Monitoring of Selected Herbicides, Antibiotics, Steroids, and Industrial Chemicals in Water by ELISA

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Organic Water Contaminants

• Greater Than 90,000 compounds in commerce
• Some manufactured and used in large quantities
• Examples: agrochemicals, pharmaceuticals, surfactants, flame retardants, personal care products, commercial and industrial products,
• Usage has increased over the years
• Enter water sources by a variety of environmental pathways
TARGETED COMPOUNDS

- **Herbicides**
  - Acetochlor
  - Atrazine

- **Steroid Hormones**
  - Estradiol (E2)
  - Ethenyl Estradiol (EE2)
  - Estrone (E1)

- **Antibiotics**
  - Sulfamethazine

- **Surfactants**
  - Alkylphenols
  - Alkylphenol ethoxylates
  - Alkyl ethoxylates

- **Industrial Chemicals**
  - Bisphenol A
Description of Water Samples

DWT Facility-NJ
- Urban drainage basin serving ~ 850,000 people
- >50 STPs discharge effluent to 2 streams from which DWT withdraws raw water
- Conventional treatment:
  1) Screening
  2) Addition of powdered AC
  3) Addition of acid/base, pH
  4) Coagulation
  5) Flocculation
  6) Sedimentation
  7) Primary Desinfection
  8) Filtration
  9) Secondary Desinfection
  10) Add caustic soda, pH

Sampling Methods
- Twelve water samples collected
- Over a 3 week period (July-Aug ’03)
- Six locations in the facility
  1) Raw
  2) Raw and recycled
  3) Settled
  4) Disinfected
  5) Filtered
  6) Finished
Purpose of the Project

To determine variation of pharmaceuticals, antibiotics, household chemicals, hormones, and industrial waste products going in and coming out of the plan during low and high flow in the wet and dry season.
ANALYTICAL METHODS

• Very sensitive methods required sensitivities down to ng/ml, pg/mL
  - HPLC
  - LC-MS
  - GC-MS
  - Immunoassay (ELISA)
An immunoassay is an analytical method which uses antibodies as reagents to quantitate specific analytes.
Immunoassay

- $6 Billion Industry Worldwide
- 2.5 Billion Tests Sold Annually
- Highly Quantitative
- Regulatory Approved
- Flexible Test Formats
- Diverse Markets and Applications
Immunoassay Markets

- Clinical
- Agricultural
- Environmental
- Food
- Industrial
- Pharmaceutical
- Veterinary
- Water Quality
Competitive ELISA

Assay Conjugate

Target Analyte

Capture Antibody

Detector and Analyte compete to bind with antibody
Competitive ELISA

I. No analyte - high detection signal

II. Analyte present - detection signal reduced
Competitive ELISA Curve

Competitive Immunoassay Data

Percent of Maximum Absorbance

Concentration of Analyte

0.1 1 10 100 1000
1. Separate the rack. Add 200 or 250 µL of either Standards, Control or Samples to the bottom of each test tube. **NOTE:** Same chosen volume should be added for standards and samples.

2. Add 250 µL of Atrazine Enzyme Conjugate to each test tube.

3. Add 500 µL of Mixed Magnetic Particles to each test tube. Vortex.

4. Incubate for 15 minutes.

5. Combine the rack and magnetic base. Seat all tubes. Wait 2 minutes.

6. Invert the combined rack. Blot gently.

7. Add 1 mL of Washing Solution. Wait 2 minutes. Invert the combined rack. Blot gently. Repeat this step.

8. Separate the rack. Add 500 µL of Color Reagent to each test tube. Vortex.


10. Add 500 µL of Stopping Solution to each test tube. Read OD 450.
**SPE Procedure, Hormones**

### C18 SPE Cleanup & Enrichment
- <Sample Loading> Filtered sample: 1L (up to 20ml/min)
- <Washing> Distilled water: 5ml (up to 20ml/min)
- <Drying> Vacuuming: 1minute
- <Washing> Hexane: 5ml (up to 20ml/min)
- <Elution> Dichloromethane: 5mL (up to 3ml/min)

### Aminopropyl SPE Separation & Enrichment
- <Sample Loading> 1ml (up to 3ml/minute)
  - *Salvage the filtrate, ca. 1ml.
- <Elution> methanol: 5mL (up to 3ml/minute)
  - *Add the eluate, ca. 5mL, to the filtrate.
- Methanol solution (ca. 6ml, unretained E1 + eluted E1)

### Evaporation
- 10% methanol

### ELISA Sample Preparation
- 1mL aqueous solution (10% methanol)
  1) Residue in 100% methanol (100μl) under mixing
  2) Distilled water (900μl) without stirring
Herbicide Analysis by ELISA, NJ Samples

Atrazine Analysis by ELISA

Acetochlor Analysis by ELISA
Sulfamethazine Analysis by ELISA, NJ Samples

Sulfamethazine Analysis by ELISA

Sample date

Concentration (ppb)

07/22/03 07/27/03 08/01/03 08/06/03 08/11/03

Finished
Disinfected
Filtered
Settled
R & R
Raw
E1, E2, EE2 Analysis by ELISA, FL Samples

Florida & Miscellaneous Sample Analysis by Hormone ELISA

- Estrone
- EE2
- E2

Sample ID

Concentration (ppt)
## E2 ELISA vs. GC, CO Samples

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Estradiol Microtiter Plate ELISA
Atrazine Magnetic Particle Reagents
Conclusion

- ELISA Technologies, can be used to screen large number of samples timely and cost-effectively.
- Can be use for screening and for quantitation of analytes in water monitoring programs and to efficiently monitor the various stages of treatment at DWT facilities.
- Additional work is currently being performed with surfactants, bisphenol A, as well as comparison to GC values.
Acknowledgements

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Disclaimer: Mention of a particular product during this presentation does not constitute an endorsement by the USGS.