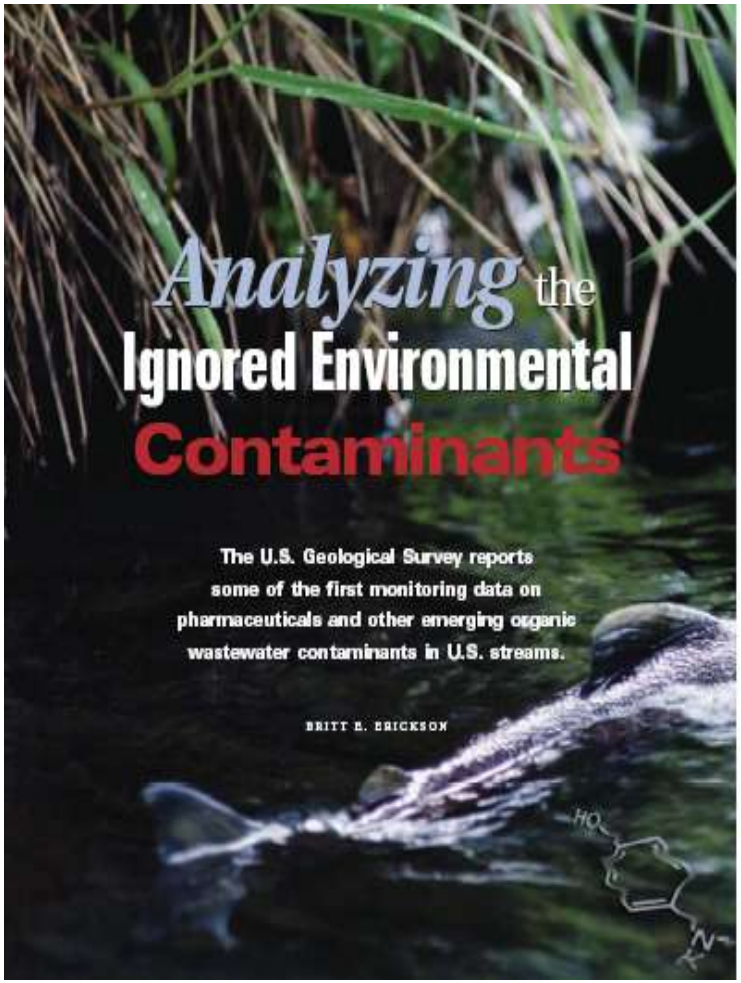
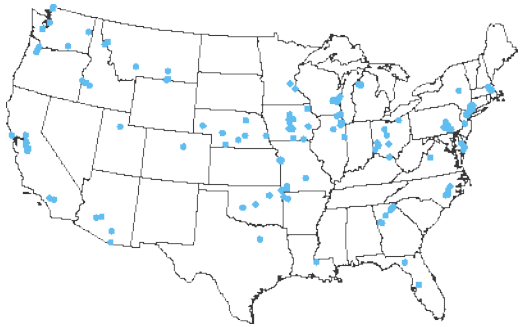
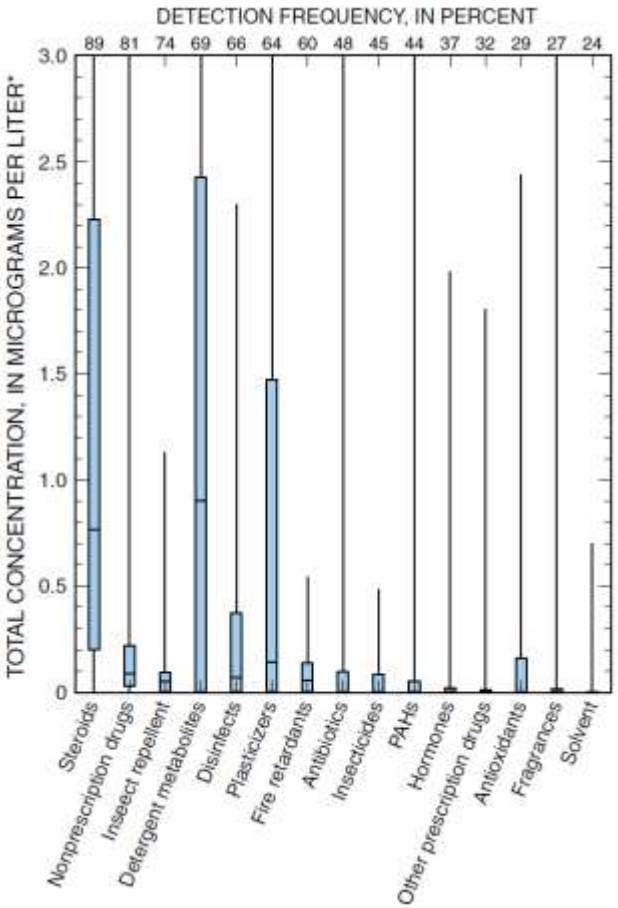
Water Center/Water Sciences Laboratory
School of Natural Resources

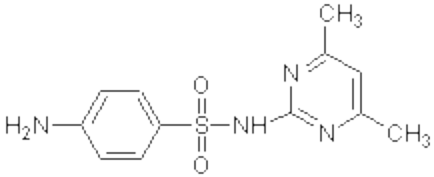
PHARMACEUTICALS AND STEROID HORMONES IN SOILS, SLUDGE AND WATER

DANIEL D SNOW

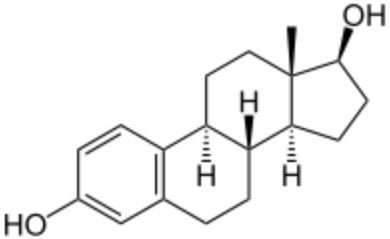








Sulfamethazine



17β-estradiol

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. **Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance.** Environ. Sci. Tech. Mar 15;36(6):1202-11

Wastewater “Micro-contaminants”

- Wastewater typically characterized by solids, oxygen demand, nutrients, bacteria, and metals
- “New” trace level (sub-ppb) contaminants include pharmaceuticals, steroids, personal care products, plasticizers, surfactants, flame retardants, metabolites
- New chemicals of concern - <u>Advances in measuring technology</u>
- Understand occurrence and control risks to water quality

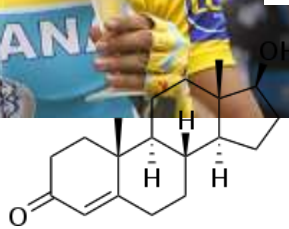
Why are traces of these contaminants a concern?

- Pharmaceuticals or “drugs”
  - Substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease
  - Any substance that, when absorbed into the body of a living organism, alters normal bodily function
- Steroid Hormones

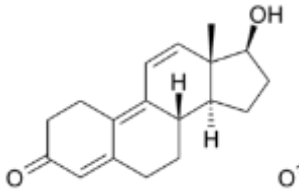
Tour Champ Contador Tests Positive for Steroid

Published September 30, 2010| Associated Press

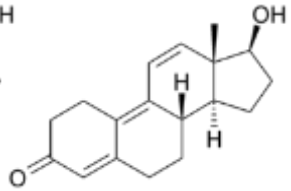
A World Anti-Doping Agency-accredited lab in Cologne, Germany, found a "very small concentration" of the muscle-building and fat-burning drug clenbuterol in the Spanish rider's sample on July 21 at the Tour, according to a statement from the UCI.



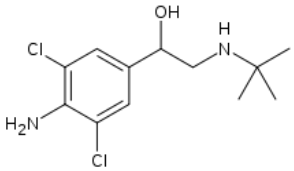
Testosterone



17beta-trenbolone

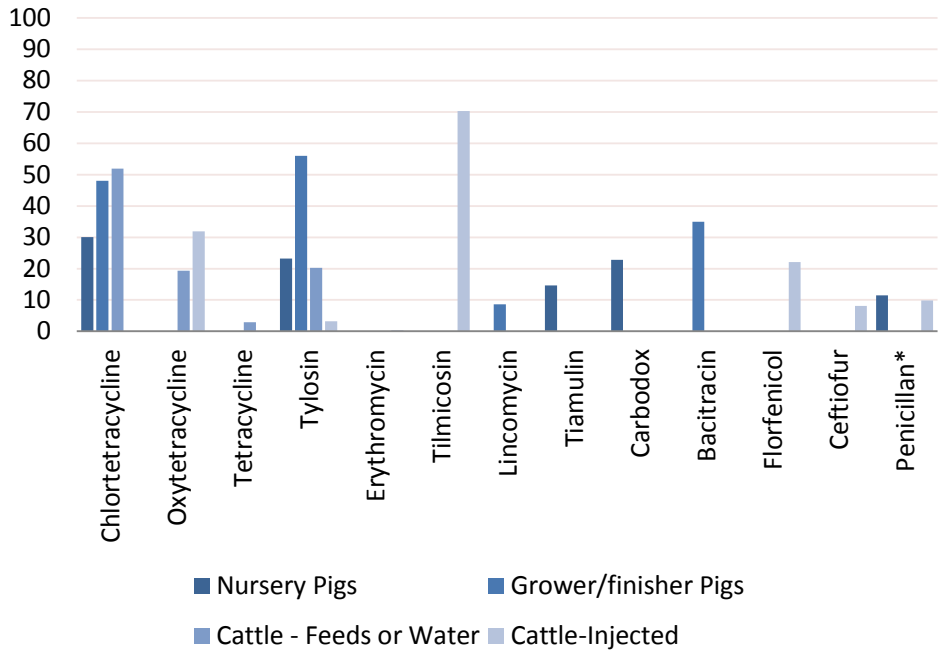


nandrolone

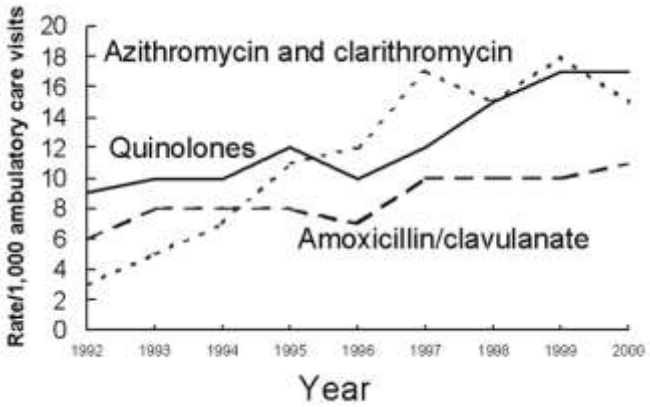


clenbuterol

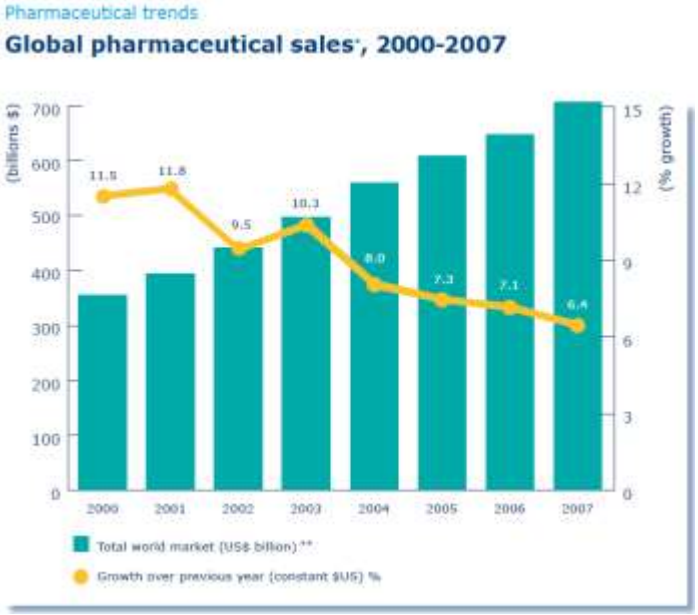
Veterinary Antibiotic Use in the U.S.



USDA (1999) National Animal Health Monitoring Survey - 1990-1997



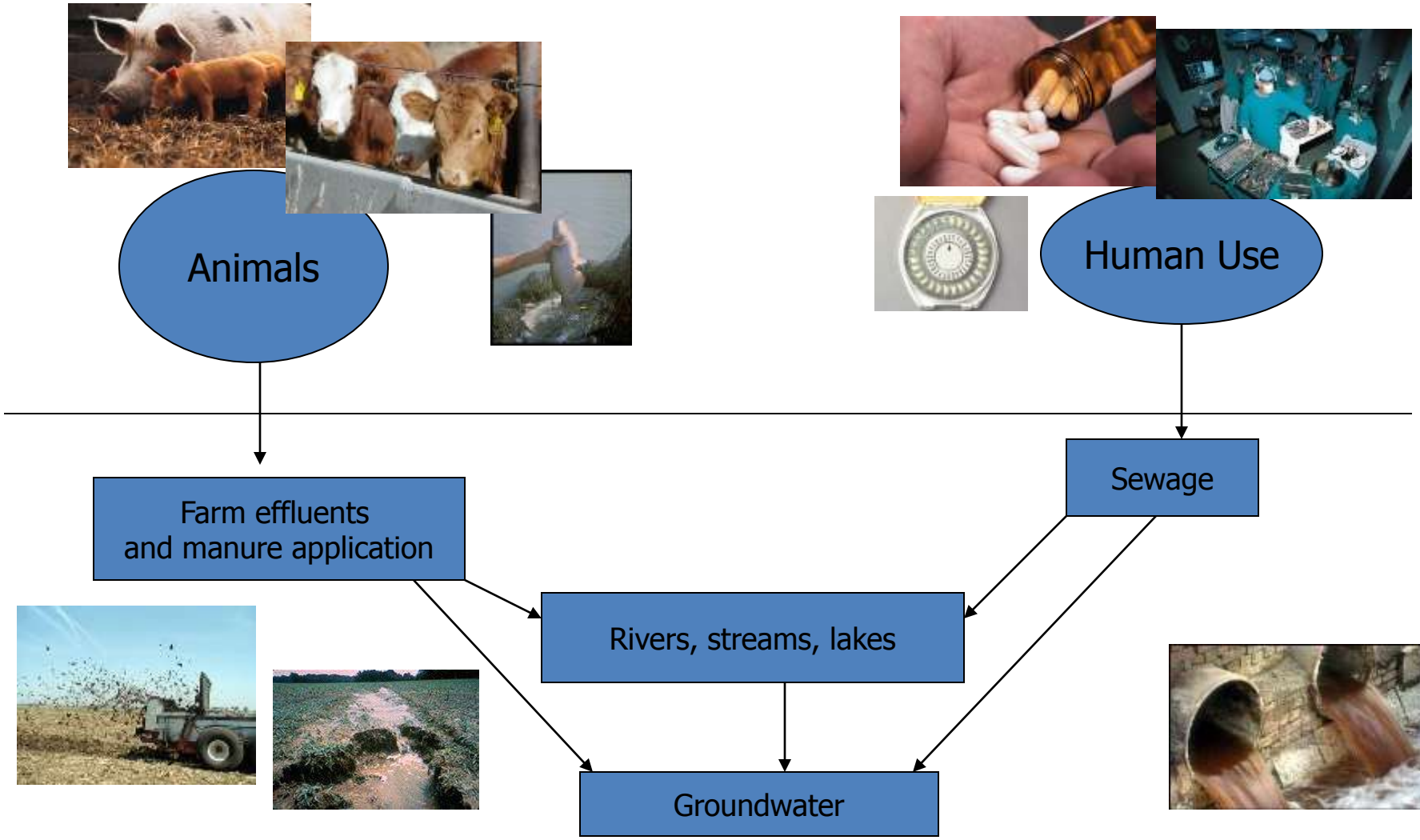
McCaig LF, Besser RE, Hughes JM. Antimicrobial-drug prescrption in ambulatory care settings, United States, 1992-2000. Emerg Infect Dis. 2003 Apr .



~10,000 tons/year antibiotics used annually

Tetracyclines and penicillin first used in 1950s for disease prevention and growth promotion in livestock production

Multiple inputs of drugs to the environment

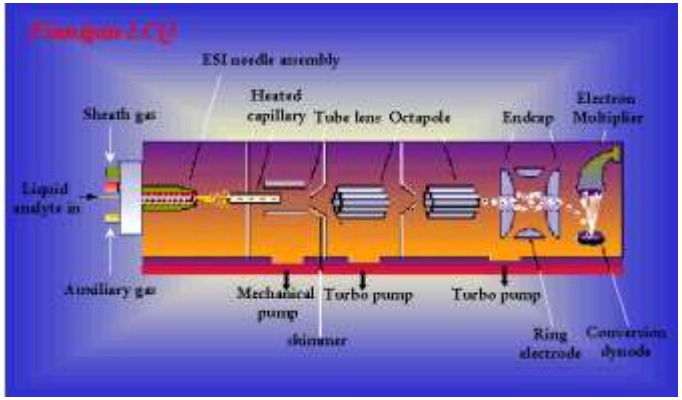


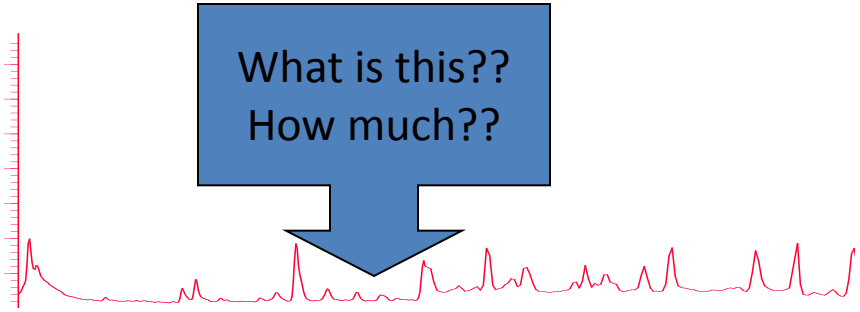
Most Wastewater Treatment Systems not designed to removed these substances

Will field application of municipal bio-solids, manure or wastewater result in measureable concentrations of pharmaceuticals and steroid hormones in the environment?

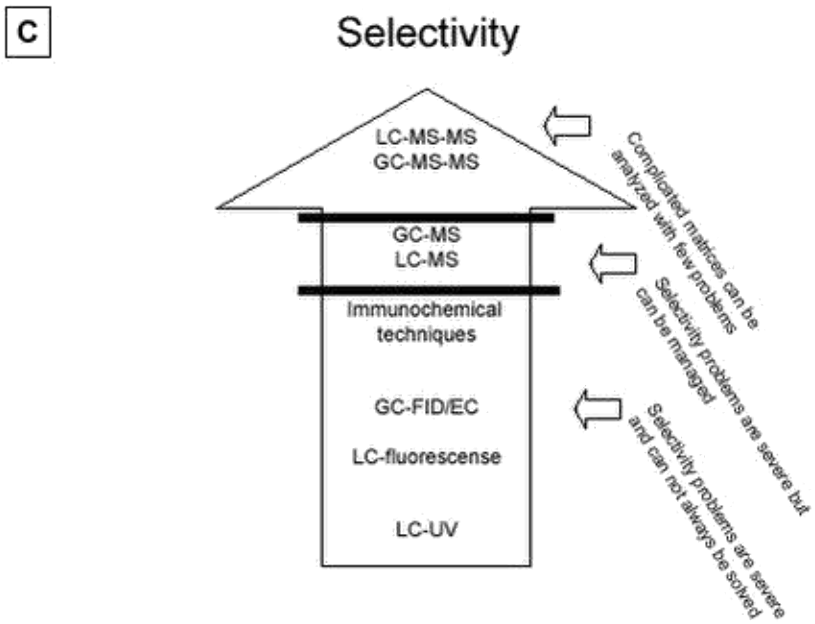
How do we measure these chemicals?

- Water soluble compounds in a complex matrix!!
- LC columns have low resolving power
- Conventional detectors not sensitive enough
- Mass Spectrometer (MS) most sensitive and specific detector available
  - Single Quadrupole
  - Ion Trap MS
  - Triple Quadruple

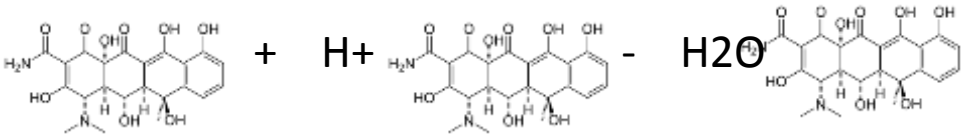




Method requirements depend on confidence needed for use of data



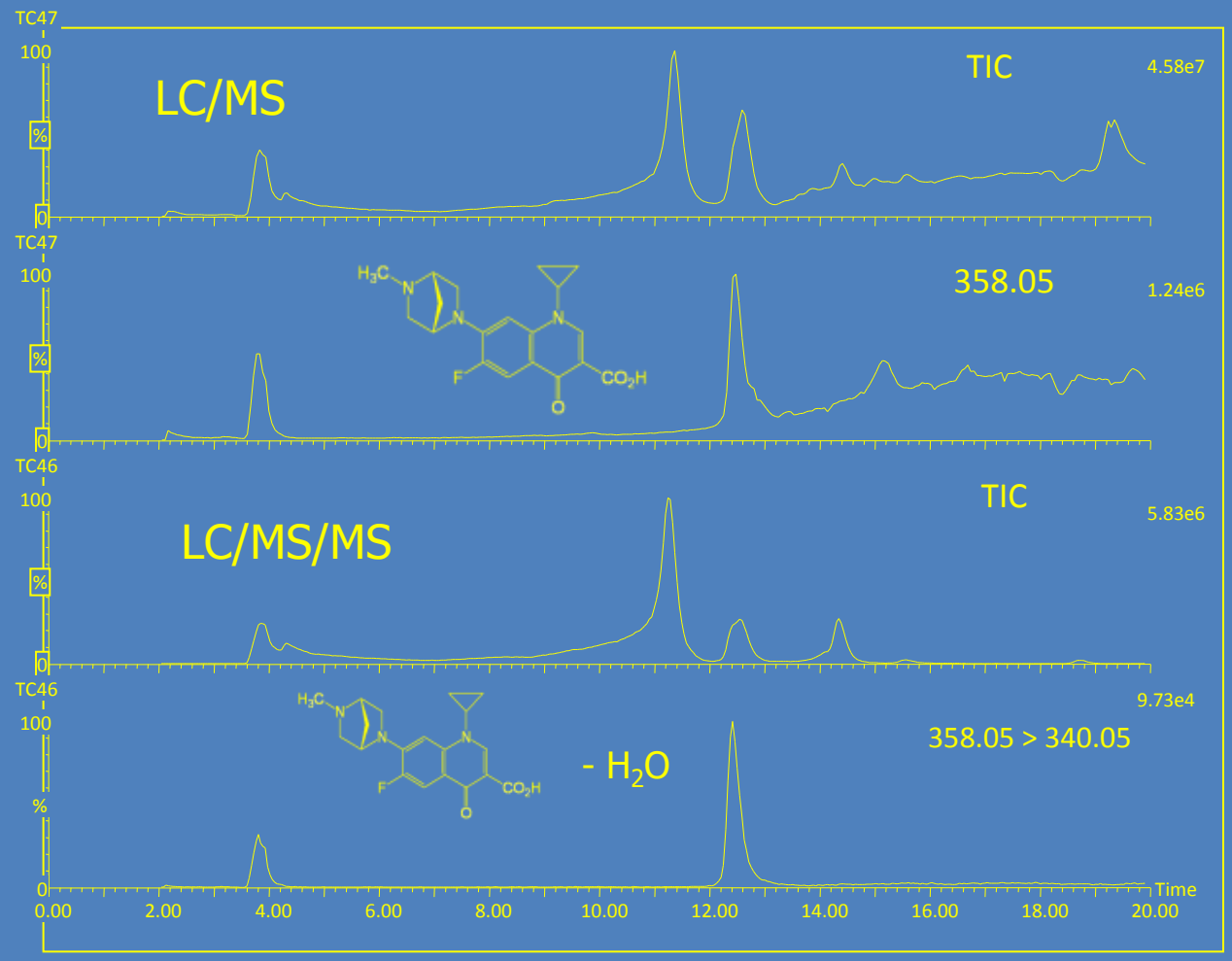
- Increased selectivity required for improved confidence in detection
- MS-MS methods higher selectivity and higher cost
- Matrix still a problem



MW=460 MH+ = 461 MH+(-H2O)=443

Ingerslev and Halling-Sørensen (2003) Evaluation of Analytical Chemical Methods for Detection of Estrogens in the Environment

Advantage of MS/MS



Single Quadrupole

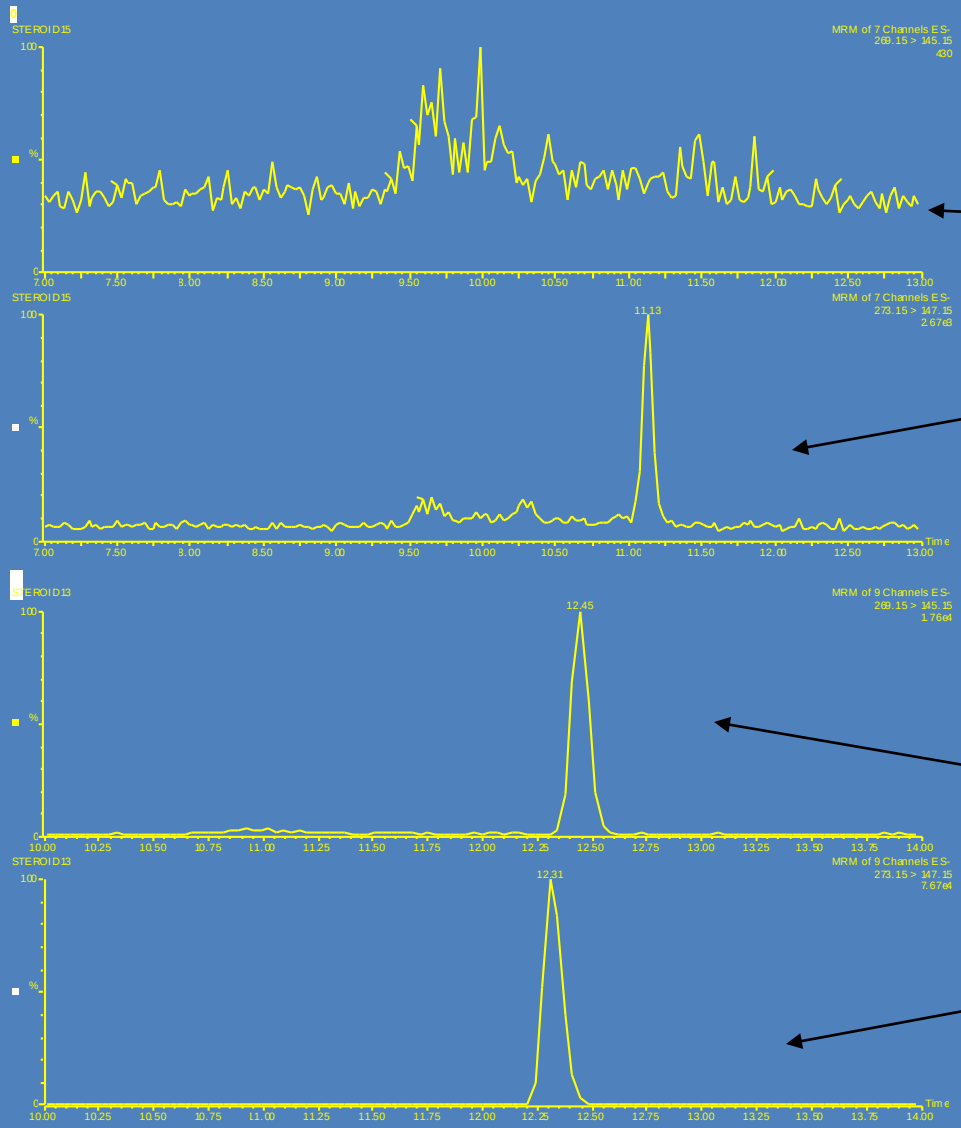
Selected Ion Monitoring

Tandem Quadrupole

Selected Reaction Monitoring

SUPPRESSION

Estrone in Wastewater POCIS extract



Estrone MRM

D_2-17βestradiol (IS)

Same extract after Florisil clean-up

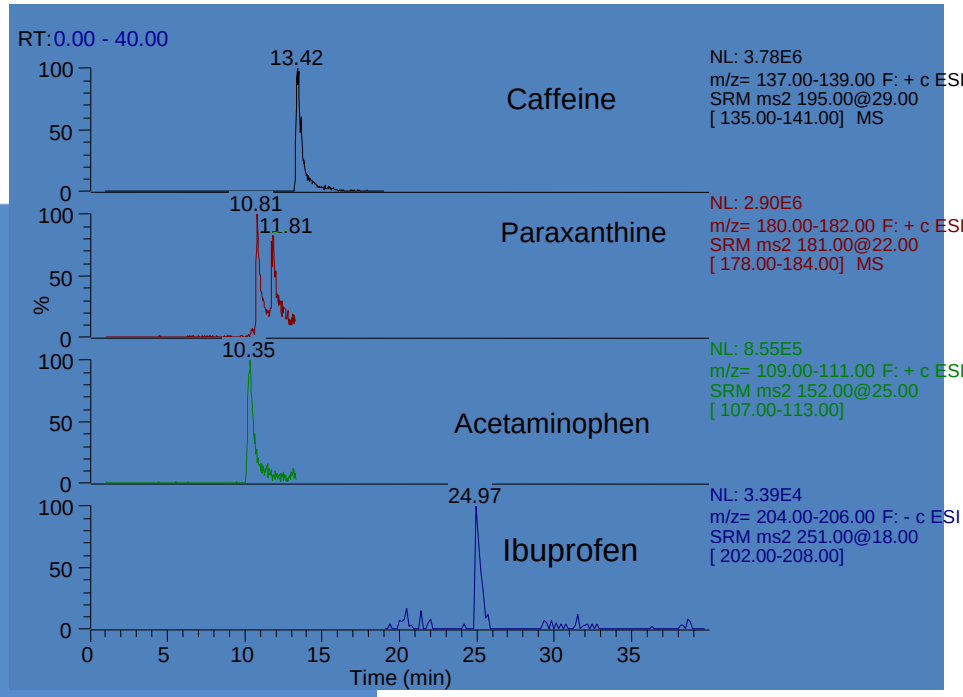
Estrone MRM

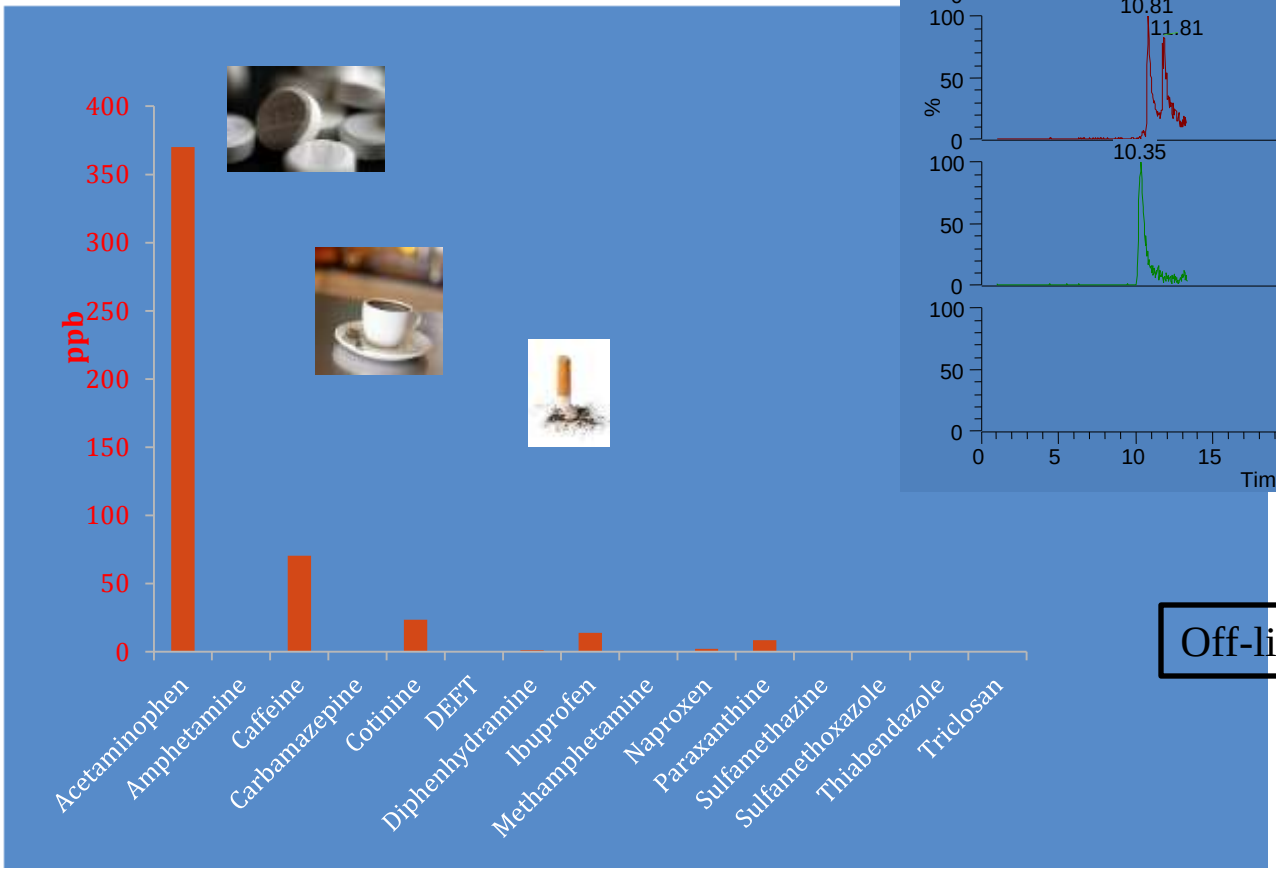
D_2-17βestradiol (IS)

Examples from Nebraska

- Septic effluent
- Municipal wastewater effluent
- Livestock wastewater
- Field treated with manure
- Feeding pen run-off

LC/MS analysis of surface water suspected to be impacted by on-site wastewater source





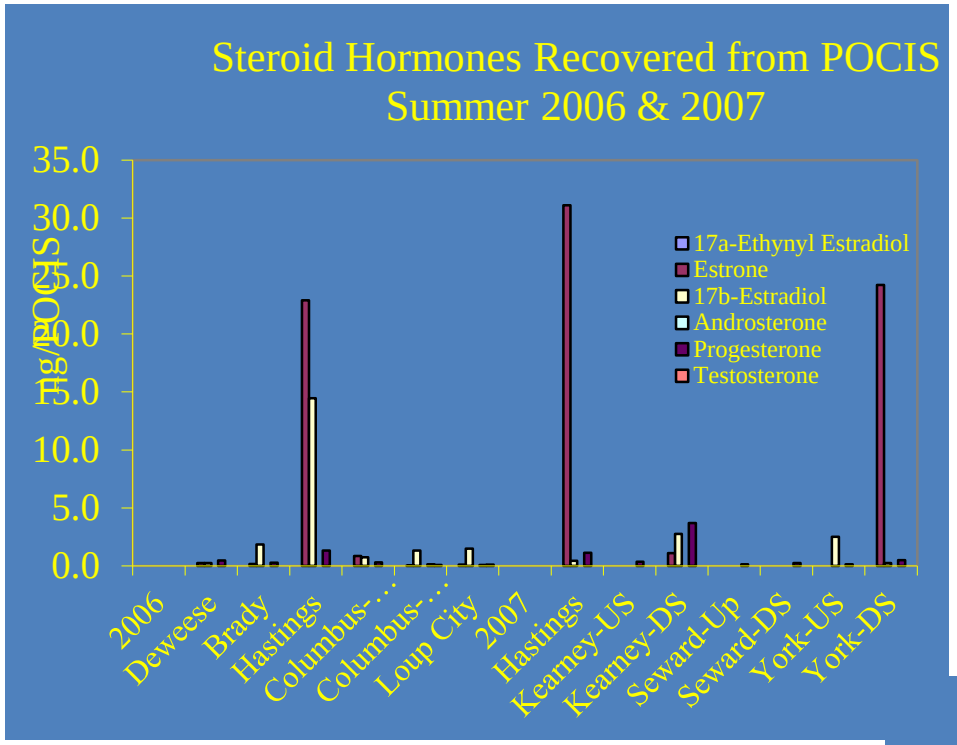
Off-line SPE LC/MS/MS Ion Trap

Polar organic contaminant integrative samplers

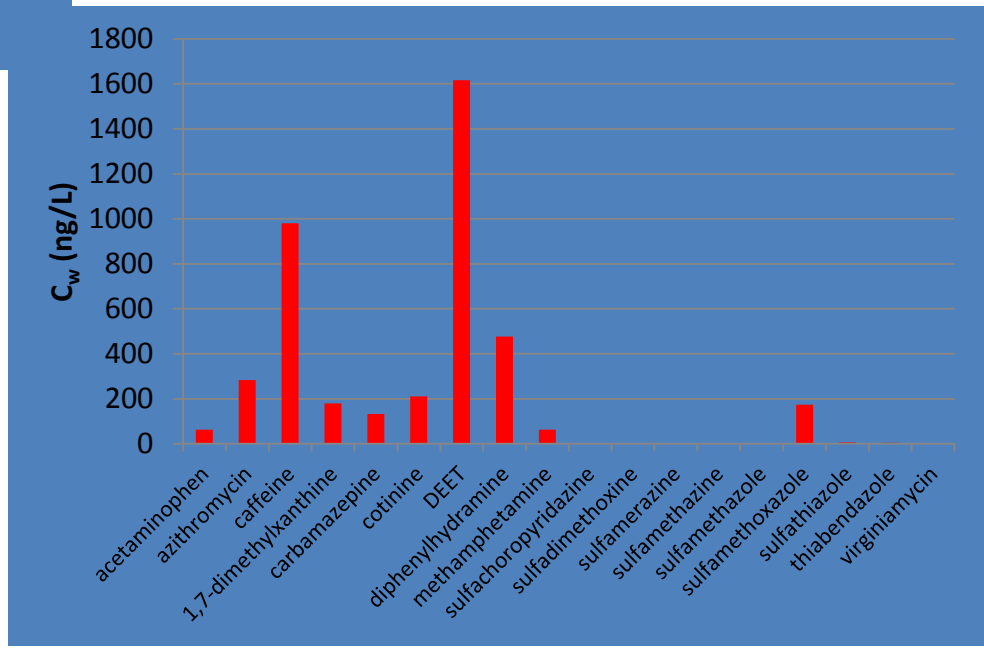
Solid sorbent inside polyethersulfone (PES) membrane “sandwich”

Two different sorbent mixtures used depending on the type of contaminants trapped

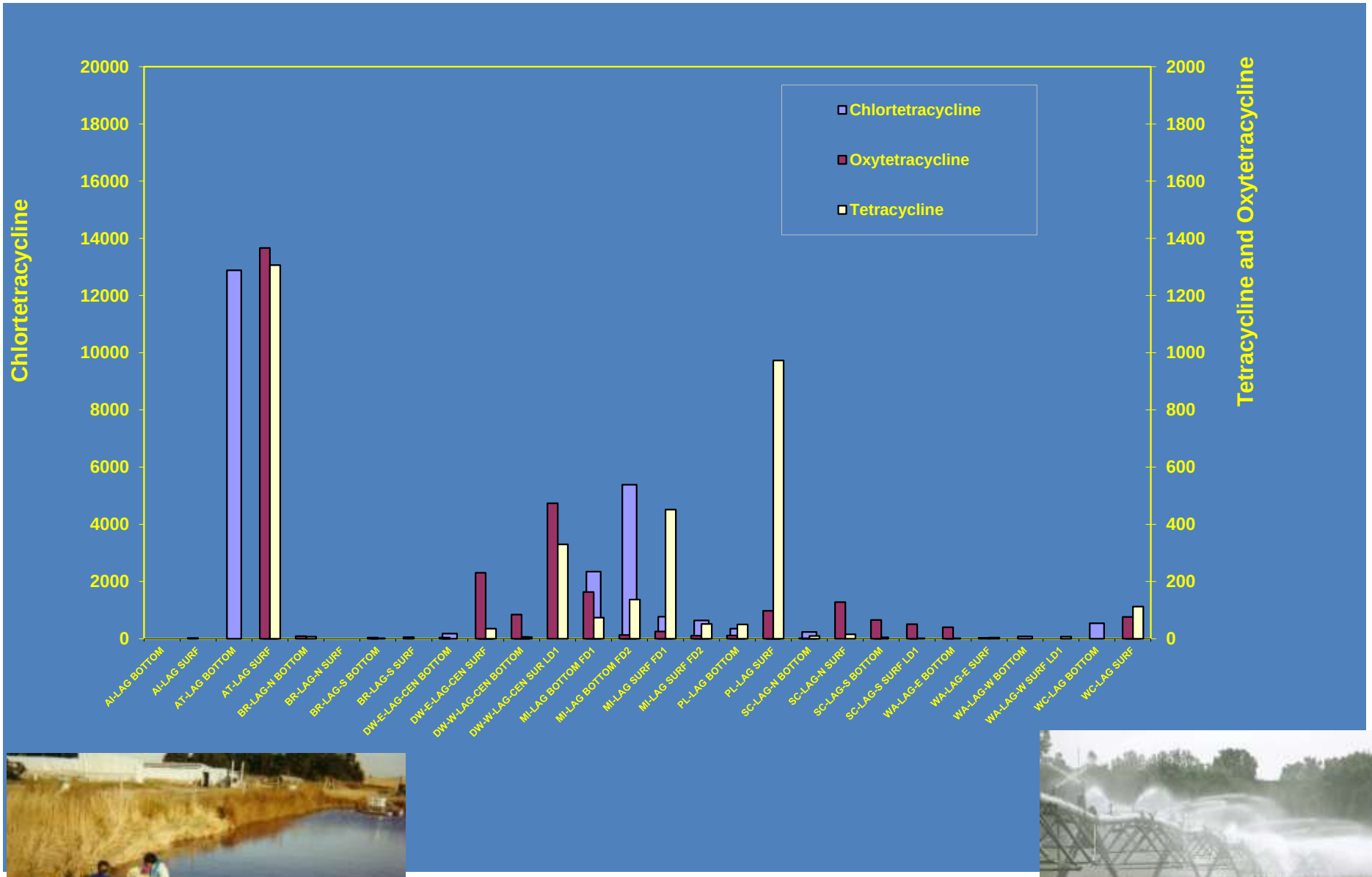
Alvarez DA, Petty JD, Huckins JN, Jones-Lepp TL, Getting DT, et al. (2004) DEVELOPMENT OF A PASSIVE, IN SITU, INTEGRATIVE SAMPLER FOR HYDROPHILIC ORGANIC CONTAMINANTS IN AQUATIC ENVIRONMENTS. Environmental Toxicology and Chemistry: Vol. 23, No. 7 pp. 1640–1648



Sellin, Marlo K., Daniel D. Snow, Debbie L. Akerly, and Alan S. Kolok. 2009. Estrogenic Compounds Downstream From Three Small Cities in Eastern Nebraska: Occurrence and Biological Effect. JAWRA Journal of the American Water Resources Association. 45(1), 14 - 21



Bartelt-Hunt, Shannon L. Daniel D. Snow, Teyona Damon, Johnette Shockley, Kyle Hoagland. 2009. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. Environmental Pollution. 157(3): 786-791



Zhu, J., D.D. Snow, D.A. Cassada, S.J. Monson, and R.F. Spalding 2001. Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid phase extraction and liquid chromatography-tandem mass spectrometry. Journal of Chromatography. 928:177-186

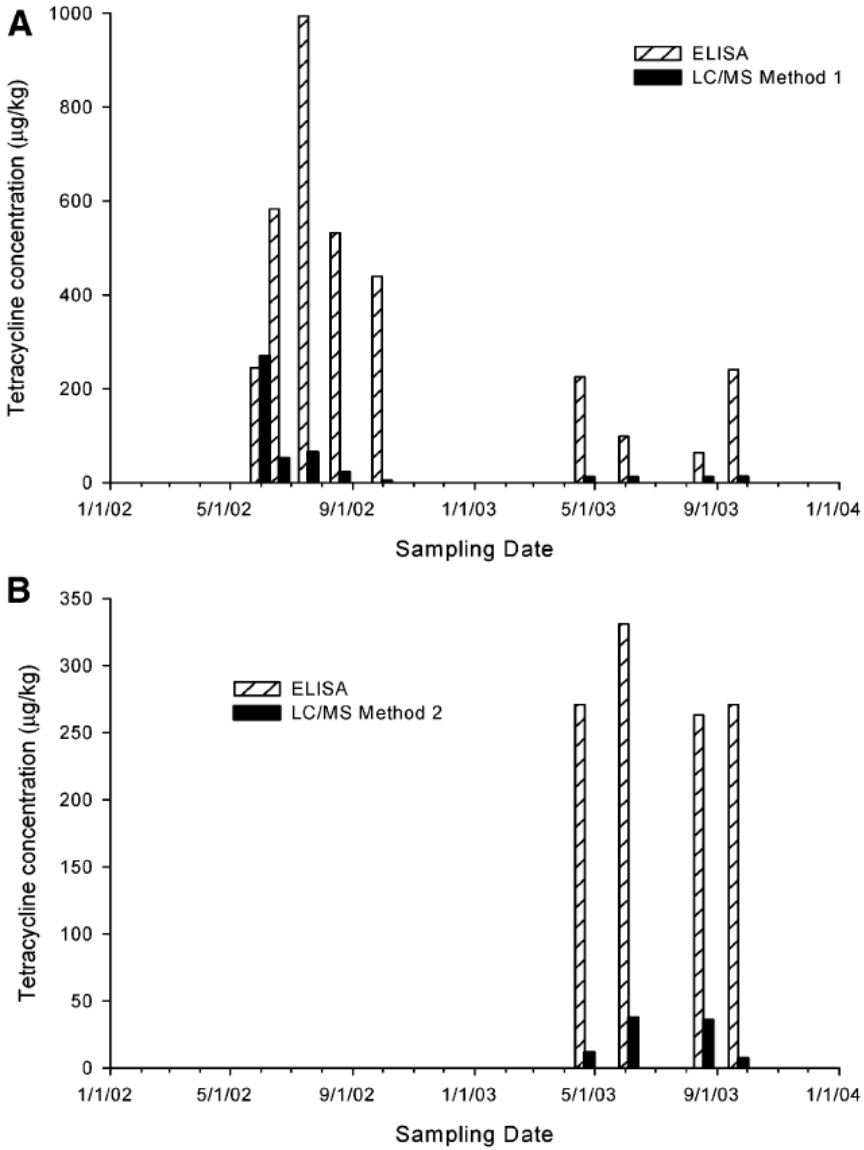
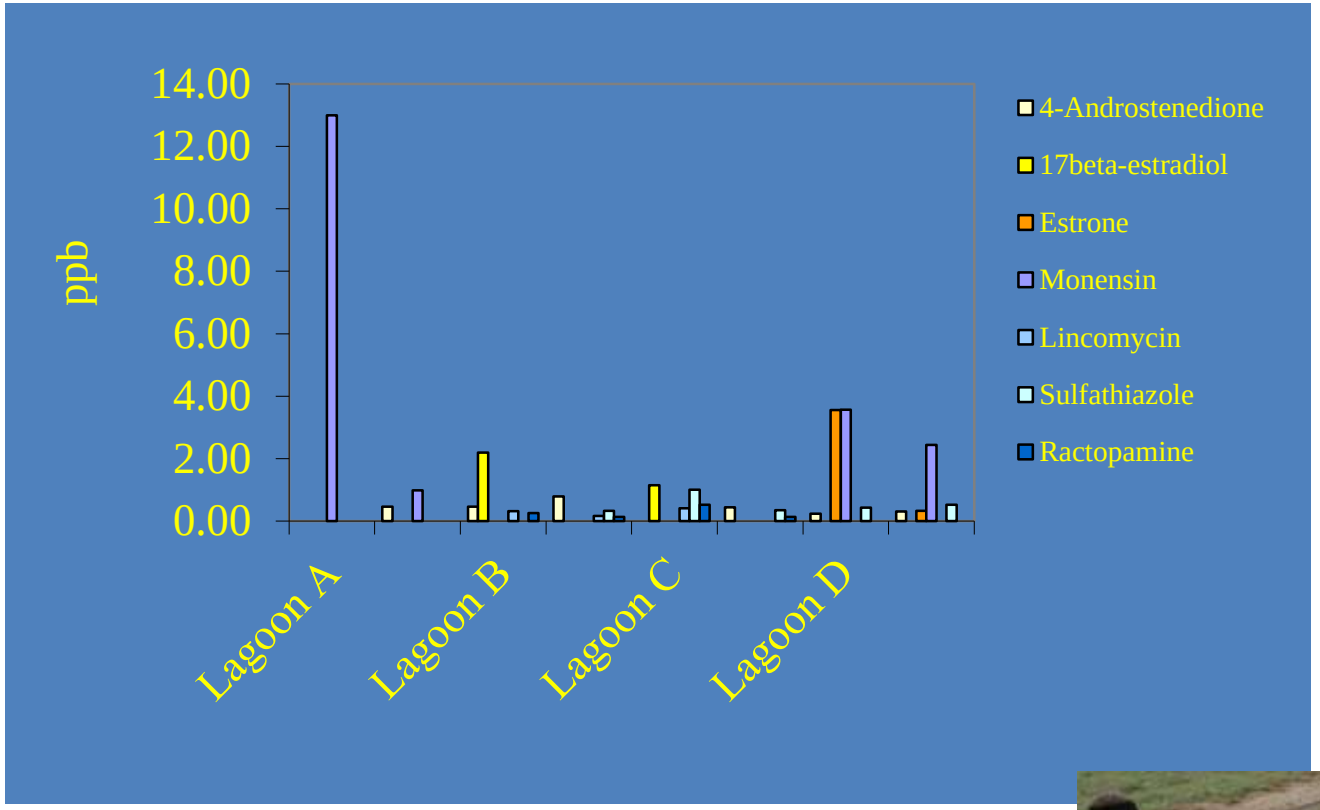


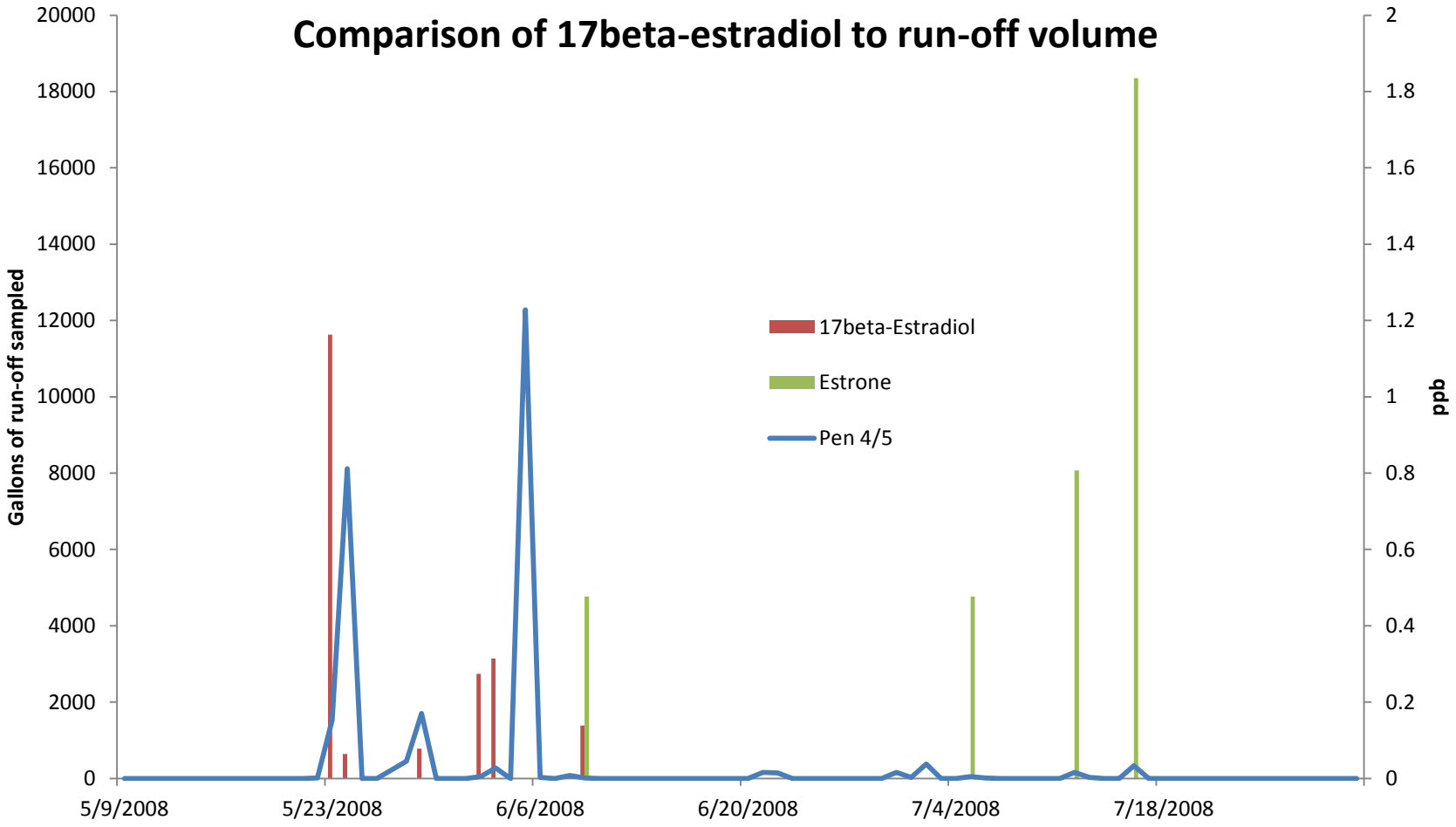
Figure 3. Comparison of LC-MS and ELISA results from the analysis of surface soil samples from a plot that received manure in 2002 (**A**) and a second manure application in 2003 (**B**).

Aga, D. S.; O'Connor, S.; Ensley, S.; Payero, J. O.; Snow, D.; Tarkalson, D., Determination of the Persistence of Tetracycline Antibiotics and Their Degradates in Manure-Amended Soil Using Enzyme-Linked Immunosorbent Assay and Liquid Chromatographyâˆ’Mass Spectrometry. *Journal of Agricultural and Food Chemistry* ***2005, 53, (18), 7165-7171.***

Is the occurrence of wastewater compounds related to type of livestock?



Seasonal variation of estrogen levels in feeding pen run-off



Pharmaceuticals and steroid hormones in soils, sludge and water

- Bioactive chemicals released to the environment through multiple pathways
- Specific compounds detected probably related to source, use, treatment technology
- Chemicals occur at very low concentrations in complex matrixes