Effects of Cattle Manure Handling and Management on Fate and Transport of Hormones in the Feedlot and the Field

EPA-G2006-STAR-M1: Fate and Effects of Hormones in Waste From Concentrated Animal Feeding Operations (CAFOS)

Research Team

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University of Nebraska Lincoln

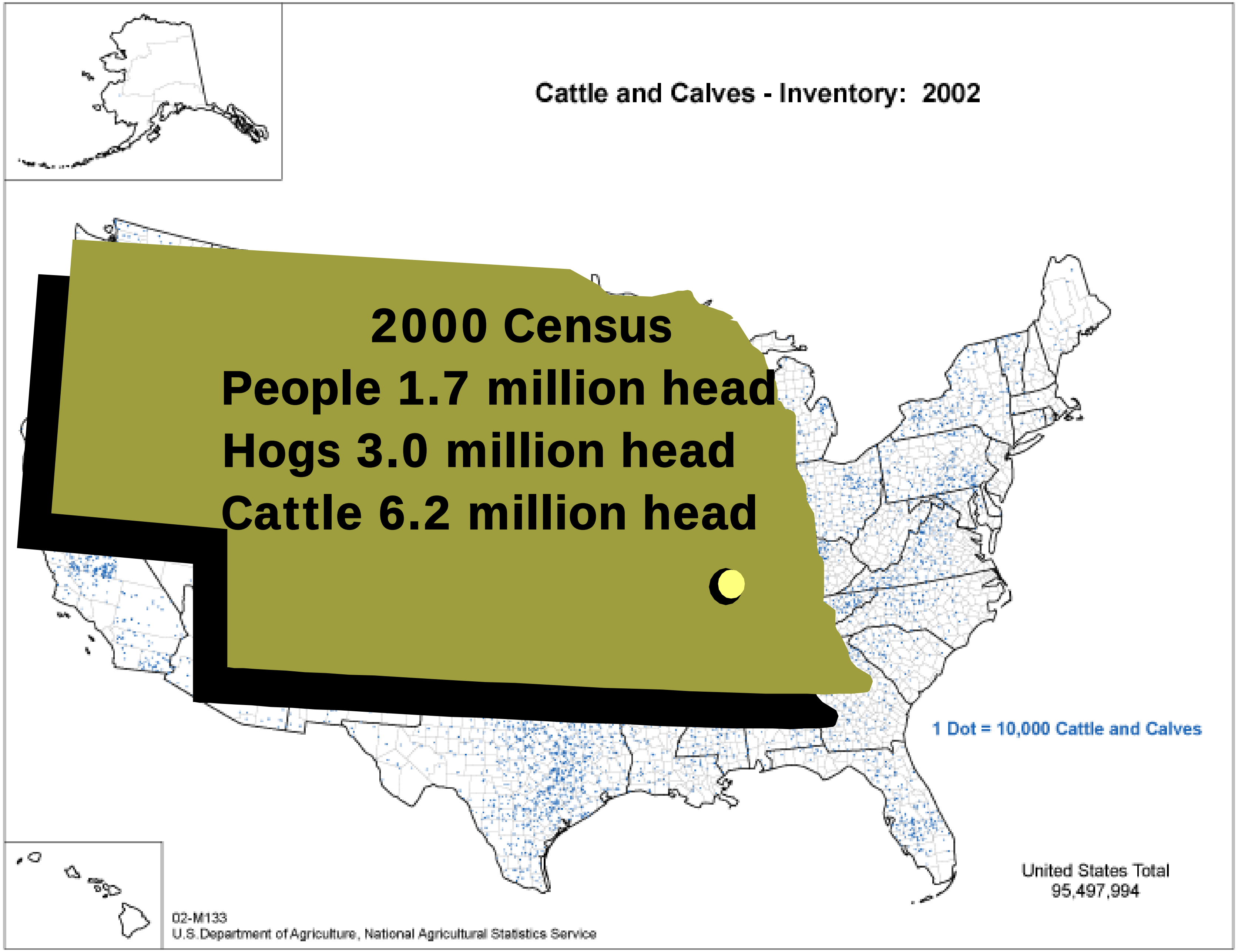
Pioneering new frontiers.

EPA Request for Proposals Purpose of Program

- “Characterize the occurrence, magnitude, and extent of the impact of natural and synthetic steroid hormones in liquid and solid animal waste from concentrated animal feeding operations (CAFOs) on the environment and human health”
- “Determine the impact of current CAFO waste management strategies (i.e. storage and disposal) on the transport, fate, and effects of steroid hormones originating from CAFOs”

Background

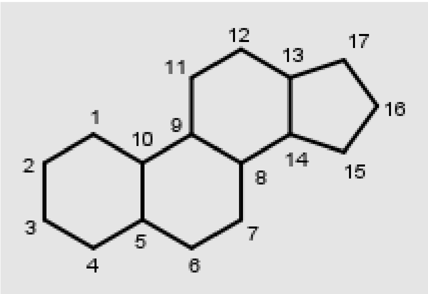
- Estimated 1.3 million livestock operations in the U.S
- Roughly 20% confined generate ~500 million tons of waste annually
- Large facilities have limited land available for effective use of the generated waste as fertilizer
- Potential for natural and synthetic steroid hormones in livestock waste to reach groundwater and surface waters through many pathways (e.g. surface runoff, leaching from holding tanks and lagoons and composting facilities)



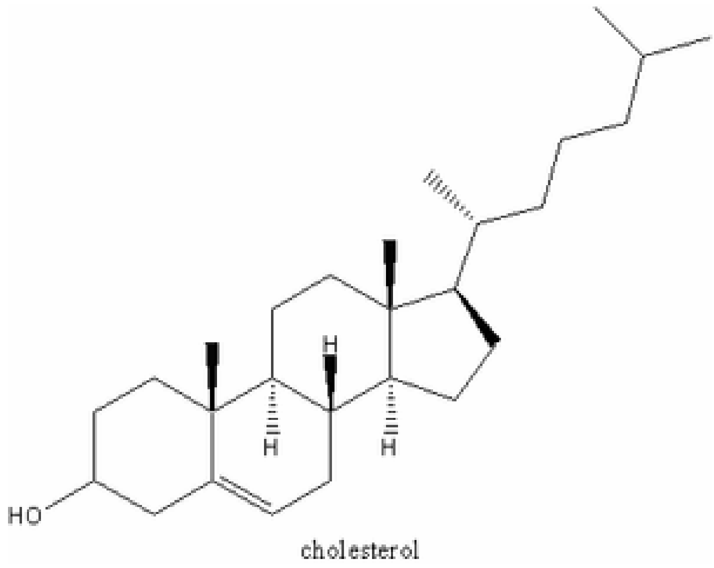
Cattle and Calves - Inventory: 2002
2000 Census
People 1.7 million head
Hogs 3.0 million head
Cattle 6.2 million head
1 Dot = 10,000 Cattle and Calves
United States Total
95,497,994
02-M133
U.S. Department of Agriculture, National Agricultural Statistics Service

Steroid Hormones

- Steroids (terpinoid lipids) that act as hormones
- Hormone is a chemical messenger from one cell or group of cells to another (3 classes)
- Natural steroid hormones are generally synthesized from cholesterol

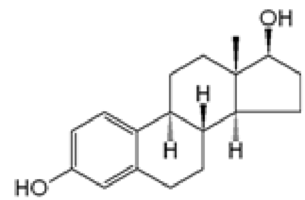


General chemical structure

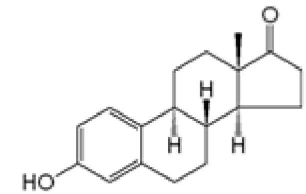


Steroid Hormones in Cattle Waste

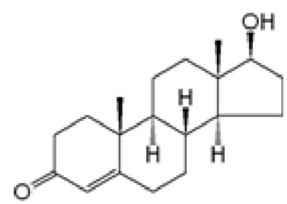
- Endogenous (natural)
  - Estrodiol
  - Estrone
  - Estriol
  - Testosterone
  - Androstenedione
  - Progesterone
- Exogenous (synthetic)
  - Trenbolone
  - Zearalonol
  - Melengestrol Acetate



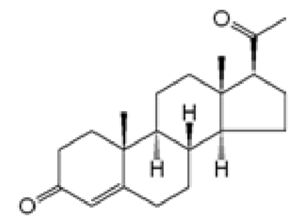
17β-estradiol



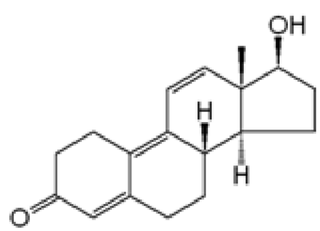
estrone



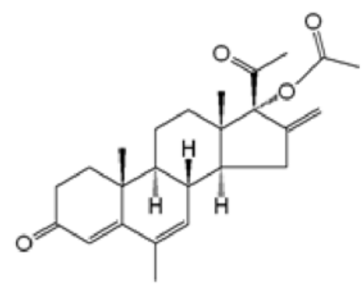
Testosterone



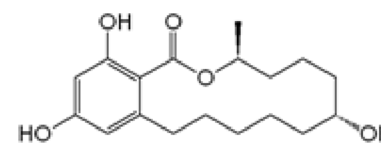
Progesterone



17β-trenbolone



Melengestrol acetate

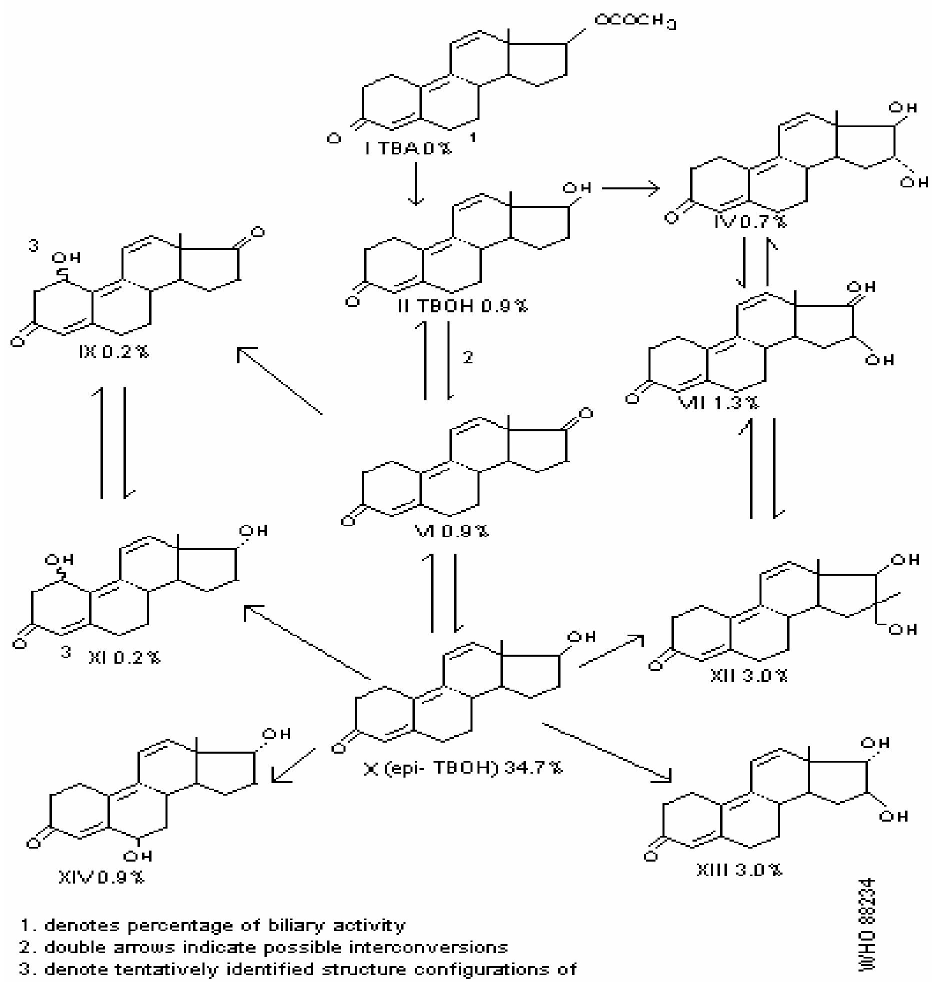


α-zearalonol

Steroid hormones excreted in livestock manure

- Levels are highly variable
  - up to 1 μg/g
  - <100 ng/g
  - depends on compound, sex, diet, reproductive status
- Conjugated forms can be converted back to free hormones

Figure 2. Biliary 3-ketotrienic metabolites in the bile of a heifer



1. denotes percentage of biliary activity
2. double arrows indicate possible interconversions
3. denote tentatively identified structure configurations of 1-hydroxyls of compounds IX and XI are unspecified

Chemical Properties

Common Name	***Aqueous solubility (µg/L)@ 25ºC***	**Log P_{ow}**
Estrogens		
17β-estradiol	12,960	4.01
Estrone	12,420	3.13
Estriol	13,250	2.45
Androgens		
Testosterone	5,570	3.23
Androstenedione	27,000	2.9
Androsterone	8,750	3.69
Progestins		
Progesterone	6,600	4.0
Synthetic Steroids/progestins		
Trenbolone	20,000	--
Zearalanol	--	3.9
Melengestrol Acetate	1,060	3.67
Ethinylestradiol	483	3.67

Atrazine solubility = 30,000 µg/L Log Pow=2.3

Persistence

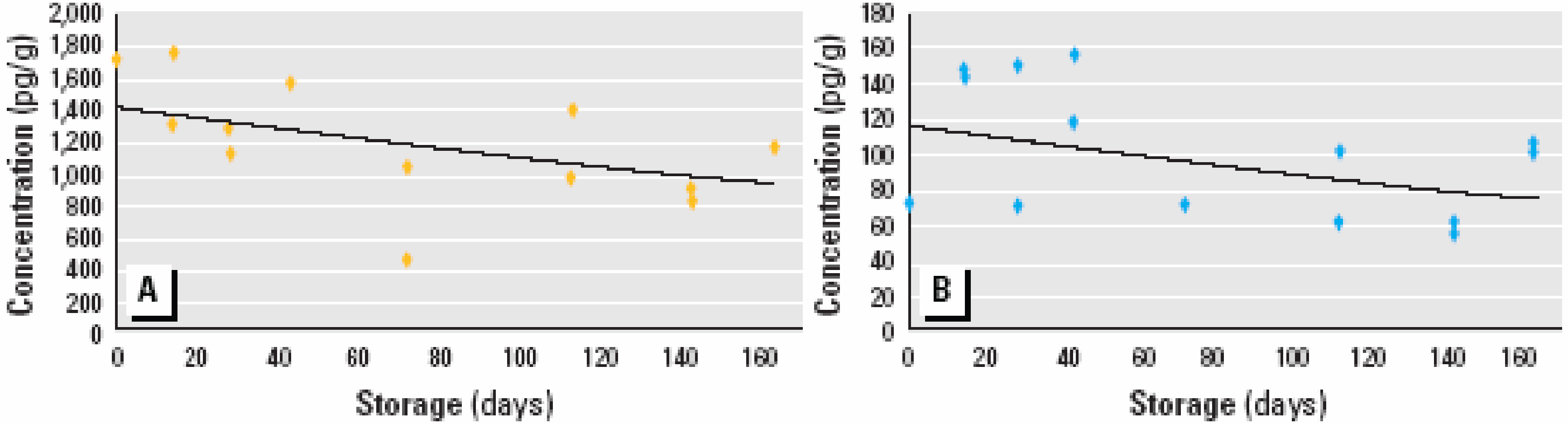


Figure 4. Degradation of (*A*) TbOH-17α, (*B*) TbOH-17β, and (*C*) TbO in liquid manure during storage.

"The Fate of Trenbolone Acetate and Melengestrol Acetate after Application as Growth Promoters in Cattle: Environmental Studies" ***Bettina Schiffer, Andreas Daxenberger, Karsten Meyer, and Heinrich H.D. Meyer.*** Environmental Health Perspectives • VOLUME 109 | NUMBER 11 | November 2001.

Project Objectives

1) *Quantify hormones in various stages of the manure pathway in cattle feedlots.*
2) *Determine the effects of different handling practices of cattle feedlot wastes on the stability and availability of hormones.*
3) *Determine the effects of different land application strategies on the fate and transport of hormones in vadose zone soils.*
4) *Determine if grasses grown in conservation buffers assimilate hormones.*

Environmental Fate and Transport of Steroid Hormones

Implant 36 mg Z
140 mg TBA
16mg E2

Feed Supplement

0.45 mg/day MGA

Fresh Manure ~25kg/day
~0.5 mg/day excretion
~ 20 ug/kg maximum

Accumulation & degradation

Run-off to wastewater lagoon

Degradation in stockpiled and composted manure

Run-off and leaching from fertilized fields

Uptake in grasses

145 day feeding period

Research Hypotheses

1) Hormone levels in manure are greater for cattle treated with hormones compared to untreated cattle.
2) Composting manure will facilitate the degradation of hormones in manure compared to stockpiling.
3) Hormone losses in runoff will be greater when manure is surface applied compared to when it is incorporated into the soil
4) Hormone losses in runoff will be related to the duration and timing of rainfall events with respect to land application of manure
5) Grasses commonly used in conservation buffer strips will assimilate hormones
6) Hormones will persist in soils and will leach through soils toward groundwater

Project Tasks

- Task 1: Survey existing feedlots and agricultural fields to determine the fate of hormones in the manure handling pathway over a climatic gradient.
- Task 2: Quantity the fate of hormones as influenced by manure handling practices such as stockpiling, composting, and runoff retention basins.
- Task 3: Conduct runoff studies with controlled rainfall simulators to determine the effect of manure application strategies on hormone transport.
- Task 4: Quantify the uptake of hormones applied in manures to selected grass species commonly used in grass buffer strips.
- Task 5: Determine the fate and transport of hormones in vadose zone soil after land application of manure.

Task 1. Survey of hormones in pens, run-off basins, stockpiles and fertilized cropland

- Where: Four University of Nebraska feedlots and manure-fertilized crop land
  - Collect manure from 3 pens and 2 stockpiles
  - Composite liquid from 2 run-off basins
  - Collect 12 composite samples at 3 depths from fields having received manure application within 1 month, 1 year, 2 year, and 3 years
- Collect data on feeding strategies, pen cleaning history, implant history, and manure handling

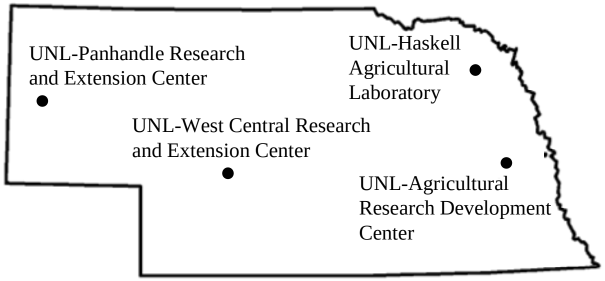
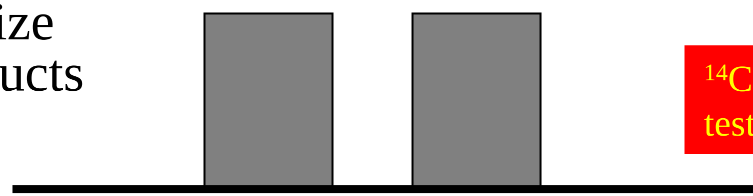


Figure 2. Locations of University of Nebraska Feedlots that will be used for Task 1.

Task 2. Measure differences in cattle waste as influence by handling practices

- Where: Haskell Ag Lab
- 96 heifers split between six pens
- 48 will receive implants (TBA, Z, E) and MGA supplement
- Composite pen surface samples and fresh manure collected at 0, 7, 45, and 125 days after implanting
- Waste removed after 150 days, stockpiled and composted
- Samples of stockpiled and composted manure will be collected to determine changes in hormone levels
- Reactors used to characterize degradation rates and products

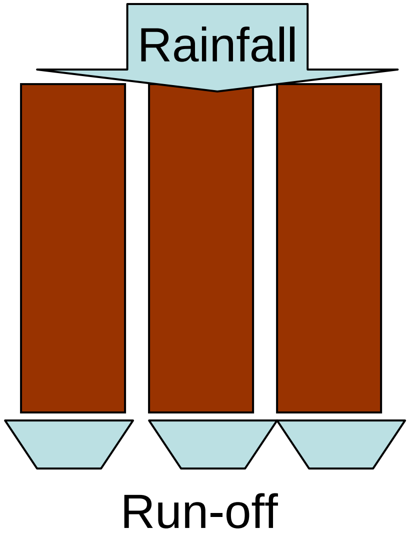


^{14}C-labelled estradiol, testosterone, trenbolone

Aerobic vs anaerobic bioreactors

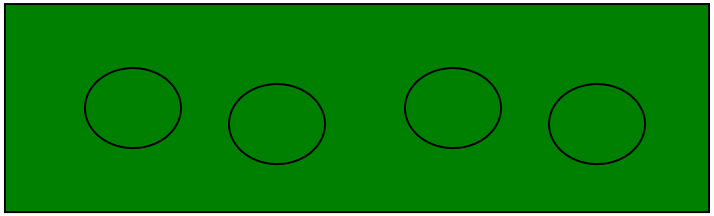
Task 3. Effect of tillage on hormones levels in field run-off

- Where: Haskell Ag Lab
- Five manure sources
    - treated compost and stockpile manure
    - untreated compost and stockpile manure
    - control with no manure applied
- Compare surface application (no-till), single disk incorporation, mold-board plow
- Simulated rainfall



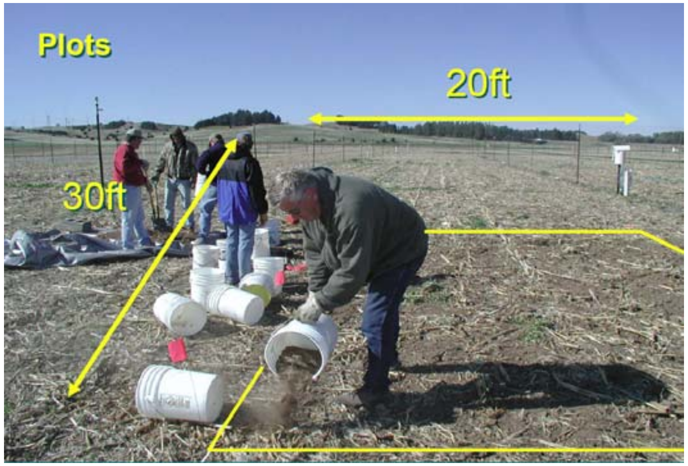
Task 4. Uptake of hormones applied in manures to selected grass species commonly used in grass buffer strips

- Where: Haskell Ag Lab
- Hormone concentrations measured in buffer strip grasses receiving manure from steroid treated cattle
- Grass harvested after 30, 60, 90, 180, and 365 days after manure application

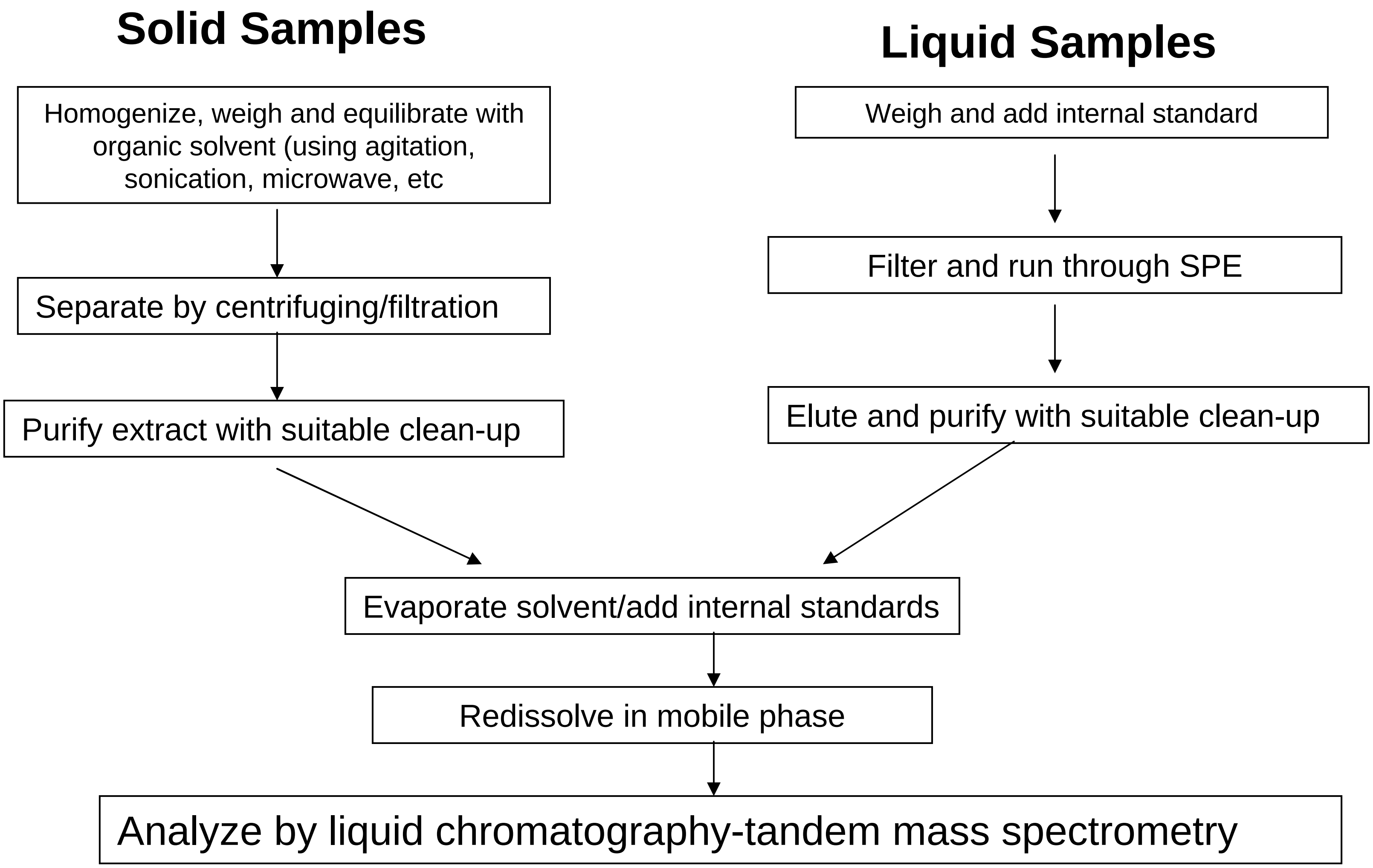


Task 5. Fate and transport of hormones in vadose zone soil

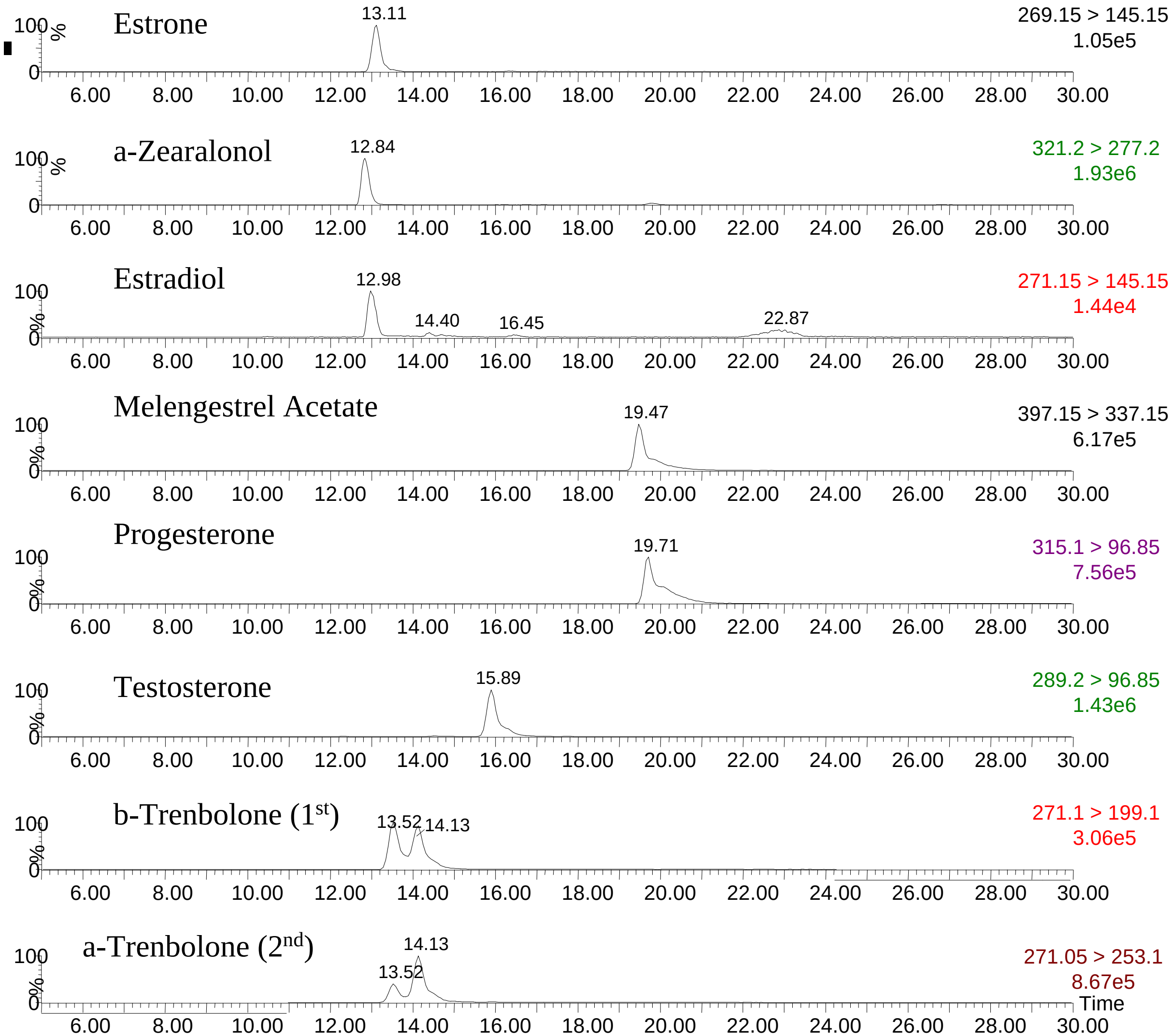
- Where: WREC North Platte
- Lysimeter-instrumented test plots will be fertilized with stockpiled and composted manure from treated cattle
- Soil water will be collected from lysimeters over a 6-month period



Method Development: Extraction and analysis of steroid hormones



LC/MS/MS of Steroid Hormones

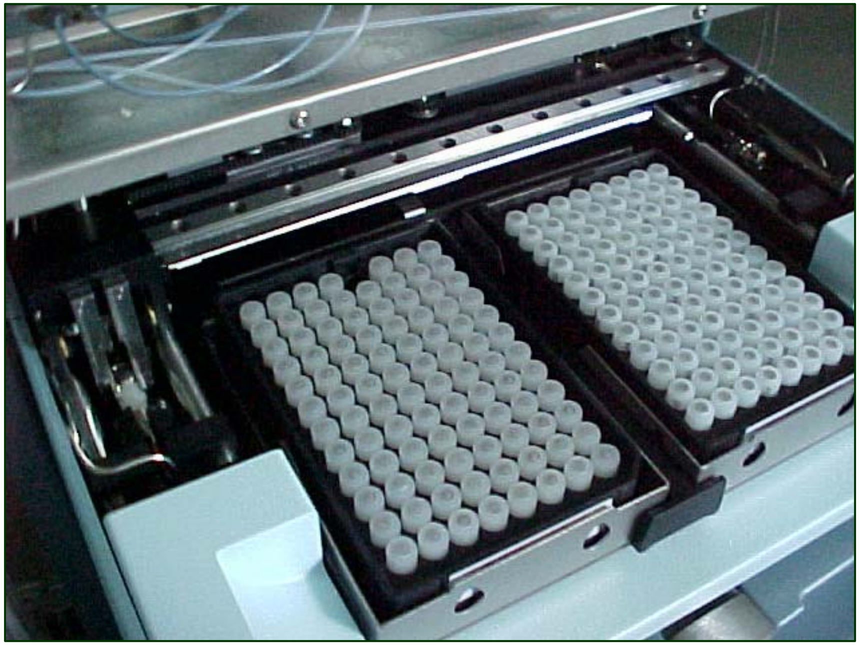


Quattro micro triple quadrupole

LC/MS/MS Detection Limits

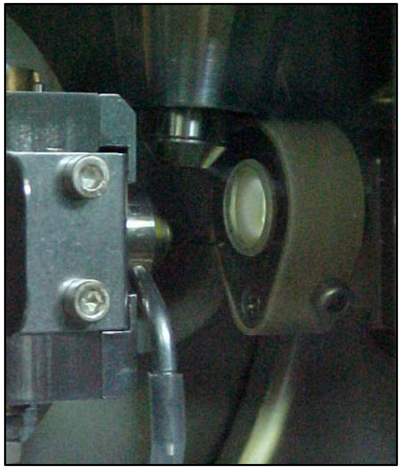
Compound	Direct Injection ppb	on-line SPE ppb
17β-estradiol	1.07	0.0036
Estrone	0.77	0.0034
17α-ethynyl estradiol	0.92	0.0056
Estriol	1.00	0.0064
α-Zearalonol	0.64	0.0032
Testosterone	1.46	0.0011
11-Ketotestosterone	0.58	0.0040
4-Androstenedione	1.71	0.0005
Progesterone	1.00	0.0008
Melengestrel Acetate	1.03	0.0016
17β-trenbolone	0.93	0.0018
Androsterone	3.16	0.0038

Spark Holland Symbiosys
Environ Extraction System



LC/MS/MS of steroid hormones

- Steroids
  - difficult to ionize by electrospray (ESI)
  - matrix effects severe
- Atmospheric pressure photoionization (APPI)
  - more selective
  - improved ionization efficiency
  - better for steroid analysis



Preliminary On-Column Detection Limit (pg)

Compound	ESI	Recovery	APPI	Recovery
Testosterone	36.5	116 %	30.5	97 %
11-Ketotestosterone	14.5	91 %	13.8	103 %
4-Androstenedione	42.8	118 %	33.5	156 %
Progesterone	25.0	99 %	23.3	104 %
Melengestrol Acetate	25.8	96 %	20.5	125 %
17β-Trenbolone	23.3	154 %	18.0	142 %

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