FATE OF HORMONES IN TILE-DRAINED FIELDS & IMPACT TO AQUATIC ORGANISMS UNDER DIFFERENT ANIMAL WASTE LAND-APPLICATION PRACTICES

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EPA STAR CAFO Kickoff Meeting
Chicago, IL
August 20-22, 2007

Background…

- Midwest agricultural soils are typically fertile, but poorly drained
- To enhance productivity, drainage is facilitated by networks of artificial, subsurface drains ~ 1 m below the soil surface that can rapidly move excessive soil water (and associated constituents) to surface waterways
- Preferential flow paths to tile drain systems develop reducing the effects of soil attenuation on transport

Purdue's Research Focus to Address:

- Hormone persistence in manure- and lagoon effluent-applied fields
- Subsequent attenuation of hormone release from manure- and effluent-treated fields
- Contribution of tile-drained fields on hormone loads to the aquatic systems
- Impact of hormone mixtures relevant to animal wastes on aquatic species.

Objective 1

Quantify the mass of hormones discharged from tile-drained agricultural fields under different manure and lagoon effluent application practices. **We hypothesize that tile-drain discharge of manure-borne hormones to receiving waters will occur primarily in the first rain events after land-application.**

- **Purdue's Water Quality Field Station (WQFS)**
- **Purdue University Animal Science Farm**

Water Quality Field Station (WQFS)

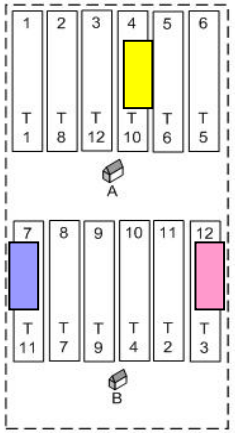
- Established in 1992 to evaluate the impact of regional crop management practices on subsurface water quality from tile drain systems
- Predominantly Drummer silty clay loam soils typical of the Midwest
- Tile drain systems (0.1 m d tiles placed at 0.9 m)
- 48 treatment plots (10.8 x 48 m) - 12 treatment plots randomly assigned within each of four blocks or replicates
- Leachate collection system allowing recovery of soil drainage water into tile lines from a hydrologically isolated volume of soil.

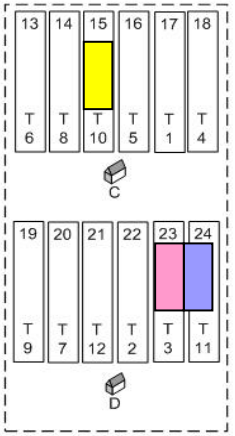
WQFS Field Layout

* The # at the top is the plot #.

* The # at the bottom is the treatment number.

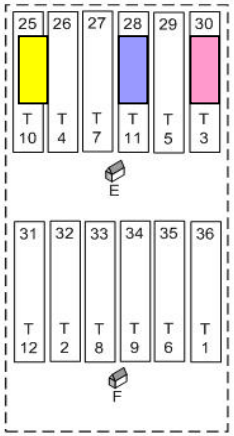
BLOCK I

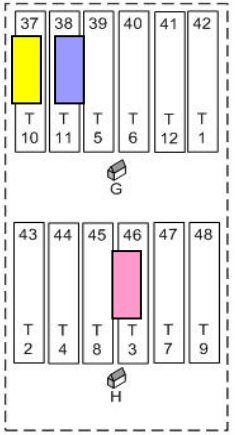




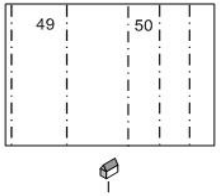
BLOCK II

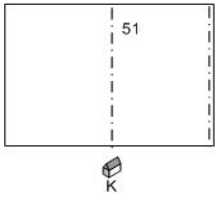
BLOCK III

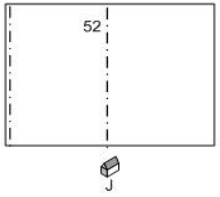


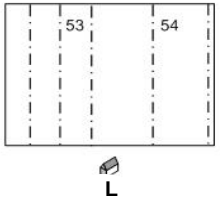


BLOCK VI





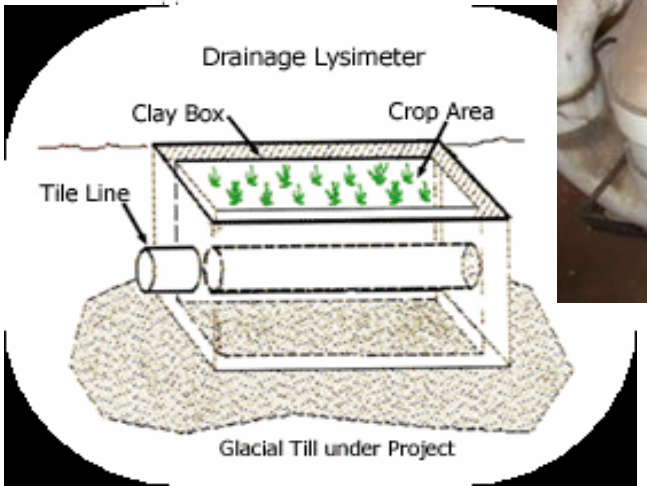


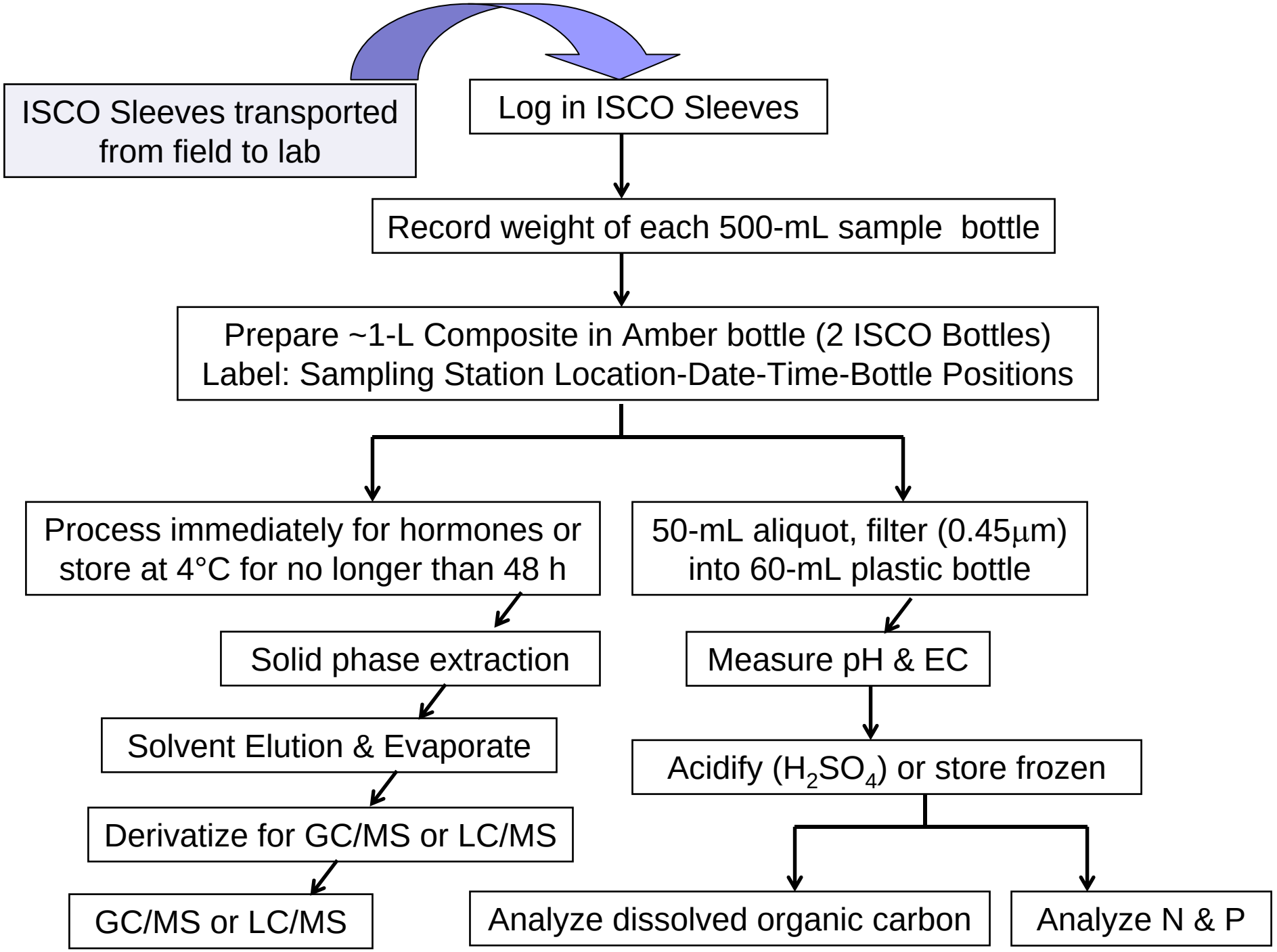


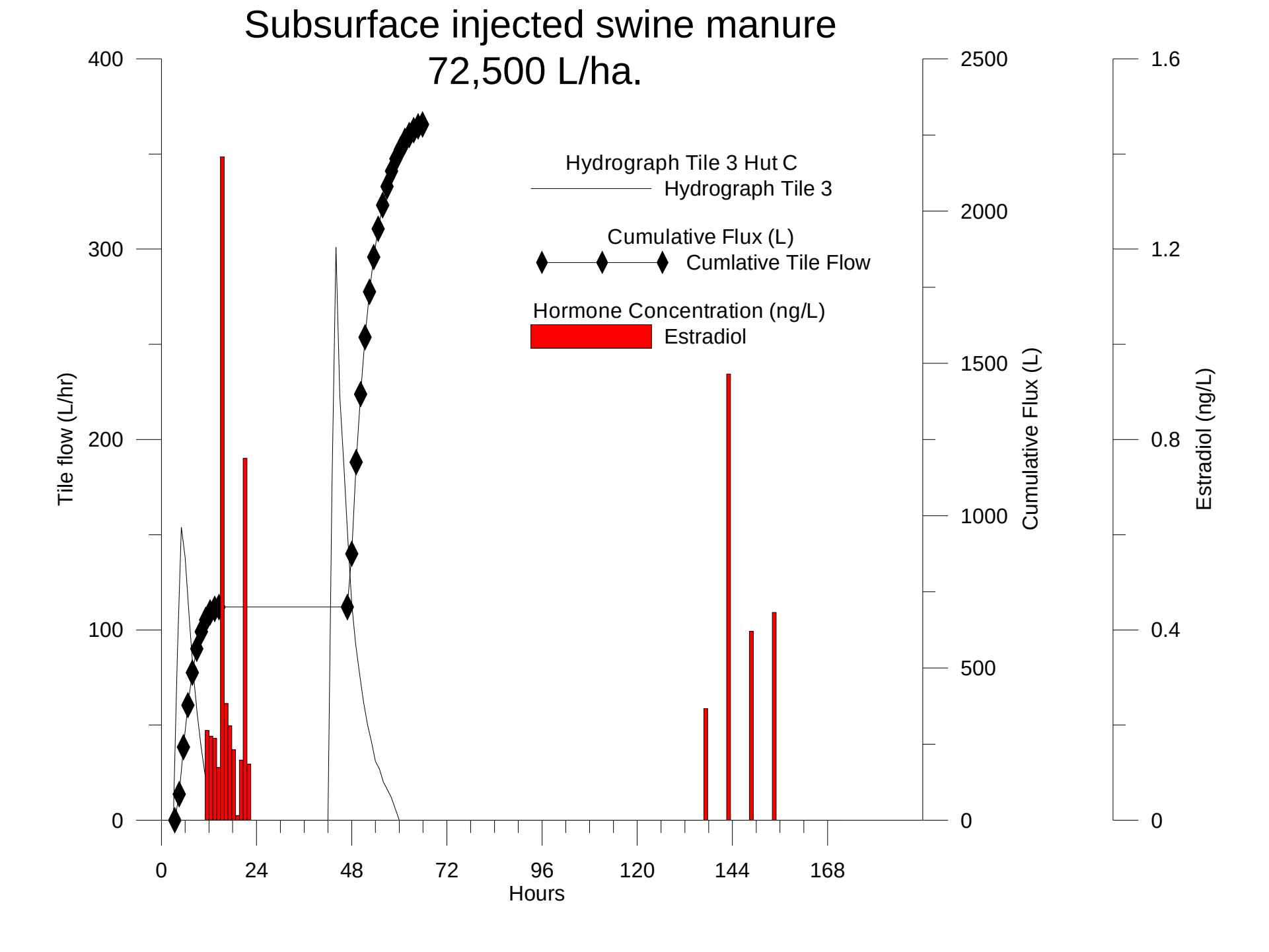
Fall Manure

Spring Manure

Inorganic N

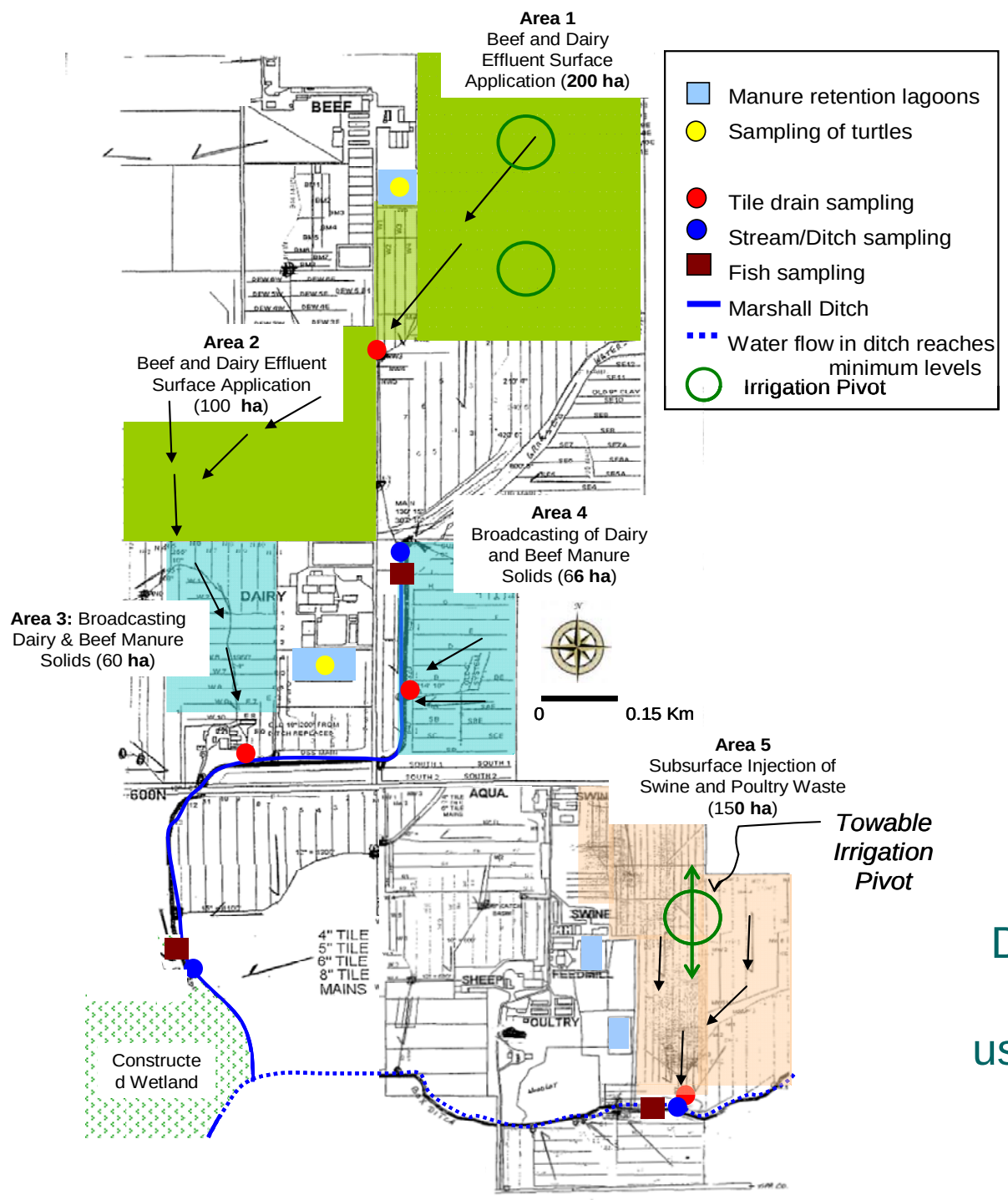



ISCO Sleeves transported from field to lab
Log in ISCO Sleeves
Record weight of each 500-mL sample bottle
Prepare ~1-L Composite in Amber bottle (2 ISCO Bottles)
Label: Sampling Station Location-Date-Time-Bottle Positions
Process immediately for hormones or store at 4°C for no longer than 48 h
50-mL aliquot, filter (0.45μm) into 60-mL plastic bottle
Solid phase extraction
Measure pH & EC
Solvent Elution & Evaporate
Acidify (H_2SO_4) or store frozen
Derivatize for GC/MS or LC/MS
GC/MS or LC/MS
Analyze dissolved organic carbon
Analyze N & P


Subsurface injected swine manure
72,500 L/ha.
Hydrograph Tile 3 Hut C
Hydrograph Tile 3
Cumulative Flux (L)
Cumlative Tile Flow
Hormone Concentration (ng/L)
Estradiol
Tile flow (L/hr)
Cumulative Flux (L)
Estradiol (ng/L)
Hours
0
24
48
72
96
120
144
168
0
100
200
300
400
0
500
1000
1500
2000
2500
0
0.4
0.8
1.2
1.6

Animal Science Research & Education Center (ASREC)

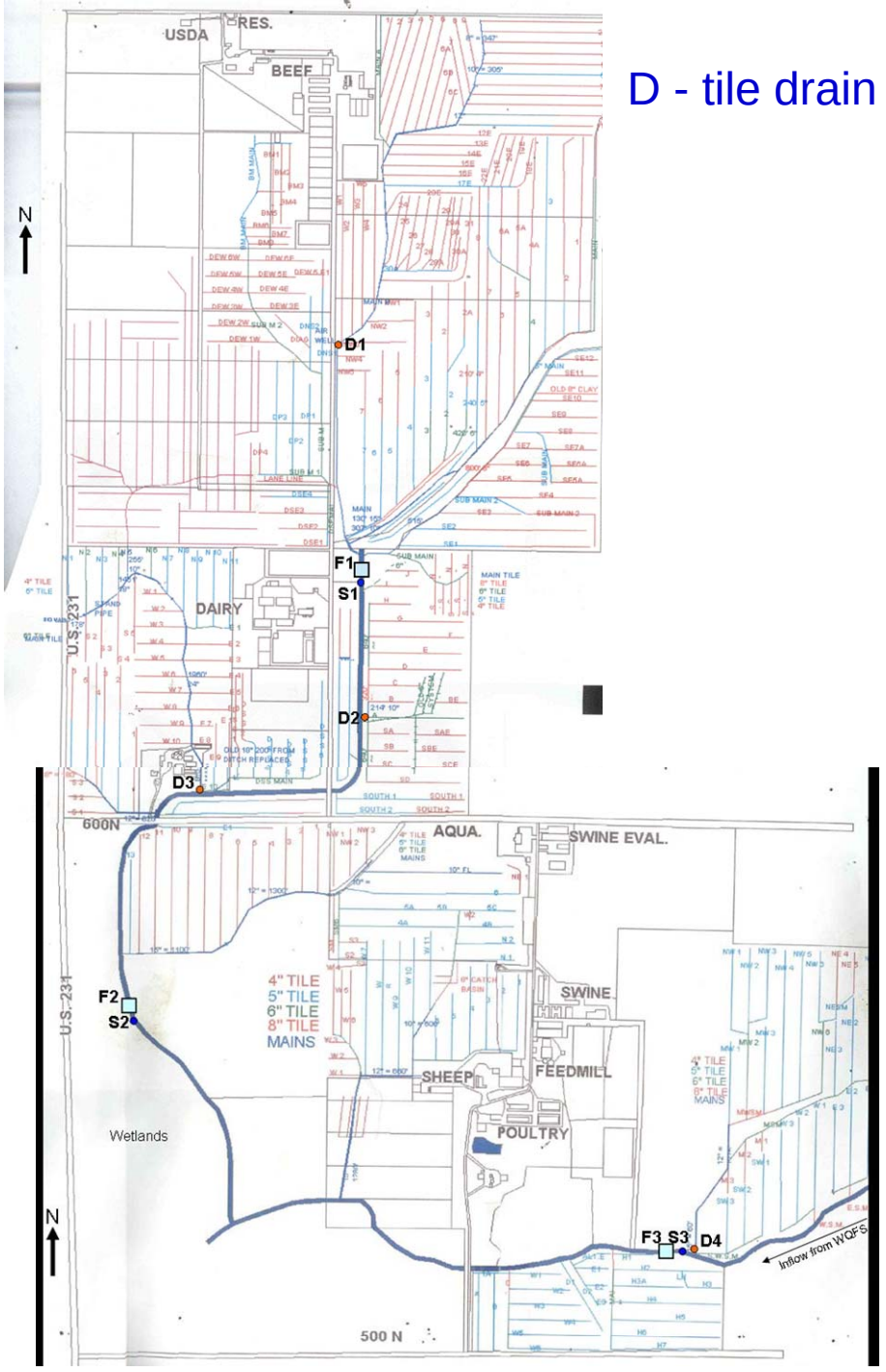
- Several animal species are housed, fed, and bred at the farm, each in their own designated unit.
- Various effluent and manure management application methods are practiced on several hundred acres of tile-drained cropland.
- Type, timing, location and amount of waste applied well documented.
- Beef wastes washed from manure pits into two sequential lagoons.
- Dairy wastes washed into an above ground separator followed by an above ground storage facility then into two sequential waste lagoons.
- Each dairy and beef lagoon has a pumpable or spreadable capacity of ~ 3,000,000 gallons.
- Swine and poultry manure pits are washed into three sequential waste lagoons each that have capacities from 700,000 to 5,500,000 galloons.



Animal wastes are land-applied through:

- lagoon effluent irrigation via fixed or towable pivots
- broadcasting of dairy and beef solids or spreading of liquid manure slurries
- subsurface injection of primarily swine manure from the manure pits

Decisions as to which fields receive animal wastes are usually controlled by N needs of the crops being grown.

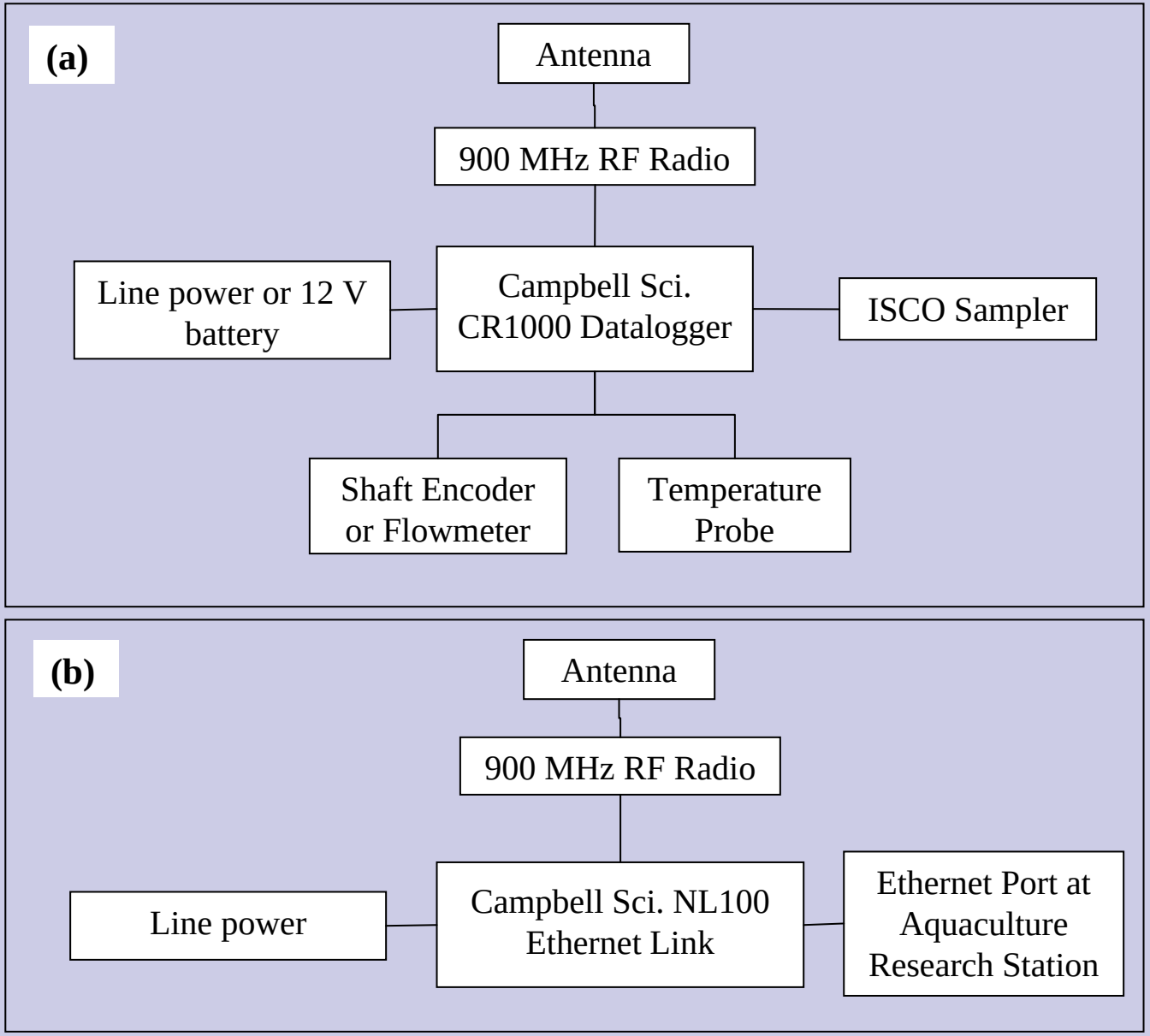


D - tile drain sampling; S - stream sampling; F - fish studies

ID	Comments
D1	Drainage from beef and dairy effluent (Area 1) application to corn and soybean fields
D2	Drainage from beef and dairy broadcasting (Area 4) application to primarily corn and some soybean fields
D3	Drainage from beef and dairy effluent (Area 2) and solids broadcasting (Area 3) application to primarily corn and some soybean fields
D4	Drainage from subsurface injection of swine manure (Area 5) & some surface application of poultry lagoon effluent to corn fields
S1	Co-located with F1
S2	Will capture Over-land Flow during heavy rain event
S3	Will capture Over-land Flow during heavy rain event

(A)Tile discharge into stream/ditch
(B) Sampling & Field Fish Studies hut
(C) Submerged V-Notch weir

(flow monitored with Marsh McBirney 3000 Flo-tote Electromagnetic Level/Velocity Sensor; circular dam approach in tile drains)



Schematic of (a) basic instruments to be installed at each site, and (b) hardware to be installed at the Aquaculture Research Station.

Objective 2

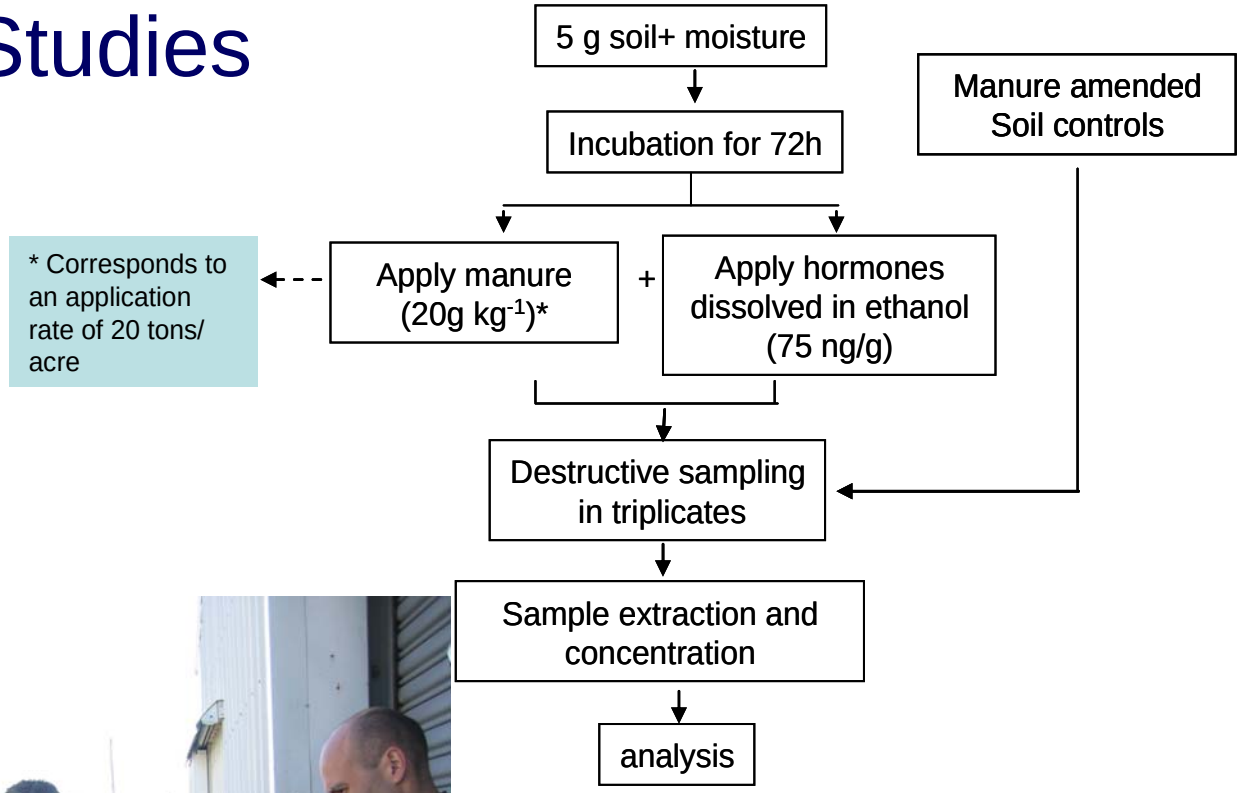
Assess hormone persistence in fields under different manure and lagoon effluent application practices. **We hypothesize that hormone degradation in manure- or effluent-amended soils will be rapid compared to persistence in typical anaerobic storage conditions (e.g., manure pit, lagoon), but may be measurably slower when subsurface injected compared to surface applied.**

Field Soil Studies

- Sample field soils at the WQFS before, after, and one additional time for hormone analysis (12 core composites in N and S ends of each plot outside of drainage area)
- Selected soil and stream sediment sampling at the ASREC
- Selected degradation studies quantifying the effect of temperature, moisture content, and pesticides
- Quantifying hormones in manure and lagoons for 3 months after beef implanting (TBA & estradiol combo)

Supporting Lab Studies

- Degradation studies quantifying the effect T, moisture, and pesticides
- Quantifying hormones in manure & lagoons for 3 mo after beef implanting (TBA & estradiol combo)



Objective 3

Evaluate the impacts of these hormone loads (relevant levels and mixtures) on aquatic organisms. **We propose that under certain management and environmental conditions, mixtures of synthetic and natural hormones discharged by CAFOs can be sufficient and persistent enough in aquatic systems to induce irreversible gonadal changes in sensitive life-stages of fish and other aquatic organisms that will persist in adult stages resulting in altered reproduction and population-level effects.**

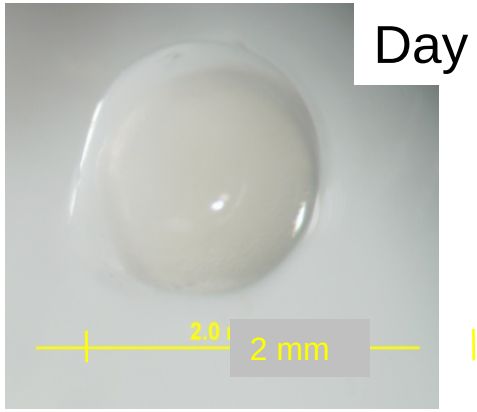
Rationale

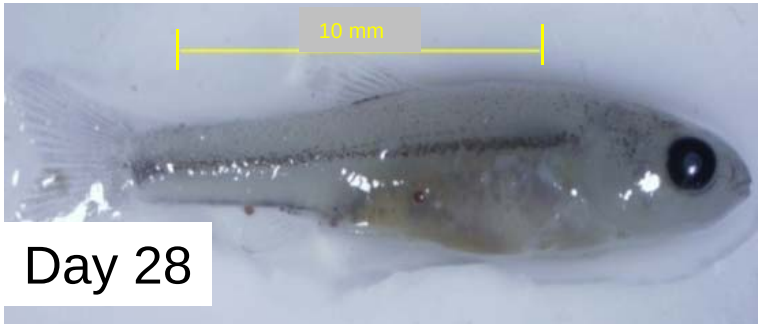
- Animal wastes generated from CAFOs may be significant sources of compounds with endocrine activities
- Chemicals of concern include:
  - Natural sex steroids
  - Natural and synthetic steroids used to promote growth (testosterone, trenbolone acetate, estradiol, zeranol, progesterone, and melengestrol acetate)
  - Phytoestrogens (formononetin, daidzein, and equol)
- However, very little is known about the environmental impact of discharges released from CAFOs

Laboratory Studies (Years 1 – 3)

We will evaluate developmental and reproductive effects on early-life stages of fathead minnows after exposures to:

- Natural sex steroids (estrogens and androgens)
- Synthetic and non-aromatizable androgen (17ß-trenbolone)
- Plant derived weak estrogens (zeranol and equol)





- **Gonad histology (sex differentiation)**
- **qPCR and microarrays (Gene expression)**

Field Studies (Years 1 – 3)

Fish will be collected using backpack electrofishing a minimum of four times per year during the spring and summer, to cover most of the spawning and reproductive season

- Sites:
  - Several points downstream from CAFO
  - Agricultural ditch with no CAFO impact
  - Control site (forested site, no agricultural impact)
- Endpoints will be measured at different levels of biological organization, including individual, population, and community responses

Field Studies (Years 1 – 3)

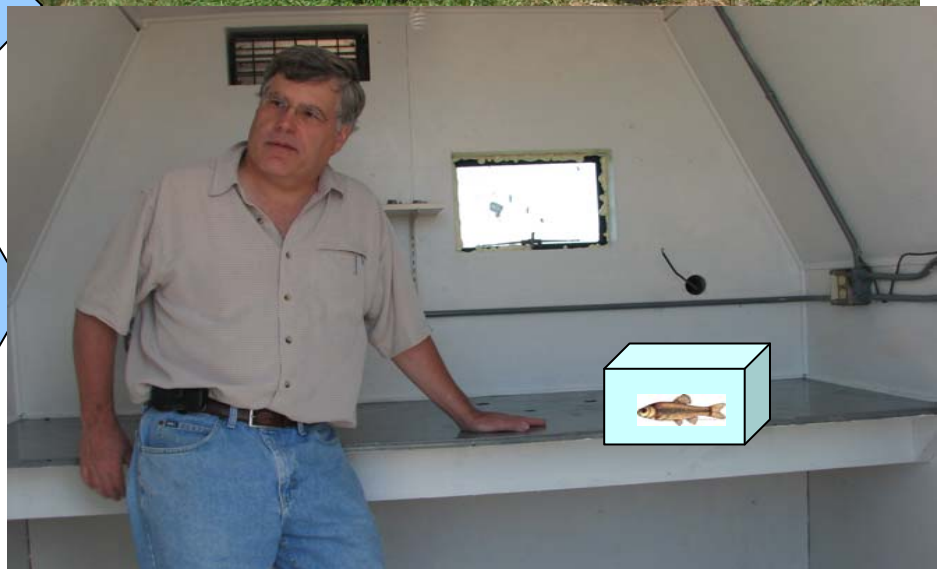
Fish sampling at Martell Forest, control Site

“In situ” Ditch Studies (Years 2 – 3)

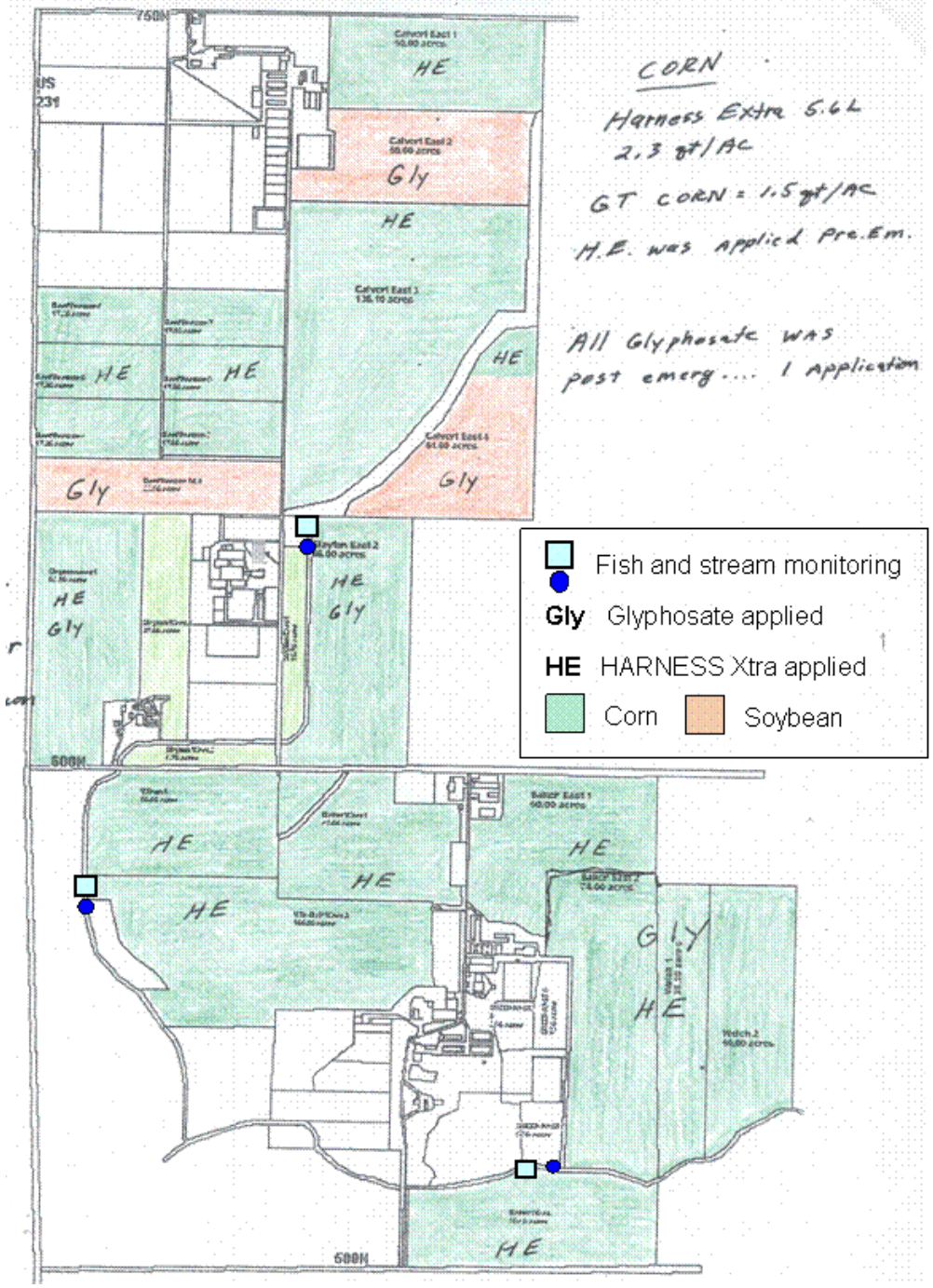
- Early-life stages of fathead minnows will be exposed to ditch (CAFO) waters on a flow-trough exposure system built on site and positioned at the edge of the ditch
- For controls, we will expose fry in clean water at the Baker Aquaculture Facility
- At the end of these exposures, we will collect fry and evaluate the same endpoints (gonad histology and gene expression) as already described

Fish Studies

Well with submersed pump that moves ditch water into the shed.



Inside of shed showing shelve where 10 glass aquaria will be positioned to receive ditch water.



What about presence of other chemicals, besides steroids associated, with CAFOs?

- Herbicides (Atrazine and glyphosate)
- Nitrate

Purdue Project Team

- Linda S. Lee (PI)
- Marisol Sepulveda (Co-PI, Aquatic effects)
- Chad Jafvert (Flow Measurements & Sampling Station Design)
- Byron Jenkinson (Sampling Station Design & Retrieval)
- Steve Sassman (Analytical Chemist, hormones)
- Judy Lindell (N, P)
- Sylvie Brouder (WQFS Support)
- Ron Turco (Soil Microbiologist)
- Suresh Rao & Eileen Kladivko (Modeling Support)
- ASREC & AGRY Farm Staff
- Graduate Students:

  Bushra Khan (AGRY), Nadia Carmosini (AGRY), Heather Preisendanz (CIVIL), Yong Sang Kim (Civil), TBA (FNR)
- Undergraduates