

US EPA ARCHIVE DOCUMENT

Assessing occurrence, persistence and biological effects of hormones released from livestock waste

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Overall Project Goal

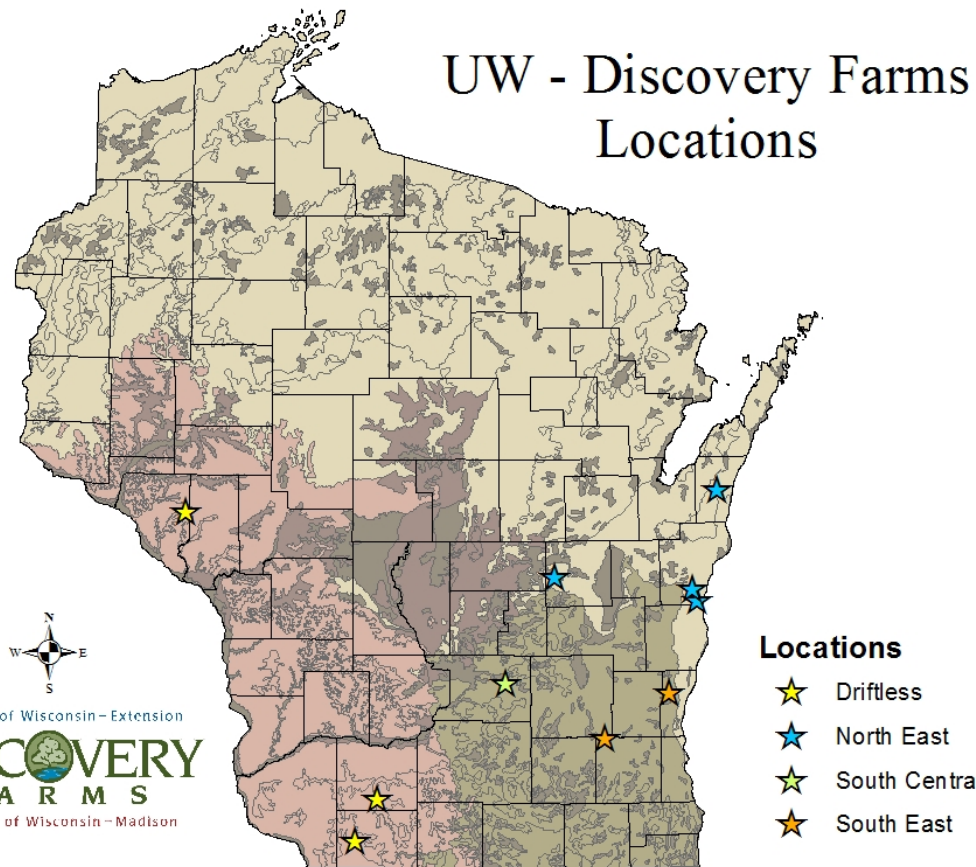
To determine the presence, persistence and biological effects of natural and synthetic hormones that may be released into the environment from CAFOs, and evaluate the effects of different animal waste disposal practices on the fate and activity of these compounds.

Specific Objectives

- Characterize the environmental transport and fate of natural and synthetic steroid hormones that accompany discharges and the disposal of animal wastes from CAFOs in Wisconsin.
- Evaluate how various animal waste handling/management strategies (e.g., lagoon storage and spraying of liquid manure vs. deep-stacking and field application of solid manure) impact the transport, fate, potential exposure, and associated effects of discharged steroid hormones.
- Investigate the biological effects associated with steroid hormones in animal waste from CAFOs.

Experimental Approach

Partnering with Discovery Farms



Established to support and facilitate research on best management practices at operating private farms

Experimental Approach

Study Farm Characteristics and Settings

Location (WI)	Animals	Setting	Monitoring Infrastructure
Lafayette Co. 300 acres	600 steer finishing (confined).	Un-glaciated. Black silt loam over brown silt loam. 6% slopes.	3-surface water flumes and auto-samplers in farm fields & waterways. Met. station.
Buffalo Co. 1010 acres	180 cows, 80-100 steers, 32,000 pullets	Un-glaciated. Silty soils. Moderate slopes.	2-gauged streams, 2-automated water samplers. Meteorology station.
Dunn Co. 852 acres	10,000 hog finishing	Forested silty soils over sandstone. Moraine.	Extensive soil sampling. Lake on-site.
Iowa Co. 480 acres	120 cow (organic grass-based dairy)	Un-glaciated. Silty soils. Moderate slopes.	2-stream + 1-field monitoring station. Two ponds, stream, springs, on-site.
Kewaunee Co. 4000 acres	1400 dairy cows, permitted CAFO	Thin silt loam over clay loam over fractured dolomite. 4% Slopes.	3-surface, 2-tile drain-flumes and auto-samplers in farm fields & waterways. Met. Sta.
Dane Co. 2040 acres	150-200 steer finishing (semi-confined).	Silty prairie soils – ground moraine. Shallow slopes.	Soil Monitoring Meteorology station.

Proposed CAFO-Hormone Sampling Program

- Establish hormone use profile of each study farm
- Establish waste handling practices at each study farm
- Validate hormone “fingerprint” in primary sources
- Determine hormone levels in receptors under **baseline hydrologic conditions and various waste management practices and farm crop/tillage operations.**
- Determine hormone levels in receptors under **event hydrologic conditions and various waste management practices and farm crop/tillage operations.**

Experimental Approach

Field Sampling Strategy

- Tile Drains/Field Streams
 - Automated samplers-event driven
- Shallow Soil- and Ground Water
 - Multi-level piezometers to monitor movement into the shallow ground water system
- Soils
- Waste Storage Facilities
- Field-applied Manure Slurry



Experimental Approach

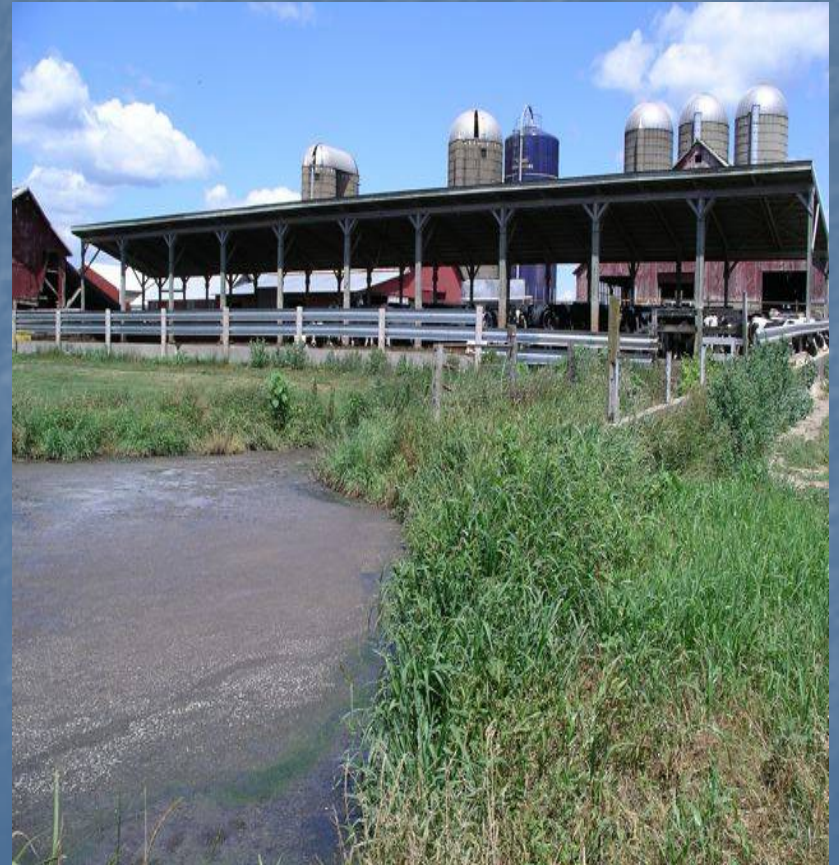
Receiving Streams -Watersheds

- Stream Samples
 - Baseline
 - Automated samplers-event driven
- Stream Sediments
 - <128 micron fraction
- Algae
- Native Fish

Experimental Approach

Assessing Waste Management/Utilization Effects on Hormones

- CAFO vs. Grazing
- Till vs. no-till
- Crop variety and type
- Soil type and slope



Experimental Approach

Target Hormones

Biological Effect	Origin	Hormone [CAS #]
Estrogen	Natural	17 β -Estradiol [50-28-2]
		17 α -Estradiol [57-90-1]
		Estrone [53-16-7]
		Estriol [50-27-1]
	Synthetic	Zeranol (α -zearalanol) [26538-44-3]
		α -Zearalenol [36455-72-8]
		Zearalanon [5975-78-0]
		Zearalenon [17924-92-4]
	Taleranol (β -zearalanol) [42422-68-4]	
Androgen	Natural	Testosterone [58-22-0]
		5 α -Androstan-17 β -ol-3-one (DHT) [521-18-6]
		Androsterone [53-41-8]
		5 α -Androstane-3,17-dione [846-46-8]
		4-Androstene-3, 17-dione [63-05-8]
		11 β -Hydroxy-etiocholanolone [739-26-4]
		1-Dehydrotestosterone (Boldenone) [846-48-0]
		17 β -nortestosterone [434-22-0]
	Synthetic	17 β -trenbolone [10161-33-8]
		17 α -trenbolone [80657-17-6]
Progestin	Natural	Progesterone [57-83-0]
	Synthetic	Melengestrol acetate [2919-66-6]



Experimental Approach Chemical Analyses



- Water Sample Extraction-Evaluating 2 SPE techniques
 - 3M Empore extraction disks (SDB, C18)
 - HLB cartridges
- Solid Extraction
 - Automated Solvent Exchange (ASE) followed by SPE extraction

Experimental Approach Chemical Analyses

- HPLC-MS-MS
- Method based on Vanderford et al. 2003

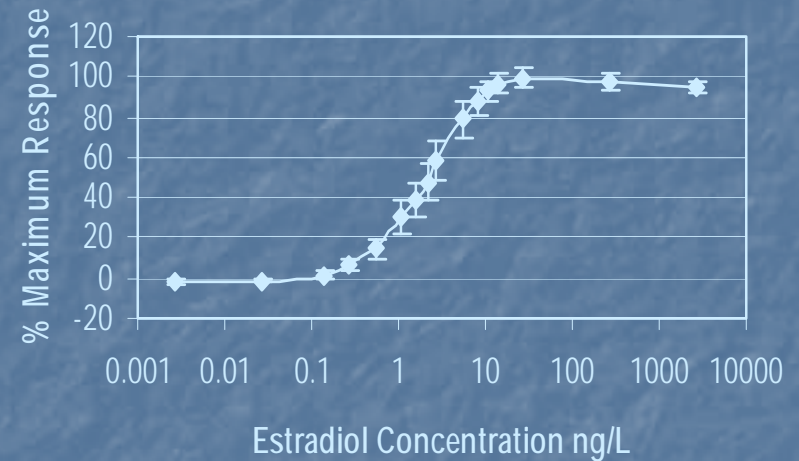
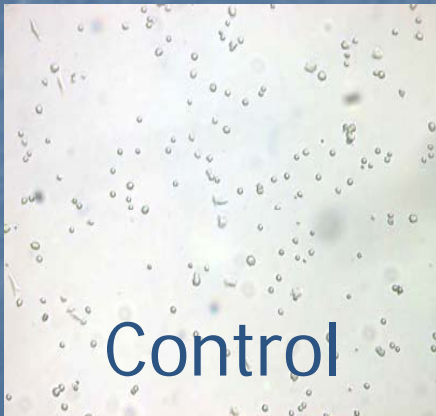
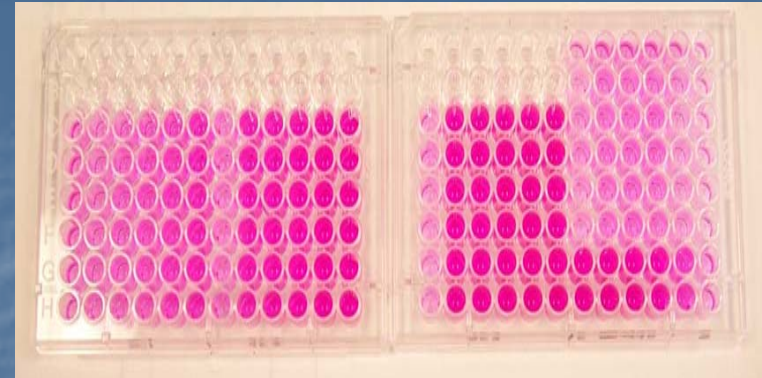


Experimental Approach Chemical Analyses

- Exploring preservation techniques
- Exploring recoveries in various matrices

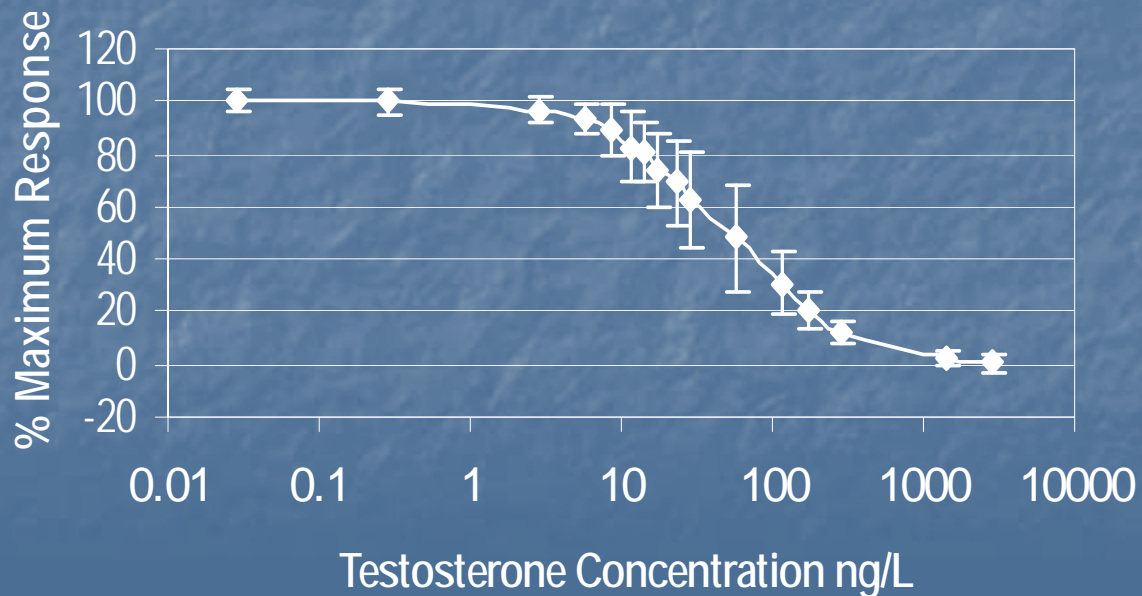
Bioassay: E-Screen

- MCF-7 breast cancer cell line proliferates in response to estrogenic compounds
- Incubate 5 days with sample
- Protein dye to indicate cell #
- + control E2 spiked samples



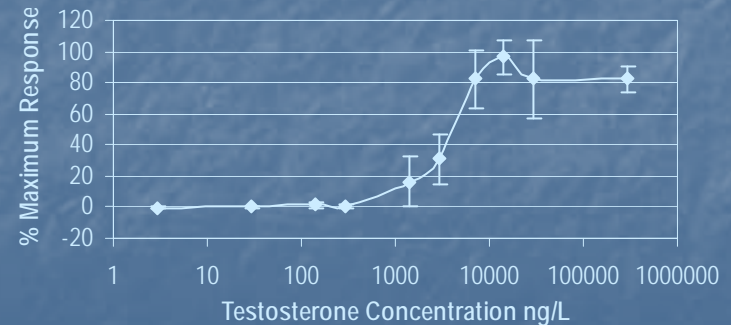
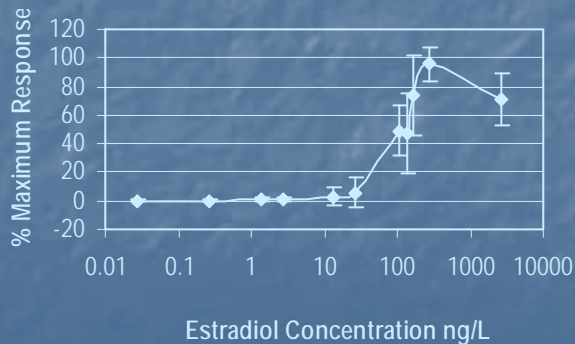
Bioassay: A-Screen

- MCF-7 breast cancer cell line transfected with androgen receptor. Inhibits proliferation in response to androgenic compounds
- Incubate 5 days with sample
- Protein dye to indicate cell #
- + control: MCF-7 cells



Transgenic Yeast Assays

- Human ER, AR or PR and corresponding response element linked to β -galactosidase DNA stably transfected into yeast cell
- Incubate overnight with sample (4 hrs PR)
- Lyse yeast and perform β -galactosidase assay
- Normalize activity to yeast density (OD_{600})

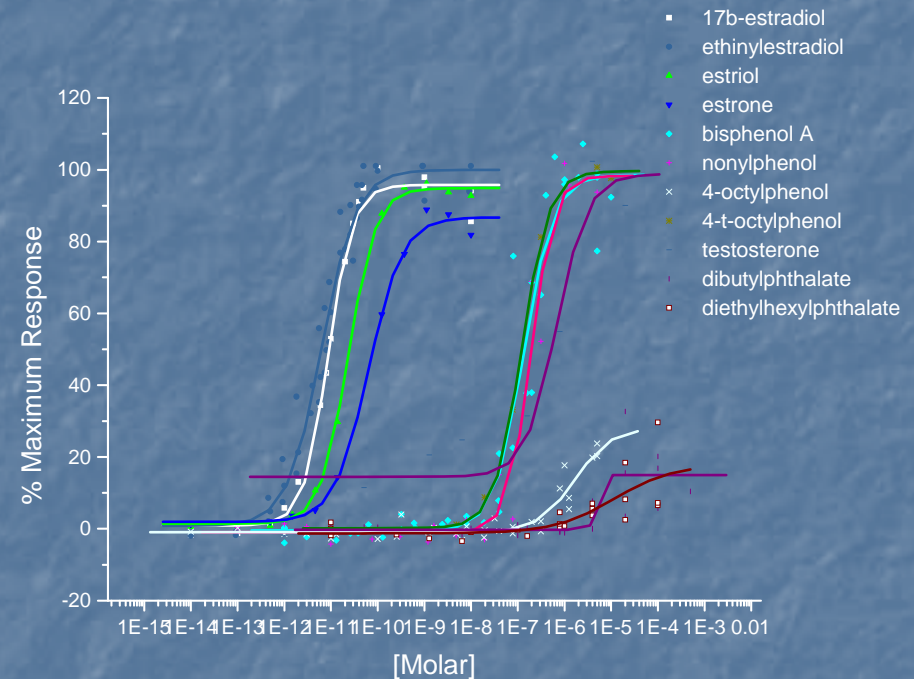


Bioassay comparison

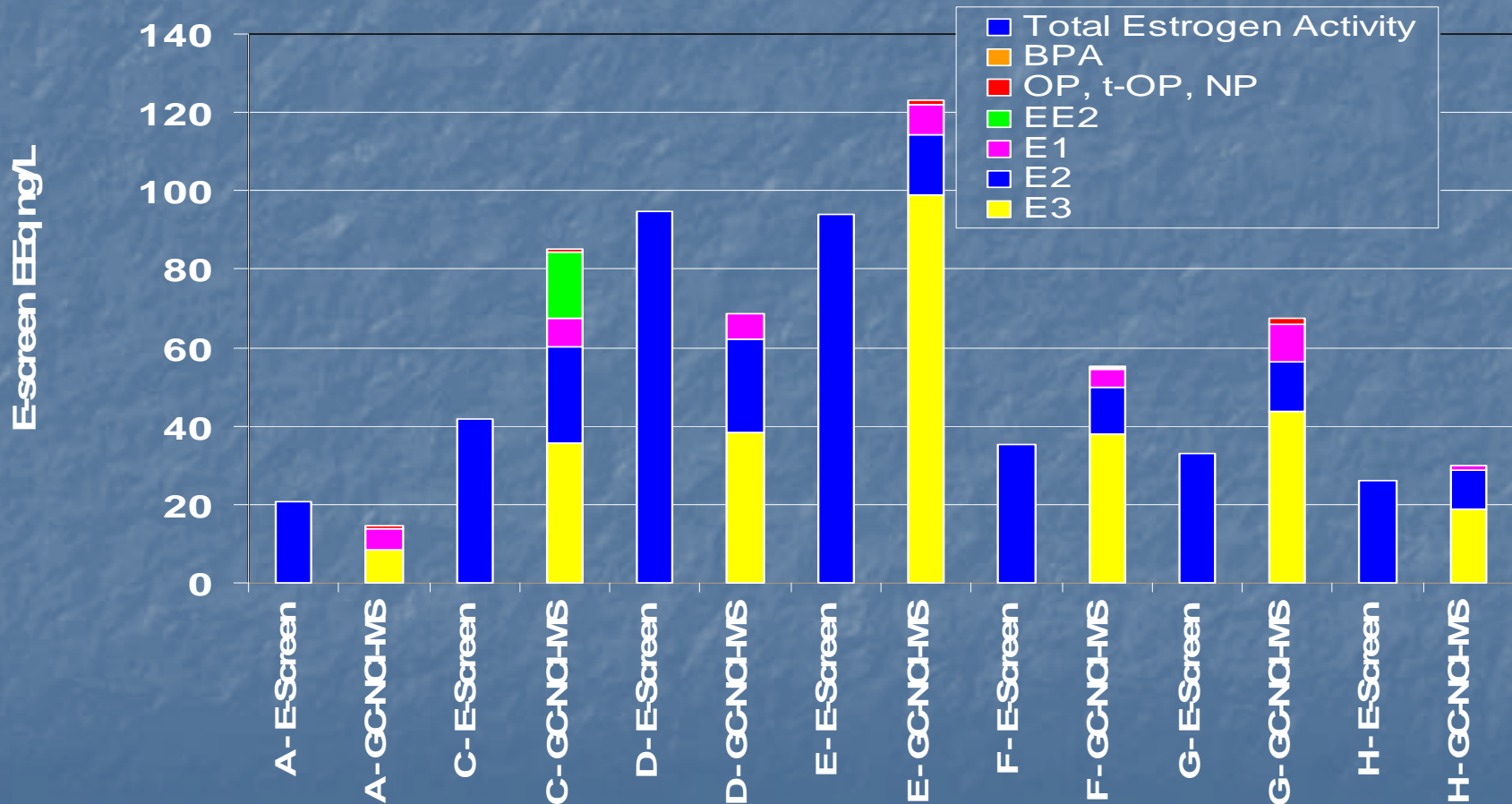
Bioassay	Reference cmpd	EC50 ng/L	Lowest std ng/L
E-screen	Estradiol	2.0	.27
ER-yeast	Estradiol	61	14
A-screen	DHT	22	14
AR-yeast	DHT	1016	290
PR-yeast	Progesterone	3145	32

Comparing Chemical and Biological Analyses

- Bioassay results reported as “activities”
- Need to convert chemical analyses to expected activities in order to compare results
- Each chemical has unique potency
- Assuming additive response



Comparison of Bioassay and Chemistry Results



HPLC Fractionation: TIE-type approach

Samples where activity and chemical analyses are markedly different can be subjected to fractionation followed by bioassay to help identify active components

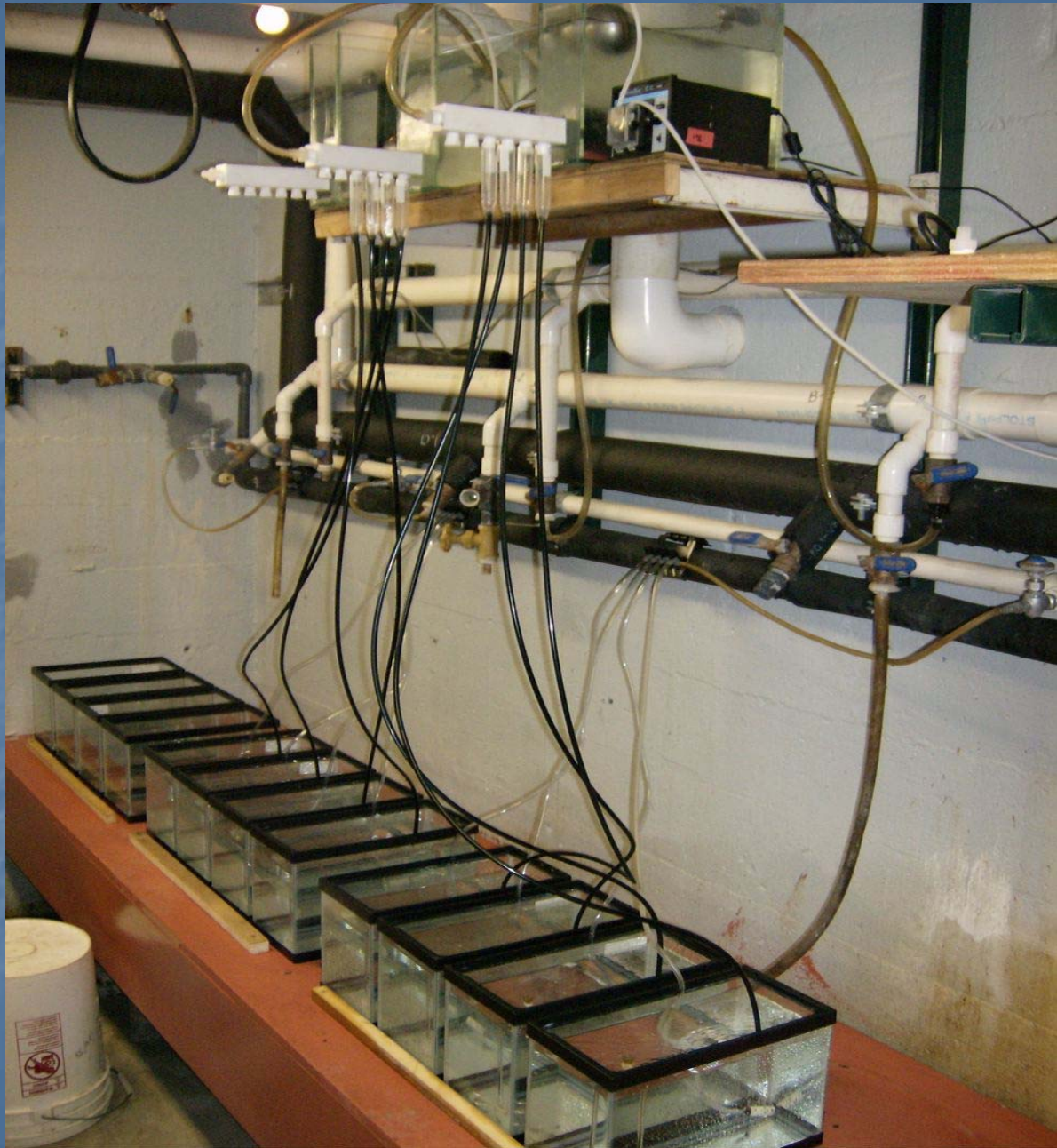
- Interferences



Whole fish Assays

Partial life-cycle testing of specific hormones

- Hormones, metabolites or relevant mixtures depending on data gaps
- Expose breeding fathead minnows
- Endpoints include fecundity and secondary sex characteristics, plasma VTG, hormones.



Gene Expression

1) Expose adult FHM to sample extracts showing activity in cell-based bioassays

and

2) Perform full/partial life-cycle tests with CAFO effluent



↓ 24 hrs

Remove liver and gonads



Extract RNA → cDNA → Determine expression of biomarkers

Gene Expression

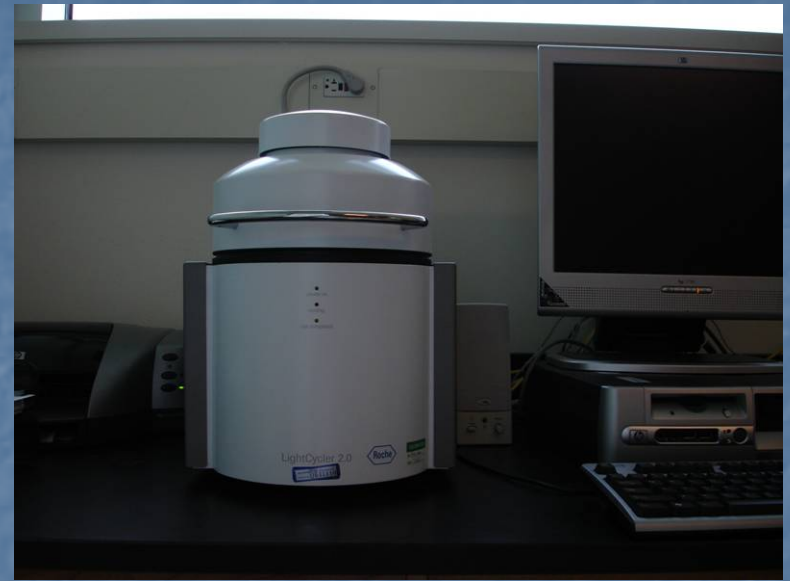
Estrogenic Biomarkers:

- Vitellogenin (\uparrow males)
- Zona Pellucida (ZP3)
- other?

Androgenic Biomarkers :

- Vitellogenin (\downarrow females)
- other?

Progesteronic ?



Expected Results:

The project will determine the potential for environmental release of hormones:

- under various waste management practices
- degradation of hormones and associated activities
- the persistence of residuals in transport from CAFOs
- the relative importance of different natural and synthetic hormones to the biological activity of CAFO wastes and effluents.

The results will assist CAFO operators to optimize management practices that mitigate environmental problems associated with hormones discharged from CAFOs, and help regulators with risk assessment as biologically relevant chemicals will be identified, quantified, and ranked.

Ultimately, the proposed project will help protect sensitive aquatic environments, native species, and humans from wastes associated with CAFOs.