIZATIONS + + +
C
 DATA YEAR /'YEAR'/
 DATA MNTH /'MNTH'/
 DATA DAY /' DAY'/
 DATA CONC /'CONC'/
C
C + + + OUTPUT FORMATS + + +
2000 FORMAT('Application [',I3,'] chem [',I1,
 1 '] on julday [',I3,'] year [',I2,'] zone [',I2,']')
2001 FORMAT('Application [',I3,'] chem [',I1,
 1 '] on julday [',I3,'] year [',I2,'] zone [',I2,']')
2002 FORMAT('ERROR, Application [',I3,'] failed ideal soil conditions')
2010 FORMAT('Nitrogen application [',I3,'] on julday [',I3,'] year [',
 $ I2,'] zone [',I2,']')
2020 FORMAT('ERROR, Nitrogen application [',I3,'] failed ideal soil ',
 $ 'conditions')
C
C + + + END SPECIFICATIONS + + +
C
```

```
 R0 = 0.0
 APPLY = .FALSE.
 MESAGE = 'PRZM'
 CALL SUBIN(MESAGE)
C get unit numbers used for input and output
 FLPS= LPRZOT
 FLCN= LPRZOT
C
C in restart mode
 IF(IPRZM.NE.1)THEN
 CALL RSTGET (LPRZRS,IPRZM)
 CALL RSTGT1 (RSTFG,LPRZRS,IPRZM)
 ENDIF
C
C use dates passed as input rather than on input file
 ISTYR = RSDAT(1)
 ISMON = RSDAT(2)
 ISDAY = RSDAT(3)
 IEYR = REDAT(1)
 IEMON = REDAT(2)
 IEDAY = REDAT(3)
C
C check temperature simulation flag
 IF ((ITFLAG .EQ. 1).or.(ITFLAG .EQ. 2)) THEN
 DO 178 K=1,NCHEM
  CALL KHCORR(SPT,HENRYK(K),ENPY(K),NCOM2,OLDKH)
  DO 177 I=1,NCOM2
  OKH(K,I) = OLDKH(I)
177 CONTINUE
178 CONTINUE
 ELSE
 DO 189 K=1,NCHEM
  DO 188 I=1,NCOM2
  OKH(K,I) = HENRYK(K)
  KH(K,I) = HENRYK(K)
188 CONTINUE
189 CONTINUE
 ENDIF
C
 IF(KDFLAG.EQ.3)CALL AGEKD
C
 NMCDAY = (ITSAFT-1)*NLDLT
 DO 200 IY=ISTYR,IEYR
 IF (MOD(IY,4) .NE. 0 .OR. MOD(IY,100) .EQ. 0) THEN
  LEAP=1
  LDAY=365
 ELSE
  LEAP=2
  LDAY=366
 ENDIF
 IF (IY .EQ. IEYR) LDAY=IEDAY+CNDMO(LEAP,IEMON)
C
 FDAY=1
 IF (IY .EQ. ISTYR)THEN
  FDAY=ISDAY+CNDMO(LEAP,ISMON)
 ENDIF
C
```

```
 EYRFG = 0
C
C counter for VADOFT link
 ILDLT = 0
C set input accumulator for GLOMAS
CJMC determine time period for each decay rate if DK2FLG=1
 DO 39 K = 1,NCHEM
  IF(DK2FLG.EQ.1)THEN
  DKSTRT(K)=DKDAY(K)+CNDMO(LEAP,DKMNTH(K))
  DKEND(K)=DKSTRT(K)+DKNUM(K)
  IF(DKEND(K).GT.365)DKEND(K)=DKEND(K)-LDAY
  ENDIF
  PTAP(K) = 0.
 39 CONTINUE
C
C begin daily loop
 DO 100 JULDAY=FDAY,LDAY
  NMCDAY = NMCDAY + 1
  ILDLT = ILDLT + 1
  IF (JULDAY .EQ. LDAY) THEN
  EYRFG = 1
  ENDIF
  IF (JULDAY.EQ.1) DAYCNT = 0
  DAYCNT = DAYCNT + 1
  rngcnt=rngcnt+1
  elpsed=int((float(rngcnt)/float(dycnt))*100.)
cwinter
c 1
  call wdialogputprogressbar(idf_progress1,elpsed,0)
  if(itype.eq.3)then
  close(156)
  close(157)
  close(158)
cwinter
c 1
  call iosdeletefile('*.cnc')
  stop
  endif
cwinter
c 4
  call wmessagepeek(itype,message)
  if(itype.ne.NoMessage)then
  call wdialogshow(-1,-1,0,semimodeless)
  endif
  DO 40 J=1,12
  JP1= J+ 1
  IF (JULDAY.GT.CNDMO(LEAP,J) .AND.
 1  JULDAY.LE.CNDMO(LEAP,JP1)) MONTH = J
40 CONTINUE
  DOM=JULDAY-CNDMO(LEAP,MONTH)
  MNTHP1 = MONTH + 1
C
  SSFLAG = 0
  IF (IY.EQ.SAYR .AND. JULDAY.EQ.SAVAL) THEN
C  time for a special action
  CALL ACTION (LPRZIN,LPRZOT,MODID(3))
  END IF
```

```
C  get BUFFER data
   IF((buffbf.eq.1))then
   CALL GETBUF
   ENDIF
C  get met data
   CALL GETMET(
 I IY,JULDAY,MONTH,DOM,LMETEO,LSPTIC,LNITAD,FWDMS,
 I LDAY,RSTFG,NITRON,SEPTON,LIRRG1,LHRMET,
 O RETCOD)
C
   IF(THRFL2.GT.0.0)THEN
   PRECIP=PRECIP+THRFL2
   ENDIF

C  grow some crops
   CALL PLGROW(IRDAY)
C
   APDEP = 0.0
   AINF(1)= 0.0
   THRUFL = 0.0
   IF((IRFILE.EQ.0).AND.(IRTYPE.GT.0).AND.
 *  (IRNONE.NE.4).AND.(RZI.EQ.1))THEN
C  need to do irrigation
   IRRR=0.0
   CALL IRRIG
   ELSEIF(IRFILE.EQ.0)THEN
   IF (IRNONE .EQ. 4) THEN
   IF (IRDAY) THEN
   IRRR=0.0
   CALL IRRIG
   ELSE
   GOTO 555
   ENDIF
   ENDIF
   ENDIF
C
C  calculate surface hydrology factors
C
555 CONTINUE
   IF(IRFILE.EQ.0)THEN
   CALL HYDROL (LPRZOT,MODID(3),RODPTH,CURVN)
   ELSEIF((IRFILE.EQ.1).or.(IRFILE.EQ.2))THEN
   CALL HYDROL2 (LPRZOT,MODID(3),RODPTH,CURVN)
   ENDIF
C
C  calculate et
   IF (IPEIND.LE.2)THEN
   CALL EVPOTR
   ELSEIF ((IPEIND.GT.2).AND.(IPEIND.LT.7))THEN
   CALL EVPOTR2
   ELSEIF (IPEIND.GE.7)THEN
   CALL EVPOTR3
   ENDIF
C
   IF (HSWZT .EQ. 0) THEN
C  hydraulics with unrestricted drainage
   CALL HYDR1
```

```
  ELSEIF (HSWZT .EQ. 1) THEN
C  hydraulics with restricted drainage
  CALL HYDR2
  ELSEIF (HSWZT .EQ. 2) THEN
  isim1=0
C  hydraulics with restricted drainage
  CALL HYDR3(isim1)
  ENDIF
C
  IF (DSPFLG .EQ. 1) THEN
  CALL DSPINIT
  ENDIF
C
  IF (SEPTON) THEN
C  introduce septic effluent into soil column
  CALL SEPTIN
  END IF
C
  if((buffbf.eq.1))THEN
  CALL APPBUF
  ENDIF
C
  IF(ERFLAG.GT.0)THEN
  IF(LEAP.EQ.1)THEN
  IF(UCFLG.EQ.0)THEN
  CFAC=USLEC(NCROP,IUSLEC)
  N1=MNGN(NCROP,IUSLEC)
  IF(JULDAY.EQ.JUSLEC(NCROP,IUSLEC))UCFLG=2
  ISCOND=IUSLEC
  IF(UCFLG.EQ.2)IUSLEC=IUSLEC+1
  ELSEIF(UCFLG.EQ.1)THEN
  CFAC=USLEC(NCROP,IUSLEC)
  N1=MNGN(NCROP,IUSLEC)
  IF(JULDAY.EQ.JUSLEC(NCROP,IUSLEC))UCFLG=2
  ISCOND=IUSLEC
  IF(UCFLG.EQ.2)IUSLEC=IUSLEC+1
  ELSE
  IF(JULDAY.EQ.(JUSLEC(NCROP,IUSLEC)))THEN
   CFAC=USLEC(NCROP,IUSLEC)
   N1=MNGN(NCROP,IUSLEC)
   ISCOND=IUSLEC
   IUSLEC=IUSLEC+1
   IF(IUSLEC.GT.NUSLEC(NCROP))IUSLEC=1
  ENDIF
  ENDIF
  ELSE
  LPAD=0
  IF(UCFLG.EQ.0)THEN
  CFAC=USLEC(NCROP,IUSLEC)
  N1=MNGN(NCROP,IUSLEC)
  IF(JULDAY.GT.59)LPAD=1
  IF(JULDAY.EQ.JUSLEC(NCROP,IUSLEC)+LPAD)UCFLG=2
  ISCOND=IUSLEC
  ELSEIF(UCFLG.EQ.1)THEN
  CFAC=USLEC(NCROP,IUSLEC)
  N1=MNGN(NCROP,IUSLEC)
  IF(JULDAY.GT.59)LPAD=1
```

```
  IF(JULDAY.EQ.JUSLEC(NCROP,IUSLEC)+LPAD)UCFLG=2
  ISCOND=IUSLEC
  ELSE
  IF(JULDAY.GT.59)LPAD=1
  IF(JULDAY.EQ.(JUSLEC(NCROP,IUSLEC))+LPAD)THEN
   CFAC=USLEC(NCROP,IUSLEC)
   N1=MNGN(NCROP,IUSLEC)
   ISCOND=IUSLEC
   IUSLEC=IUSLEC+1
   IF(IUSLEC.GT.NUSLEC(NCROP))IUSLEC=1
  ENDIF
  ENDIF
  ENDIF
  ENDIF
c
  SEDL= 0.0
  ELTT= 0.0
  IF (RUNOF .GT. 0.0 .AND. ERFLAG .GE. 1) THEN
C  calc loss of chem due to erosion
  CALL EROSN
  END IF
CJMC
  IF(DK2FLG.EQ.1)THEN
  CALL DKINIT
  ELSEIF(DK2FLG.EQ.2)THEN
  CALL HUCALC
  ELSEIF(DK2FLG.EQ.3)THEN
  ENDIF
CJMC
C
  IF (NITRON) THEN
C  perform nitrogen simulation
  CALL SLTEMP (LPRZOT,MODID(3))
  CALL ZIPR (3*NCOM2,R0,SOILAP)
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND. IY.EQ.IAPYR(NAPPC)) THEN
C  need to perform ag nitrogen application
  IF ((FRMFLG .GE. 1).AND.(FRMFLG.LE.3)) THEN
C  check for appropriate soil moisture
  CALL FARM (RODPTH,APPLY,CURVN)
  IF (APPLY) THEN
C   make ag nitrogen application
   WRITE(MESAGE,2010) NAPPC,IAPDY(NAPPC),
 $     IAPYR(NAPPC),IPRZM
   CALL PZSCRN(1,MESAGE)
   CALL NITRAP (FECHO)
   NAPPC= NAPPC+ 1
   WIN = 0
  ELSE
C   soil moisture not right for application, try again tomorrow
   WIN = WIN + 1
   IF (WIN .GT. WINDAY(NAPPC)) THEN
C   beyond window of opportunity
   WRITE(MESAGE,2020) NAPPC
   IERROR= 2150
   FATAL = .TRUE.
   CALL ERRCHK(IERROR,MESAGE,FATAL)
   ELSE
```

```
C   try to apply tomorrow
   IAPDY(NAPPC) = IAPDY(NAPPC) + 1
   ENDIF
  ENDIF
  ELSE
  WRITE(MESAGE,2010) NAPPC,IAPDY(NAPPC),
 $    IAPYR(NAPPC),IPRZM
  CALL PZSCRN(1,MESAGE)
  CALL NITRAP (FECHO)
  NAPPC= NAPPC+ 1
  ENDIF
  ENDIF
  IF (MCARLO) THEN
  CALL NITR (IY,MONTH,DOM,FECHO,IPRZM,MODID(13))
  ELSE
  CALL NITR (IY,MONTH,DOM,LPRZOT,IPRZM,MODID(13))
  END IF
C  perform mass balance for nitrogen constituents
  CALL NITBAL (APDEP,IPRZM)
  IF (ECHOLV .GE. 3) THEN
  CALL OUTHYD (LPRZOT,LTMSRS,MODID(3),MODID(5),SEPTON)
  IF (ITEM3 .EQ. CONC .AND. (STEP3 .EQ. DAY .OR. (STEP3
 1  .EQ. MNTH .AND. JULDAY .EQ. CNDMO(LEAP,MNTHP1)) .OR.
 2  (STEP3 .EQ. YEAR .AND. JULDAY .EQ. CNDMO(LEAP,13)))
 3  .AND. FLCN.GT.0) CALL OUTCNI (LPRZOT,MODID(6))
  CALL OUTNIT (FLPS,MODID(13),SEPTON)
  IF (NPLOTS .GT. 0) THEN
C  output time-series
  HEADER = HEADER + 1
  IF (HEADER .EQ. 1) SRNFG = 1
  CALL OUTTSR (SRNFG,EYRFG,LPRZOT,LTMSRS,LWDMS,
 I   MODID(3),MODID(5))
  ENDIF
  ENDIF
C  store PRZM nitrogen fluxes for vadoft, start w/ammonia
  PRZMPF(IPRZM,ILDLT,1) = PRZMPF(IPRZM,ILDLT,1) +
 $    NCFX2(BASEND,1)/1.0E5
C  nitrate
  PRZMPF(IPRZM,ILDLT,2) = PRZMPF(IPRZM,ILDLT,2) +
 $    NCFX4(BASEND,1)/1.0E5
C  combine the two organic species
  PRZMPF(IPRZM,ILDLT,3) = PRZMPF(IPRZM,ILDLT,3) +
 $    (NCFX13(BASEND,1) +
 $    NCFX15(BASEND,1))/1.0E5
  ELSE
C  perform pesticide simulation
  DO 1000 J=1,NCOM2
  SRCFLX(1,J)=0.0
  SRCFLX(2,J)=0.0
  SRCFLX(3,J)=0.0
  DKFLUX(1,J)=0.0
  DKFLUX(2,J)=0.0
  DKFLUX(3,J)=0.0
  TRFLUX(1,J)=0.0
  TRFLUX(2,J)=0.0
  TRFLUX(3,J)=0.0
1000 CONTINUE
```

```
C
C Begin Chemical Loop
C
  DO 95 K=1, NCHEM
  ELTERM(K) = ELTT*FEQ(K,1)*KD(K,1)
  DO 74 I=1,NCOM2
  SOILAP(K,I) = 0.0
  DKBIO(K,I) = 0.0
74  CONTINUE
C
CJMC
  IF (((ITFLAG .EQ. 1).OR.(ITFLAG.EQ.2)).AND.
 *  (QFAC(K).GT.0.0)) THEN
  IF (K .EQ. 1) CALL SLTEMP (LPRZOT,MODID(3))
  IF(K.EQ.1)CALL Q10DK
  CALL KHCORR (SPT,HENRYK(K),ENPY(K),NCOM2,OLDKH)
  DO 75 I=1, NCOM2
   KH(K,I) = OLDKH(I)
75  CONTINUE
  ELSEIF (((ITFLAG .EQ. 1).OR.(ITFLAG.EQ.2)).AND.
 *  (QFAC(K).LE.0.0)) THEN
  IF (K .EQ. 1) CALL SLTEMP (LPRZOT,MODID(3))
  CALL KHCORR (SPT,HENRYK(K),ENPY(K),NCOM2,OLDKH)
  DO 76 I=1, NCOM2
   KH(K,I) = OLDKH(I)
76  CONTINUE
  IF((DK2FLG.EQ.1).AND.(K.EQ.1))CALL DKINIT
  ELSEIF(DK2FLG.EQ.1)THEN
  IF(K.EQ.1)CALL DKINIT
  ENDIF
CJMC
C
  PLNTAP(K) = 0.0
C
  IF(DK2FLG.EQ.2)THEN
  IF((HU_ACCUM(K).GE.HUTARGET(K)).AND.(HUFLG(K).EQ.1))THEN
   WRITE(187,'(I3,1X,I2,1X,53I8)')IAPDY(NAPPC-1),
 *     IY,HU_ACCUM(K),NHORIZ,
 *   (INT(FLOAT(HUCNT(K))*DEGFAC(J)),J=1,NHORIZ)
   HUFLG(K)=0
   HUCNT(K)=0
  ELSEIF(HUFLG(K).EQ.1)THEN
   HU_ACCUM(K)=HU_ACCUM(K)+INT(HU2)
   HUCNT(K)=HUCNT(K)+1
  ENDIF
  ENDIF
c
  IF ((FRMFLG .EQ. 6)) THEN
  IF(JULDAY.EQ.IAPDY(NAPPC+1).AND.(NCLNC.NE.NAPPC))THEN
   NAPPC= NAPPC+ 1
  ENDIF
  ENDIF
c
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND. IY.EQ.IAPYR(NAPPC))THEN
  ttapp=0
  IF ((FRMFLG .GE. 1).AND.(FRMFLG.LE.3)) THEN
C   added new statement for farm option -jam 4/24/91
```

```
   CALL FARM (RODPTH,APPLY,CURVN)
   IF (APPLY) THEN
   AOFF(K)=0
   WRITE(MESAGE,2000) NAPPC,K,IAPDY(NAPPC),
 $     IAPYR(NAPPC),IPRZM
   CALL PZSCRN(1,MESAGE)
   CALL PESTAP(K)
   PTAP(K) = PTAP(K) +
 *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
   ENDIF
  ELSEIF ((FRMFLG .EQ. 4)) THEN
   AOFF(K)=0
   WRITE(MESAGE,2001) NAPPC,K,IAPDY(NAPPC),
 $    IAPYR(NAPPC),IPRZM
   CALL PZSCRN(1,MESAGE)
   if((julday.eq.1).and.(fdfrmflg.eq.0))then
   CALL PESTAP(K)
   fdfrmflg=1
   elseif(tapp(k,nappc).gt.0.0)then
   DO I=1,NCMPTS
   soilap(k,i)=soilap2(k,i)
   pestr(k,i) = pestr(k,i)+
 1   (SOILAP(k,i)/(DELX(i)*theto(i)))
   SPESTR(k,i) = (pcncx(k,i)+
 1   (SOILAP(k,i)/(DELX(i)*THETO(i))))*
 1   (THETO(i)/(THETO(i)
 1   +feq(k,i)*KD(k,i)*BD(i)
 1   +(THETAS(i)-THETO(i))*KH(k,i)))
   soilap2(k,i)=0.0
   ENDDO
   endif
C   global mass balance
   PTAP(K) = PTAP(K) +
 *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
   NAPPC= 1
  ELSEIF ((FRMFLG .EQ. 5)) THEN
   AOFF(K)=0
   WRITE(MESAGE,2001) NAPPC,K,IAPDY(NAPPC),
 $    IAPYR(NAPPC),IPRZM
   CALL PZSCRN(1,MESAGE)
   CALL PESTAP(K)
C   global mass balance
   PTAP(K) = PTAP(K) +
 *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
   NAPPC= 1
  ELSEIF ((FRMFLG .EQ. 6).OR.(FRMFLG .EQ. 7)) THEN
   AOFF(K)=0
   WRITE(MESAGE,2001) NAPPC,K,IAPDY(NAPPC),
 $    IAPYR(NAPPC),IPRZM
   CALL PZSCRN(1,MESAGE)
   CALL PESTAP(K)
C   global mass balance
   PTAP(K) = PTAP(K) +
 *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
  ELSE
   AOFF(K)=0
   WRITE(MESAGE,2001) NAPPC,K,IAPDY(NAPPC),
```

```
 $    IAPYR(NAPPC),IPRZM
   CALL PZSCRN(1,MESAGE)
   CALL PESTAP(K)
C   global mass balance
   PTAP(K) = PTAP(K) +
 *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
  ENDIF
  IF(DK2FLG.EQ.2)THEN
   HU_ACCUM(K)=0
   HUFLG(K)=1
  ELSEIF(DK2FLG.EQ.3)THEN
   CALL HU_T12UPDATE(K)
  ENDIF
  ELSEIF ((FRMFLG .EQ. 8)) THEN
   ttapp=1
   CALL PESTAP(K)
  ENDIF
C
c jmc 6/17/96 fam=2 signifies that some applications were foliar
  IF (FAM.EQ.2)then
  if(ptrflg.eq.0)then
   CALL PLPEST(K)
  elseif(ptrflg.eq.1)then
   CALL PLPEST2(K)
  endif
  endif
C
  CNDBDY(K) = DAIR(K)/0.5
  CONDUC(K) = CNDBDY(K)
C
C  When canopy develops, resistance type approach is used
C  to estimate the volatilization flux and concentration
C  retains in the canopy
C
  IF (HEIGHT .GT. 5.0) THEN
  ZCH = HEIGHT/100.0
  IF (ITFLAG .EQ. 0) THEN
   ATEMP(1)= 15.0
  ELSE
   ATEMP(1)= UBT
  ENDIF
  ATEMP(2)= TEMP
  PWIND(1)= 0.0
  PWIND(2)= WIND*36.0*24.0
  URH = PWIND(2)
  IF ((ITFLAG .EQ. 1).or.(ITFLAG .EQ. 2)) THEN
   ZRH= ZWIND
  ELSE
   ZRH= 2.0
  ENDIF
C
C  CONDUC was being calculated after the following
C  if then statement. It should be calculated right
C  after the call CANOPY statement. Change made by
C  PV @ AQUA TERRA Consultants, 10/93
C
  IF(HENRYK(K).GT.0.0.AND.URH.GT.0.0)THEN
```

```
    CALL CANOPY(ATEMP,PWIND,ZRH,ZCH,URH,TOTCR,CRCNC)
    CONDUC(K) = 1.0 / (1.0/CNDBDY(K) + TOTCR)
   ELSE
    TOTCR=0.0
   ENDIF
C   CONDUC(K) = 1.0 / (1.0/CNDBDY(K) + TOTCR)
   ENDIF
C
C   Include calls to biodegradation subroutines here
C
   IF (BIOFLG .EQ. 1) THEN
   CALL BIODEG(K,DKBIO)
   ENDIF
C
C   end of biodegradation
C
   IF ((MCFLAG.EQ.0.OR.MCFLAG.EQ.3.OR.VLFLAG.EQ.0)
  1   .AND.(MCFLAG.NE.2)) THEN
   CALL SLPST0 (LPRZOT, MODID(3), K, DKBIO)
   ELSE
   IF(MCFLAG.EQ.1)THEN
    CALL MOC(K)
    CALL SLPST1 (LPRZOT,MODID(3),K, DKBIO)
   ELSEIF(MCFLAG.EQ.2)THEN
    CALL SLPST3 (LPRZOT,MODID(3),K, DKBIO)
   ENDIF
   END IF
C
C
C   calculate correction for dissolved to total solute conc.
   CALL MASBAL (APDEP,K,IPRZM,RODPTH)
C
   CALL FCSCNC(K)
   CALL FCSMSB(K)
   CALL FCSHYD(K)
   CALL FCSSOILCNC(IY,MONTH,DOM,K)
C
   IF (MCOFLG .EQ. 0 .AND. ECHOLV .GE.3) THEN
   if(mcflag.ne.3)then
    IF (ITEM3 .EQ. CONC .AND.
  1   (STEP3 .EQ. DAY .OR.(STEP3 .EQ. MNTH .AND.
  1   JULDAY .EQ. CNDMO(LEAP,MNTHP1)) .OR.
  2   (STEP3 .EQ. YEAR .AND.
  2   JULDAY .EQ. CNDMO(LEAP,13))).AND. FLCN.GT.0)then
    CALL OUTCNC (LPRZOT,MODID(6),K)
    ENDIF
C    Determine if a write to files MODOUT.DAT
C    or SNAPSHOT.DAT is required
    CALL OUTRPT (LPRZOT,MODID(7),MODID(8),K)
   elseif(mcflag.eq.3)then
    IF (ITEM3 .EQ. CONC .AND.
  1   (STEP3 .EQ. DAY .OR.(STEP3 .EQ. MNTH .AND.
  1   JULDAY .EQ. CNDMO(LEAP,MNTHP1)) .OR.
  2   (STEP3 .EQ. YEAR .AND.
  2   JULDAY .EQ. CNDMO(LEAP,13))).AND. FLCN.GT.0)then
    CALL OUTCNC2 (LPRZOT,MODID(6),K)
    ENDIF
```

```
  endif
C
  ENDIF
C
  IF (ECHOLV .GE. 3) THEN
  IF (K .EQ. 1) CALL OUTHYD (
 I    LPRZOT,LTMSRS,MODID(3),MODID(5),SEPTON)
  if(mcflag.ne.3)then
   CALL OUTPST (FLPS,MODID(4),K)
  elseif(mcflag.eq.3)then
   IF (K .EQ. NCHEM) THEN
   CALL OUTPST2(FLPS,MODID(4),K)
   endif
  endif
  ENDIF
  PRZMPF(IPRZM,ILDLT,K) = PRZMPF(IPRZM,ILDLT,K) +
 1  DFFLUX(K,BASEND) + ADFLUX(K,BASEND)
CPRH  DAFLUX(IPRZM,1,ILDLT,K) = DFFLUX(K,1) + ADFLUX(K,1) +
CPRH 1  PVFLUX(K,1)
C
  pctot(k)=0.0
  s2tot(k)=0.0
  DO 90 I=1,NCOM2
CPRH  DAFLUX(IPRZM,I+1,ILDLT,K) = DFFLUX(K,I) + ADFLUX(K,I) +
CPRH 1  PVFLUX(K,I)
  SPESTR(K,I)=sngl(X(I))
C  store SPESTR for this zone (for use w/ MASCOR)
  PESTR(K,I)=((SPESTR(K,I)*(THETN(I)+
 *   FEQ(K,I)*KD(K,I)*BD(I)+
 *   (THETAS(I)-THETN(I))*KH(K,I)))/THETN(I))+
 *   (s2(k,i)*BD(i))/thetn(i)
  pcncx(k,i)=(((spestr(k,i)*(THETN(I)+
 *   FEQ(K,I)*KD(K,I)*BD(I)+
 *   (THETAS(I)-THETN(I))*KH(K,I))))/thetn(i))
90  CONTINUE
C
C  last value of DAFLUX and ZPESTR is same as
C  in last compartment
CPRH  DAFLUX(IPRZM,NCOM2+2,ILDLT,K) = DFFLUX(K,NCOM2) +
CPRH 1  ADFLUX(K,NCOM2) + PVFLUX(K,NCOM2)
C
  IF (NPLOTS .GT. 0 .AND. K .EQ. NCHEM) THEN
   HEADER = HEADER + 1
   IF (HEADER .EQ. 1) SRNFG = 1
  IF(ECHOLV .GE.3)then
   if(mcflag.ne.3)then
   CALL OUTTSR
 1  (SRNFG,EYRFG,LPRZOT,LTMSRS,LWDMS,MODID(3),MODID(5))
   elseif(mcflag.eq.3)then
   CALL OUTTSR2
 1  (SRNFG,EYRFG,LPRZOT,LTMSRS,LWDMS,MODID(3),MODID(5))
   endif
  ENDIF
  ENDIF
C
C  new code added for EXAMS
  IF (ERFLAG.GT.0 .AND. IPRZM.EQ.1) THEN
```

```
  IF ((EXMFLG.GT.0) .AND. (K.EQ.NCHEM))then
   if(mcflag.ne.3)then
   CALL PRZEXM(K)
   elseif(mcflag.eq.3)then
   K1=1
   CALL PRZEXM2(K1)
   endif
  endif
  ENDIF
C  end of code added for EXAMS
C
C  new code added for ADAM
   IF ((ADMFLGON.GT.0) .AND. (K.EQ.1)) CALL PRZADM(K)
C
  SRNFG = 0
  IF ((ITFLAG .EQ. 1).or.(ITFLAG .EQ. 2)) THEN
  DO 92 I=1,NCOM2
   OKH(K,I) = KH(K,I)
92  CONTINUE
  ENDIF
C
  if(frmflg.eq.8)then
  sumdepi=0.0
  summan=0.0
  KLIN = 0
  DDLN = 0.0
115  CONTINUE
   KLIN = KLIN + 1
   DDLN = DDLN + DELX(KLIN)
  IF (DDLN .LT. depi(k,nappc)) GO TO 115
  DEPICNT = KLIN
  do p=1,mancpt
   SPESTR(K,P)=sngl(X(P))
   PESTR(K,P)=((SPESTR(K,P)*(THETN(P)+
 *   FEQ(K,P)*KD(K,P)*BD(P)+
 *    (THETAS(P)-THETN(P))*KH(K,P)))/THETN(P))+
 *    (s2(k,P)*BD(P))/thetn(P)
   summan=summan+pestr(k,p)*delx(p)*thetn(p)
  enddo
  do QQ=(mancpt-depicnt)+1,mancpt
   SPESTR(K,QQ)=sngl(X(QQ))
   PESTR(K,QQ)=((SPESTR(K,QQ)*(THETN(QQ)+
 *   FEQ(K,QQ)*KD(K,QQ)*BD(QQ)+
 *    (THETAS(QQ)-THETN(QQ))*KH(K,QQ)))/THETN(QQ))+
 *    (s2(k,QQ)*BD(QQ))/thetn(QQ)
   sumdepi=sumdepi+PESTR(K,QQ)*delx(qq)*thetn(qq)
  enddo
  if(julday.eq.1)then
  endif
  DO 690 I=1,NCOM2
   x(i)=0.0
   SPESTR(K,I)=sngl(X(I))
C   store SPESTR for this zone (for use w/ MASCOR)
   PESTR(K,I)=((SPESTR(K,I)*(THETN(I)+
 *    FEQ(K,I)*KD(K,I)*BD(I)+
 *    (THETAS(I)-THETN(I))*KH(K,I)))/THETN(I))+
 *     (s2(k,i)*BD(i))/thetn(i)
```

```
   pcncx(k,i)=(((spestr(k,i)*(THETN(I)+
 *     FEQ(K,I)*KD(K,I)*BD(I)+
 *    (THETAS(I)-THETN(I))*KH(K,I))))/thetn(i))
690  CONTINUE
  summan=summan-sumdepi
  endif
95   CONTINUE
C
C End Chemical Loop
C
  IF ((FRMFLG .GE. 1).AND.(FRMFLG.LE.3)) THEN
  IF (APPLY) THEN
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND.
 $    IY.EQ.IAPYR(NAPPC)) THEN
   NAPPC= NAPPC+ 1
   WIN = 0
  ENDIF
  ELSE
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND.
 $    IY.EQ.IAPYR(NAPPC)) THEN
   WIN = WIN + 1
   IF (WIN .GT. WINDAY(NAPPC)) THEN
   WRITE(MESAGE,2002)NAPPC
   IERROR = 2150
   FATAL = .TRUE.
   CALL ERRCHK(IERROR,MESAGE,FATAL)
   ELSE
   IAPDY(NAPPC) = IAPDY(NAPPC) + 1
   ENDIF
  ENDIF
  ENDIF
  ELSEIF ((FRMFLG .EQ. 4)) THEN
  NAPPC= 1
  do i=1,ncmpts
  TAPP(1,1)=tapp(1,1)+soilap2(k,i)
  enddo
  IF(TAPP(1,1).GT.0.0)IAPDY(NAPPC) = JULDAY + 1
  IF (LEAP .EQ. 2 .AND. JULDAY .EQ. 366)THEN
  IF(TAPP(1,1).GT.0.0)IAPDY(NAPPC) = 1
  IAPYR(NAPPC)=IY+1
  ENDIF
  IF (LEAP .EQ. 1 .AND. JULDAY .EQ. 365)THEN
  IF(TAPP(1,1).GT.0.0)IAPDY(NAPPC) = 1
  IAPYR(NAPPC)=IY+1
  ENDIF
  ELSEIF ((FRMFLG .EQ. 5)) THEN
  NAPPC= 1
  IF (JULDAY.EQ.CLNDY(NCLNC) .AND. IY.EQ.CLNYR(NCLNC))THEN
  CLNPAD=2
  CALL FRM5CLN(CLNPAD)
  ELSEIF(JULDAY.EQ.CLNDY(NCLNC)+1
 *   .AND. IY.EQ.CLNYR(NCLNC))THEN
  CLNPAD=1
  CALL FRM5CLN(CLNPAD)
  NCLNC= NCLNC + 1
  ENDIF
  IAPDY(NAPPC) = JULDAY + 1
```

```
 IF (LEAP .EQ. 2 .AND. JULDAY .EQ. 366)THEN
 IAPDY(NAPPC)=1
 IAPYR(NAPPC)=IY+1
 ENDIF
 IF (LEAP .EQ. 1 .AND. JULDAY .EQ. 365)THEN
 IAPDY(NAPPC)=1
 IAPYR(NAPPC)=IY+1
 ENDIF
 ELSEIF ((FRMFLG .EQ. 6)) THEN
 IF (JULDAY.EQ.CLNDY(NCLNC) .AND. IY.EQ.CLNYR(NCLNC))THEN
 TAPP(1,NAPPC)=0.0
 NCLNC=NCLNC+1
 ENDIF
 IF(JULDAY.EQ.IAPDY(NAPPC+1).AND.(NCLNC.NE.NAPPC))THEN
 NAPPC= NAPPC+ 1
 ENDIF
 IAPDY(NAPPC) = JULDAY + 1
 IF (LEAP .EQ. 2 .AND. JULDAY .EQ. 366)THEN
 IAPDY(NAPPC)=1
 IAPYR(NAPPC)=IY+1
 ENDIF
 IF (LEAP .EQ. 1 .AND. JULDAY .EQ. 365)THEN
 IAPDY(NAPPC)=1
 IAPYR(NAPPC)=IY+1
 ENDIF
 ELSEIF ((FRMFLG .EQ. 7).or.(FRMFLG .EQ. 8)) THEN
 IF ((JULDAY.EQ.IAPDY(NAPPC)).AND.(IY.EQ.IAPYR(NAPPC))
*      .and.(pwin.eq.0)) THEN
 PWIN=PWIN+1
 IAPDY(NAPPC) = JULDAY + 1
 ELSEIF((PWIN.NE.WINDAY(NAPPC)).and.(PWIN.NE.0))THEN
 PWIN=PWIN+1
 IAPDY(NAPPC) = JULDAY + 1
 ELSEIF(PWIN.EQ.WINDAY(NAPPC))THEN
 PWIN=0
 NAPPC=NAPPC+1
 ENDIF
 IF (JULDAY.EQ.CLNDY(NCLNC) .AND. IY.EQ.CLNYR(NCLNC))THEN
 summan=0.0
 CLNPAD=2
 CALL FRM5CLN(CLNPAD)
 ELSEIF(JULDAY.EQ.CLNDY(NCLNC)+1
*   .AND. IY.EQ.CLNYR(NCLNC))THEN
 summan=0.0
 CLNPAD=1
 CALL FRM5CLN(CLNPAD)
 NCLNC= NCLNC + 1
 ENDIF
 ELSE
 IF (JULDAY.EQ.IAPDY(NAPPC) .AND. IY.EQ.IAPYR(NAPPC))THEN
 NAPPC= NAPPC+ 1
 IF((buffbf.eq.1))then
  NAPPC= 1
 ENDIF
 ENDIF
 ENDIF
 END IF
```

```
C
C  water flux to EXESUP
  PRZMWF(IPRZM,ILDLT) = PRZMWF(IPRZM,ILDLT) + AINF(BASEND)
C
C  transfer results to Monte Carlo arrays
  IF(MCARLO) THEN
  MCTFLG = .TRUE.
  CALL MCPRZ(
 I MCTFLG,IPRZM,NMCDAY)
  ENDIF
C
  IF((KDFLAG.EQ.2).OR.(KDFLAG.EQ.3))THEN
  if(mcflag.ne.3)then
  CALL PZFRND
  elseif(mcflag.eq.3)then
  CALL PZFRND2
  endif
  ENDIF
CJMC
100 CONTINUE
200 CONTINUE
 IF(IPRZM.NE.1)THEN
 IF (RSTFG .EQ. 1 .OR. RSTFG .EQ. 2) THEN
C
C  Save state of system for next execution
  CALL RSTPUT (LPRZRS,IPRZM)
  CALL RSTPT1 (LPRZRS,IPRZM)
 ENDIF
 ENDIF
C
 CALL SUBOUT
C
 RETURN
 END
```

RSMISC.FOR

```
 SUBROUTINE FRM5CLN(CLNPAD)
C
C + + + PURPOSE + + +
C switches half-life when FRMFLG=5
C Modification date: 3/11/96 waterborne
C
C + + + PARAMETERS + + +
 INCLUDE 'PPARM.INC'
C
C + + + COMMON BLOCKS + + +
 INCLUDE 'CHYDR.INC'
 INCLUDE 'CPEST.INC'
 INCLUDE 'CCROP.INC'
 INCLUDE 'CMISC.INC'
C
C + + + LOCAL VARIABLES + + +
 REAL*4 TTHKNS,MODFC,TNT
 INTEGER I,J,JB,IB,IBM1,K,L,M
 INTEGER CLNPAD
```

```
 CHARACTER*80 MESAGE
C
C + + + EXTERNALS + + +
 EXTERNAL SUBIN,ERRCHK,SUBOUT
C
C
C + + + END SPECIFICATIONS + + +
C
 MESAGE = 'FRM5CLN'
 CALL SUBIN(MESAGE)
C
 DO 650 L=1,NCHEM
 IF(CLNPAD.EQ.1)THEN
C  assign horizon soil profile values
C  to individual soil layers
  IB = NHORIZ
  TNT = 0.0
  TTHKNS = THKNS(IB)
  DO 160 J = 1, NCOM2
  IBM1= IB - 1
  JB = NCOM2 - J + 1
  TNT = TNT + DELX(JB)
  MODFC = 0.0
  IF (TNT .LE. TTHKNS+.01) THEN
  DWRATE(L,JB) = DWRAT1(L,IB)
  DSRATE(L,JB) = DSRAT1(L,IB)
  DGRATE(L,JB) = DGRAT1(L,IB)
  IF(L.EQ.2)THEN
  DKRW12(JB)=DKW112(IB)
  DKRS12(JB)=DKS112(IB)
  ELSEIF(L.EQ.3)THEN
  DKRW13(JB)=DKW113(IB)
  DKRW23(JB)=DKW123(IB)
  DKRS13(JB)=DKS113(IB)
  DKRS23(JB)=DKS123(IB)
  ENDIF
  ELSE
  MODFC=(TNT-TTHKNS)/DELX(JB)
  DWRATE(L,JB)=DWRAT1(L,IB)*(1.-MODFC)+DWRAT1(L,IBM1)*MODFC
  DSRATE(L,JB)=DSRAT1(L,IB)*(1.-MODFC)+DSRAT1(L,IBM1)*MODFC
  DGRATE(L,JB)=DGRAT1(L,IB)*(1.-MODFC)+DGRAT1(L,IBM1)*MODFC
  IF(L.EQ.2)THEN
  DKRW12(JB)=DKW112(IB)*(1.-MODFC)+DKW112(IBM1)*MODFC
  DKRS12(JB)=DKS112(IB)*(1.-MODFC)+DKS112(IBM1)*MODFC
  ELSEIF(L.EQ.3)THEN
  DKRW13(JB)=DKW113(IB)*(1.-MODFC)+DKW113(IBM1)*MODFC
  DKRW23(JB)=DKW123(IB)*(1.-MODFC)+DKW123(IBM1)*MODFC
  DKRS13(JB)=DKS113(IB)*(1.-MODFC)+DKS113(IBM1)*MODFC
  DKRS23(JB)=DKS123(IB)*(1.-MODFC)+DKS123(IBM1)*MODFC
  ENDIF
  IB=IB-1
  TTHKNS=TTHKNS+THKNS(IB)
  ENDIF
160 CONTINUE
  DKSTAT(L)=1
 ELSEIF(CLNPAD.EQ.2)THEN
C  assign horizon soil profile values
```

```
C  to individual soil layers
   IB = NHORIZ
   TNT = 0.0
   TTHKNS = THKNS(IB)
   IF(CLNPCT(NCLNC).GE.1.0)THEN
   DO J=1,IB
   DWRAT2(L,J)=5.0
   DSRAT2(L,J)=5.0
   DGRAT2(L,J)=5.0
   ENDDO
   ELSE
   DO J=1,IB
   DWRAT2(L,J)=-ALOG(1.0-CLNPCT(NCLNC))
   DSRAT2(L,J)=-ALOG(1.0-CLNPCT(NCLNC))
   DGRAT2(L,J)=-ALOG(1.0-CLNPCT(NCLNC))
   ENDDO
   ENDIF
   DO 165 J = 1, NCOM2
   IBM1= IB - 1
   JB = NCOM2 - J + 1
   TNT = TNT + DELX(JB)
   MODFC = 0.0
   IF(JB.GT.CLNCMP(NCLNC))THEN
   IF (TNT .LE. TTHKNS+.01) THEN
   DWRATE(L,JB) = DWRAT1(L,IB)
   DSRATE(L,JB) = DSRAT1(L,IB)
   DGRATE(L,JB) = DGRAT1(L,IB)
   IF(L.EQ.2)THEN
    DKRW12(JB)=DKW112(IB)
    DKRS12(JB)=DKS112(IB)
   ELSEIF(L.EQ.3)THEN
    DKRW13(JB)=DKW113(IB)
    DKRW23(JB)=DKW123(IB)
    DKRS13(JB)=DKS113(IB)
    DKRS23(JB)=DKS123(IB)
   ENDIF
   ELSE
   MODFC=(TNT-TTHKNS)/DELX(JB)
   DWRATE(L,JB)=DWRAT1(L,IB)*(1.-MODFC)+
  *    DWRAT1(L,IBM1)*MODFC
   DSRATE(L,JB)=DSRAT1(L,IB)*(1.-MODFC)+
  *    DSRAT1(L,IBM1)*MODFC
   DGRATE(L,JB)=DGRAT1(L,IB)*(1.-MODFC)+
  *    DGRAT1(L,IBM1)*MODFC
   IF(L.EQ.2)THEN
    DKRW12(JB)=DKW112(IB)*(1.-MODFC)+DKW112(IBM1)*MODFC
    DKRS12(JB)=DKS112(IB)*(1.-MODFC)+DKS112(IBM1)*MODFC
   ELSEIF(L.EQ.3)THEN
    DKRW13(JB)=DKW113(IB)*(1.-MODFC)+DKW113(IBM1)*MODFC
    DKRW23(JB)=DKW123(IB)*(1.-MODFC)+DKW123(IBM1)*MODFC
    DKRS13(JB)=DKS113(IB)*(1.-MODFC)+DKS113(IBM1)*MODFC
    DKRS23(JB)=DKS123(IB)*(1.-MODFC)+DKS123(IBM1)*MODFC
   ENDIF
   IB=IB-1
   TTHKNS=TTHKNS+THKNS(IB)
   ENDIF
   ELSEIF(JB.LE.CLNCMP(NCLNC))THEN
```

```
    IF (TNT .LE. TTHKNS+.01) THEN
    DWRATE(L,JB) = DWRAT2(L,IB)
    DSRATE(L,JB) = DSRAT2(L,IB)
    DGRATE(L,JB) = DGRAT2(L,IB)
    IF(L.EQ.2)THEN
     DKRW12(JB)=DKW212(IB)
     DKRS12(JB)=DKS212(IB)
    ELSEIF(L.EQ.3)THEN
     DKRW13(JB)=DKW213(IB)
     DKRW23(JB)=DKW223(IB)
     DKRS13(JB)=DKS213(IB)
     DKRS23(JB)=DKS223(IB)
    ENDIF
    ELSE
    MODFC=(TNT-TTHKNS)/DELX(JB)
    DWRATE(L,JB)=DWRAT2(L,IB)*(1.-MODFC)+
   *   DWRAT2(L,IBM1)*MODFC
    DSRATE(L,JB)=DSRAT2(L,IB)*(1.-MODFC)+
   *   DSRAT2(L,IBM1)*MODFC
    DGRATE(L,JB)=DGRAT2(L,IB)*(1.-MODFC)+
   *   DGRAT2(L,IBM1)*MODFC
    IF(L.EQ.2)THEN
     DKRW12(JB)=DKW212(IB)*(1.-MODFC)+DKW212(IBM1)*MODFC
     DKRS12(JB)=DKS212(IB)*(1.-MODFC)+DKS212(IBM1)*MODFC
    ELSEIF(L.EQ.3)THEN
     DKRW13(JB)=DKW213(IB)*(1.-MODFC)+DKW213(IBM1)*MODFC
     DKRW23(JB)=DKW223(IB)*(1.-MODFC)+DKW223(IBM1)*MODFC
     DKRS13(JB)=DKS213(IB)*(1.-MODFC)+DKS213(IBM1)*MODFC
     DKRS23(JB)=DKS223(IB)*(1.-MODFC)+DKS223(IBM1)*MODFC
    ENDIF
    IB=IB-1
    TTHKNS=TTHKNS+THKNS(IB)
    ENDIF
    ENDIF
165 CONTINUE
  ENDIF
650 CONTINUE
C
  CALL SUBOUT
C
  RETURN
  END
```

Appendix 10. Calculation of Manure Depth

The uniform depth of manure produced each day on feedlot is estimated as 0.18 cm as shown below.

Daily manure excreted (Appendix 3 of 2014 EA)	=	$27.3\,\frac{kg}{head}$
Stocking density	=	$270\,\frac{head}{ac}$
Total daily manure excreted	=	$27.3\,\frac{kg}{head} \times 270\,\frac{head}{ac}$
	=	$7371\,\frac{kg}{acre}$
Density of fresh (as-excreted) beef cattle manure [53]	=	$63\,\frac{lb}{ft^3}$
	=	$1000\,\frac{kg}{m^3}$
Depth of daily manure excreted	=	$7371\,\frac{kg}{acre} \div 1000\,\frac{kg}{m^3} \times \frac{1\ acre}{4046.825\ m^2}$
	=	$0.0018\ m$
	=	$0.18\ cm$

Appendix 11. Cropland Application Rates of Manure

Cropland application rates of estradiol and trenbolone residues in manure were calculated using a ratio between the phosphorus (P_2O_5) in the manure and the amounts of TBA and EB released from one or more implants during a feedlot cycle. If only a portion of an implant is released during a cattle production cycle, only the amount released during the cattle cycle is used to calculate the amount in the applied manure.

An example calculation to determine the total application amount of trenbolone residues in manure is presented below using the Choice-Plus-117 TBA scenario (release rates and durations are from Table 11). The same approach is used for other re-implantation scenarios and for EB by substituting the number of days and timing in which overlapping (additive) release occurs and using the daily release rates and durations of release of EB or TBA from Table 10 or Table 11, respectively.

Choice – Daily excretion rate per unit	=	$0.7388 \frac{mg}{d}$
Choice – Days Active in Cycle	=	$117d$
Plus - Daily excretion rate per unit	=	$1.4777 \frac{mg}{d}$
Plus – Days of Release in Cycle	=	$57d$
Cycle Duration	=	$117d$
Time Weighted Averaged Release Rate	=	$\left(0.7388 \frac{mg}{d} \times 117d + 1.4777 \frac{mg}{d} \times 57d\right) \div 117d$
		$1.4587 \frac{mg}{d}$
Daily P_2O_5 Excreted	=	$0.0747 \frac{kg\ P_2O_5}{d}$
Ratio of Trenbolone to P_2O_5 in Manure	=	$1.4587 \frac{mg}{d} \div 0.0747 \frac{kg\ P_2O_5}{d}$
	=	$19.5275 \frac{mg}{kg\ P_2O_5}$
Total P_2O_5 Application from Manure	=	$35.986 \frac{kg\ P_2O_5}{ac}$
Total Chemical Applied from Manure	=	$35.986 \frac{kg\ P_2O_5}{ac} \times 19.5275 \frac{mg}{kg\ P_2O_5}$
	=	$702.7170 \frac{mg}{ac}$
	=	$7021.7170 \frac{mg}{ac} \times 0.001 \frac{g}{mg} \times 2.47105 \frac{ac}{ha}$
	=	$1.7364 \frac{g}{ha}$
Percent Application Applied as a solid	=	90%
Number of solid applications	=	2

Solid Rate Per Application	=	$1.7364 \frac{g}{ha} \times 90\% \div 2$
	=	$0.7814 \frac{g}{ha}$
Percent Application Applied as a liquid	=	10%
Number of liquid applications	=	4
Liquid Rate Per Application	=	$1.7364 \frac{g}{ha} \times 10\% \div 4$
	=	$0.0434 \frac{g}{ha}$

Application rates of trenbolone and estradiol residues in manure at various times in a cattle production cycle for several implantation scenarios are summarized in Table 34 and Table 35.

Table 34. Mass of Trenbolone Applied to Cropland, Feedlot, and Pasture (g/ha)

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Implant(s)	Rate	
Choice	0.879	0.396	0.022	0-116	Choice	0.4929	0.00478
Plus	1.759	0.792	0.044	0-116	Plus	0.9859	0.00478
ONE-F	0.975	0.439	0.024	0-211	ONE-F	0.5466	0.00478
ONE-G	0.732	0.329	0.018	0-211	ONE-G	0.4100	0.00478
Choice-Plus-117	1.736	0.781	0.043	0-59	Choice	0.4929	0.00478
				60-116	Choice + Plus	1.4788	
Choice-Plus-177	1.744	0.785	0.044	0-59	Choice	0.4929	0.00478
				60-116	Choice + Plus	1.4788	
				117-176	Plus	0.9859	
Choice-Choice	1.308	0.589	0.033	0-59	Choice	0.4929	0.00478
				60-116	Choice + Choice	0.9858	
Choice-ONE-F	1.355	0.610	0.034	0-59	Choice	0.4929	0.00478
				60-116	ONE-F	0.5466	
Plus-Plus-117	2.616	1.177	0.065	0-59	Plus	0.9859	0.00478
				60-116	Plus + Plus	1.9718	
Plus-Plus-177	2.326	1.046	0.058	0-59	Plus	0.9859	0.00478
				60-116	Plus + Plus	1.9718	
				117-176	Plus	0.9859	
Plus-Choice	2.188	0.984	0.055	0-59	Plus	0.9859	0.00478
				60-116	Plus + Choice	1.4788	
Plus-ONE-F	2.234	1.005	0.056	0-59	Plus	0.9859	0.00478
				60-116	Plus + ONE-F	1.5325	
ONE-F-Plus-211	1.567	0.705	0.039	0-139	ONE-F	0.5466	0.00478
				140-210	ONE-F + Plus	1.5325	
ONE-F-Plus-257	1.602	0.721	0.040	0-139	ONE-F	0.5466	0.00478

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Implant(s)	Rate	
				140-210	ONE-F + Plus	1.5325	
				211-256	Plus	0.9859	
ONE-F-Choice	1.271	0.572	0.032	0-139	ONE-F	0.5466	0.00478
				140-210	ONE-F + Choice	1.0395	
ONE-F-ONE-F	1.303	0.587	0.033	0-139	ONE-F	0.5466	0.00478
				140-210	ONE-F + ONE-F	1.0932	
ONE-G-Plus-211	1.323	0.596	0.033	0-139	ONE-G	0.4100	0.00478
				140-210	ONE-G + Plus	1.3959	
ONE-G-Plus-257	1.401	0.631	0.035	0-139	ONE-G	0.4100	0.00478
				140-210	ONE-G + Plus	1.3959	
				211-256	Plus	0.9859	
ONE-G-CHOICE	1.027	0.462	0.026	0-139	ONE-G	0.4100	0.00478
				140-210	ONE-G + Choice	0.9029	
ONE-G-ONE-F	1.060	0.477	0.026	0-139	ONE-G	0.4100	0.00478
				140-210	ONE-G + ONE-F	0.9566	
Choice-Plus-Plus-177	2.311	1.040	0.058	0-59	Choice	0.4929	0.00478
				60-116	Choice + Plus	1.4788	
				117-119	Plus	0.9859	
				120-176	Plus + Plus	1.9718	
Choice-Plus-Plus-237	2.171	0.977	0.054	0-59	Choice	0.4929	0.00478
				60-116	Choice + Plus	1.4788	
				117-119	Plus	0.9859	
				120-176	Plus + Plus	1.9718	
				177-236	Plus	0.9859	
Choice-Choice-Plus-177	1.729	0.778	0.043	0-59	Choice	0.4929	0.00478
				60-116	Choice + Choice	0.9858	
				117-119	Choice	0.4929	
				120-176	Choice + Plus	1.4788	
Choice-Choice-Plus-237	1.737	0.782	0.043	0-59	Choice	0.4929	0.00478
				60-116	Choice + Choice	0.9858	
				117-119	Choice	0.4929	
				120-176	Choice + Plus	1.4788	
				177-236	Plus	0.9859	
Choice-ONE-F-Plus-271	1.600	0.720	0.040	0-59	Choice	0.4929	0.00478
				60-116	Choice + ONE-F	1.0395	
				117-199	ONE-F	0.5466	
				199-270	ONE-F + Plus	1.5325	
Choice-ONE-F-Plus-317	1.623	0.730	0.041	0-59	Choice	0.4929	0.00478
				60-116	Choice + ONE-F	1.0395	
				117-199	ONE-F	0.5466	
				200-270	ONE-F + Plus	1.5325	
				271-316	Plus	0.9859	
Plus-Plus-Plus-177	2.892	1.301	0.072	0-59	Plus	0.9859	0.00478
				60-116	Plus + Plus	1.9718	
				117-119	Plus	0.9859	
				120-176	Plus + Plus	1.9718	
Plus-Plus-Plus-237	2.605	1.172	0.065	0-59	Plus	0.9859	0.00478
				60-116	Plus + Plus	1.9718	
				117-119	Plus	0.9859	
				120-176	Plus + Plus	1.9718	
				177-236	Plus	0.9859	

Table 35. Mass of Estradiol Applied to Cropland, Feedlot, and Pasture (g/ha)

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Implant(s)	Rate	
Choice	0.879	0.396	0.022	0-140	Choice	0.0478	0.00044
Plus	1.759	0.792	0.044	0-140	Plus	0.0956	0.00044
ONE-F	0.975	0.439	0.024	0-266	ONE-F	0.0506	0.00044
ONE-G	0.732	0.329	0.018	0-266	ONE-G	0.0380	0.00044
Choice-Plus-141	1.736	0.781	0.043	0-59	Choice	0.0478	0.00044
				60-140	Choice + Plus	0.1434	
Choice-Plus-201	1.744	0.785	0.044	0-59	Choice	0.0478	0.00044
				60-140	Choice + Plus	0.1434	
				141-200	Plus	0.0956	
Choice-Choice	1.308	0.589	0.033	0-59	Choice	0.0478	0.00044
				60-140	Choice + Choice	0.0956	
Choice-ONE-F	1.355	0.610	0.034	0-59	Choice	0.0478	0.00044
				60-140	ONE-F	0.0506	
Plus-Plus-141	2.616	1.177	0.065	0-59	Plus	0.0956	0.00044
				60-140	Plus + Plus	0.1912	
Plus-Plus-201	2.326	1.046	0.058	0-59	Plus	0.0956	0.00044
				60-140	Plus + Plus	0.1912	
				141-200	Plus	0.0956	
Plus-Choice	2.188	0.984	0.055	0-59	Plus	0.0956	0.00044
				60-140	Plus + Choice	0.1434	
Plus-ONE-F	2.234	1.005	0.056	0-59	Plus	0.0956	0.00044
				60-140	Plus + ONE-F	0.1462	
ONE-F-Plus-267	1.567	0.705	0.039	0-139	ONE-F	0.0506	0.00044
				140-266	ONE-F + Plus	0.1462	
ONE-F-Plus-281	1.602	0.721	0.040	0-139	ONE-F	0.0506	0.00044
				140-266	ONE-F + Plus	0.1462	
				267-280	Plus	0.0956	
ONE-F-Choice	1.271	0.572	0.032	0-139	ONE-F	0.0506	0.00044
				140-266	ONE-F + Choice	0.0984	
ONE-F-ONE-F	1.303	0.587	0.033	0-139	ONE-F	0.0506	0.00044
				140-266	ONE-F + ONE-F	0.1013	
ONE-G-Plus-267	1.323	0.596	0.033	0-139	ONE-G	0.0380	0.00044
				140-266	ONE-G + Plus	0.1336	
ONE-G-Plus-281	1.401	0.631	0.035	0-139	ONE-G	0.0380	0.00044
				140-266	ONE-G + Plus	0.1336	
				267-280	Plus	0.0956	
ONE-G-Choice	1.027	0.462	0.026	0-139	ONE-G	0.0380	0.00044
				140-266	ONE-G + Choice	0.0858	
ONE-G-ONE-F	1.060	0.477	0.026	0-139	ONE-G	0.0380	0.00044
				140-266	ONE-G + ONE-F	0.0886	
Choice-Plus-Plus-201	2.311	1.040	0.058	0-59	Choice	0.0478	0.00044
				60-119	Choice + Plus	0.1434	
				120-140	Choice + Plus + Plus	0.2390	
				141-200	Plus + Plus	0.1912	
Choice-Plus-Plus-261	2.171	0.977	0.054	0-59	Choice	0.0478	0.00044
				60-119	Choice + Plus	0.1434	
				120-140	Choice + Plus + Plus	0.2390	
				141-200	Plus + Plus	0.1912	
				201-260	Plus	0.0956	
Choice-Choice-Plus-201	1.729	0.778	0.043	0-59	Choice	0.0478	0.00044
				60-119	Choice + Choice	0.0956	
				120-140	Choice + Choice + Plus	0.1912	
				141-200	Choice + Plus	0.1434	

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Implant(s)	Rate	
Choice-Choice-Plus-261	1.737	0.782	0.043	0-59	Choice	0.0478	0.00044
				60-119	Choice + Choice	0.0956	
				120-140	Choice + Choice + Plus	0.1912	
				141-200	Choice + Plus	0.1434	
				201-260	Plus	0.0956	
Choice-ONE-F-Plus-327	1.600	0.720	0.040	0-59	Choice	0.0478	0.00044
				60-140	Choice + ONE-F	0.0984	
				141-199	ONE-F	0.0506	
				199-326	ONE-F + Plus	0.1462	
Choice-ONE-F-Plus-341	1.623	0.730	0.041	0-59	Choice	0.0478	0.00044
				60-140	Choice + ONE-F	0.0984	
				141-199	ONE-F	0.0506	
				200-326	ONE-F + Plus	0.1462	
				327-340	Plus	0.0956	
Plus-Plus-Plus-327	2.892	1.301	0.072	0-59	Plus	0.0956	0.00044
				60-119	Plus + Plus	0.1912	
				120-140	Plus + Plus + Plus	0.2868	
				141-200	Plus + Plus	0.1912	
Plus-Plus-Plus-341	2.605	1.172	0.065	0-59	Plus	0.0956	0.00044
				60-119	Plus + Plus	0.1912	
				120-140	Plus + Plus + Plus	0.2868	
				141-200	Plus + Plus	0.1912	
				201-260	Plus	0.0956	

Appendix 12. Sensitivity Analysis of Modeling Results

Choice-Plus scenarios (Choice-Plus-117 for trenbolone and Choice-Plus-141 for estradiol) were used to compare the effects of tillage and scraping efficiency upon PECs. PECs were calculated for a base scenario assuming no till cropping practices and 70% scraping efficiency. Results are summarized in Table 36 below.

Table 36. Results of Sensitivity Analyses for Choice-Plus

	Trenbolone		Estradiol	
Scenario	**21d PEC (ng/L)**	**RQ**	**21d PEC (ng/L)**	**RQ**
Base Scenario (No Till, 70% Scraping)	0.88	0.44	0.09	0.07
No Till, 90% Scraping	0.88	0.44	0.09	0.07
Conventional Tillage, 70% Scraping	0.75	0.37	0.08	0.06

To assess the effect of different tillage practices upon runoff of trenbolone and estradiol residues, a sensitivity analysis was conducted by comparing conventional tillage versus no till practices. Reduced tillage was not evaluated because it is intermediate between no till and conventional till.

As shown by results in Table 36, changing the base scenario from no till to conventional tillage produced lower 90th percentile PECs and RQs. For trenbolone, the PEC decreased from 0.88 ng/L for no till to 0.75 ng/L for conventional tillage with a corresponding reduction in RQ. For estradiol, PECs likewise decreased from 0.08 ng/L to 0.06 with a corresponding reduction in RQ.

Regarding scraping efficiency, a default value of 70% was established as a conservative value for the percentage of manure removed from a feedlot after completing a cattle production cycle. It was assumed that manure was not mounded or removed during the cycle and that manure was present at a uniform depth. PECs were calculated with values of 70% and 90%.

As shown by results in Table 36, PECs and RQs for trenbolone and estradiol were the same for both scraping values. There were two reasons for this. First, the 21-day maximum annual value occurred towards the end of a cycle so that effects due to scraping were minimized. And second, most of the residues in feedlot runoff are contributed by residues in the topmost 2 cm of the manure pack. With an average depth of 0.18 cm of manure produced each per day, cattle production cycles greater than 37 days will leave at least 2 cm of residual manure if 70% of manure is removed between cycles (111 days if 90% is removed). For comparison, the shortest re-implant scenario evaluated in this EA for was 117 days for trenbolone. A scraping value of 70% is used in this EA to be conservative even though cattle are typically held in feedlots for several months.

Appendix 13. Exposure Assessment of Trenbolone and Estradiol in Surface Water Associated with the use of Synovex® Brand Implants and Re-Implants in Beef Cattle Sensitivity Analysis of Modeling Results

TITLE

EXPOSURE ASSESSMENT OF TRENBOLONE AND ESTRADIOL IN SURFACE WATER ASSOCIATED WITH THE USE OF SYNOVEX® BRAND IMPLANTS AND RE-IMPLANTS IN BEEF CATTLE

DATA REQUIREMENTS

Not Applicable

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COMPLETION DATE

October 21, 2021

PERFORMED BY

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PROJECT ID

Waterborne Study Number: 734.06

Zoetis Study Number: A5X0Z-US-21-807

GOOD LABORATORY PRACTICE STATEMENT

This study, titled "Exposure Assessment of Trenbolone and Estradiol in Surface Water Associated with the use of Synovex® Brand Implants and Re-implants in Beef Cattle," contains the results of model simulations using information obtained from a variety of government and literature sources. The work was neither applicable nor conducted under Good Laboratory Practice (GLP) as defined by 21 CFR Part 58 for products registered by the U.S Food and Drug Administration and GLPs by the Organization for Economic Cooperation and Development (OEC) for the European Union.

21-Oct-2021

W. Martin Williams
Facility Management

Date

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GENERAL INFORMATION

Report Title: Exposure Assessment of Trenbolone and Estradiol in Surface Water Associated with the use of Synovex® Brand Implants and Re-implants in Beef Cattle

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LIST OF ABBREVIATIONS AND ACRONYMS

AFO	Animal feeding operations less than 1,000 head
APEX	Agricultural Policy / Environmental eXtender model
AU	Animal Unit
CAFO	Animal feeding operations over 1,000 head
CVM	Center for Veterinary Medicine (Organization in the United States Food & Drug Administration)
DOF	Days on Feed
EA	Environmental Assessment
EB	Estradiol benzoate
EDC	Endocrine disrupting compounds
EXAMS	Exposure Analysis Modeling System
IA DNR	Iowa Department of Natural Resources
PCA	Percent Crop Area – Fraction of a watershed represented by a specific crop or land use (e.g., feedlot, manured cropland, or pasure).
PEC	Predicted environmental concentration
PNEC	Predicted no-effect concentration
PRZM	Pesticide Root Zone Model
PRZM-3.12	PRZM verison 3.12
PRZM5	PRZM version 5
RQ	Risk quotient
SynovexPRZM	Modified version of winPRZM used for assessment
TBA	Trenbolone acetate
USDA NASS	United States Department of Agriculture National Agricultural Statistics Service
USEPA	United States Environmental Protection Agency's Office of Pesticide Programs
VVWM	Variable Volume Water Model
winPRZM	European version of PRZM

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1.0 EXECUTIVE SUMMARY

Zoetis is seeking current and future registrations for Synovex® products that would allow the administration of four products (CHOICE, PLUS, ONE Feedlot (ONE), ONE Grower (ONEg)) to beef steers and heifers fed in confinement (feedlot) using different implant and re-implant combinations. Synovex products are extended-release growth promoting implants. These products contain different amounts of trenbolone acetate (TBA) and estradiol benzoate (EB) and have different rates and durations of release of each active.

This exposure assessment contains predicted environmental concentrations (PECs) of trenbolone and estradiol resulting from runoff from feedlot manure, pasture manure, and manured cropland for a hypothetical realistic intense-use watershed in Iowa using a modeling framework established by the U.S. Environmental Protection Agency (USEPA) for pesticide registration. This watershed scenario was determined to have the highest exposure in a previous national assessment conducted for Synovex ONE Feedlot in 2014 (Zoetis, 2014) and thus represents a reasonable worst-case scenario addressing feedlot use of Synovex products in the United States. A geospatial analysis was conducted for the current environmental assessment (EA) using the 2017 Agricultural Census (USDA NASS 2017) to confirm that the Iowa scenario remains relevant and highly conservative. The feedlot cattle density, pasture cattle density, and estimated manured cropland were updated to reflect 2018 Iowa Department of Natural Resources (IA DNR) surveys.

Predicted environmental concentrations (PECs) of trenbolone and estradiol were determined for the Iowa watershed under different implant combinations (order and identity of Synovex products administered) and re-implantation regimens (times when the second and sometimes third implant are administered and total number of days on feed). The concentrations were estimated from combined contributions from feedlots, pasture, and manured cropland discharging to surface water in the watershed under assumptions of 25% of feedlots with <1000 Animal Units (AUs) do not control runoff and thus reflect worst-case concentrations. For each re-implantation combination, the regimen producing the highest environmental exposure was selected for modeling. If this maximum exposure regimen produced acceptable PECs, all re-implantation times within the dosing window and all grow out periods (total days on feed, DOF, for implanted animals) have been addressed.

For environmental assessment purposes, risk quotients (RQ) were determined for each simulated re-implantation combination and regimen, with the RQ equal to the PEC divided by the predicted no effect concentration (PNEC). For both trenbolone and estradiol, PECs were calculated separately assuming that 25% of feedlot AFOs do not control runoff. Two risk quotients were calculated for trenbolone assuming that: (1) 5% of trenbolone-related residues were conservatively assigned with biological activity equivalent to17β-trenbolone and the remaining 95% with activity equivalent to 17α-trenbolone, and (2) 8% assigned to 17β-trenbolone and 92% to 17α-trenbolone. A single risk quotient was calculated for estradiol residues in cattle excreta with 100% of the estradiol-related residues conservatively assigned with biological activity equivalent to 17β-estradiol.

The PEC used for the risk assessment is the 90th percentile 21-day average concentration calculated from a rolling average assessment of the annual maximum series from a 30-year simulation. The 90th centile concentrations correspond to a 10-year return period. Trenbolone PECs ranged from

0.41 ng/L (ONEg single implant) to 1.51 ng/L (PLUS with two re-implants of PLUS at 60 and 120 days and grow-out period of 177 days). Estradiol PECs ranged from 0.04 (ONEg single implant) to 0.16 ng/L (PLUS with two re-implants of PLUS at 60 and 120 days and grow-out period of 201 days) with 25% of AFOs contributing runoff.

RQs were calculated using the 21-day chronic no effect concentration of 3.2 ng/L for 17α-trenbolone, 0.25 ng/L for 17β-trenbolone, 25 ng/L for 17α-estradiol and 1.4 ng/L for 17β-estradiol. The highest PECs and RQs for trenbolone were associated with the most aggressive re-implant scenario, PLUS-PLUS-PLUS-177 in which all feedlot cattle are implanted with PLUS on days 0, 60 and 120; reside in the feedlot for 177 days on feed; the next cattle production cycle beginning immediately; and repeated cattle production cycles with the same scenario occur over a 30-year modeling period. The RQs associated with the PLUS-PLUS-PLUS-177 scenario are 0.75 and 0.92 under assumptions of 5% and 8% excretion of 17β-trenbolone respectively, indicating no Synovex implant and re-implant condition that exceeds the level of concern based upon the 90th percentile 21-day average concentration annual maximum series.

Similarly, RQs for estradiol were calculated conservatively assuming that 100% of estradiol-related residues are excreted in the form of 17β-estradiol. The RQ for the PLUS-PLUS-PLUS-201 scenario is 0.11, thus indicating that all Synovex implant combinations and re-implant conditions are under levels of concern.

In addition to results based on the 90th percentile 21-day annual maximum series, daily RQs were calculated and graphed to identify and visualize any events in the 30-year modeling period that exceeded the 21-day PNEC. Scenarios with events exceeding the RQ threshold of 1.0 are identified by number of events in the 30-year modeling period and the date, magnitude (maximum and average daily RQ), and duration of each event. For trenbolone, two implant regimens had a single event occurrence with a daily RQ ≥ 1.0 over the 30-year simulation under the assumption of 5% excretion of 17β-trenbolone. For 8% excretion of 17β-trenbolone, five implant scenarios had a single event with an RQ ≥ 1.0 and one implant regimen had two events. Daily RQs were less than 1.0 for all estradiol implant treatment regimes.

2.0 INTRODUCTION AND OBJECTIVES

In 2014, an environmental assessment (EA) was prepared for Synovex ONE extended-release, growth-promoting implant for feedlot and pasture beef steers and heifers (Zoetis, 2014). The purpose of the EA was to assess the fate, effects, and overall impact of the environmentally relevant metabolites excreted in cattle implanted with Synovex ONE. The EA addressed the potential for runoff and leaching of EB and TBA residues from excreted manure from feedlot cattle, pasture cattle, and manure applied to cropland.

The 2014 assessment for ONE was patterned after the tiered process used by the USEPA's Office of Pesticide Programs to evaluate pesticides for registration and re-registration (USEPA, 2011). Mixed-use watersheds (feedlot, pasture, and cropland) were simulated using USEPA's "Index Reservoir" scenario (USEPA 2020). The Pesticide Root Zone Model (PRZM) which is used by USEPA to simulate chemical losses from fields from runoff and erosion, was found suitable for simulating manure applications to cropland, but it could not directly simulate daily loadings of chemical applications to pasture and feedlots. Therefore, modifications were made to a version of

PRZM to simulate daily constant concentrations of EB and TBA in feedlots and daily applications of EB and TBA in certain months of the year to pasture.

Zoetis is seeking current and future registrations that would allow for repeated administration of different Synovex products (ONE Feedlot (ONE), ONE Grower (ONEg), CHOICE, and PLUS) to beef steers and heifers using different combinations of Synovex products. All implants contain different amounts of TBA and EB and are released for different durations. Table 1 shows the trenbolone and estradiol release rates and durations for different Synovex implants. In support of the current registration, this report describes the updated methodology to derive predicted environmental concentrations of trenbolone and estradiol resulting from their runoff from feedlot manure, pasture manure, and manured cropland following the methodology used in the Iowa watershed from the 2014 EA (Zoetis, 2014). Predicted concentrations are provided for various re-implant scenarios to illustrate the range of predicted concentrations from the model and covering more aggressive re-implant schemes that yield higher potential environmental exposures.

Note: ONE Grower is currently under CVM review (INAD 006-242). Upon approval, ONE Grower will replace ONE Grass for use in both feedlot and pasture cattle (CVM letter I-006242-G-406-OT dated September 1, 2020). Because the work described in this report was conducted during this transition, modeling and supporting documents may refer to both products. The two product names are interchangeable for the purposes of this work.

3.0 PROBLEM FORMULATION

Synovex brand products are administered as implants and/or re-implants to beef cattle. Active metabolites of EB and TBA are excreted in manure and urine during the implant pay-out period, where they have the potential to enter the aquatic environment through runoff or leaching. Potential sources include: 1) the manure/urine contained in feedlots, 2) manure/urine deposited on pastureland, and 3) manure applied as fertilizer to crop fields. When all these sources and exposure pathways exist in a watershed, it could potentially result in an aggregate exposure.[1] In addition, the trenbolone and estradiol metabolites entering the environment may also contribute to an already existing load of Endocrine Disrupting Compounds (EDCs), including synthetic and naturally occurring estrogens and androgens; ultimately, resulting in a cumulative exposure.[2]

4.0 METHODS AND MATERIALS

4.1 Assessment framework

The 2014 assessment for Synovex ONE-Feedlot was patterned after the aquatic risk assessment process used by the USEPA to evaluate pesticides for registration and re-registration (Zoetis, 2014). USEPA conducts ecological risk assessments using a "standard pond" scenario and drinking water assessments using an "Index Reservoir" scenario (USEPA, 2020). The "Index Reservoir" scenario was used for the Synovex EA because it can be modified to represent a mixed-use watershed containing feedlot, pasture, and cropland. In keeping with USEPA's approach to

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

[2] For this EA, cumulative exposure is defined as aggregate exposures to multiple EDCs.

practice of a conservative risk assessment, the following assumptions were made in the 2014 risk assessment:

- Risk assessment focused on areas of the United States with a high potential for trenbolone and estradiol exposure to aquatic organisms.
- Modeling addressed trenbolone and estradiol residue runoff from feedlots, pasture cattle, and cropland treated with manure under the assumption that all cattle in the watershed received implants.
- The assumption that 20% of the trenbolone residues excreted in cattle urine and feces would be in the form of 17β-trenbolone.
  Note: Assumptions of 5% and 8% 17β-trenbolone are examined in the current assessment based upon additional data regarding the composition and relative androgenic activity of trenbolone-related metabolites in cattle excreta acquired since the 2014 EA.
- No decrease in excreted daily masses of trenbolone and estradiol due to metabolism in the treated animal.
- High feedlot stocking density: 270 head/acre.
- Fully stocked feedlot every day of the year.
- High pasture stocking density: 3.15 head/acre and that all pasture animals are implanted with Synovex ONE Grass/Grower.
- No degradation of residues in manure in the feedlot.
- Cropland application rate calculated from daily release rate of trenbolone or estradiol, phosphorus application rate for corn grain, and daily phosphorus excretion rate in beef cattle manure.
- All manure generated on feedlots is applied to croplands in the watershed up to the total acreage of cropland in the watershed. 90% of the manure generated is applied to croplands as solid manure and 10% is applied as liquid manure applied with irrigation water.
- Solid manure is applied two times a year, in the spring and fall, before and after corn crop cycle and liquid manure is applied four times a year with irrigation water during the crop cycle.
- Curve number[3] for feedlots used in NRCS curve number method is 95, representative of high runoff potential.
- 25% of the Animal Feeding Operations with less than 1,000 head (AFOs) in the watershed were assumed to be directly releasing to nearby streams on a nationwide level, which was rounded up from 17% based on the calculations in Appendix 9 of the 2014 EA.
- PECs were based on 90th percentile annual maximum, 21-day concentration from the model simulations, corresponding to a 10-year recurrence interval.

The revised assessment follows the same framework. Materials and methods used in the 2014 EA have been updated to reflect current information related to beef cattle statistics, environmental fate properties of trenbolone and estradiol, and model technology.

Because previous modeling was conducted for a single implant with constant release of TBA and EB, a new mixed-use watershed model (build-up model) was developed to derive PECs for

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[3] Runoff curve numbers (CN) are used in the PRZM model to determine the amount of rainfall that becomes runoff. The CN reflects both the soil properties and land cover of an area.

trenbolone and estradiol metabolites in feedlot cattle receiving more than one implant. Simulations were performed examining different implant product combinations (including two and three implants per cycle), different schedules and dose rates of animal treatment, and the accumulation and removal of trenbolone and estradiol metabolite residues in the feedlot over time. PECs include aggregate environmental inputs resulting from runoff from feedlot manure, pasture manure, and manure applied to cropland.

The resulting model was implemented to calculate predicted environmental concentrations (PECs) for several representative and conservative implantation scenarios. Modeling was conducted for combinations with CHOICE, PLUS, ONE, and ONEg using different lead and follow-on implants and re-implant timings. Single implant scenarios for CHOICE, PLUS, ONE, and ONEg were modeled for comparison. As with the 2014 Synovex ONE EA, PECs and resulting RQs were calculated separately for the 90th percentile 21-day moving average annual maximum series assuming 25% of AFOs contributed runoff. In addition, daily RQ values also were evaluated over the 30-year model period to identify any events with RQ values greater than 1.0 and the corresponding maximum daily RQ, average RQ, and duration for any such events.

4.2 Watershed confirmation

The model scenario used for the 2014 assessment was selected by conducting a national geospatial analysis to identify regions of high potential vulnerability of surrogate estradiol and trenbolone compounds to runoff or erosion into surface waters. The analysis considered areas of high beef cattle density, high density of feedlots, and normal annual precipitation. Based on this information, five regions were initially selected from five states: Iowa, Texas, Ohio, Michigan, and Pennsylvania. The Lyon/Sioux county region of Iowa was chosen for this process because it represented the 98th percentile or greater in terms of beef cattle density at the county level. In this region, operations with greater risk (<500 head) were in the 99th percentile or greater consistently since 2002 (Table 2).

The 2014 assessment relied on the 2007 Census of Agriculture released by the United States Department of Agriculture National Agricultural Statistics Service (USDA NASS) for cattle and feedlot statistics. The current 2017 Census of Agriculture (USDA NASS, 2017) was assessed to confirm the selected study regions remain relevant from a beef cattle density perspective (see Table 2). The results indicate that all study regions remain relevant, and the Lyon and Sioux Iowa counties continue to rank similarly, and their selection remain justified. For completeness, Table 2 presents the 2002, 2007, 2012 and 2017 censuses.

For further confirmation that the Iowa counties represent high potential exposure, model results were compared to predictions for counties with high feedlot cattle density in Texas, Ohio, Michigan, and Pennsylvania (see Appendix A).

For the Iowa study region, the most recent IA DNR Feedlot location database (IA DNR, 2018) was acquired to examine any changes in watershed-level feedlot cattle densities. A comparison (Figure 1) of feedlot counts in the IA DNR database with the 2017 Census of Agriculture indicate the IA DNR database to be a more conservative representation of actual beef cattle counts passing through a facility given the database represents “permitted cattle”. Likewise, the most recent USDA NASS Cropland Data Layer (USDA NASS, 2018) was used to recalculate updated pasturelands with which pasture cattle may be stocked. The following table summarizes the critical factors affecting the selected watershed in Lyon and Sioux counties, indicating higher AFO beef head, CAFO (concentrated animal feeding operation) beef head, pasture cattle beef head, AFO feedlots, cropland manured, and pastureland.

Assessment	AFO beef head	CAFO beef head	Pasture Cattle Beef head	% AFO Feedlot	% Cropland Manured	% Pastureland
2014 EA	5,373	10,410	1,525	0.094%	56.6%	2.29%
2021 EA	6,130	17,800	2,743	0.108%	88.7%	4.12%

Based on the 2017 Census of Agriculture, the region and county selection remain justified. The revised EA reflects the more recent, higher density of AFO cattle, pasture cattle, and cropland.

4.3 Model technology

At the time of the 2014 EA, USEPA was using the models PRZM version 3.12 (PRZM-3.12) (Suárez, 2005) to simulate chemical mass balance in the terrestrial environment and the Exposure Analysis Modeling System (EXAMS) version 2.98 (Burns, 2004) to simulate chemical mass balance in the aquatic environment. PRZM-3.12 was found suitable for simulating manure applications to cropland, but it did not have the ability to simulate daily loadings of chemical applications to pasture and feedlots. Therefore, modifications were made to PRZM at the time of the 2014 EA to simulate daily applications of estradiol and trenbolone for certain months of the year to pasture and daily constant concentration of estradiol and trenbolone on feedlots. In addition, the manure erosion equation from the Agricultural Policy / Environmental eXtender model (APEX) model (Williams et al., 2006) was incorporated into the model.

The European version of PRZM, version 4.73, (winPRZM) developed under the European Commission's FOCUS DG SANTE (the Forum for the co-ordination of pesticide fate models and their use) (FOCUS, 2015a,b) was the source model for the modifications used in the 2014 EA because familiarity and ownership of the code by the authors. At the time of the assessment the cropland scenario with winPRZM was tested against PRZM-3.12 prior to and after the addition of the modifications and was found to reproduce the same results (within 0.26%).

For the revised EA, additional modifications were required for winPRZM to model trenbolone and estradiol releases associated with re-implant scenarios. It was important to change the amount of trenbolone and estradiol to correspond to implant schedules, doses, and payout periods. While doing the modifications, a more realistic method was used to simulate feedlot management practices as described below.

This updated version of winPRZM (hereafter referred to as SynovexPRZM) can accommodate different schedules and dose rates of animal treatment and accumulation of manure and trenbolone and estradiol residues in the feedlot over time. In addition, the model can simulate the removal of manure from feedlots that occurs during periodic scraping. User inputs include the date, dose, payout period (the duration in which residues are released from the animal), and the amount of medicine excreted in manure each day (or application rate).

Using a daily time step, the model calculates the build-up of manure based on daily rate of excretion from cattle. Each day, the mass of trenbolone or estradiol is added to residues remaining from the previous time step. The mass added is estimated as the combined release rates of actively releasing implants during the grow out period multiplied by feedlot stocking density (270 head/ac) (Table 3). Total drug is assumed to be uniformly mixed in the manure pile to account for the mixing that occurs from cattle movement in the feedlot. Mixing occurs in the active (upper 10 cm) of the manure pile. Once the manure pile exceeds 10 cm, residues below 10 cm are assumed buried and unavailable for mixing as was assumed in the 2014 EA. After that point in time, a depth of manure equal to the daily addition is buried and the daily addition of veterinary medicine mass is mixed to the active manure pile.

For illustration, a graphical representation of the mass of trenbolone in the top 10 cm of manure layer is shown in Figure 2 for three re-implantation examples: (1) CHOICE followed by a PLUS implant with a 60-day re-implantation and 117 day grow out interval (CHOICE-PLUS-117), (2) PLUS followed by a second PLUS implant at 60 days and 177 day grow out interval (PLUS-PLUS-177), and (3) PLUS followed by a second PLUS implant at 60 days, a third PLUS implant at 120 days, and a 237 day grow out interval (PLUS-PLUS-PLUS-237). The image shows three full cycles for CHOICE-PLUS-117, two cycles for PLUS-PLUS-177, and one and a half cycles for PLUS-PLUS-PLUS-237 during a 1-year period.

All scenarios begin their first implantation at the same time, the cycle's day 0 (4/15/1961 for cycle 1). The PLUS-PLUS-177 and PLUS-PLUS-PLUS-237 scenarios release a higher mass of trenbolone residues due to the PLUS implant having a greater release rate than the CHOICE implant. The second implant on day 60 (6/14/1961 for cycle 1) shows an increase of daily addition of trenbolone in the top cm manure layer. On day 118 (8/10/1961 for cycle 1), a decrease in the total mass is shown for PLUS-PLUS-177 and PLUS-PLUS-PLUS-237 as the initial PLUS implant is no longer actively releasing. On day 120 (8/13/1961 for cycle 1), the PLUS-PLUS-PLUS-237 scenario deviates from the PLUS-PLUS-177 scenario as the third PLUS implant occurs. A decrease in total mass at the end of each cycle is clearly shown. CHOICE-PLUS-117 occurs first on day 117 (8/9/1961 for cycle 1), then PLUS-PLUS-177 on day 177 (10/8/1961 for cycle 1), and lastly PLUS-PLUS-PLUS-237 on day 237 (12/7/1961 for cycle 1). At this point for each scenario, the accumulation cycle begins anew. Conceptually the same process occurs for estradiol mass in the manure layer, however the shape of the profiles and timings are different due to the different durations of release of EB from ear implants than TBA.

The same approach was applied for each re-implantation scenario using the average rates and durations of release of trenbolone and estradiol from each implant along with the order and timing of re-implantation as described in Section 4.5, Implant Scenarios.

Note: As of the date of this report, USEPA has replaced PRZM-3.12 and EXAMS with PRZM5 (Young and Fry, 2020) and the Variable Volume Water Model, VVWM (Young, 2019). To determine the suitability of continued use of the SynovexPRZM as the basis of assessment for trenbolone and estradiol, the PECs resulting from the Iowa cropland scenario generated by SynovexPRZM have been compared to those with PRZM5 and found to be similar (within 0.71%). Simulations contained in the current EA have been conducted with SynovexPRZM and VVWM.

4.4 Environmental fate properties

Environmental fate properties used in the previous EA were based on literature available at the time. A comprehensive literature search was conducted for the period from 2014 (time of the Synovex ONE EA) to present to determine if properties should be updated for the current EA. Several published articles were identified by key word search and relevant articles were reviewed. No articles were found to justify a revision of environmental fate properties. Details and results of the data review are provided in Maples-Reynolds and Green (2019a,b) and Maples-Reynolds (2021). Environmental fate properties used in the 2014 and revised EA are summarized in Table 4.

4.5 Implant scenarios

A variety of re-implant conditions are being sought under the concept of an umbrella EA in which all re-implantation scenarios with acceptable risk assessments would be addressed under a single unifying EA. Re-implantation scenarios were selected for trenbolone and estradiol each to illustrate the versatility and range of predicted concentrations of trenbolone and estradiol as a function of lead implant, the subsequent implant(s), re-implant schedule, grow-out days (total residence time in the feedlot, days on feed), number of cycles per year, number of overlap days per cycle, and the number of days and amounts of TBA and EB released from cattle in excreta in terms of trenbolone and estradiol equivalents. For comparison, modeling output was generated for each of the implant products when used as single implants (no-reimplantation) for the entire residence time of cattle in feedlots, 365 days per year.

Grow out periods vary from production cycle to production cycle to meet the objectives of the producer for specific cattle. In selecting the scenarios for modeling a re-implant combination, the regimen (re-implant time and grow out period) predicted to produce the highest environmental exposure was identified. If this regimen produces an acceptable risk quotient (RQ = PEC / PNEC), all re-implantation times within the dosing window and all grow out periods have been addressed. In all scenarios, grow out begins at time 0 for the first implant and there are no vacancies in the feedlot, i.e., cattle are immediately restocked between production cycles. The predicted highest-exposure regimens for trenbolone and estradiol for each implant combination were separately modeled because TBA and EB have different rates and durations of release. The durations of release of TBA and EB from CHOICE and PLUS are 117 and 141 days, respectively. The durations of release of TBA and EB from ONE and ONEg are 211 and 267 days, respectively. See Table 1.

Greater potential environmental exposure occurs at the earliest re-implantation times because these regimens produce the longest duration of overlapping daily release of TBA and EB. For CHOICE or PLUS as the initial implant, the earliest re-implant time is 60 (60 to 120 days in the proposed claim). For combinations with ONE or ONEg as the initial implant, the earliest re-implantation time is 140 days.

In determining the grow out time, the objective was to maximize the number of days of overlapping exposure of TBA and EB from two or more implants over the 30-year modeling period, which will be different for TBA and EB. A second consideration was whether the next implant releases greater daily amounts of TBA and EB than the preceding implant.

For combinations in which the terminal implant releases similar or lower daily amounts of TBA and EB than the first implant, the grow out period corresponds to the last day of overlapping exposure. After this time, drug release is from the terminal implant only. Greatest potential environmental exposure occurs by starting the next production cycle immediately, thereby increasing the total number of production cycles and maximizing the total number of days of overlapping release over a thirty-year period. Immediately restocking the feedlot with freshly implanted animals negates or is greater than the contribution from leftover TBA or EB not released from the previous implants. For CHOICE-CHOICE, CHOICE-ONEg, and CHOICE-ONE two-implant combinations in which the second implant is administered on Day 60, grow out periods that produce the greatest number of days of overlapping release are 117 days on feed for trenbolone and 141 days for estradiol.

For combinations in which the terminal implant (usually PLUS) has higher daily release of TBA and EB than the preceding implant, maximum potential exposure may either occur by increasing the grow out interval until the terminal implant has been depleted (117 days for trenbolone and 141 days for estradiol for PLUS) or by concluding the grow out period on the last day of overlapping exposure. Both scenarios/regimens are modeled to define the higher-exposure scenario for that implant combination.

Model results for the complete set of re-implant combinations for trenbolone with assumptions of (1) 17β-trenbolone composition of excreted residues of 5% or 8% and (2) percent AFOs directly releasing to streams of 25% are provided in Appendix C. Model results for the complete set of re-implant combinations for estradiol assuming the percent AFOs directly releasing to streams of 25% also are provided in Appendix C. A sub-set of treatment combinations that were modeled are shown in Table 5. This subset includes representative worst-case regimens in terms of dose, re-implant timing, grow out period, and cycles per year. This subset also includes examples that illustrate the impact of single implantation vs. re-implantation for different Synovex products and combinations. It was assumed for the base case that animals were restocked the following day, that all manure produced by animals in each production cycle remains in the feedlot and thus available for runoff, that the lot was scraped with 70% efficiency at the end of the grow-out period immediately before restocking with fresh animals, and that no till practices were used for manure applied to cropland. The latter two assumptions are conservative as it is common feedlot practice to remove or mound some or all the manure in the lot periodically which lowers the total amount of residues present in the feedlot available for potential runoff and because a variety of tillage practices are used to incorporate manure into cropland.

Note: Section 5.3 illustrates the impact of tillage practices and scraping efficiency, respectively, on TBA runoff resulting PEC.

4.6 Model Input Parameters

Model production involves individual simulations of SynovexPRZM for each of the three sources of trenbolone and estradiol residue: feedlot, pasture, and manured cropland. Each simulation is run for 30 consecutive years using historical weather data for the region. The time series output of trenbolone and estradiol in runoff, from both dissolved chemical and in eroded manure from each simulation are multiplied by the proportions of feedlot, pasture, and manured cropland in the watershed (PCA factors) to provide a total mass of trenbolone and estradiol entering the reservoir on each day of the 30-year simulation. The resulting buildups of trenbolone and estradiol in the reservoir are simulated with VVWM. The maximum 21-day concentration for each year are ranked and the 90th percentile concentration is used as the PEC for the risk assessment. The annual maximum 21-day concentration is calculated from a rolling average. The 90th percentile of the annual maximum series corresponds to a 10-year return period.

The required inputs used in SynovexPRZM and VVWM models were selected based on conservative assumptions as described in the 2014 EA (Zoetis, 2014). A detailed list of inputs used in SynovexPRZM's input file is listed in Appendix B. Table 4 lists the physical and chemical properties of trenbolone and estradiol used in SynovexPRZM and VVWM runs. All changes to inputs from the 2014 EA are discussed in the following sections. Weather, crop, soil, runoff, and erosion parameters were kept the same from the previous EA (Zoetis, 2014).

4.6.1 Manure Depth

The depth of manure generated each day on feedlot is estimated as 0.18 cm (as shown in the equations below).

Daily manure excreted[4] $= 27.3\,\frac{kg}{head}$

Stocking density $= 270\,\frac{head}{ac}$

Total daily manure excreted $= 27.3\,\frac{kg}{head} \times 270\,\frac{head}{ac}$

$= 7371\,\frac{kg}{acre}$

Density of fresh (as-excreted) beef cattle manure[5] $= 63\,\frac{lb}{ft^3}$

$= 1000\,\frac{kg}{m^3}$

Depth of daily manure excreted $= 7371\,\frac{kg}{acre} \div 1000\,\frac{kg}{m^3} \times \frac{1\,acre}{4046.825\,m^2}$

$= 0.0018\,m$

$= 0.18\,cm$

4.6.2 Application Method and Timing

Cropland scenarios were kept the same as the 2014 EA. Planting and application scenarios for each year of the 30-year simulation are in the table below. The cropland was split assuming 90% used solid application and 10% used liquid.

Date	Action
5/4	Manure Application – Solid
5/25	Crop Emergence
5/30	Manure Application – Liquid
6/30	Manure Application – Liquid
7/24	Crop Maturation

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[4] Appendix 3 of 2014 EA (Zoetis, 2014)

[5] Manure Characteristics; MWPS-18, Section 1, Second Edition

7/30	Manure Application – Liquid
8/30	Manure Application – Liquid
10/19	Crop Harvest
10/26	Manure Application – Solid

4.6.3 Application Rates

Cropland rates were calculated using a ratio between the P_2O_5 produced in the manure and the amount of total chemical released from both implants during a feedlot cycle. If only a portion of the implant is released, the fraction of what is released is used to calculate the amount in the applied manure. An example calculation to find the total application amount of using the CHOICE-PLUS-117 TBA scenario is presented below (release rates and durations are from Table 1).

CHOICE - Daily excretion rate per unit	=	$0.7388\frac{mg}{d}$
CHOICE – Days Active in Cycle	=	$117d$
PLUS - Daily excretion rate per unit	=	$1.4777\frac{mg}{d}$
PLUS - Days Active in Cycle	=	$57d$
Cycle Duration	=	$117d$
Time Weighted Averaged Release Rate	=	$\left(0.7388\frac{mg}{d}\times 117d + 1.4777\frac{mg}{d}\times 57d\right)\div 117d$
		$1.4587\frac{mg}{d}$
Daily P_2O_5 Excreted	=	$0.0747\frac{kg\ P_2O_5}{d}$
Ratio of Trenbolone to P_2O_5 in Manure	=	$1.4587\frac{mg}{d}\div 0.0747\frac{kg\ P_2O_5}{d}$
	=	$19.5275\frac{mg}{kg\ P_2O_5}$
Total P_2O_5 Application from Manure	=	$35.986\frac{kg\ P_2O_5}{ac}$
Total Chemical Applied from Manure	=	$35.986\frac{kg\ P_2O_5}{ac}\times 19.5275\frac{mg}{kg\ P_2O_5}$
	=	$702.7170\frac{mg}{ac}$
	=	$7021.7170\frac{mg}{ac}\times 0.001\frac{g}{mg}\times 2.47105\frac{ac}{ha}$
	=	$1.7364\frac{g}{ha}$
Percent Application Applied as a solid	=	90%
Number of solid applications	=	2
Solid Rate Per Application	=	$1.7364\frac{g}{ha}\times 90\% \div 2$
	=	$0.7814\frac{g}{ha}$
Percent Application Applied as a liquid	=	10%
Number of liquid applications	=	4

$$\text{Liquid Rate Per Application} = 1.7364\frac{g}{ha} \times 10\% \div 4$$
$$= 0.0434\frac{g}{ha}$$

In feedlots, each implant had a constant daily mass of trenbolone and estradiol released for the pay-out period of the implant or re-implant. During periods of overlap the application reflected the total daily release of all active implants. Using the CHOICE-PLUS-117 TBA scenario as an example, CHOICE was applied on day 0 and the first 60 days (days 0-59) only released the CHOICE implant, the PLUS implant was applied on day 60 and for 57 days (days 60-116) both the CHOICE and PLUS implants were released. Scraping occurs on day 116 ending the cycle. The result is that 60 days of potential release from the PLUS implant did not occur in this scenario. However, because animals are immediately re-stocked to begin the next production cycle, TBA and EB are present and released from animals every day throughout the 30-year modeling period. Note, since day 0 is considered part of a cycle a 117-day cycle ends on day 116 (i.e., occurs from day 0 to day 116). In contrast for the CHOICE-PLUS-177 scenario, similar calculations were performed but with full release of the TBA from the second implant before commencing the next production cycle.

No changes were made to the pasture simulations from the 2014 EA as there is no reimplantation for the pasture cattle. For implanted animals in pasture, daily excretion occurs from April 1st to October 28th every year.

Application rates of trenbolone and estradiol residues for each scenario and land use are presented in Table 6 and Table 7, respectively.

4.6.4 Cropland Tillage Parameters

No till parameters were used for the cropland SynovexPRZM runs. A breakdown of tillage practices in Iowa and two major counties (Lyon and Sioux) are in the following table (Source: USDA NASS (2017).

	Iowa	**Lyon**	**Sioux**
Total Cropland (ac)	26,545,960	318,213	453,455
No Till (ac)	8,196,199 (31%)	66,480 (21%)	89,870 (20%)
Reduced Tillage (ac)	10,132,599 (38%)	146,956 (46%)	211,087 (47%)
Conventional Tillage (ac)	5,018,129 (19%)	93,967 (30%)	132,188 (29%)

To assess the effects that different tillage practices have on runoff of trenbolone and estradiol, a sensitivity analysis was conducted using the conventional tillage method and it was compared to the no till simulations. Reduced tillage was not considered as it is a medium set up between no-till and conventional till and therefore conventional tillage would always have the bigger difference from no-till. The different inputs used for the tillage options are in the following table. These values were taken from the PRZM3 manual (Suárez, 2005).

	No Till	Conventional Tillage
Curve Number Change	-10%	0%
RUSLE C Factors	No Till	Conventional Till
Solid Application Depth (cm)	5	15
Liquid Application Depth (cm)	5	5

Model scenarios used in the EA represented no-till practices in the cropland SynovexPRZM runs because the practice results in the highest runoff of TB and EA (results of both practices are discussed in Section 5 and Appendix D).

4.6.5 Feedlot Scraping Efficiency

Feedlot scraping was simulated in a conservative matter by only simulating scraping to occur on the last day of the cycle and only 70% of the total manure is removed. In reality, scraping can occur multiple times throughout a single cycle and is most likely to have a scraping efficiency of 90% or more (Clay, 2020). To determine the effects of scraping, a sensitivity analysis was conducted with the higher 90% efficiency and compared to the 70% efficiency runs. Results of both scaping efficiencies are discussed in Section 5 and Appendix D.

4.6.6 Source Contributions

Time series loadings predicted by SynovexPRZM for feedlots, cropland, and pasture were scaled to reflect the fraction of the watershed of each source, resulting in PCA factors of 0.108%, 88.7%, and 4.12%, respectively (see Section 4.2). Remaining area was assumed to be cropland not treated with manure.

Feedlot contributions were reduced further to reflect the fraction of feedlots having direct runoff discharging to surface water. In modeling direct runoff from feedlots, it was assumed that all CAFOs are in compliance with the Clean Water Act and are not discharging to surface water. In the 2014 EA, 25% of small and medium AFOs were assumed to directly discharge to surface waters (i.e., they are significant contributors of pollutants to surface waters). This value was based on an estimated 17% of AFOs on a nationwide basis have runoff that may directly enter surface water. Based on CVM guidance (I-012466-Q-0046-OT, I-012467-Q-0048-OT, I-012468-Q-0045-OT), a value of 25% was modeled for the present assessment.

5.0 RESULTS AND DISCUSSION

For environmental risk assessment, a deterministic risk quotient (RQ) approach was used in the 2014 Synovex ONE EA (Zoetis 2014). RQ was calculated to be the PEC produced from the model divided by the PNEC. The PEC value was calculated as the 90th centile of the annual maximum series for consistency with USEPA procedures (USEPA 2020). PNEC values for trenbolone and estradiol are described in Sections 6.3.2 and 6.3.3 of the 2014 Synovex ONE EA and were the following values:

- 17α-estradiol: 25 ng/L
- 17β-estradiol: 1.4 ng/L
- 17α-trenbolone: 3.2 ng/L
- 17β-trenbolone: 0.25-0.5 ng/L. (For conservatism, 0.25 ng/L was used in RQ calculations.)

To calculate RQs for trenbolone, either 5% or 8% of the total trenbolone-related residues excreted by cattle were assigned a biological activity equivalent to 17β-trenbolone (most potent metabolite) and the remainder was assigned an activity equivalent to 17α-trenbolone (less potent metabolite). RQs for trenbolone were calculated as the sum of independent RQs for 17β-trenbolone and 17α-trenbolone based upon the fraction of the total residues attributed to 17β-trenbolone (f_β) and to 17α-trenbolone (f_α), see equation below.

$$RQ = RQ_\alpha + RQ_\beta = \frac{PEC \times f_\alpha}{PNEC_\alpha} + \frac{PEC \times f_\beta}{PNEC_\beta}$$

For estradiol, all residue was assigned an activity equivalent to 17β-estradiol, the most potent metabolite. RQs were calculated the same way as above but the equation is simplified as shown below.

$$RQ = \frac{PEC}{PNEC_\beta}$$

5.1 Standard Scenarios – Annual Maximum Series

The PECs and RQs for trenbolone and estradiol in the Iowa watershed for selected scenarios are summarized for selected re-implant combinations and regimens. Results for trenbolone with 5% and 8% 17β-trenbolone are in Table 8. Estradiol results are shown in Table 9. Results for additional re-implant scenarios are provided in Appendix C.

PECs for trenbolone and estradiol residues were estimated from the combined contributions from feedlots, pasture, and manured cropland discussed in Section 4.6.6. In the discussion that follows in this section, RQ values for trenbolone are provided for selected examples with 8% 17β-trenbolone. RQs corresponding to 5% 17β-trenbolone follow a similar pattern with lower values. Because RQ values for estradiol are always lower than trenbolone in each scenario, trenbolone is the focus of this exercise.

The trenbolone CHOICE-PLUS-117 scenario had a PEC of 0.88 ng/L and 0.54 RQ for 8% 17β-trenbolone. The extended CHOICE-PLUS scenario (CHOICE-PLUS-177) for trenbolone allowed for the higher-dose second implant (PLUS) to completely release and created a third stage where only the second implant was releasing into the environment (see Table 6 for trenbolone and Table 7 for estradiol). The extended scenario had a slightly increased PEC of 0.91 ng/L and RQ of 0.56 compared to the shorter grow out period.

A lower dosage second implant scenario (CHOICE-CHOICE) was also simulated. For trenbolone, the PEC decreased from the CHOICE-PLUS-117 scenario to 0.68 ng/L and the RQ reduced to 0.41. Similarly, a lower dosage second implant scenario using ONE-Feedlot was simulated (CHOICE-ONE). This scenario also showed a decrease for trenbolone vs. the CHOICE-PLUS scenario with a PEC of 0.71 ng/L and RQ of 0.43.

The above re-implant scenarios were also compared with single implant scenarios to determine the effects of re-implantation. For trenbolone, the CHOICE, PLUS, ONE, and ONEg single implant scenarios resulted in PECs of 0.48, 0.95, 0.55, and 0.41 ng/L and RQs of 0.29, 0.58, 0.33 and 0.25, respectively. Trenbolone and estradiol PEC and RQ results for the above re-implant scenarios with Choice as lead implant were approximately two times the results produced for a single CHOICE implant and similar to a single PLUS implant.

A similar comparison was conducted with PLUS as the first implant. The scenarios of PLUS-PLUS-117 for trenbolone and PLUS-PLUS-141 for estradiol produced the highest PEC and RQs of the entire simulation set among two-implant combinations. The trenbolone PLUS-PLUS-117 scenario had a PEC of 1.36 ng/L and RQ of 0.83. The trenbolone extended scenario (PLUS-PLUS-177) had a slightly lower PEC of 1.26 ng/L compared to the shorter grow out period and an RQ of 0.77.

The scenarios in which the 2nd implant had lower daily release rates of TBA and EB showed a similar pattern as the CHOICE scenarios. For trenbolone, the PLUS-CHOICE scenario showed a decreased exposure with a PEC of 1.16 ng/L and an RQ of 0.70. The PLUS-ONE scenario for trenbolone also showed a decreased exposure with PEC of 1.18 ng/L with an RQ of 0.72.

Four three-implant combinations were also compared. For trenbolone, the CHOICE-PLUS-PLUS-177 scenario had a PEC of 1.17 ng/L and an RQ of 0.71. The CHOICE-CHOICE-PLUS-177 and CHOICE-ONE-PLUS-271 scenarios both had lower PECs than the CHOICE-PLUS-PLUS-177 scenario. The PLUS-PLUS-PLUS-177 scenario had the highest PEC of 1.51 ng/L and RQ of 0.92. As this and all other scenarios are below the 1.0 threshold, all re-implant combinations can be considered acceptable based on the numerous conservative assumptions on which this is EA is based.

5.2 Standard Scenarios – Daily Risk Quotients

Daily RQs were also calculated to characterize the occurrence of any events in which PEC exceeds PNEC. Selected scenarios for trenbolone with events in which RQ exceeded 1.0 are summarized in Table 10 for illustration. There were no such events for estradiol. Results for all scenarios with excursion events are shown in Appendix C.

To visualize daily risk, images were created across the 30-year model period for representative implant combinations (see Figure 3 through Figure 13). The left panel of each image shows the daily RQ values over the 30-year modeling period of each simulation. The yearly maximum is a black hollow circle and the 90th percentile of the yearly maximum is a red circle. The purple dashed line indicates a RQ of 1.0 to identify periods of time when the RQ exceeds 1.0. The duration associated with these events are indicated above the event. The right panel plots all RQ values as a cumulative distribution function and the fraction of all values less than 1.0 is plotted at the top left of the panel. Images for all runs are in Appendix C.

May 6, 1983 marks the initiation of the events with the highest daily RQs. The peak daily RQs were caused by a rainfall 3.76 cm. Figure 14 shows the daily PEC and daily rainfall for three example scenarios. for the month prior to the rainfall event and five months after. The rainfall event occurred towards the end of the current cycle for all scenarios: day 101 for CHOICE-PLUS-

117, day 92 for PLUS-PLUS-177, and day 236 for PLUS-PLUS-PLUS-237. For PLUS-PLUS-PLUS-237, the peak day occurred the day before scraping and therefore the day with the largest amount of build up for that cycle.

5.3 Sensitivity Analysis

The base CHOICE-PLUS scenarios (CHOICE-PLUS-117 for trenbolone and CHOICE-PLUS-141 for estradiol) were also used to compare effects of tillage and scraping efficiency.

	Trenbolone			Estradiol	
Scenario	**21d PEC (ng/L)**	**RQ (5% beta)**	**RQ (8% beta)**	**21d PEC (ng/L)**	**RQ (100% beta)**
Base Scenario (No Till, 70% Scraping)	0.88	0.44	0.54	0.09	0.07
No Till, 90% Scraping	0.88	0.44	0.54	0.09	0.07
Conventional Tillage, 70% Scraping	0.75	0.37	0.46	0.08	0.06

Scraping efficiency was increased to 90% using data from Clay (2020) indicating this is a more realistic value. For both estradiol and trenbolone, no changes to PEC or RQ were shown.

Changing the base scenario to a conventional tillage approach produced lower PEC and RQ for trenbolone and estradiol and therefore decreased the number, magnitude, and duration of events in in which daily RQs exceeded 1 versus no till applications. For trenbolone, the PEC decreased to 0.75 ng/L with a corresponding reduction in RQ. For estradiol, a smaller influence was demonstrated, with PEC decreasing to 0.08 ng/L and RQ reducing to 0.06.

6.0 SUMMARY AND CONCLUSIONS

An aquatic exposure assessment was conducted for trenbolone and estradiol associated with the use of Synovex® brand implants in beef cattle. The assessment utilized a modeling framework established by the 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.

The assessment was conducted for a high exposure environment based on a national geospatial analysis that combined normal annual precipitation with areas of high beef cattle production and density of feedlots. 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.

A literature review was conducted to bring the state of knowledge on the environmental fate and toxicity of TBA and EB up to date. The national geospatial analysis was updated using 2017 Agricultural Census to confirm watershed selection. The PCA factors were updated to reflect 2018 Iowa Department of Natural Resources surveys.

Other conservative assumptions from the 2014 EA have been carried forward into this current EA:

- Modeling addressed trenbolone and estradiol residues runoff from feedlots, pasture cattle, and cropland treated with manure under the assumption that all cattle in the watershed were assumed to receive implants.
- No decrease in excreted daily masses of trenbolone or estradiol due to metabolism in the treated animal.
- High feedlot stocking density: 270 head/acre.
- Fully stocked feedlot every day of the year.
- High pasture stocking density: 3.15 head/acre and that all pasture animals are implanted with Synovex ONE Grass/Grower.
- No degradation of residues in manure in the feedlot.
- Cropland application rate calculated from daily release rate of trenbolone or estradiol, phosphorus application rate for corn grain, and daily phosphorus excretion rate in beef cattle manure.
- All manure generated on feedlots is applied to croplands in the watershed up to the total acreage of cropland in the watershed. 90% of the manure generated is applied to croplands as solid manure and 10% is applied as liquid manure applied with irrigation water.
- Solid manure is applied two times a year- spring and fall before and after corn crop cycle and liquid manure is applied 4 times a year with irrigation water during the crop cycle.
- Curve number for feedlots used in NRCS curve number method is 95, representative of high runoff potential.
- 25% of the Animal Feeding Operations with less than 1,000 head (AFOs) in the watershed were assumed to be directly releasing to nearby streams on a nationwide level, which was rounded up from 17% based on the calculations in Appendix 9 of the 2014 EA.
- PECs were based on 90th percentile annual maximum, 21-day concentration from the model simulations, corresponding to a 10-year recurrence interval.

All cropland simulations were conducted under no-till reduces the depth of incorporation of manure which increases the amount of TBA and EB available for runoff.

RQs for trenbolone were calculated under a 25% AFO assumption and two assumptions for trenbolone based upon the amount excreted in the form of 17β-trenbolone (5% and 8% with remaining residues assigned to 17α-trenbolone). For trenbolone, the highest PECs and risk quotients for multi-implant regimens were produced for the PLUS-PLUS-PLUS-177 scenario with an initial implant of PLUS, re-implantations of PLUS after 60 and 120 days, and a grow-out period of 177 days. The RQs associated with PLUS-PLUS-PLUS-177 are 0.75 and 0.92 assuming 5% and 8% excretion of 17β-trenbolone, respectively. As these and all other scenarios are below the 1.0 threshold, all re-implant combinations can be considered acceptable based on the numerous conservative assumptions on which this is EA is based.

Risk quotients for estradiol were calculated conservatively assuming that 100% of estradiol-related residues are excreted in the form of 17β-estradiol. As with trenbolone, the highest PECs and risk quotients for the two-implant regimens were produced for PLUS-PLUS-PLUS-201 with an initial implant of PLUS, re-implantations of PLUS after 60 and 120 days, and a grow-out period of 201 days. The RQ for estradiol associated with PLUS-PLUS-PLUS-201 is 0.11 with no daily events in which RQ > 1.0, thus indicating that all Synovex implants and re-implants conditions are under levels of concern for estradiol.

Daily RQs were plotted for the select scenarios in Figure 3 through Figure 13 and all implant regimes in Appendix C. For trenbolone, two implant regimes had a single event occurrence with a daily RQ ≥ 1.0 over the 30-year simulation under the assumption of 5% excretion of 17β-trenbolone. The highest RQ was associated with PLUS-PLUS-PLUS-177 with a peak daily RQ of 1.11 and mean RQ of 1.05 over the duration of the event. For 8% excretion of 17β-trenbolone, five implant scenarios had a single event with an RQ ≥ 1.0 and one implant regime (PLUS-PLUS-PLUS-177) had two events. The highest occurrence with PLUS-PLUS-PLUS-177 had a peak RQ of 1.35 and a mean RQ of 1.17 during the second event. Daily RQs were less than 1.0 for all estradiol implant treatment regimes

Final RQs for various re-implant scenarios will be determined after the Center for Veterinary Medicine (CVM) completes its review of cattle metabolism data submitted for trenbolone (I-012466-P-0047-NV, I-012467-P-0049-NV, and I-012468-P-0046-NV) to establish the value for the 17β-trenbolone composition of excreta.

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TABLES

Table 1. Trenbolone acetate (TBA) and Estradiol (EB) release rates and duration for various Synovex products

Trenbolone acetate (TBA) release rates for various Synovex products					
Implant	**Total Dose (mg TBA)**	**Estimated Duration of Release (days) [C]**	**Metabolite Adjustment Factor [F]**	**Average Daily Release of TBA (mg/d) [B]**	**Average Daily Release of Trenbolone (mg/d) [270.4/312.4] [A]**
ONE Feedlot	200	211	None (1.000)	0.9466	0.8193
ONE Grass/Grower	150	Same as ONE Feedlot	None (1.000)	0.7100 [D]	0.6145
PLUS	200	117	None (1.000)	1.7073	1.4777
CHOICE	100	Same as PLUS	None (1.000)	0.8537 [E]	0.7388

[A] Stoichiometric conversion from trenbolone acetate to 17α- or 17β-trenbolone. Ratio = 0.86556.
[B] Average daily release values for TBA are from Appendix 13.4 of the 2014 Synovex ONE EA. Assumed constant average daily release of TBA from implant(s) over the entire duration in the feedlot.
[C] Estimated duration of release = total mg TBA in implant (200 mg for PLUS and ONE Feedlot, 150 mg for ONE Grass/Grower, and 100 mg for CHOICE) ÷ average daily release rate of TBA.
[D] Coated pellets: ONE Feedlot = 8 pellets = 200 mg vs. ONE Grass/Grower = 6 pellets = 150 mg. Multiplied rate for Feedlot by 0.75 for ONE Grass/Grower.
[E] Uncoated pellets: PLUS = 8 pellets = 200 mg vs. CHOICE = 4 pellets = 100 mg. Multiplied rate for PLUS by 0.5 for CHOICE.
[F] A metabolic adjustment factor was applied in Appendix 6 of the 2014 Synovex ONE EA for trenbolone residues. For conservatism, no adjustment factor is applied when calculating PECs. The percentages of metabolites assigned to 17β- and 17α-trenbolone are applied in the Risk Quotient calculation. All trenbolone-related metabolite residues are assumed to be at least as potent as 17α-trenbolone. No metabolites are discounted.

Estradiol benzoate (EB) release rates for various Synovex products					
Implant	**Total Dose (mg EB)**	**Estimated Duration of Release (days) [C]**	**Metabolite Adjustment Factor [F]**	**Average Daily Release of EB (mg/d) [B]**	**Average Daily Release of Estradiol (mg/d) [272.38/376.49] [A]**
ONE Feedlot	28	267	None (1.000)	0.1049	0.07590
ONE Grass/Grower	21	Same as ONE-Feedlot	None (1.000)	0.0787 [D]	0.05692
PLUS	28	141	None (1.000)	0.1980	0.1433
CHOICE	14	Same as PLUS	None (1.000)	0.0990 [E]	0.07163

[A] Stoichiometric conversion from Estradiol Benzoate (EB) to 17α- or 17β-estradiol (E2). Ratio = 0.7235.
[B] Average daily release values for EB are from Appendix 13.4 of the 2014 Synovex ONE EA. Assumed constant average daily release of EB from implant(s) over the entire duration in the feedlot.
[C] Estimated duration of release = total mg EB in implant (28 mg for PLUS and ONE Feedlot, 21 mg for ONE Grass/Grower, and 14 mg for CHOICE) ÷ average daily release rate of EB.
[D] Coated pellets: ONE-Feedlot = 8 pellets = 28 mg vs. ONE Grass/Grower = 6 pellets = 21 mg. Multiplied rate for Feedlot by 0.75 for ONE Grass/Grower.
[E] Uncoated pellets: PLUS = 8 pellets = 28 mg vs. CHOICE = 4 pellets = 14 mg. Multiplied rate for PLUS by 0.5 for CHOICE.
[F] No metabolic adjustment factor was applied in Appendix 6 of the 2014 Synovex ONE EA for estradiol.

Table 2. Relative Ranking of Selected Study Regions through time 2002, 2007, 2012, and 2017.

2002 Ag Census (for historical reference)

State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Manure Density Rank	Pasture Cattle Density Rank
		(n=1,953)	(n=700)	(n=1,772)	(n=2,795)	(n=3,019)
Iowa	Lyon	98.7%	90.4%	99.7%	98.9%	83.1%
Iowa	Sioux	99.6%	97.1%	100.0%	99.9%	89.7%
Michigan	Huron	96.9%	91.8%	99.2%	96.3%	68.6%
Ohio	Mercer	92.9%	0.0%	97.2%	99.6%	68.8%
Pennsylvania	Lancaster	96.0%	78.1%	99.9%	100.0%	95.1%
Texas	Castro	99.8%	99.1%	69.3%	90.4%	99.3%

2007 Ag Census (from Zoetis Study No. A7X0R-US-12-003)

State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Manure Density Rank	Pasture Cattle Density Rank
		(n=1,380)	(n=290)	(n=1,345)	(n=2,887)	(n=3,019)
Iowa	Lyon	98.9%	96.8%	99.7%	99.6%	92.5%
Iowa	Sioux	99.5%	98.4%	99.8%	99.9%	97.5%
Michigan	Huron	95.5%	89.6%	98.1%	97.9%	75.3%
Ohio	Mercer	96.0%	88.4%	99.4%	99.8%	88.4%
Pennsylvania	Lancaster	94.9%	72.8%	99.6%	100.0%	94.9%
Texas	Castro	99.9%	99.6%	74.1%	95.4%	99.7%

2012 Ag Census

State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Manure Density Rank	Pasture Cattle Density Rank
		(n=1,203)	(n=602)	(n=836)	(n=2,852)	(n=3,019)
Iowa	Lyon	98.9%	95.5%	99.5%	99.9%	92.6%
Iowa	Sioux	99.8%	98.8%	99.6%	100.0%	97.1%
Michigan	Huron	97.0%	94.3%	98.2%	98.5%	85.6%
Ohio	Mercer	94.5%	85.1%	94.8%	99.7%	78.4%
Pennsylvania	Lancaster	94.4%	82.8%	99.8%	100.0%	95.7%
Texas	Castro	99.6%	99.3%	0.0%	91.3%	98.4%

2017 Ag Census

State	County	Feedlot Density Rank	>500 Head Feedlot Density Rank	<500 Head Feedlot Density Rank	Acres Manure Density Rank	Pasture Cattle Density Rank*
		(n=1,104)	(n=645)	(n=826)	(n=2,884)	(n=3,019)
Iowa	Lyon	99.0%	98.4%	99.2%	99.9%	92.6%
Iowa	Sioux	99.6%	99.6%	99.6%	99.9%	97.1%
Michigan	Huron	94.9%	96.5%	98.8%	98.7%	85.6%
Ohio	Mercer	95.1%	93.5%	97.9%	99.5%	78.4%
Pennsylvania	Lancaster	91.1%	90.9%	99.3%	99.8%	95.7%
Texas	Castro	99.3%	99.7%	0.0%	94.7%	98.4%

* based on 2012 Ag Census as pasture cattle have no material impact on RQs

Table 3. Revised method to estimate application rate for constant concentration feedlot model

Implant	Daily TBA Excretion Rate (g/ha) *	Daily EB Excretion Rate (g/ha) *
ONE Feedlot	0.5466	0.0506
ONE Grass/Grower	0.4100	0.0380
PLUS	0.9859	0.0956
CHOICE	0.4929	0.0478

* Example calculations using PLUS for TBA

Daily excretion rate per unit (from Table 1) $= 1.4777\,\frac{mg}{head}$

Daily excretion rate per area $= 1.4777\,\frac{mg}{head} \times 270\,\frac{head}{ac}$

$$= 398.979\,\frac{mg}{ac}$$

$$= 398.979\,\frac{mg}{ac} \times 0.001\,\frac{g}{mg} \times 2.47105\,\frac{ac}{ha}$$

$$= 0.9859\,\frac{g}{ha}$$

Table 4. Physical and Chemical Properties of Trenbolone and Estradiol 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 K_{OC}	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 5. Selected Implant Scenarios for Trenbolone and Estradiol*

Trenbolone Scenario	Implant 1 (on day 0)	Implant 2 (Implant day)	Implant 3 (Implant day)	Days Per Production Cycle	Production Cycles Per Year
CHOICE	CHOICE	--	--	117	3.12
PLUS	PLUS	--	--	117	3.12
ONEg	ONEg	--	--	211	1.73
ONE	ONE	--	--	211	1.73
CHOICE-PLUS-117	CHOICE	PLUS (60)	--	117	3.12
CHOICE-PLUS-177	CHOICE	PLUS (60)	--	177	2.06
CHOICE-CHOICE	CHOICE	CHOICE (60)	--	117	3.12
CHOICE-ONE	CHOICE	ONE (60)	--	117	3.12
PLUS-PLUS-117	PLUS	PLUS (60)	--	117	3.12
PLUS-PLUS-177	PLUS	PLUS (60)	--	177	2.06
PLUS-CHOICE	PLUS	CHOICE (60)	--	117	3.12
PLUS-ONE	PLUS	ONE	--	117	3.12
ONE-PLUS-257	ONE	PLUS (140)	--	257	1.42
ONE-ONE	ONE	ONE (140)	--	211	1.73
CHOICE- PLUS-PLUS-177	CHOICE	PLUS (60)	PLUS (120)	177	2.06
CHOICE-CHOICE-PLUS-177	CHOICE	CHOICE (60)	PLUS (120)	177	2.06
CHOICE-ONE-PLUS-271	CHOICE	ONE (60)	PLUS (200)	271	1.35
PLUS-PLUS-PLUS-177	PLUS	PLUS (60)	PLUS (120)	177	2.06

Estradiol Scenario	Implant 1 (on day 0)	Implant 2 (Implant day)	Implant 3 (Implant day)	Days Per Production Cycle	Production Cycles Per Year
CHOICE	CHOICE	--	--	141	2.59
PLUS	PLUS	--	--	141	2.59
ONE	ONE	--	--	267	1.37
ONEg	ONEg	--	--	267	1.37
CHOICE-PLUS-141	CHOICE	PLUS (60)	--	141	2.59
CHOICE-PLUS-201	CHOICE	PLUS (60)	--	201	1.82
CHOICE-CHOICE	CHOICE	CHOICE (60)	--	141	2.59
CHOICE-ONE	CHOICE	ONE (60)	--	141	2.59
PLUS-PLUS-141	PLUS	PLUS (60)	--	141	2.59
PLUS-PLUS-201	PLUS	PLUS (60)	--	201	1.82
PLUS-CHOICE	PLUS	CHOICE (60)	--	141	2.59
PLUS-ONE	PLUS	ONE	--	141	2.59
ONE-PLUS-281	ONE	PLUS (140)	--	281	1.30
ONE-ONE	ONE	ONE (140)	--	267	1.37
CHOICE- PLUS-PLUS-201	CHOICE	PLUS (60)	PLUS (120)	201	1.82
CHOICE-CHOICE-PLUS-201	CHOICE	CHOICE (60)	PLUS (120)	201	1.82
CHOICE-ONE-PLUS-327	CHOICE	ONE (60)	PLUS (200)	327	1.12
PLUS-PLUS-PLUS-201	PLUS	PLUS (60)	PLUS (120)	201	1.82

* Complete list of scenarios modeled and their output is provided in Appendix D.

Table 6. Application Scenario Rates for Trenbolone (units in g/ha)

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Active Implant(s)*	Rate	
CHOICE	0.879	0.396	0.022	0-116	CHOICE	0.4929	0.00478
PLUS	1.759	0.792	0.044	0-116	PLUS	0.9859	0.00478
ONE	0.975	0.439	0.024	0-211	ONE	0.5466	0.00478
ONEg	0.732	0.329	0.018	0-211	ONEg	0.4100	0.00478
CHOICE-PLUS-117	1.736	0.781	0.043	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+PLUS	1.4788	
CHOICE-PLUS-177	1.744	0.785	0.044	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+PLUS	1.4788	
				117-176	PLUS	0.9859	
CHOICE-CHOICE	1.308	0.589	0.033	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+CHOICE	0.9858	
CHOICE-ONE	1.355	0.610	0.034	0-59	CHOICE	0.4929	0.00478
				60-116	ONE	0.5466	
PLUS-PLUS-117	2.616	1.177	0.065	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+PLUS	1.9718	
PLUS-PLUS-177	2.326	1.046	0.058	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+PLUS	1.9718	
				117-176	PLUS	0.9859	
PLUS-CHOICE	2.188	0.984	0.055	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+CHOICE	1.4788	
PLUS-ONE	2.234	1.005	0.056	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+ONE	1.5325	
ONE-PLUS-257	1.602	0.721	0.040	0-139	ONE	0.5466	0.00478
				140-210	ONE+PLUS	1.5325	
				211-256	PLUS	0.9859	
ONE-ONE	1.303	0.587	0.033	0-139	ONE	0.5466	0.00478
				140-210	ONE+ONE	1.0932	
CHOICE-PLUS-PLUS-177	2.311	1.040	0.058	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+PLUS	1.4788	
				117-119	PLUS	0.9859	
				120-176	PLUS+PLUS	1.9718	
CHOICE-CHOICE-PLUS-177	1.729	0.778	0.043	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+CHOICE	0.9858	
				117-119	CHOICE	0.4929	
				120-176	CHOICE+PLUS	1.4788	
CHOICE-ONE-PLUS-271	1.600	0.720	0.040	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+ONE	1.0395	
				117-199	ONE	0.5466	
				199-270	ONE+PLUS	1.5325	
PLUS-PLUS-PLUS-177	2.892	1.301	0.072	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+PLUS	1.9718	
				117-119	PLUS	0.9859	
				120-176	PLUS+PLUS	1.9718	

* ONE-Feedlot is denoted ONE and ONE-Grower is denoted ONEg to save space

Table 7. Application Scenario Rates for Estradiol (units in g/ha)

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Active Implant(s)*	Rate	
CHOICE	0.879	0.396	0.022	0-140	CHOICE	0.0478	0.00044
PLUS	1.759	0.792	0.044	0-140	PLUS	0.0956	0.00044
ONE	0.975	0.439	0.024	0-266	ONE	0.0506	0.00044
ONEg	0.732	0.329	0.018	0-266	ONEg	0.0380	0.00044
CHOICE-PLUS-141	1.736	0.781	0.043	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+PLUS	0.1434	
CHOICE-PLUS-201	1.744	0.785	0.044	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+PLUS	0.1434	
				141-200	PLUS	0.0956	
CHOICE-CHOICE	1.308	0.589	0.033	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+CHOICE	0.0956	
CHOICE-ONE	1.355	0.610	0.034	0-59	CHOICE	0.0478	0.00044
				60-140	ONE	0.0506	
PLUS-PLUS-141	2.616	1.177	0.065	0-59	PLUS	0.0956	0.00044
				60-140	PLUS+PLUS	0.1912	
PLUS-PLUS-201	2.326	1.046	0.058	0-59	PLUS	0.0956	0.00044
				60-140	PLUS+PLUS	0.1912	
				141-200	PLUS	0.0956	
PLUS-CHOICE	2.188	0.984	0.055	0-59	PLUS	0.0956	0.00044
				60-140	PLUS+CHOICE	0.1434	
PLUS-ONE	2.234	1.005	0.056	0-59	PLUS	0.0956	0.00044
				60-140	PLUS+ONE	0.1462	
ONE-PLUS-281	1.602	0.721	0.040	0-139	ONE	0.0506	0.00044
				140-266	ONE+PLUS	0.1462	
				267-280	PLUS	0.0956	
ONE-ONE	1.303	0.587	0.033	0-139	ONE	0.0506	0.00044
				140-266	ONE+ONE	0.1013	
CHOICE-PLUS-PLUS-201	2.311	1.040	0.058	0-59	CHOICE	0.0478	0.00044
				60-119	CHOICE+PLUS	0.1434	
				120-140	CHOICE+PLUS+PLUS	0.2390	
				141-200	PLUS+PLUS	0.1912	
CHOICE-CHOICE-PLUS-201	1.729	0.778	0.043	0-59	CHOICE	0.0478	0.00044
				60-119	CHOICE+CHOICE	0.0956	
				120-140	CHOICE+CHOICE+PLUS	0.1912	
				141-200	CHOICE+PLUS	0.1434	
CHOICE-ONE-PLUS-327	1.600	0.720	0.040	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+ONE	0.0984	
				141-199	ONE	0.0506	
				199-326	ONE+PLUS	0.1462	
PLUS-PLUS-PLUS-201	2.892	1.301	0.072	0-59	PLUS	0.0956	0.00044
				60-119	PLUS+PLUS	0.1912	
				120-140	PLUS+PLUS+PLUS	0.2868	
				141-200	PLUS+PLUS	0.1912	

* ONE-Feedlot is denoted ONE and ONE-Grower is denoted ONEg to save space

Table 8. Results for Trenbolone Scenarios

Application Scenario	21d PEC (ng/L)	5% 17β-trenbolone		8% 17β-trenbolone	
		RQ	Event Count	RQ	Event Count
CHOICE	0.48	0.24	0	0.29	0
PLUS	0.95	0.47	0	0.58	0
ONE	0.55	0.27	0	0.33	0
ONEg	0.41	0.21	0	0.25	0
CHOICE-PLUS-117	0.88	0.44	0	0.54	0
CHOICE-PLUS-177	0.91	0.45	0	0.56	0
CHOICE-CHOICE	0.68	0.34	0	0.41	0
CHOICE-ONE	0.71	0.35	0	0.43	0
PLUS-PLUS-117	1.36	0.68	1	0.83	1
PLUS-PLUS-177	1.26	0.63	0	0.77	1
PLUS-CHOICE	1.16	0.58	0	0.70	1
PLUS-ONE	1.18	0.59	0	0.72	1
ONE-PLUS-257	0.88	0.44	0	0.53	0
ONE-ONE	0.72	0.36	0	0.44	0
CHOICE-PLUS-PLUS-177	1.17	0.58	0	0.71	1
CHOICE-CHOICE-PLUS-177	0.89	0.44	0	0.54	0
CHOICE-ONE-PLUS-271	0.93	0.46	0	0.57	0
PLUS-PLUS-PLUS-177	1.51	0.75	1	0.92	2

Table 9. Results for Estradiol Scenarios

Application Scenario	21d PEC (ng/L)	RQ	Event Count
CHOICE	0.05	0.03	0
PLUS	0.09	0.07	0
ONE	0.05	0.04	0
ONEg	0.04	0.03	0
CHOICE-PLUS-141	0.09	0.07	0
CHOICE-PLUS-201	0.09	0.07	0
CHOICE-CHOICE	0.07	0.05	0
CHOICE-ONE	0.07	0.05	0
PLUS-PLUS-141	0.14	0.10	0
PLUS-PLUS-201	0.13	0.09	0
PLUS-CHOICE	0.12	0.08	0
PLUS-ONE	0.12	0.08	0
ONE-PLUS-281	0.09	0.06	0
ONE-ONE	0.07	0.05	0
CHOICE-PLUS-PLUS-201	0.12	0.09	0
CHOICE-CHOICE-PLUS-201	0.09	0.07	0

Application Scenario	21d PEC (ng/L)	RQ	Event Count
CHOICE-ONE-PLUS-327	0.10	0.07	0
PLUS-PLUS-PLUS-201	0.16	0.11	0

Table 10. Trenbolone Scenario Events where RQ exceeds 1.0

Scenario	Event	Begin Date	Peak RQ	Arith. Avg. RQ	Duration (days)
5% 17β-trenbolone					
PLUS-PLUS-117	1	5/6/1983	1	1	1
PLUS-PLUS-PLUS-177	1	5/6/1983	1.11	1.05	8
8% 17β-trenbolone					
PLUS-CHOICE	1	5/6/1983	1.04	1.02	3
PLUS-PLUS-117	1	5/6/1983	1.23	1.11	15
PLUS-PLUS-177	1	5/6/1983	1.15	1.08	10
PLUS-ONE	1	5/6/1983	1.06	1.03	5
CHOICE-PLUS-PLUS-177	1	5/6/1983	1.03	1.02	2
PLUS-PLUS-PLUS-177	1	10/30/1979	1.17	1.09	14
	2	5/6/1983	1.35	1.17	23

FIGURES

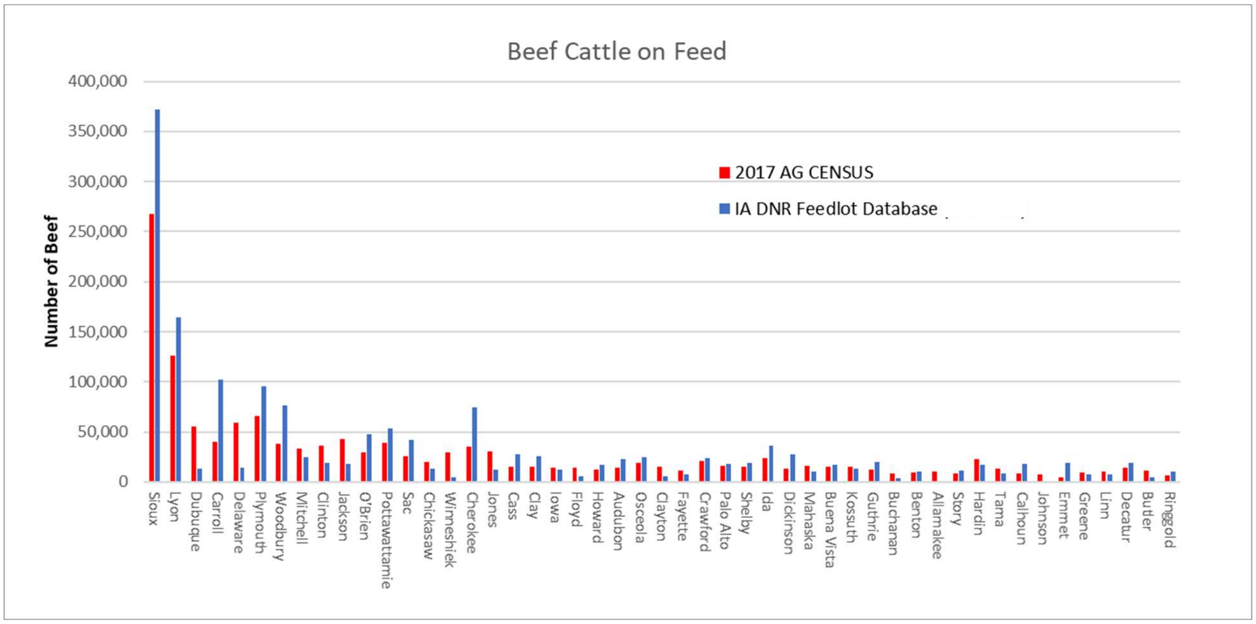

Figure 1. Feedlot beef cattle counts from Iowa DNR Feedlot database and 2017 Census of Agriculture.

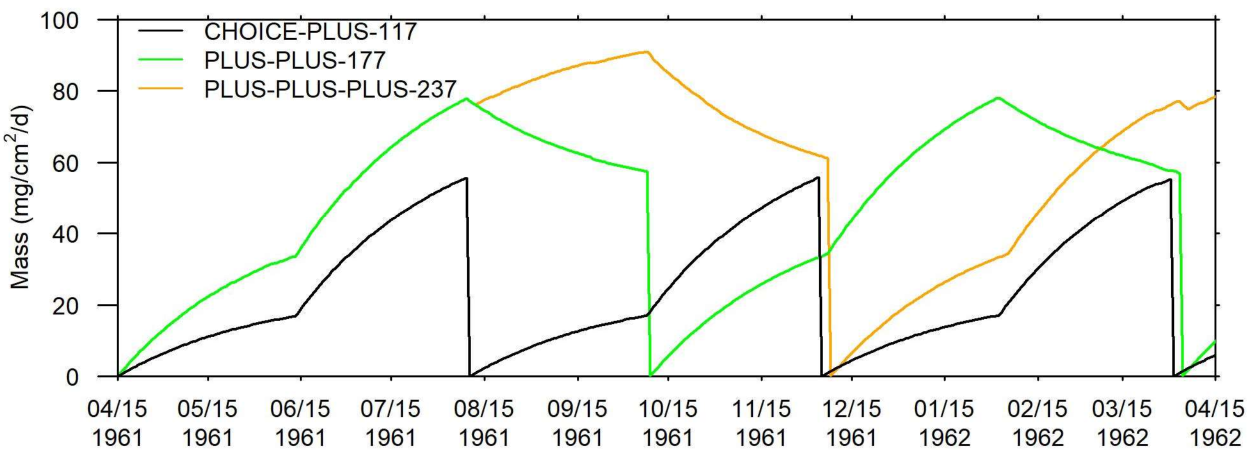

Figure 2. Time series plot of total mass of trenbolone in top 10 cm manure layer in feedlot for CHOICE-PLUS, PLUS-PLUS-177, and PLUS-PLUS-PLUS-237 implant combinations with 25% AFO value (4/15/1961 to 4/15/1962)

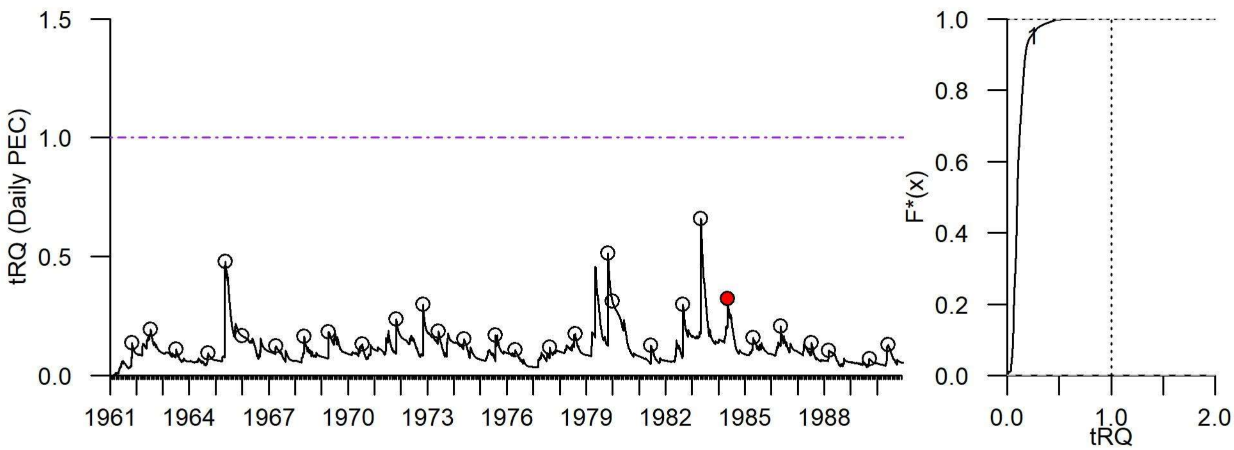

Figure 3. Daily trenbolone RQs for CHOICE-PLUS-117 with 5% beta

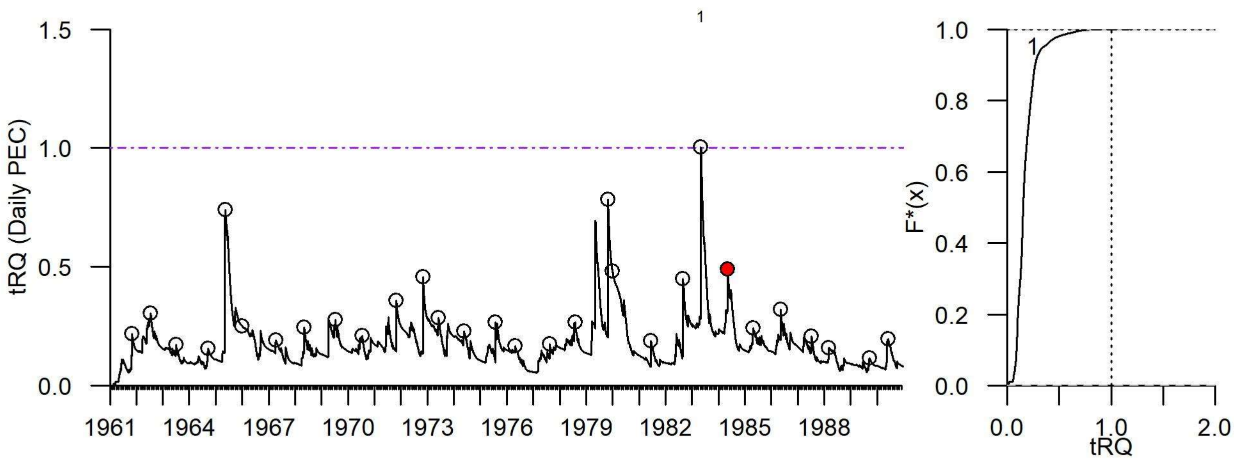

Figure 4. Daily trenbolone RQs for PLUS-PLUS-117 with 5% beta

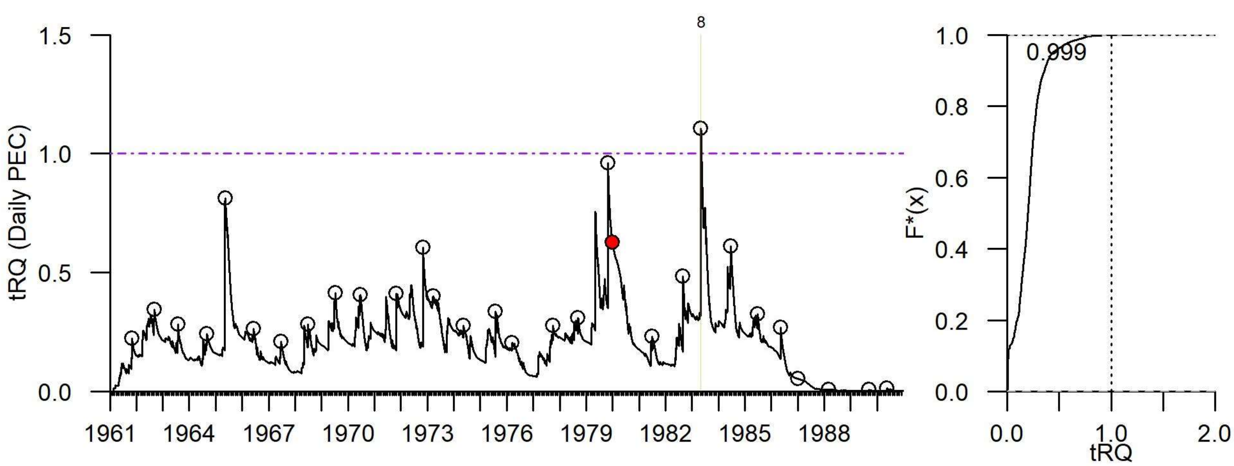

Figure 5. Daily trenbolone RQs for PLUS-PLUS-PLUS-177 with 5% beta

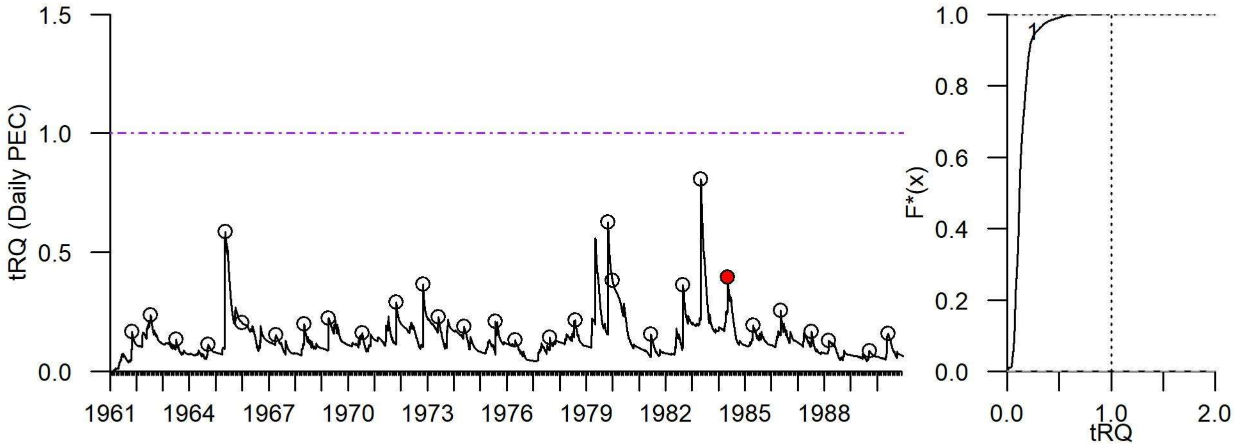

Figure 6. Daily trenbolone RQs for CHOICE-PLUS-117 with 8% beta

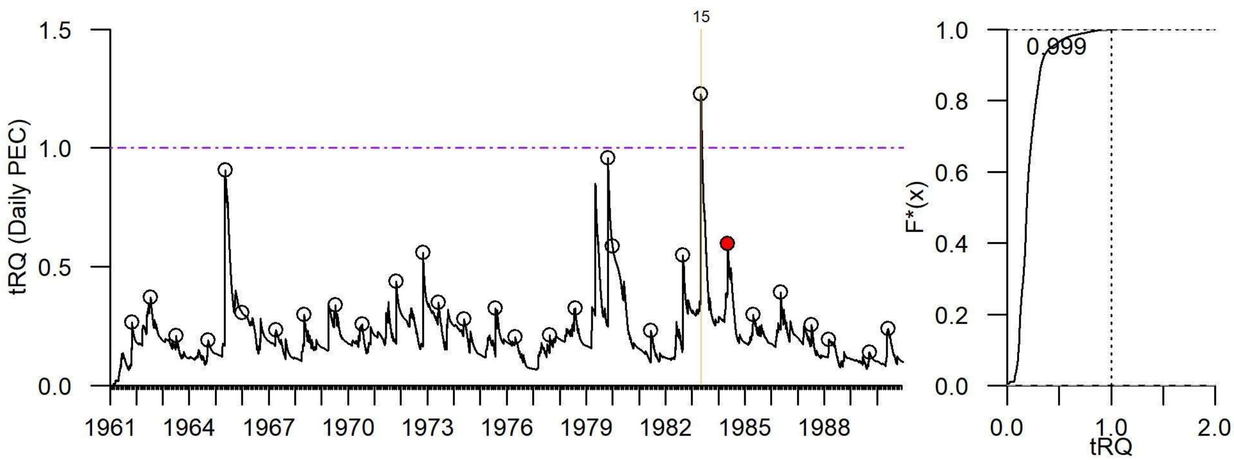

Figure 7. Daily trenbolone RQs for PLUS-PLUS-117 with 8% beta

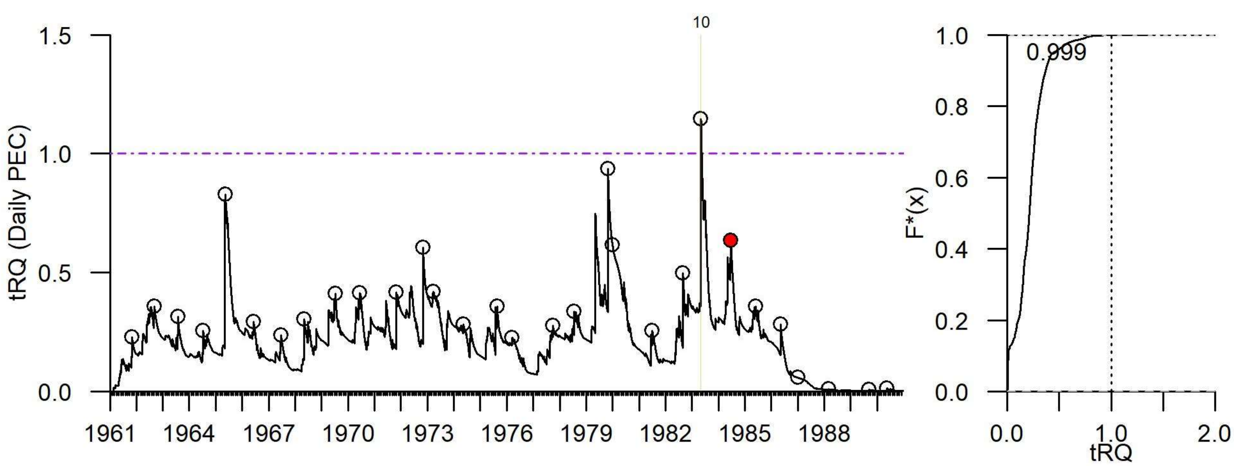

Figure 8. Daily trenbolone RQs for PLUS-PLUS-177 with 8% beta

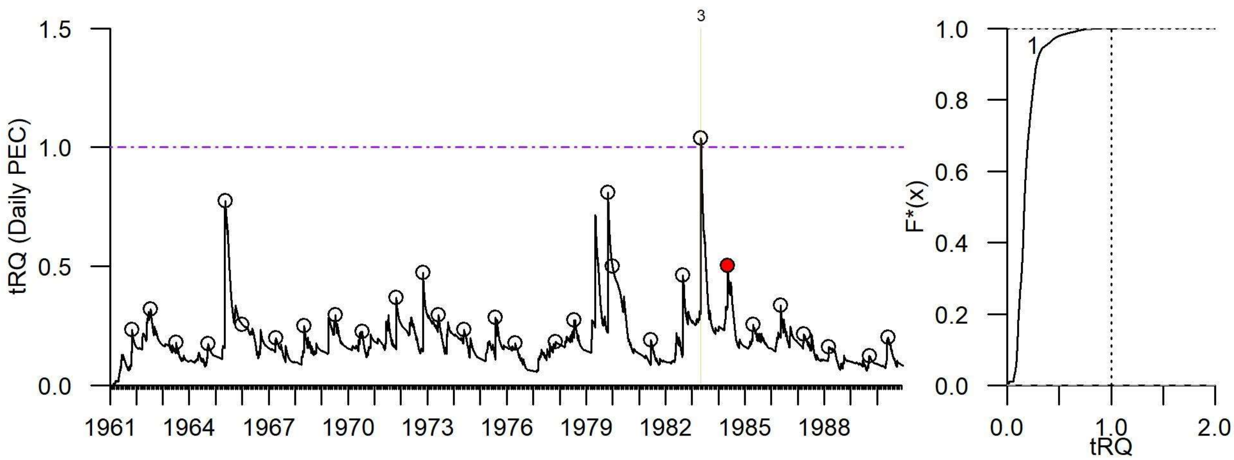

Figure 9. Daily trenbolone RQs for PLUS-CHOICE with 8% beta

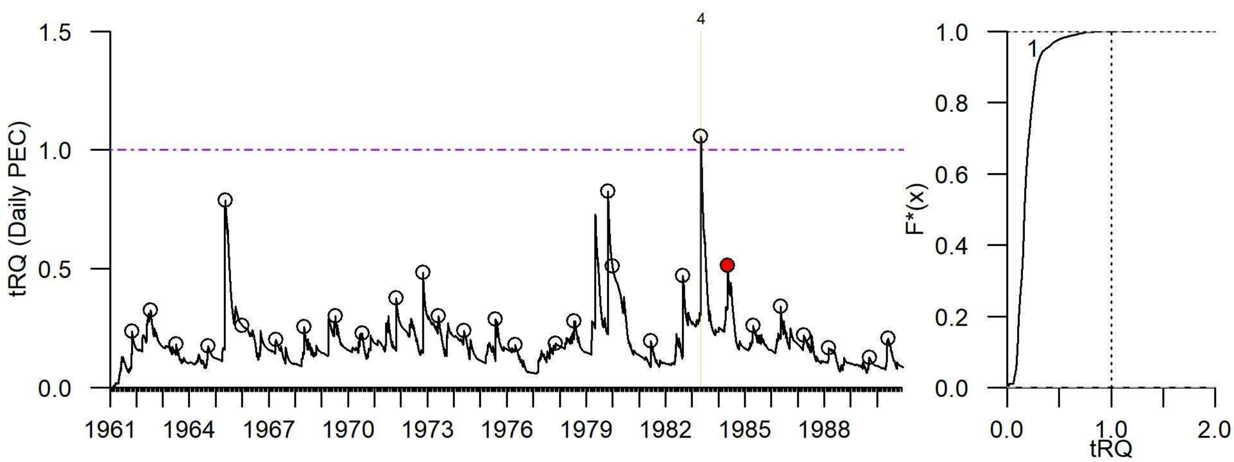

Figure 10. Daily trenbolone RQs for PLUS-ONE with 8% beta

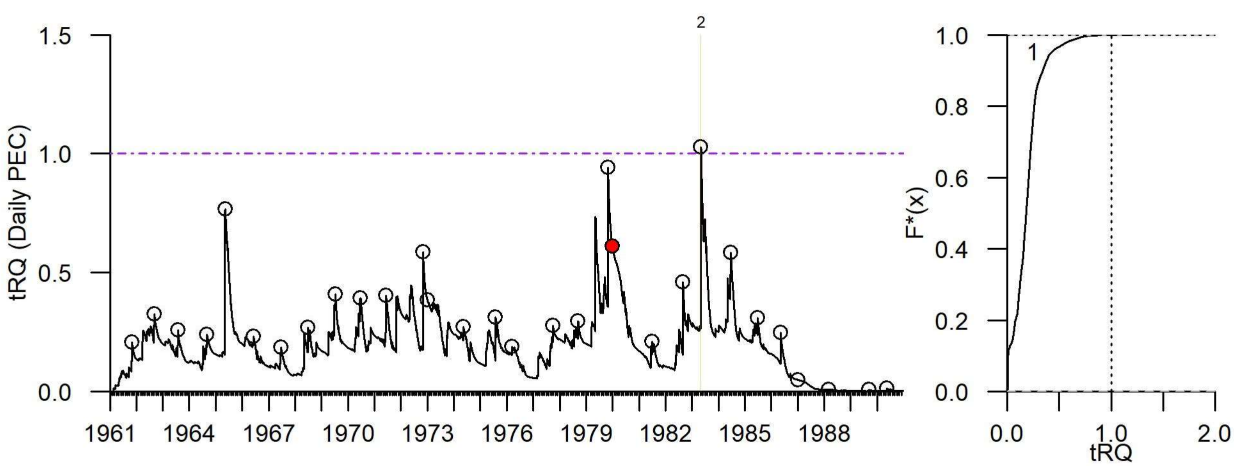

Figure 11. Daily trenbolone RQs for CHOICE-PLUS-PLUS-177 with 8% beta

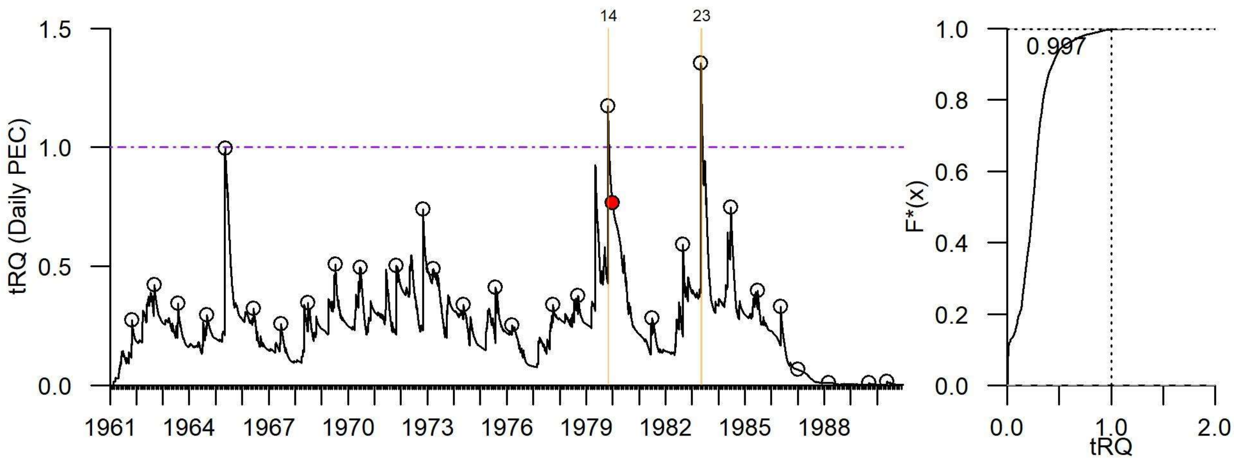

Figure 12. Daily trenbolone RQs for PLUS-PLUS-PLUS-177 with 8% beta

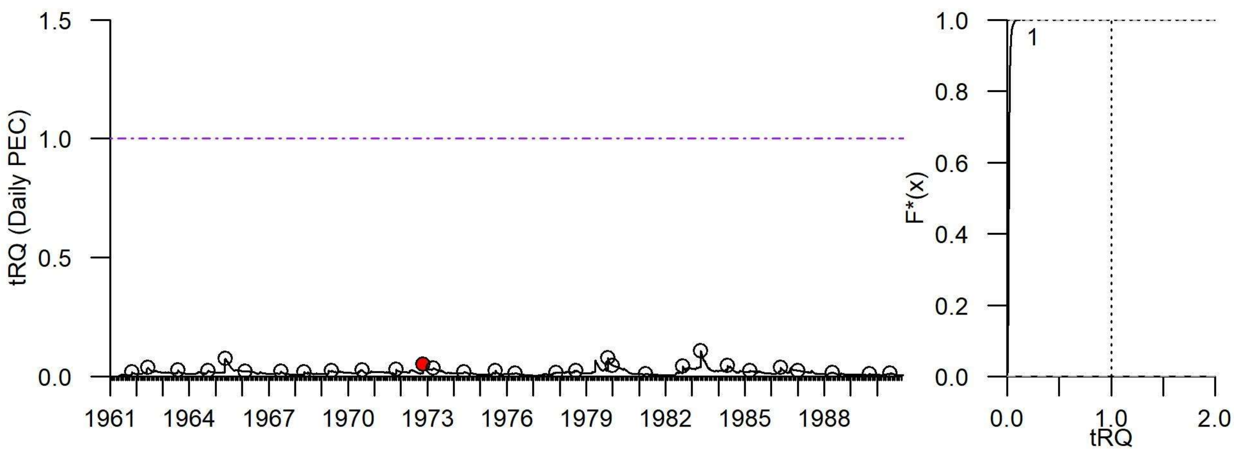

Figure 13. Daily estradiol RQs for CHOICE-PLUS-141

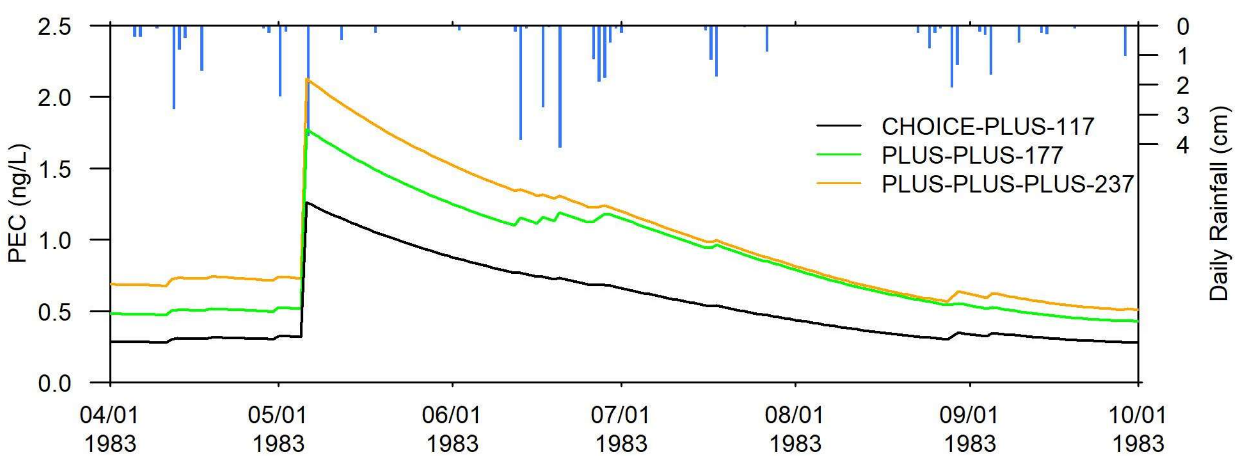

Figure 14. Daily trenbolone PEC for the period around the peak for risk (5/6/1983)

Appendix A - Scenario Comparison Between Five Selected Regions

The Environmental Safety Team of the Center for the Veterinary Medicine recommended a sensitivity analysis for direct runoff of feedlot manure prior to scraping to verify that the Iowa mixed-use watershed remained the highest exposure scenario (FDA, 2018). To assess this, an example scenario (CHOICE-PLUS-60/180 with implantation of CHOICE on Day 0, implantation of PLUS on Day 60, and 180 day grow out) was run for trenbolone for all 5 selected regions in the 2014 EA (Huron County, MI; Mercer County, OH; Lancaster County, PA; Castro County, TX; and Lyon-Sioux Counties, IA). using the region's weather file and 2014 EA's PCAs (see table below).

PCA values from 2014 EA	IA	MI	OH	PA	TX
Feedlot	0.094%	0.032%	0.068%	0.04%	0.003%
Applied Cropland	56.62%	20.10%	29.13%	9.41%	53.59%
Pasture	2.29%	4.05%	2.93%	2.67%	4.36%

The USEPA weather stations used in the assessment were: IA-Sioux City, SD (w14944.dvf), Pennsylvania-Harrisburg, PA (w14751.dvf), Ohio-Dayton, OH (w93815.dvf), Michigan-Flint, MI (w14826.dvf), and Texas-Amarillo, TX (w23047.dvf).

To evaluate the trenbolone loadings originating solely from the feedlots, the only area considered to include Synovex in manure was the AFO. All other areas of the watershed were considered cropland. The SynovexPRZM cropland run was kept the same across regions except again for the weather file. PCAs were based off the data in the table below.

As the initial comment was pertaining specifically to feedlots, only the feedlot TB flux values were considered. Results in the table below showed IA and OH had the highest, and similar, 21-day PEC value than the other three sites.

Region	21-day PEC Trenbolone (ng/L)
IA	0.286
MI	0.124
OH	0.283
PA	0.153
TX	0.007

To further confirm IA being the most at risk site, the simulations were repeated for both IA and OH including the TB flux from all three land types. The results in the table below show IA's 21-day PEC value increased significantly from the original assessment and OH did not. Thus, the IA scenario remains the most conservative.

Region	21-day PEC Trenbolone (ng/L)
IA	0.541
MI	0.160
OH	0.328
PA	0.158

TX	0.189

The comparison was based on the PCAs from the 2014 EA because of the intensive labor involved in updating watershed specific PCAs for all land uses. However, 2017 the Census of Agriculture shows a higher increase in total cattle on feed in Lyon-Sioux Counties from the 2007 Census compared to the other states (see table below); and therefore the IA scenario would stand out as even more if a worst-case scenario if the intensive effort had been performed.

	MI	**OH**	**PA**	**TX**	**IA**
	Huron County	**Mercer County**	**Lancaster County**	**Castro County**	**Lyon + Sioux Counties**
Acres	317,161	303,801	629,314	582,814	869,295
Cattle on feed 2007	45,367	28,448	43,349	341,694	303,244
Cattle on feed 2017	50,691	28,977	29,786	272,913*	394,153
% change 2007-2017	11.7%	1.9%	-31.3%	NA*	30.0%

Cattle on feed (beef feedlot cattle) is the sum of cattle of feed >500 head and cattle on feed <500 head from the 2007 and 2017 Census of Agriculture.
*2,231 cattle on feed <500 head reported in Castro County, TX, in 2007 Census of Agriculture. All subsequent years not disclosed for confidentiality due to limited farms

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USDA NASS (2019). County- and State-level Census of Agriculture tables for years 2002, 2007, 2012, 2017. Washington, DC. http://quickstats.nass.usda.gov/. Accessed April 2019.

Zoetis (2014). Environmental Assessment for Synovex® ONE (Estradiol Benzoate and Trenbolone Acetate Extended Release Implant) Feedlot and Grass for Beef Steers and Heifers. PNU-0090851, PNU-0023173. 29 May 2014. 401p.

Appendix B - PRZM and VVWM Input Parameter Values and Rationale

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	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	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 (USLE K)	12 t/ha (RSDM)	0.37 (USLE K)	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	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 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	Table 5.9 PRZM manual
Max areal coverage % (COVMAX)	97	0	100	Depends upon crop canopy
Curve Number (CN)	82 79 82	95 95 95	77 71 77[6]	CN for fallow, cropping and residue. Table 5.16 PRZM manual. Conservative assumption for feedlot.
Max canopy height at maturation -cm (HTMAX)	122	0	300	Table 5.16 PRZM manual

[6] Numbers for No Till. For conventional tillage the values: 86 79 86 were used.

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[7]	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. 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	1/yr	Scenario Based	6/yr (2 solid, 4 liquid)	
Farm flag	6	4	0	4- feedlot and 6- pasture; Flag used in modified PRZM
Application date	1-Apr	Scenario Based	Solid: 4-May, 26-Oct Liquid: 30-May, 30-Jun, 30-Jul, 30-Aug	
Application stop date	28 Oct for T and 26 Dec for E	Scenario Based	N/A	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[8]	5 cm for surface application (pond water and pasture), 10 cm for feedlot and 15 cm for solid manure application

[7] Numbers for No Till. For conventional tillage the values: .536 .550 .528 .377 .193 .117 .109 .114 .117 .117 .117 .165 .186 .016 .040 .046 .334 .337 .341 .345 .352 .366 .381 .485 .485 .536 were used

[8] Numbers for No Till. For conventional tillage solid applications were 15 cm and liquid applications 5 cm were used.

Parameter	Pasture	Feedlot	Crop	Rationale/source
Application rate-kg/ha	Refer Table 6 and Table 7	Refer Table 6 and Table 7	Refer Table 6 and Table 7	
Application efficiency	1	1	1	USEPA Standard Scenario
Spray drift	0	0	0	Not applicable
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. Conservative assumption.
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	

Parameter	Pasture	Feedlot	Crop	Rationale/source
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	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)

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Meilke, L.N., N.P. Swanson, and T.M. McCalla (1974). Soil Profile Concentration of Cattle Feedlots. J. Environ. Quality. Vol. 3, no. 1.

Appendix C – Predicted Environmental Concentrations and Risk Quotients Associated with All Simulated Implant Regimens

Implant regimens and cattle cycling assumptions are listed in the table below

Implant Scenario	Implant 1 (on day 0)	Implant 2 (Implant day)	Implant 3 (Implant day)	Days Per Production Cycle	Production Cycles Per Year
Trenbolone Scenarios					
CHOICE	CHOICE	--	--	117	3.12
PLUS	PLUS	--	--	117	3.12
ONE	ONE	--	--	211	1.73
ONEg	ONEg	--	--	211	1.73
CHOICE-PLUS-117	CHOICE	PLUS (60)	--	117	3.12
CHOICE-PLUS-177	CHOICE	PLUS (60)	--	177	2.06
CHOICE-CHOICE	CHOICE	CHOICE (60)	--	117	3.12
CHOICE-ONE	CHOICE	ONE (60)	--	117	3.12
PLUS-PLUS-117	PLUS	PLUS (60)	--	117	3.12
PLUS-PLUS-177	PLUS	PLUS (60)	--	177	2.06
PLUS-CHOICE	PLUS	CHOICE (60)	--	117	3.12
PLUS-ONE	PLUS	ONE	--	117	3.12
ONE-PLUS-211	ONE	PLUS (140)	--	211	1.73
ONE-PLUS-257	ONE	PLUS (140)	--	257	1.42
ONE-CHOICE	ONE	CHOICE (140)	--	211	1.73
ONE-ONE	ONE	ONE (140)	--	211	1.73
ONEg-PLUS-211	ONE	PLUS (140)	--	211	1.73
ONEg-PLUS-257	ONE	PLUS (140)	--	257	1.42
ONEg-CHOICE	ONE	CHOICE (140)	--	211	1.73
ONEg-ONE	ONE	ONE (140)	--	211	1.73
CHOICE- PLUS-PLUS-177	CHOICE	PLUS (60)	PLUS (120)	177	2.06
CHOICE- PLUS-PLUS-237	CHOICE	PLUS (60)	PLUS (120)	237	1.54
CHOICE-CHOICE-PLUS-177	CHOICE	CHOICE (60)	PLUS (120)	177	2.06
CHOICE-CHOICE-PLUS-237	CHOICE	CHOICE (60)	PLUS (120)	237	1.54
CHOICE-ONE-PLUS-271	CHOICE	ONE (60)	PLUS (200)	271	1.35
CHOICE-ONE-PLUS-317	CHOICE	ONE (60)	PLUS (200)	317	1.15
PLUS-PLUS-PLUS-177	PLUS	PLUS (60)	PLUS (120)	177	2.06
PLUS-PLUS-PLUS-237	PLUS	PLUS (60)	PLUS (120)	237	1.54
Estradiol Scenarios					
CHOICE	CHOICE	--	--	141	2.59
PLUS	PLUS	--	--	141	2.59
ONE	ONE	--	--	267	1.37
ONEg	ONEg	--	--	267	1.37
CHOICE-PLUS-141	CHOICE	PLUS (60)	--	141	2.59
CHOICE-PLUS-201	CHOICE	PLUS (60)	--	201	1.82
CHOICE-CHOICE	CHOICE	CHOICE (60)	--	141	2.59
CHOICE-ONE	CHOICE	ONE (60)	--	141	2.59
PLUS-PLUS-141	PLUS	PLUS (60)	--	141	2.59
PLUS-PLUS-201	PLUS	PLUS (60)	--	201	1.82
PLUS-CHOICE	PLUS	CHOICE (60)	--	141	2.59
PLUS-ONE	PLUS	ONE	--	141	2.59
ONE-PLUS-267	ONE	PLUS (140)	--	267	1.37

Implant Scenario	Implant 1 (on day 0)	Implant 2 (Implant day)	Implant 3 (Implant day)	Days Per Production Cycle	Production Cycles Per Year
ONE-PLUS-281	ONE	PLUS (140)	--	281	1.30
ONE-CHOICE	ONE	CHOICE (140)	--	267	1.37
ONE-ONE	ONE	ONE (140)	--	267	1.37
ONEg-PLUS-267	ONE	PLUS (140)	--	267	1.37
ONEg-PLUS-281	ONE	PLUS (140)	--	281	1.30
ONEg-CHOICE	ONE	CHOICE (140)	--	267	1.37
ONEg-ONE	ONE	ONE (140)	--	267	1.37
CHOICE- PLUS-PLUS-201	CHOICE	PLUS (60)	PLUS (120)	201	1.82
CHOICE- PLUS-PLUS-261	CHOICE	PLUS (60)	PLUS (120)	261	1.40
CHOICE-CHOICE-PLUS-201	CHOICE	CHOICE (60)	PLUS (120)	201	1.82
CHOICE-CHOICE-PLUS-261	CHOICE	CHOICE (60)	PLUS (120)	261	1.40
CHOICE-ONE-PLUS-327	CHOICE	ONE (60)	PLUS (200)	327	1.12
CHOICE-ONE-PLUS-341	CHOICE	ONE (60)	PLUS (200)	341	1.07
PLUS-PLUS-PLUS-201	PLUS	PLUS (60)	PLUS (120)	201	1.82
PLUS-PLUS-PLUS-261	PLUS	PLUS (60)	PLUS (120)	261	1.40

This represents the full range of exposure patterns. Each of these scenarios had a unique application rate scenario with respect to mass of trenbolone or estradiol applied to cropland, feedlot, and pasture. The application rates are listed below (all rates in g/ha)

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Active Implant(s)*	Rate	
Trenbolone Scenarios							
CHOICE	0.879	0.396	0.022	0-116	CHOICE	0.4929	0.00478
PLUS	1.759	0.792	0.044	0-116	PLUS	0.9859	0.00478
ONE	0.975	0.439	0.024	0-211	ONE	0.5466	0.00478
ONEg	0.732	0.329	0.018	0-211	ONEg	0.4100	0.00478
CHOICE-PLUS-117	1.736	0.781	0.043	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+PLUS	1.4788	
CHOICE-PLUS-177	1.744	0.785	0.044	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+PLUS	1.4788	
				117-176	PLUS	0.9859	
CHOICE-CHOICE	1.308	0.589	0.033	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+CHOICE	0.9858	
CHOICE-ONE	1.355	0.610	0.034	0-59	CHOICE	0.4929	0.00478
				60-116	ONE	0.5466	
PLUS-PLUS-117	2.616	1.177	0.065	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+PLUS	1.9718	
PLUS-PLUS-177	2.326	1.046	0.058	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+PLUS	1.9718	
				117-176	PLUS	0.9859	
PLUS-CHOICE	2.188	0.984	0.055	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+CHOICE	1.4788	

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Active Implant(s)*	Rate	
PLUS-ONE	2.234	1.005	0.056	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+ONE	1.5325	
ONE-PLUS-211	1.567	0.705	0.039	0-139	ONE	0.5466	0.00478
				140-210	ONE+PLUS	1.5325	
ONE-PLUS-257	1.602	0.721	0.040	0-139	ONE	0.5466	0.00478
				140-210	ONE+PLUS	1.5325	
				211-256	PLUS	0.9859	
ONE-CHOICE	1.271	0.572	0.032	0-139	ONE	0.5466	0.00478
				140-210	ONE+CHOICE	1.0395	
ONE-ONE	1.303	0.587	0.033	0-139	ONE	0.5466	0.00478
				140-210	ONE+ONE	1.0932	
ONEg-PLUS-211	1.323	0.596	0.033	0-139	ONEg	0.4100	0.00478
				140-210	ONEg+PLUS	1.3959	
ONEg-PLUS-257	1.401	0.631	0.035	0-139	ONEg	0.4100	0.00478
				140-210	ONEg+PLUS	1.3959	
				211-256	PLUS	0.9859	
ONEg-CHOICE	1.027	0.462	0.026	0-139	ONEg	0.4100	0.00478
				140-210	ONEg+CHOICE	0.9029	
ONEg-ONE	1.060	0.477	0.026	0-139	ONEg	0.4100	0.00478
				140-210	ONEg+ONE	0.9566	
CHOICE-PLUS-PLUS-177	2.311	1.040	0.058	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+PLUS	1.4788	
				117-119	PLUS	0.9859	
				120-176	PLUS+PLUS	1.9718	
CHOICE-PLUS-PLUS-237	2.171	0.977	0.054	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+PLUS	1.4788	
				117-119	PLUS	0.9859	
				120-176	PLUS+PLUS	1.9718	
				177-236	PLUS	0.9859	
CHOICE-CHOICE-PLUS-177	1.729	0.778	0.043	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+CHOICE	0.9858	
				117-119	CHOICE	0.4929	
				120-176	CHOICE+PLUS	1.4788	
CHOICE-CHOICE-PLUS-237	1.737	0.782	0.043	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+CHOICE	0.9858	
				117-119	CHOICE	0.4929	
				120-176	CHOICE+PLUS	1.4788	
				177-236	PLUS	0.9859	
CHOICE-ONE-PLUS-271	1.600	0.720	0.040	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+ONE	1.0395	
				117-199	ONE	0.5466	
				199-270	ONE+PLUS	1.5325	
CHOICE-ONE-PLUS-317	1.623	0.730	0.041	0-59	CHOICE	0.4929	0.00478
				60-116	CHOICE+ONE	1.0395	
				117-199	ONE	0.5466	
				200-270	ONE+PLUS	1.5325	
				271-316	PLUS	0.9859	
PLUS-PLUS-PLUS-177	2.892	1.301	0.072	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+PLUS	1.9718	

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Active Implant(s)*	Rate	
				117-119	PLUS	0.9859	
				120-176	PLUS+PLUS	1.9718	
PLUS-PLUS-PLUS-237	2.605	1.172	0.065	0-59	PLUS	0.9859	0.00478
				60-116	PLUS+PLUS	1.9718	
				117-119	PLUS	0.9859	
				120-176	PLUS+PLUS	1.9718	
				177-236	PLUS	0.9859	
Estradiol Scenarios							
CHOICE	0.879	0.396	0.022	0-140	CHOICE	0.0478	0.00044
PLUS	1.759	0.792	0.044	0-140	PLUS	0.0956	0.00044
ONE	0.975	0.439	0.024	0-266	ONE	0.0506	0.00044
ONEg	0.732	0.329	0.018	0-266	ONEg	0.0380	0.00044
CHOICE-PLUS-141	1.736	0.781	0.043	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+PLUS	0.1434	
CHOICE-PLUS-201	1.744	0.785	0.044	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+PLUS	0.1434	
				141-200	PLUS	0.0956	
CHOICE-CHOICE	1.308	0.589	0.033	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+CHOICE	0.0956	
CHOICE-ONE	1.355	0.610	0.034	0-59	CHOICE	0.0478	0.00044
				60-140	ONE	0.0506	
PLUS-PLUS-141	2.616	1.177	0.065	0-59	PLUS	0.0956	0.00044
				60-140	PLUS+PLUS	0.1912	
PLUS-PLUS-201	2.326	1.046	0.058	0-59	PLUS	0.0956	0.00044
				60-140	PLUS+PLUS	0.1912	
				141-200	PLUS	0.0956	
PLUS-CHOICE	2.188	0.984	0.055	0-59	PLUS	0.0956	0.00044
				60-140	PLUS+CHOICE	0.1434	
PLUS-ONE	2.234	1.005	0.056	0-59	PLUS	0.0956	0.00044
				60-140	PLUS+ONE	0.1462	
ONE-PLUS-267	1.567	0.705	0.039	0-139	ONE	0.0506	0.00044
				140-266	ONE+PLUS	0.1462	
ONE-PLUS-281	1.602	0.721	0.040	0-139	ONE	0.0506	0.00044
				140-266	ONE+PLUS	0.1462	
				267-280	PLUS	0.0956	
ONE-CHOICE	1.271	0.572	0.032	0-139	ONE	0.0506	0.00044
				140-266	ONE+CHOICE	0.0984	
ONE-ONE	1.303	0.587	0.033	0-139	ONE	0.0506	0.00044
				140-266	ONE+ONE	0.1013	
ONEg-PLUS-267	1.323	0.596	0.033	0-139	ONEg	0.0380	0.00044
				140-266	ONEg+PLUS	0.1336	
ONEg-PLUS-281	1.401	0.631	0.035	0-139	ONEg	0.0380	0.00044
				140-266	ONEg+PLUS	0.1336	
				267-280	PLUS	0.0956	
ONEg-CHOICE	1.027	0.462	0.026	0-139	ONEg	0.0380	0.00044
				140-266	ONEg+CHOICE	0.0858	
ONEg-ONE	1.060	0.477	0.026	0-139	ONEg	0.0380	0.00044
				140-266	ONEg+ONE	0.0886	
CHOICE-PLUS-PLUS-201	2.311	1.040	0.058	0-59	CHOICE	0.0478	0.00044
				60-119	CHOICE+PLUS	0.1434	
				120-140	CHOICE+PLUS+PLUS	0.2390	

Application Scenario	Cropland			Feedlot			Pasture
	Total	Solid	Liquid	Cycle Days	Active Implant(s)*	Rate	
				141-200	PLUS+PLUS	0.1912	
CHOICE-PLUS-PLUS-261	2.171	0.977	0.054	0-59	CHOICE	0.0478	0.00044
				60-119	CHOICE+PLUS	0.1434	
				120-140	CHOICE+PLUS+PLUS	0.2390	
				141-200	PLUS+PLUS	0.1912	
				201-260	PLUS	0.0956	
CHOICE-CHOICE-PLUS-201	1.729	0.778	0.043	0-59	CHOICE	0.0478	0.00044
				60-119	CHOICE+CHOICE	0.0956	
				120-140	CHOICE+CHOICE+PLUS	0.1912	
				141-200	CHOICE+PLUS	0.1434	
CHOICE-CHOICE-PLUS-261	1.737	0.782	0.043	0-59	CHOICE	0.0478	0.00044
				60-119	CHOICE+CHOICE	0.0956	
				120-140	CHOICE+CHOICE+PLUS	0.1912	
				141-200	CHOICE+PLUS	0.1434	
				201-260	PLUS	0.0956	
CHOICE-ONE-PLUS-327	1.600	0.720	0.040	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+ONE	0.0984	
				141-199	ONE	0.0506	
				199-326	ONE+PLUS	0.1462	
CHOICE-ONE-PLUS-341	1.623	0.730	0.041	0-59	CHOICE	0.0478	0.00044
				60-140	CHOICE+ONE	0.0984	
				141-199	ONE	0.0506	
				200-326	ONE+PLUS	0.1462	
				327-340	PLUS	0.0956	
PLUS-PLUS-PLUS-327	2.892	1.301	0.072	0-59	PLUS	0.0956	0.00044
				60-119	PLUS+PLUS	0.1912	
				120-140	PLUS+PLUS+PLUS	0.2868	
				141-200	PLUS+PLUS	0.1912	
PLUS-PLUS-PLUS-341	2.605	1.172	0.065	0-59	PLUS	0.0956	0.00044
				60-119	PLUS+PLUS	0.1912	
				120-140	PLUS+PLUS+PLUS	0.2868	
				141-200	PLUS+PLUS	0.1912	
				201-260	PLUS	0.0956	

* ONE-Feedlot is denoted ONE and ONE-Grower is denoted ONEg to save space

Each scenario was tested using the 70% scraping efficiency and no tillage "standard" scenario. Trenbolone was tested at a 5% and 8% beta and estradiol was tested at 100% beta. The results are in the tables on the following pages (first trenbolone, then estradiol).

Trenbolone

Application Scenario	21d PEC (ng/L)	5% 17β-trenbolone		8% 17β-trenbolone	
		RQ	Event Count	RQ	Event Count
CHOICE	0.48	0.24	0	0.29	0
PLUS	0.95	0.47	0	0.58	0
ONE	0.55	0.27	0	0.33	0
ONEg	0.41	0.21	0	0.25	0
CHOICE-PLUS-117	0.88	0.44	0	0.54	0

Application Scenario	21d PEC (ng/L)	5% 17β-trenbolone		8% 17β-trenbolone	
		RQ	Event Count	RQ	Event Count
CHOICE-PLUS-177	0.91	0.45	0	0.56	0
CHOICE-CHOICE	0.68	0.34	0	0.41	0
CHOICE-ONE	0.71	0.35	0	0.43	0
PLUS-PLUS-117	1.36	0.68	1	0.83	1
PLUS-PLUS-177	1.26	0.63	0	0.77	1
PLUS-CHOICE	1.16	0.58	0	0.70	1
PLUS-ONE	1.18	0.59	0	0.72	1
ONE-PLUS-211	0.85	0.42	0	0.52	0
ONE-PLUS-257	0.88	0.44	0	0.53	0
ONE-CHOICE	0.70	0.35	0	0.43	0
ONE-ONE	0.72	0.36	0	0.44	0
ONEg-PLUS-211	0.72	0.36	0	0.44	0
ONEg-PLUS-257	0.76	0.38	0	0.46	0
ONEg-CHOICE	0.57	0.28	0	0.34	0
ONEg-ONE	0.58	0.29	0	0.35	0
CHOICE-PLUS-PLUS-177	1.17	0.58	0	0.71	1
CHOICE-PLUS-PLUS-237	1.08	0.54	0	0.66	1
CHOICE-CHOICE-PLUS-177	0.89	0.44	0	0.54	0
CHOICE-CHOICE-PLUS-237	0.88	0.44	0	0.53	0
CHOICE-ONE-PLUS-271	0.93	0.46	0	0.57	0
CHOICE-ONE-PLUS-317	0.91	0.45	0	0.55	0
PLUS-PLUS-PLUS-177	1.51	0.75	1	0.92	2
PLUS-PLUS-PLUS-237	1.37	0.68	1	0.83	3

Estradiol

Application Scenario	21d PEC (ng/L)	RQ	Event Count
CHOICE	0.05	0.03	0
PLUS	0.09	0.07	0
ONE	0.05	0.04	0
ONEg	0.04	0.03	0
CHOICE-PLUS-141	0.09	0.07	0
CHOICE-PLUS-201	0.09	0.07	0
CHOICE-CHOICE	0.07	0.05	0
CHOICE-ONE	0.07	0.05	0
PLUS-PLUS-141	0.14	0.10	0
PLUS-PLUS-201	0.13	0.09	0
PLUS-CHOICE	0.12	0.08	0
PLUS-ONE	0.12	0.08	0
ONE-PLUS-267	0.09	0.06	0

Application Scenario	21d PEC (ng/L)	RQ	Event Count
ONE-PLUS-281	0.09	0.06	0
ONE-CHOICE	0.07	0.05	0
ONE-ONE	0.07	0.05	0
ONEg-PLUS-267	0.08	0.06	0
ONEg-PLUS-281	0.07	0.05	0
ONEg-CHOICE	0.06	0.04	0
ONEg-ONE	0.06	0.04	0
CHOICE-PLUS-PLUS-201	0.12	0.09	0
CHOICE-PLUS-PLUS-261	0.08	0.06	0
CHOICE-CHOICE-PLUS-201	0.09	0.07	0
CHOICE-CHOICE-PLUS-261	0.07	0.05	0
CHOICE-ONE-PLUS-327	0.10	0.07	0
CHOICE-ONE-PLUS-341	0.10	0.07	0
PLUS-PLUS-PLUS-201	0.16	0.11	0
PLUS-PLUS-PLUS-261	0.10	0.07	0

None of the scenarios had a PEC with an RQ > 1.0. Multiple trenbolone and no estradiol scenarios had events where the RQ reached above 1.0 for any particular day using all three set ups and both AFO percentages. Descriptions of the events where the RQ exceeded 1.0 are in the table below.

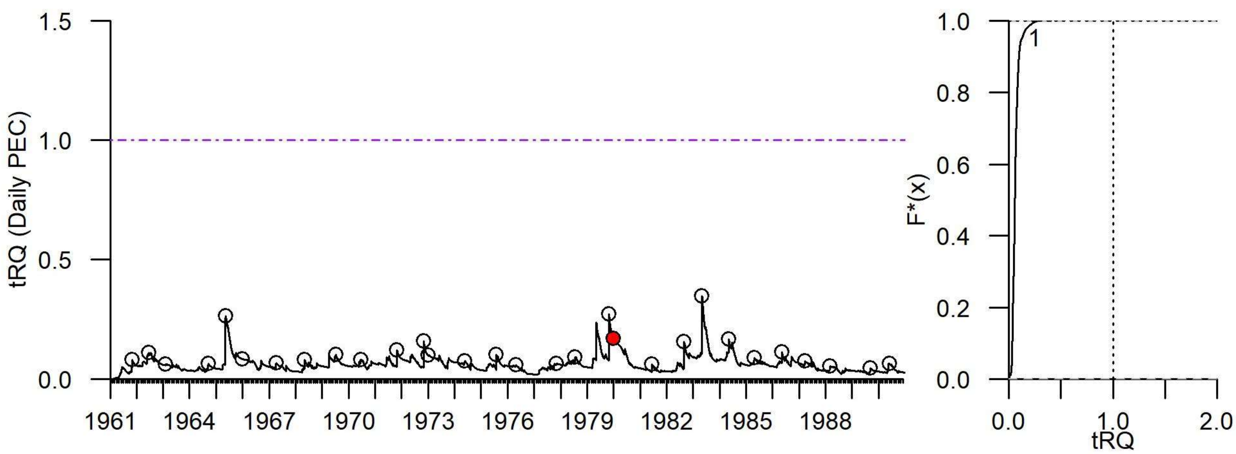
Trenbolone – 5% 17β-trenbolone

Scenario	Event	Begin Date	Peak RQ	Arith. Avg. RQ	Duration (days)
PLUS-PLUS-117	1	5/6/1983	1	1	1
PLUS-PLUS-PLUS-177	1	5/6/1983	1.11	1.05	8
PLUS-PLUS-PLUS-237	1	5/6/1983	1.14	1.07	10

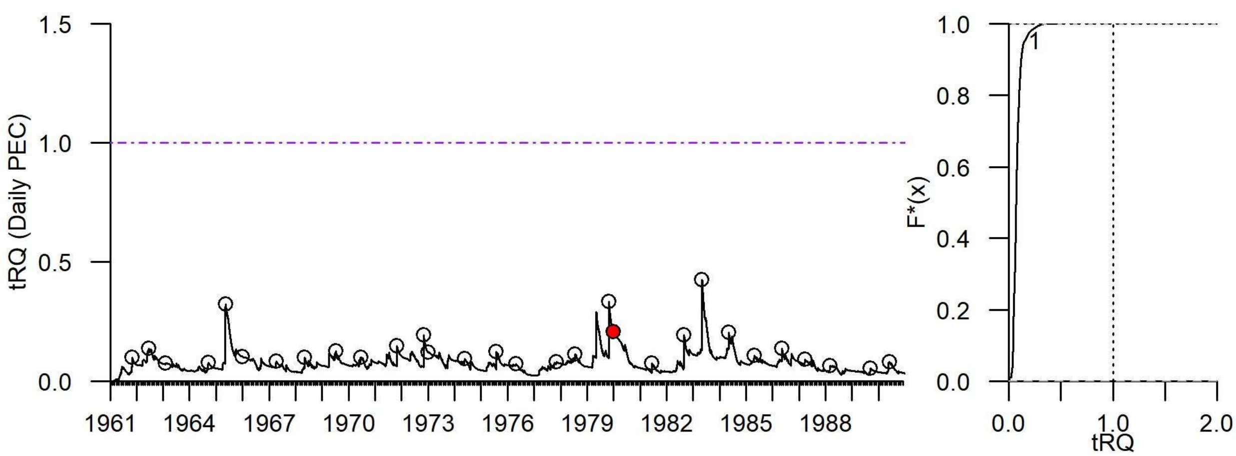
Trenbolone – 8% 17β-trenbolone

Scenario	Event	Begin Date	Peak RQ	Arith. Avg. RQ	Duration (days)
PLUS-CHOICE	1	5/6/1983	1.04	1.02	3
PLUS-PLUS-117	1	5/6/1983	1.23	1.11	15
PLUS-PLUS-177	1	5/6/1983	1.15	1.08	10
PLUS-ONE	1	5/6/1983	1.06	1.03	5
CHOICE-PLUS-PLUS-177	1	5/6/1983	1.03	1.02	2
CHOICE-PLUS-PLUS-237	1	5/6/1983	1.18	1.09	13
PLUS-PLUS-PLUS-177	1	10/30/1979	1.17	1.09	14
	2	5/6/1983	1.35	1.17	23
PLUS-PLUS-PLUS-237	1	5/9/1979	1.04	1.02	5
	2	10/30/1979	1.07	1.04	7
	3	5/6/1983	1.39	1.18	27

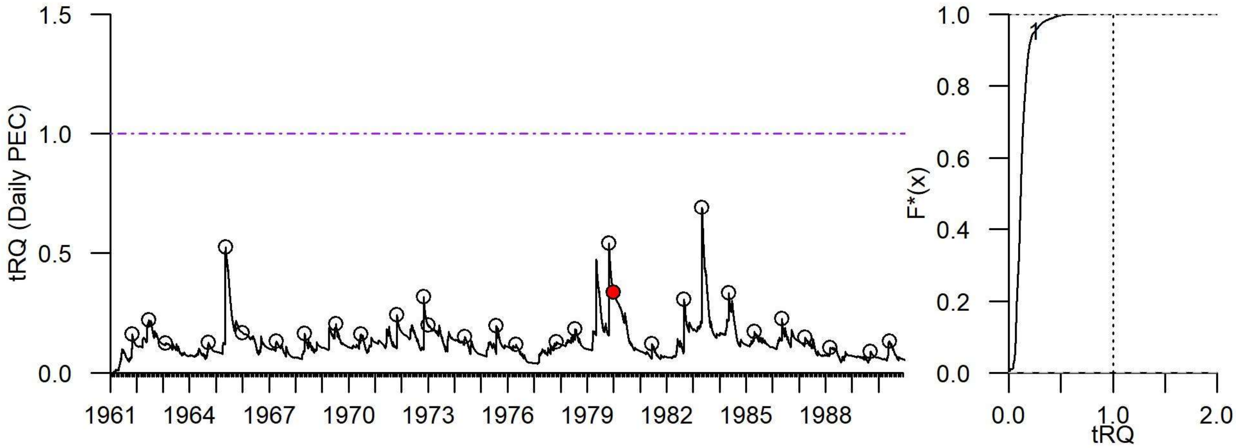
For each scenario, an image was created to visualize the daily RQ across the 30-year model period. On the left panel, the image shows the daily RQ value for the simulation. The yearly maximum is a black hollow circle and the 90th percentile of the yearly maximum is a red circle. The purple dashed line indicates a RQ of 1.0 and periods of time where the RQ exceeds 1.0 are shaded with the duration of the event listed above the shaded area. The right panel plots all RQ values as a cumulative distribution function and the fraction of all values less than 1.0 is plotted at the top left of the panel. Images for each simulation set are shown in the attached document.



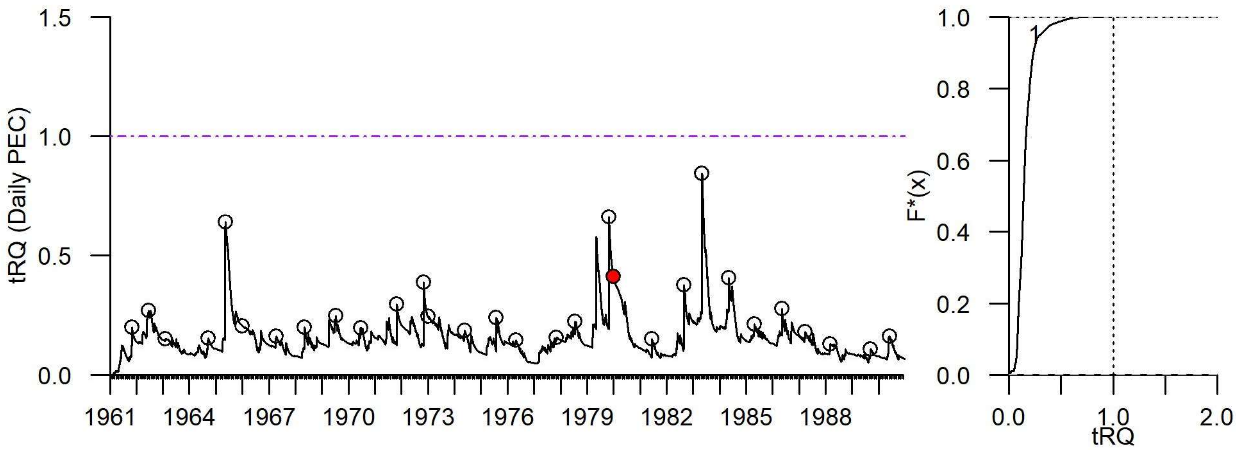
Daily Trenbolone RQs – CHOICE – 5% beta



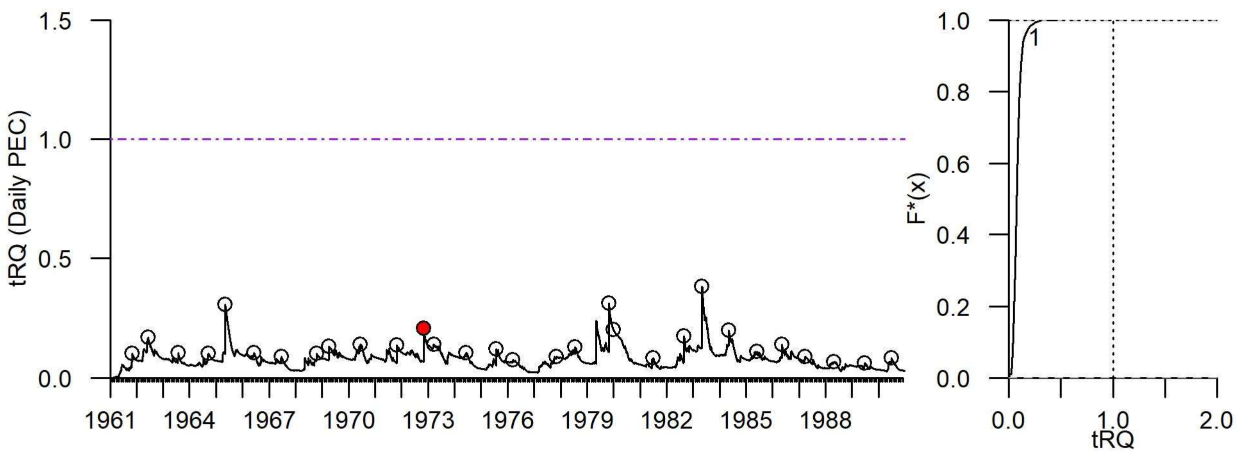
Daily Trenbolone RQs – CHOICE – 8% beta



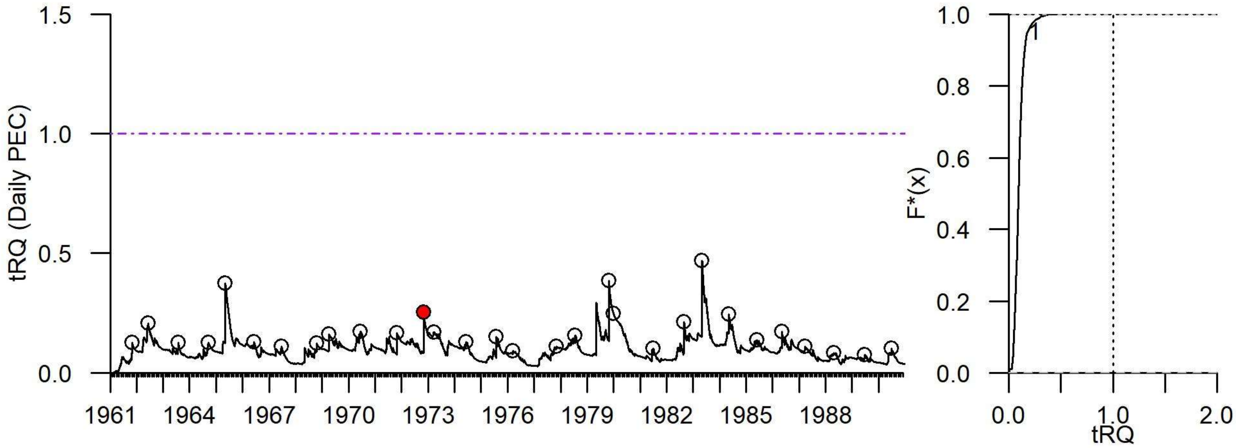
Daily Trenbolone RQs – PLUS – 5% beta



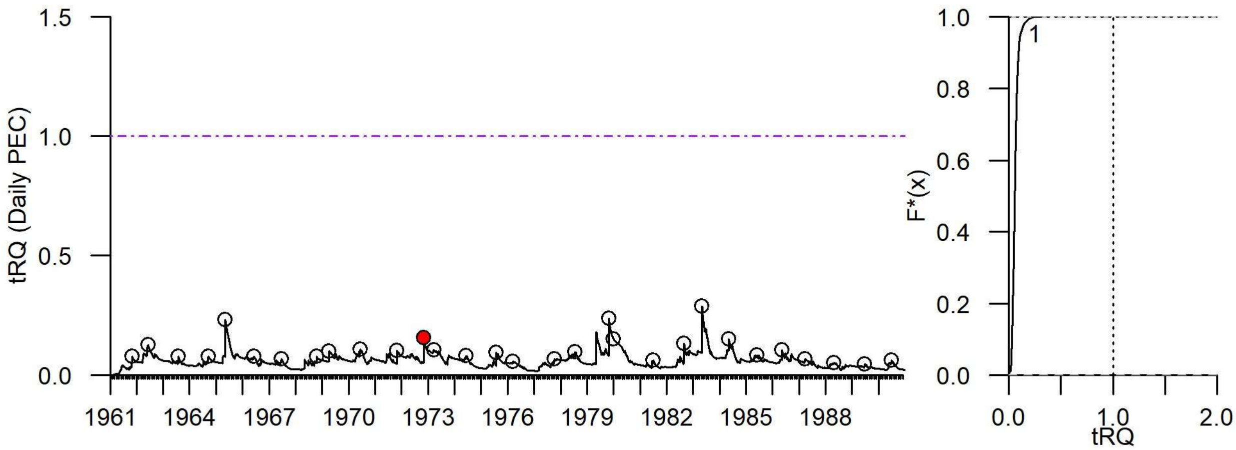
Daily Trenbolone RQs – PLUS – 8% beta



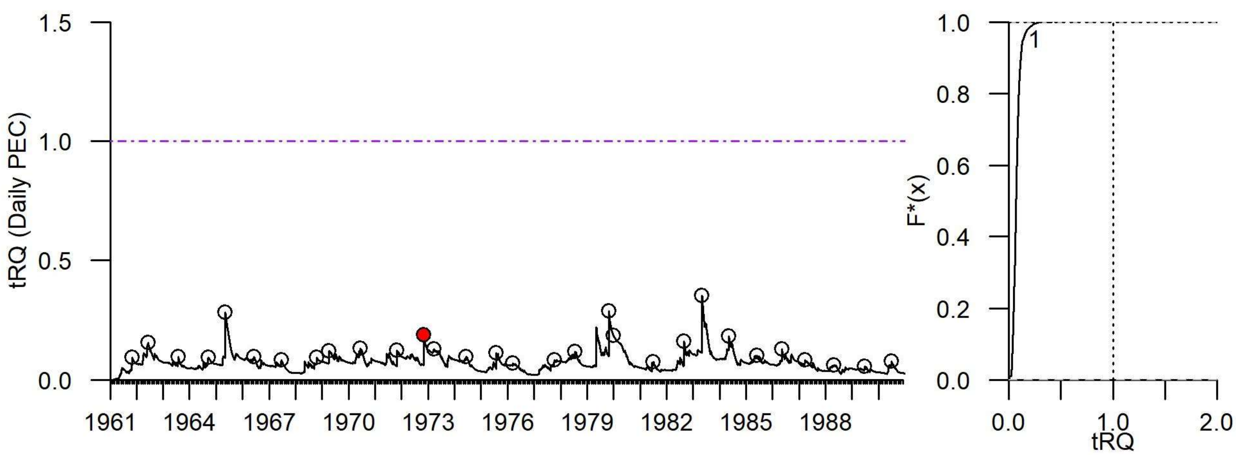
Daily Trenbolone RQs – ONE – 5% beta



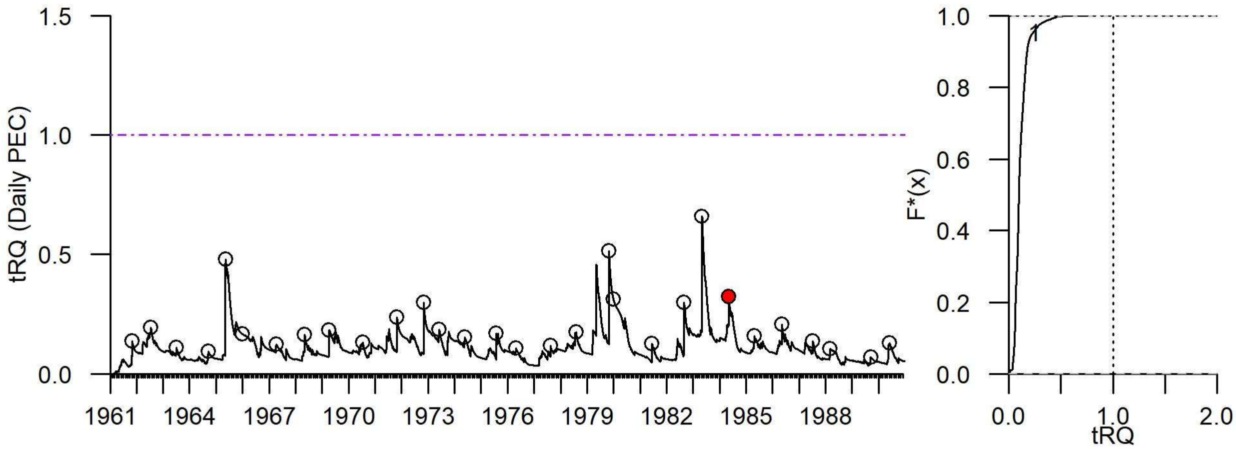
Daily Trenbolone RQs – ONE – 8% beta



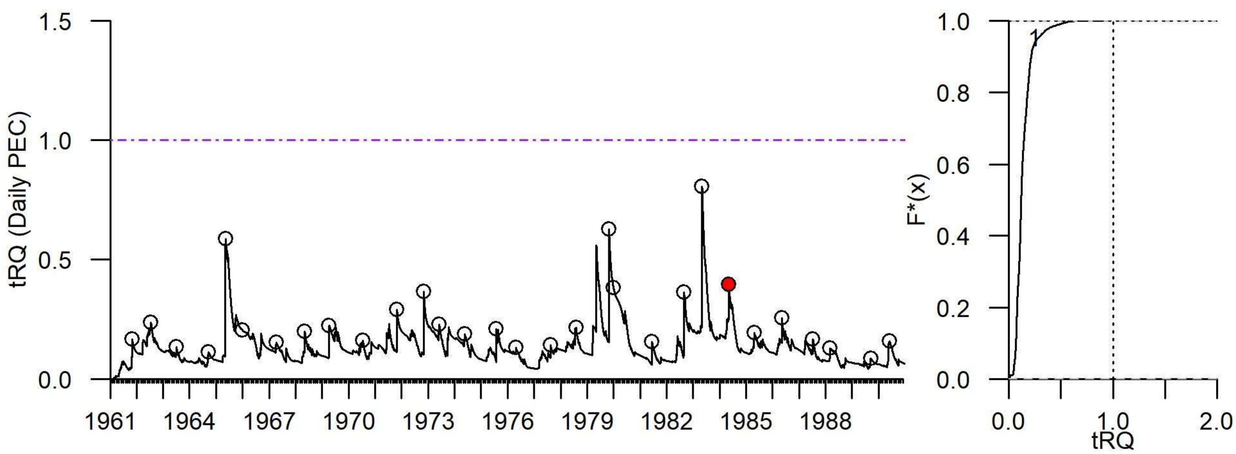
Daily Trenbolone RQs – ONEg – 5% beta



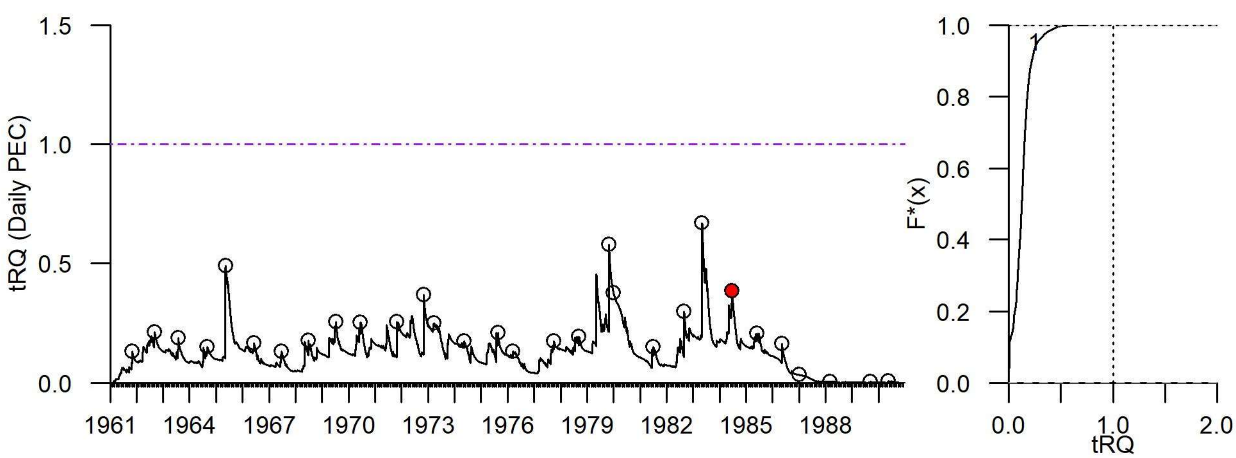
Daily Trenbolone RQs – ONEg – 8% beta



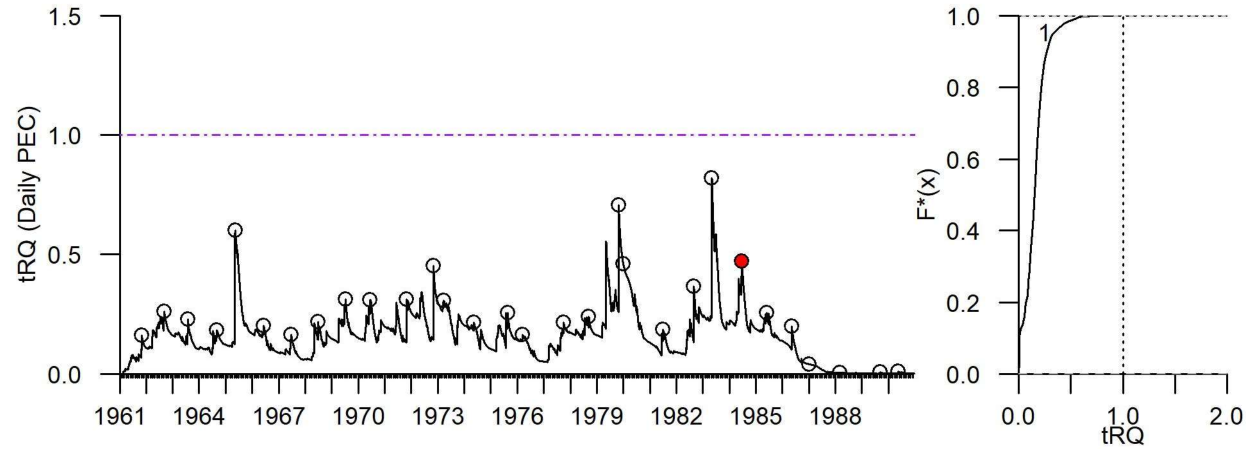
Daily Trenbolone RQs – CHOICE-PLUS-117 – 5% beta



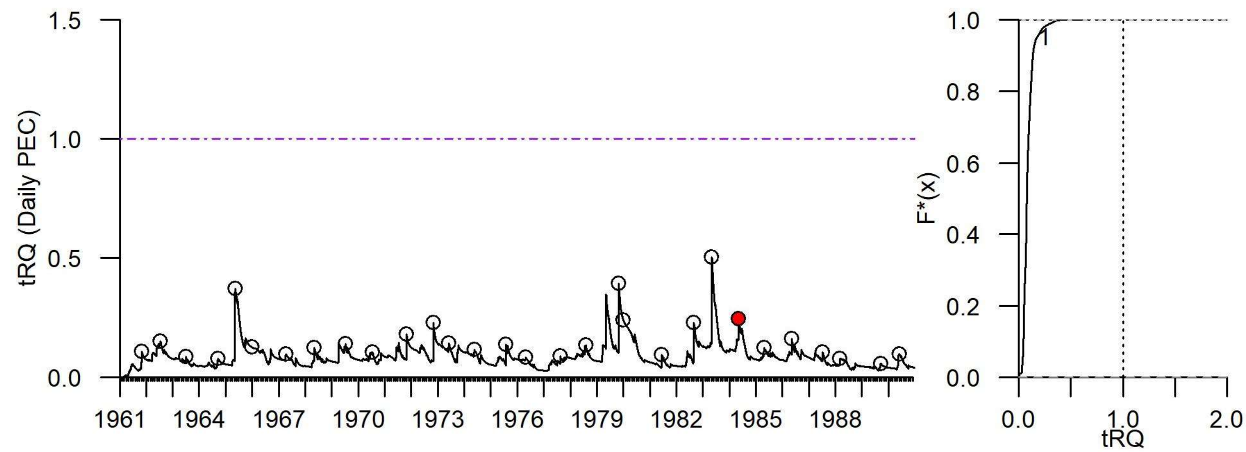
Daily Trenbolone RQs – CHOICE-PLUS-117 – 8% beta



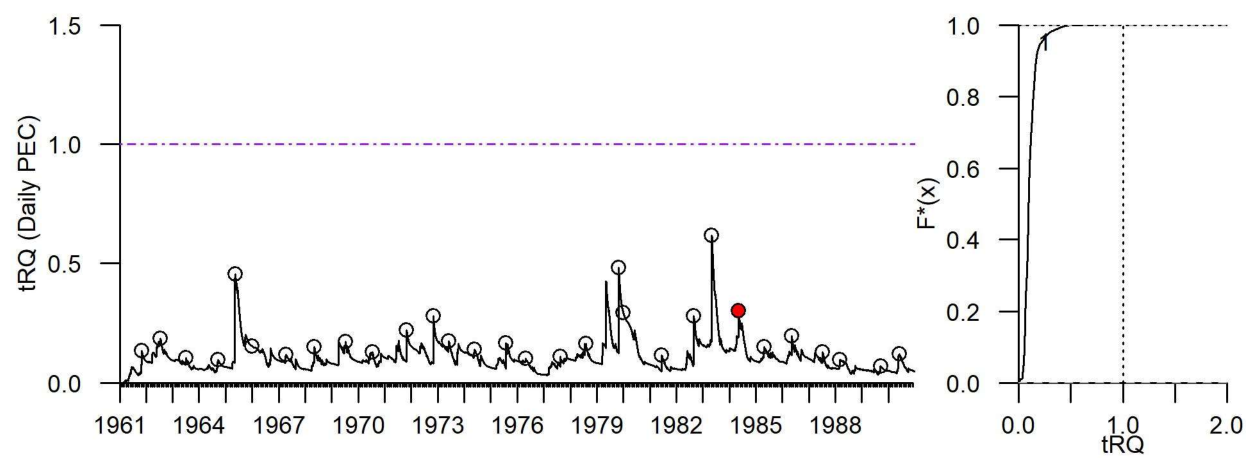
Daily Trenbolone RQs – CHOICE-PLUS-177 – 5% beta



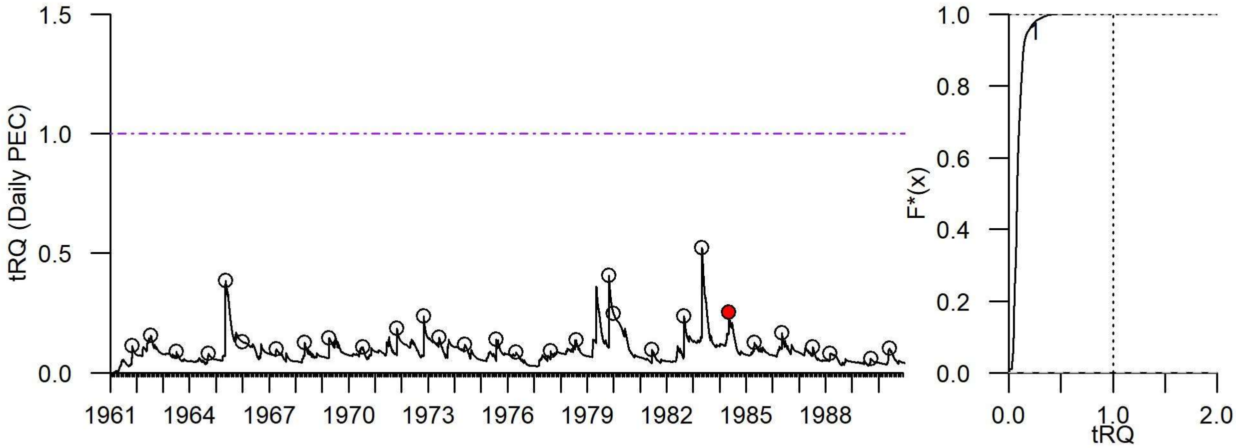
Daily Trenbolone RQs – CHOICE-PLUS-177 – 8% beta



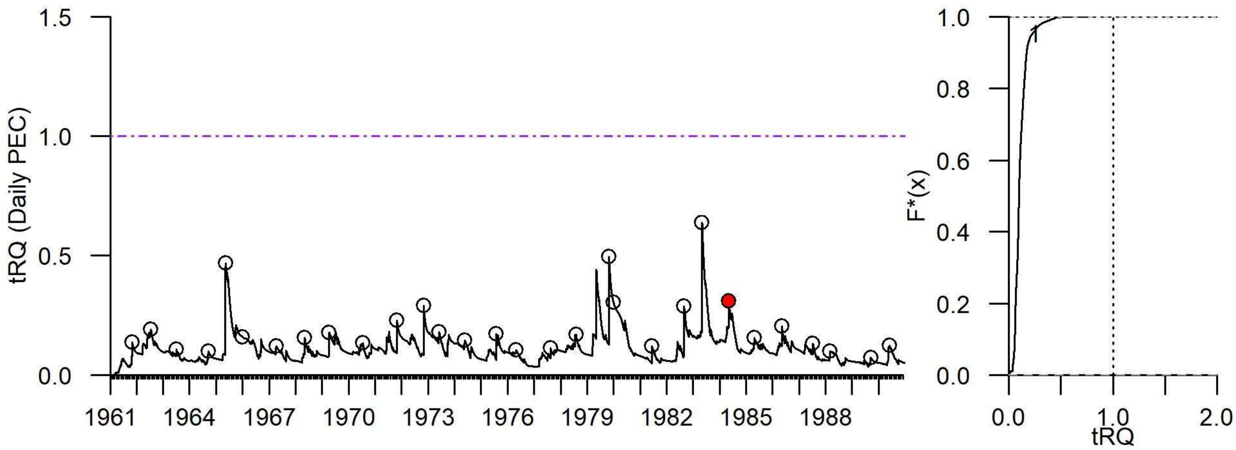
Daily Trenbolone RQs – CHOICE-CHOICE – 5% beta



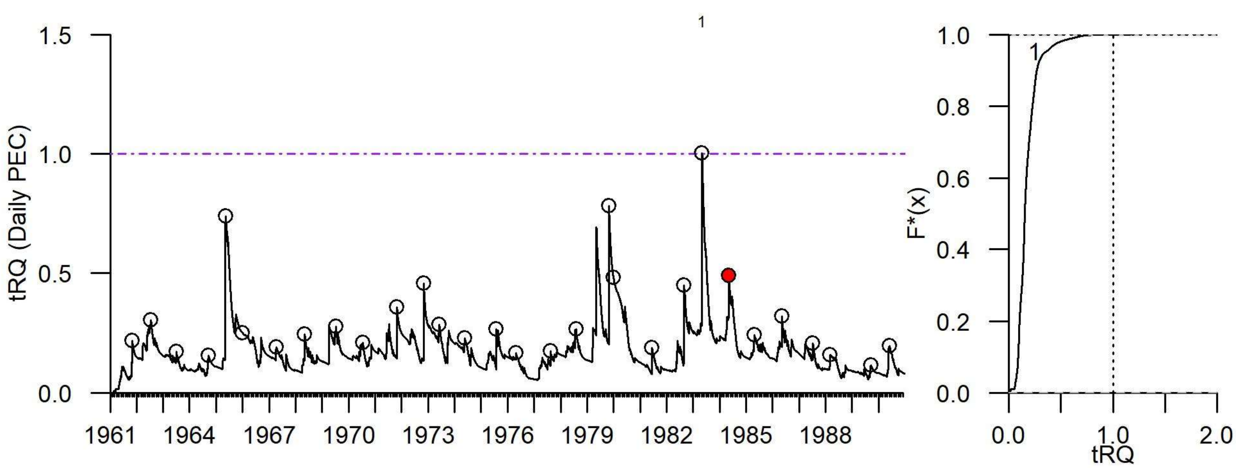
Daily Trenbolone RQs – CHOICE-CHOICE – 8% beta



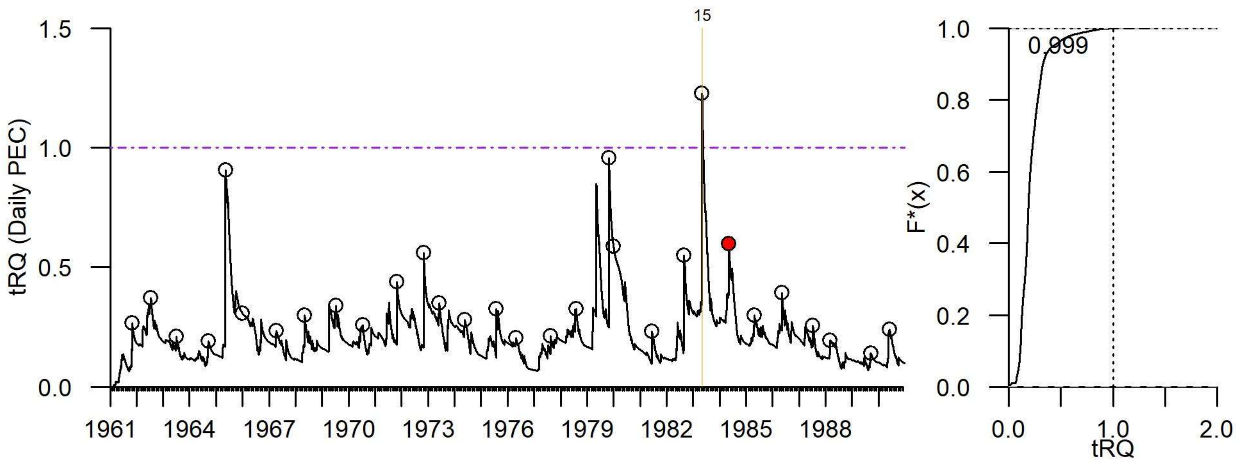
Daily Trenbolone RQs – CHOICE-ONE – 5% beta



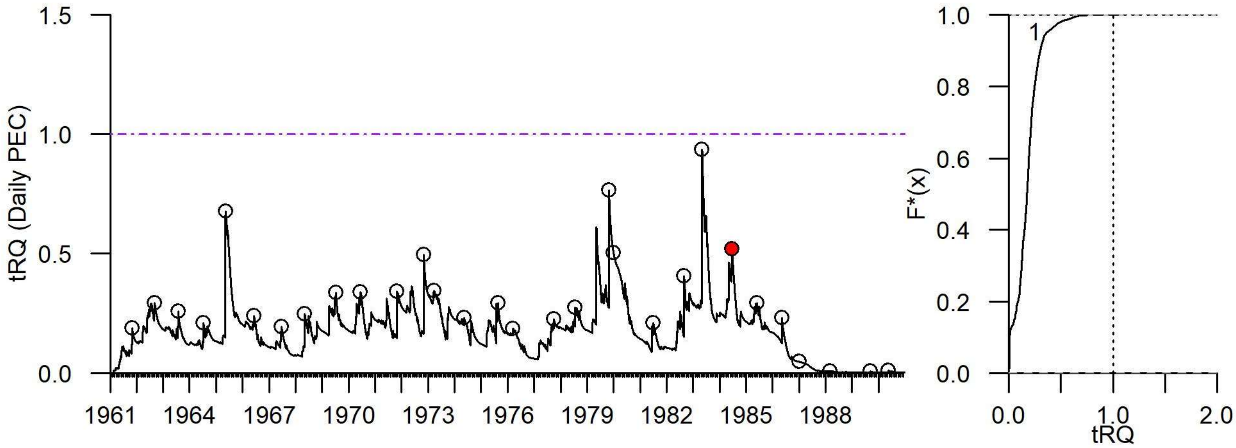
Daily Trenbolone RQs – CHOICE-ONE – 8% beta



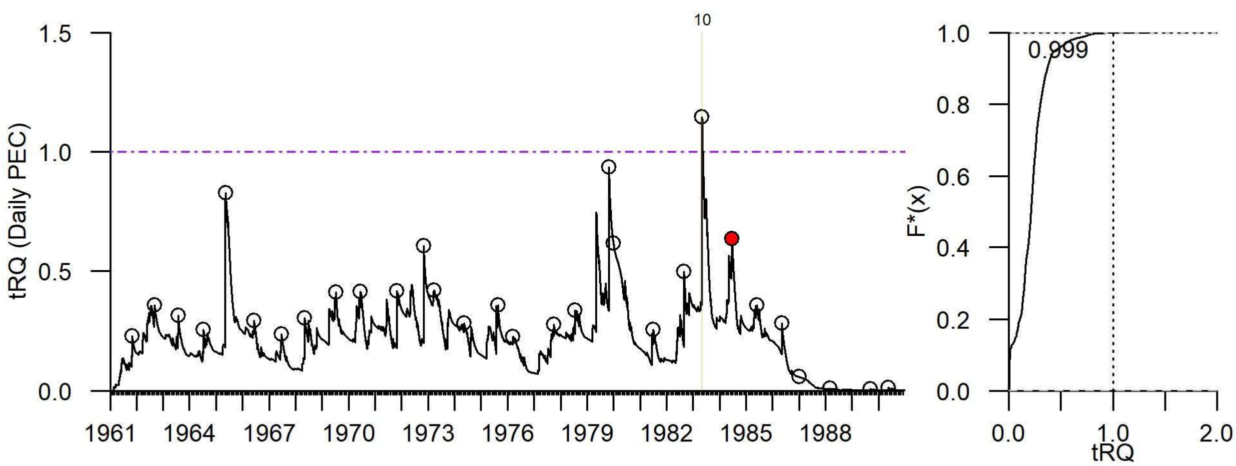
Daily Trenbolone RQs – PLUS-PLUS-117 – 5% beta



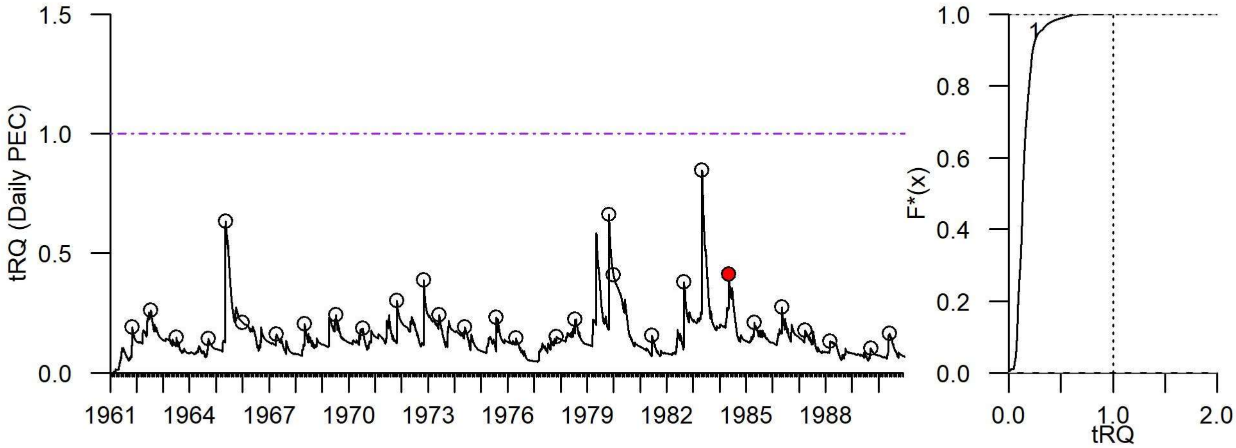
Daily Trenbolone RQs – PLUS-PLUS-117 – 8% beta



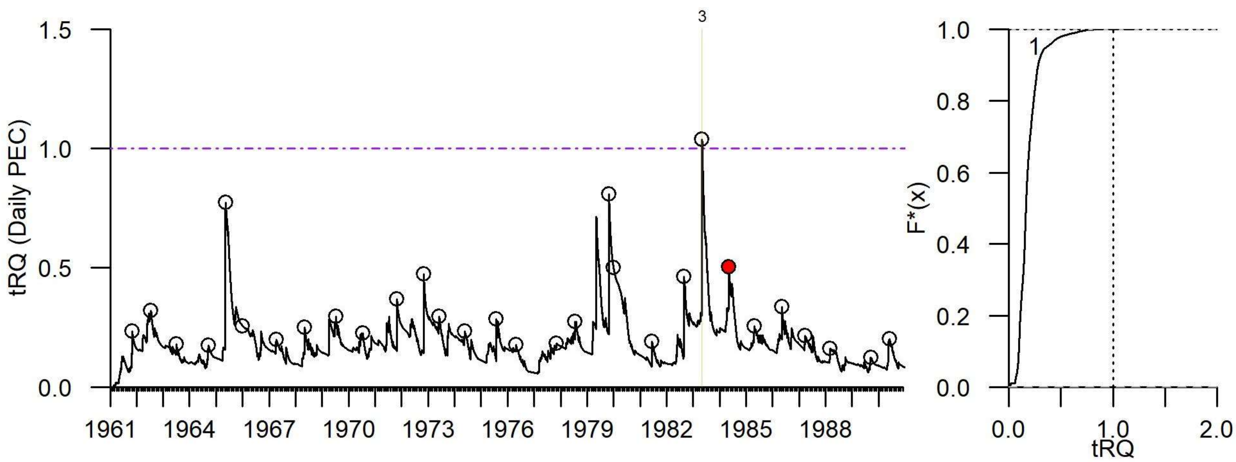
Daily Trenbolone RQs – PLUS-PLUS-177 – 5% beta



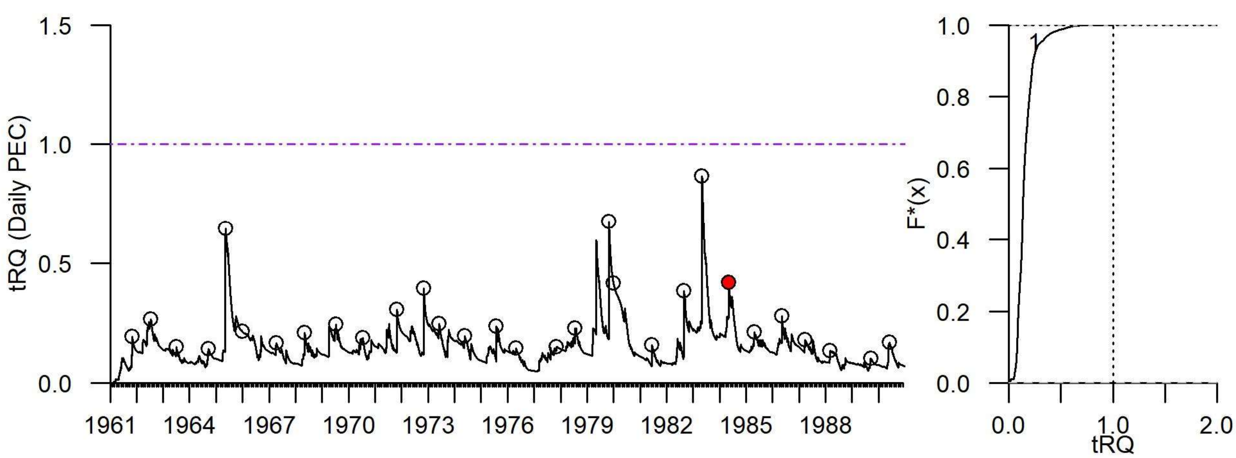
Daily Trenbolone RQs – PLUS-PLUS-177 – 8% beta



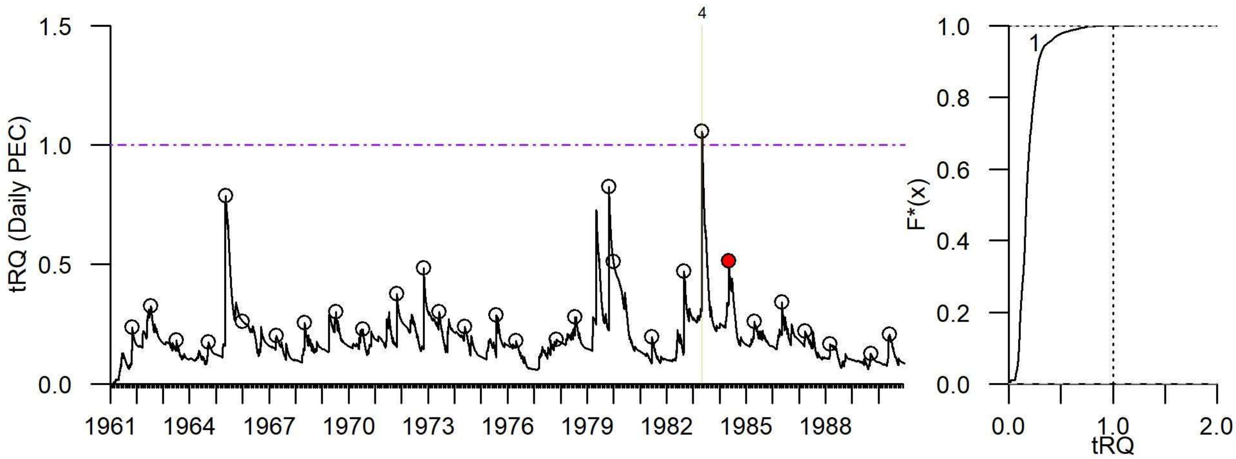
Daily Trenbolone RQs – PLUS-CHOICE – 5% beta



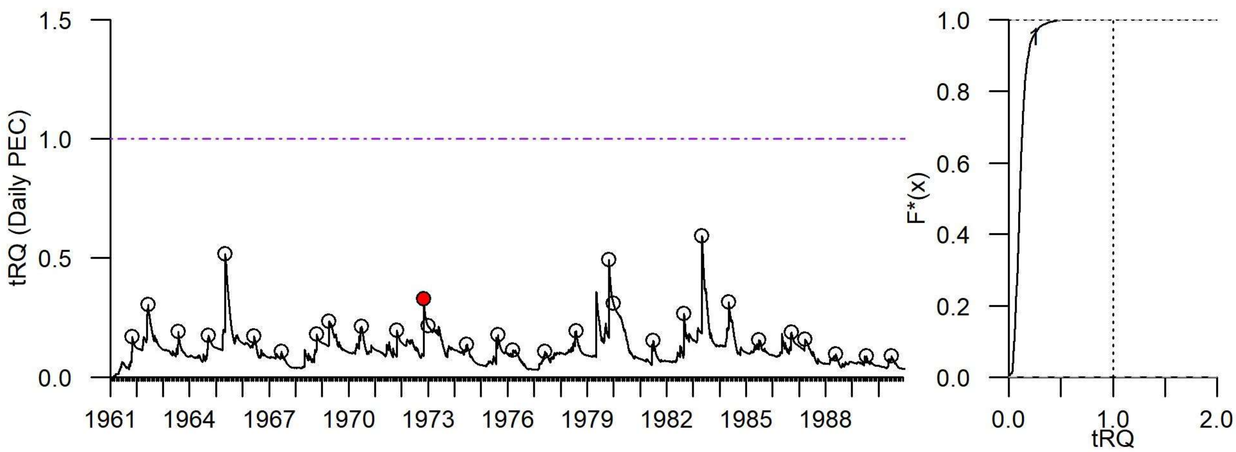
Daily Trenbolone RQs – PLUS-CHOICE – 8% beta



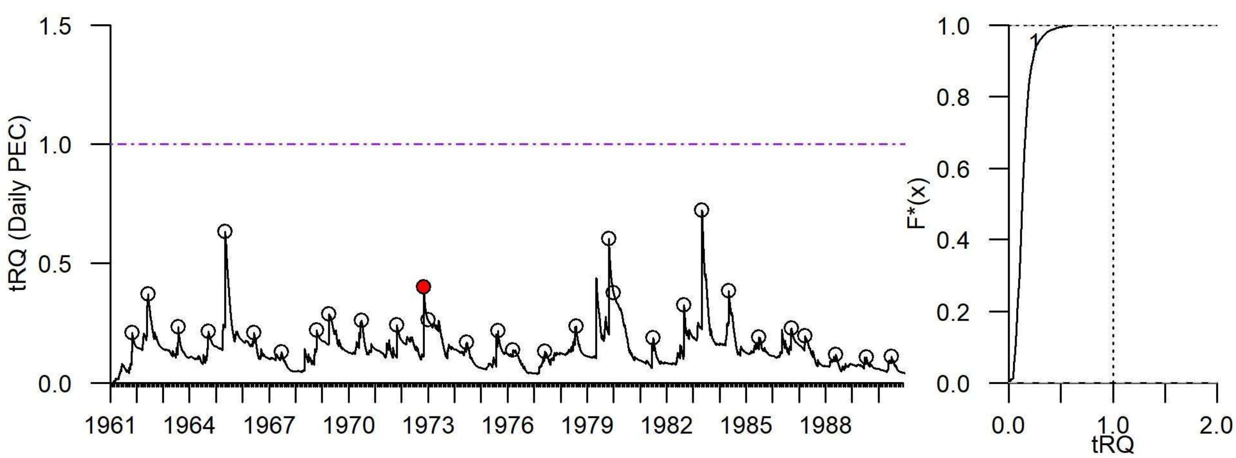
Daily Trenbolone RQs – PLUS-ONE – 5% beta



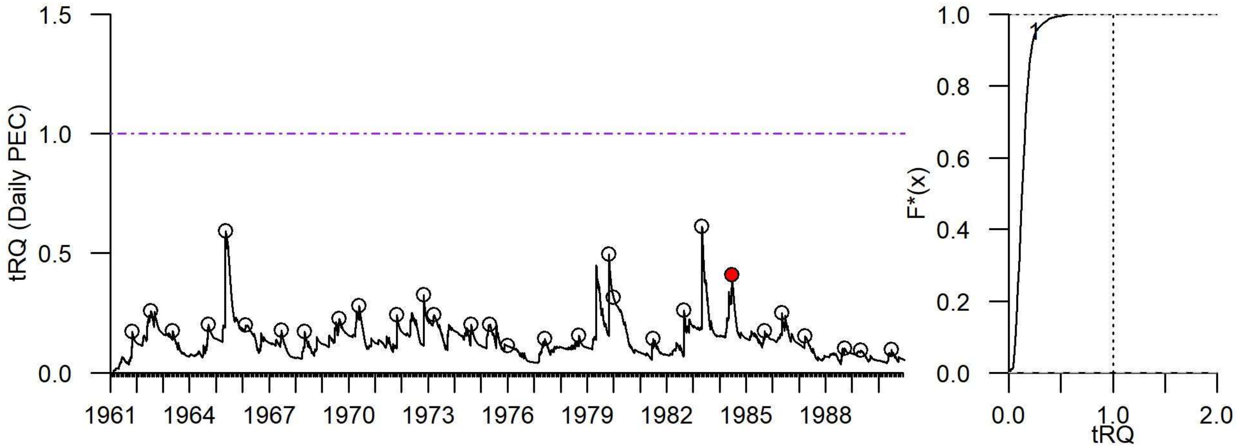
Daily Trenbolone RQs – PLUS-ONE – 8% beta



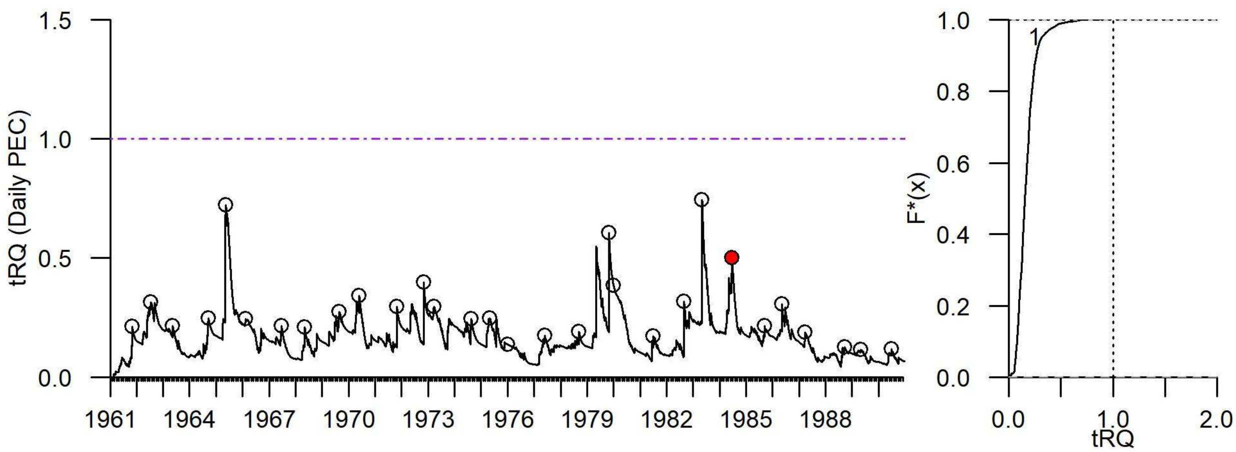
Daily Trenbolone RQs – ONE-PLUS-211 – 5% beta



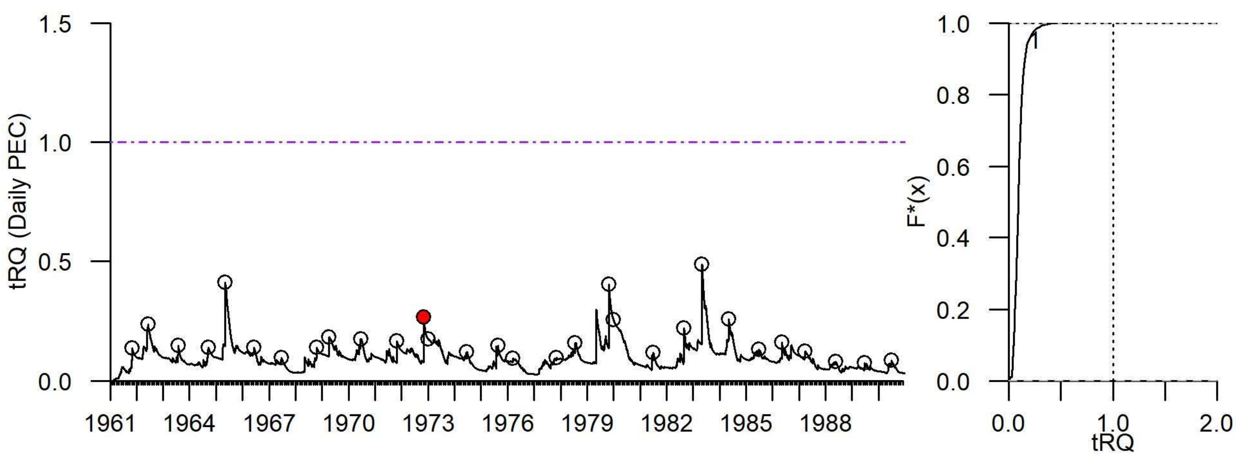
Daily Trenbolone RQs – ONE-PLUS-211 – 8% beta



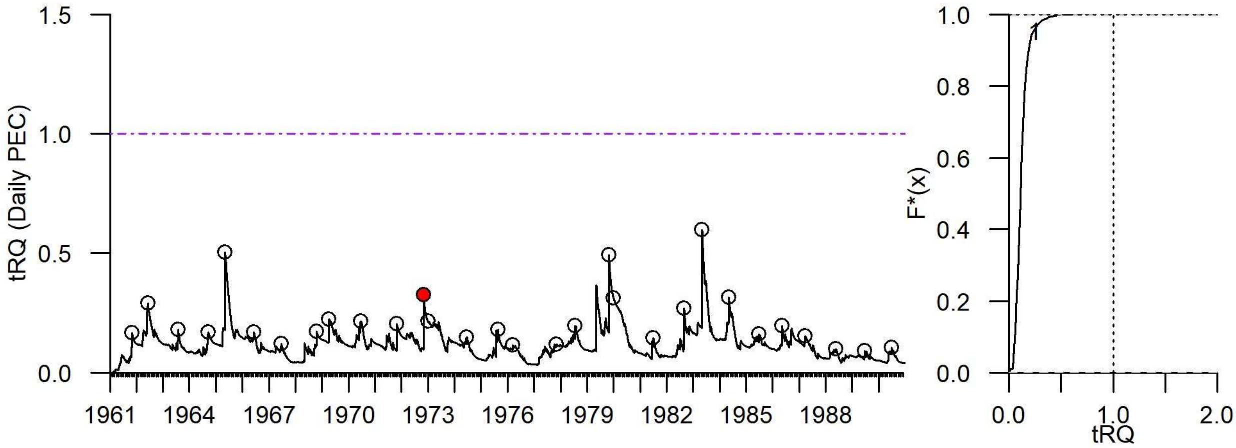
Daily Trenbolone RQs – ONE-PLUS-257 – 5% beta



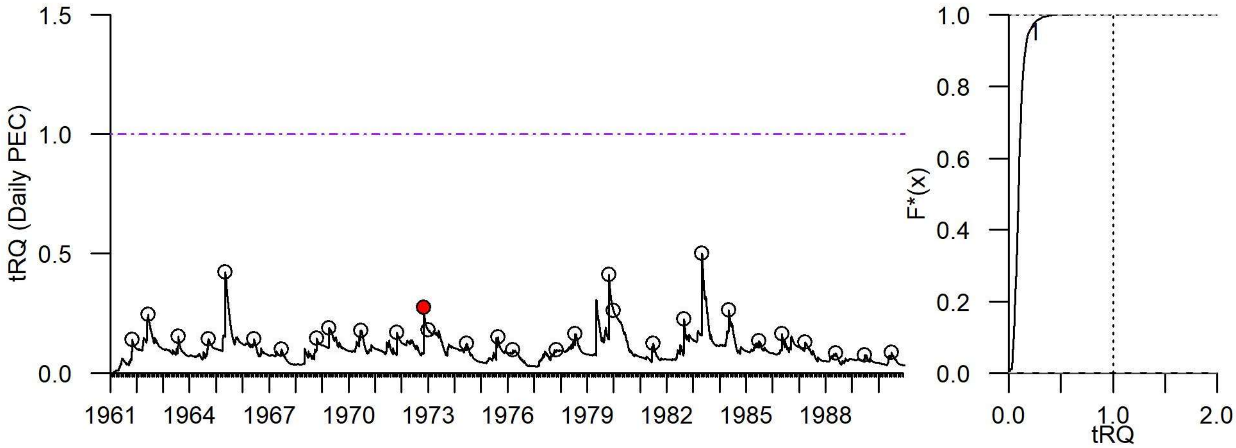
Daily Trenbolone RQs – ONE-PLUS-257 – 8% beta



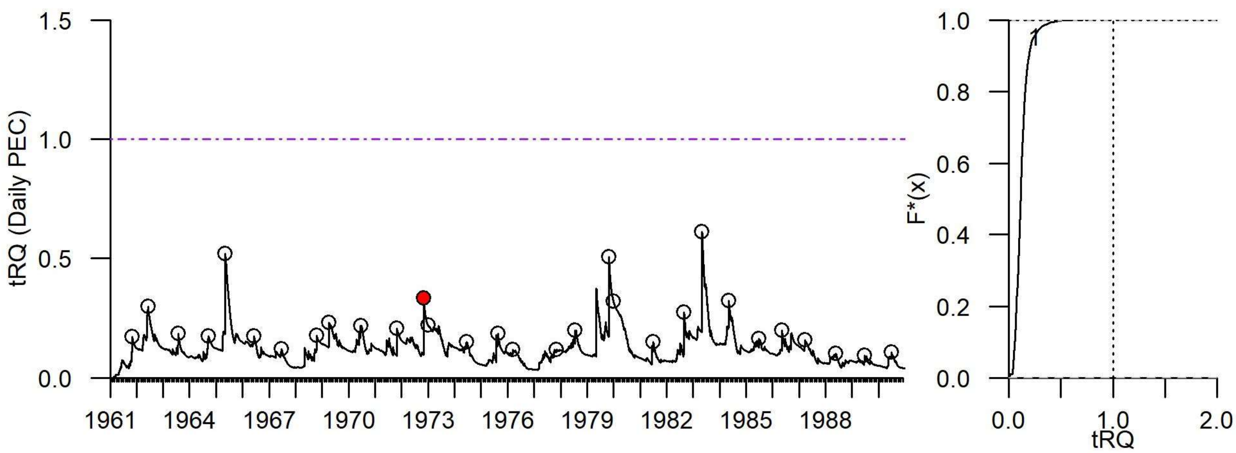
Daily Trenbolone RQs – ONE-CHOICE – 5% beta



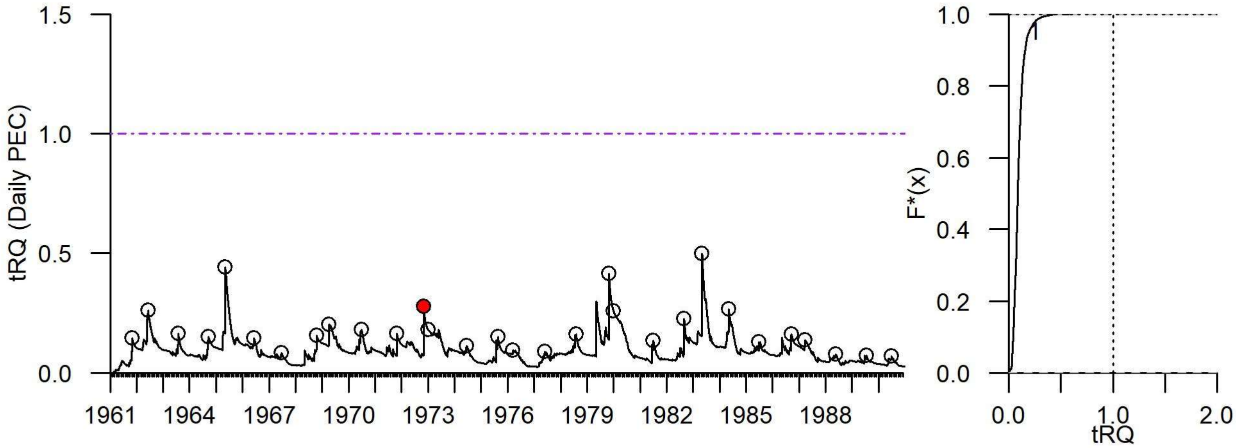
Daily Trenbolone RQs – ONE-CHOICE – 8% beta



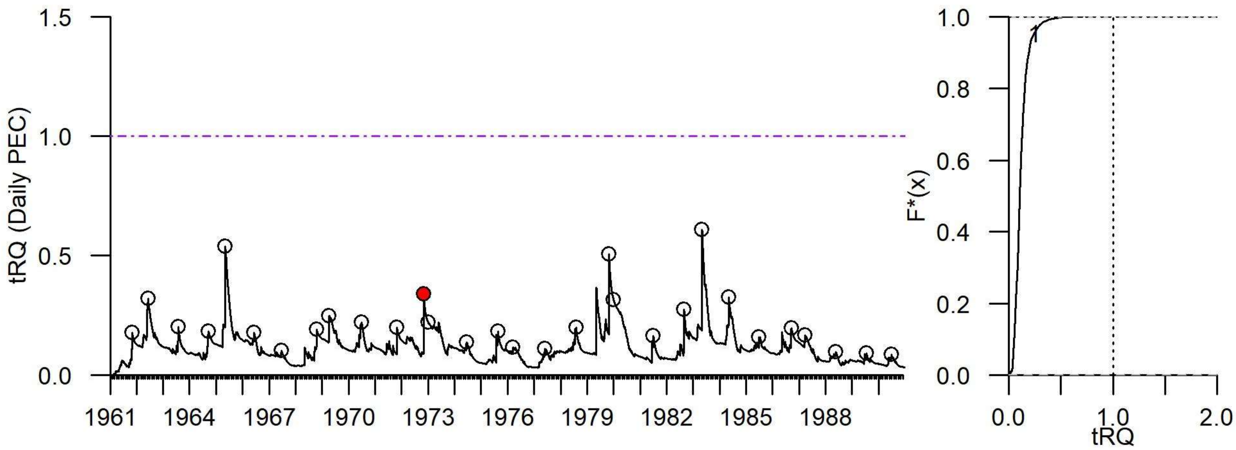
Daily Trenbolone RQs – ONE-ONE – 5% beta



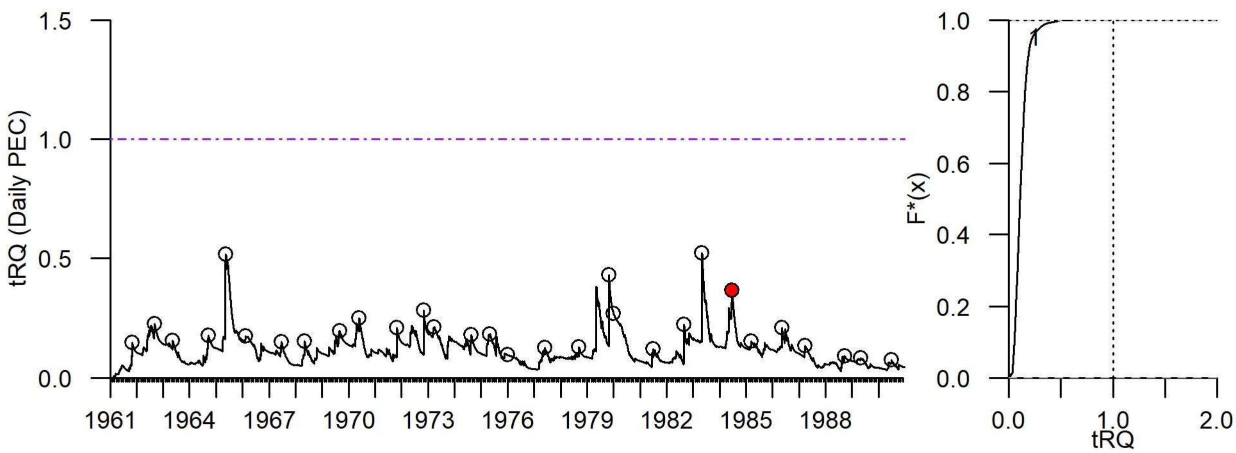
Daily Trenbolone RQs – ONE-ONE – 8% beta



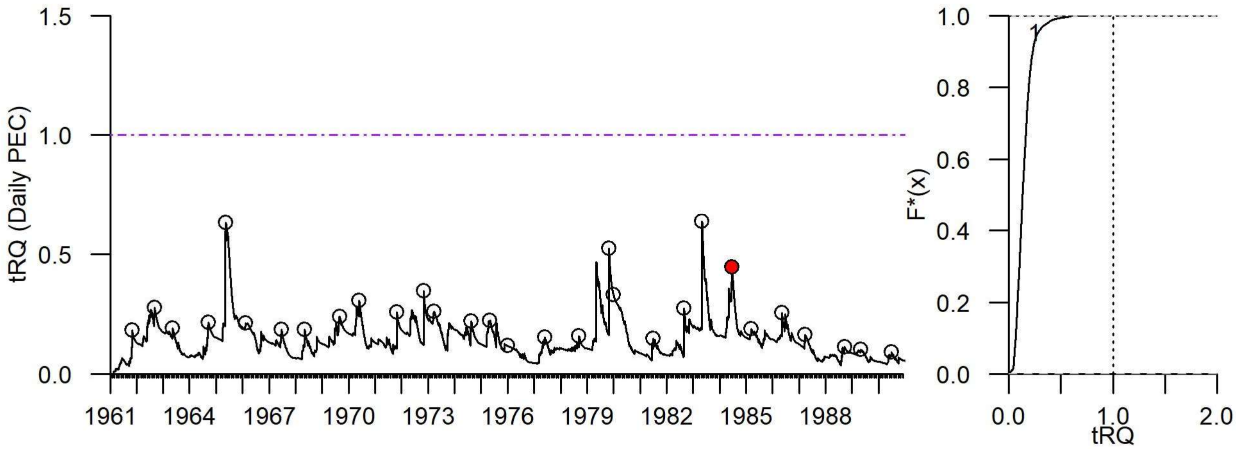
Daily Trenbolone RQs – ONEg-PLUS-211 – 5% beta



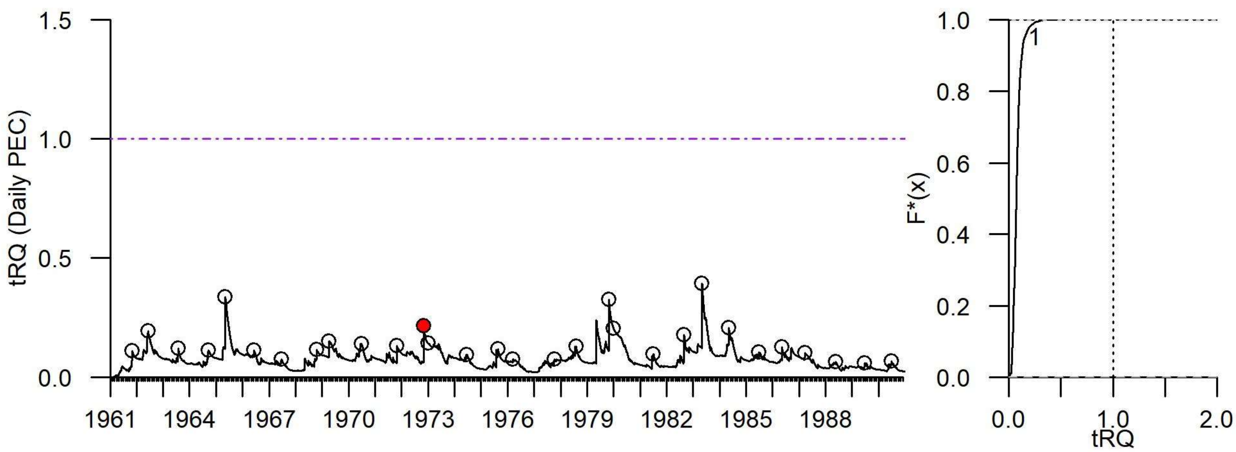
Daily Trenbolone RQs – ONEg-PLUS-211 – 8% beta



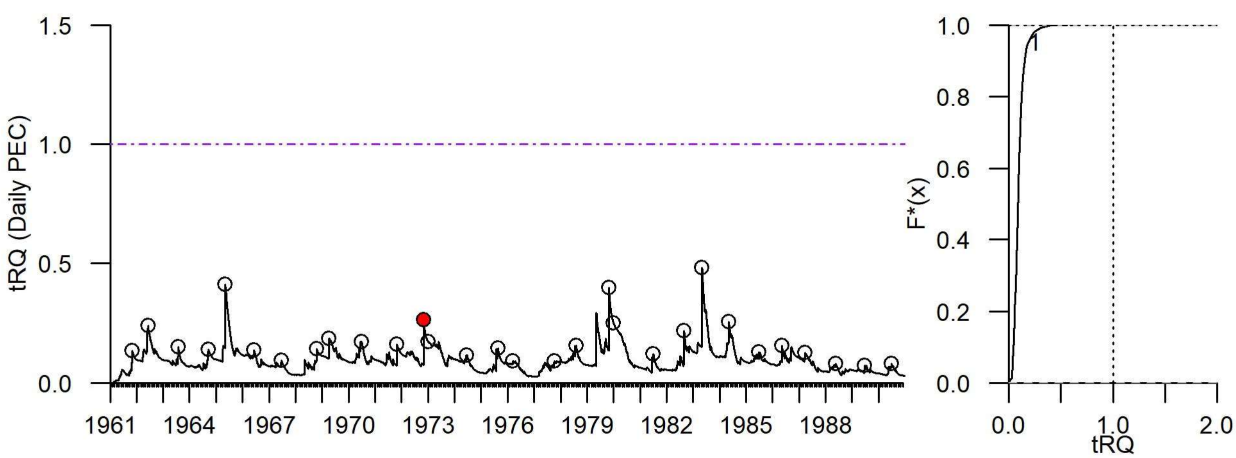
Daily Trenbolone RQs – ONEg-PLUS-257 – 5% beta



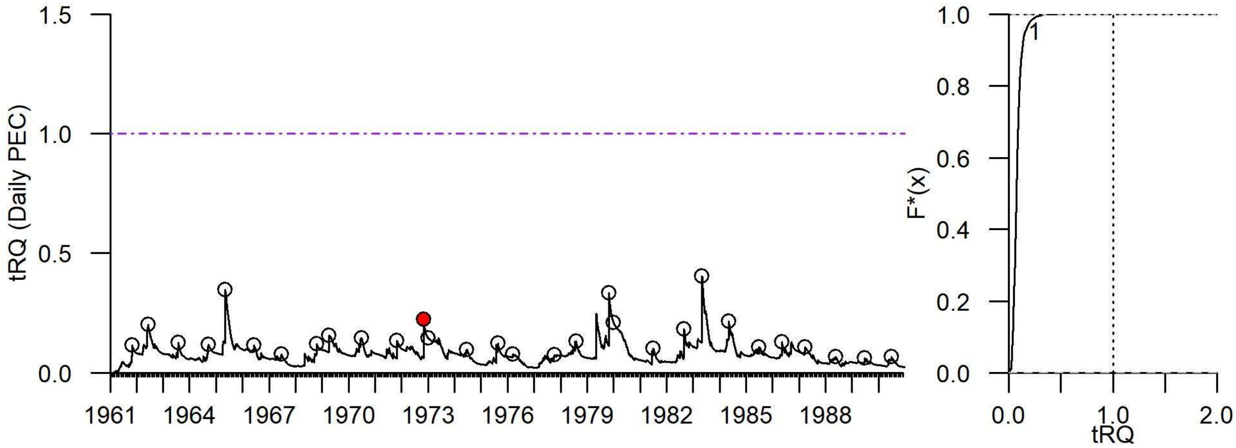
Daily Trenbolone RQs – ONEg-PLUS-257 – 8% beta



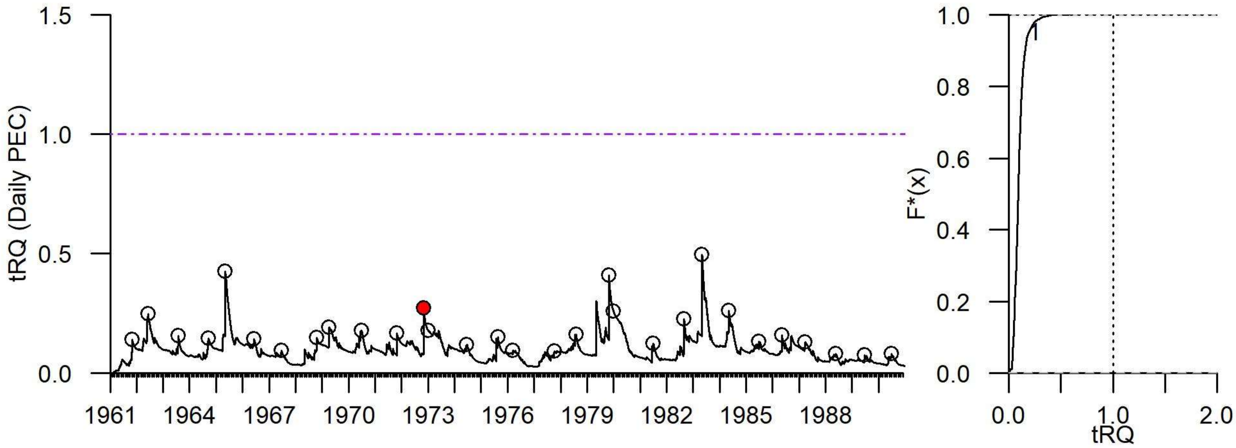
Daily Trenbolone RQs – ONEg-CHOICE – 5% beta



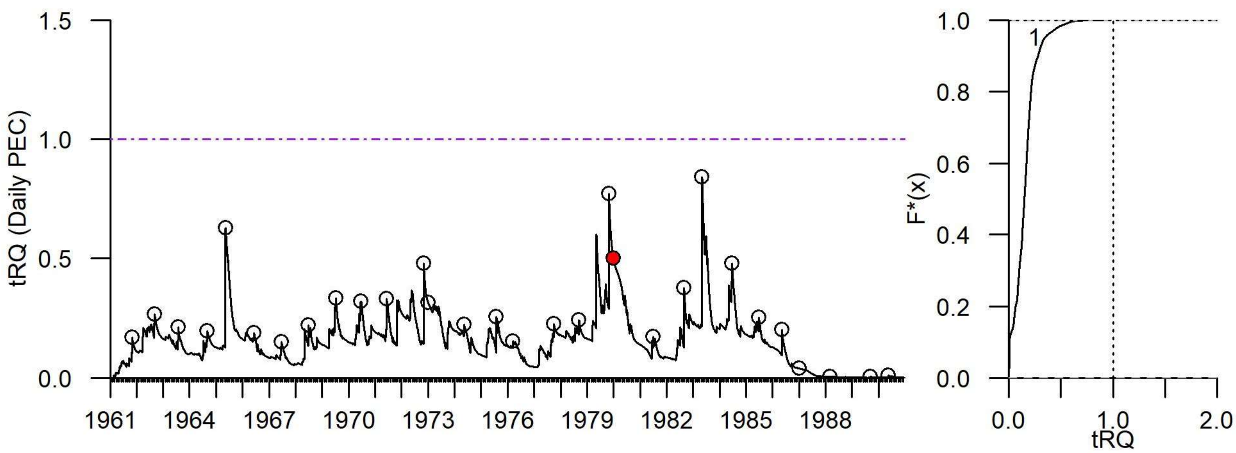
Daily Trenbolone RQs – ONEg-CHOICE – 8% beta



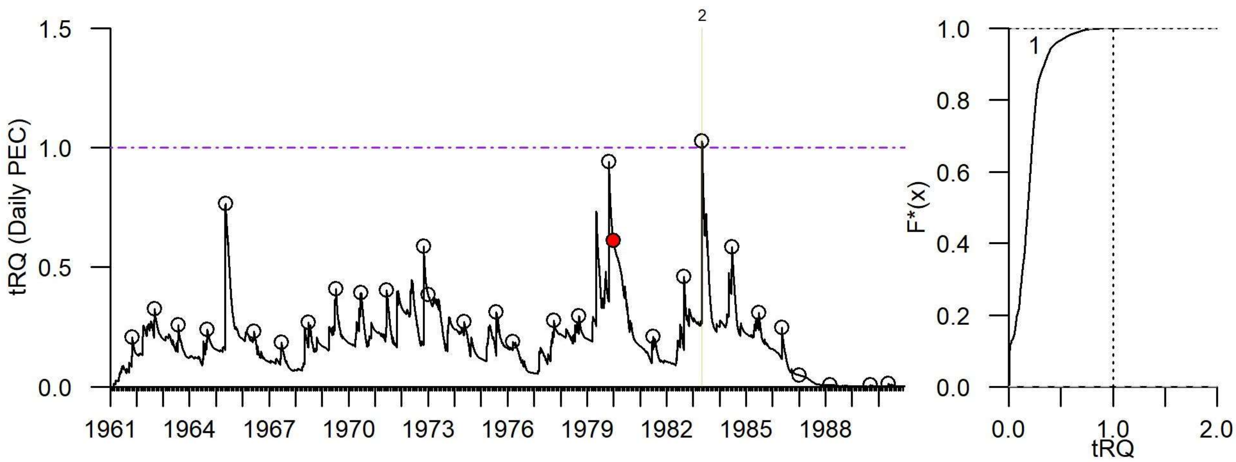
Daily Trenbolone RQs – ONEg-ONE – 5% beta



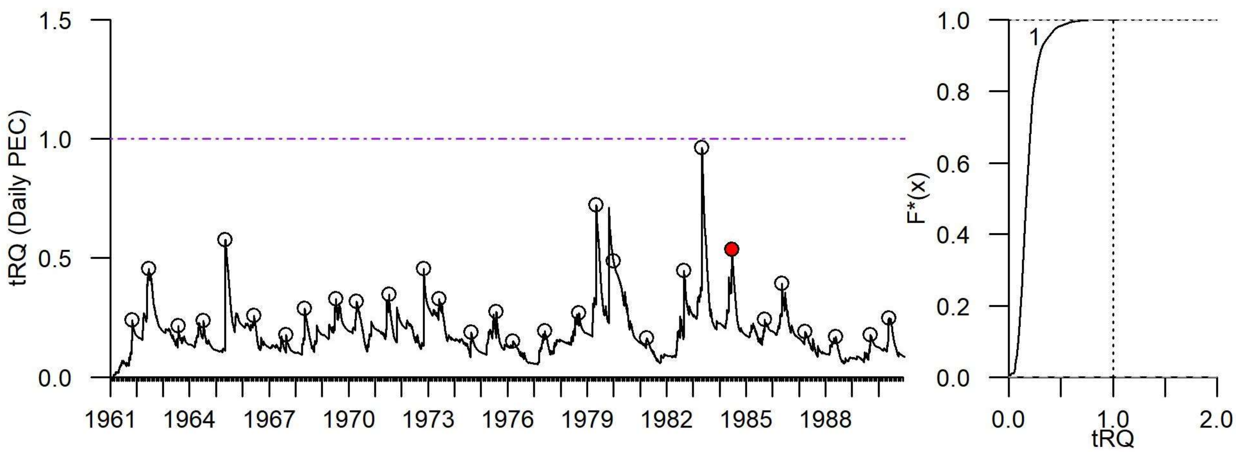
Daily Trenbolone RQs – ONEg-ONE – 8% beta



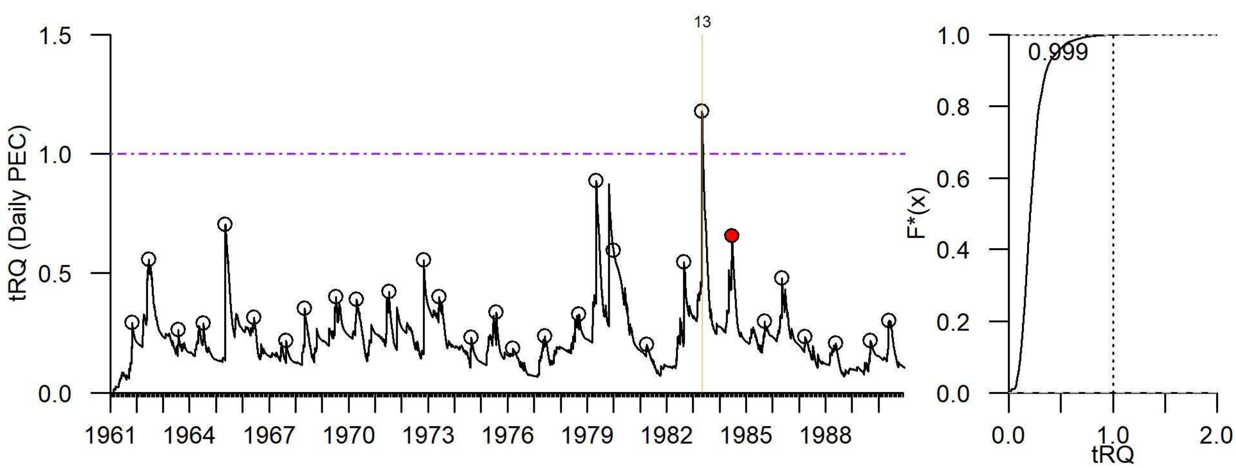
Daily Trenbolone RQs – CHOICE-PLUS-PLUS-177 – 5% beta



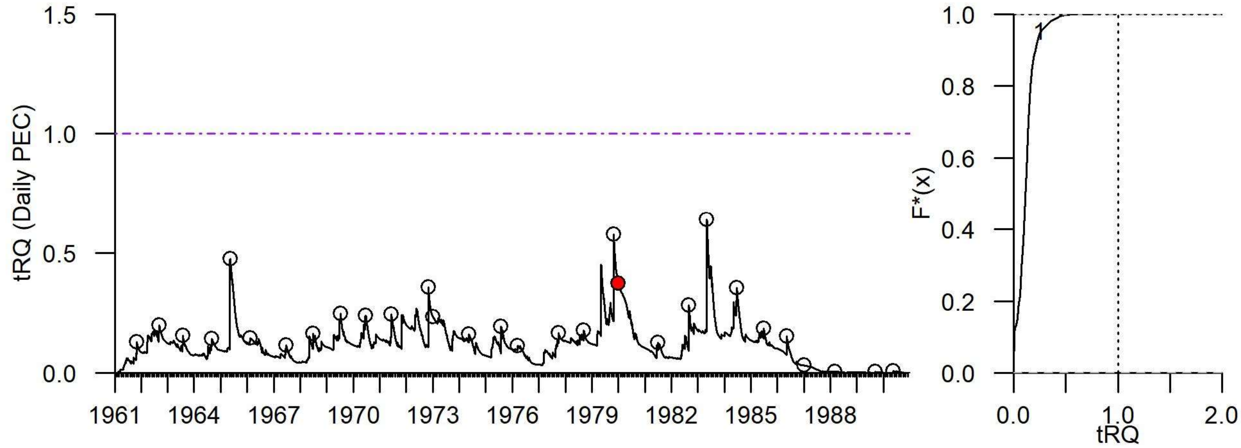
Daily Trenbolone RQs – CHOICE-PLUS-PLUS-177 – 8% beta



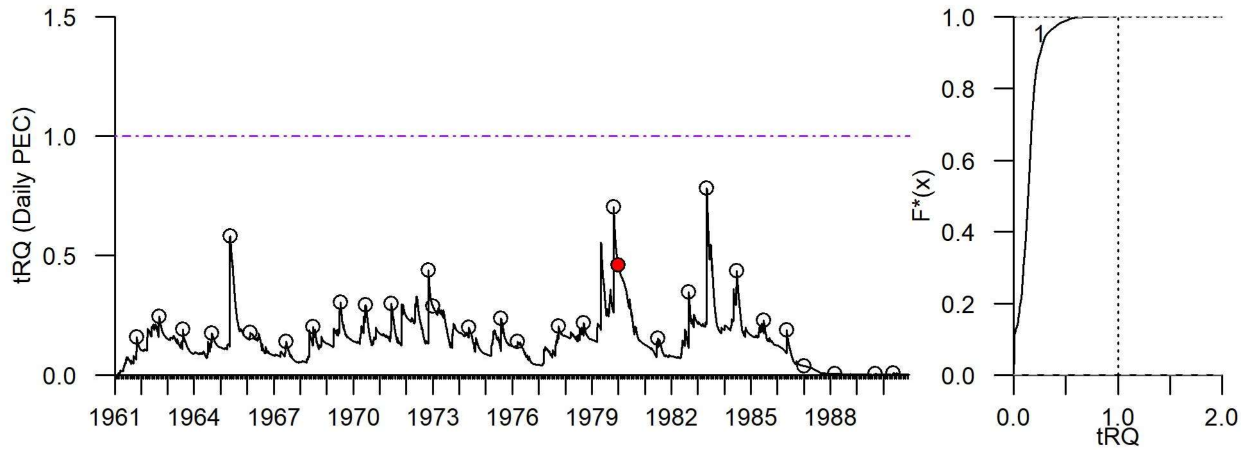
Daily Trenbolone RQs – CHOICE-PLUS-PLUS-237 – 5% beta



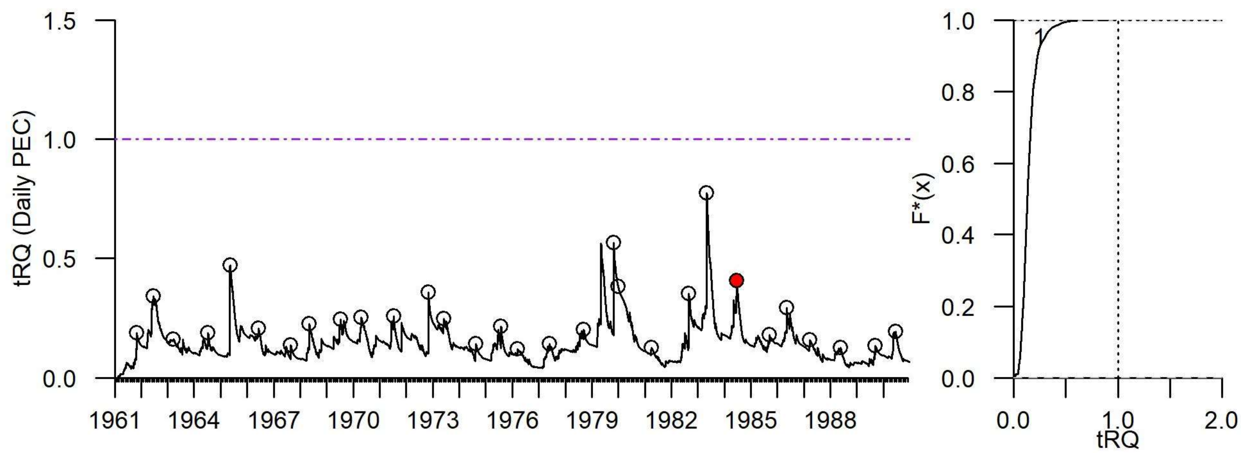
Daily Trenbolone RQs – CHOICE-PLUS-PLUS-237 – 8% beta



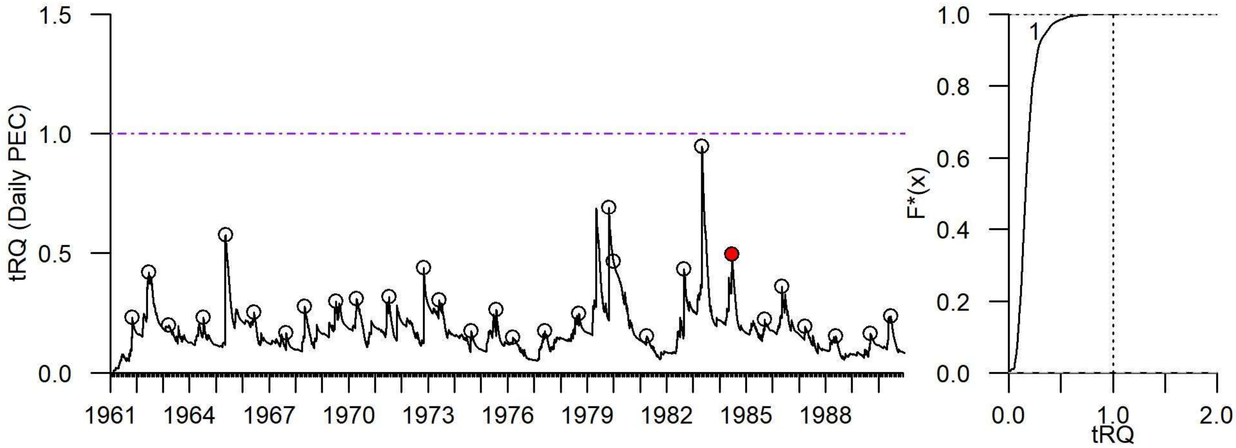
Daily Trenbolone RQs – CHOICE-CHOICE-PLUS-177 – 5% beta



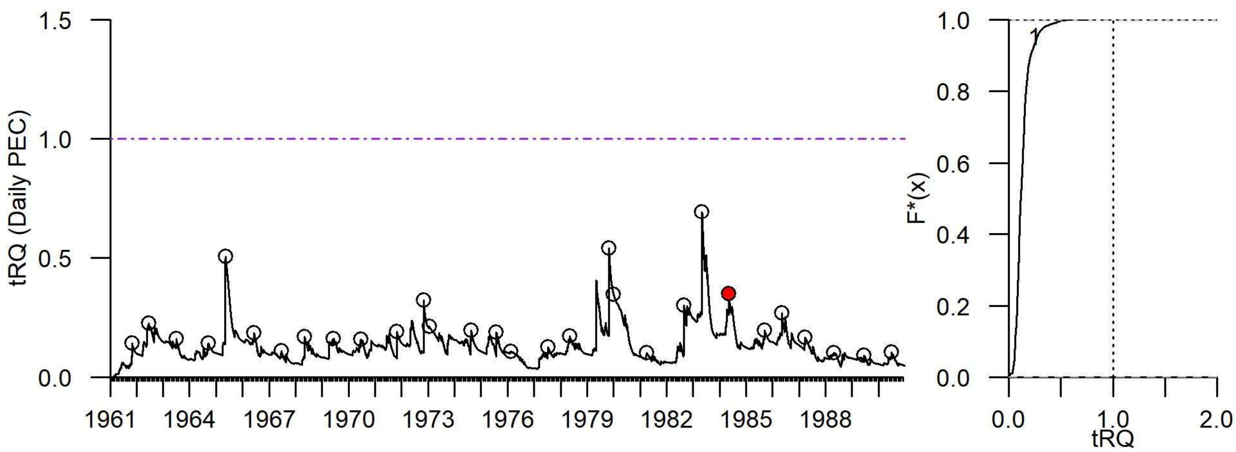
Daily Trenbolone RQs – CHOICE-CHOICE-PLUS-177 – 8% beta



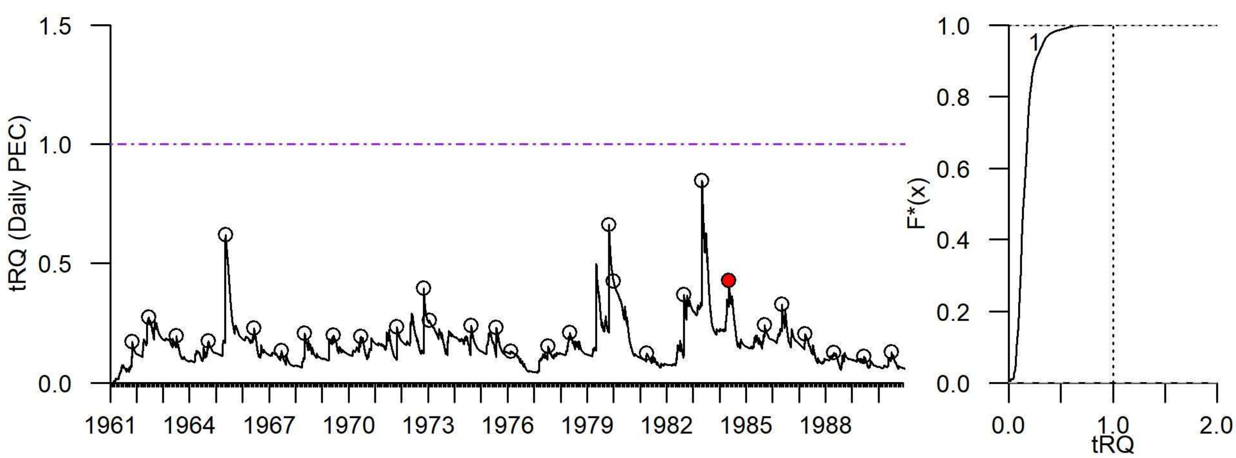
Daily Trenbolone RQs – CHOICE-CHOICE-PLUS-237 – 5% beta



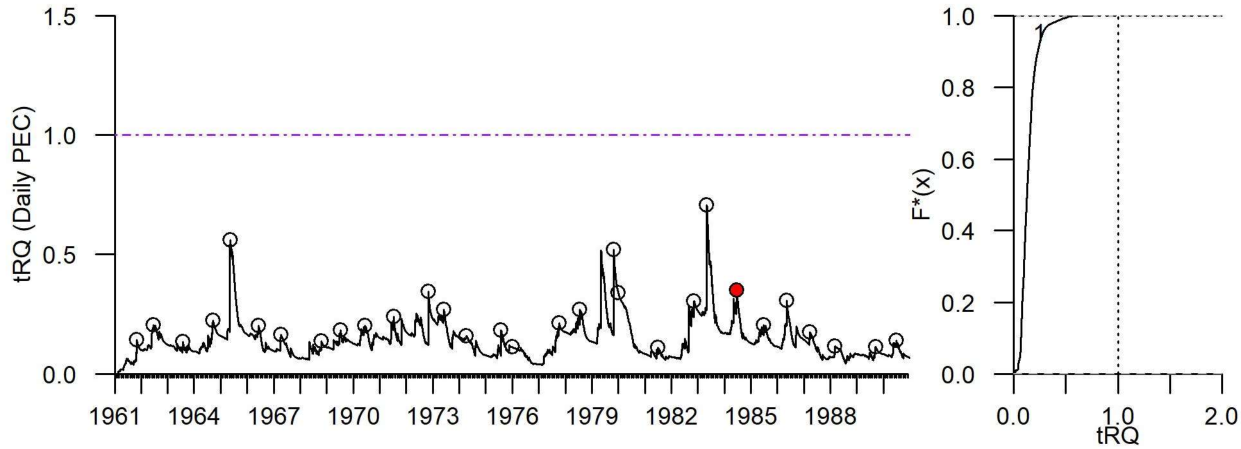
Daily Trenbolone RQs – CHOICE-CHOICE-PLUS-237 – 8% beta



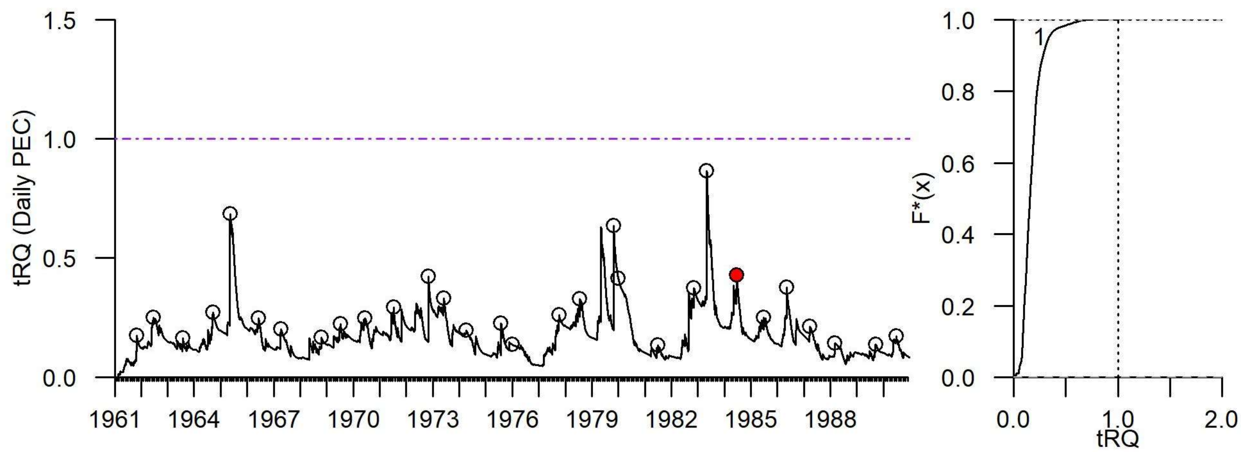
Daily Trenbolone RQs – CHOICE-ONE-PLUS-271 – 5% beta



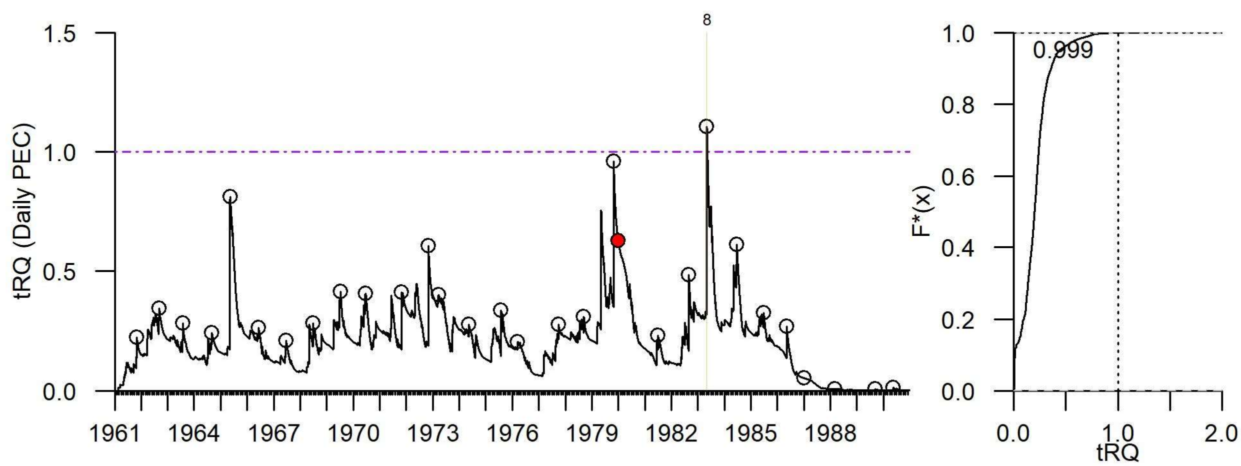
Daily Trenbolone RQs – CHOICE-ONE-PLUS-271 – 8% beta



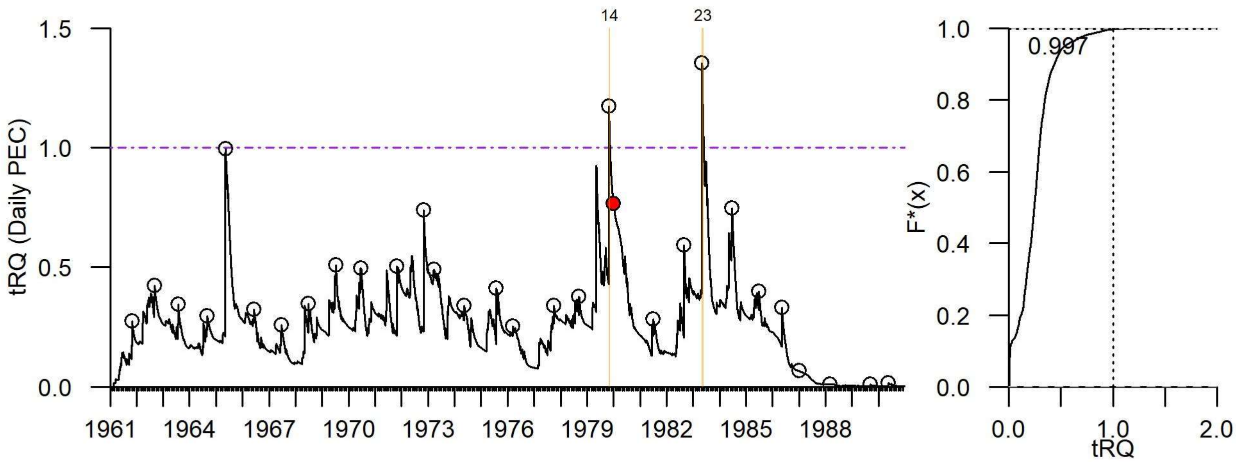
Daily Trenbolone RQs – CHOICE-ONE-PLUS-317 – 5% beta



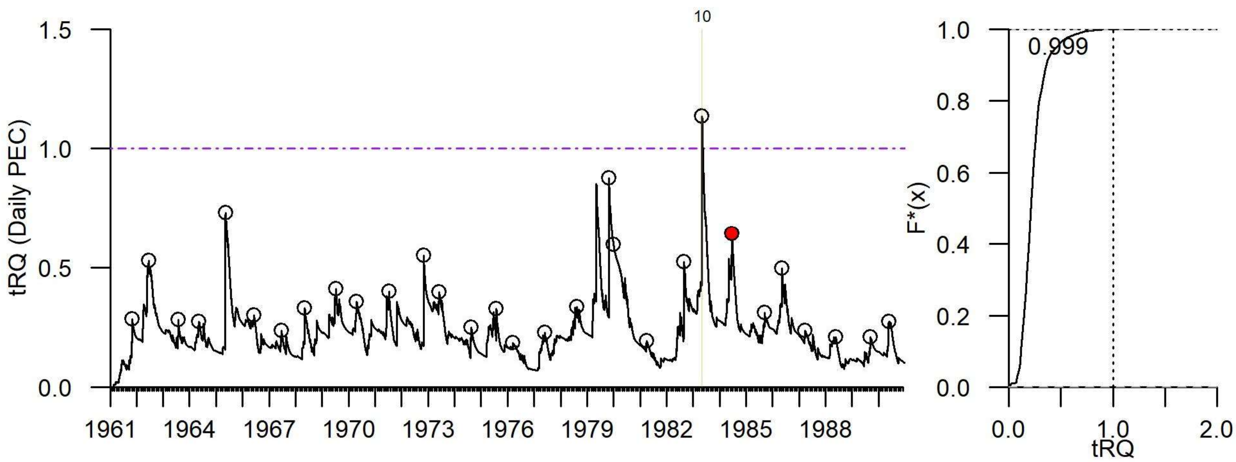
Daily Trenbolone RQs – CHOICE-ONE-PLUS-317 – 8% beta



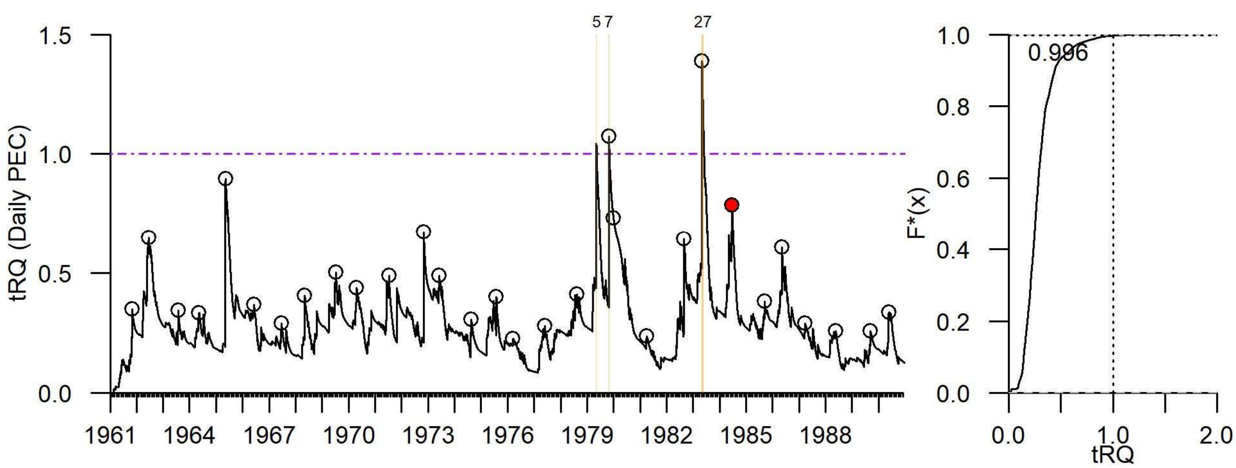
Daily Trenbolone RQs – PLUS-PLUS-PLUS-177 – 5% beta



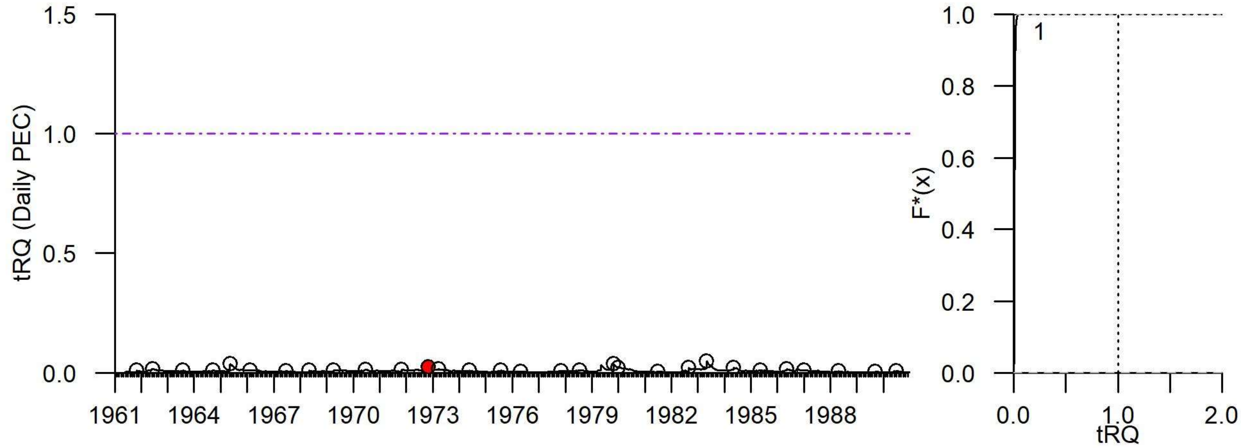
Daily Trenbolone RQs – PLUS-PLUS-PLUS-177 – 8% beta



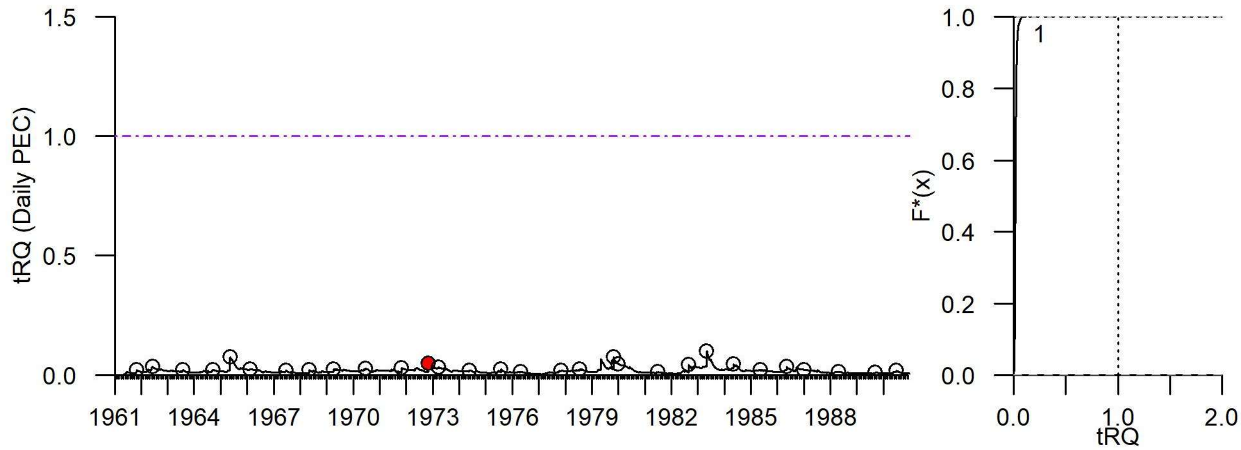
Daily Trenbolone RQs – PLUS-PLUS-PLUS-237 – 5% beta



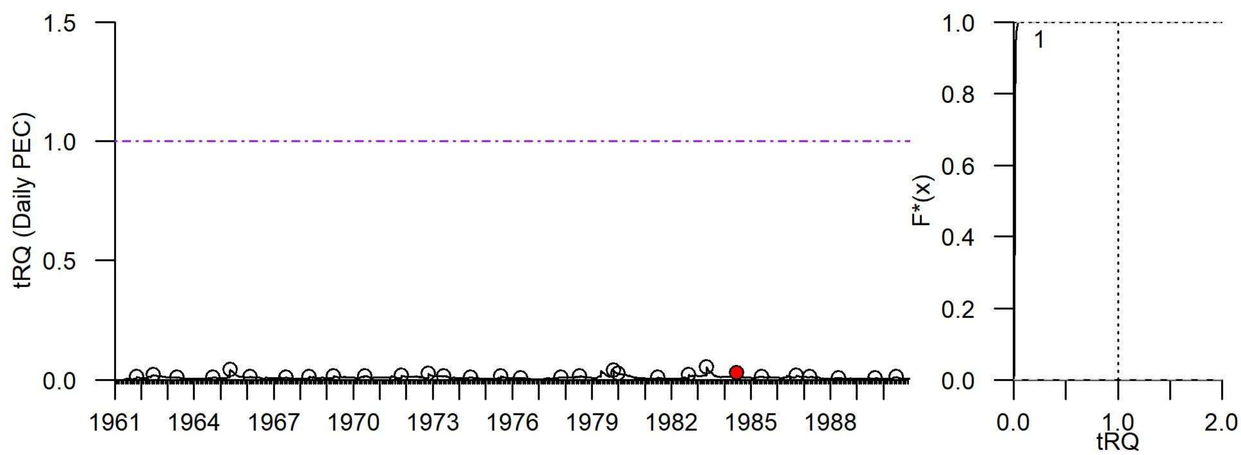
Daily Trenbolone RQs – PLUS-PLUS-PLUS-237 – 8% beta



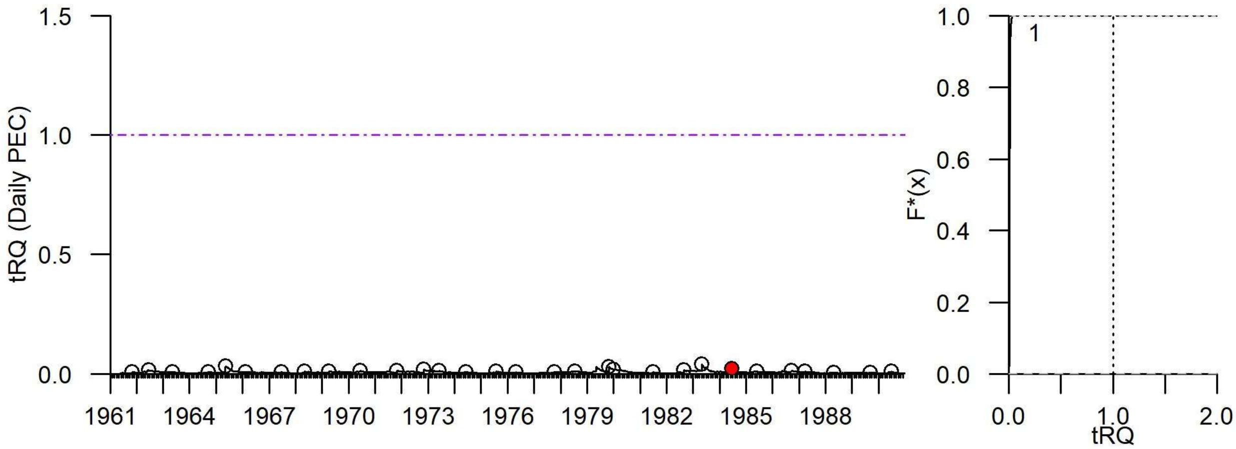
Daily Estradiol RQs – CHOICE



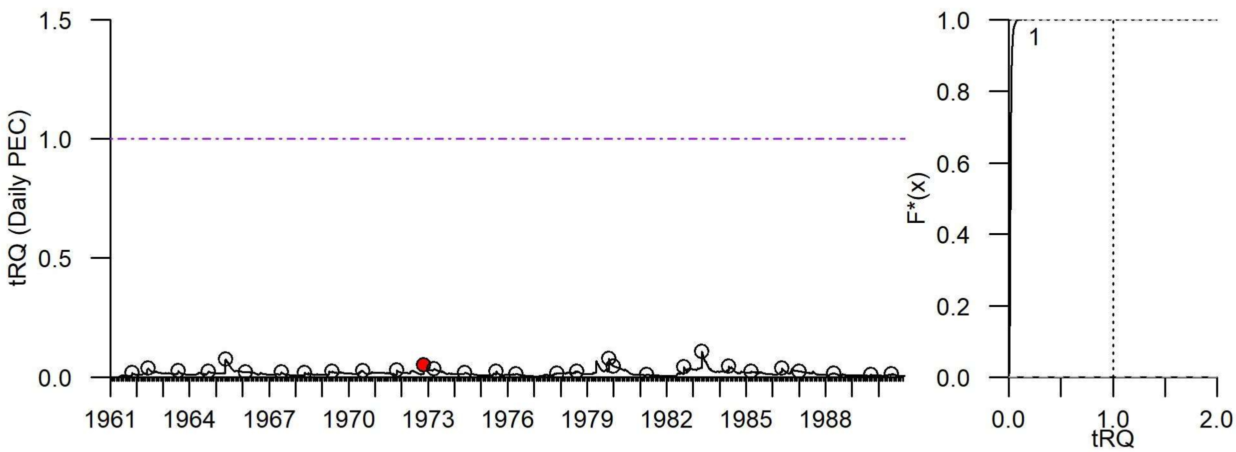
Daily Estradiol RQs – PLUS



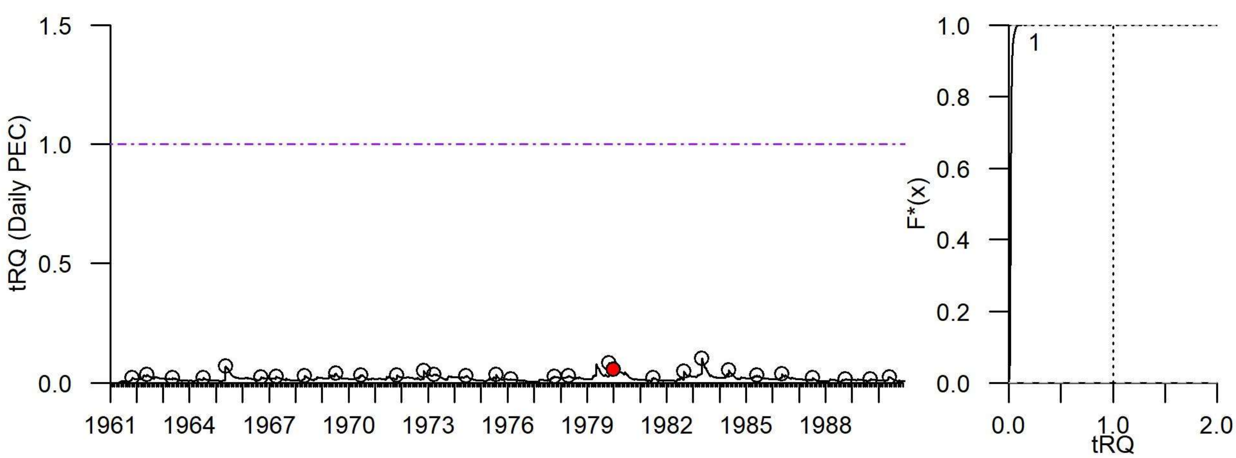
Daily Estradiol RQs – ONE



Daily Estradiol RQs – ONEg



Daily Estradiol RQs – CHOICE-PLUS-141



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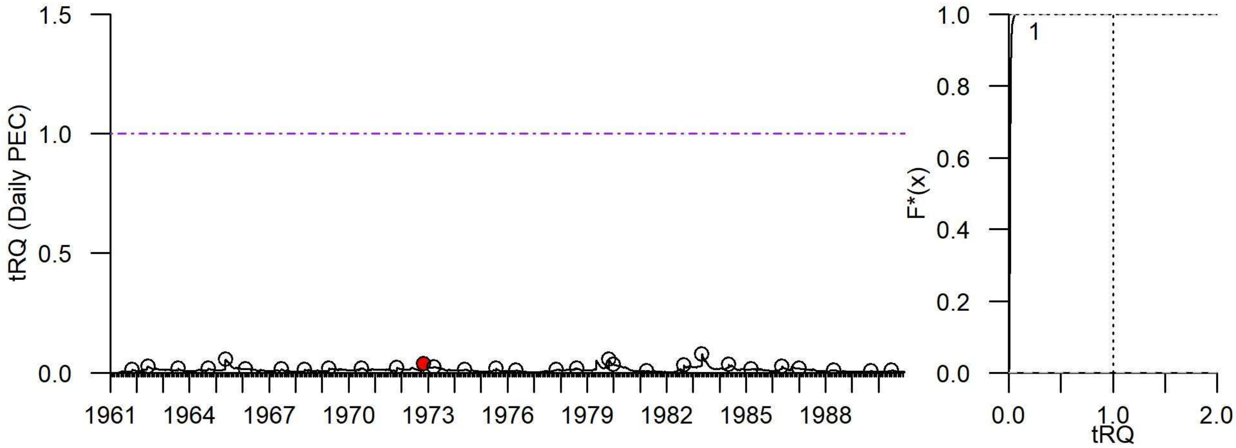
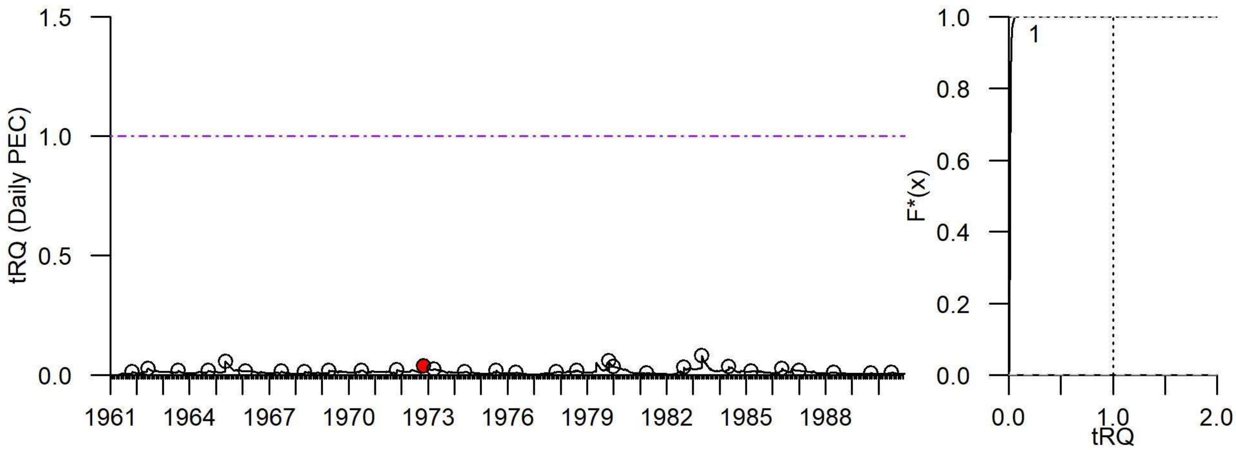
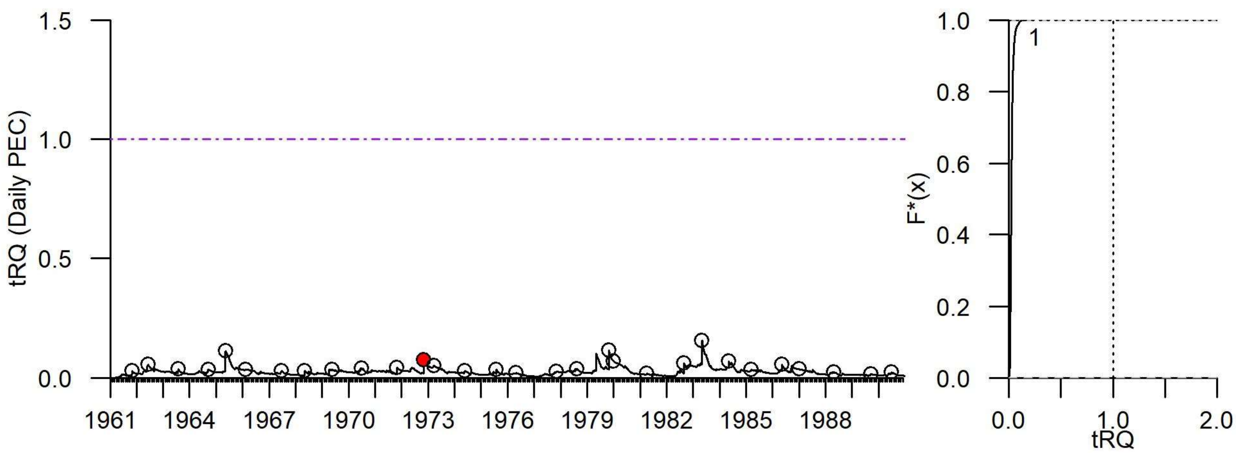


Daily Estradiol RQs – CHOICE-CHOICE



Daily Estradiol RQs – CHOICE-ONE



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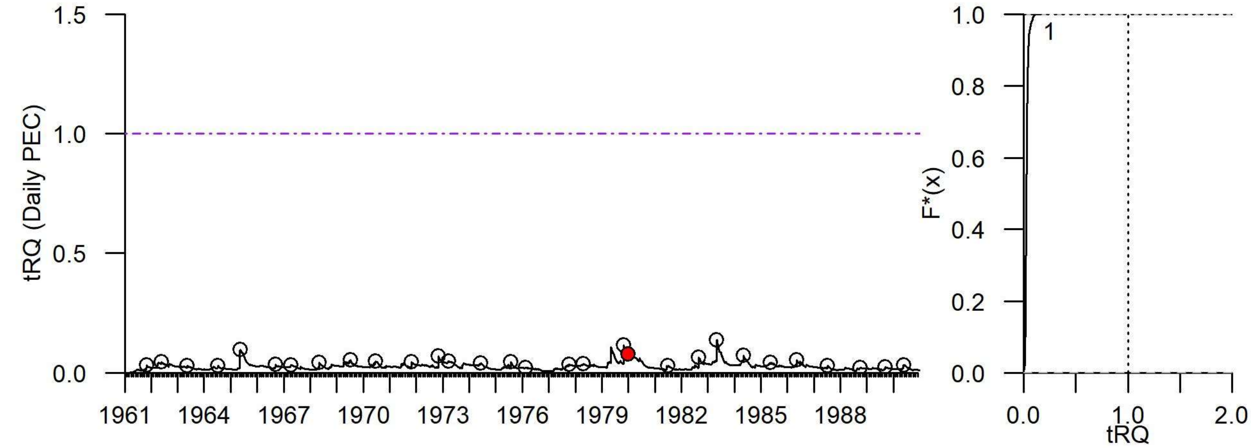

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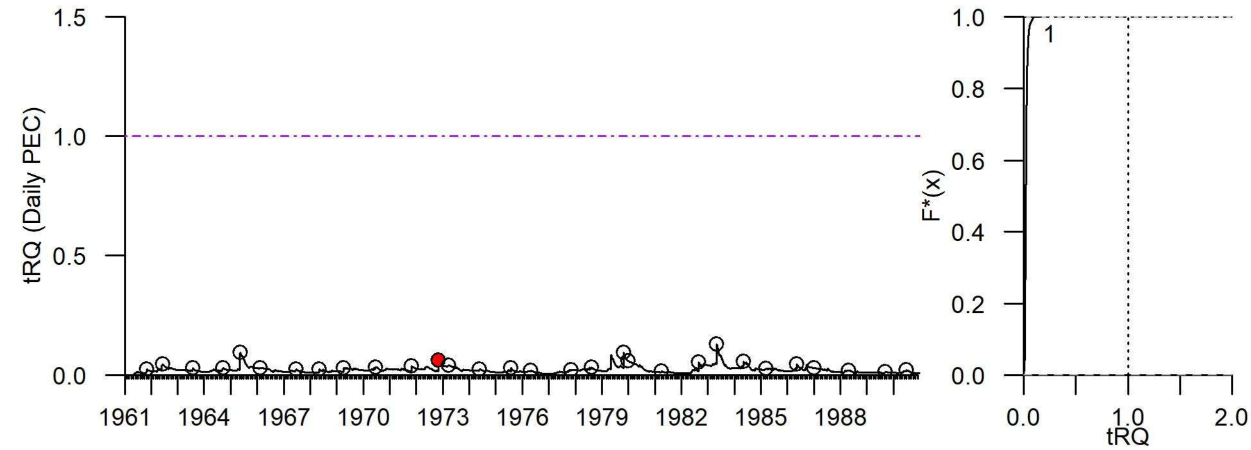
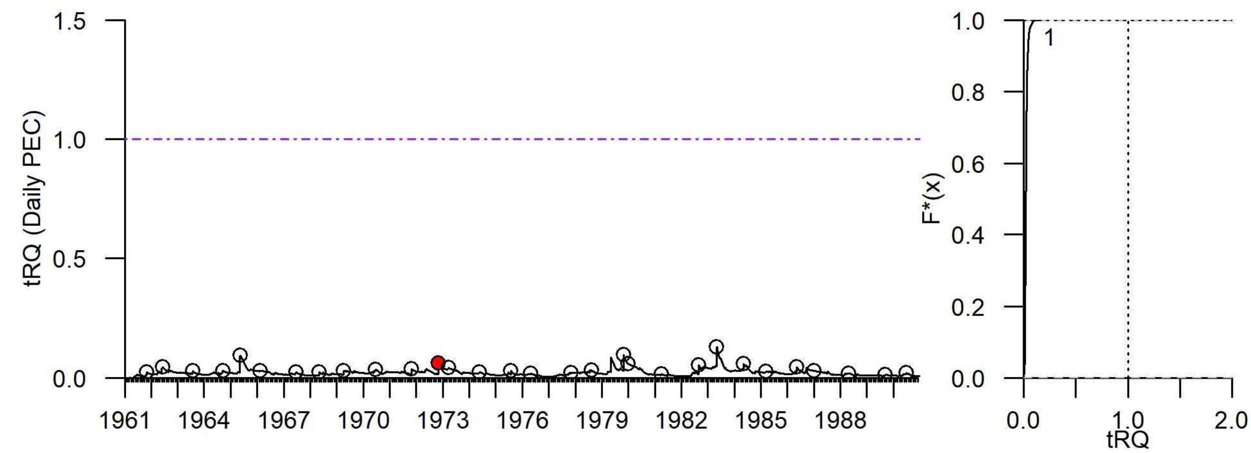
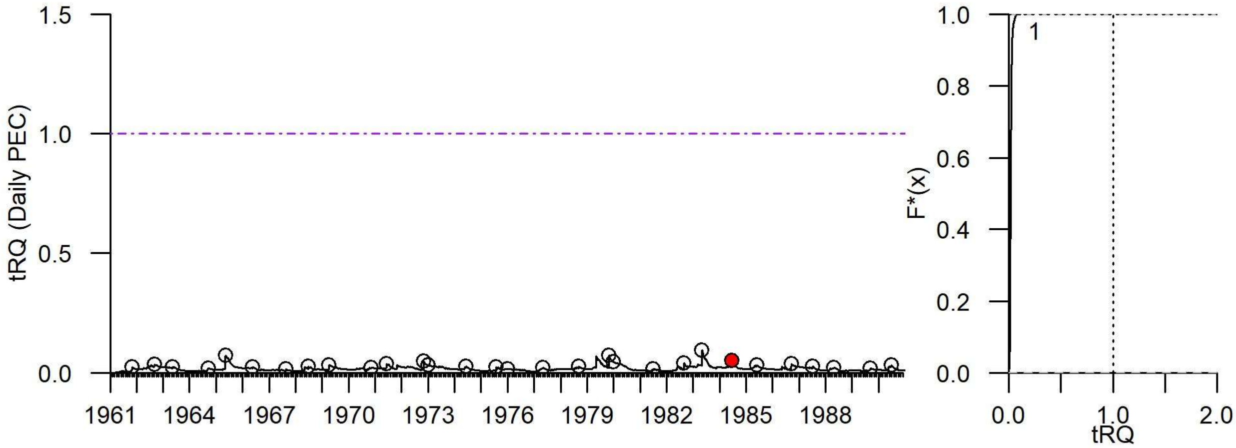


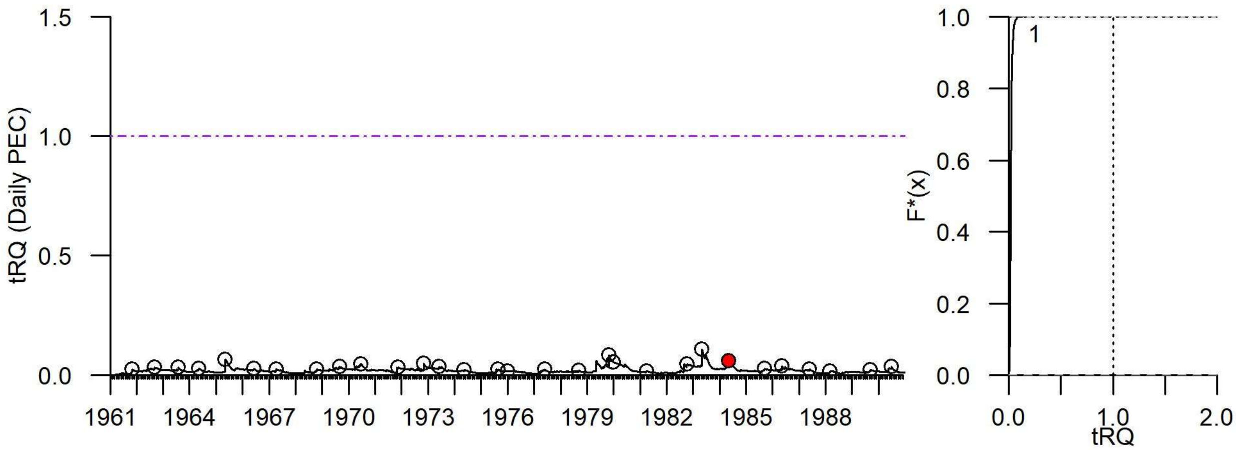
Daily Estradiol RQs – PLUS-CHOICE



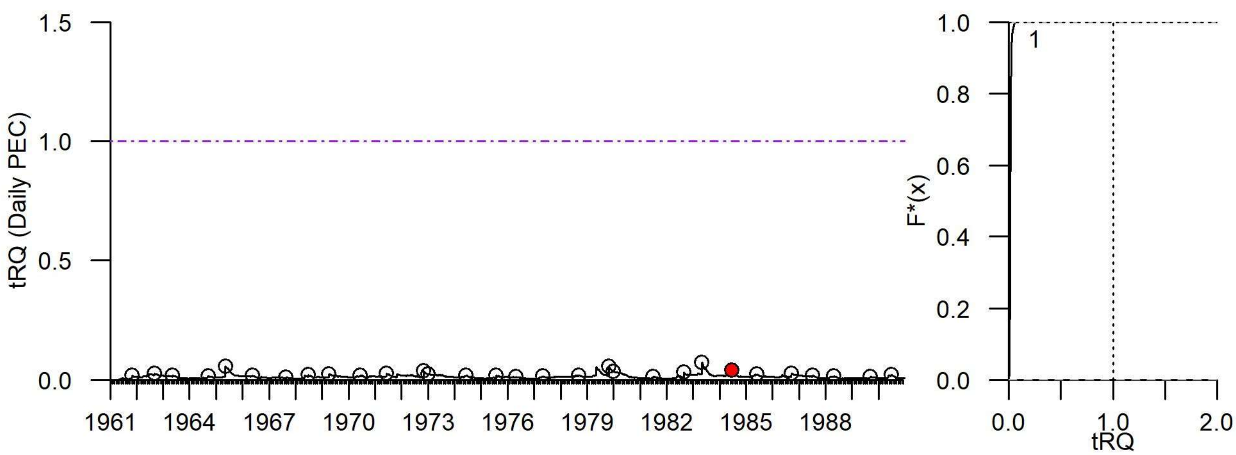
Daily Estradiol RQs – PLUS-ONE



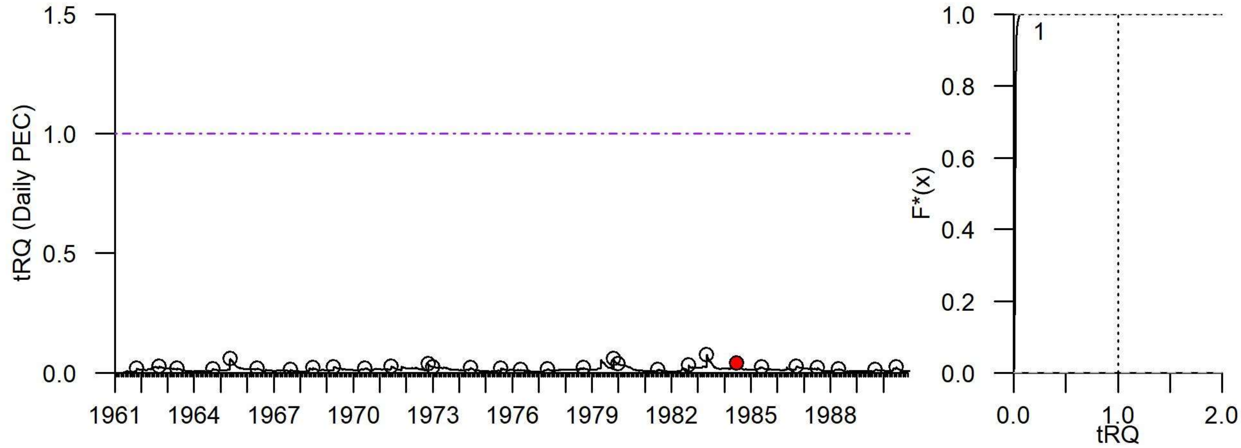
Daily Estradiol RQs – ONE-PLUS-267



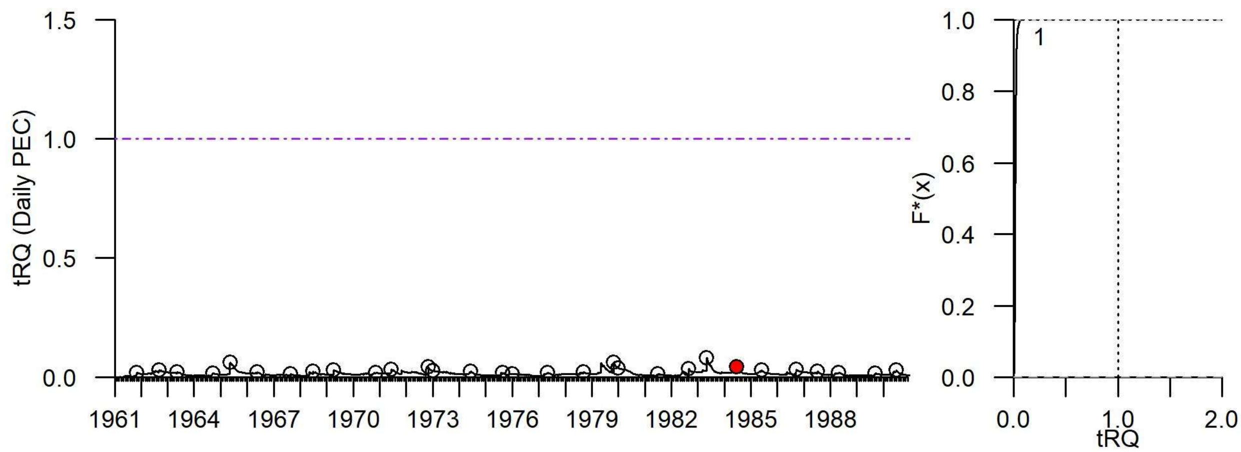
Daily Estradiol RQs – ONE-PLUS-281



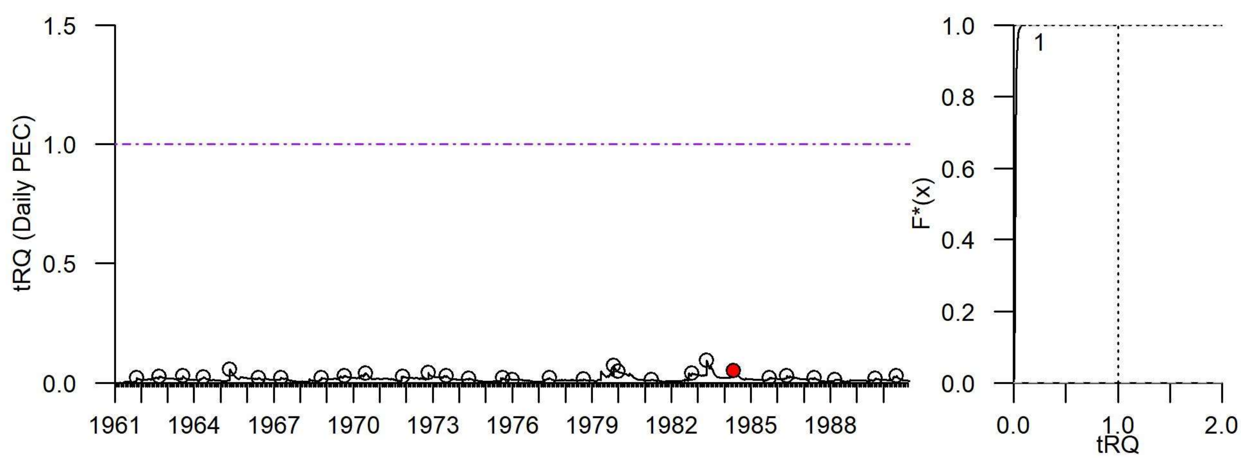
Daily Estradiol RQs – ONE-CHOICE



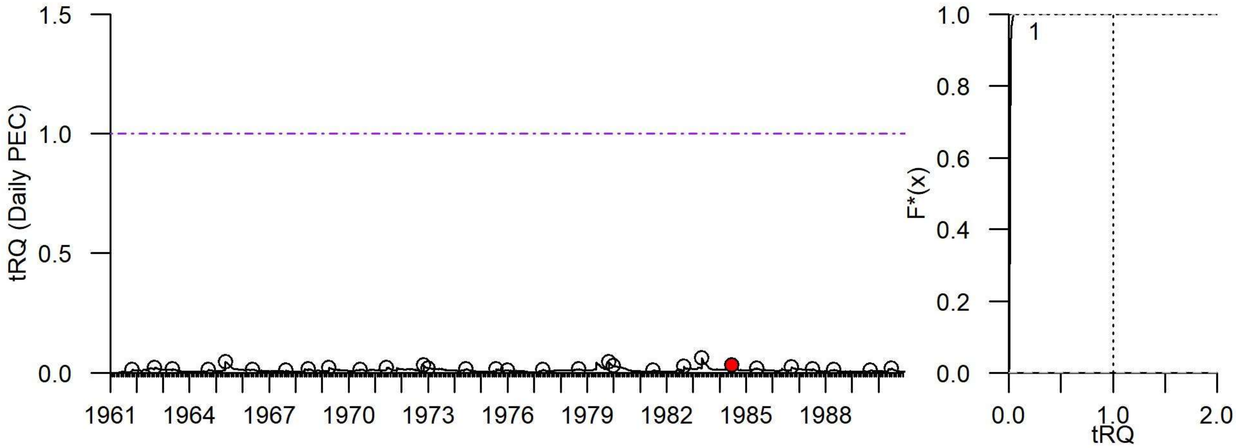
Daily Estradiol RQs – ONE-ONE



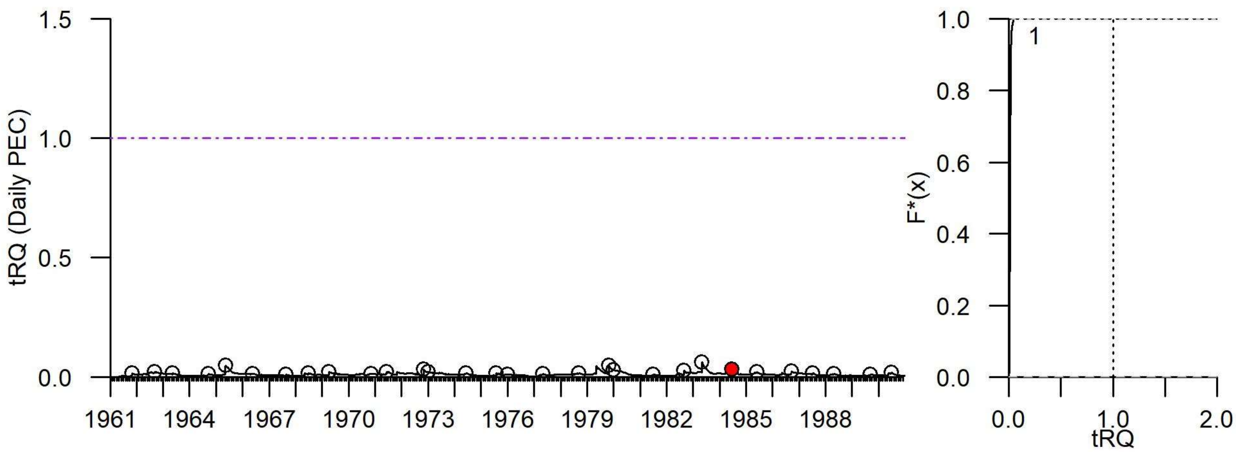
Daily Estradiol RQs – ONEg-PLUS-267



Daily Estradiol RQs – ONEg-PLUS-281



Daily Estradiol RQs – ONEg-CHOICE



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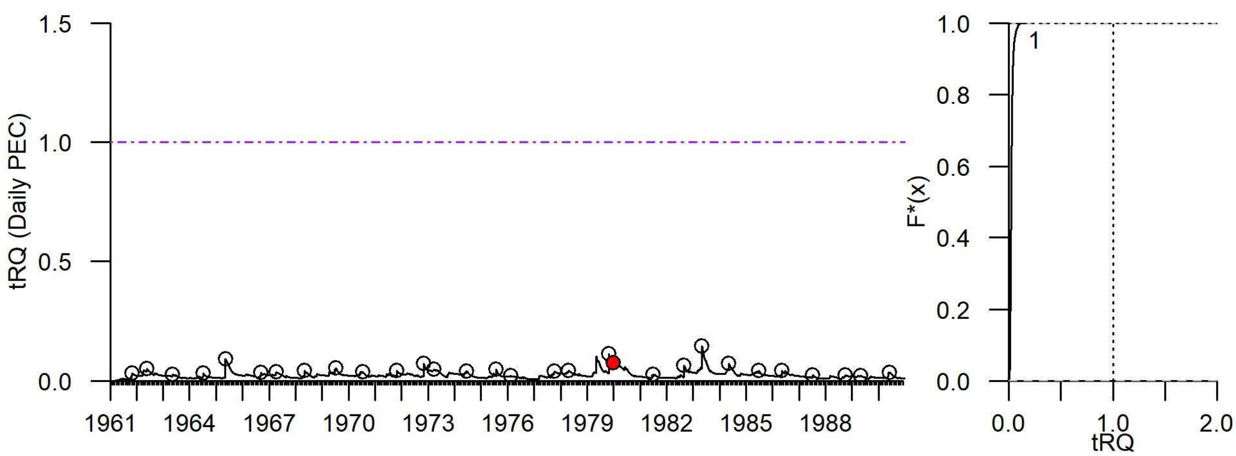
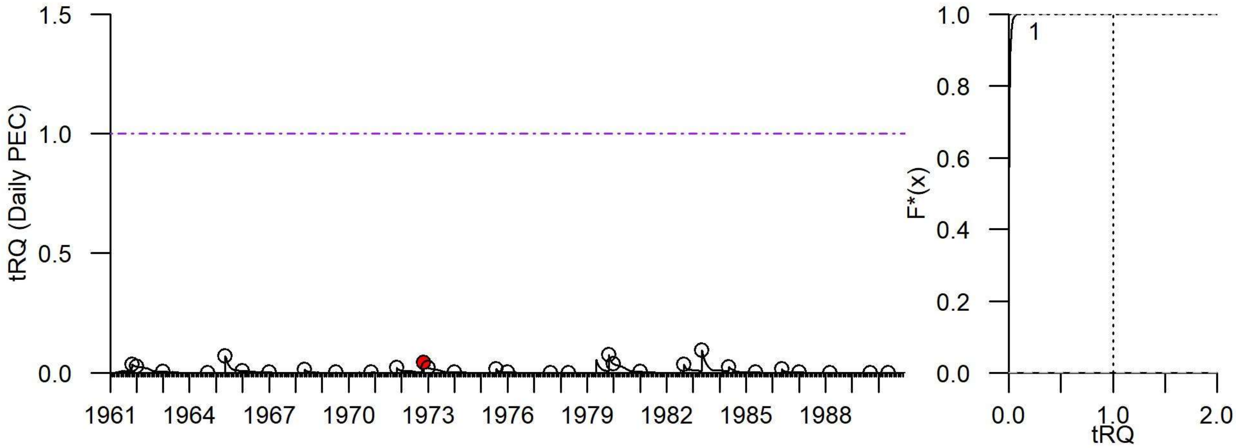
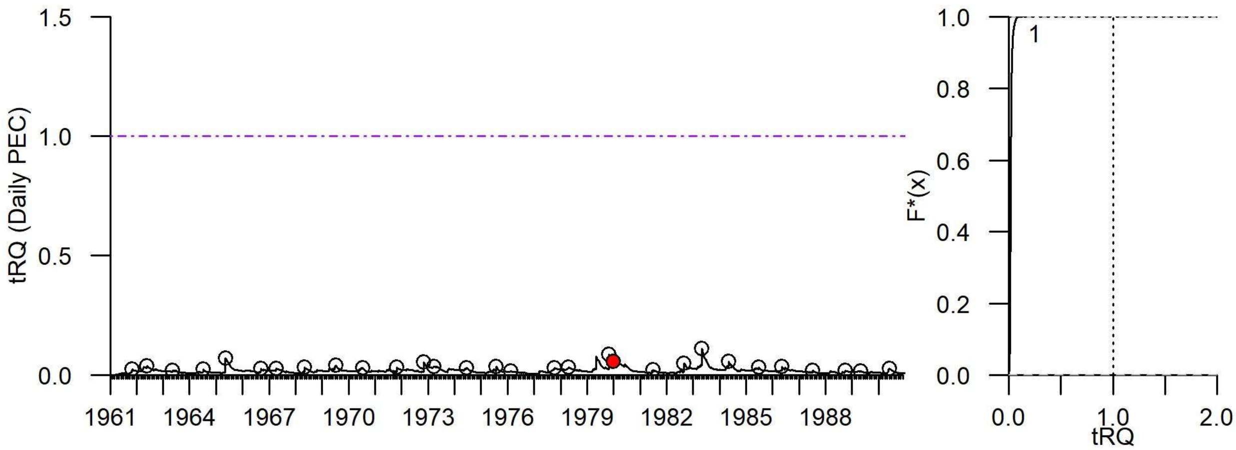


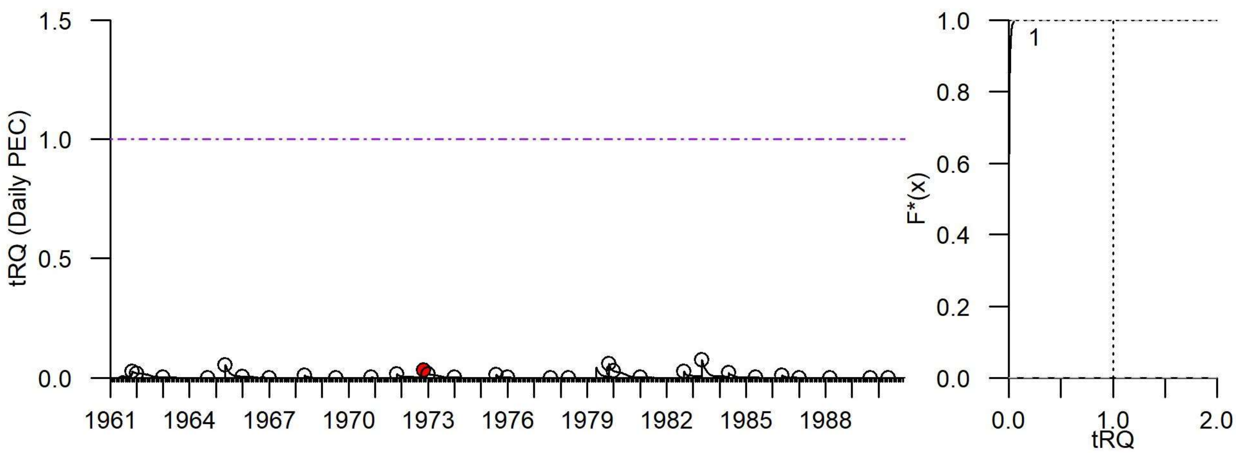
Daily Estradiol RQs – CHOICE-PLUS-PLUS-201



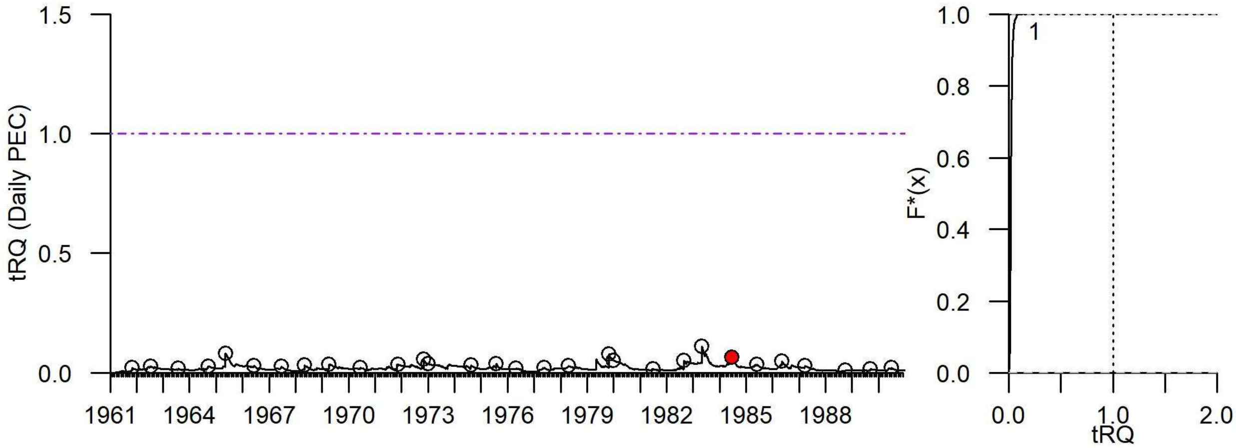
Daily Estradiol RQs – CHOICE-PLUS-PLUS-261



Daily Estradiol RQs – CHOICE-CHOICE-PLUS-201



Daily Estradiol RQs – CHOICE-CHOICE-PLUS-261



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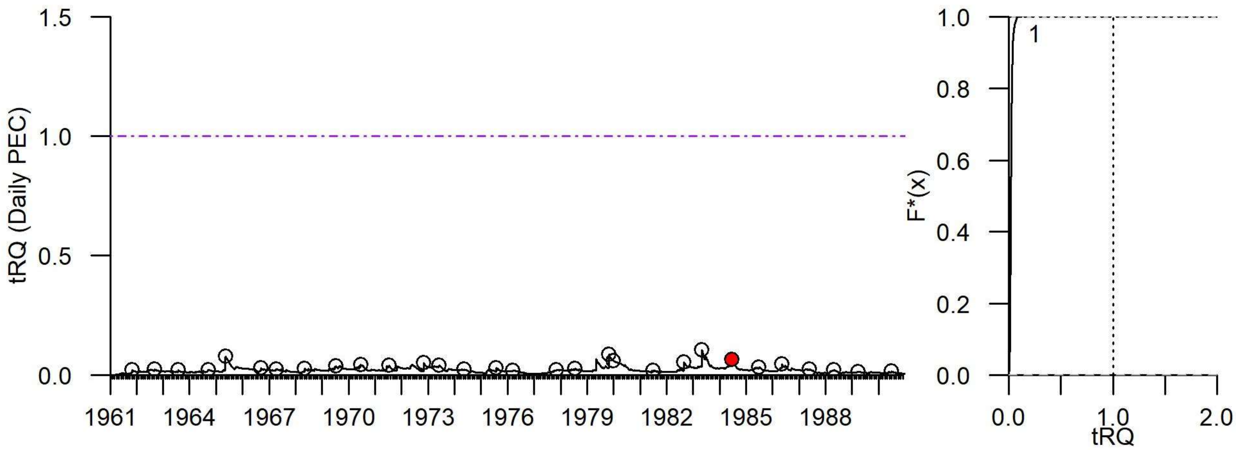
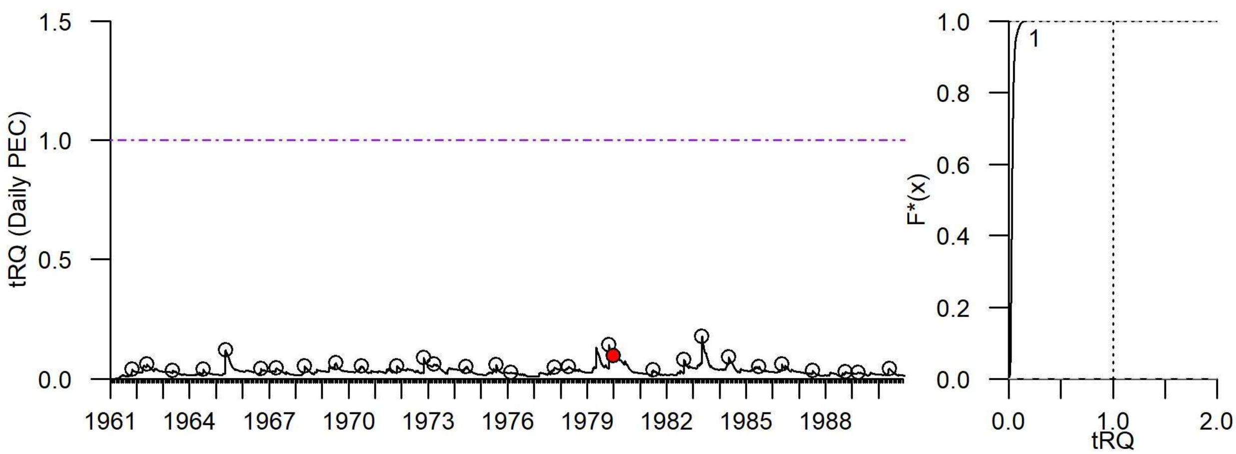
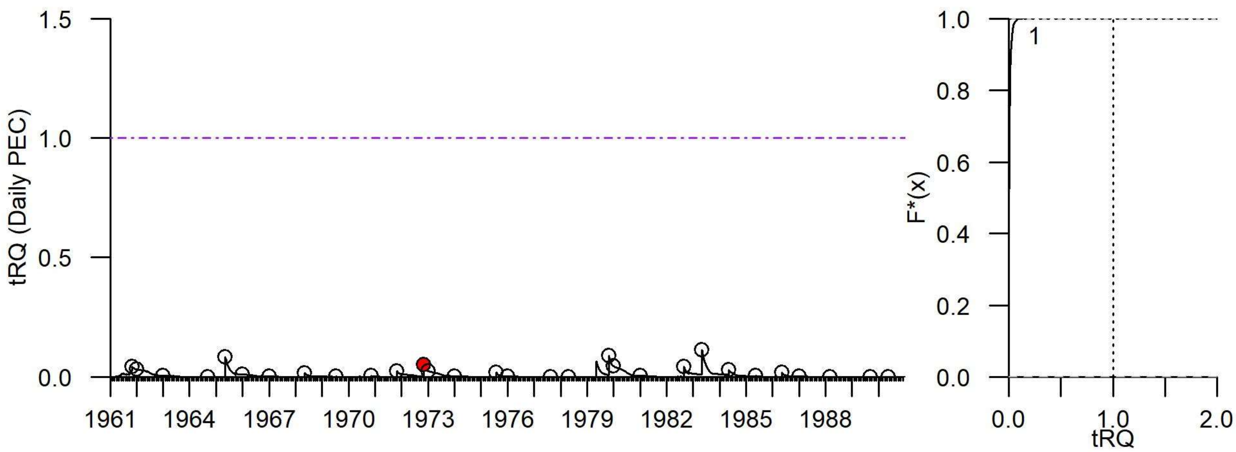


Daily Estradiol RQs – CHOICE-ONE-PLUS-341



Daily Estradiol RQs – PLUS-PLUS-PLUS-201



Daily Estradiol RQs – PLUS-PLUS-PLUS-261

Appendix D – Example PRZM .INP file for CHOICE-PLUS-177 cropland run

```
FOCUS PRZM Groundwater Tool v3.5.2 (Dec., 2010)         WINPRZM4.51
Simulation Location: Iowa        Crop: corn
    0.76    0.36       0    17.5       1       1
       4
    0.37    3.73     0.8  8550.2               3     3.7   33080
       1
       1    0.25      90     100       3  77  71  77        0     300
       1      26
2505 0106 1606 0107 1607 0108 1608 0109 1609 0110 1610 2010 0111 1611 0112 1612
.307 .263 .141 .089 .086 .092 .097 .102 .104 .104 .104 .016 .017 .017 .017 .017
.014 .014 .014 .014 .014 .014 .014 .014 .014 .014 .014 .014 .014 .014 .014 .014
  71   71   71   71   71   71   71   71   71   71   71   77   77   77   77   77
0101 1601 0102 1602 0103 1603 0104 1604 0105 1605
.230 .228 .227 .226 .227 .233 .245 .267 .267 .307
.014 .014 .014 .014 .014 .014 .014 .014 .014 .014
  77   77   77   77   77   77   77   77   77   77
       5
  250561  240761  191061       1
  250562  240762  191062       1
  250563  240763  191063       1
  250564  240764  191064       1
  250565  240765  191065       1
Chemical Input Data:
      30       1       0       0
Parent Template
  040561  0 4   5 0.7849 1.00 0.00
  300561  0 4   5 0.0436 1.00 0.00
  300661  0 4   5 0.0436 1.00 0.00
  300761  0 4   5 0.0436 1.00 0.00
  300861  0 4   5 0.0436 1.00 0.00
  261061  0 4   5 0.7849 1.00 0.00
  040562  0 4   5 0.7849 1.00 0.00
  300562  0 4   5 0.0436 1.00 0.00
  300662  0 4   5 0.0436 1.00 0.00
  300762  0 4   5 0.0436 1.00 0.00
  300862  0 4   5 0.0436 1.00 0.00
  261062  0 4   5 0.7849 1.00 0.00
  040563  0 4   5 0.7849 1.00 0.00
  300563  0 4   5 0.0436 1.00 0.00
  300663  0 4   5 0.0436 1.00 0.00
  300763  0 4   5 0.0436 1.00 0.00
  300863  0 4   5 0.0436 1.00 0.00
  261063  0 4   5 0.7849 1.00 0.00
  040564  0 4   5 0.7849 1.00 0.00
  300564  0 4   5 0.0436 1.00 0.00
  300664  0 4   5 0.0436 1.00 0.00
  300764  0 4   5 0.0436 1.00 0.00
  300864  0 4   5 0.0436 1.00 0.00
  261064  0 4   5 0.7849 1.00 0.00
  040565  0 4   5 0.7849 1.00 0.00
  300565  0 4   5 0.0436 1.00 0.00
  300665  0 4   5 0.0436 1.00 0.00
  300765  0 4   5 0.0436 1.00 0.00
  300865  0 4   5 0.0436 1.00 0.00
```

```
261065  0 4   5 0.7849 1.00 0.00
     0       0       0
   Soil Series:                         C
    152          0   0   1   0   0   0   0   0   0
     0       0       0
     4     912
     4
     1      10     1.4    0.31       0       0       0
      0.2310490.231049       0
          0.1    0.31    0.12    0.93       0
     2       5     1.4    0.31       0       0       0
      0.2310490.231049       0
            1    0.31    0.12    0.93       0
     3     125    1.37   0.279       0       0       0
      0.2310490.231049       0
            5   0.279   0.079    0.14       0
     4      12    1.48   0.309       0       0       0
      0.2310490.231049       0
            6   0.309   0.119    0.14       0
     0
   WATR    YEAR      10    PEST    YEAR      10    CONC    YEAR      10   1
     8     DAY
   PRCP    TSER   0   0
   RUNF    TSER   0   0
   INFL    TSER   1   1
   ESLS    TSER   0   0
   RFLX    TSER   0   0
   EFLX    TSER   0   0
   TPST    TSUM   1 100  1.0E5
   TPAP    TSER   1  50  1.0
```

Appendix E – Example PRZM .INP file for CHOICE-PLUS-177 feedlot run

```
FOCUS PRZM Groundwater Tool v3.5.2 (Dec., 2010)         WINPRZM4.51
Simulation Location: Iowa        Crop: none
    0.76    0.36       0   17.50       1       1
       5
      12     1.0     1.0   172.8       0       3     4.0     600
       1
       1     0.0    0.00       0       1  95  95  95       0    0.00
       1      24
0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608
1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00
.050 .050 .050 .050 .050 .050 .050 .050 .050 .050 .050 .050 .050 .050 .050 .050
  95   95   95   95   95   95   95   95   95   95   95   95   95   95   95   95
0109 1609 0110 1610 0111 1611 0112 1612
1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00
.050 .050 .050 .050 .050 .050 .050 .050
  95   95   95   95   95   95   95   95
       1
  010161  020161  311265       1
Chemical Input Data:
     35        1       8       0     100
Parent Template
  010161 43 4 0.181.4788 1.00 0.00
  140261 59 4 0.180.9859 1.00 0.00
  150461 59 4 0.180.4929 1.00 0.00
  140661 56 4 0.181.4788 1.00 0.00
  100861 59 4 0.180.9859 1.00 0.00
  091061 59 4 0.180.4929 1.00 0.00
  081261 23 4 0.181.4788 1.00 0.00
  010162 32 4 0.181.4788 1.00 0.00
  030262 59 4 0.180.9859 1.00 0.00
  040462 59 4 0.180.4929 1.00 0.00
  030662 56 4 0.181.4788 1.00 0.00
  300762 59 4 0.180.9859 1.00 0.00
  280962 59 4 0.180.4929 1.00 0.00
  271162 34 4 0.181.4788 1.00 0.00
  010163 21 4 0.181.4788 1.00 0.00
  230163 59 4 0.180.9859 1.00 0.00
  240363 59 4 0.180.4929 1.00 0.00
  230563 56 4 0.181.4788 1.00 0.00
  190763 59 4 0.180.9859 1.00 0.00
  170963 59 4 0.180.4929 1.00 0.00
  161163 45 4 0.181.4788 1.00 0.00
  010164 10 4 0.181.4788 1.00 0.00
  120164 59 4 0.180.9859 1.00 0.00
  120364 59 4 0.180.4929 1.00 0.00
  110564 56 4 0.181.4788 1.00 0.00
  070764 59 4 0.180.9859 1.00 0.00
  050964 59 4 0.180.4929 1.00 0.00
  041164 56 4 0.181.4788 1.00 0.00
  010165 58 4 0.180.9859 1.00 0.00
  010365 59 4 0.180.4929 1.00 0.00
  300465 56 4 0.181.4788 1.00 0.00
  260665 59 4 0.180.9859 1.00 0.00
  250865 59 4 0.180.4929 1.00 0.00
```

```
241065 56 4 0.181.4788 1.00 0.00
201265 11 4 0.180.9859 1.00 0.00
    0.       1    0.00
   62
140461     100    0.70
081061     100    0.70
030462     100    0.70
270962     100    0.70
230363     100    0.70
160963     100    0.70
110364     100    0.70
040964     100    0.70
280265     100    0.70
240865     100    0.70
  Soil Series:                         C
   152           0    0    1    0    0    0    0    0    0
     0       0        0
     4     912
     4
     1      10     0.85   0.450       0       0       0
           0.0      0.0       0
           0.1    0.450   0.140    38.0       0
     2      10     1.25   0.321       0       0       0
           0.0      0.0       0
             1    0.321   0.202    31.0       0
     3     120     1.37   0.279       0       0       0
       0.23105 0.23105        0
             5    0.279   0.079    0.14       0
     4      12     1.48   0.309       0       0       0
       0.23105 0.23105        0
             6    0.309   0.119    0.14       0
     0
  WATR    YEAR       10    PEST      DAY       1    CONC    YEAR      10   1
     8     DAY
  PRCP    TSER   0   0
  RUNF    TSER   0   0
  INFL    TSER   1   1
  RFLX    TSER   0   0
  EFLX    TSER   0   0
  ESLS    TSER   0   0
  TPST    TSUM   1 100  1.0E5
  TPAP    TSER   1 100  1.0
```

Appendix F – Example PRZM .INP file for CHOICE-PLUS-177 pasture run

```
FOCUS PRZM Groundwater Tool v3.5.2 (Dec., 2010)         WINPRZM4.51
Simulation Location: Iowa        Crop: pasture
    0.76    0.36       0   17.50       1       3
       4
    0.37    3.73     1.0   172.8               3     6.0     600
       1
       1     0.2      43      97       3  82  79  82       0     122
       1      24
0101 1601 0102 1602 0103 1603 0104 1604 0105 1605 0106 1606 0107 1607 0108 1608
.001 .001 .001 .001 .001 .001 .001 .001 .001 .001 .001 .001 .001 .001 .001 .001
.110 .110 .110 .110 .110 .110 .110 .110 .110 .110 .110 .110 .110 .110 .110 .110
  82   82   82   82   82   82   79   79   79   79   79   79   79   79   79   79
0109 1609 0110 1610 0111 1611 0112 1612
.001 .001 .001 .001 .001 .001 .001 .001
.110 .110 .110 .110 .110 .110 .110 .110
  79   79   79   79   79   82   82   82
       5
  010441  150461  151161       1
  010462  150462  151162       1
  010463  150463  151163       1
  010464  150464  151164       1
  010465  150465  151165       1
Chemical Input Data:
       5       1       6       0
Parent Template
  010461  0 4 5.0 .00478 1.00 0.00
  010462  0 4 5.0 .00478 1.00 0.00
  010463  0 4 5.0 .00478 1.00 0.00
  010464  0 4 5.0 .00478 1.00 0.00
  010465  0 4 5.0 .00478 1.00 0.00
      0.       1    0.00
      5
  281061
  281062
  281063
  281064
  281065
    Soil Series:                           C
     152          0   0   1   0   0   0   0   0   0
       0       0       0
       4     912
       4
       1      10     1.4    0.31       0       0       0
        0.2310490.231049       0
             0.1    0.31    0.12    0.93       0
       2       5     1.4    0.31       0       0       0
        0.2310490.231049       0
               1    0.31    0.12    0.93       0
       3     125    1.37   0.279       0       0       0
        0.2310490.231049       0
               5   0.279   0.079    0.14       0
       4      12    1.48   0.309       0       0       0
        0.2310490.231049       0
               6   0.309   0.119    0.14       0
```

```
   0
WATR    YEAR      10    PEST    YEAR      10    CONC    YEAR      10   1
   8     DAY
PRCP    TSER   0   0
RUNF    TSER   0   0
INFL    TSER   1   1
ESLS    TSER   0   0
RFLX    TSER   0   0
EFLX    TSER   0   0
TPST    TSUM   1 100  1.0E5
TPAP    TSER   1  50  1.0
```

Appendix G – Alterations to the SynovexPRZM Source Code

Note: Changes from PRZM including both those from 2014 and 2019 are marked in red text

Changes to RDPRZM Subroutine in RSINP2.FOR

```
RECORD 13
1010 FORMAT(10I8)
 READ(MESAGE,1010,END=910,ERR=920) NAPS,NCHEM,FRMFLG,DK2FLG,MANCPT

 Multiple FRMFLG options added:
 FRMFLG=5, Feedlot-Constant soil concentration
 FRMFLG=6, Pasture
 FRMFLG=7, Obsolete, Superceded by FRMFLG 8
 FRMFLG=8, Feedlot-Manure mixing zone

 MANCPT: Manure mixing zone entered
  as number of manure (soil) compartments FRMFLG=8)

 Note: NAPS should be set to 1 for FRMFLG=5

            DEPI transforms when FRMFLG=8 to signify the depth
            added/subtracted per day of manure

RECORD 18.2 IF((FRMFLG.EQ.5).OR.(FRMFLG.EQ.6).OR.
  (FRMFLG.EQ.7).OR.(FRMFLG.EQ.8))THEN
 1010 FORMAT(10I8)
 READ(MESAGE,1010,END=910,ERR=920) NCLND

 NCLND: Number of scraping events (total for sn)

RECORD 18.5 IF((FRMFLG.EQ.5).OR.(FRMFLG.EQ.6).OR.
  (FRMFLG.EQ.7).OR.(FRMFLG.EQ.8))THEN
 READ(MESAGE,'(2X,3I2,I8,F8.0)',END=910,ERR=92
 1 CPD,CPM,CLNYR(I),CLNCMP(I),CLNPCT(I) (re 1 to NCLND)

 CPD: Clean Day
 CPM: Clean Month
 CLNYR: Clean Year
 CLNCMP: Number of manure compartments to remoLG 5,7,8)
 CLNPCT: Percent removal of mass from compartmFLG 5,7,8)
```

RSPRZ1.FOR

```
 SUBROUTINE PRZM
 I   (RSTFG, NUMFIL, MCARLO, SEPTON, NITRON,
 I   MODID, RSDAT, REDAT, LPRZRS,
 I   LPRZOT, LPRZIN, LWDMS,
 I   LMETEO, LSPTIC, LNITAD, LIRRG1,LHRMET,
 I   LTMSRS, SRNFG, BASEND, IPRZM, ITSAFT, NLDLT)
C
C + + + PURPOSE + + +
C called by EXESUP to execute PRZM
C Modification date: 2/18/92 JAM
C
```

```
cwinter
c 2
 USE WINTERACTER
 TYPE(WIN_MESSAGE) MESSAGE
C
cwinter
c 1
 include 'resource.inc'
 INCLUDE 'CDAYS.INC'
C
C + + + DUMMY ARGUMENTS + + +
 INTEGER SRNFG,BASEND,RSTFG,NUMFIL,IPRZM,ITSAFT,NLDLT,
 1  KLIN,DEPICNT
 INTEGER RSDAT(3),REDAT(3),LPRZRS,LPRZOT,LIRRG1,LHRMET,
 1  LPRZIN,LMETEO,LSPTIC,LNITAD,LTMSRS,LWDMS,K1
 LOGICAL MCARLO,SEPTON,NITRON,APPLY
 CHARACTER*3 MODID(NUMFIL)
 REAL CURVN,DDLN
 INTEGER*4 RODPTH
C
C + + + ARGUMENT DEFINITIONS + + +
C RSTFG - restart starting flag
C NUMFIL - max. number of open files
C MCARLO - flag for Monte Carlo on
C SEPTON - septic effluent on flag
C NITRON - nitrogen modeling on flag
C MODID - model id (pest,conc,water)
C RSDAT - restart starting date
C REDAT - restart ending date
C LPRZRS - unit number for przm restart file
C LPRZOT - unit number for przm output file
C LPRZIN - unit number for przm input file
C LMETEO - unit number for meteorlogical file
C LSPTIC - unit number for septic effluent file
C LNITAD - unit number for nitrogen atmospheric deposition
C LTMSRS - unit number for time series file
C LWDMS - unit number for WDM file
C SRNFG - starting run flag
C BASEND - base node for PRZM
C IPRZM - current przm zone
C ITSAFT - current time step
C NLDLT - maximum days in a time step (31)
C
C + + + PARAMETERS + + +
C
 INCLUDE 'PPARM.INC'
 INCLUDE 'PMXPDT.INC'
 INCLUDE 'PMXNSZ.INC'
 INCLUDE 'PMXZON.INC'
C
C + + + COMMON BLOCKS + + +
C
 INCLUDE 'CMET.INC'
 INCLUDE 'CMISC.INC'
 INCLUDE 'CVMISC.INC'
 INCLUDE 'CPRZST.INC'
 INCLUDE 'CHYDR.INC'
```

```
      INCLUDE 'CPEST.INC'
      INCLUDE 'CCROP.INC'
      INCLUDE 'CIRGT.INC'
      INCLUDE 'CECHOT.INC'
      INCLUDE 'CPTAP.INC'
      INCLUDE 'CFILEX.INC'
      INCLUDE 'CBIO.INC'
      INCLUDE 'EXAM.INC'
      INCLUDE 'CNITR.INC'
C
C + + + LOCAL VARIABLES + + +
C
      INTEGER J,I,LDAY,FDAY,JP1,MNTHP1,EYRFG,
     1  K, NMCDAY,LPAD,elpsed,ITYPE,CLNPAD,P,QQ
      INTEGER FLPS,FLCN
      REAL  ATEMP(2),PWIND(2),R0,pctot(3),s2tot(3),
     1  OLDKH(NCMPTS),ZCH,URH,ZRH,TOTCR
      REAL*8 DKBIO(3,NCMPTS),PP2
      CHARACTER*4 YEAR,MNTH,DAY,CONC
      INTEGER ILDLT,IERROR,isim1
      LOGICAL MCTFLG,IRDAY,FATAL
      CHARACTER*80 MESAGE
C
C + + + INTRINSICS + + +
C
      INTRINSIC MOD
C
C + + + EXTERNALS + + +
C
      EXTERNAL SUBIN,RSTGET,RSTGT1,KHCORR,ACTION,GETMET,PLGROW
      EXTERNAL IRRIG,HYDROL,EVPOTR,HYDR1,HYDR2,EROSN,SLTEMP,FARM
      EXTERNAL PZSCRN,PESTAP,PLPEST,CANOPY,BIODEG,SLPST0,SLPST1
      EXTERNAL MOC,MASBAL,OUTCNC,OUTRPT,OUTPST,OUTHYD,OUTTSR
      EXTERNAL MCPRZ,RSTPUT,RSTPT1,SUBOUT,PRZEXM,ERRCHK
      EXTERNAL SEPTIN,NITR,NITRAP,NITBAL,OUTCNI,OUTNIT,ZIPR
C
C + + + DATA INITIALIZATIONS + + +
C
      DATA YEAR /'YEAR'/
      DATA MNTH /'MNTH'/
      DATA DAY /' DAY'/
      DATA CONC /'CONC'/
C
C + + + OUTPUT FORMATS + + +
2000 FORMAT('Application [',I3,'] chem [',I1,
     1 '] on julday [',I3,'] year [',I2,'] zone [',I2,']')
2001 FORMAT('Application [',I3,'] chem [',I1,
     1 '] on julday [',I3,'] year [',I2,'] zone [',I2,']')
2002 FORMAT('ERROR, Application [',I3,'] failed ideal soil conditions')
2010 FORMAT('Nitrogen application [',I3,'] on julday [',I3,'] year [',
     $ I2,'] zone [',I2,']')
2020 FORMAT('ERROR, Nitrogen application [',I3,'] failed ideal soil ',
     $ 'conditions')
C
C + + + END SPECIFICATIONS + + +
C
      R0 = 0.0
```

```
 APPLY = .FALSE.
 MESAGE = 'PRZM'
 CALL SUBIN(MESAGE)
C get unit numbers used for input and output
 FLPS= LPRZOT
 FLCN= LPRZOT
C
C in restart mode
 IF(IPRZM.NE.1)THEN
 CALL RSTGET (LPRZRS,IPRZM)
 CALL RSTGT1 (RSTFG,LPRZRS,IPRZM)
 ENDIF
C
C use dates passed as input rather than on input file
 ISTYR = RSDAT(1)
 ISMON = RSDAT(2)
 ISDAY = RSDAT(3)
 IEYR = REDAT(1)
 IEMON = REDAT(2)
 IEDAY = REDAT(3)
C
C check temperature simulation flag
 IF ((ITFLAG .EQ. 1).or.(ITFLAG .EQ. 2)) THEN
 DO 178 K=1,NCHEM
  CALL KHCORR(SPT,HENRYK(K),ENPY(K),NCOM2,OLDKH)
  DO 177 I=1,NCOM2
  OKH(K,I) = OLDKH(I)
177 CONTINUE
178 CONTINUE
 ELSE
 DO 189 K=1,NCHEM
  DO 188 I=1,NCOM2
  OKH(K,I) = HENRYK(K)
  KH(K,I) = HENRYK(K)
188 CONTINUE
189 CONTINUE
 ENDIF
C
 IF(KDFLAG.EQ.3)CALL AGEKD
C
 NMCDAY = (ITSAFT-1)*NLDLT
 DO 200 IY=ISTYR,IEYR
 IF (MOD(IY,4) .NE. 0 .OR. MOD(IY,100) .EQ. 0) THEN
  LEAP=1
  LDAY=365
 ELSE
  LEAP=2
  LDAY=366
 ENDIF
 IF (IY .EQ. IEYR) LDAY=IEDAY+CNDMO(LEAP,IEMON)
C
 FDAY=1
 IF (IY .EQ. ISTYR)THEN
  FDAY=ISDAY+CNDMO(LEAP,ISMON)
 ENDIF
C
 EYRFG = 0
```

```
C
C counter for VADOFT link
 ILDLT = 0
C set input accumulator for GLOMAS
CJMC determine time period for each decay rate if DK2FLG=1
 DO 39 K = 1,NCHEM
  IF(DK2FLG.EQ.1)THEN
  DKSTRT(K)=DKDAY(K)+CNDMO(LEAP,DKMNTH(K))
  DKEND(K)=DKSTRT(K)+DKNUM(K)
  IF(DKEND(K).GT.365)DKEND(K)=DKEND(K)-LDAY
  ENDIF
  PTAP(K) = 0.
 39 CONTINUE
C
C begin daily loop
 DO 100 JULDAY=FDAY,LDAY
  NMCDAY = NMCDAY + 1
  ILDLT = ILDLT + 1
  IF (JULDAY .EQ. LDAY) THEN
  EYRFG = 1
  ENDIF
  IF (JULDAY.EQ.1) DAYCNT = 0
  DAYCNT = DAYCNT + 1
  rngcnt=rngcnt+1
  elpsed=int((float(rngcnt)/float(dycnt))*100.)
cwinter
c 1
  call wdialogputprogressbar(idf_progress1,elpsed,0)
  if(itype.eq.3)then
  close(156)
  close(157)
  close(158)
cwinter
c 1
  call iosdeletefile('*.cnc')
  stop
  endif
cwinter
c 4
  call wmessagepeek(itype,message)
  if(itype.ne.NoMessage)then
  call wdialogshow(-1,-1,0,semimodeless)
  endif
  DO 40 J=1,12
  JP1= J+ 1
  IF (JULDAY.GT.CNDMO(LEAP,J) .AND.
 1  JULDAY.LE.CNDMO(LEAP,JP1)) MONTH = J
40 CONTINUE
  DOM=JULDAY-CNDMO(LEAP,MONTH)
  MNTHP1 = MONTH + 1
C
  SSFLAG = 0
  IF (IY.EQ.SAYR .AND. JULDAY.EQ.SAVAL) THEN
C  time for a special action
  CALL ACTION (LPRZIN,LPRZOT,MODID(3))
  END IF
C  get BUFFER data
```

```
      IF((buffbf.eq.1))then
      CALL GETBUF
      ENDIF
C     get met data
      CALL GETMET(
     I IY,JULDAY,MONTH,DOM,LMETEO,LSPTIC,LNITAD,FWDMS,
     I LDAY,RSTFG,NITRON,SEPTON,LIRRG1,LHRMET,
     O RETCOD)
C
      IF(THRFL2.GT.0.0)THEN
      PRECIP=PRECIP+THRFL2
      ENDIF

C     grow some crops
      CALL PLGROW(IRDAY)
C
      APDEP = 0.0
      AINF(1)= 0.0
      THRUFL = 0.0
      IF((IRFILE.EQ.0).AND.(IRTYPE.GT.0).AND.
     * (IRNONE.NE.4).AND.(RZI.EQ.1))THEN
C     need to do irrigation
      IRRR=0.0
      CALL IRRIG
      ELSEIF(IRFILE.EQ.0)THEN
      IF (IRNONE .EQ. 4) THEN
      IF (IRDAY) THEN
      IRRR=0.0
      CALL IRRIG
      ELSE
      GOTO 555
      ENDIF
      ENDIF
      ENDIF
C
C     calculate surface hydrology factors
C
555   CONTINUE
      IF(IRFILE.EQ.0)THEN
      CALL HYDROL (LPRZOT,MODID(3),RODPTH,CURVN)
      ELSEIF((IRFILE.EQ.1).or.(IRFILE.EQ.2))THEN
      CALL HYDROL2 (LPRZOT,MODID(3),RODPTH,CURVN)
      ENDIF
C
C     calculate et
      IF (IPEIND.LE.2)THEN
      CALL EVPOTR
      ELSEIF ((IPEIND.GT.2).AND.(IPEIND.LT.7))THEN
      CALL EVPOTR2
      ELSEIF (IPEIND.GE.7)THEN
      CALL EVPOTR3
      ENDIF
C
      IF (HSWZT .EQ. 0) THEN
C     hydraulics with unrestricted drainage
      CALL HYDR1
      ELSEIF (HSWZT .EQ. 1) THEN
```

```
C  hydraulics with restricted drainage
   CALL HYDR2
   ELSEIF (HSWZT .EQ. 2) THEN
   isim1=0
C  hydraulics with restricted drainage
   CALL HYDR3(isim1)
   ENDIF
C
   IF (DSPFLG .EQ. 1) THEN
   CALL DSPINIT
   ENDIF
C
   IF (SEPTON) THEN
C  introduce septic effluent into soil column
   CALL SEPTIN
   END IF
C
   if((buffbf.eq.1))THEN
   CALL APPBUF
   ENDIF
C
   IF(ERFLAG.GT.0)THEN
   IF(LEAP.EQ.1)THEN
   IF(UCFLG.EQ.0)THEN
   CFAC=USLEC(NCROP,IUSLEC)
   N1=MNGN(NCROP,IUSLEC)
   IF(JULDAY.EQ.JUSLEC(NCROP,IUSLEC))UCFLG=2
   ISCOND=IUSLEC
   IF(UCFLG.EQ.2)IUSLEC=IUSLEC+1
   ELSEIF(UCFLG.EQ.1)THEN
   CFAC=USLEC(NCROP,IUSLEC)
   N1=MNGN(NCROP,IUSLEC)
   IF(JULDAY.EQ.JUSLEC(NCROP,IUSLEC))UCFLG=2
   ISCOND=IUSLEC
   IF(UCFLG.EQ.2)IUSLEC=IUSLEC+1
   ELSE
   IF(JULDAY.EQ.(JUSLEC(NCROP,IUSLEC)))THEN
    CFAC=USLEC(NCROP,IUSLEC)
    N1=MNGN(NCROP,IUSLEC)
    ISCOND=IUSLEC
    IUSLEC=IUSLEC+1
    IF(IUSLEC.GT.NUSLEC(NCROP))IUSLEC=1
   ENDIF
   ENDIF
   ELSE
   LPAD=0
   IF(UCFLG.EQ.0)THEN
   CFAC=USLEC(NCROP,IUSLEC)
   N1=MNGN(NCROP,IUSLEC)
   IF(JULDAY.GT.59)LPAD=1
   IF(JULDAY.EQ.JUSLEC(NCROP,IUSLEC)+LPAD)UCFLG=2
   ISCOND=IUSLEC
   ELSEIF(UCFLG.EQ.1)THEN
   CFAC=USLEC(NCROP,IUSLEC)
   N1=MNGN(NCROP,IUSLEC)
   IF(JULDAY.GT.59)LPAD=1
   IF(JULDAY.EQ.JUSLEC(NCROP,IUSLEC)+LPAD)UCFLG=2
```

```
  ISCOND=IUSLEC
  ELSE
  IF(JULDAY.GT.59)LPAD=1
  IF(JULDAY.EQ.(JUSLEC(NCROP,IUSLEC))+LPAD)THEN
   CFAC=USLEC(NCROP,IUSLEC)
   N1=MNGN(NCROP,IUSLEC)
   ISCOND=IUSLEC
   IUSLEC=IUSLEC+1
   IF(IUSLEC.GT.NUSLEC(NCROP))IUSLEC=1
  ENDIF
  ENDIF
  ENDIF
  ENDIF
c
  SEDL= 0.0
  ELTT= 0.0
  IF (RUNOF .GT. 0.0 .AND. ERFLAG .GE. 1) THEN
C  calc loss of chem due to erosion
  CALL EROSN
  END IF
CJMC
  IF(DK2FLG.EQ.1)THEN
  CALL DKINIT
  ELSEIF(DK2FLG.EQ.2)THEN
  CALL HUCALC
  ELSEIF(DK2FLG.EQ.3)THEN
  ENDIF
CJMC
C
  IF (NITRON) THEN
C  perform nitrogen simulation
  CALL SLTEMP (LPRZOT,MODID(3))
  CALL ZIPR (3*NCOM2,R0,SOILAP)
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND. IY.EQ.IAPYR(NAPPC)) THEN
C  need to perform ag nitrogen application
  IF ((FRMFLG .GE. 1).AND.(FRMFLG.LE.3)) THEN
C  check for appropriate soil moisture
  CALL FARM (RODPTH,APPLY,CURVN)
  IF (APPLY) THEN
C   make ag nitrogen application
   WRITE(MESAGE,2010) NAPPC,IAPDY(NAPPC),
 $     IAPYR(NAPPC),IPRZM
   CALL PZSCRN(1,MESAGE)
   CALL NITRAP (FECHO)
   NAPPC= NAPPC+ 1
   WIN = 0
  ELSE
C   soil moisture not right for application, try again tomorrow
   WIN = WIN + 1
   IF (WIN .GT. WINDAY(NAPPC)) THEN
C   beyond window of opportunity
   WRITE(MESAGE,2020) NAPPC
   IERROR= 2150
   FATAL = .TRUE.
   CALL ERRCHK(IERROR,MESAGE,FATAL)
   ELSE
C   try to apply tomorrow
```

```
    IAPDY(NAPPC) = IAPDY(NAPPC) + 1
    ENDIF
   ENDIF
   ELSE
   WRITE(MESAGE,2010) NAPPC,IAPDY(NAPPC),
  $     IAPYR(NAPPC),IPRZM
   CALL PZSCRN(1,MESAGE)
   CALL NITRAP (FECHO)
   NAPPC= NAPPC+ 1
   ENDIF
   ENDIF
   IF (MCARLO) THEN
   CALL NITR (IY,MONTH,DOM,FECHO,IPRZM,MODID(13))
   ELSE
   CALL NITR (IY,MONTH,DOM,LPRZOT,IPRZM,MODID(13))
   END IF
C  perform mass balance for nitrogen constituents
   CALL NITBAL (APDEP,IPRZM)
   IF (ECHOLV .GE. 3) THEN
   CALL OUTHYD (LPRZOT,LTMSRS,MODID(3),MODID(5),SEPTON)
   IF (ITEM3 .EQ. CONC .AND. (STEP3 .EQ. DAY .OR. (STEP3
  1  .EQ. MNTH .AND. JULDAY .EQ. CNDMO(LEAP,MNTHP1)) .OR.
  2  (STEP3 .EQ. YEAR .AND. JULDAY .EQ. CNDMO(LEAP,13)))
  3  .AND. FLCN.GT.0) CALL OUTCNI (LPRZOT,MODID(6))
   CALL OUTNIT (FLPS,MODID(13),SEPTON)
   IF (NPLOTS .GT. 0) THEN
C  output time-series
   HEADER = HEADER + 1
   IF (HEADER .EQ. 1) SRNFG = 1
   CALL OUTTSR (SRNFG,EYRFG,LPRZOT,LTMSRS,LWDMS,
  I   MODID(3),MODID(5))
   ENDIF
   ENDIF
C  store PRZM nitrogen fluxes for vadoft, start w/ammonia
   PRZMPF(IPRZM,ILDLT,1) = PRZMPF(IPRZM,ILDLT,1) +
  $    NCFX2(BASEND,1)/1.0E5
C  nitrate
   PRZMPF(IPRZM,ILDLT,2) = PRZMPF(IPRZM,ILDLT,2) +
  $    NCFX4(BASEND,1)/1.0E5
C  combine the two organic species
   PRZMPF(IPRZM,ILDLT,3) = PRZMPF(IPRZM,ILDLT,3) +
  $     (NCFX13(BASEND,1) +
  $    NCFX15(BASEND,1))/1.0E5
   ELSE
C  perform pesticide simulation
   DO 1000 J=1,NCOM2
   SRCFLX(1,J)=0.0
   SRCFLX(2,J)=0.0
   SRCFLX(3,J)=0.0
   DKFLUX(1,J)=0.0
   DKFLUX(2,J)=0.0
   DKFLUX(3,J)=0.0
   TRFLUX(1,J)=0.0
   TRFLUX(2,J)=0.0
   TRFLUX(3,J)=0.0
1000 CONTINUE
C
```

```
C Begin Chemical Loop
C
  DO 95 K=1, NCHEM
  ELTERM(K) = ELTT*FEQ(K,1)*KD(K,1)
  DO 74 I=1,NCOM2
  SOILAP(K,I) = 0.0
  DKBIO(K,I) = 0.0
74  CONTINUE
C
CJMC
  IF (((ITFLAG .EQ. 1).OR.(ITFLAG.EQ.2)).AND.
 *   (QFAC(K).GT.0.0)) THEN
  IF (K .EQ. 1) CALL SLTEMP (LPRZOT,MODID(3))
  IF(K.EQ.1)CALL Q10DK
  CALL KHCORR (SPT,HENRYK(K),ENPY(K),NCOM2,OLDKH)
  DO 75 I=1, NCOM2
   KH(K,I) = OLDKH(I)
75  CONTINUE
  ELSEIF (((ITFLAG .EQ. 1).OR.(ITFLAG.EQ.2)).AND.
 *   (QFAC(K).LE.0.0)) THEN
  IF (K .EQ. 1) CALL SLTEMP (LPRZOT,MODID(3))
  CALL KHCORR (SPT,HENRYK(K),ENPY(K),NCOM2,OLDKH)
  DO 76 I=1, NCOM2
   KH(K,I) = OLDKH(I)
76  CONTINUE
  IF((DK2FLG.EQ.1).AND.(K.EQ.1))CALL DKINIT
  ELSEIF(DK2FLG.EQ.1)THEN
  IF(K.EQ.1)CALL DKINIT
  ENDIF
CJMC
C
  PLNTAP(K) = 0.0
C
  IF(DK2FLG.EQ.2)THEN
  IF((HU_ACCUM(K).GE.HUTARGET(K)).AND.(HUFLG(K).EQ.1))THEN
   WRITE(187,'(I3,1X,I2,1X,53I8)')IAPDY(NAPPC-1),
 *      IY,HU_ACCUM(K),NHORIZ,
 *    (INT(FLOAT(HUCNT(K))*DEGFAC(J)),J=1,NHORIZ)
   HUFLG(K)=0
   HUCNT(K)=0
  ELSEIF(HUFLG(K).EQ.1)THEN
   HU_ACCUM(K)=HU_ACCUM(K)+INT(HU2)
   HUCNT(K)=HUCNT(K)+1
  ENDIF
  ENDIF
c
  IF ((FRMFLG .EQ. 6)) THEN
  IF(JULDAY.EQ.IAPDY(NAPPC+1).AND.(NCLNC.NE.NAPPC))THEN
   NAPPC= NAPPC+ 1
  ENDIF
  ENDIF
c
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND. IY.EQ.IAPYR(NAPPC))THEN
  ttapp=0
  IF ((FRMFLG .GE. 1).AND.(FRMFLG.LE.3)) THEN
C   added new statement for farm option -jam 4/24/91
   CALL FARM (RODPTH,APPLY,CURVN)
```

```
    IF (APPLY) THEN
    AOFF(K)=0
    WRITE(MESAGE,2000) NAPPC,K,IAPDY(NAPPC),
  $      IAPYR(NAPPC),IPRZM
    CALL PZSCRN(1,MESAGE)
    CALL PESTAP(K)
    PTAP(K) = PTAP(K) +
  *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
    ENDIF
   ELSEIF ((FRMFLG .EQ. 4)) THEN
    AOFF(K)=0
    WRITE(MESAGE,2001) NAPPC,K,IAPDY(NAPPC),
  $     IAPYR(NAPPC),IPRZM
    CALL PZSCRN(1,MESAGE)
    if((julday.eq.1).and.(fdfrmflg.eq.0))then
    CALL PESTAP(K)
    fdfrmflg=1
    elseif(tapp(k,nappc).gt.0.0)then
    DO I=1,NCMPTS
    soilap(k,i)=soilap2(k,i)
    pestr(k,i) = pestr(k,i)+
  1   (SOILAP(k,i)/(DELX(i)*theto(i)))
    SPESTR(k,i) = (pcncx(k,i)+
  1   (SOILAP(k,i)/(DELX(i)*THETO(i))))*
  1   (THETO(i)/(THETO(i)
  1   +feq(k,i)*KD(k,i)*BD(i)
  1   +(THETAS(i)-THETO(i))*KH(k,i)))
    soilap2(k,i)=0.0
    ENDDO
    endif
C    global mass balance
    PTAP(K) = PTAP(K) +
  *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
    NAPPC= 1
   ELSEIF ((FRMFLG .EQ. 5)) THEN
    AOFF(K)=0
    WRITE(MESAGE,2001) NAPPC,K,IAPDY(NAPPC),
  $     IAPYR(NAPPC),IPRZM
    CALL PZSCRN(1,MESAGE)
    CALL PESTAP(K)
C    global mass balance
    PTAP(K) = PTAP(K) +
  *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
    NAPPC= 1
   ELSEIF ((FRMFLG .EQ. 6).OR.(FRMFLG .EQ. 7)) THEN
    AOFF(K)=0
    WRITE(MESAGE,2001) NAPPC,K,IAPDY(NAPPC),
  $     IAPYR(NAPPC),IPRZM
    CALL PZSCRN(1,MESAGE)
    CALL PESTAP(K)
C    global mass balance
    PTAP(K) = PTAP(K) +
  *   (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
   ELSE
    AOFF(K)=0
    WRITE(MESAGE,2001) NAPPC,K,IAPDY(NAPPC),
  $     IAPYR(NAPPC),IPRZM
```

```
   CALL PZSCRN(1,MESAGE)
   CALL PESTAP(K)
C   global mass balance
   PTAP(K) = PTAP(K) +
 *    (TAPP(K,NAPPC)*APPEFF(K,NAPPC))-PLNTAP(K)
  ENDIF
  IF(DK2FLG.EQ.2)THEN
   HU_ACCUM(K)=0
   HUFLG(K)=1
  ELSEIF(DK2FLG.EQ.3)THEN
   CALL HU_T12UPDATE(K)
  ENDIF
  ELSEIF ((FRMFLG .EQ. 8)) THEN
   ttapp=1
   CALL PESTAP(K)
  ENDIF
C
c jmc 6/17/96 fam=2 signifies that some applications were foliar
  IF (FAM.EQ.2)then
  if(ptrflg.eq.0)then
   CALL PLPEST(K)
  elseif(ptrflg.eq.1)then
   CALL PLPEST2(K)
  endif
  endif
C
  CNDBDY(K) = DAIR(K)/0.5
  CONDUC(K) = CNDBDY(K)
C
C  When canopy develops, resistance type approach is used
C  to estimate the volatilization flux and concentration
C  retains in the canopy
C
  IF (HEIGHT .GT. 5.0) THEN
  ZCH = HEIGHT/100.0
  IF (ITFLAG .EQ. 0) THEN
   ATEMP(1)= 15.0
  ELSE
   ATEMP(1)= UBT
  ENDIF
  ATEMP(2)= TEMP
  PWIND(1)= 0.0
  PWIND(2)= WIND*36.0*24.0
  URH = PWIND(2)
  IF ((ITFLAG .EQ. 1).or.(ITFLAG .EQ. 2)) THEN
   ZRH= ZWIND
  ELSE
   ZRH= 2.0
  ENDIF
C
C  CONDUC was being calculated after the following
C  if then statement. It should be calculated right
C  after the call CANOPY statement. Change made by
C  PV @ AQUA TERRA Consultants, 10/93
C
  IF(HENRYK(K).GT.0.0.AND.URH.GT.0.0)THEN
   CALL CANOPY(ATEMP,PWIND,ZRH,ZCH,URH,TOTCR,CRCNC)
```

```
    CONDUC(K) = 1.0 / (1.0/CNDBDY(K) + TOTCR)
   ELSE
    TOTCR=0.0
   ENDIF
C   CONDUC(K) = 1.0 / (1.0/CNDBDY(K) + TOTCR)
   ENDIF
C
C  Include calls to biodegradation subroutines here
C
   IF (BIOFLG .EQ. 1) THEN
   CALL BIODEG(K,DKBIO)
   ENDIF
C
C  end of biodegradation
C
   IF ((MCFLAG.EQ.0.OR.MCFLAG.EQ.3.OR.VLFLAG.EQ.0)
  1  .AND.(MCFLAG.NE.2)) THEN
   CALL SLPST0 (LPRZOT, MODID(3), K, DKBIO)
   ELSE
   IF(MCFLAG.EQ.1)THEN
    CALL MOC(K)
    CALL SLPST1 (LPRZOT,MODID(3),K, DKBIO)
   ELSEIF(MCFLAG.EQ.2)THEN
    CALL SLPST3 (LPRZOT,MODID(3),K, DKBIO)
   ENDIF
   END IF
C
C
C  calculate correction for dissolved to total solute conc.
   CALL MASBAL (APDEP,K,IPRZM,RODPTH)
C
   CALL FCSCNC(K)
   CALL FCSMSB(K)
   CALL FCSHYD(K)
   CALL FCSSOILCNC(IY,MONTH,DOM,K)
C
   IF (MCOFLG .EQ. 0 .AND. ECHOLV .GE.3) THEN
   if(mcflag.ne.3)then
    IF (ITEM3 .EQ. CONC .AND.
  1  (STEP3 .EQ. DAY .OR.(STEP3 .EQ. MNTH .AND.
  1  JULDAY .EQ. CNDMO(LEAP,MNTHP1)) .OR.
  2  (STEP3 .EQ. YEAR .AND.
  2  JULDAY .EQ. CNDMO(LEAP,13))).AND. FLCN.GT.0)then
    CALL OUTCNC (LPRZOT,MODID(6),K)
    ENDIF
C    Determine if a write to files MODOUT.DAT
C    or SNAPSHOT.DAT is required
    CALL OUTRPT (LPRZOT,MODID(7),MODID(8),K)
   elseif(mcflag.eq.3)then
    IF (ITEM3 .EQ. CONC .AND.
  1  (STEP3 .EQ. DAY .OR.(STEP3 .EQ. MNTH .AND.
  1  JULDAY .EQ. CNDMO(LEAP,MNTHP1)) .OR.
  2  (STEP3 .EQ. YEAR .AND.
  2  JULDAY .EQ. CNDMO(LEAP,13))).AND. FLCN.GT.0)then
    CALL OUTCNC2 (LPRZOT,MODID(6),K)
    ENDIF
   endif
```

```
C
   ENDIF
C
   IF (ECHOLV .GE. 3) THEN
   IF (K .EQ. 1) CALL OUTHYD (
  I     LPRZOT,LTMSRS,MODID(3),MODID(5),SEPTON)
   if(mcflag.ne.3)then
    CALL OUTPST (FLPS,MODID(4),K)
   elseif(mcflag.eq.3)then
    IF (K .EQ. NCHEM) THEN
    CALL OUTPST2(FLPS,MODID(4),K)
    endif
   endif
   ENDIF
   PRZMPF(IPRZM,ILDLT,K) = PRZMPF(IPRZM,ILDLT,K) +
  1  DFFLUX(K,BASEND) + ADFLUX(K,BASEND)
CPRH   DAFLUX(IPRZM,1,ILDLT,K) = DFFLUX(K,1) + ADFLUX(K,1) +
CPRH 1  PVFLUX(K,1)
C
   pctot(k)=0.0
   s2tot(k)=0.0
   DO 90 I=1,NCOM2
CPRH   DAFLUX(IPRZM,I+1,ILDLT,K) = DFFLUX(K,I) + ADFLUX(K,I) +
CPRH 1  PVFLUX(K,I)
   SPESTR(K,I)=sngl(X(I))
C  store SPESTR for this zone (for use w/ MASCOR)
   PESTR(K,I)=((SPESTR(K,I)*(THETN(I)+
  *    FEQ(K,I)*KD(K,I)*BD(I)+
  *    (THETAS(I)-THETN(I))*KH(K,I)))/THETN(I))+
  *    (s2(k,i)*BD(i))/thetn(i)
   pcncx(k,i)=(((spestr(k,i)*(THETN(I)+
  *    FEQ(K,I)*KD(K,I)*BD(I)+
  *    (THETAS(I)-THETN(I))*KH(K,I))))/thetn(i))
90  CONTINUE
C
C  last value of DAFLUX and ZPESTR is same as
C  in last compartment
CPRH   DAFLUX(IPRZM,NCOM2+2,ILDLT,K) = DFFLUX(K,NCOM2) +
CPRH 1  ADFLUX(K,NCOM2) + PVFLUX(K,NCOM2)
C
   IF (NPLOTS .GT. 0 .AND. K .EQ. NCHEM) THEN
    HEADER = HEADER + 1
    IF (HEADER .EQ. 1) SRNFG = 1
   IF(ECHOLV .GE.3)then
    if(mcflag.ne.3)then
    CALL OUTTSR
  1  (SRNFG,EYRFG,LPRZOT,LTMSRS,LWDMS,MODID(3),MODID(5))
    elseif(mcflag.eq.3)then
    CALL OUTTSR2
  1  (SRNFG,EYRFG,LPRZOT,LTMSRS,LWDMS,MODID(3),MODID(5))
    endif
   ENDIF
   ENDIF
C
C  new code added for EXAMS
   IF (ERFLAG.GT.0 .AND. IPRZM.EQ.1) THEN
   IF ((EXMFLG.GT.0) .AND. (K.EQ.NCHEM))then
```

```
    if(mcflag.ne.3)then
    CALL PRZEXM(K)
    elseif(mcflag.eq.3)then
    K1=1
    CALL PRZEXM2(K1)
    endif
   endif
   ENDIF
C   end of code added for EXAMS
C
C   new code added for ADAM
   IF ((ADMFLGON.GT.0) .AND. (K.EQ.1)) CALL PRZADM(K)
C
   SRNFG = 0
   IF ((ITFLAG .EQ. 1).or.(ITFLAG .EQ. 2)) THEN
   DO 92 I=1,NCOM2
    OKH(K,I) = KH(K,I)
92  CONTINUE
   ENDIF
C
   if(frmflg.eq.8)then
   sumdepi=0.0
   summan=0.0
   KLIN = 0
   DDLN = 0.0
115  CONTINUE
    KLIN = KLIN + 1
    DDLN = DDLN + DELX(KLIN)
   IF (DDLN .LT. depi(k,nappc)) GO TO 115
   DEPICNT = KLIN
   do p=1,mancpt
    SPESTR(K,P)=sngl(X(P))
    PESTR(K,P)=((SPESTR(K,P)*(THETN(P)+
  *   FEQ(K,P)*KD(K,P)*BD(P)+
  *   (THETAS(P)-THETN(P))*KH(K,P)))/THETN(P))+
  *   (s2(k,P)*BD(P))/thetn(P)
    summan=summan+pestr(k,p)*delx(p)*thetn(p)
   enddo
   do QQ=(mancpt-depicnt)+1,mancpt
    SPESTR(K,QQ)=sngl(X(QQ))
    PESTR(K,QQ)=((SPESTR(K,QQ)*(THETN(QQ)+
  *   FEQ(K,QQ)*KD(K,QQ)*BD(QQ)+
  *   (THETAS(QQ)-THETN(QQ))*KH(K,QQ)))/THETN(QQ))+
  *   (s2(k,QQ)*BD(QQ))/thetn(QQ)
    sumdepi=sumdepi+PESTR(K,QQ)*delx(qq)*thetn(qq)
   enddo
   if(julday.eq.1)then
   endif
   DO 690 I=1,NCOM2
    x(i)=0.0
    SPESTR(K,I)=sngl(X(I))
C    store SPESTR for this zone (for use w/ MASCOR)
    PESTR(K,I)=((SPESTR(K,I)*(THETN(I)+
  *    FEQ(K,I)*KD(K,I)*BD(I)+
  *   (THETAS(I)-THETN(I))*KH(K,I)))/THETN(I))+
  *    (s2(k,i)*BD(i))/thetn(i)
    pcncx(k,i)=(((spestr(k,i)*(THETN(I)+
```

```
 *    FEQ(K,I)*KD(K,I)*BD(I)+
 *   (THETAS(I)-THETN(I))*KH(K,I))))/thetn(i))
690  CONTINUE
  summan=summan-sumdepi
  endif
95  CONTINUE
C
C End Chemical Loop
C
  IF ((FRMFLG .GE. 1).AND.(FRMFLG.LE.3)) THEN
  IF (APPLY) THEN
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND.
 $   IY.EQ.IAPYR(NAPPC)) THEN
   NAPPC= NAPPC+ 1
   WIN = 0
  ENDIF
  ELSE
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND.
 $   IY.EQ.IAPYR(NAPPC)) THEN
   WIN = WIN + 1
   IF (WIN .GT. WINDAY(NAPPC)) THEN
   WRITE(MESAGE,2002)NAPPC
   IERROR = 2150
   FATAL = .TRUE.
   CALL ERRCHK(IERROR,MESAGE,FATAL)
   ELSE
   IAPDY(NAPPC) = IAPDY(NAPPC) + 1
   ENDIF
  ENDIF
  ENDIF
  ELSEIF ((FRMFLG .EQ. 4)) THEN
  NAPPC= 1
  do i=1,ncmpts
  TAPP(1,1)=tapp(1,1)+soilap2(k,i)
  enddo
  IF(TAPP(1,1).GT.0.0)IAPDY(NAPPC) = JULDAY + 1
  IF (LEAP .EQ. 2 .AND. JULDAY .EQ. 366)THEN
  IF(TAPP(1,1).GT.0.0)IAPDY(NAPPC) = 1
  IAPYR(NAPPC)=IY+1
  ENDIF
  IF (LEAP .EQ. 1 .AND. JULDAY .EQ. 365)THEN
  IF(TAPP(1,1).GT.0.0)IAPDY(NAPPC) = 1
  IAPYR(NAPPC)=IY+1
  ENDIF
  ELSEIF ((FRMFLG .EQ. 5)) THEN
  NAPPC= 1
  IF (JULDAY.EQ.CLNDY(NCLNC) .AND. IY.EQ.CLNYR(NCLNC))THEN
  CLNPAD=2
  CALL FRM5CLN(CLNPAD)
  ELSEIF(JULDAY.EQ.CLNDY(NCLNC)+1
 *  .AND. IY.EQ.CLNYR(NCLNC))THEN
  CLNPAD=1
  CALL FRM5CLN(CLNPAD)
  NCLNC= NCLNC + 1
  ENDIF
  IAPDY(NAPPC) = JULDAY + 1
  IF (LEAP .EQ. 2 .AND. JULDAY .EQ. 366)THEN
```

```
  IAPDY(NAPPC)=1
  IAPYR(NAPPC)=IY+1
  ENDIF
  IF (LEAP .EQ. 1 .AND. JULDAY .EQ. 365)THEN
  IAPDY(NAPPC)=1
  IAPYR(NAPPC)=IY+1
  ENDIF
  ELSEIF ((FRMFLG .EQ. 6)) THEN
  IF (JULDAY.EQ.CLNDY(NCLNC) .AND. IY.EQ.CLNYR(NCLNC))THEN
  TAPP(1,NAPPC)=0.0
  NCLNC=NCLNC+1
  ENDIF
  IF(JULDAY.EQ.IAPDY(NAPPC+1).AND.(NCLNC.NE.NAPPC))THEN
  NAPPC= NAPPC+ 1
  ENDIF
  IAPDY(NAPPC) = JULDAY + 1
  IF (LEAP .EQ. 2 .AND. JULDAY .EQ. 366)THEN
  IAPDY(NAPPC)=1
  IAPYR(NAPPC)=IY+1
  ENDIF
  IF (LEAP .EQ. 1 .AND. JULDAY .EQ. 365)THEN
  IAPDY(NAPPC)=1
  IAPYR(NAPPC)=IY+1
  ENDIF
  ELSEIF ((FRMFLG .EQ. 7).or.(FRMFLG .EQ. 8)) THEN
  IF ((JULDAY.EQ.IAPDY(NAPPC)).AND.(IY.EQ.IAPYR(NAPPC))
 *      .and.(pwin.eq.0)) THEN
  PWIN=PWIN+1
  IAPDY(NAPPC) = JULDAY + 1
  ELSEIF((PWIN.NE.WINDAY(NAPPC)).and.(PWIN.NE.0))THEN
  PWIN=PWIN+1
  IAPDY(NAPPC) = JULDAY + 1
  ELSEIF(PWIN.EQ.WINDAY(NAPPC))THEN
  PWIN=0
  NAPPC=NAPPC+1
  ENDIF
  IF (JULDAY.EQ.CLNDY(NCLNC) .AND. IY.EQ.CLNYR(NCLNC))THEN
  summan=0.0
  CLNPAD=2
  CALL FRM5CLN(CLNPAD)
  ELSEIF(JULDAY.EQ.CLNDY(NCLNC)+1
 *   .AND. IY.EQ.CLNYR(NCLNC))THEN
  summan=0.0
  CLNPAD=1
  CALL FRM5CLN(CLNPAD)
  NCLNC= NCLNC + 1
  ENDIF
  ELSE
  IF (JULDAY.EQ.IAPDY(NAPPC) .AND. IY.EQ.IAPYR(NAPPC))THEN
  NAPPC= NAPPC+ 1
  IF((buffbf.eq.1))then
   NAPPC= 1
  ENDIF
  ENDIF
  ENDIF
  END IF
C
```

```
C  water flux to EXESUP
  PRZMWF(IPRZM,ILDLT) = PRZMWF(IPRZM,ILDLT) + AINF(BASEND)
C
C  transfer results to Monte Carlo arrays
  IF(MCARLO) THEN
  MCTFLG = .TRUE.
  CALL MCPRZ(
 I MCTFLG,IPRZM,NMCDAY)
  ENDIF
C
  IF((KDFLAG.EQ.2).OR.(KDFLAG.EQ.3))THEN
  if(mcflag.ne.3)then
  CALL PZFRND
  elseif(mcflag.eq.3)then
  CALL PZFRND2
  endif
  ENDIF
CJMC
100 CONTINUE
200 CONTINUE
 IF(IPRZM.NE.1)THEN
 IF (RSTFG .EQ. 1 .OR. RSTFG .EQ. 2) THEN
C
C  Save state of system for next execution
  CALL RSTPUT (LPRZRS,IPRZM)
  CALL RSTPT1 (LPRZRS,IPRZM)
 ENDIF
 ENDIF
C
 CALL SUBOUT
C
 RETURN
 END
```

RSMISC.FOR

```
 SUBROUTINE FRM5CLN(CLNPAD)
C
C + + + PURPOSE + + +
C switches half-life when FRMFLG=5
C Modification date: 3/11/96 waterborne
C
C + + + PARAMETERS + + +
 INCLUDE 'PPARM.INC'
C
C + + + COMMON BLOCKS + + +
 INCLUDE 'CHYDR.INC'
 INCLUDE 'CPEST.INC'
 INCLUDE 'CCROP.INC'
 INCLUDE 'CMISC.INC'
C
C + + + LOCAL VARIABLES + + +
 REAL*4 TTHKNS,MODFC,TNT
 INTEGER I,J,JB,IB,IBM1,K,L,M
 INTEGER CLNPAD
 CHARACTER*80 MESAGE
```

```
C
C + + + EXTERNALS + + +
 EXTERNAL SUBIN,ERRCHK,SUBOUT
C
C
C + + + END SPECIFICATIONS + + +
C
 MESAGE = 'FRM5CLN'
 CALL SUBIN(MESAGE)
C
 DO 650 L=1,NCHEM
 IF(CLNPAD.EQ.1)THEN
C  assign horizon soil profile values
C  to individual soil layers
  IB = NHORIZ
  TNT = 0.0
  TTHKNS = THKNS(IB)
  DO 160 J = 1, NCOM2
  IBM1= IB - 1
  JB = NCOM2 - J + 1
  TNT = TNT + DELX(JB)
  MODFC = 0.0
  IF (TNT .LE. TTHKNS+.01) THEN
  DWRATE(L,JB) = DWRAT1(L,IB)
  DSRATE(L,JB) = DSRAT1(L,IB)
  DGRATE(L,JB) = DGRAT1(L,IB)
  IF(L.EQ.2)THEN
  DKRW12(JB)=DKW112(IB)
  DKRS12(JB)=DKS112(IB)
  ELSEIF(L.EQ.3)THEN
  DKRW13(JB)=DKW113(IB)
  DKRW23(JB)=DKW123(IB)
  DKRS13(JB)=DKS113(IB)
  DKRS23(JB)=DKS123(IB)
  ENDIF
  ELSE
  MODFC=(TNT-TTHKNS)/DELX(JB)
  DWRATE(L,JB)=DWRAT1(L,IB)*(1.-MODFC)+DWRAT1(L,IBM1)*MODFC
  DSRATE(L,JB)=DSRAT1(L,IB)*(1.-MODFC)+DSRAT1(L,IBM1)*MODFC
  DGRATE(L,JB)=DGRAT1(L,IB)*(1.-MODFC)+DGRAT1(L,IBM1)*MODFC
  IF(L.EQ.2)THEN
  DKRW12(JB)=DKW112(IB)*(1.-MODFC)+DKW112(IBM1)*MODFC
  DKRS12(JB)=DKS112(IB)*(1.-MODFC)+DKS112(IBM1)*MODFC
  ELSEIF(L.EQ.3)THEN
  DKRW13(JB)=DKW113(IB)*(1.-MODFC)+DKW113(IBM1)*MODFC
  DKRW23(JB)=DKW123(IB)*(1.-MODFC)+DKW123(IBM1)*MODFC
  DKRS13(JB)=DKS113(IB)*(1.-MODFC)+DKS113(IBM1)*MODFC
  DKRS23(JB)=DKS123(IB)*(1.-MODFC)+DKS123(IBM1)*MODFC
  ENDIF
  IB=IB-1
  TTHKNS=TTHKNS+THKNS(IB)
  ENDIF
160 CONTINUE
  DKSTAT(L)=1
 ELSEIF(CLNPAD.EQ.2)THEN
C  assign horizon soil profile values
C  to individual soil layers
```

```
      IB = NHORIZ
      TNT = 0.0
      TTHKNS = THKNS(IB)
      IF(CLNPCT(NCLNC).GE.1.0)THEN
      DO J=1,IB
      DWRAT2(L,J)=5.0
      DSRAT2(L,J)=5.0
      DGRAT2(L,J)=5.0
      ENDDO
      ELSE
      DO J=1,IB
      DWRAT2(L,J)=-ALOG(1.0-CLNPCT(NCLNC))
      DSRAT2(L,J)=-ALOG(1.0-CLNPCT(NCLNC))
      DGRAT2(L,J)=-ALOG(1.0-CLNPCT(NCLNC))
      ENDDO
      ENDIF
      DO 165 J = 1, NCOM2
      IBM1= IB - 1
      JB = NCOM2 - J + 1
      TNT = TNT + DELX(JB)
      MODFC = 0.0
      IF(JB.GT.CLNCMP(NCLNC))THEN
      IF (TNT .LE. TTHKNS+.01) THEN
      DWRATE(L,JB) = DWRAT1(L,IB)
      DSRATE(L,JB) = DSRAT1(L,IB)
      DGRATE(L,JB) = DGRAT1(L,IB)
      IF(L.EQ.2)THEN
       DKRW12(JB)=DKW112(IB)
       DKRS12(JB)=DKS112(IB)
      ELSEIF(L.EQ.3)THEN
       DKRW13(JB)=DKW113(IB)
       DKRW23(JB)=DKW123(IB)
       DKRS13(JB)=DKS113(IB)
       DKRS23(JB)=DKS123(IB)
      ENDIF
      ELSE
      MODFC=(TNT-TTHKNS)/DELX(JB)
      DWRATE(L,JB)=DWRAT1(L,IB)*(1.-MODFC)+
     *   DWRAT1(L,IBM1)*MODFC
      DSRATE(L,JB)=DSRAT1(L,IB)*(1.-MODFC)+
     *   DSRAT1(L,IBM1)*MODFC
      DGRATE(L,JB)=DGRAT1(L,IB)*(1.-MODFC)+
     *   DGRAT1(L,IBM1)*MODFC
      IF(L.EQ.2)THEN
       DKRW12(JB)=DKW112(IB)*(1.-MODFC)+DKW112(IBM1)*MODFC
       DKRS12(JB)=DKS112(IB)*(1.-MODFC)+DKS112(IBM1)*MODFC
      ELSEIF(L.EQ.3)THEN
       DKRW13(JB)=DKW113(IB)*(1.-MODFC)+DKW113(IBM1)*MODFC
       DKRW23(JB)=DKW123(IB)*(1.-MODFC)+DKW123(IBM1)*MODFC
       DKRS13(JB)=DKS113(IB)*(1.-MODFC)+DKS113(IBM1)*MODFC
       DKRS23(JB)=DKS123(IB)*(1.-MODFC)+DKS123(IBM1)*MODFC
      ENDIF
      IB=IB-1
      TTHKNS=TTHKNS+THKNS(IB)
      ENDIF
      ELSEIF(JB.LE.CLNCMP(NCLNC))THEN
      IF (TNT .LE. TTHKNS+.01) THEN
```

```
  DWRATE(L,JB) = DWRAT2(L,IB)
  DSRATE(L,JB) = DSRAT2(L,IB)
  DGRATE(L,JB) = DGRAT2(L,IB)
  IF(L.EQ.2)THEN
   DKRW12(JB)=DKW212(IB)
   DKRS12(JB)=DKS212(IB)
  ELSEIF(L.EQ.3)THEN
   DKRW13(JB)=DKW213(IB)
   DKRW23(JB)=DKW223(IB)
   DKRS13(JB)=DKS213(IB)
   DKRS23(JB)=DKS223(IB)
  ENDIF
  ELSE
  MODFC=(TNT-TTHKNS)/DELX(JB)
  DWRATE(L,JB)=DWRAT2(L,IB)*(1.-MODFC)+
 *   DWRAT2(L,IBM1)*MODFC
  DSRATE(L,JB)=DSRAT2(L,IB)*(1.-MODFC)+
 *   DSRAT2(L,IBM1)*MODFC
  DGRATE(L,JB)=DGRAT2(L,IB)*(1.-MODFC)+
 *   DGRAT2(L,IBM1)*MODFC
  IF(L.EQ.2)THEN
   DKRW12(JB)=DKW212(IB)*(1.-MODFC)+DKW212(IBM1)*MODFC
   DKRS12(JB)=DKS212(IB)*(1.-MODFC)+DKS212(IBM1)*MODFC
  ELSEIF(L.EQ.3)THEN
   DKRW13(JB)=DKW213(IB)*(1.-MODFC)+DKW213(IBM1)*MODFC
   DKRW23(JB)=DKW223(IB)*(1.-MODFC)+DKW223(IBM1)*MODFC
   DKRS13(JB)=DKS213(IB)*(1.-MODFC)+DKS213(IBM1)*MODFC
   DKRS23(JB)=DKS223(IB)*(1.-MODFC)+DKS223(IBM1)*MODFC
  ENDIF
  IB=IB-1
  TTHKNS=TTHKNS+THKNS(IB)
  ENDIF
  ENDIF
165 CONTINUE
 ENDIF
650 CONTINUE
C
 CALL SUBOUT
C
 RETURN
 END
```

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