



AGRICULTURE & D C S

Nancy Shappell

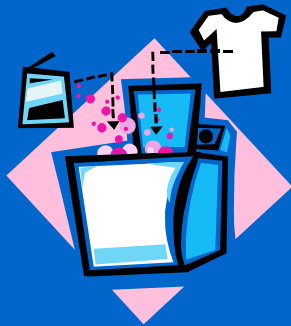
Detection of EDCs / Pharmaceuticals in Water

Kolpin et al, 2002



Detection limits as low as parts per trillion

Sources sometimes presumed



Biological Activity or Consequences
of concentrations detected ????

Infertility

Sex Reversal

Lethality

Short term effects on individuals,

Or effects on species survival, multigenerational.

For each compound, need No Observable Effect Level (NOEL)
or Lowest Observable Effect Concentration (LOEC)

Species-dependent

What species do we care about protecting?

Shortcuts – tools to monitor environment

Require development / validation / implementation

EDCs with estrogenic activity star players.



Natural EDCs from Agricultural Practices

1st : Establish Concentrations Produced

Will vary depending on : species
“state” of animal
rearing conditions
treatment of wastes
climate / temperature

Hurdles in quest for answers:

- Access to farms
- Limited research dollars
- Valid method development, expensive instrumentation
- Push for “Flashy” research – ie microarray
- Urgency detours generational studies
- Subtlety of effects, thalidomide versus lead



BUT, ARS has been working!



Investigations include:

Establishment of “basal” environment estrogenic activity

Soil studies – estrogen and testosterone retention and degradation

Effects of composting – laboratory and field studies

Composition and degradation in lagoons

Amelioration by wetlands

Destruction of estrogens by “green catalysts”

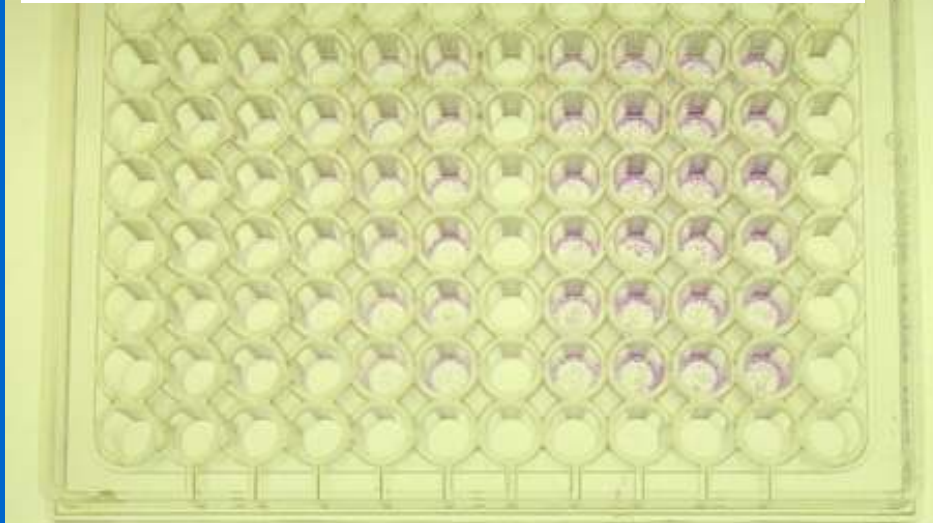




$E_2(M) 1^{-13}$

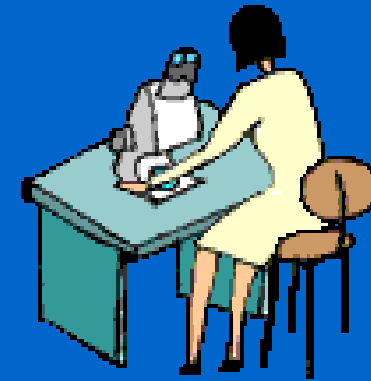
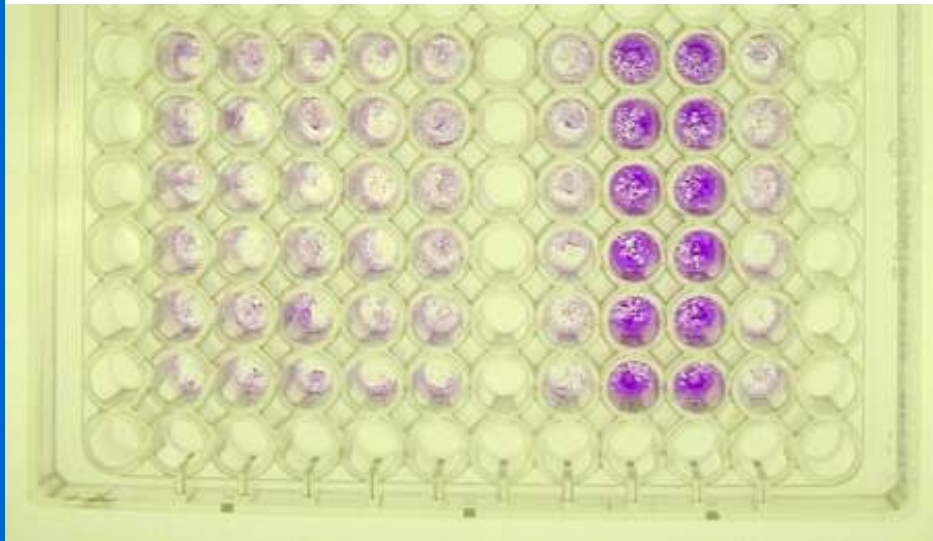
C

1^{-11}

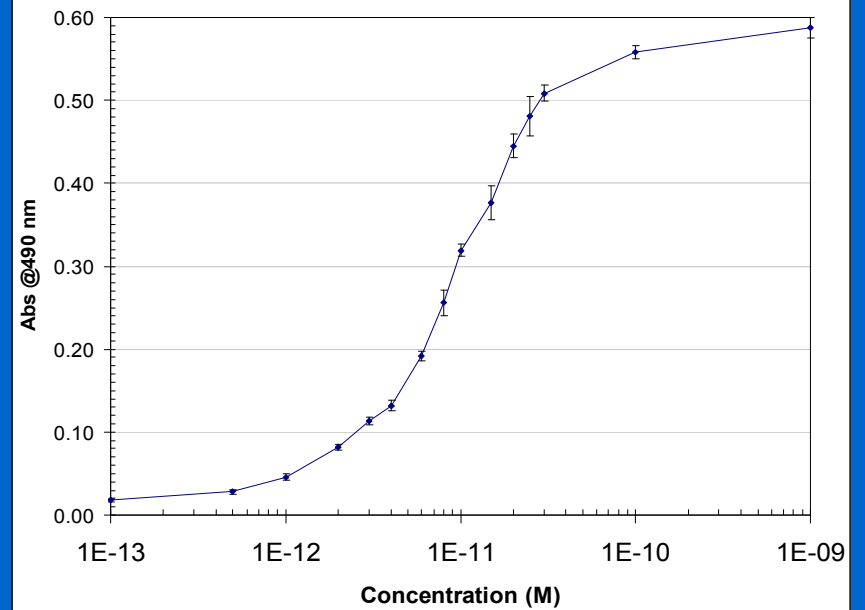


$E_2(M) 1.5^{-11}$

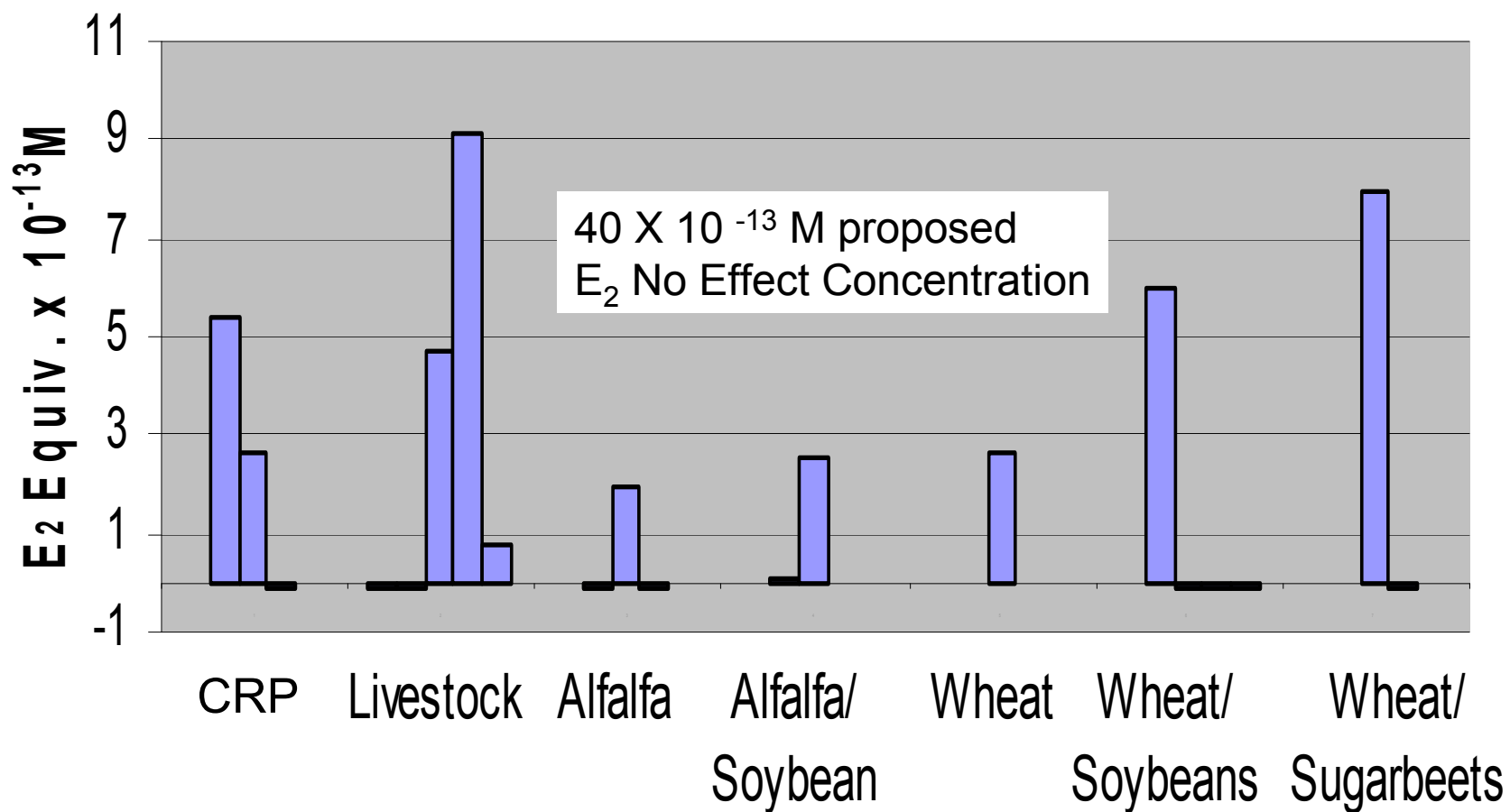
C 1^{-9} FBS 1.5^{-11}



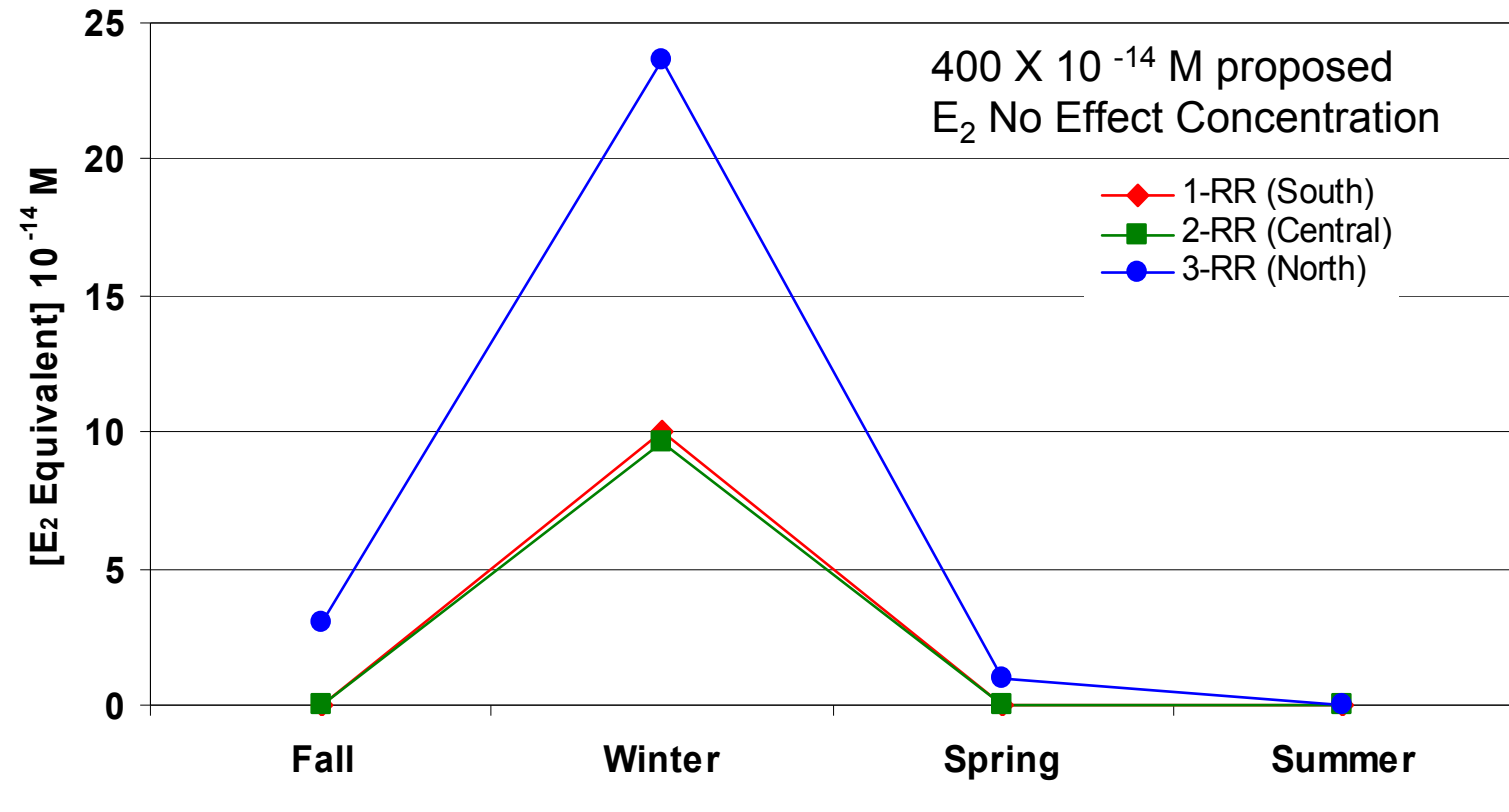
Estradiol Standard Curve



Estrogenic Activity of Wetlands by Land Use



Red River Seasonal Estrogenic Activity

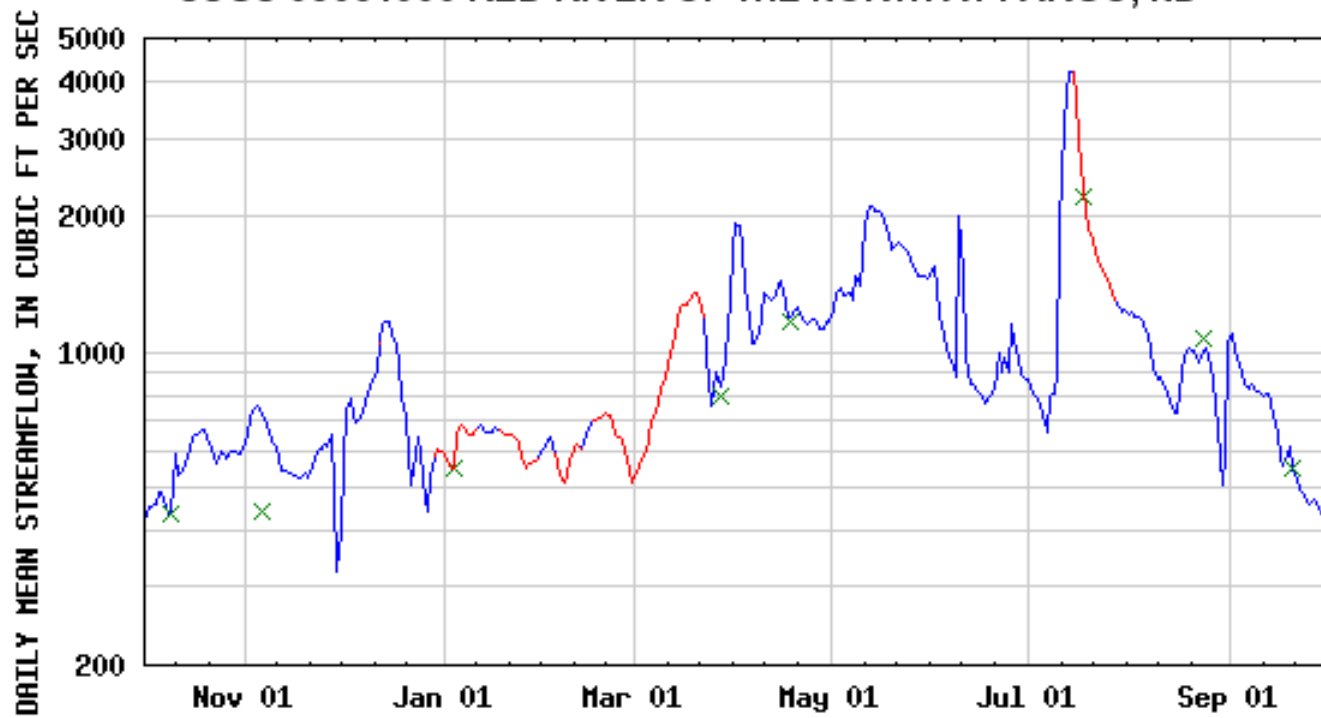


Wastewater effluent: ~12 – 32 times the peak winter value

E₂ women's blood: ~400 – 3200 times the peak winter value



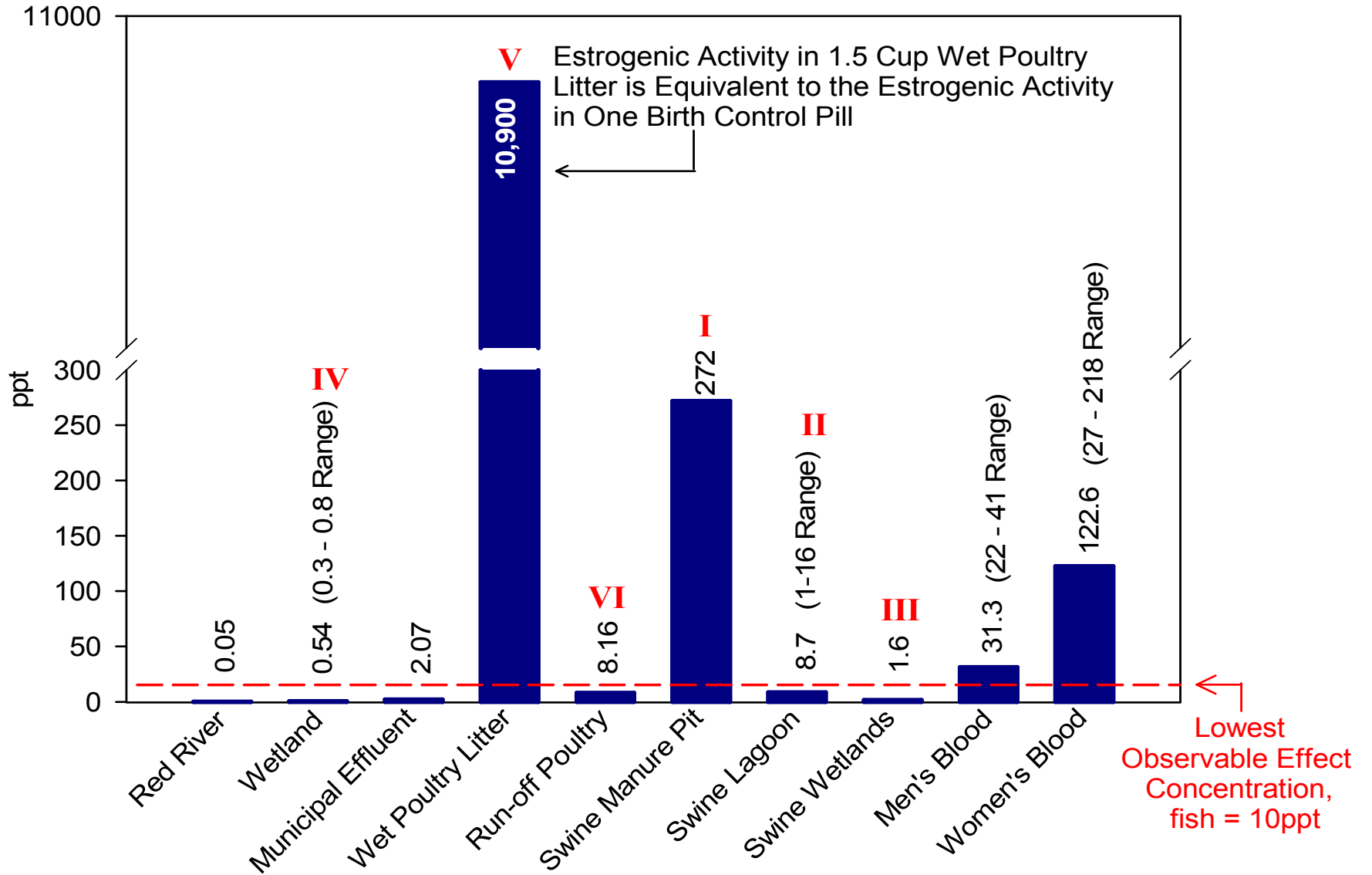
USGS 05054000 RED RIVER OF THE NORTH AT FARGO, ND



EXPLANATION

— DAILY MEAN STREAMFLOW × MEASURED STREAMFLOW — ESTIMATED STREAMFLOW

Estrogenic Activity (E-Screen)



Swine Farrowing Facility, North Carolina A & T University



**Storage Pond(lft)
Lagoon 1 (Rt)
Lagoon 2 (bhd 1)**



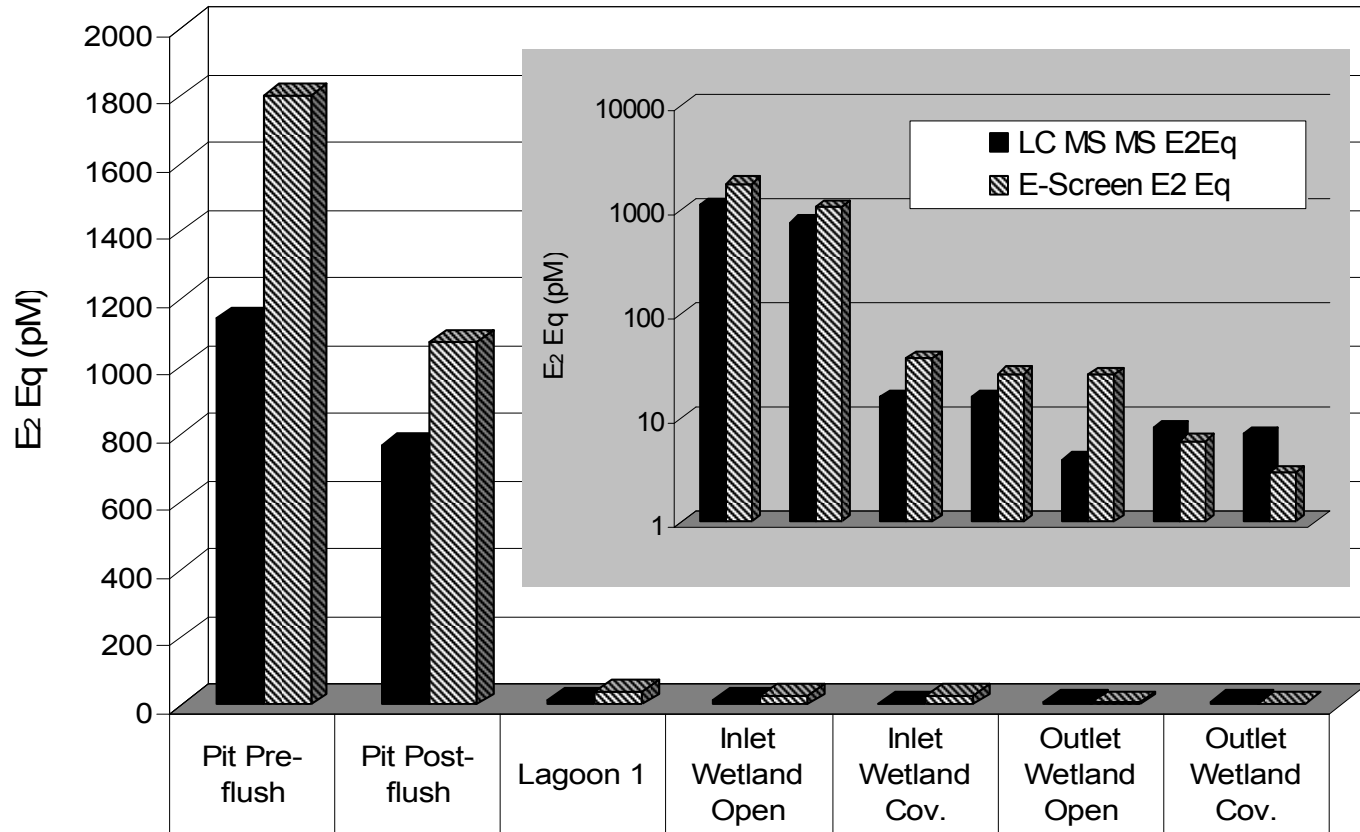
**Covered Wetland 3 and 4
looking towards storage pond**

E-Screen Evaluation of 2004 Samples
(pM Estradiol Equivalents)

Component	4/19/04	7/12/04	11/23/04
Manure Pit	843	858	NA
Lagoon 1	6.4	11.5	147
Lagoon 2	3.0	3.7	0.1
Storage Pond	2.9	0.6	2.1
Inlet Wetland Open	3.1	1.4	92.1
Inlet Wetland Covered	2.4	2.2	104.1
Outlet Wetland Open	1.8	2.8	7.8
Outlet Wetland Covered	3.6	9.2	5.5

Proposed No Effect Concentration 4 pM for E₂

July '05 : E₂ Equivalents by E-Screen vs LC MS-MS



	Pit Pre-flush	Pit Post-flush	Lagoon 1	Inlet Wetland Open	Inlet Wetland Cov.	Outlet Wetland Open	Outlet Wetland Cov.
■ LC MS MS E ₂ Eq	1141	765	16	16	4	8	7
▨ E-Screen E ₂ Eq	1802	1072	38	27	26	6	3

Findings:

Estrogenic activity of swine wastewater was greatly reduced by passage through a sequential lagoon/constructed wetland system.

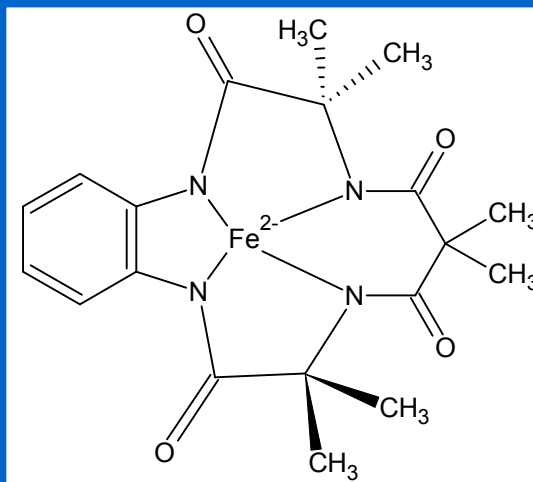
TKN was reduced by the wetland system -- but to a lesser extent than estrogenic activity (60-75 % vs 83-93%).

There was good agreement between E₂ Equivalents obtained by E-Screen and LC MS-MS.

Estrone was identified as the most persistent estrogenic compound.

Estradiol equivalents released from the wetlands were always below the Lowest Observed Effect Concentration for estradiol, and typically below the proposed No Observed Effect Concentration.

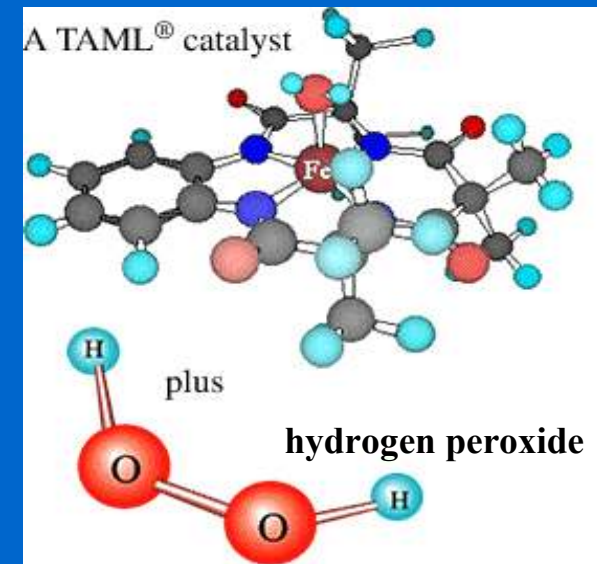
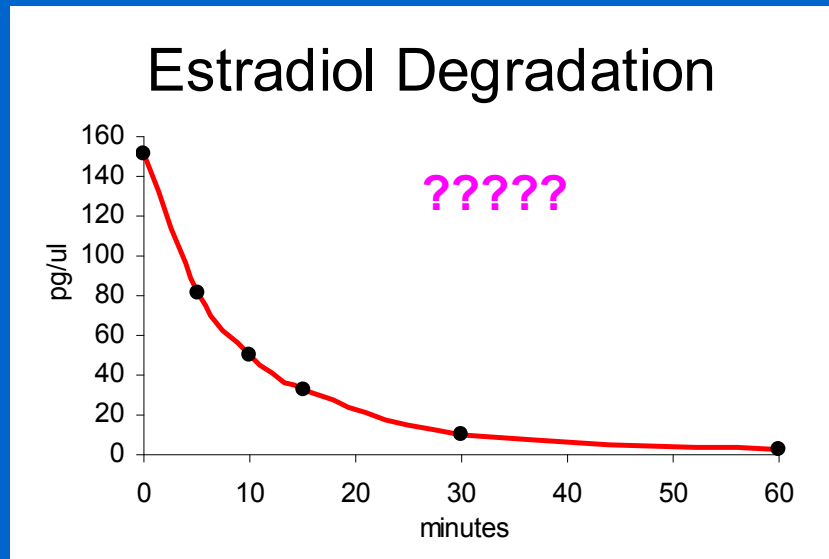
TAML® – Tetra-amido-macrocyclic ligand



Developed by scientists at Carnegie Mellon Institute

“Green Oxidant” used in conjunction with H_2O_2
for the destruction of dyes, bacterial spores, ...?

Lagoon Waste Management System



Natural Estrogenic Substrates:

Estrone

Estradiol (alpha and beta)

Estriol

were ~ 95% destroyed in 15 minutes

Synthetic Estrogenic Substrate:

Ethinyl Estradiol (birth control pill)

was ~95% destroyed in 10 minutes

Findings

TAML catalyzed the degradation of all estrogenic compounds assessed

Testosterone degradation required 1000 Xs higher TAML concentrations.

Collaborators: Horwitz, Harrington, Billey, Collins, Ro, Hunt,

Soil Fate & Transport

Hakk, Larsen, & Casey

Laboratory studies

1. Sorption- Batch Studies
2. Mobility – Miscible Displacement
3. Transformation - incubation studies with ^{14}C radiolabeled hormones

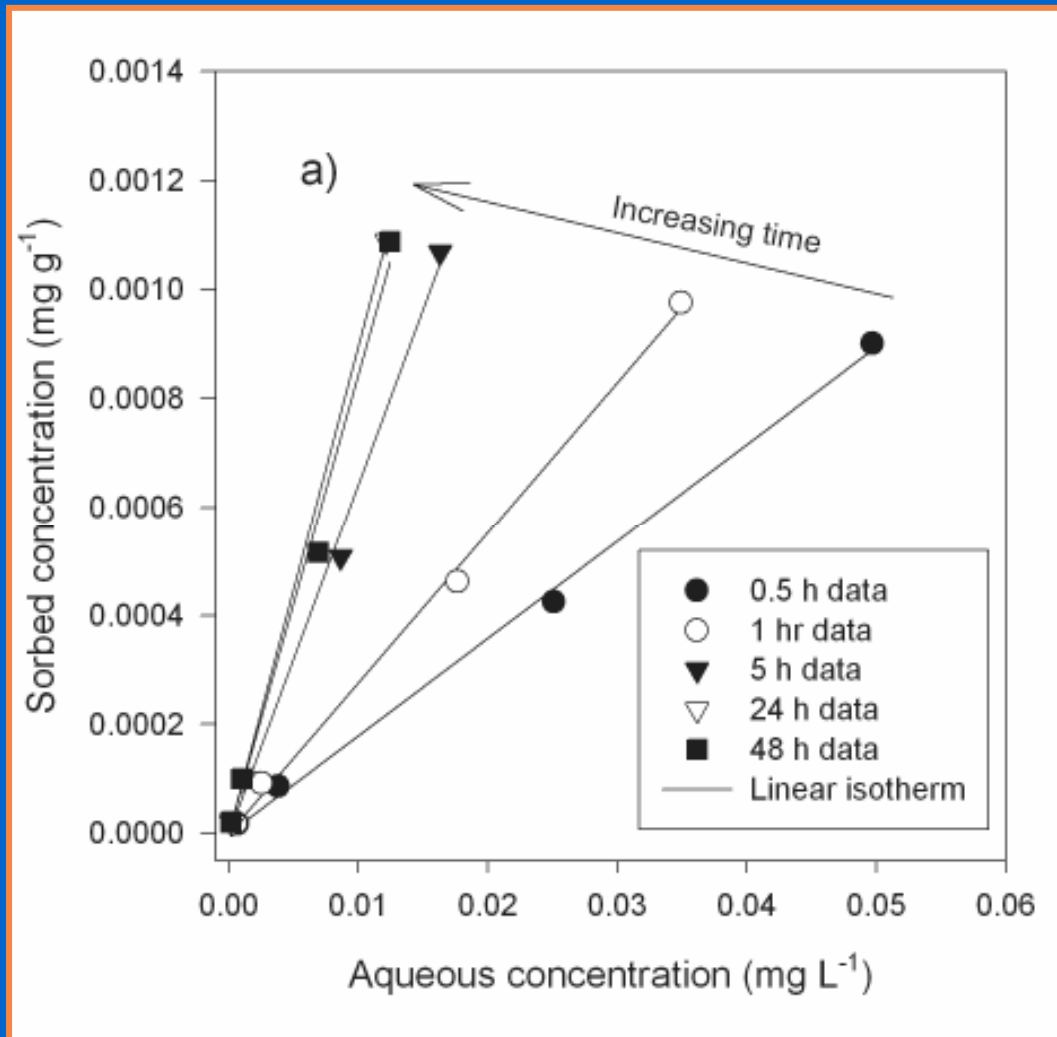
Batch Studies



Measure aqueous concentrations @
0.5, 1, 48, 72, 168h



Batch Results for Estradiol

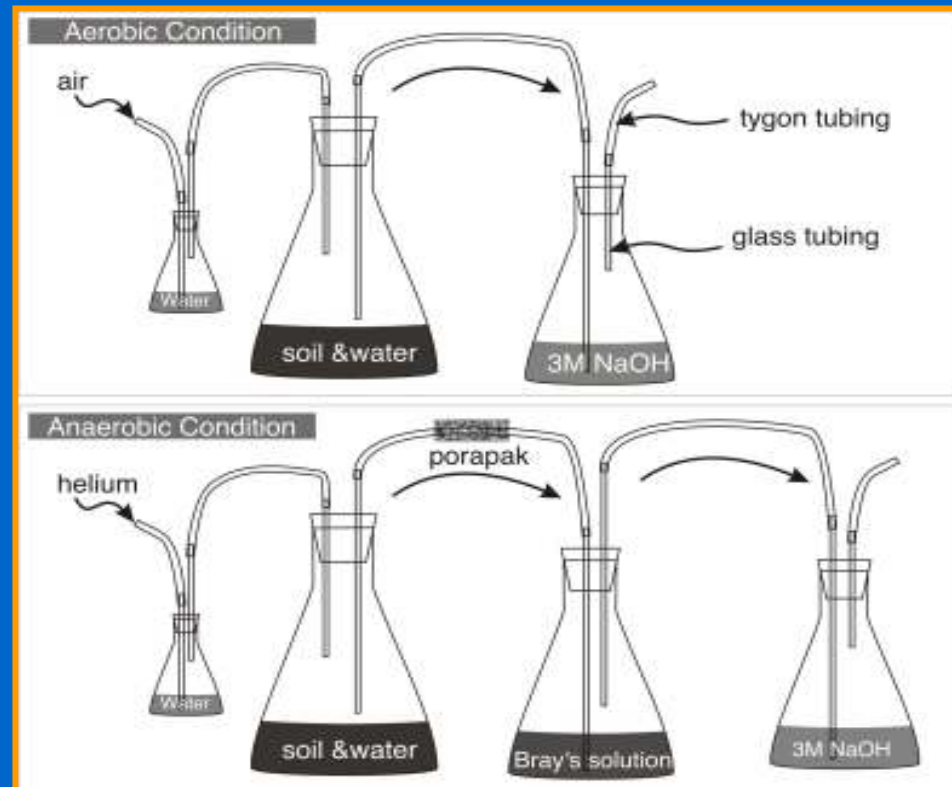


Findings:

Positive correlation between Absorption capacity and Surface area, cation exchange capacity, organic content

Incubation Studies – E2 and T with natural and sterile soils

Aerobic vs Anerobic

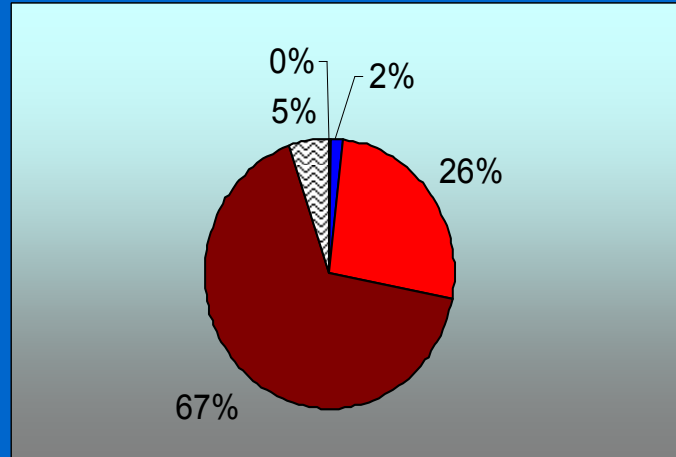
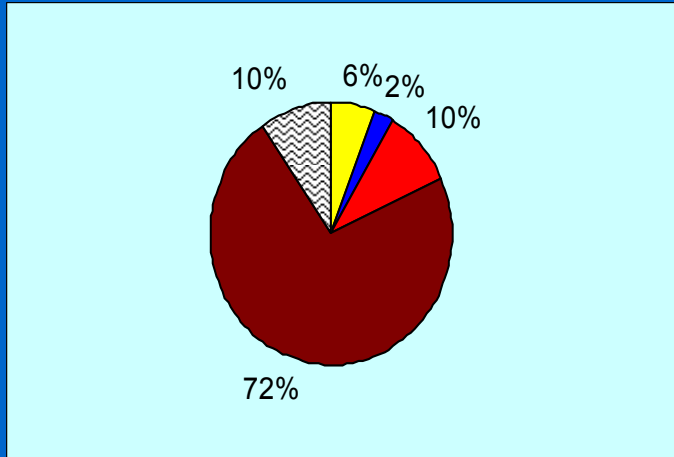


E₂ Soil Incubations

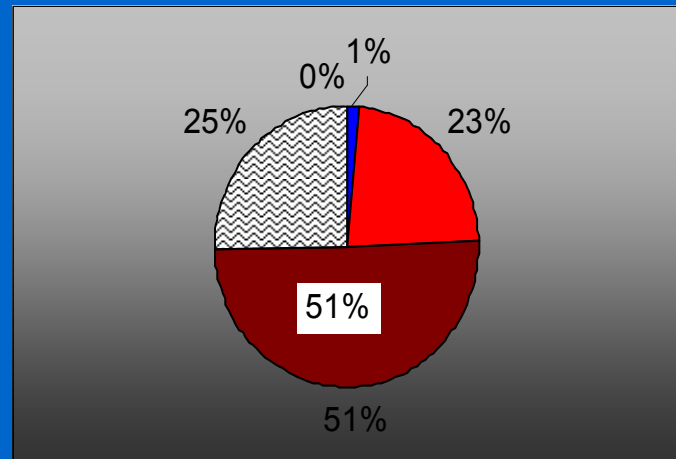
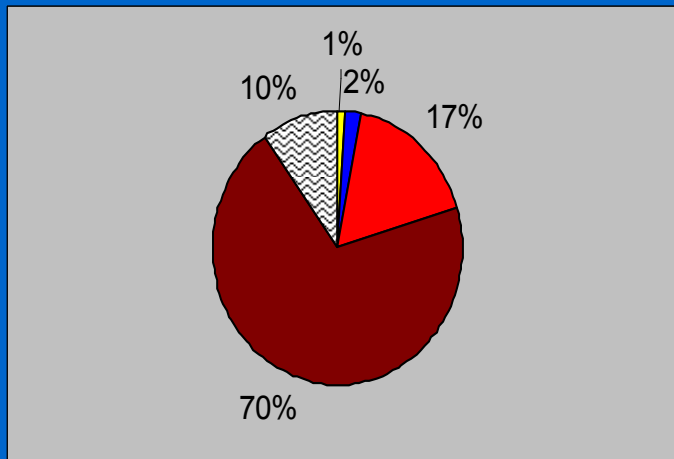
Living

Sterile

Aerobic



Anaerobic



■ ¹⁴CO₂
 ■ Water Soluble
 ■ Acetone Soluble
 ■ Non-Extractable
 Missing

Environmental Risk
Potentially Water soluble
Bound to soil
Water insoluble

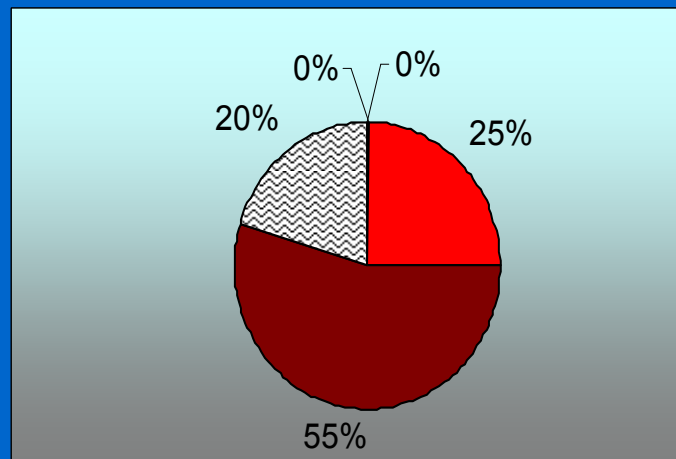
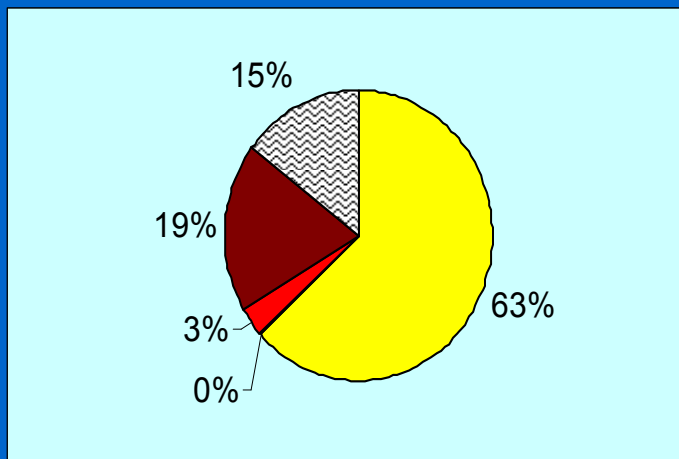
Hakk et al.

T Soil Incubations

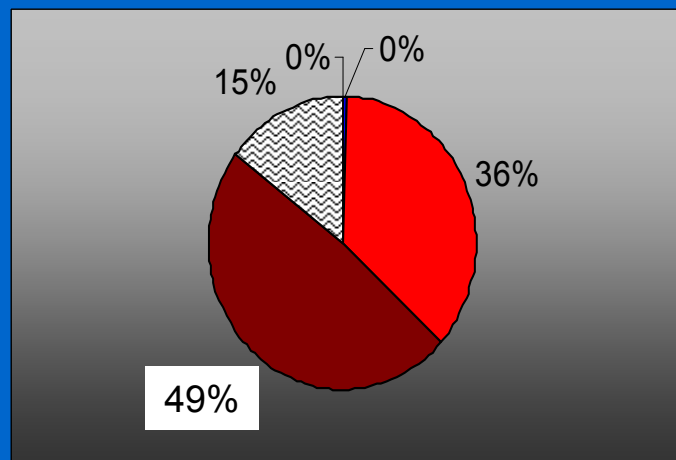
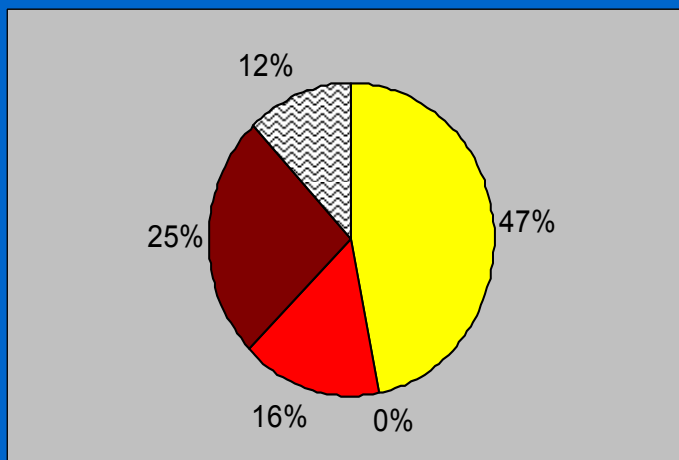
Living

Sterile

Aerobic



Anaerobic



■ $^{14}\text{CO}_2$
■ Water Soluble Environmental Risk
 ■ Acetone Soluble Potentially Water Soluble
 ■ Non-Extractable Bound to soil Water insoluble
 Missing

Hakk, et al.



Raw manure

Work in Progress
Hakk et al.



Lagoon



Compost/silage

**Animal Waste Application
And Ground Water Quality
(E₂ and T)**



We will have answers to all EDC issues when pigs can fly!