Lessons Learned from Two Decades of Research on Emerging Contaminants

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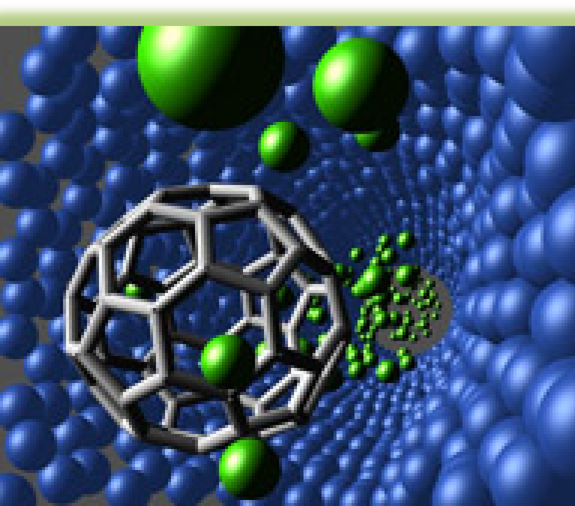
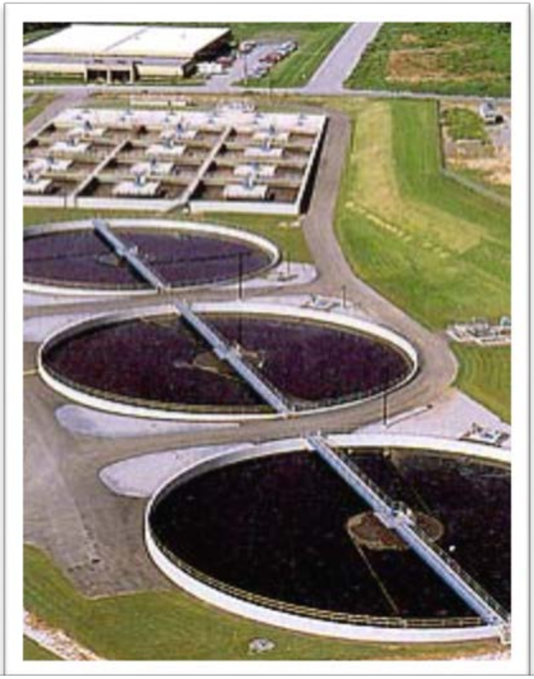
Aga Research Group: Environmental Chemistry

- Past
  - Chlorinated Pesticides
  - Brominated flame retardants
- Present
  - Antibiotics and pharmaceuticals
  - Endocrine disrupting compounds
- Future
  - Engineered nanomaterials



Fate, Effects, and Treatment of Contaminants

- Analytical Techniques
- Occurrence in Environment
- Bioaccumulation and Biotransformations
- Ecological and Human Health Impacts
- Wastewater Treatment Systems



Shedding light on potential toxins that lurk in blood and breast milk

New "one-shot" analysis quickens identification of flame retardants by five times

By Marcene Robinson

Release Date: September 16, 2015

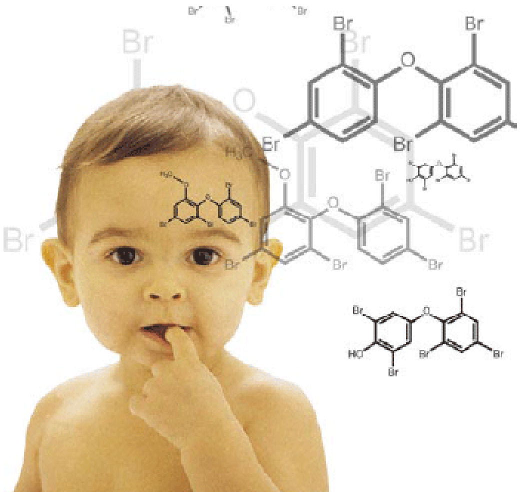
UB chemistry professor Diana Aga, left, and chemistry doctoral candidate Deena Butryn examine blood and breast milk samples using the one-shot method. Credit: Douglas Levere.

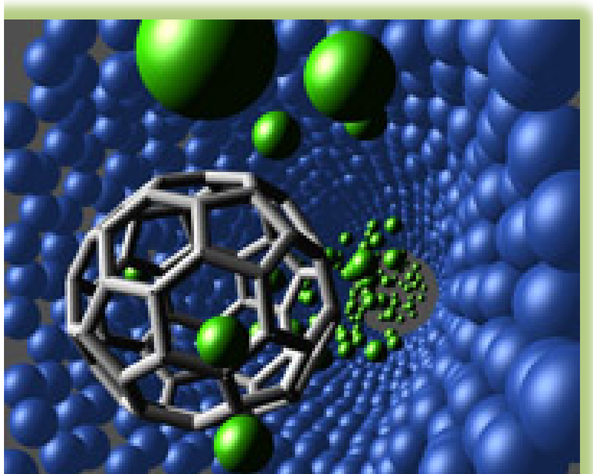
Flame

What are Emerging Contaminants

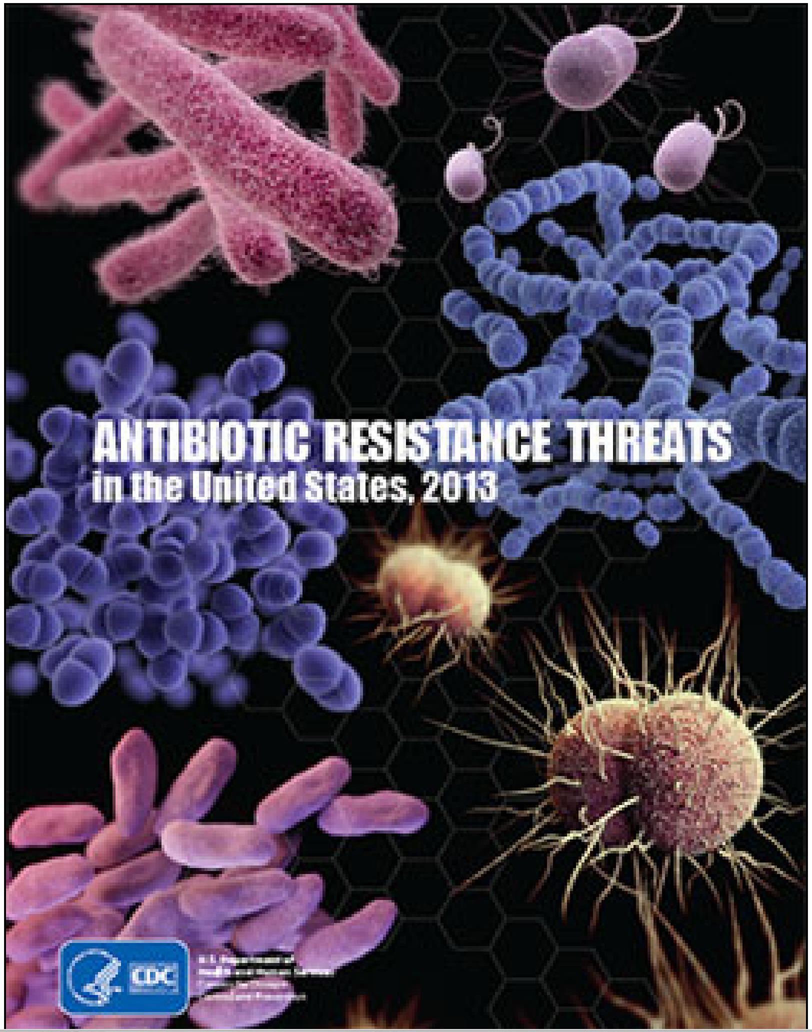
- Occurrence in the environment has been reported only in the last 20 years
- Cause adverse ecological and human health effects at very low levels
- Man-made and naturally occurring chemicals

- Antibiotics and other pharmaceuticals
- Personal care products
- Estrogens and other hormones
- New Halogenated flame retardants
- Engineered nanomaterials





Environmental Impacts of Emerging Contaminants



the WHITE HOUSE PRESIDENT BARACK OBAMA

For Immediate Release September 18, 2014

Executive Order -- Combating Antibiotic-Resistant Bacteria

Estrogen knocks out fish in whole-lake experiment

For the first time, scientists have demonstrated in a natural lake ecosystem that the synthetic estrogen found in birth control pills can cause the collapse of fathead minnow populations. The findings support lab studies, which over the past 10 years have linked natural and synthetic estrogen in wastewater to feminization of male fish but have been unable to show whet… popu… their… Fr… past… diol w… Experimental Lakes Area of north-

A researcher adds estrogen to Lake 260, a pristine lake in Canada.

western Ontario—a pristine wilderness of lakes and boreal forest on granite bedrock known as the Canadian Shield, says Karen Kidd, ecotoxicologist with the Canadian Department of Fisheries and Oceans (DFO) and coordinator of the study. Until now, Lake 260 has not been manipulated for any experiments. The average estrogen concentration, 5–6 nanograms per … ers' association.

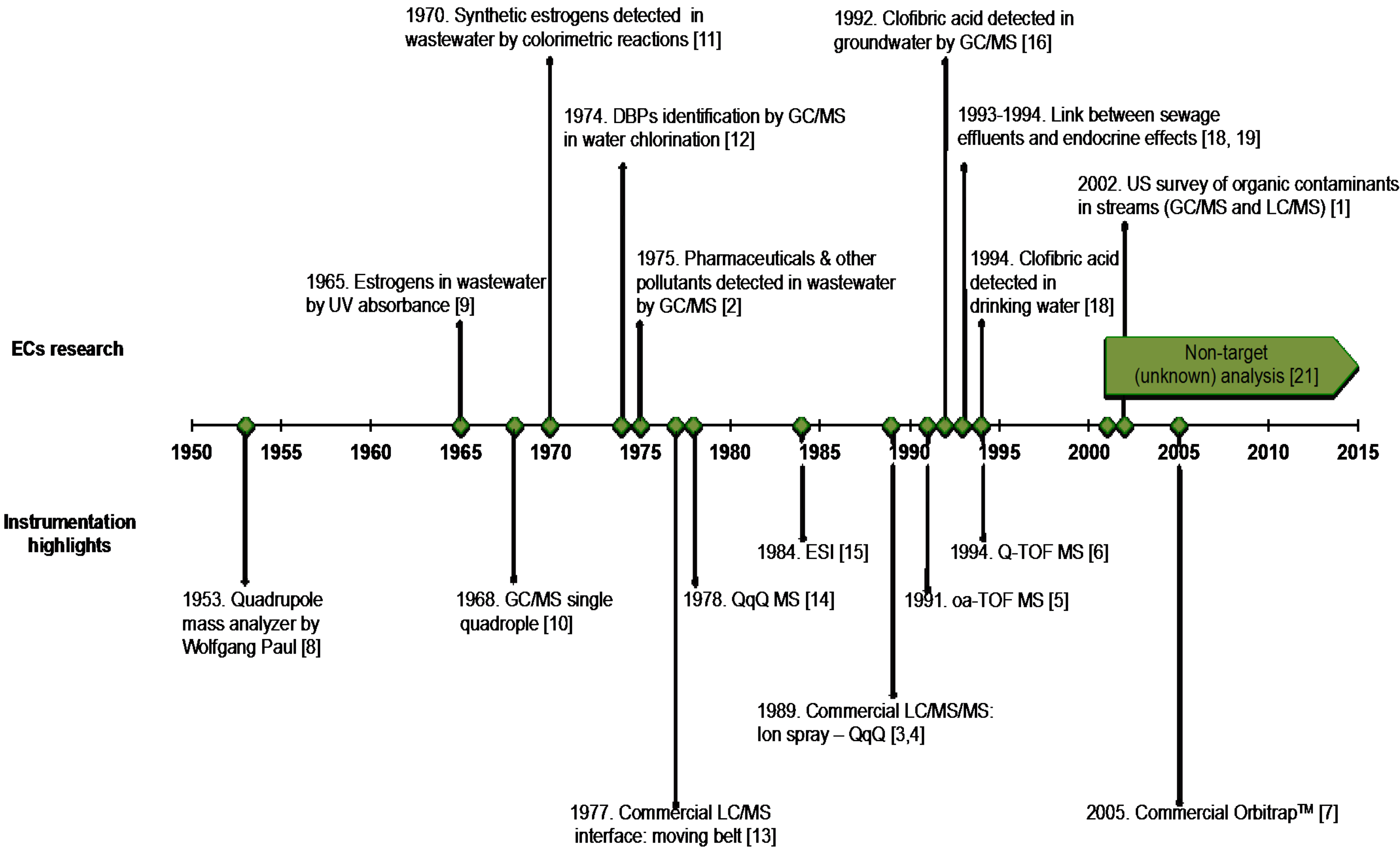
Dozens of researchers in academia and government in the United States and Canada tracked changes in the populations and physiology of fish, bacteria, phytoplankton, zooplankton, and insects in Lake 260 and several reference lakes both before and during estrogen additions, Kidd says. Although preliminary results do not yet point to dramatic changes in the lower levels of the food web, fish are clearly stressed.

Fathead minnows, which live just 2–3 years, once numbered

studies over the past 10 years have linked natural and synthetic estrogen in wastewater to feminization of male fish

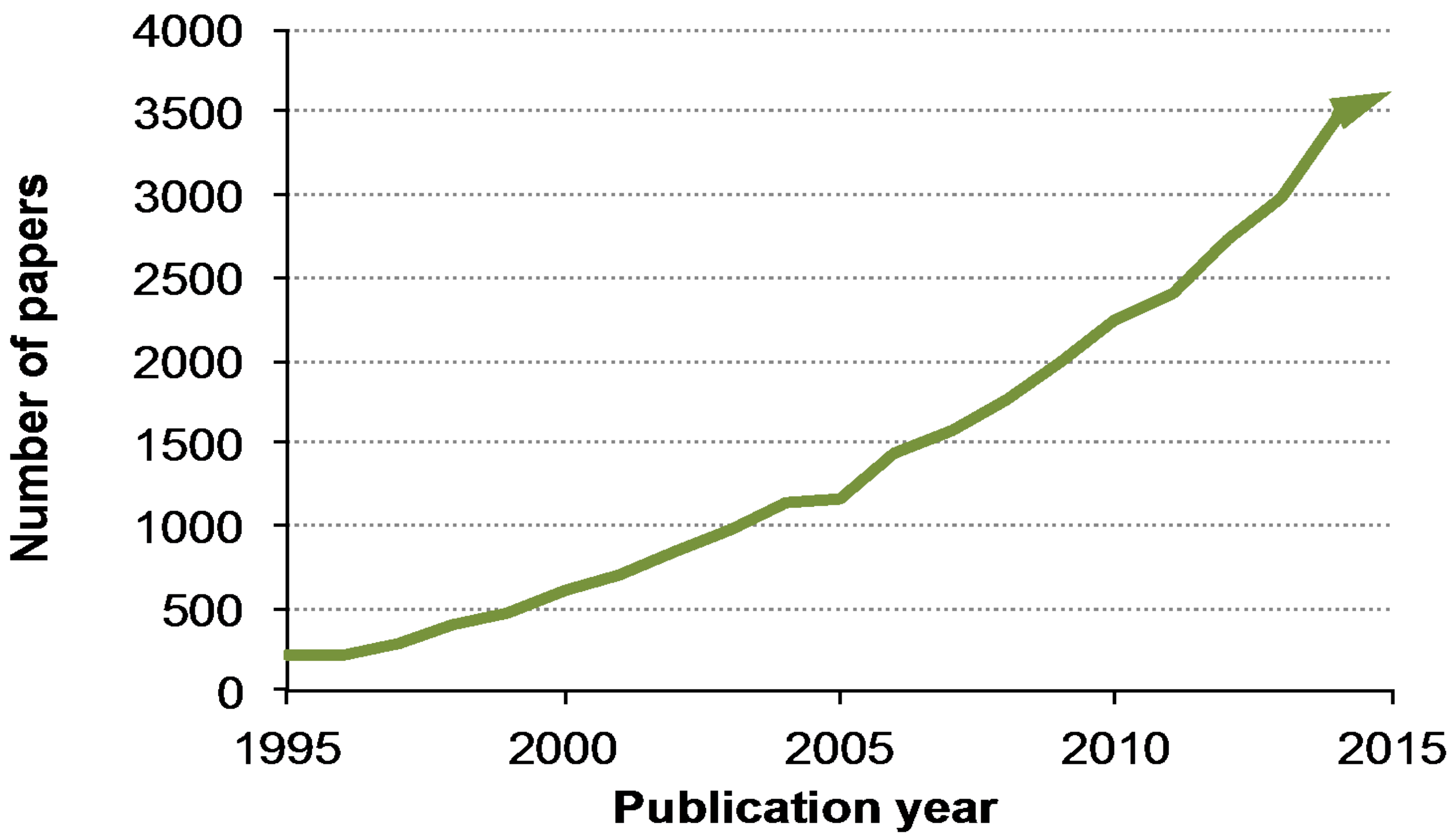
Envi. Sci. Technol. 2003, v.37, Issue 17

Important Milestones on Emerging Contaminants Research



Noguera-Oviedo, K.; Aga, D.S. *Journal of Hazardous Materials*, **2016**, *316*, 242-251

Two Decades of Publications on Emerging Contaminants



Web of Science search entry: ((pharmaceutic* OR drug OR drugs) AND (effluent*OR wastewater* OR waste water* OR river* OR stream* OR sedimen* OR (waterNEAR/8 pollut*))) OR ((emergent or emerging) NEAR/5 contamina*) OR endocrin*NEAR/4 disrupt* OR estrogenic NEAR/4 (compound* OR chemical*).

LESSON #1

Human pharmaceuticals and personal care products (PPCPs) are introduced into the environment because they are not completely eliminated in treatment systems

Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999- 2000: A National Reconnaissance

DANA W. KOLPIN*
U.S. Geological Survey, 400 S. Clinton Street, Box 1230, Iowa City, Iowa 52244

EDWARD T. FURLONG
U.S. Geological Survey, Box 25046, MS 407, Denver, Colorado 80225-0046

MICHAEL T. MEYER
U.S. Geological Survey, 4500 SW 40th Avenue, Ocala, Florida 34474

E. MICHAEL THURMAN
U.S. Geological Survey, 4821 Quail Crest Place, Lawrence, Kansas 66049

STEVEN D. ZAUGG
U.S. Geological Survey, Box 25046, MS 407, Denver, Colorado 80225-0046

LARRY B. BARBER
U.S. Geological Survey, 3215 Marine Street, Boulder, Colorado 80303

HERBERT T. BUXTON
U.S. Geological Survey, 810 Bear Tavern Road, West Trenton, New Jersey 08628

***Kolpin** et. al, Environ. Sci. Technol.*, **2002**, *36*, pp 1202–1211

Differences in Efficiencies of Wastewater Treatment Plants in Removing Pharmaceuticals

Environ. Sci. Technol. **2005**, *39*, 5816−5823

Removal of Antibiotics in Wastewater: Effect of Hydraulic and Solid Retention Times on the Fate of Tetr[...] Sludge Proc[...]

SUNGPYO KIM[...]
JAMES N. JEN[...]
A. SCOTT WE[...]
Department of Civil[...] Engineering, State U[...] Jarvis Hall, Buffalo,[...] Chemistry, State Un[...] Natural Science Cor[...]

many surface water resources that receive discharges from municipal wastewater treatment plants (WWTPs) and agricultural runoff (*3*−*6*). A recent study showed that as high as 4 μg/L tetracycline and 1.2 μg/L chlortetracycline have been detected in municipal wastewater (*7*). Further, a reconnaissance study by the United States Geological Survey

Environ. Sci. Technol. **2006**, *40*, 7367−7373

Enhanced Biodegradation of Iopromide and Trimethoprim in Nitrifying Activated Sludge†

ANGELA L. [...]
DIANA S. A[...]
Department of Ch[...] Buffalo, 608 Natu[...] New York 14260-3[...]

Iopromide (an X-[...] antibacterial drug[...] in effluents of w[...] in surface water[...]

processes available for each stage of treatment. Several reports have shown that the current wastewater treatment procedures do not completely eliminate many of these microcontaminants (*4*−*9*).

Recently, there has been an increased effort to investigate

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Available online at www.sciencedirect.com

SCIENCE DIRECT

ENVIRONMENTAL POLLUTION

Environmental Pollution 142 (2006) 295e 302

Ev[...] contam[...]

[a] Departme[...]

Abstract

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

CHEMOSPHERE

Chemosphere 68 (2007) 428–435

www.elsevier.com/locate/chemosphere

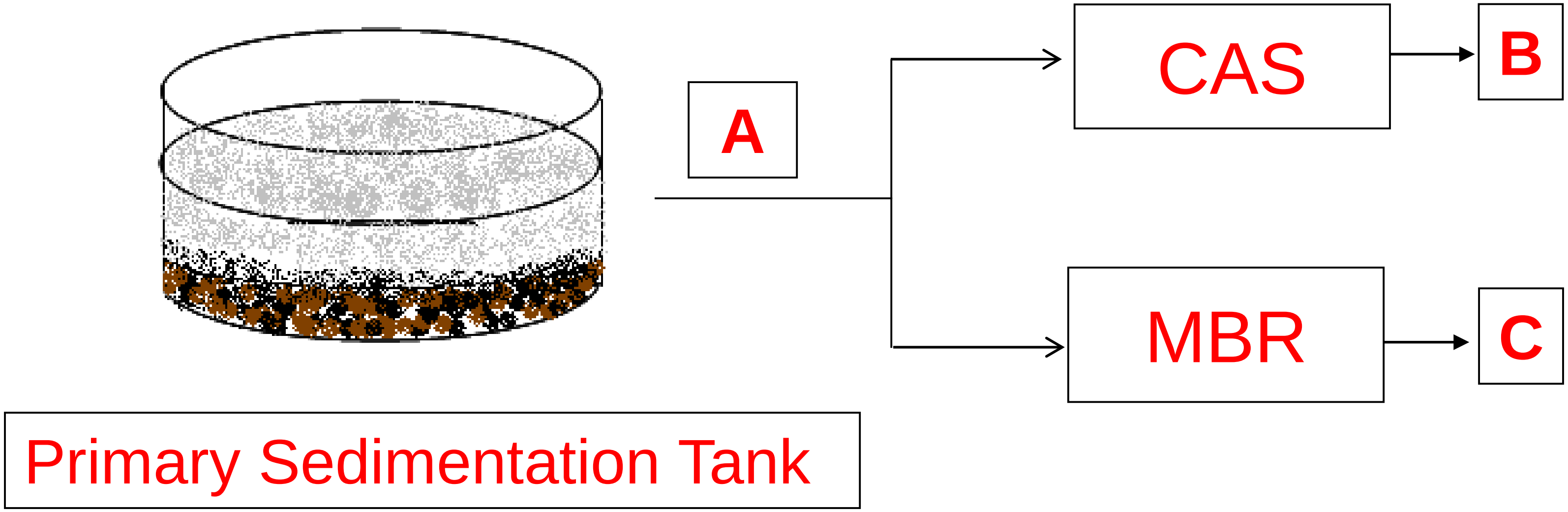
Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations

Angela L. Batt, Sungpyo Kim, Diana S. Aga *

Department of Chemistry, The State University of New York at Buffalo, 608 Natural Sciences Complex, Buffalo, NY 14260-3000, USA

Received 11 April 2006; received in revised form 21 December 2006; accepted 2 January 2007
Available online 21 February 2007

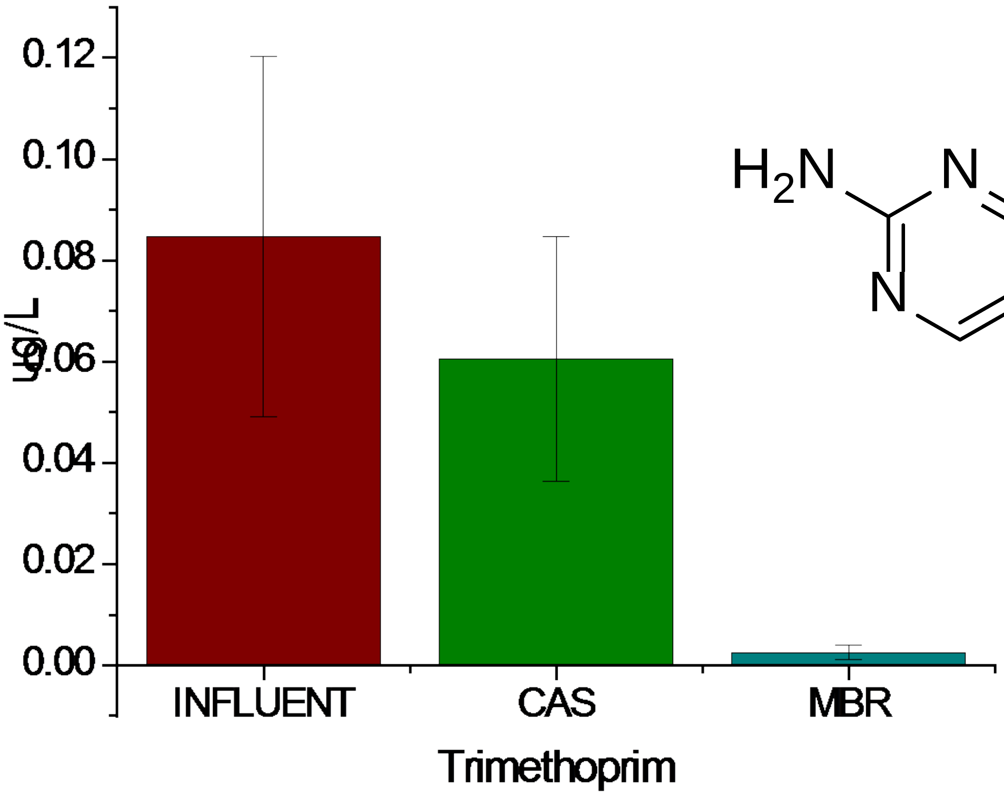
Removal in Conventional Activated Sludge (CAS) and Membrane Bioreactors (MBR)

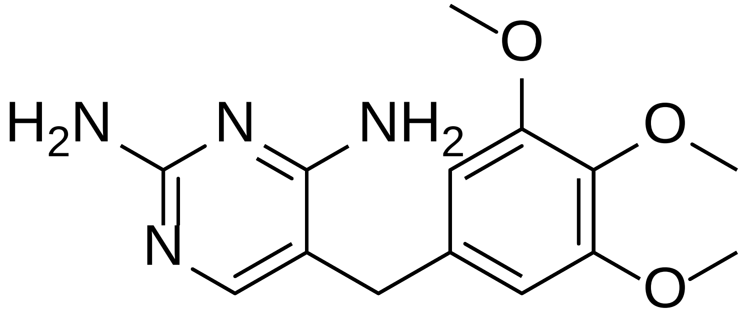


Celiz, M.D.; Pérez, S; Barcelo, D.; Aga, D.S. *Journal of Chromatographic Science*, **2009**, 47, 19-25.

Trimethoprim Removal

~ 29 % removal for conventional activated sludge (CAS) &
~ >85 % removal for membrane bioreactor (MBR)

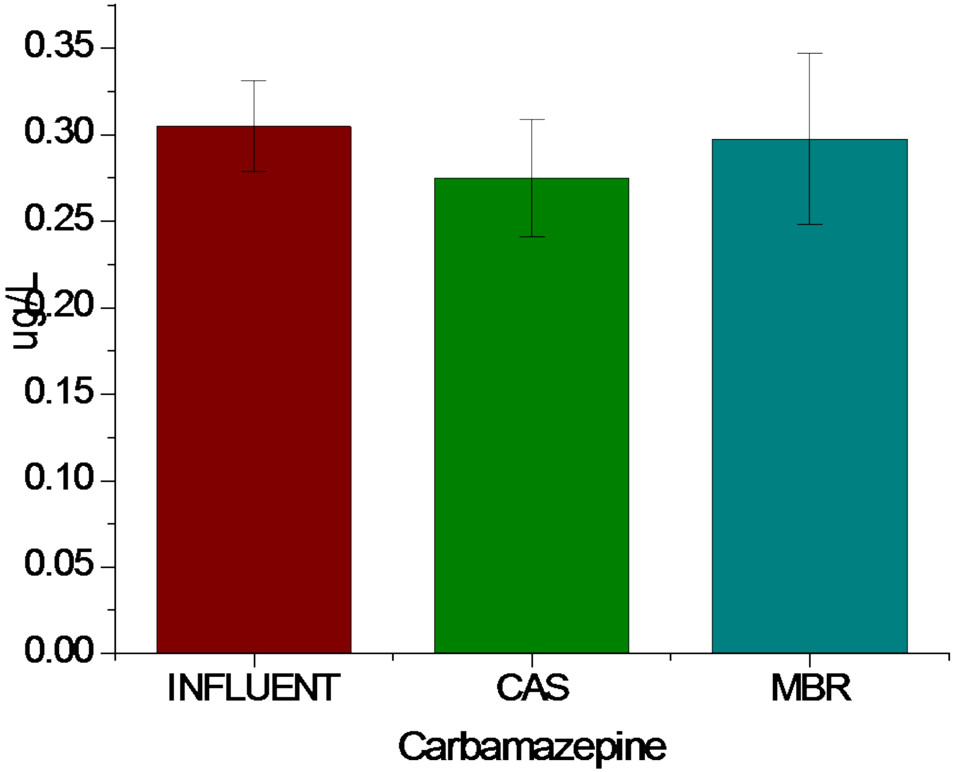


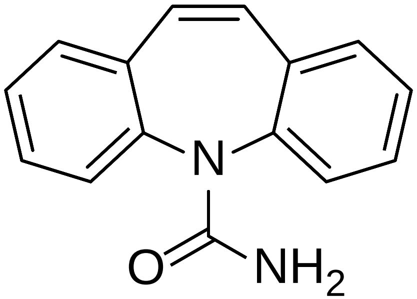


Celiz, M.D.; Pérez, S; Barcelo, D.; Aga, D.S. *Journal of Chromatographic Science*, **2009**, 47, 19-25.

Carbamazepine Removal

No significant removal in CAS or MBR



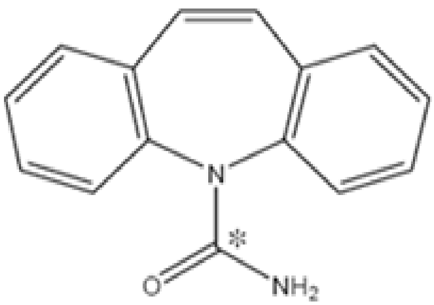


carbamazepine

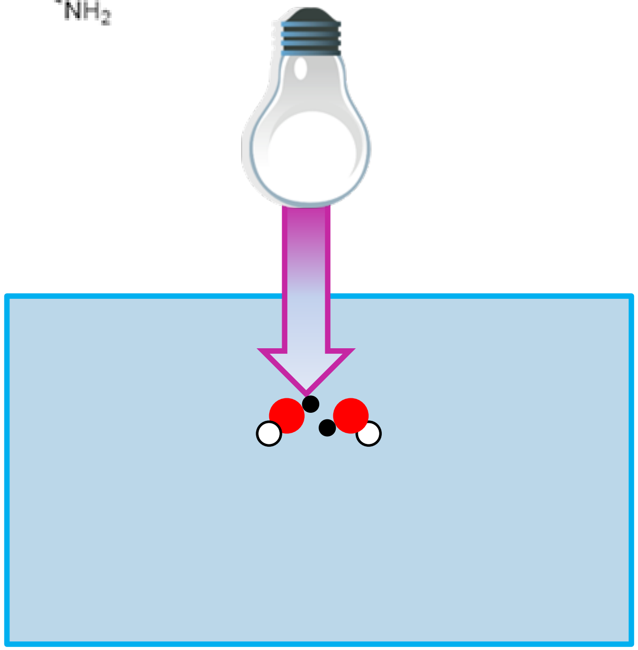
Celiz, M.D.; Pérez, S; Barcelo, D.; Aga, D.S. *Journal of Chromatographic Science*, **2009**, 47, 19-25.

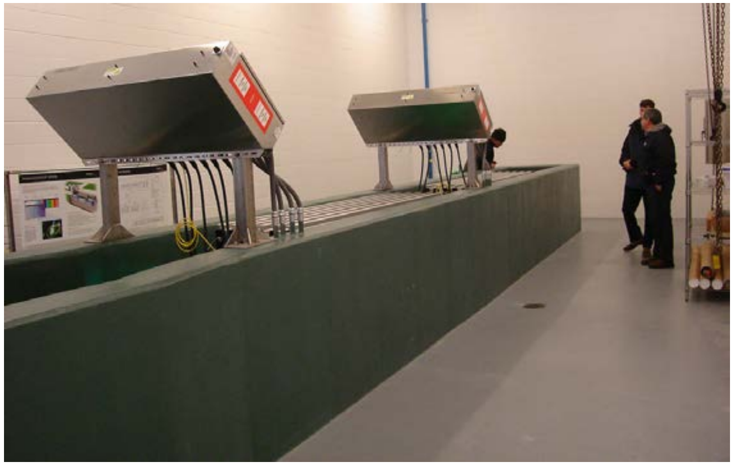
LESSON #2

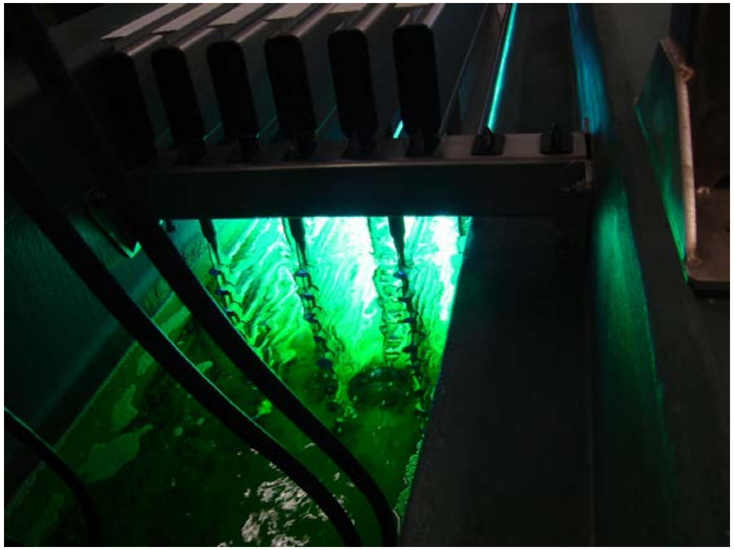
Advanced Oxidation Process (AOP) followed by Biofiltration is a very promising technology to mineralize recalcitrant pharmaceuticals



UV technologies combined with H_2O_2 generate hydroxyl radicals

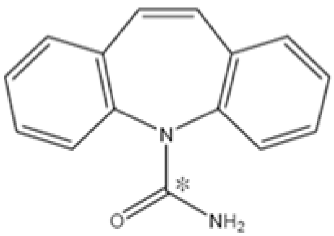


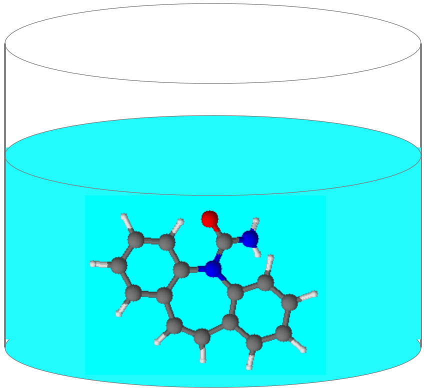


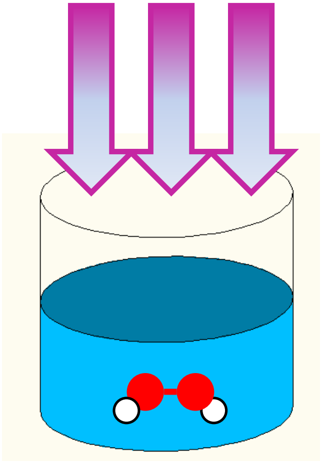


Experimental Setup for AOP of Carbamazepine

1 mg/L "cold" CBZ + 0.262 mg/L "hot" CBZ







No treatment

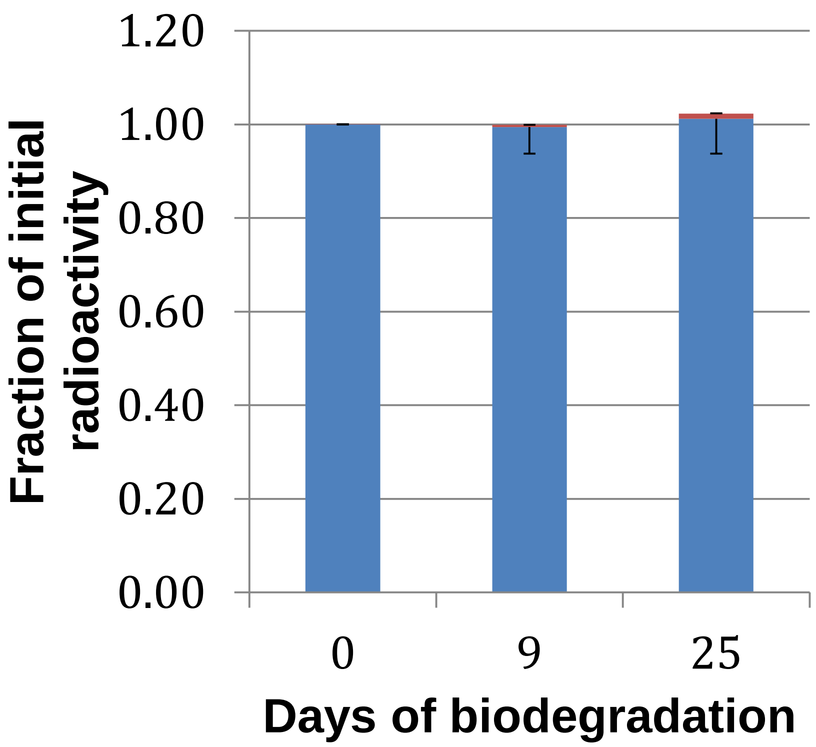
Advanced oxidation:
1800 mJ/cm^2
10 mg/L H_2O_2

Degrade H_2O_2 with bovine catalase

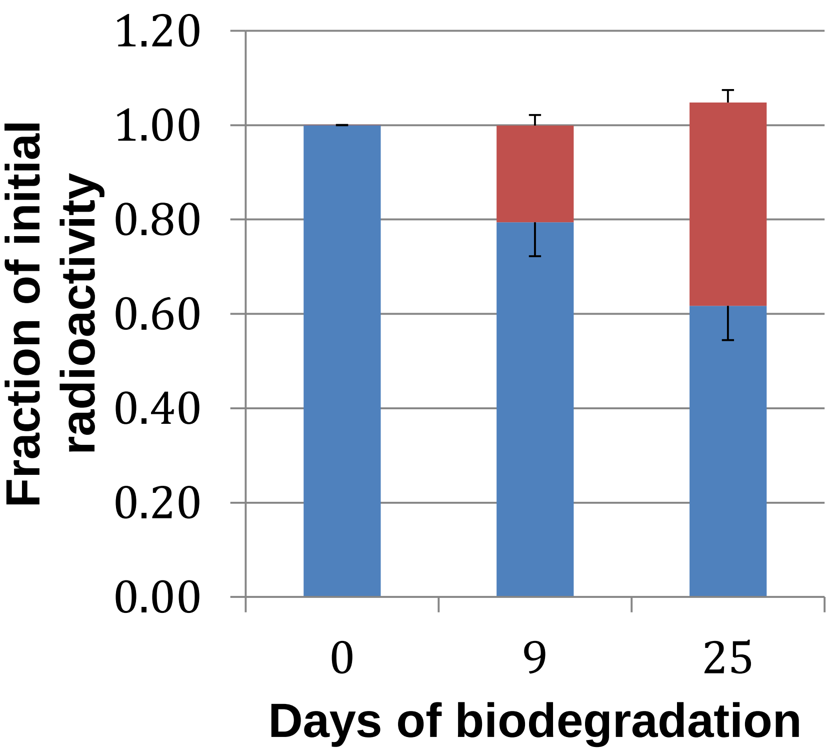
Activated sludge biodegradation (mixed liquor from WWTP)

Liquid Scintillation Counting of Samples After Biofiltration

A. Without UV/H_2O_2

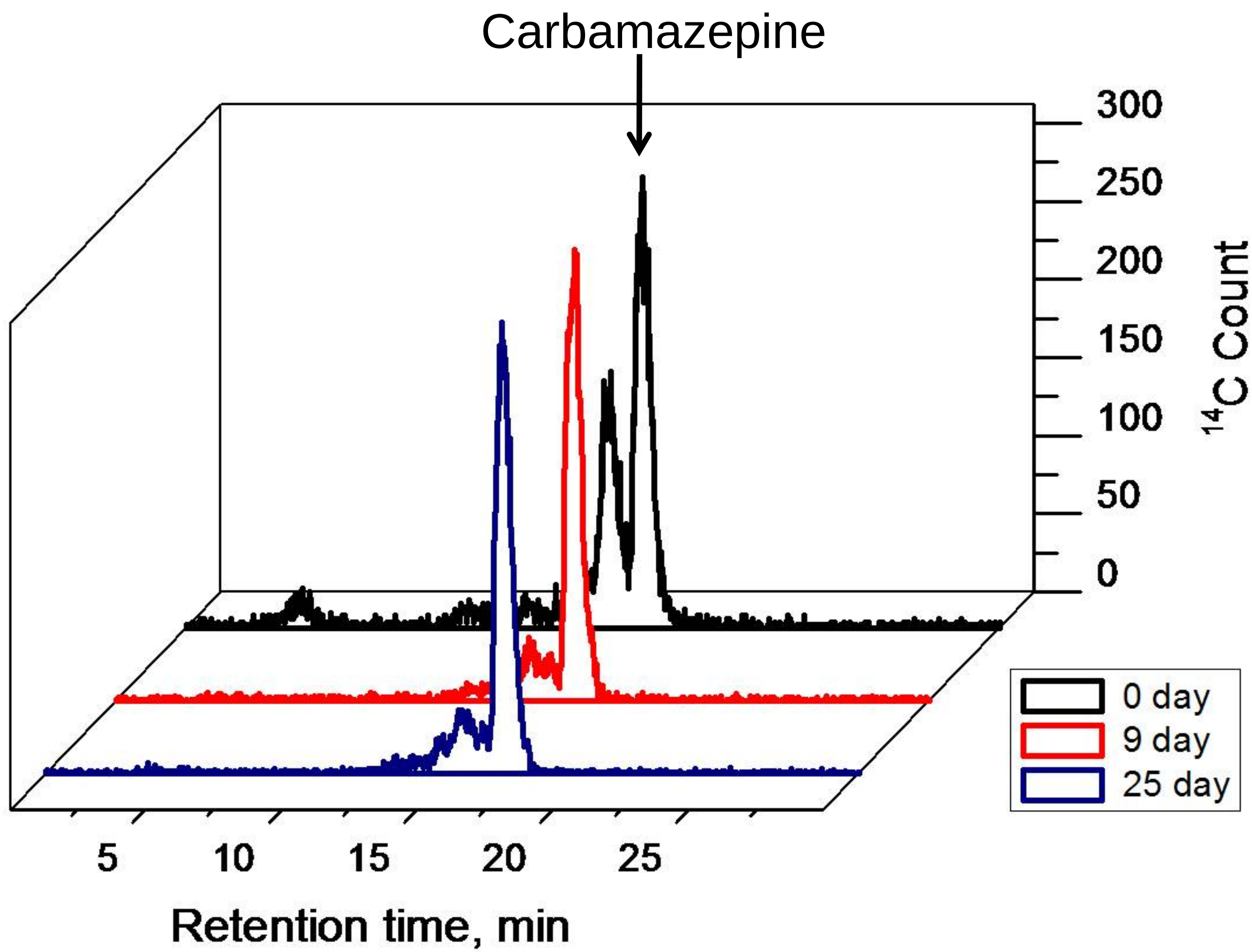


B. With UV/H_2O_2



Keen, O.S.; Baik, S.; Linden, K.G.; Aga, D.S.; Love, N.G. ***Environ. Sci. Technol*.** **2012**, *46,* 6222-27

Radiochromatograms of CBZ after AOP-Biofiltration



Keen et al. ***Environ. Sci. Technol.*** **2013, 6222-6227**

Environmental Science & Technology

Article

pubs.acs.org/est

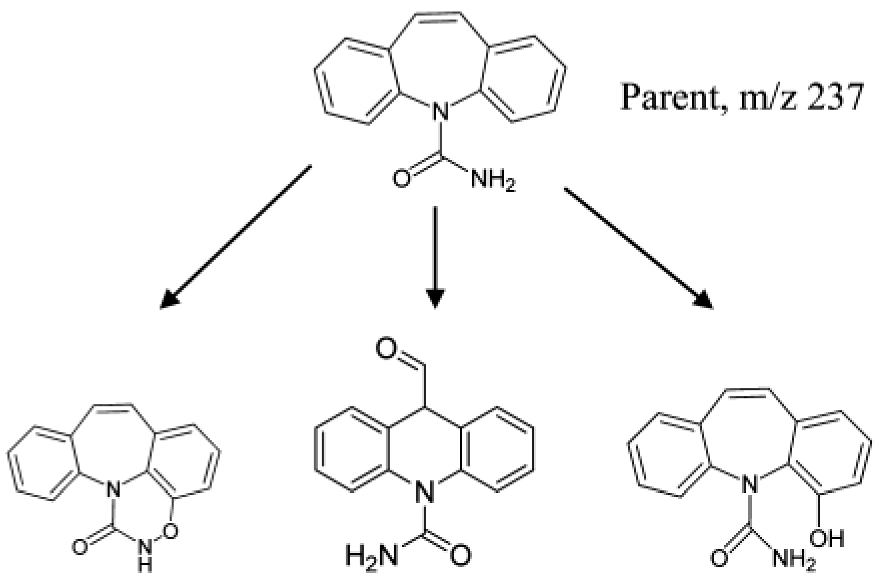
Enhanced Biodegradation of Carbamazepine after UV/H_2O_2 Advanced Oxidation

Olya S. Keen,[†] Seungyun Baik,[‡] Karl G. Linden,[*,†] Diana S. Aga,[‡] and Nancy G. Love[§]

[†]Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, Colorado 80309, United States

[‡]Chemistry Department, University at Buffalo, The State University of New York, Buffalo, New York 14260, United States

[§]Civil and Environmental Engineering Department, University of Michigan, Ann Arbor, Michigan 48109, United States

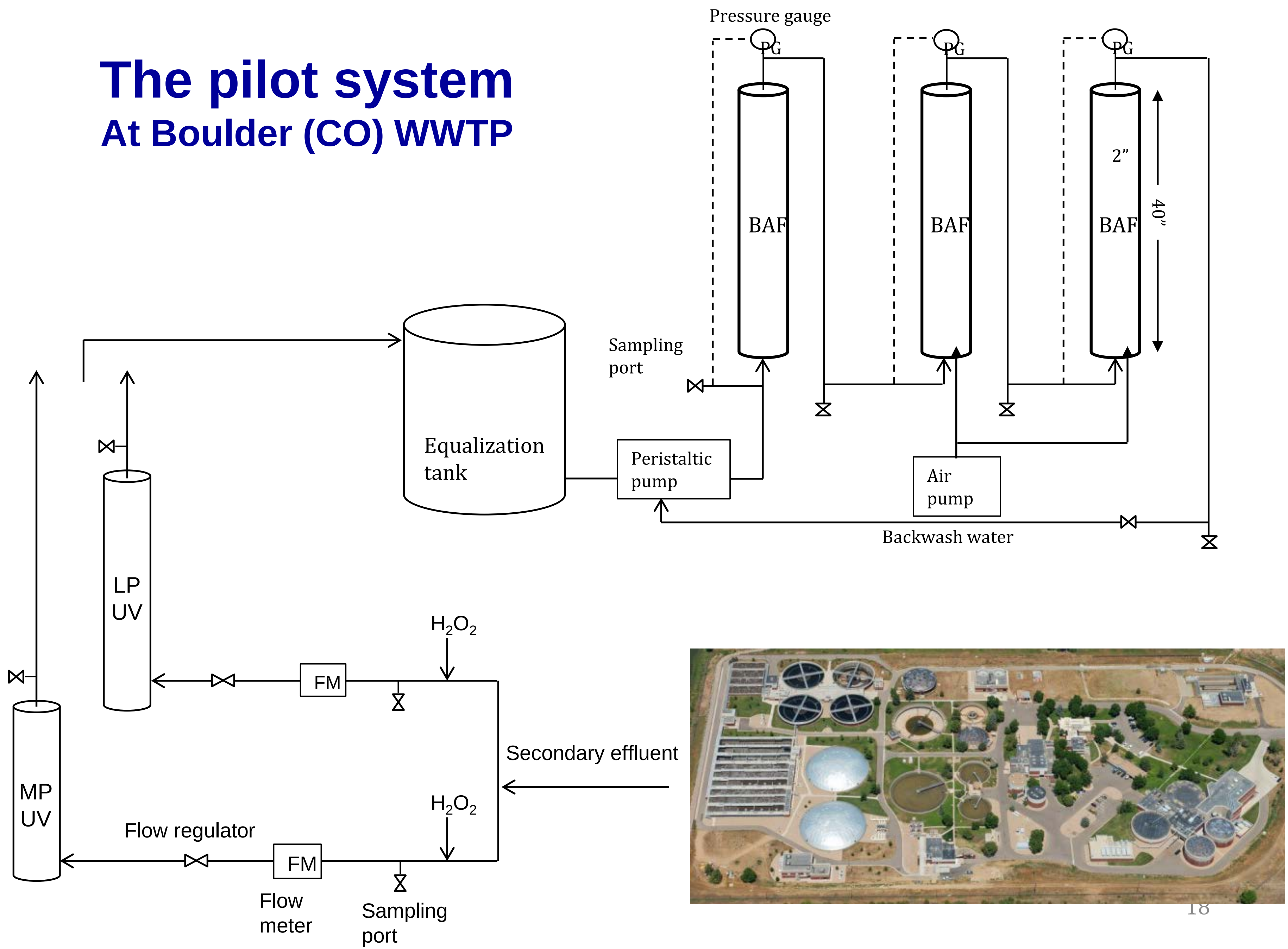


Parent, m/z 237

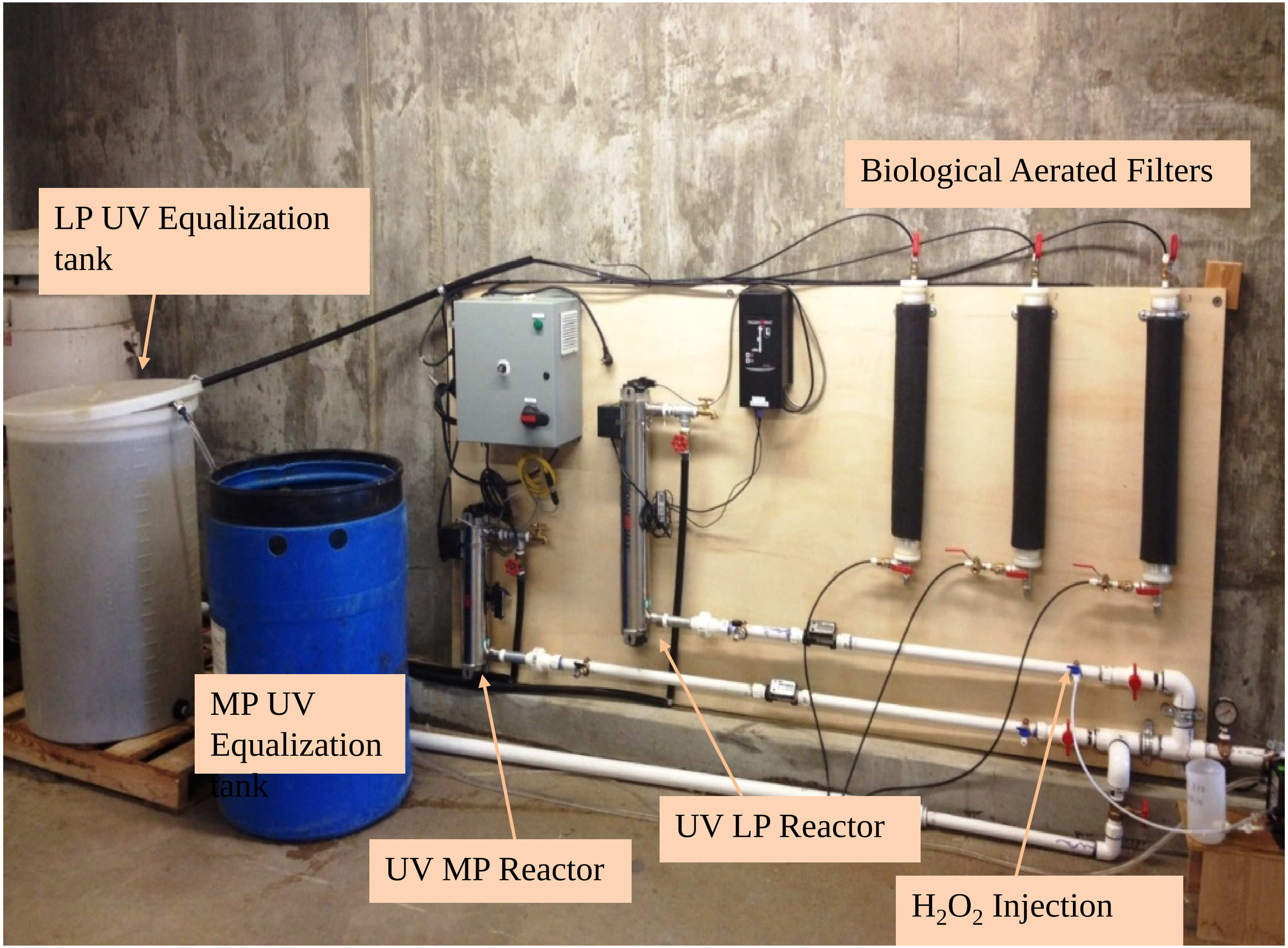
m/z 251 m/z 253 m/z 253

E. Marco-Urrea et. al. (2010) Water Research, 44,521-532
S. Chiron et. al. (2006) Env. Sci. Tech., 40, 5977-5983

The pilot system
At Boulder (CO) WWTP

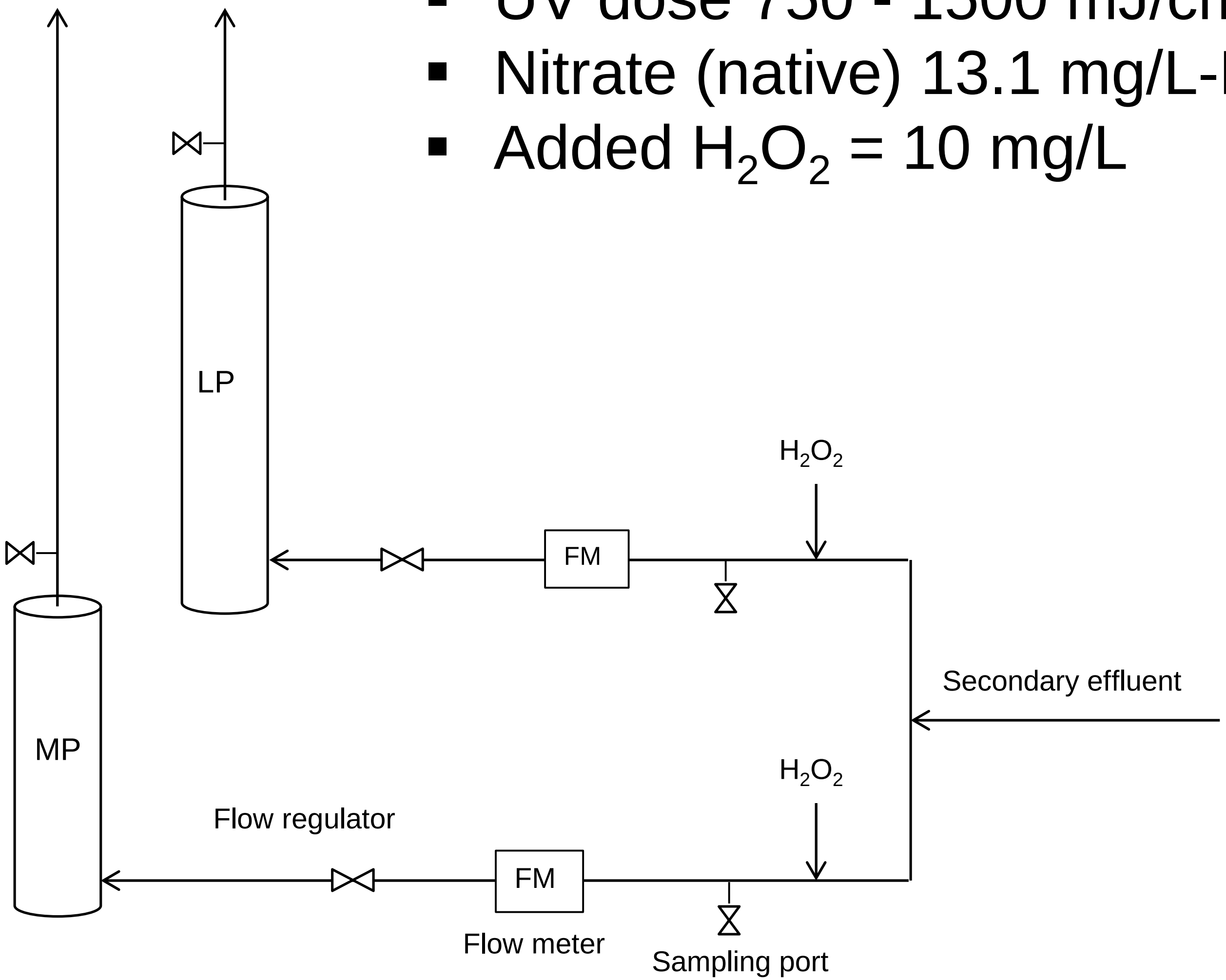


Pilot Scale System



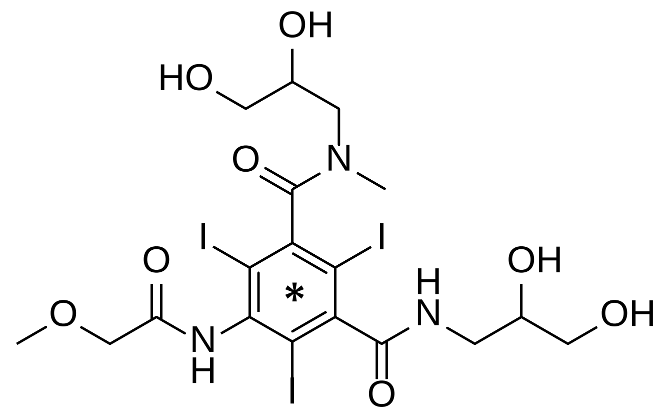
Operational Parameters

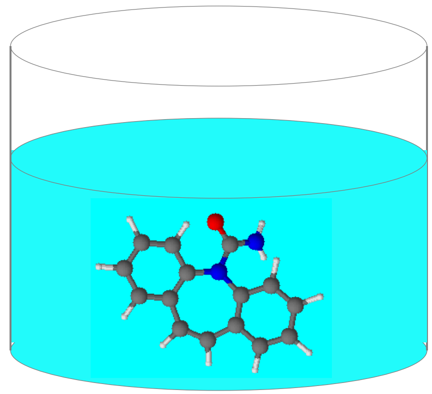
- UV dose 750 - 1500 mJ/cm^2
- Nitrate (native) 13.1 mg/L-N
- Added H_2O_2 = 10 mg/L

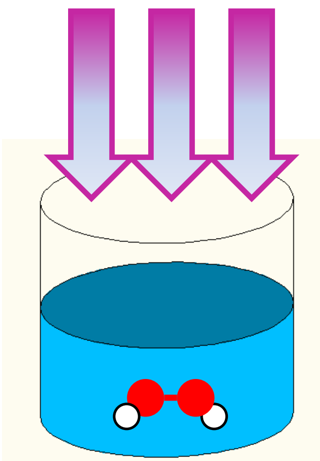


Advance Oxidation Process for Iopromide (IOP)

10 mg/L “cold” IOP + 0.3 mg/L “hot” IOP







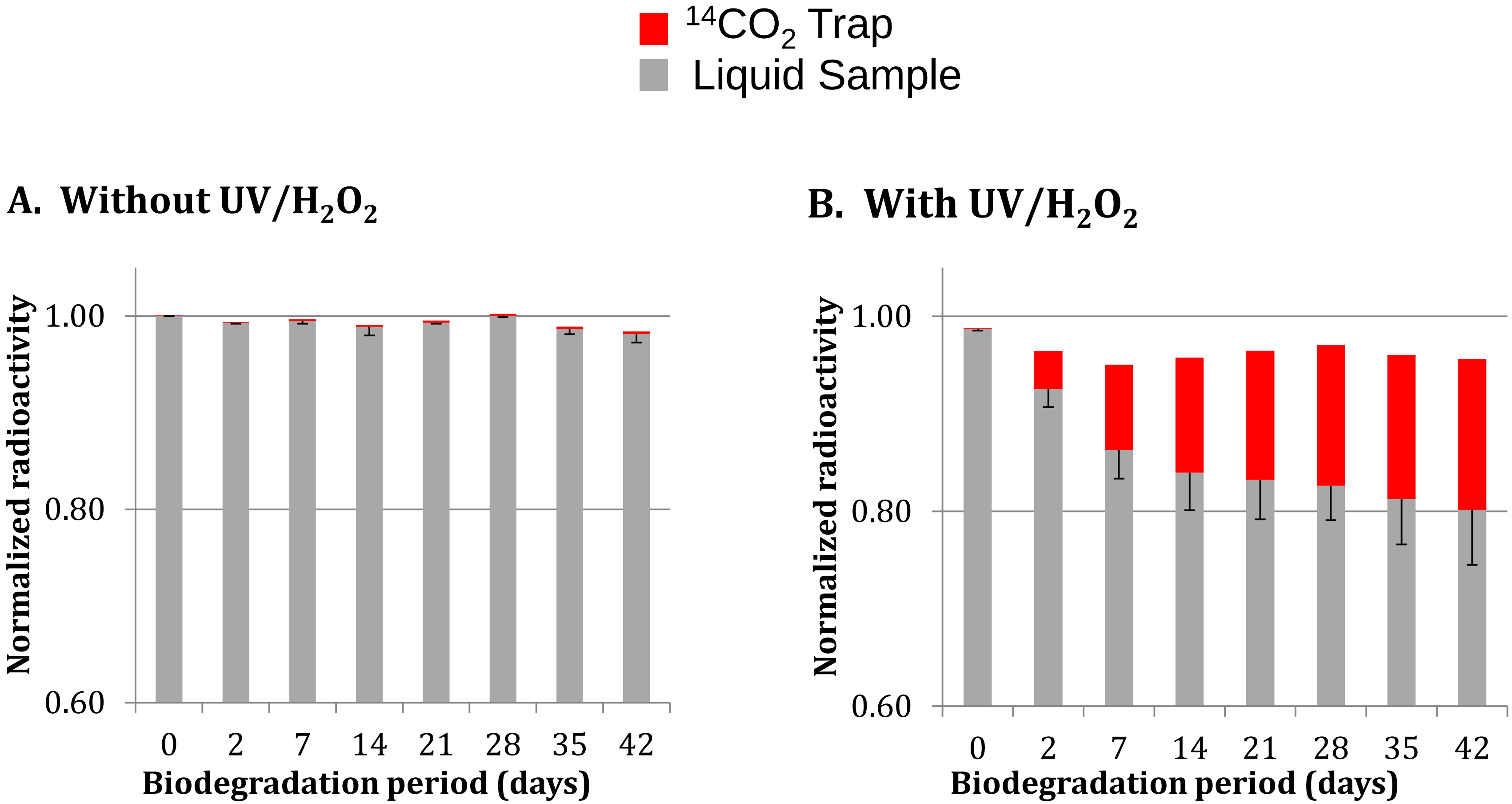
No treatment

Degrade H_2O_2 with bovine catalase

Advanced oxidation:
1800 mJ/cm^2
10 mg/L H_2O_2

Activated sludge biodegradation
(mixed liquor from WWTP)

Liquid Scintillation Counting After Biofiltration



Biodegradable Transformation Products of Iopromide

Environmental Science & Technology

Article

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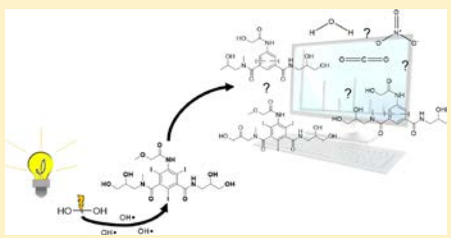
Application of Metabolite Profiling Tools and Time-of-Flight Mass Spectrometry in the Identification of Transformation Products of Iopromide and Iopamidol during Advanced Oxidation

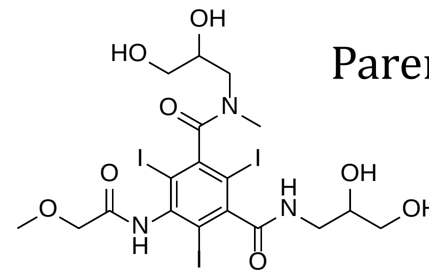
Randolph R. Singh,[†] Yaal Lester,[‡] Karl G. Linden,[‡] Nancy G. Love,[§] G. Ekin Atilla-Gokcumen,[†] and Diana S. Aga*[,†]

[†]Department of Chemistry, The State University of New York at Buffalo, Buffalo, New York 14260, United States
[‡]Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, Colorado 80309, United States
[§]Civil and Environmental Engineering Department, University of Michigan, Ann Arbor, Michigan 48109, United States

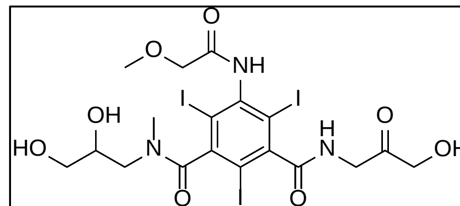
Supporting Information

ABSTRACT: The efficiency of wastewater treatment systems in removing pharmaceuticals is often assessed on the basis of the decrease in the concentration of the parent compound. However, what is perceived as "removal" during treatment may not necessarily mean mineralization of the pharmaceutical compound but simply conversion into different transformation products (TPs). Using liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (LC-QToF-MS), we demonstrated conversion of iopromide in wastewater to at least 14 TPs after an advanced oxidation process (AOP) using UV (fluence = 1500 mJ/cm^2) and H_2O_2 (10 mg/L). Due to the complexity of the wastewater matrix, the initial experiments were performed using a high concentration (10 mg/L) of iopromide in order to facilitate the identification of TPs. Despite the high concentration of iopromide used, cursory inspection of UV and mass spectra only revealed four TPs in the chromatograms of the post-AOP samples. However, the use of METLIN database and statistics-based profiling tools commonly used in metabolomics proved effective in discriminating between background signals and TPs derived from iopromide. High-resolution mass data allowed one to predict molecular formulas of putative TPs with errors below 5 ppm relative to the observed *m/z*. Tandem mass spectrometry (MS/MS) data and isotope pattern comparisons provided necessary information that allowed one to elucidate the structure of iopromide TPs. The presence of the proposed iopromide TPs was determined in unspiked wastewater from a municipal wastewater treatment plant, but no iopromide and TPs were detected. Using analogous structural modifications and oxidation that results from the AOP treatment of iopromide, the potential TPs of iopamidol (a structurally similar compound to iopromide) were predicted. The same mass fragmentation pattern observed in iopromide TPs was applied to the predicted iopamidol TPs. LC-QToF-MS revealed the presence of two iopamidol TPs in unspiked AOP-treated wastewater.

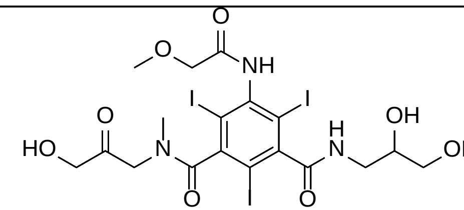




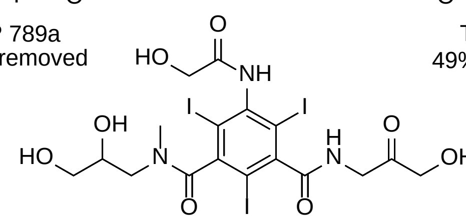
Parent Iopromide



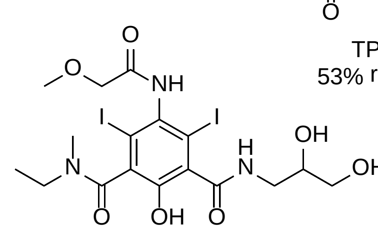
TP 789a
49% removed



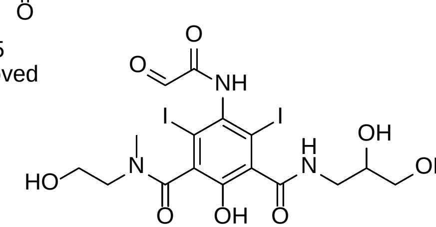
TP 789b
49% removed



TP 775
53% removed



TP 635a
40% removed



TP 635b
98% removed

Singh *et. al*, *Environmental Science and Technology* **2015,** *49*, 2983-2990

LESSON #3

Veterinary drugs are introduced into the environment through land application of manure

Table 1. Manure Production per 200 Cows*		
	Per Day (lb.)	**Per Year (LB)**
Feces and Urine	24,100	8,800,000
Total Solids	3,360	1,230,000
Volatile Solids	2,800	1,020,000
Total N	126	46,000
Total P	26	9,610
Total K	812	23,600

*does not include wastewater and bedding
*estimates may increase with milk production

http://www.epa.gov/agriculture/ag101/printdairy.html

Manure as fertilizer

- Lower cost of crop production
- Improve soil water infiltration
- Reduce soil erosion potential

2013 Sales of Antimicrobial Drugs Approved in Food-Producing Animals in the US

Class	Annual Total (Kg)
Tetracyclines	6,514,779
Ionophores (e.g. monensin)	4,434,657
Penicillins	828,721
Macrolides	563,251
Sulfonamides	384,371
Aminoglycosides	270,342
Lincosamides	236,450
Cephalosporins	28,337
Others (e.g. Polypeptides, Streptogramins)	1,527,646

US Food and Drug Administration, **2015**. *Summary Report: Antimicrobials Sold or Distributed for Use in Food- Producing Animals.*

JOURNAL OF
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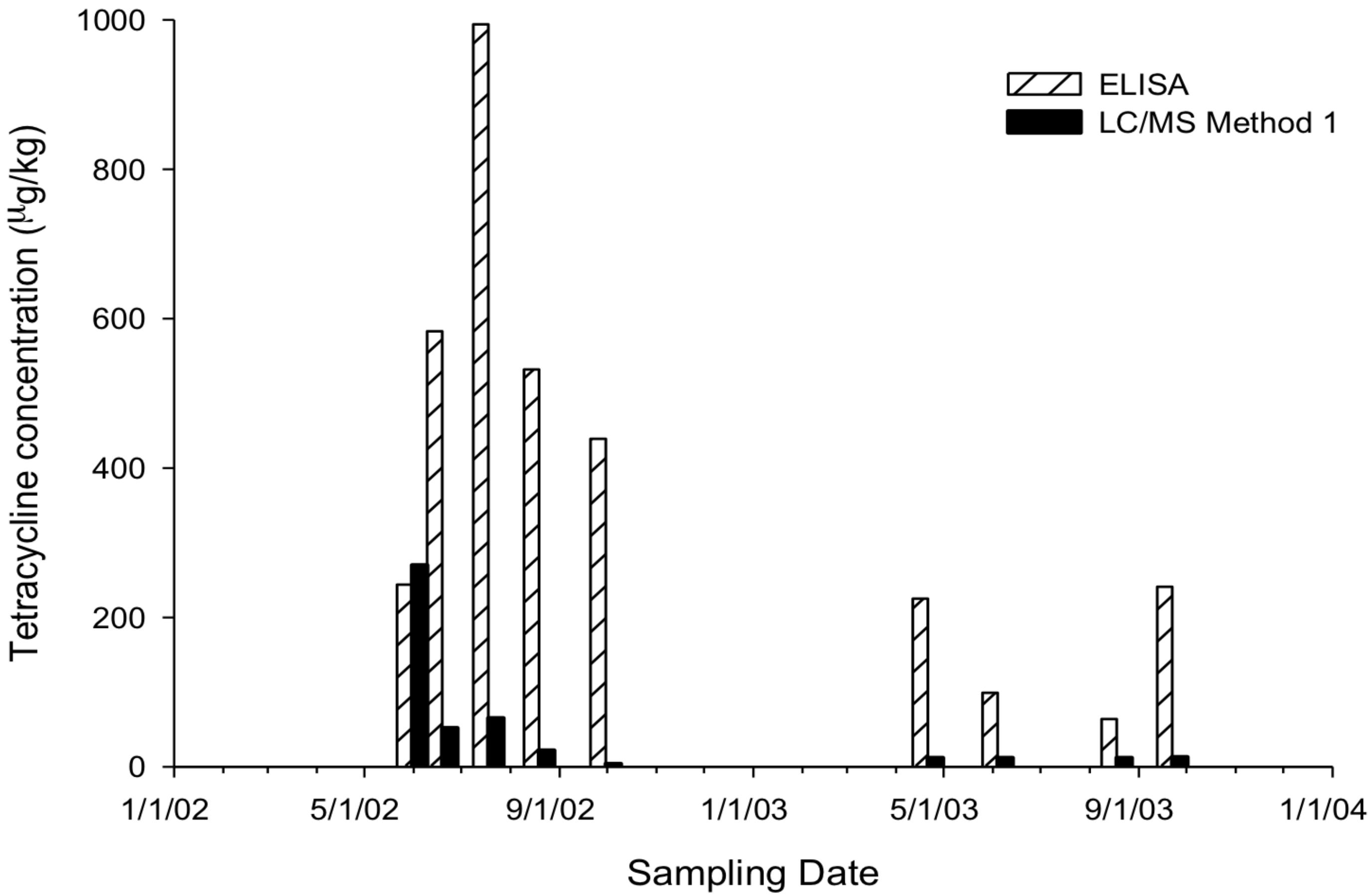
FOOD CHEMISTRY

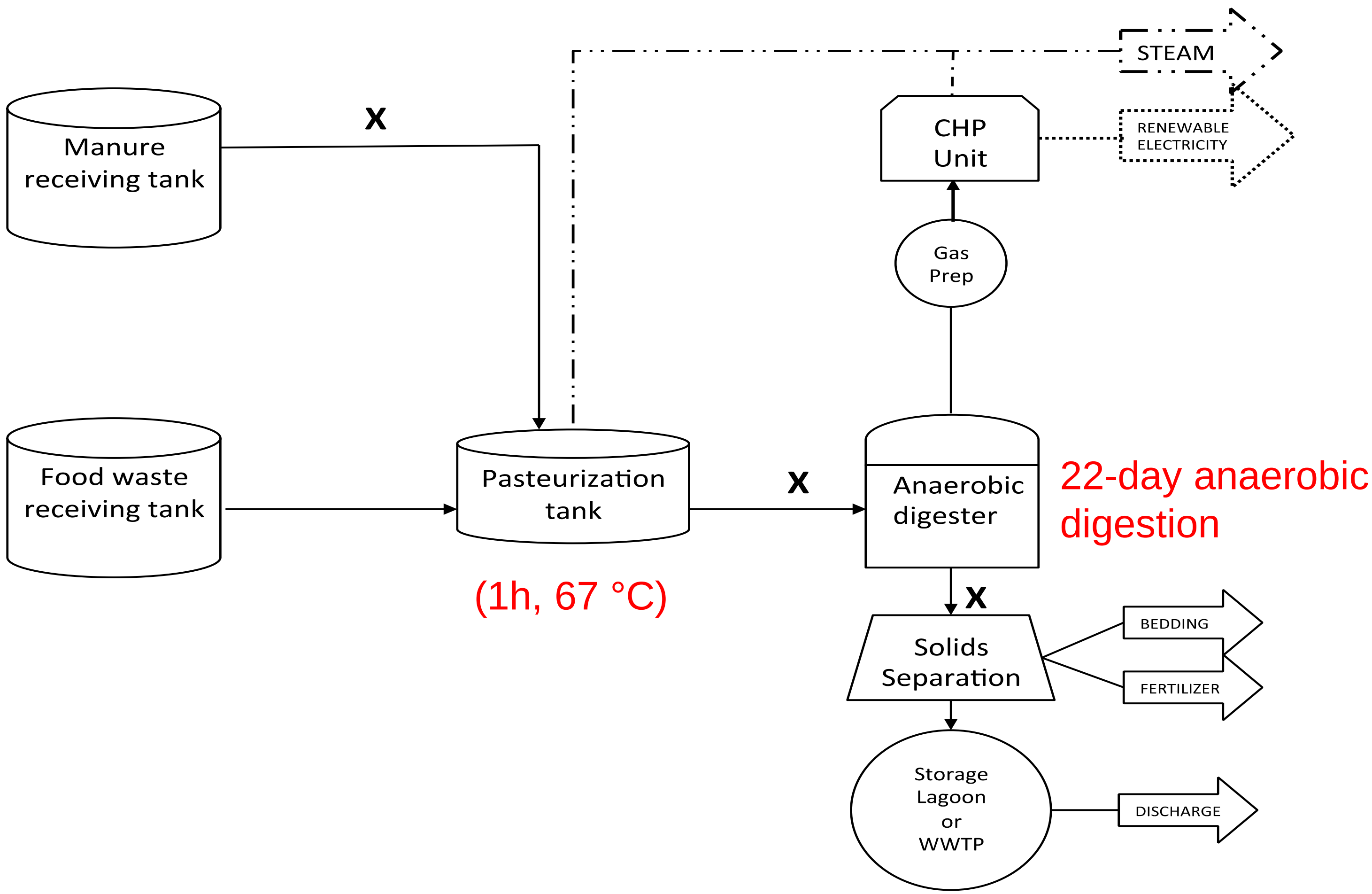


LESSON #4

Even advanced manure treatment systems do not remove antibiotics, hormones, and antibiotic resistance genes

A 2,200-cow dairy with an advanced digestion facility that includes a pasteurization step (1h, 67 °C), followed by a 22-day anaerobic digestion process.

Sampling Points in Advanced Digestion System

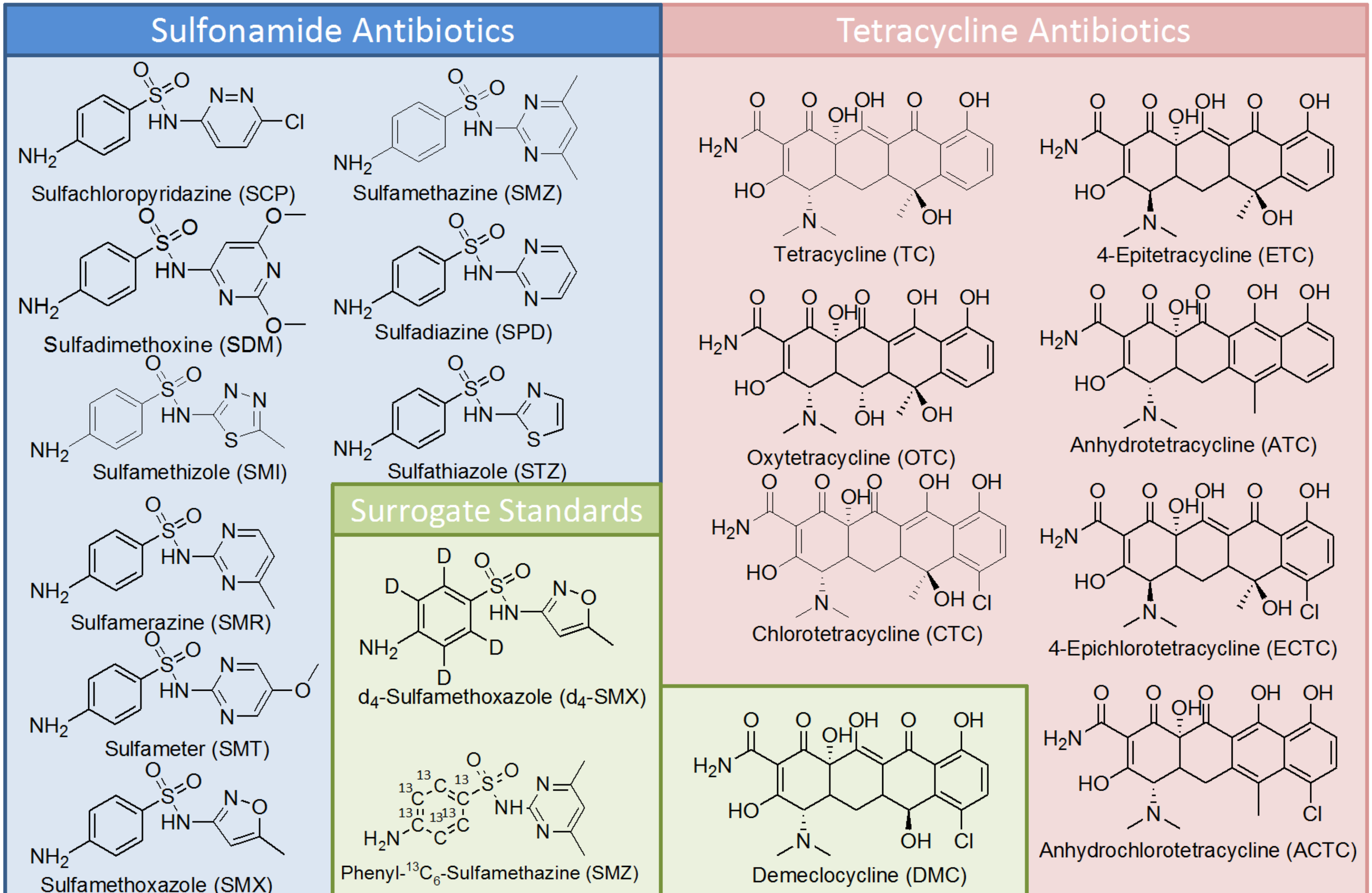


Antibiotics Used in Dairy Farms:

β-Lactams (penicillin, ampicillin, amoxicillin, ceftiofur, cephapirin)

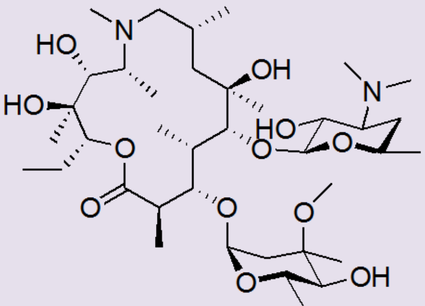
Tetracyclines (oxytetracycline), Sulfonamides

Target Analytes

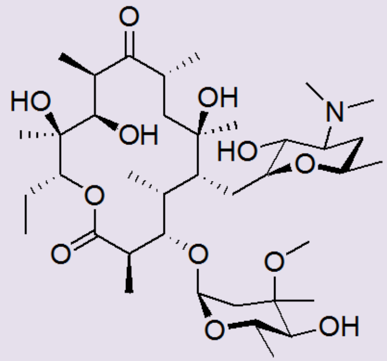


Target Analytes

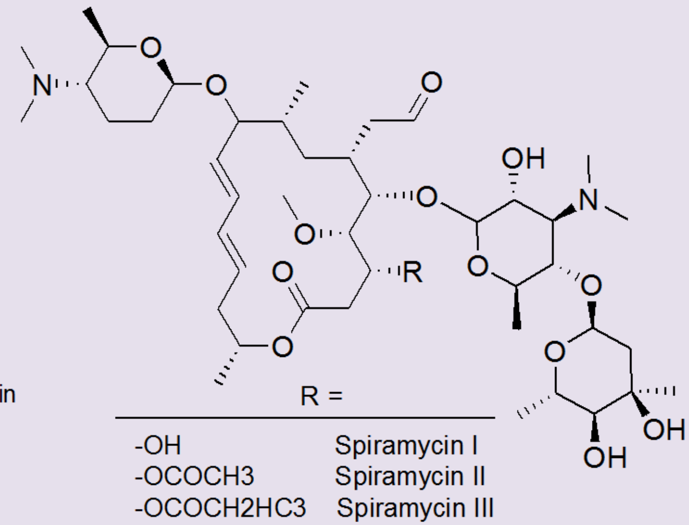
Macrolide Antibiotics

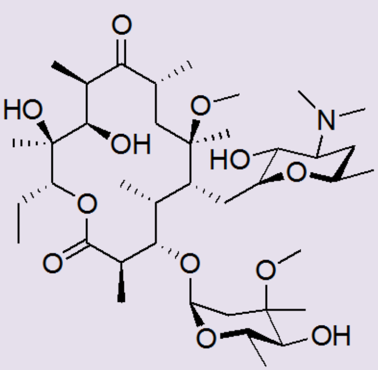


Azithromycin (AZI)

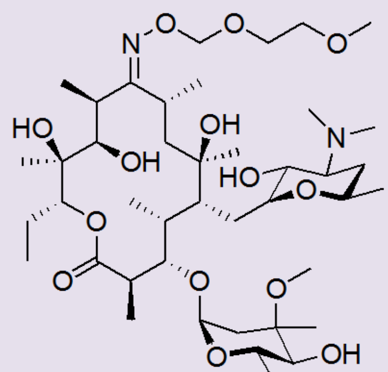


Erythromycin (ERY)
Surrogate Standard: N-Methyl [13]C Erythromycin

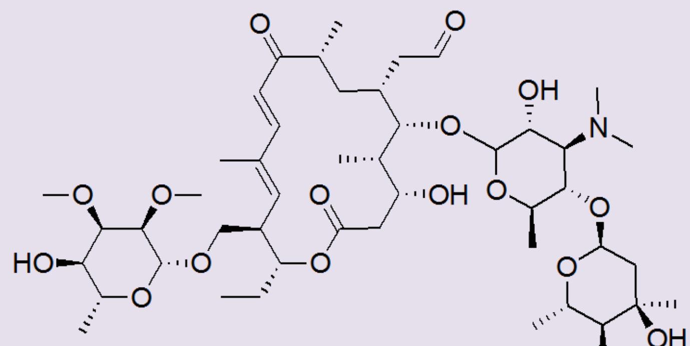




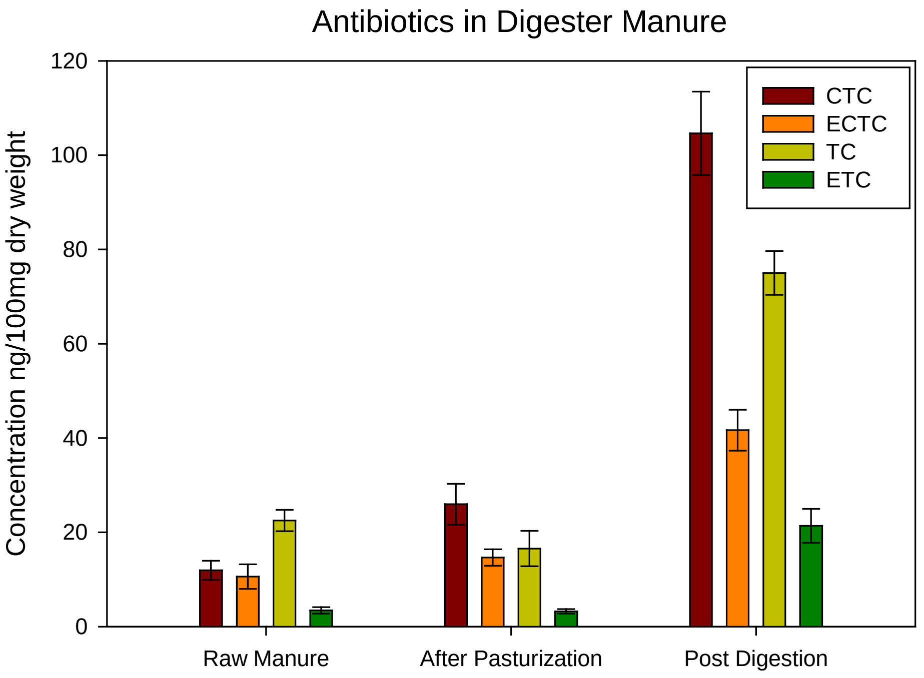
Clarithromycin (CLA)



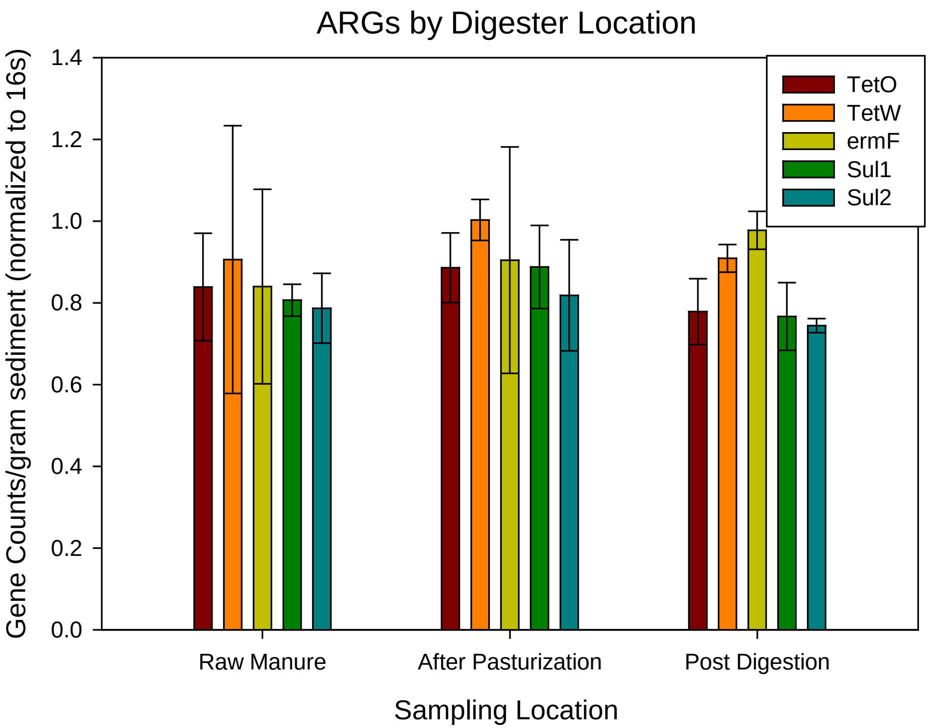
Roxithromycin (ROX)



Tylosin (TYL)



Antibiotics in Digester Manure
Concentration ng/100mg dry weight
0
20
40
60
80
100
120
CTC
ECTC
TC
ETC
Raw Manure
After Pasturization
Post Digestion



ARGs by Digester Location
Gene Counts/gram sediment (normalized to 16s)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
TetO
TetW
ermF
Sul1
Sul2
Raw Manure
After Pasturization
Post Digestion
Sampling Location

A new system that separates sand and manure solids into clean sand, usable crop nutrients and water clean enough to drink is making life a whole lot easier for Nate Hartway at McCormick Farms.

FROM MANURE SLURRY TO DRINKABLE WATER

New York dairy goes all in on nutrient recycling

BY JIM DICKRELL

Taking manure-laden sand bedding and segregating it back into clean sand, usable crop nutrients and water clean enough to drink doesn't come easy or cheap. But once the separation system is refined and working, it definitely makes life a whole lot easier for Nate Hartway, finance and environmental compliance manager for McCormick Farms, Bliss, N.Y. The farm milks 2,000 cows and farms some 8,500 acres of corn, alfalfa and potatoes. The operation spans five counties with some fields up to 40 miles from the dairy operation.

McCormick Farms installed a Livestock Water Recycling (LWR) System a year ago. And after some initial hiccups and start-up problems, the system is now operating pretty much as designed and promised.

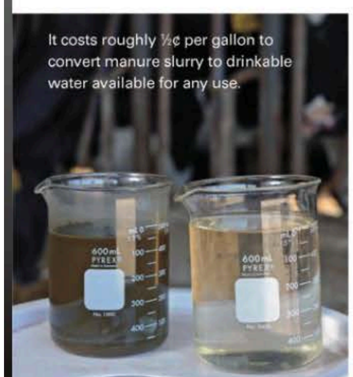
Manure-laden sand from three freestall barns is now being cleaned and can be re-bedded just a month after processing. The nutrients in the manure are being separated into nitrogen/potash and a phosphorus/organic matter components.

"Prior to installing the system, we were struggling to reclaim and clean-up the sand," Hartway says. The farm had been recycling water from one of its lagoons, but the dissolved nutrients in the lagoon water stayed in suspension and created a syrup-like goo that clogged up the liquid/solids separation press to the point where nothing came out.

About the only solution was to completely drain the lagoon and start over with fresh water. The logistics of doing that with the dairy still in operation were daunting.

After visiting dairies in New York and Michigan, Hartway and Jim McCormick, owner of McCormick Dairy, decided to go with the LWR System. "The Michigan dairy was sand bedded and had a set-up similar to us," Hartway says. "That convinced us to go this route."

Sand flushed from the freestall barns goes through a sand lane where the larger sand particles drop out. Then, the slurry is sent through a screen separator where the larger manure particles are pressed out.



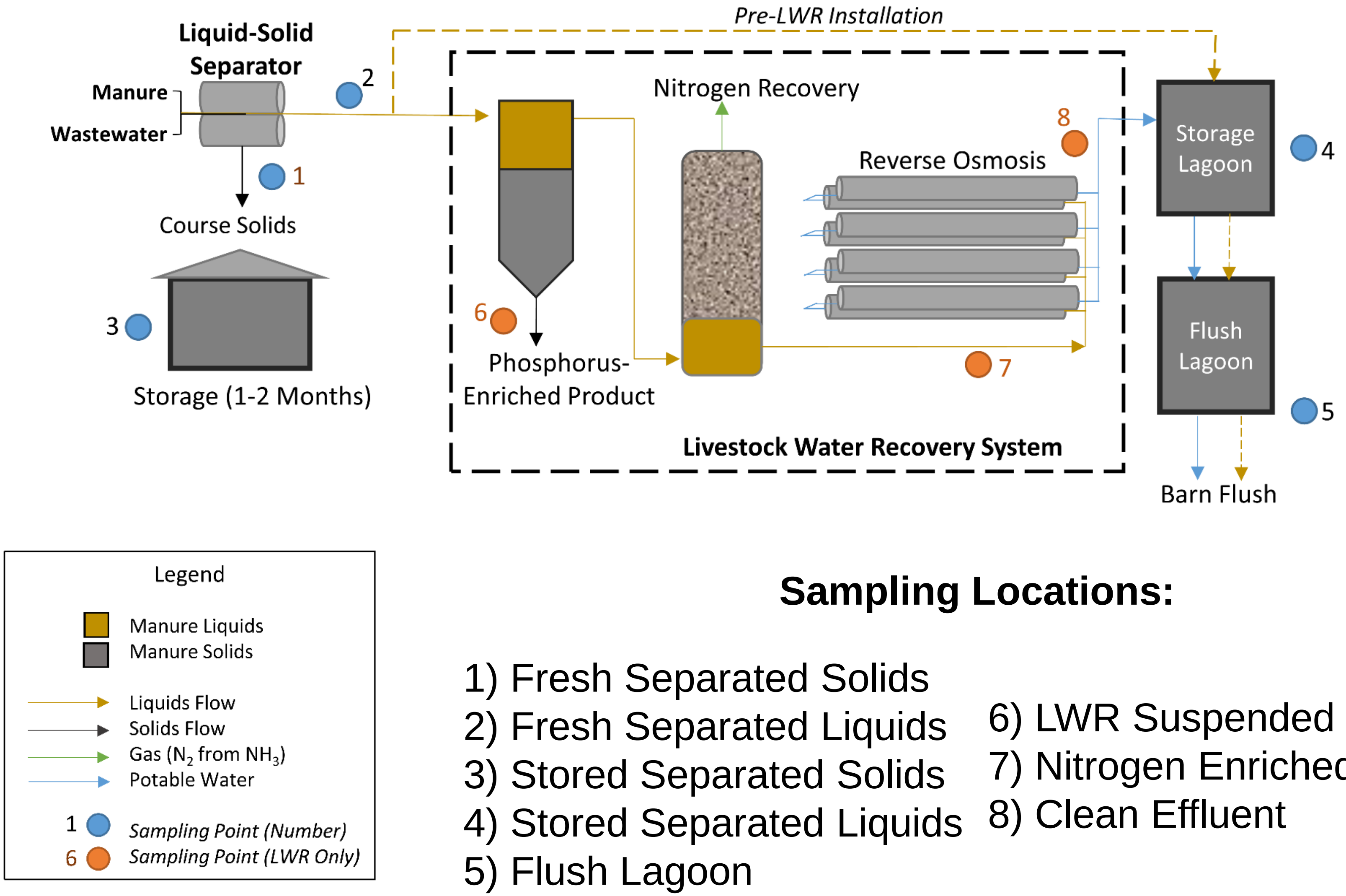
It costs roughly ½¢ per gallon to convert manure slurry to drinkable water available for any use.

PHOTOS BY PHIL MATT

Analysis of Ionophore in Manure treated by Livestock Water Recycling (LWR) Unit

The system can extract up to 70 percent of the water out of the manure while leaving the nutrients in a separate concentrated form.

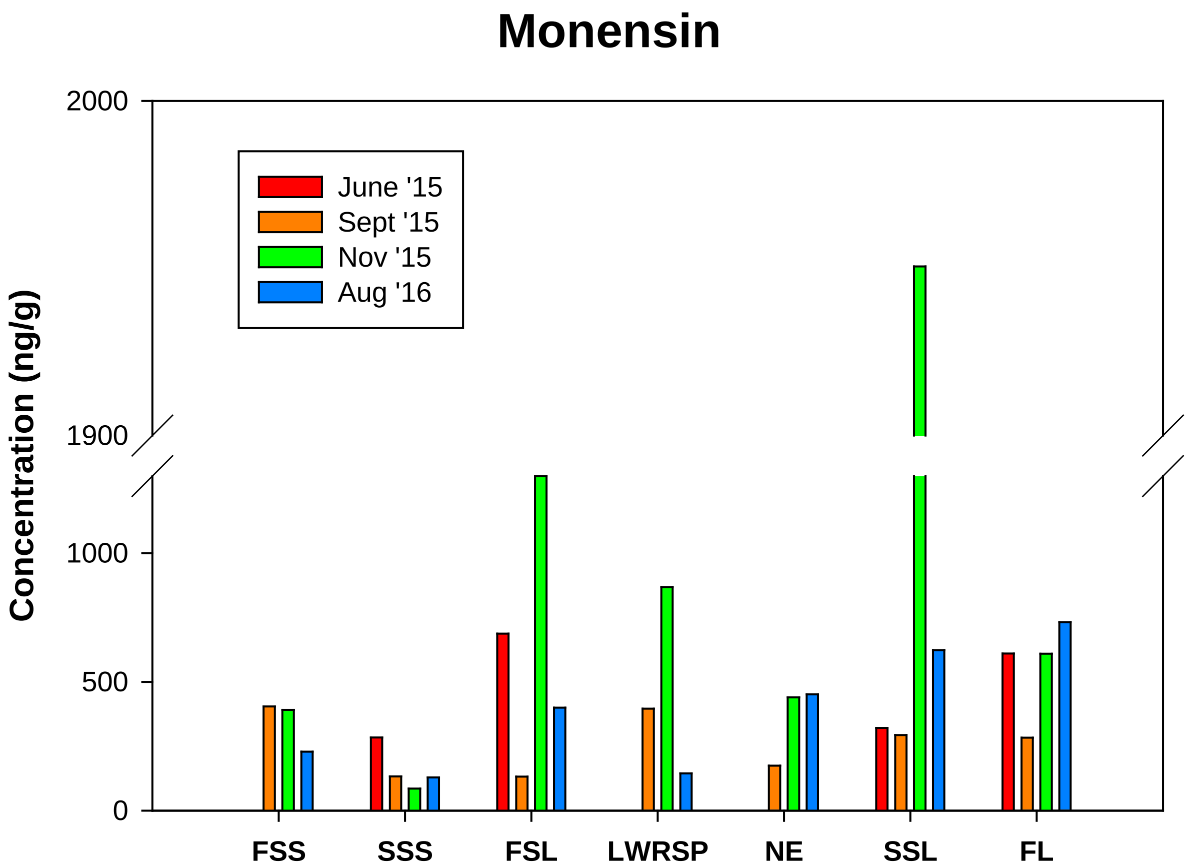
Sampling points in Dairy Farm with LWR



Sampling Locations:

1) Fresh Separated Solids
2) Fresh Separated Liquids
3) Stored Separated Solids
4) Stored Separated Liquids
5) Flush Lagoon
6) LWR Suspended Particulate
7) Nitrogen Enriched
8) Clean Effluent

Ionophore Concentrations in Solid Manure



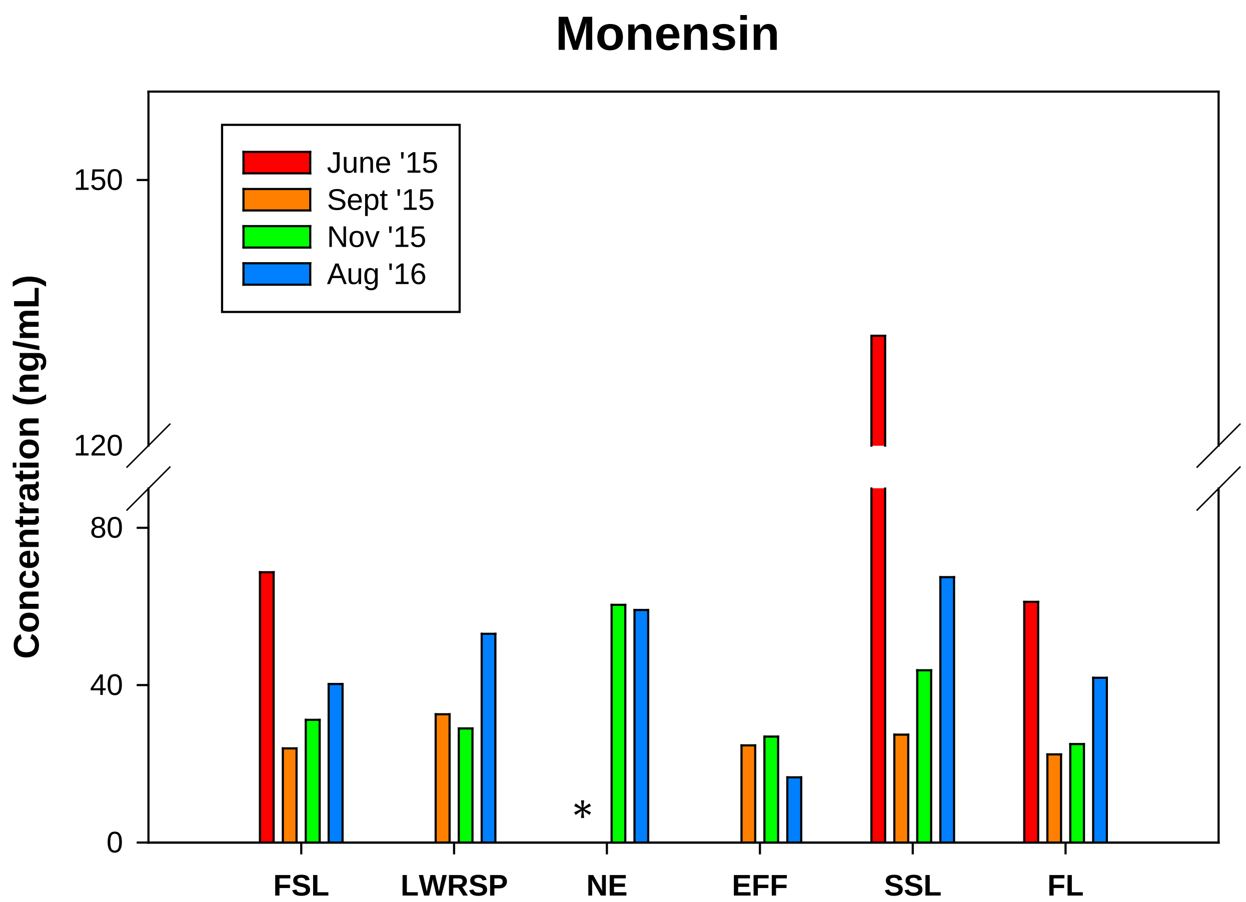
n=2 samples per condition

FSS-Fresh Separated Solids
SSS-Stored Separated Solids
FSL-Fresh Separated Liquids

LWRSP-LWR Suspended Particulate
NE-Nitrogen Enriched
SSL-Stored Separated Liquids

FL-Flush Lagoon

Ionophore Concentrations in Liquid Manure



n=2 samples per sampling site

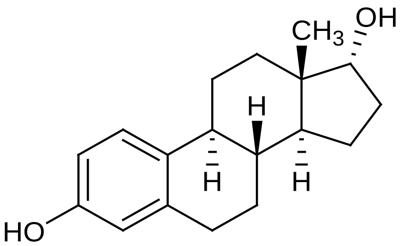
*MON at Sept NE location <LOQ

FSL-Fresh Separated Liquids
LWRSP-LWR Suspended Particulate
NE-Nitrogen Enriched

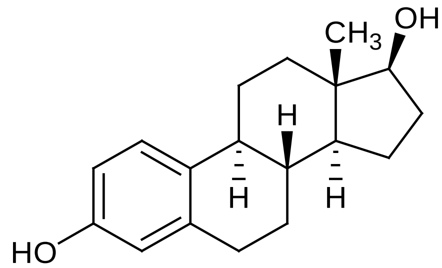
EFF-Clean Water Effluent
SSL-Stored Separated Liquids
FL-Flush Lagoon

LESSON #5

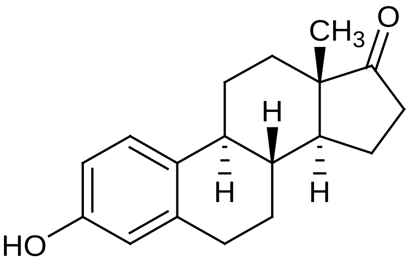
Decrease in concentration of parent compound does not always mean “removal”



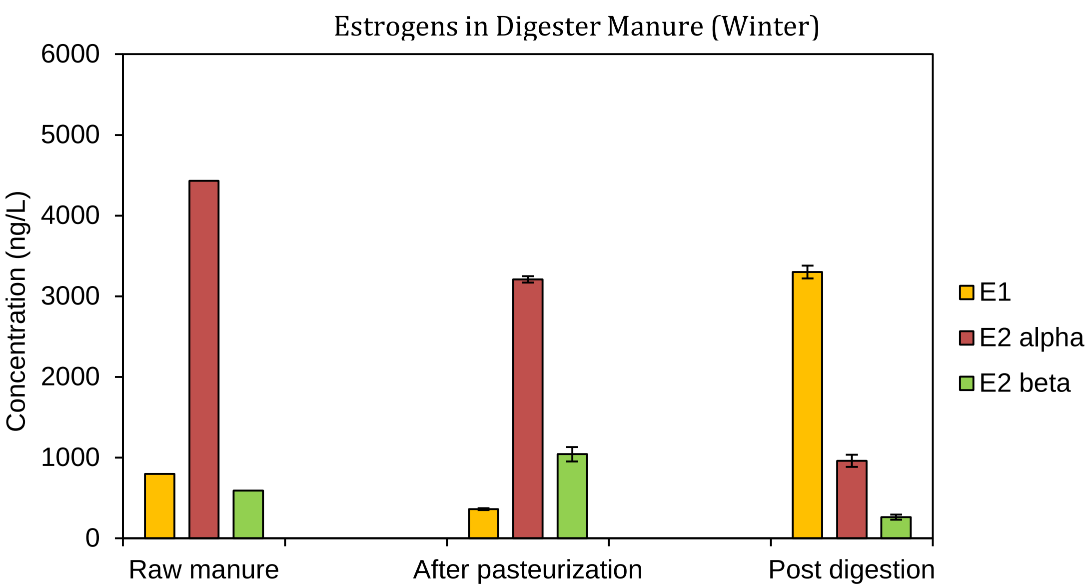
17α-estradiol **(17α-E2)**



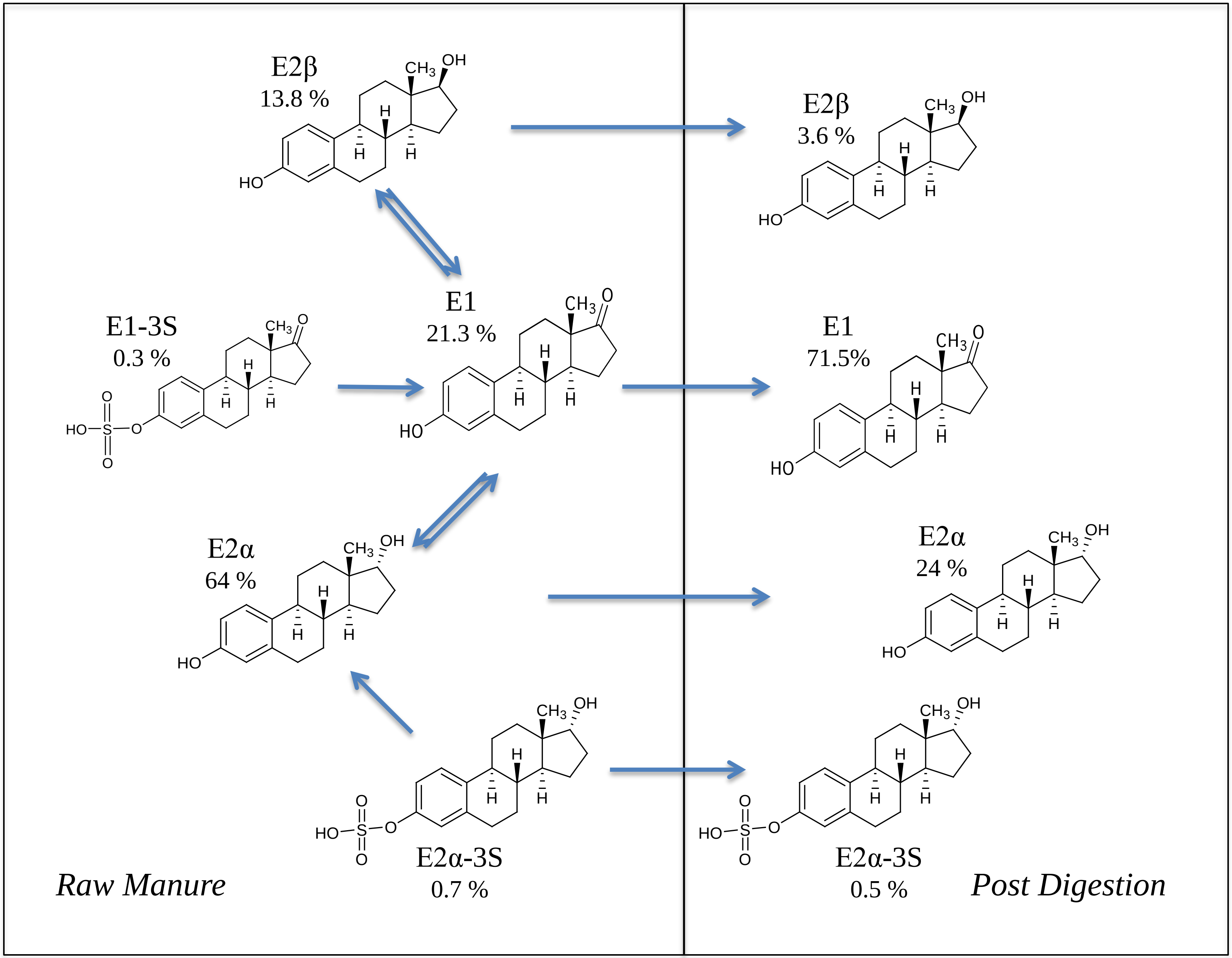
17β-estradiol **(17β-E2)**



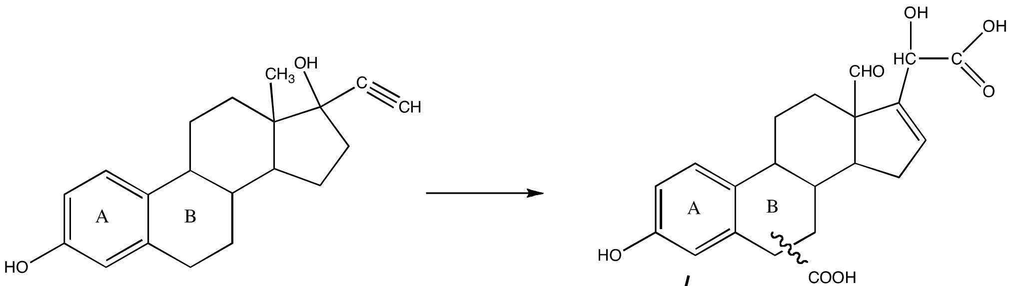
Estrone **(E1)**



Inter-conversion of Estrogens During Anaerobic Digestion



Transformation of EE2 by Ammonia Oxidizing Bacteria

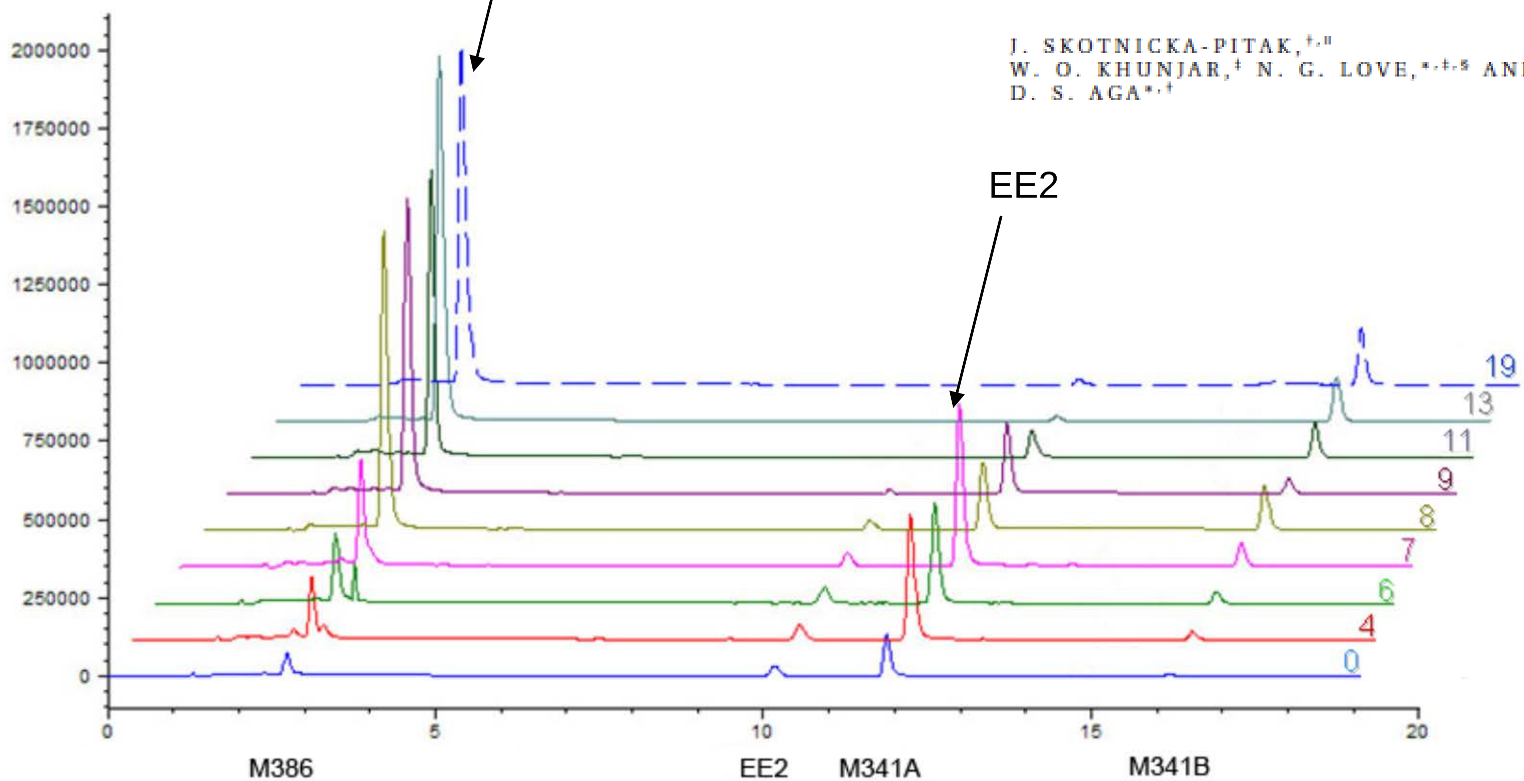


EE2

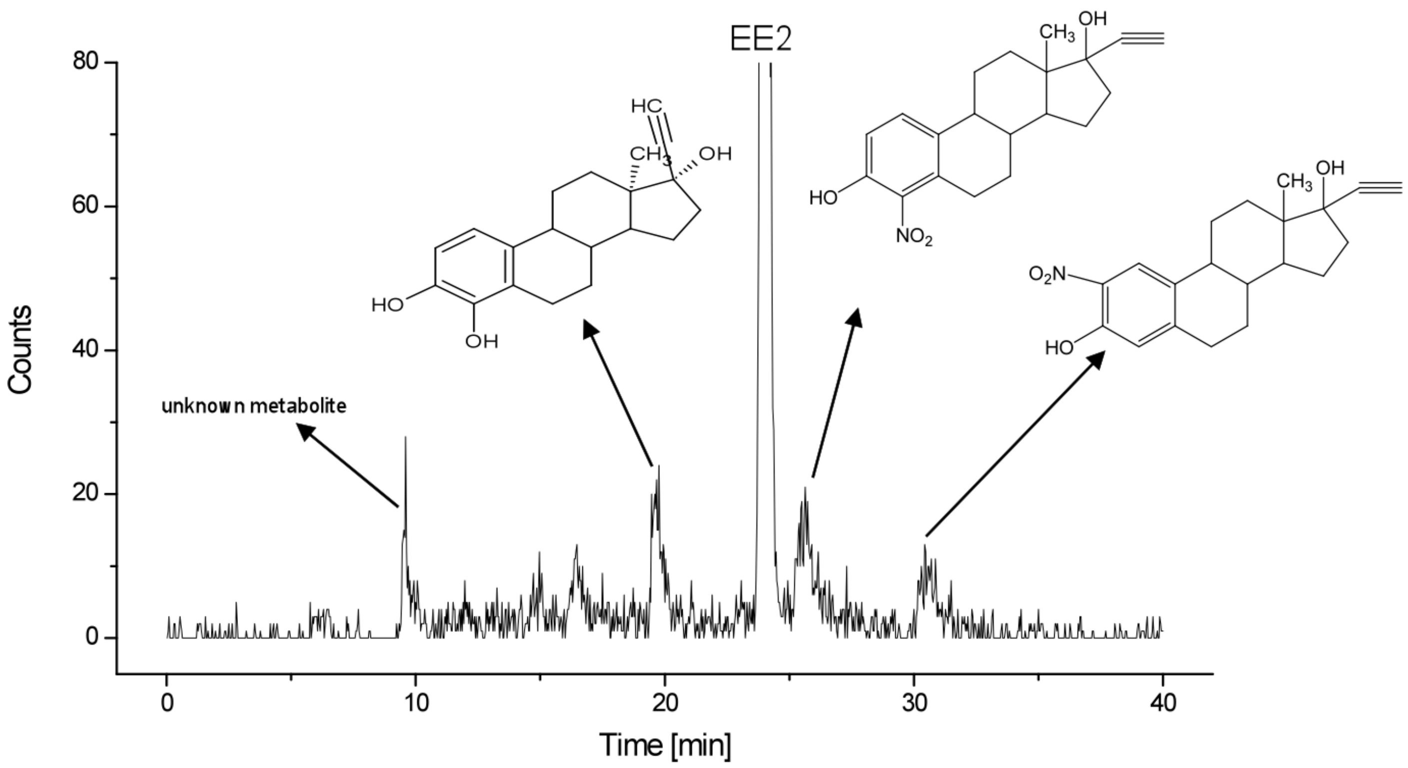
Environ. Sci. Technol. **2009**, *43*, 3549-3555

Characterization of Metabolites Formed During the Biotransformation of 17α-Ethinylestradiol by *Nitrosomonas europaea* in Batch and Continuous Flow Bioreactors

J. SKOTNICKA-PITAK,[†,‖]
W. O. KHUNJAR,[‡] N. G. LOVE,[*,‡,§] AND
D. S. AGA[*,†]



Radiochromatogram Reveals Additional Metabolites in Heterotrophic Cultures



Skotnicka-Pitak, et al. Environ. Sci. Technol. **2009**, *43*, 3549-3555

Importance of Transformation Products

Environmental Toxicology and Chemistry, Vol. 28, No. 12, pp. 2473–2484, 2009
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Critical Review

PHARMACEUTICAL METABOLITES IN THE ENVIRONMENT: ANALYTICAL CHALLENGES AND ECOLOGICAL RISKS

MARY D. CELIZ, JERRY TSO, and DIANA S. AGA*
Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York 14260, USA

Environ. Sci. Technol. 2012, 46, 10485-10486

Viewpoint

pubs.acs.org/est

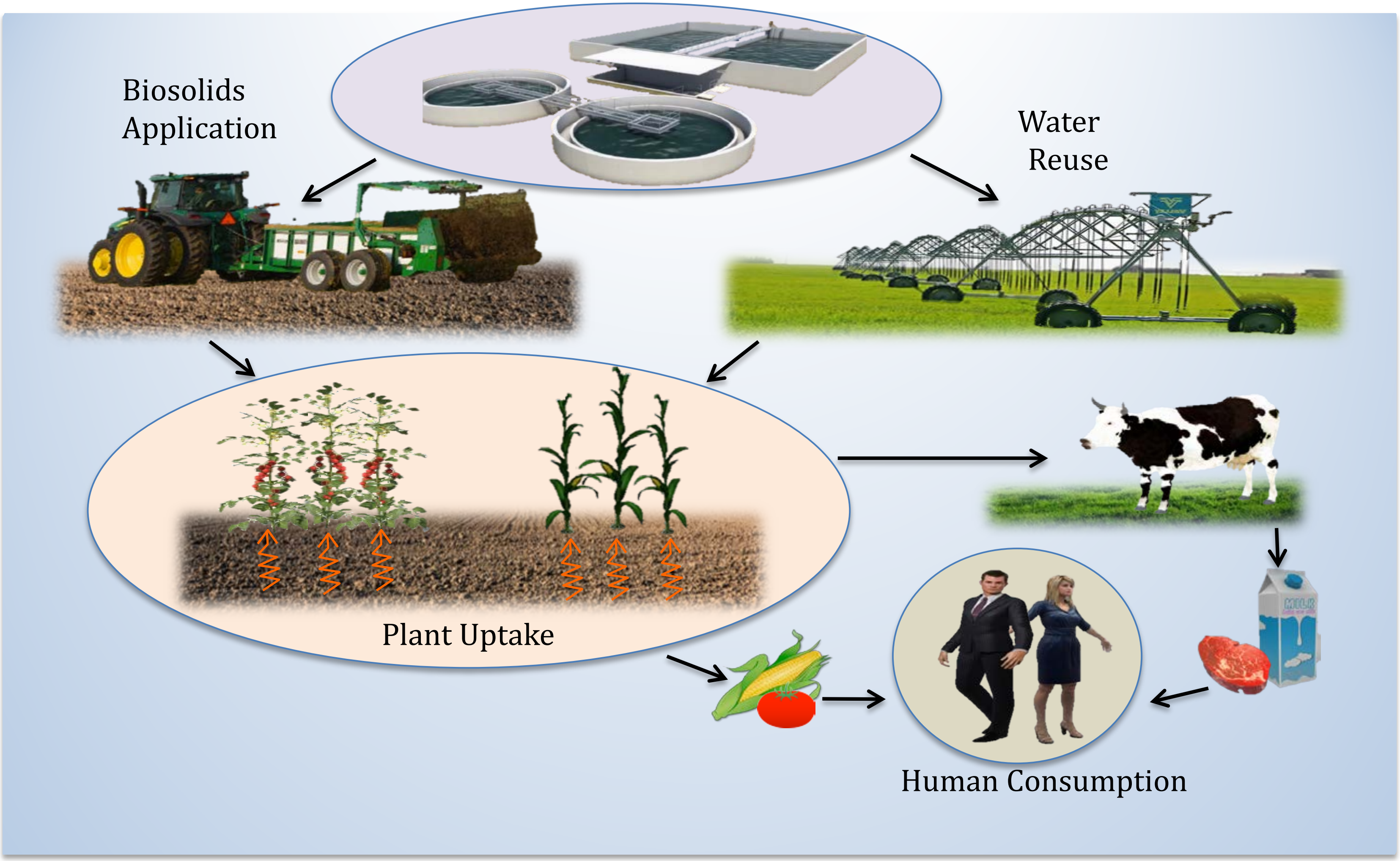
Micropollutant Fate in Wastewater Treatment: Redefining "Removal"

Lauren B. Stadler,*,† Alexi S. Ernstoff,‡ Diana S. Aga,§ and Nancy G. Love†

†Department of Civil & Environmental Engineering, University of Michigan, Ann Arbor, Michigan 48109, United States
‡Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, Michigan 48109, United States
§Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York 14260, United States

LESSON #6

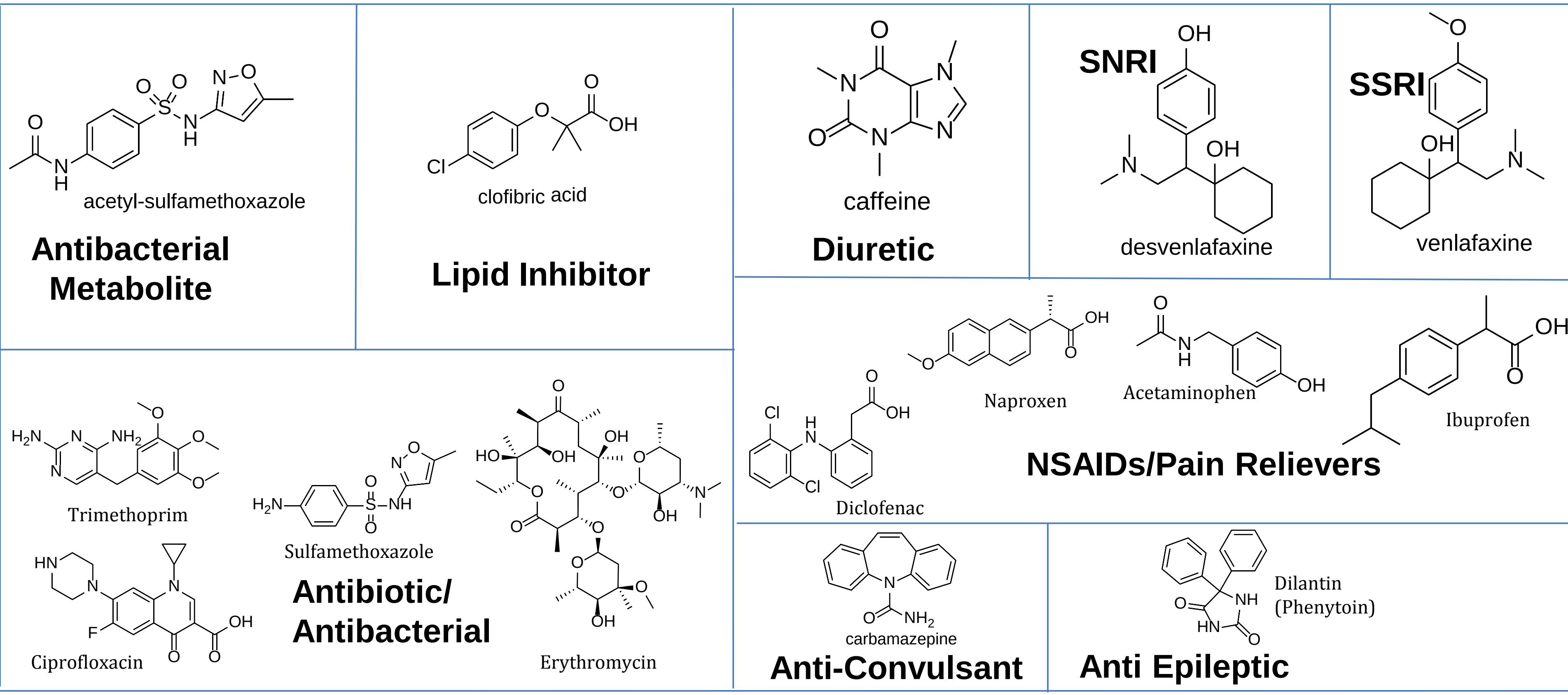
Emerging contaminants are spread in the environment and may potentially reach food products consumed by humans



Field study on plant uptake of pharmaceuticals

Pharmaceuticals Tested

Target pharmaceuticals available as OTC and prescribed medication



Use of Urine and Struvite as Fertilizer

Urine has naturally high levels of N,P,K, exact ratios are diet dependent, but ~10:1:4

<1% of the total volume of wastewater

WWTP

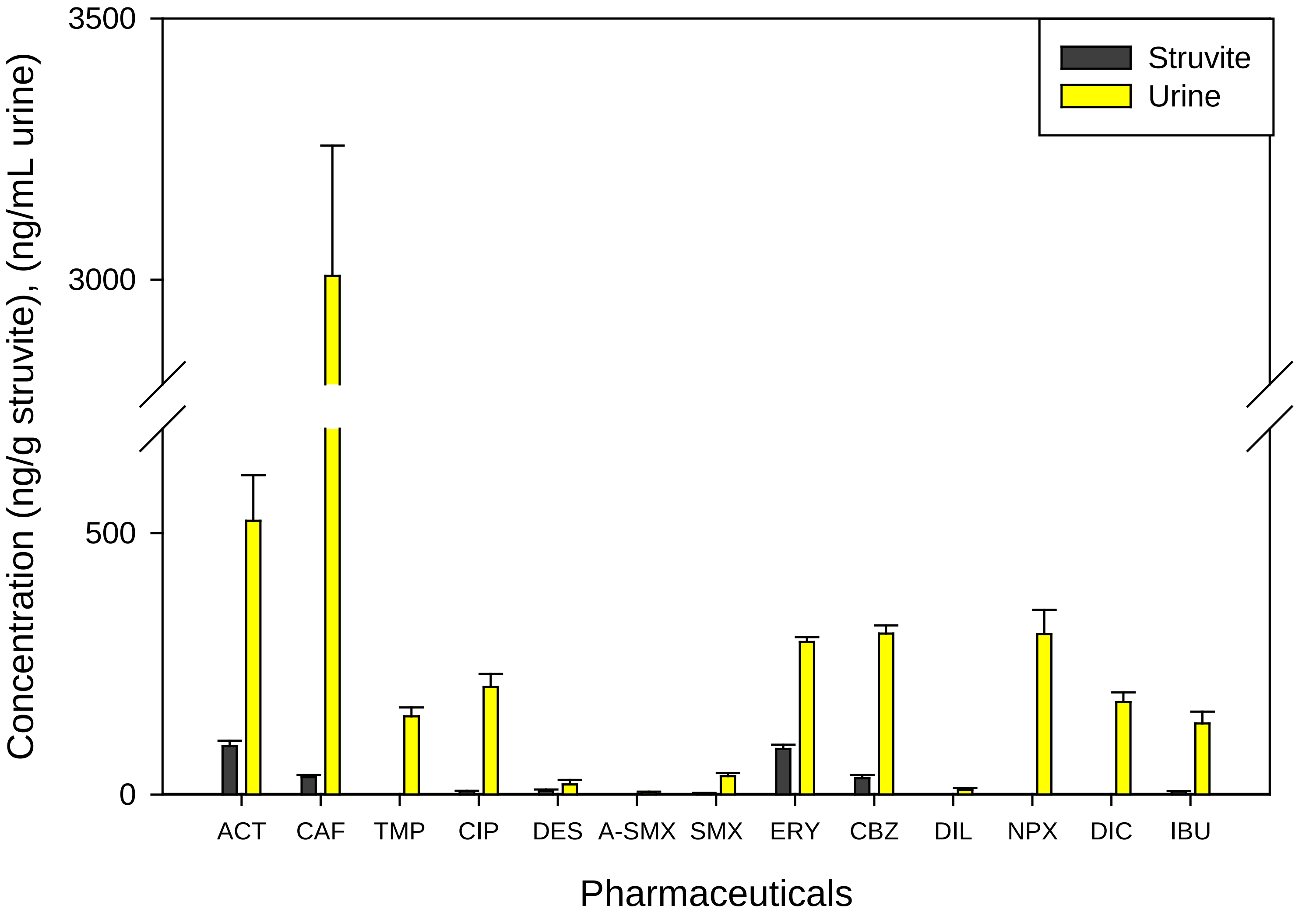
Direct use as liquid fertilizer

Precipitate as Struvite or magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) and use as solid fertilizer

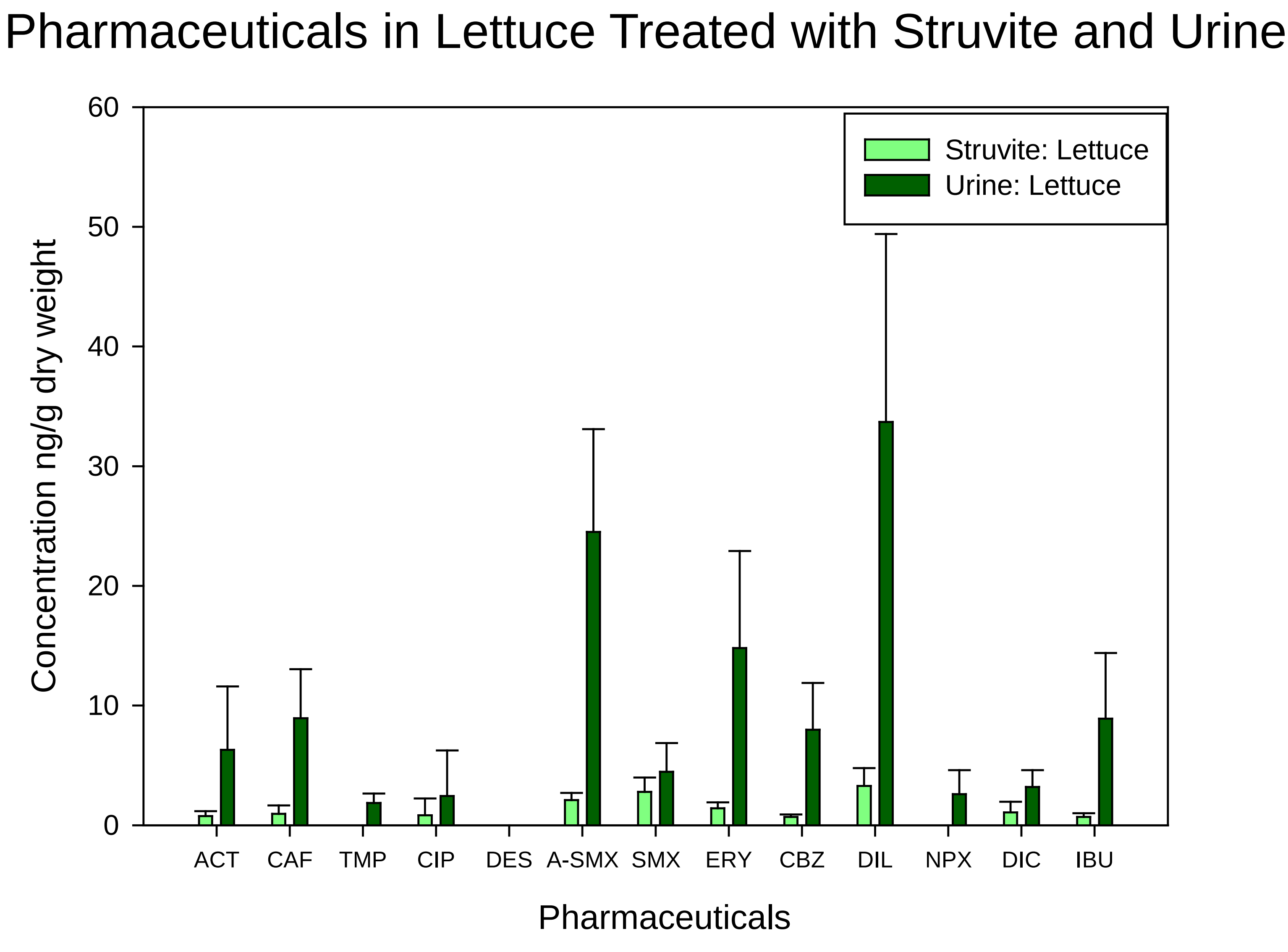
Lettuce and Carrot Sampling Set-Up

- Lettuce and carrots are sampled when “market” ready
- They are washed to simulate typical salad preparation

Pharmaceuticals in Urine and Struvite (using the same urine feedstock)

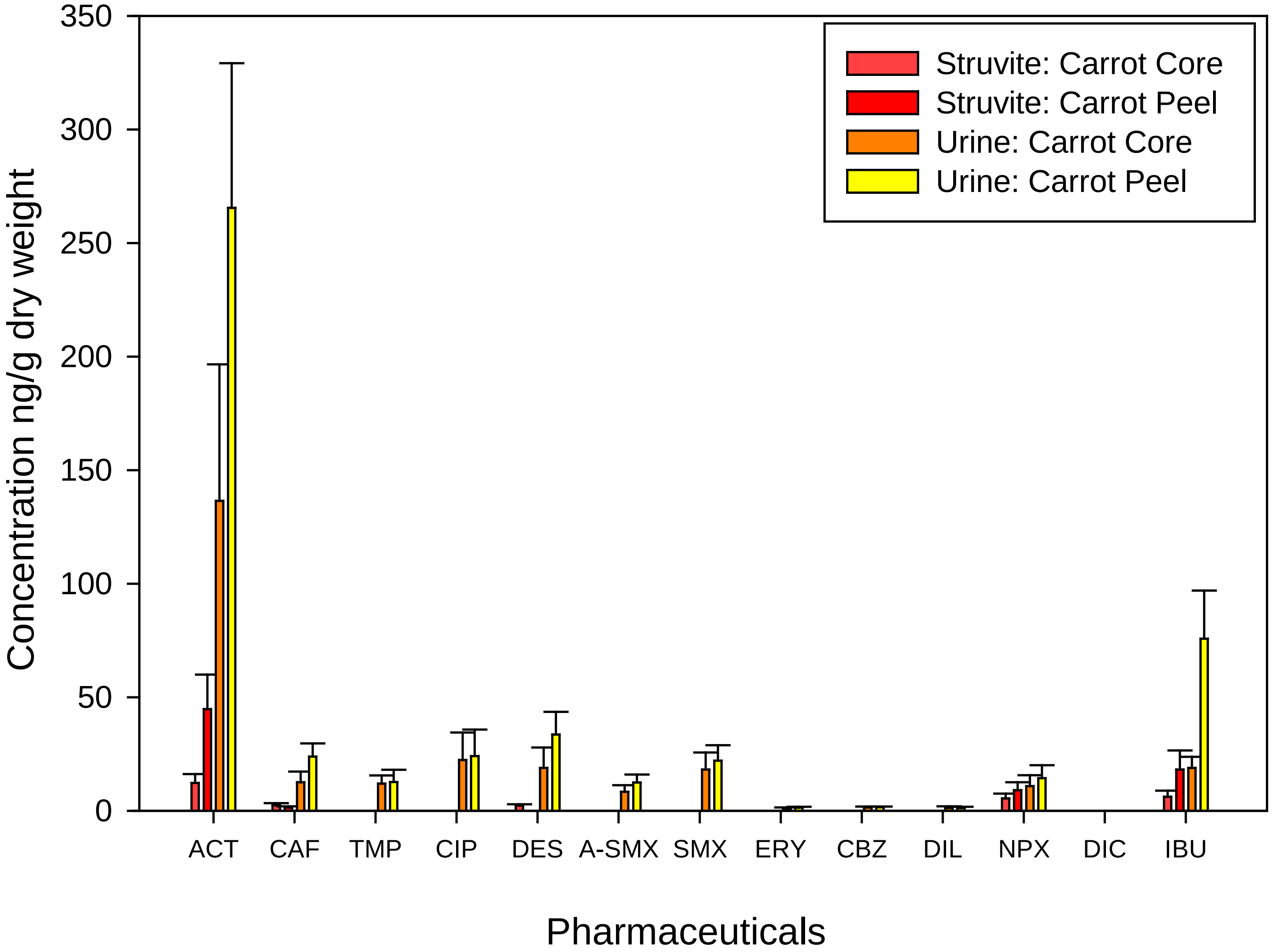


Pharmaceuticals in Lettuce



Carrot Core and Peel

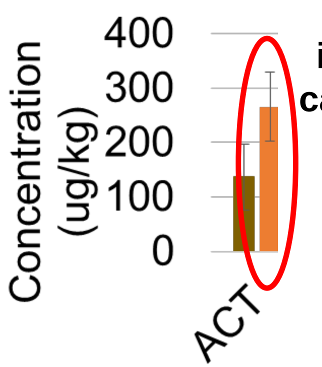
Pharmaceuticals in Carrot Treated with Struvite and Urine



Pharmaceutical levels in crops vs. tablets

265.4 ug/kg ⟶ 26.54 ug/kg

Freeze dried: Concentration is 10x because carrots are >90% water



According to USDA avg carrot weighs ~ 122 g

0.122 kg x 26.54ug/kg = **3.24 ug ACT per carrot**

Tylenol tablet ~500 mg ACT

Must consume…

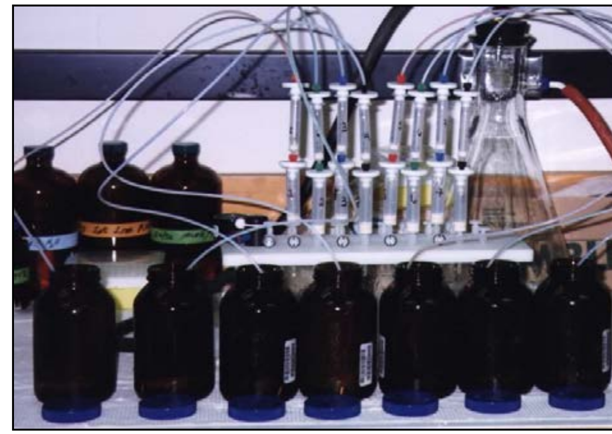
150,400 carrots =

LESSON #7

Challenges in the analysis of emerging contaminants limit our knowledge

Manure Sample Collection

Sample Preparation



Challenges in the Analysis of Antibiotics in Animal Wastes

Manure Sample Collection

Challenges in Antibiotic Analysis

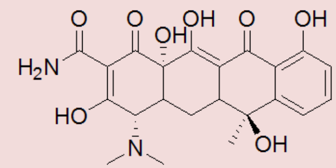
PAPER www.rsc.org/jem | Journal of Environmental Monitoring

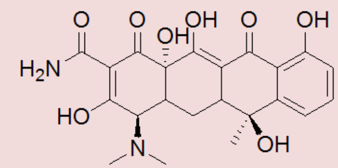
Addressing the challenges of tetracycline analysis in soil: extraction, clean-up, and matrix effects in LC-MS

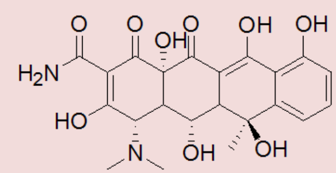
Seamus O'Connor, Jonas Locke and Diana S. Aga*

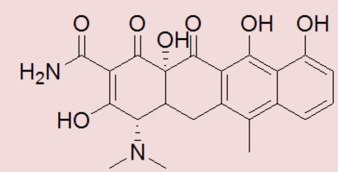
Received 31st July 2007, Accepted 31st July 2007
First published as an Advance Article on the web 14th August 2007
DOI: 10.1039/b711731k

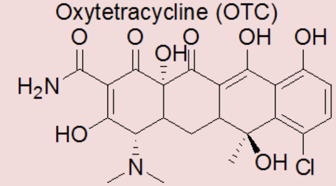
Tetracycline Antibiotics

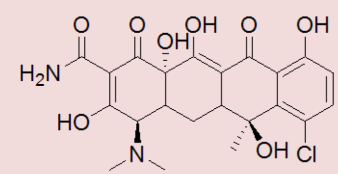

Tetracycline (TC)

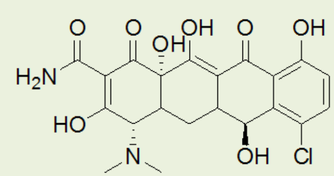

4-Epitetracycline (ETC)

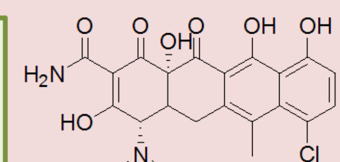

Oxytetracycline (OTC)


Anhydrotetracycline (ATC)

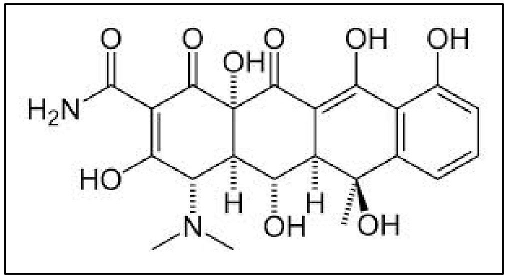

Chlorotetracycline (CTC)


4-Epichlorotetracycline (ECTC)

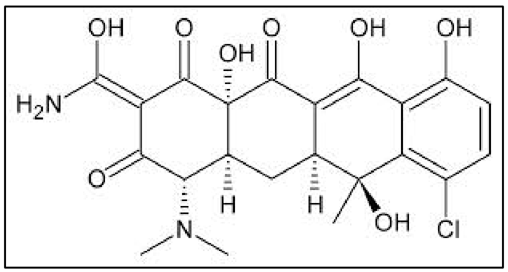

Demeclocycline (DMC)


Anhydrochlorotetracycline (ACTC

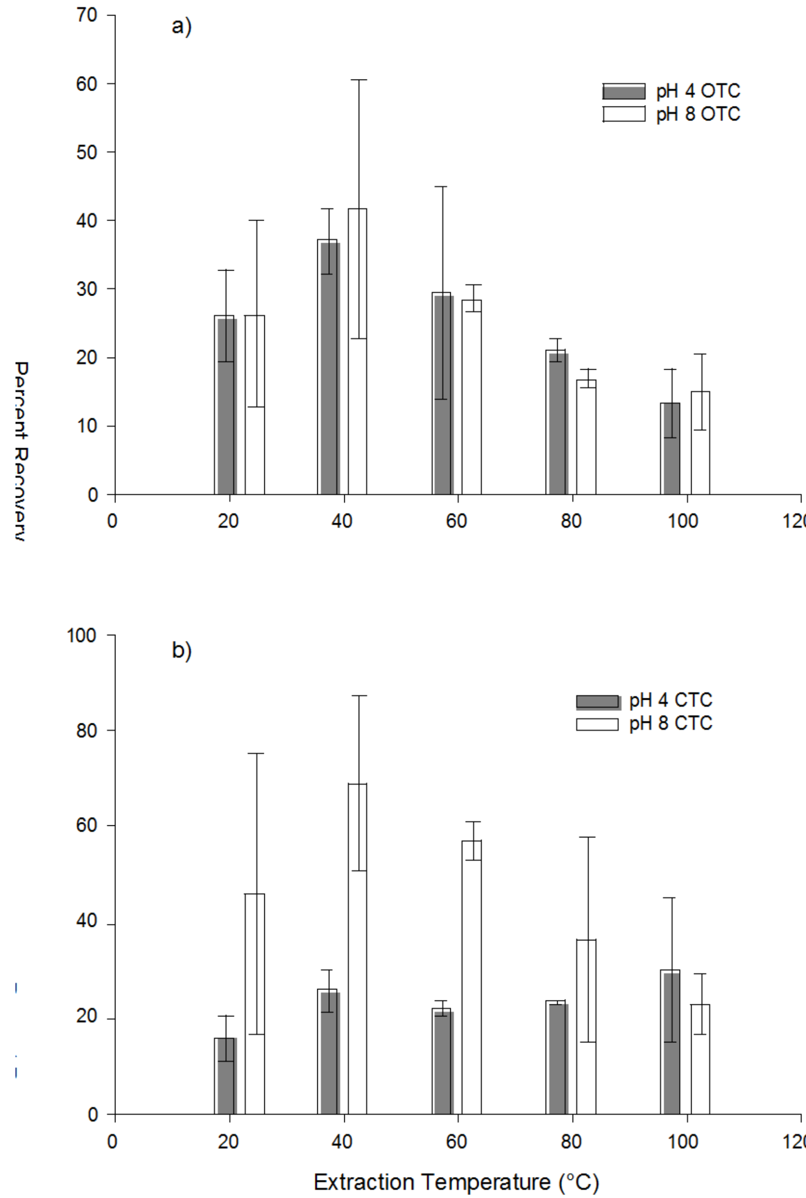
Accelerated Solvent Extraction (ASE) of Solid Samples



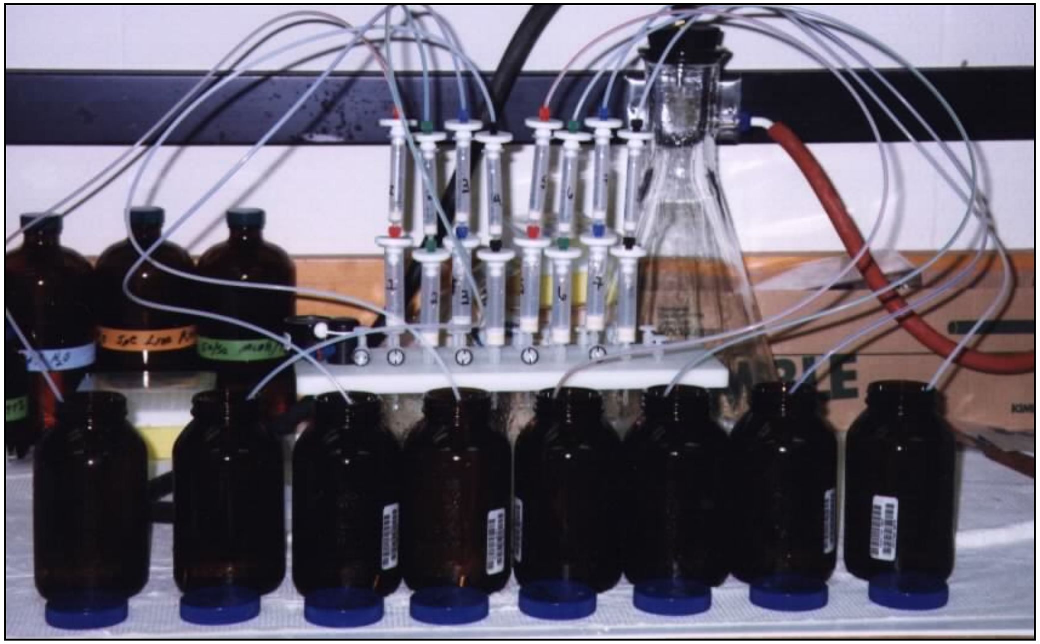
Oxytetracycline

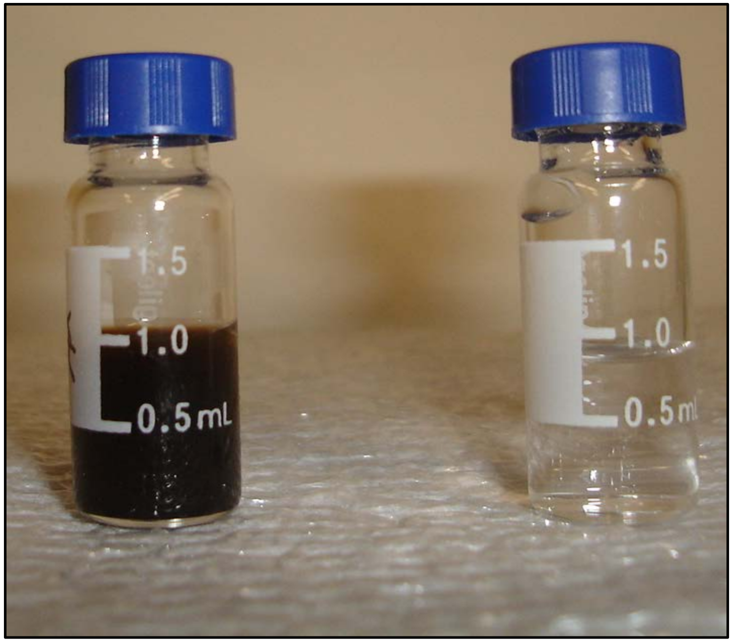


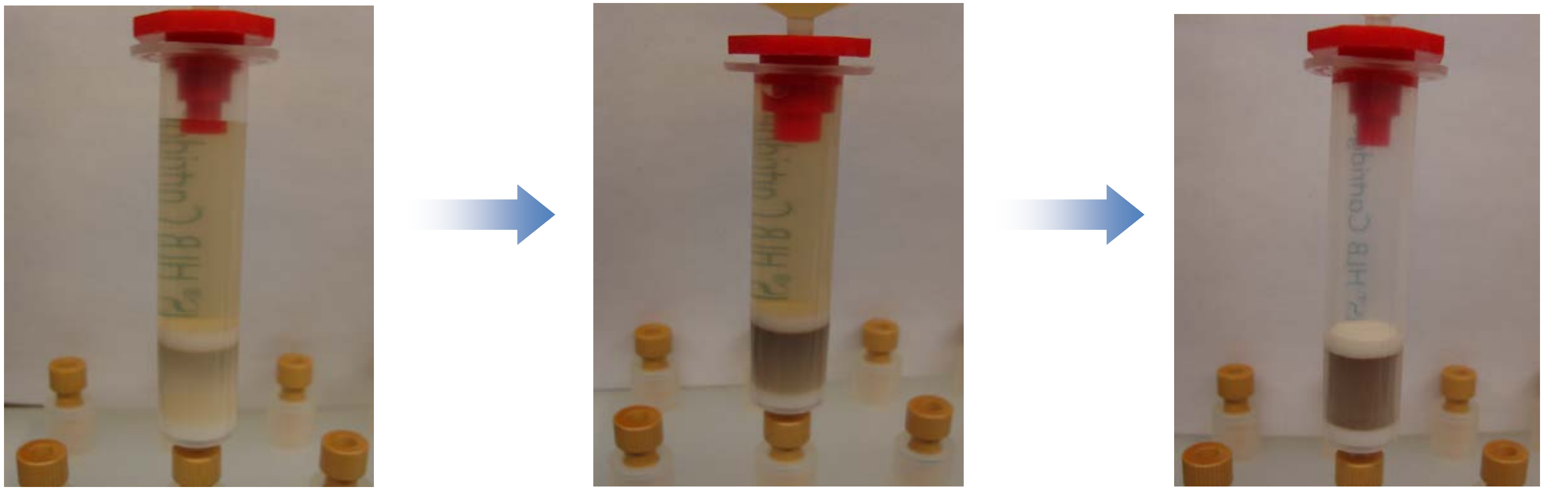
Chlortetracycline



Sample Clean-up by Solid-Phase Extraction (SPE)







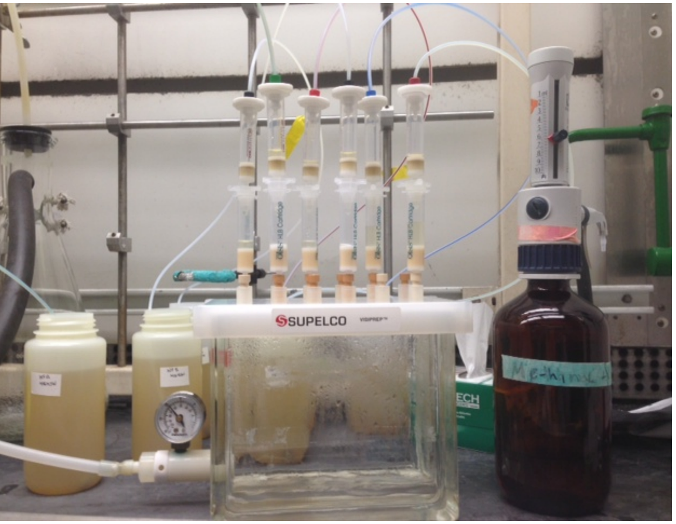
Lyophilization

Pulverization and homogenization

50µL of 500ppb standard
(natives and surrogates)

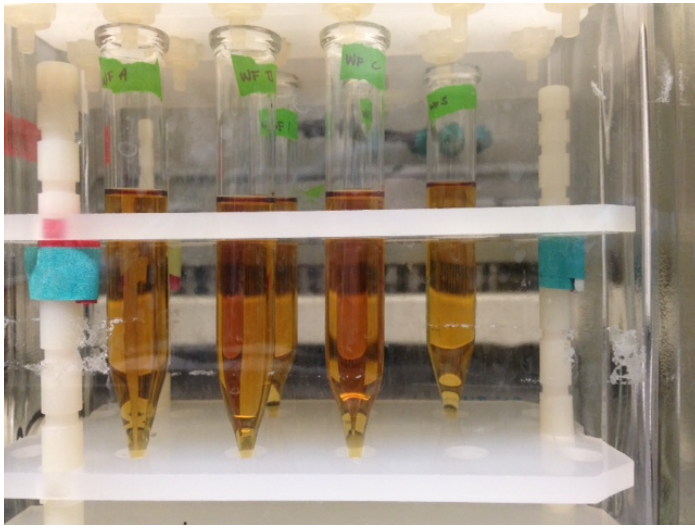
0.1 g manure
(Ho et al. 2014)

Extraction
(3x5 mL 20:30:50 ACN/MeOH/Buffer)



Dilution
(Addition of 400 mL nanopure water)

SPE Cleanup
(NH_2:HLB – 0.5-1.0 mL/min)

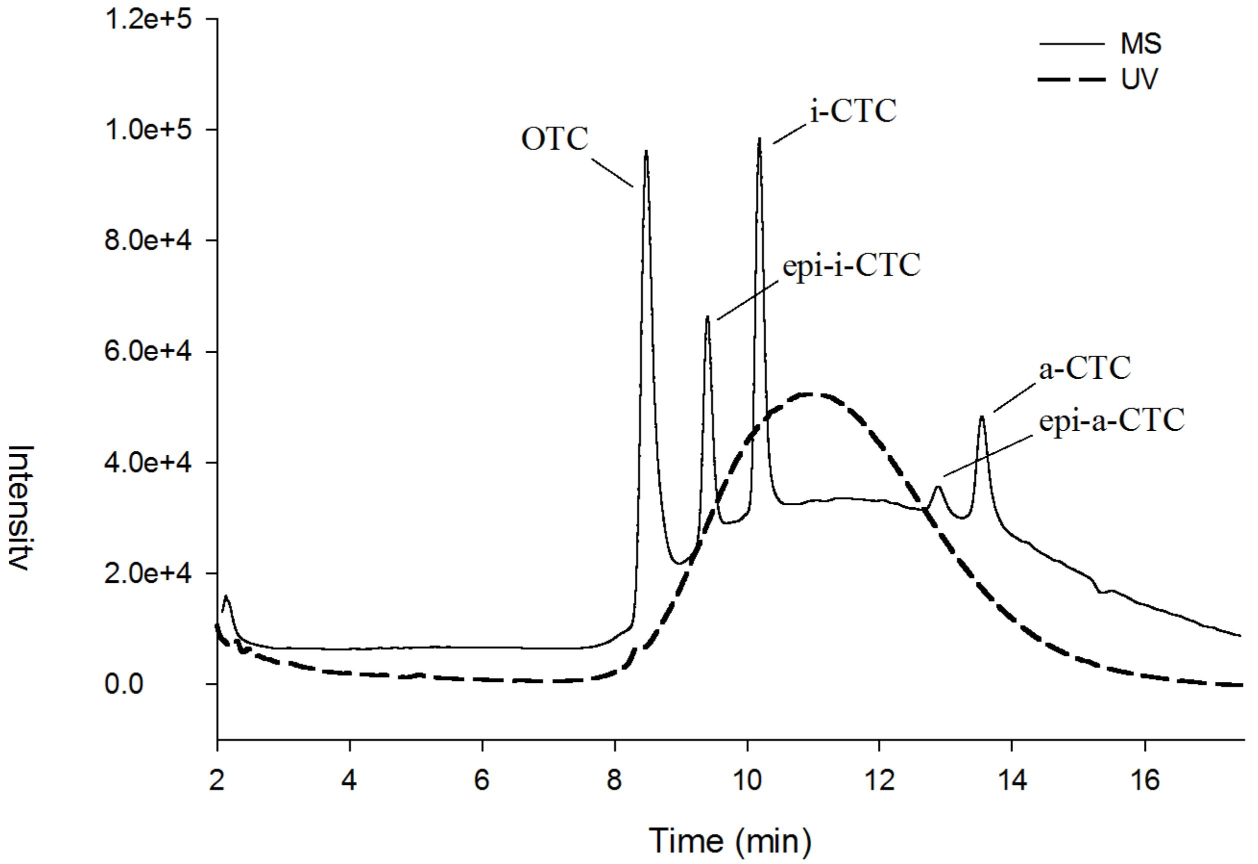


Elution
(10 mL MeOH)

Evaporation & Reconstitution
(1.0 mL in 95/5:H_2O MeOH + 0.1% HA)

LC/MS/MS Analysis
(Standard Addition and isotope Dilution)

Signal Interference from Co-extracted Matrix



O'Connor, S.; Locke, J.; Aga, D. S., Addresing the chalenges of tetracycline analysis in soil: extraction, clean-up, and matrix effects in LC-MS*. Journal of Environmental Monitoring* **2007**, 9, (11), 1254-1262.

Liquid Chromatography / Mass Spectrometry

- Single quadrupole
- Ion-trap
- Triple quadrupole
- Time of flight
- Orbitrap
- Fourier Transform Ion Cyclotron Resonance

Improved Limits of Detection (LOD) with Triple Quadrupole (QQQ) MS

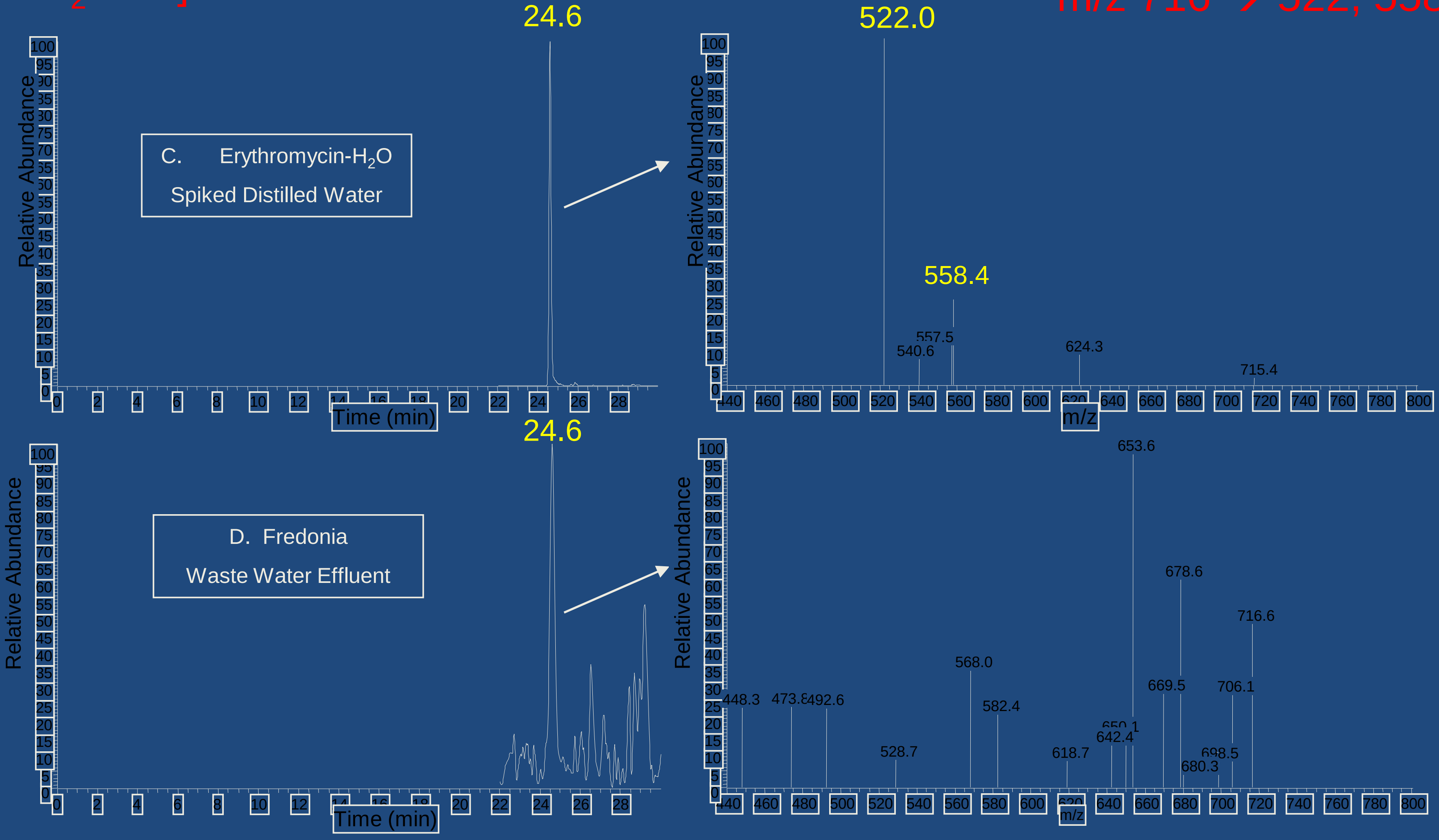
Compound	Ion-trap[1] LOD (ng/L)	QQQ LOD (ng/L)
Acetaminophen	-	2.0
Caffeine	71	2.0
Carbamazepine	-	1.0
Ciprofloxacin	30	31
Diclofenac	-	17
Erythromycin	75	9.0
Ibuprofen	-	4.0
Naproxen	-	6.0
Sulfamethoxazole	83	1.0
Trimethoprim	91	29

[1] Batt, A.L. and D.S. Aga, Simultaneous Analysis of Multiple Classes of Antibiotics by Ion Trap LC/MS/MS for Assessing Surface Water and Groundwater Contamination. *Analytical Chemistry, **2005**, 77, 2940-2947.*

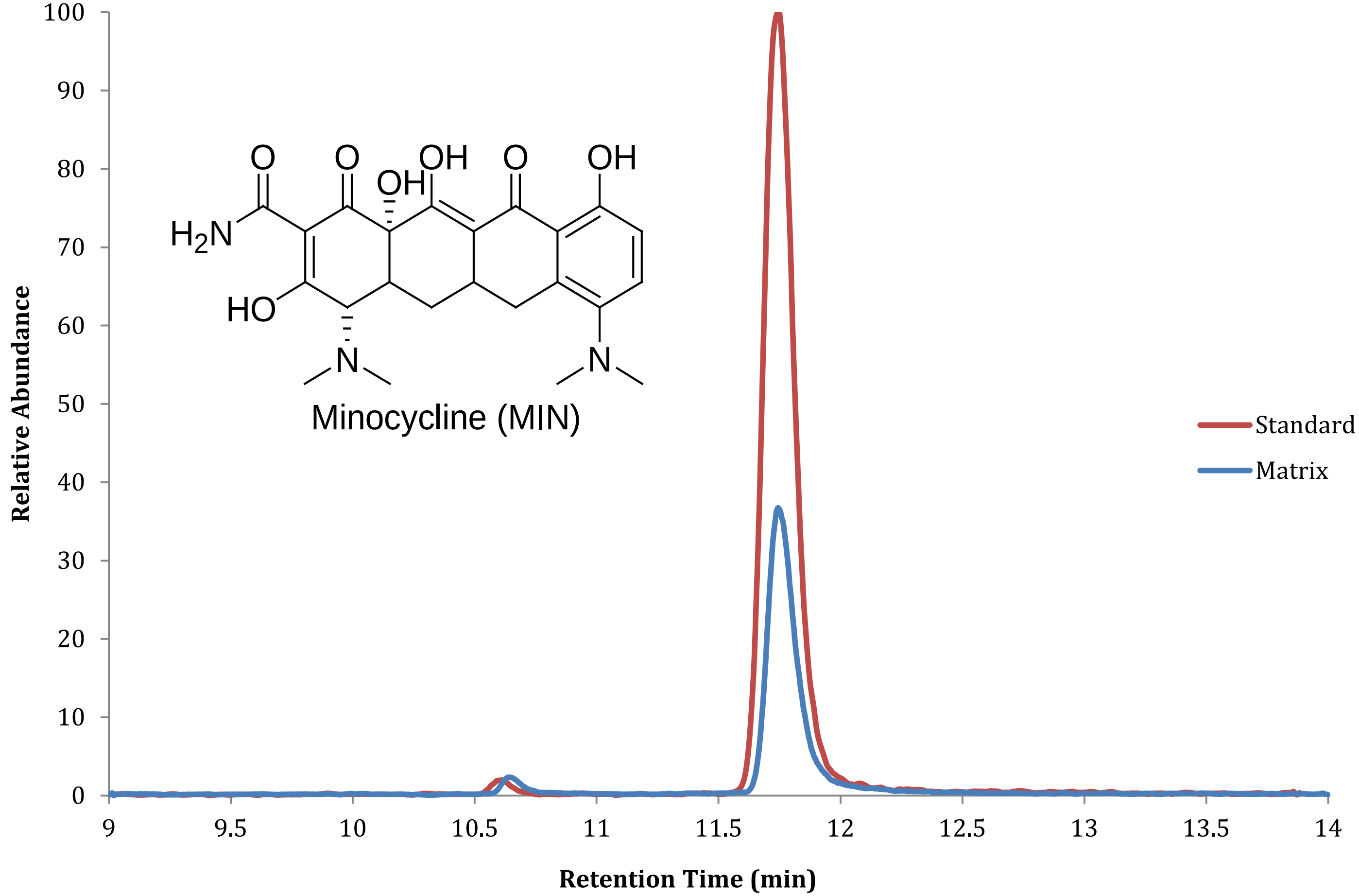
Avoiding “false positives” using MS/MS

$[M\text{-}H_2O+H]^+ = m/z\ 716$

m/z 716 → 522, 558

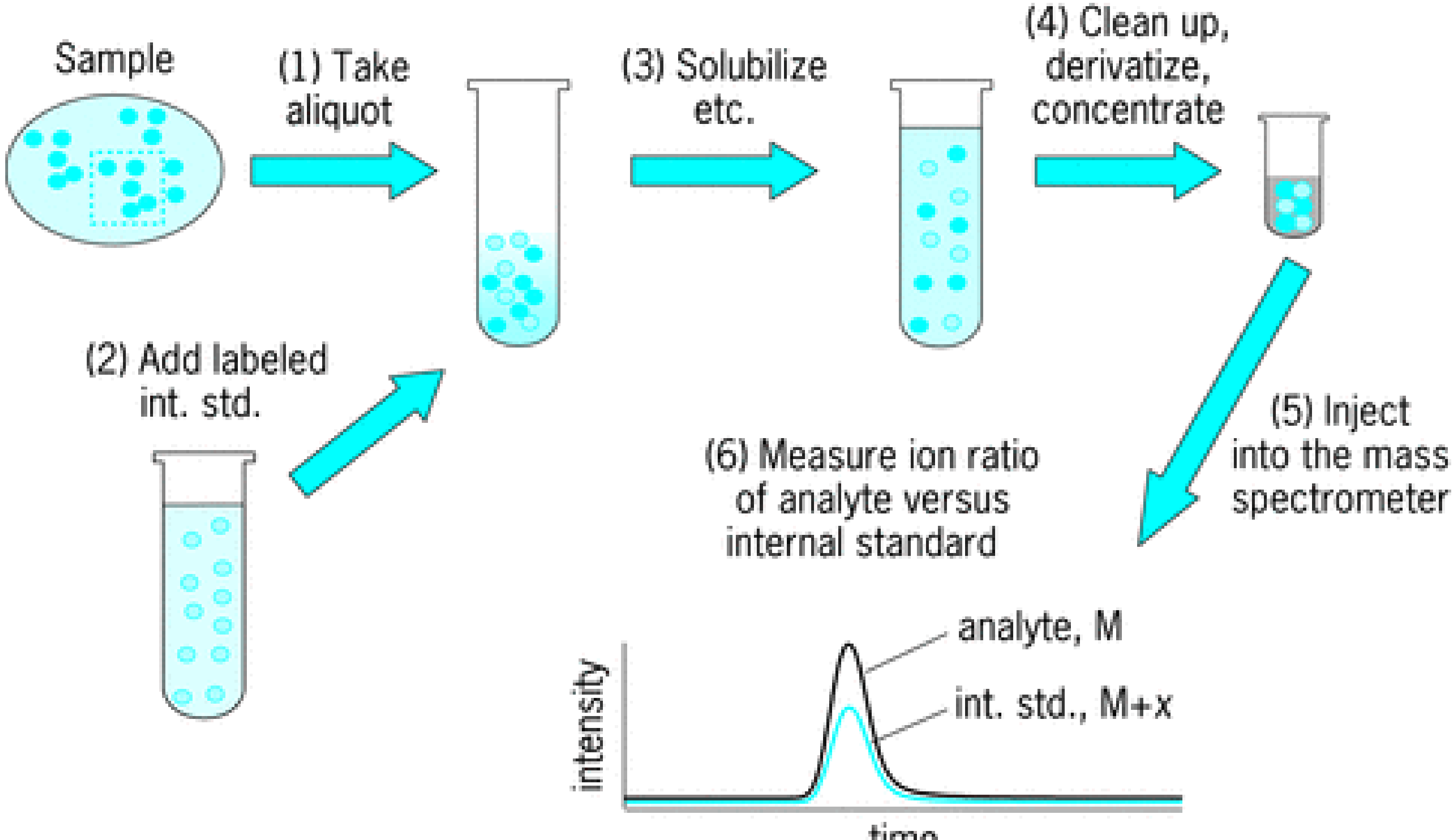


Ionization Suppression by Manure Matrix



Quantification by Isotope Dilution

Measurement of analyte mixed with known amount of isotopically-labeled analyte (^{2}H or ^{13}C)



LESSON #8: No "One-Size Fits All" Method

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

(**2011,** *59*, 2213-2222)

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Simultaneous Analysis of Free and Conjugated Estrogens, Sulfonamides, and Tetracyclines in Runoff Water and Soils Using Solid-Phase Extraction and Liquid Chromatography—Tandem Mass Spectrometry

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Estrogens From Poultry Litter

- Contains: chicken excrement, malted feathers, dead birds, food, bedding (sawdust, wood chips, peanut shells, etc.)

- Delmarva Poultry Production
  - Produces > 565,000,000 broiler chickens/yr (~7% of US total)

Image courtesy of: bayjournal.com

Image courtesy of: goyolocal.com

One-Size Fits All?

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY (**2011,** *59*, 2213-2222)
ARTICLE
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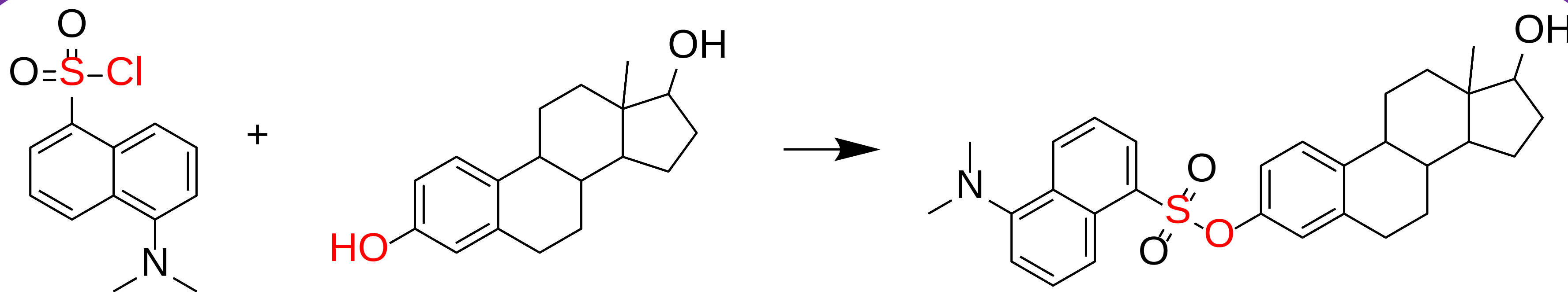
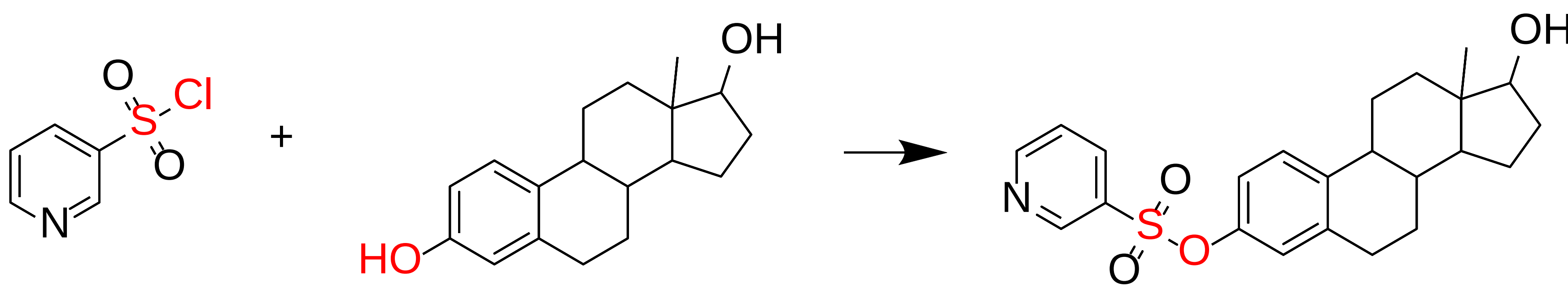
Estrogen Derivatization



Dansyl Chloride Estrogen Derivatized Estrogen

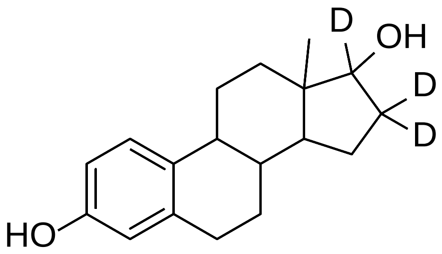


Pyridine-3-Sulfonyl (PS) Chloride Estrogen Derivatized Estrogen

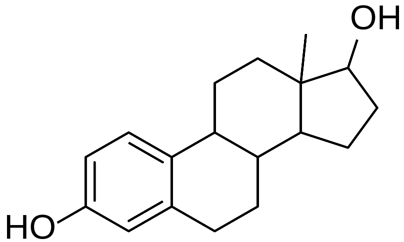
Lin, et al., *Rapid Commun. Mass Spectrom.* **2007,** *21,* 1973-1983
Szarka, et al., *Anal. Bioanal. Chem.* **2013,** *405,* 3399-3406
Xu, Spink, *Anal. Biochem.* **2008,** *375,* 105-114

Quantification by Isotope Dilution

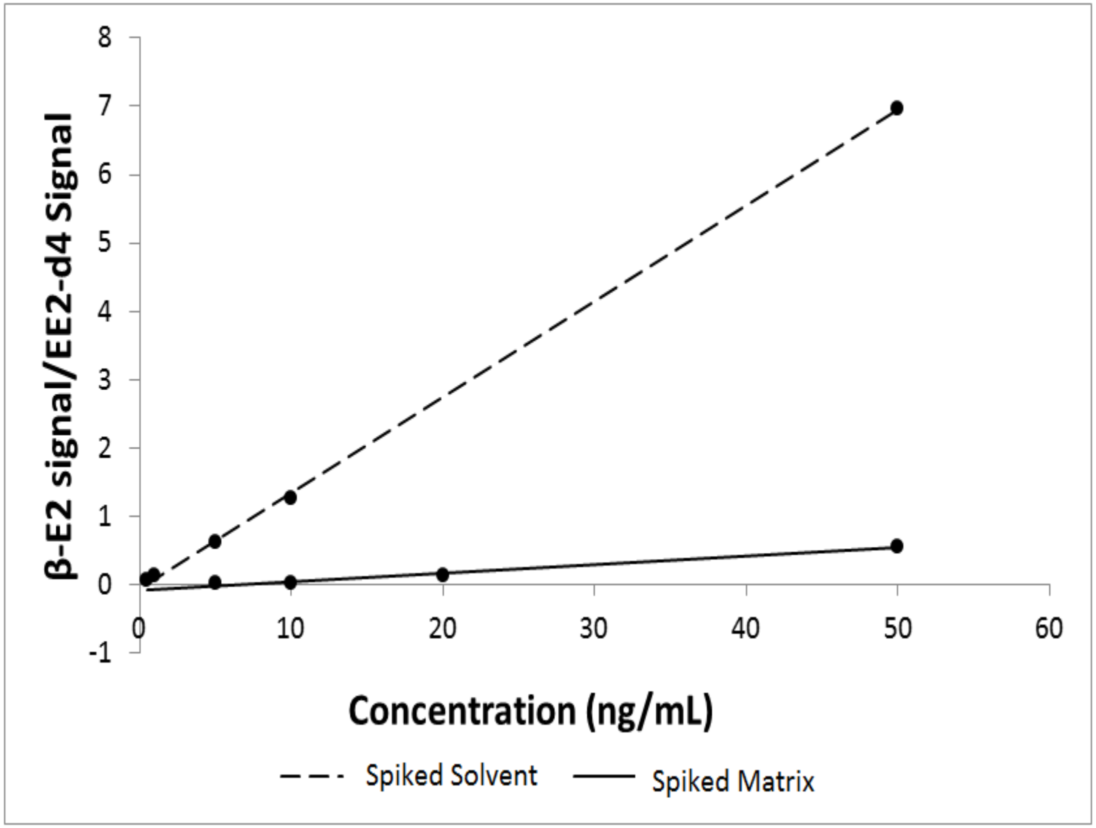
Isotope Dilution



E2-d3



E2



LESSON 9

Sometimes the "Obvious" is not the best approach in analysis

Rapid

Research Article

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(wileyonlinelibrary.com) DOI: 10.1002/rcm.6895

Analysis of trace organic pollutants in wastewater to assess biodegradation using wrong-way-round ionization in liquid chromatography/tandem mass spectrometry

Lijuan Su[1], Wendell O. Khunjar[2] and Diana S. Aga[1]*

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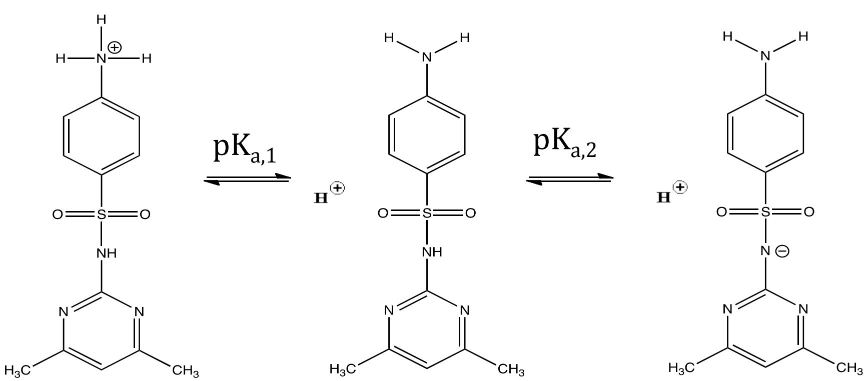
[2]Hazen and Sawyer P.C., Fairfax, VA 22030, USA



Anal. Chem. **2011**, *83*, 269–277

Wrong-Way-Round Ionization of Sulfonamides and Tetracyclines Enables Simultaneous Analysis with Free and Conjugated Estrogens by Liquid Chromatography Tandem Mass Spectrometry

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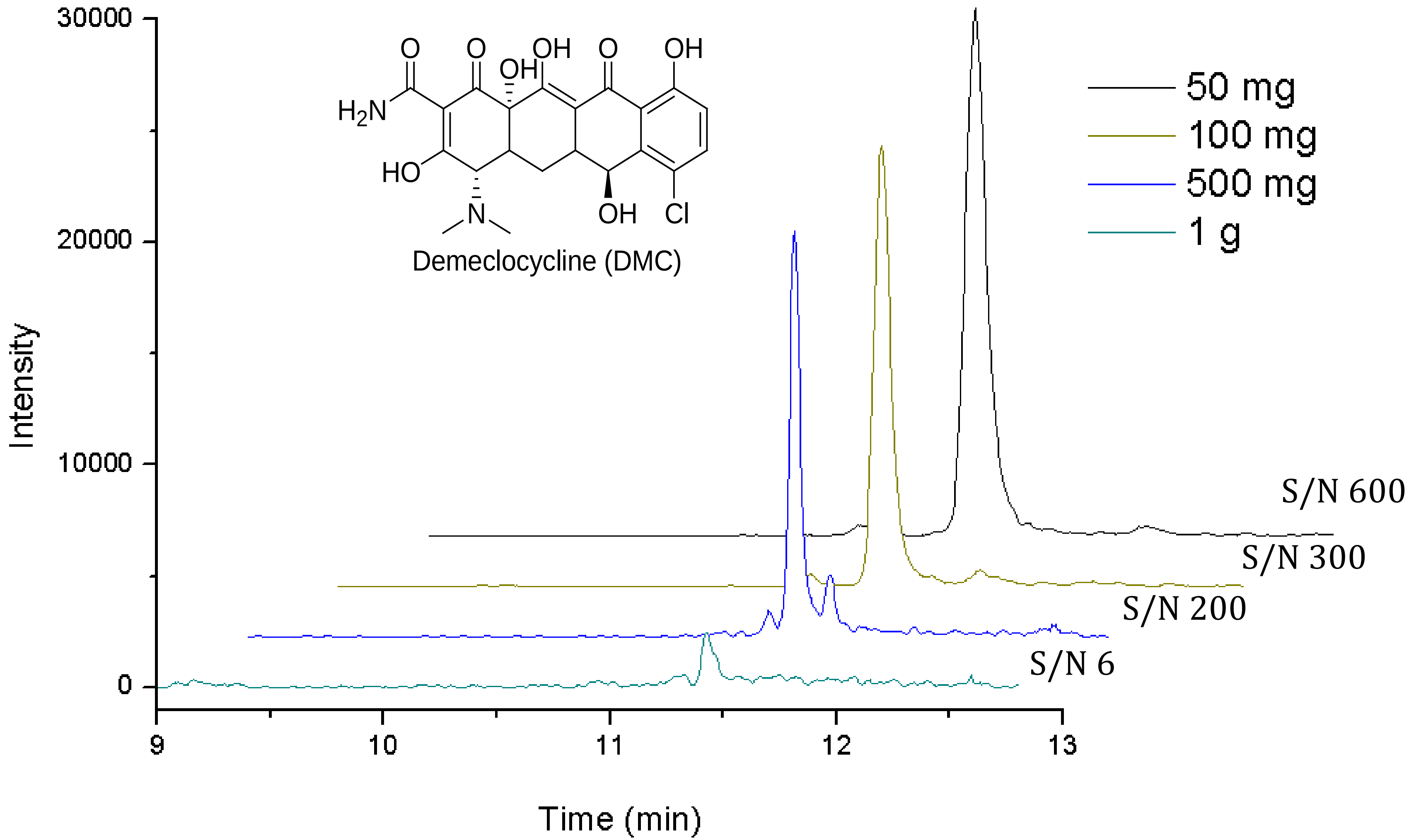
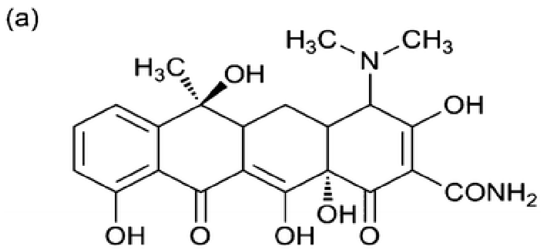
The increasing demand to monitor multiple classes of analytes has been mirrored by increased analytical cost and decreased throughput. For instance, the analyses of estrogens and antibiotics by liquid chromatography with tandem mass spectrometry (LC-MS/MS) are typically performed in two separate methods because estrogen analysis requires electrospray with negative ionization, while sulfonamide and tetracycline antibiotics are ana-

of the analytes, the mobile phase is typically adjusted with an acidic or basic modifier in order to protonate or deprotonate the analytes because it is assumed that the charge of the ion formed in ESI depends on the equilibrium solution-phase chemistry in the bulk solution.[6] Hence, for LC-MS analysis of multiple classes of compounds, needed for instance in the screening of environmental samples and in food testing, at least two separate methods are used to analyze for acidic and basic compounds. The common

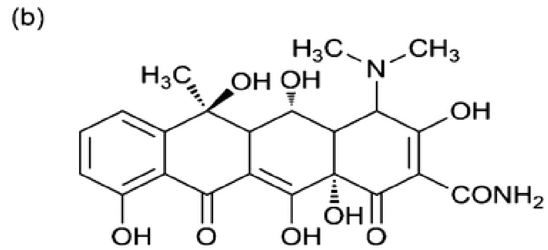
Poor LOD due to Ionization Suppression by Manure Matrix



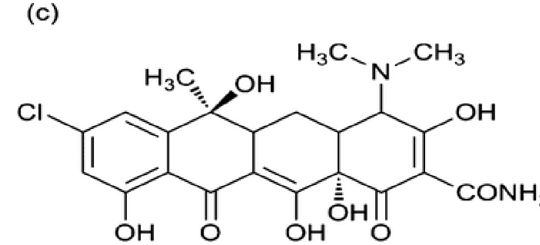
Importance of Transformation Products: Tetracyclines



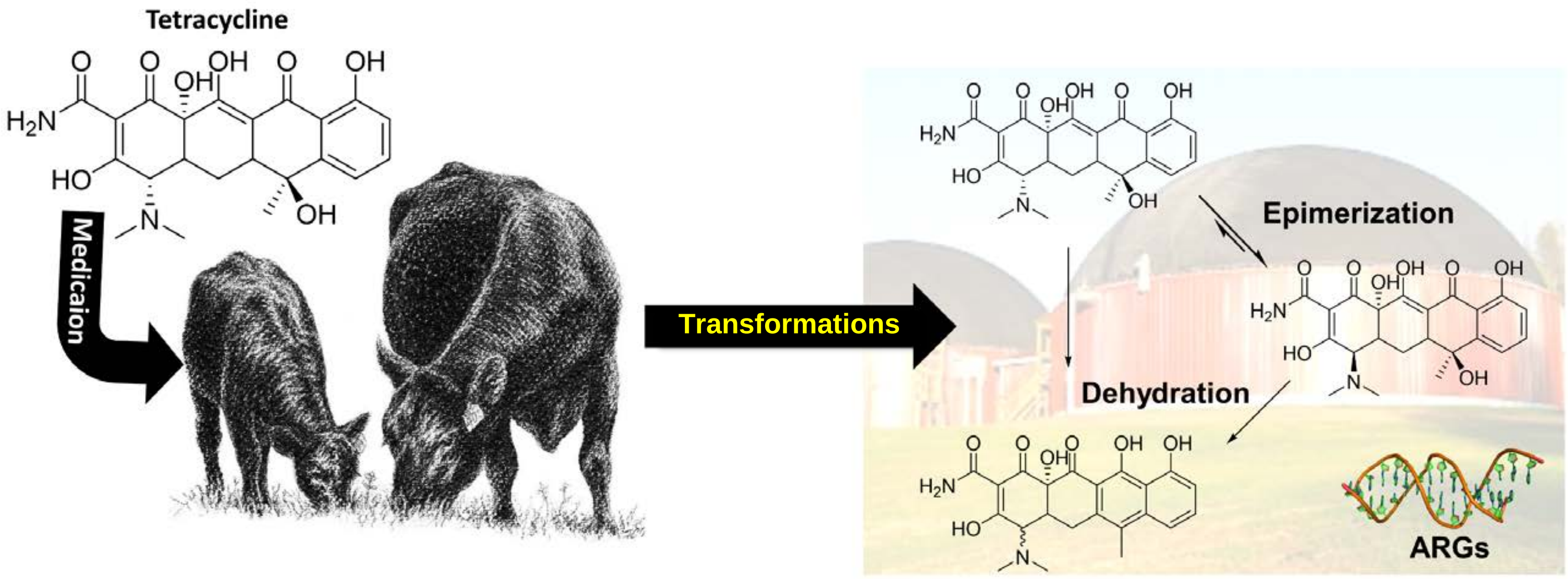
Tetracycline



Oxytetracycline



Chlortetracycline

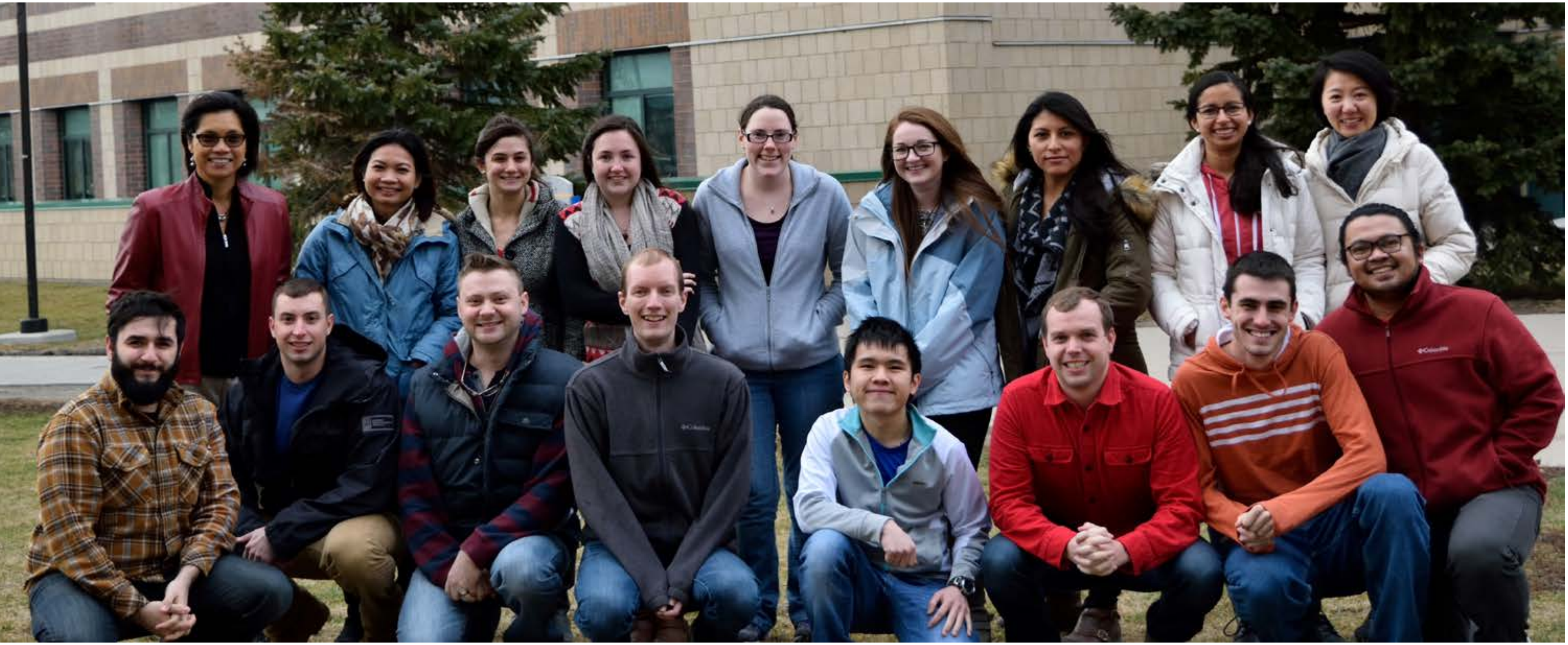


Sample Collection: Which samples are important?

LE$$ON 10

Good funding and excellent students are key to finding solutions to problems posed by emerging contaminants

- US Department of Agriculture
- US Environmental Protection Agency
- National Science Foundation
- Water Environment Research Foundation
- New York State Pollution Prevention Institute



Thank YOU!

NSF
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WHERE DISCOVERIES BEGIN

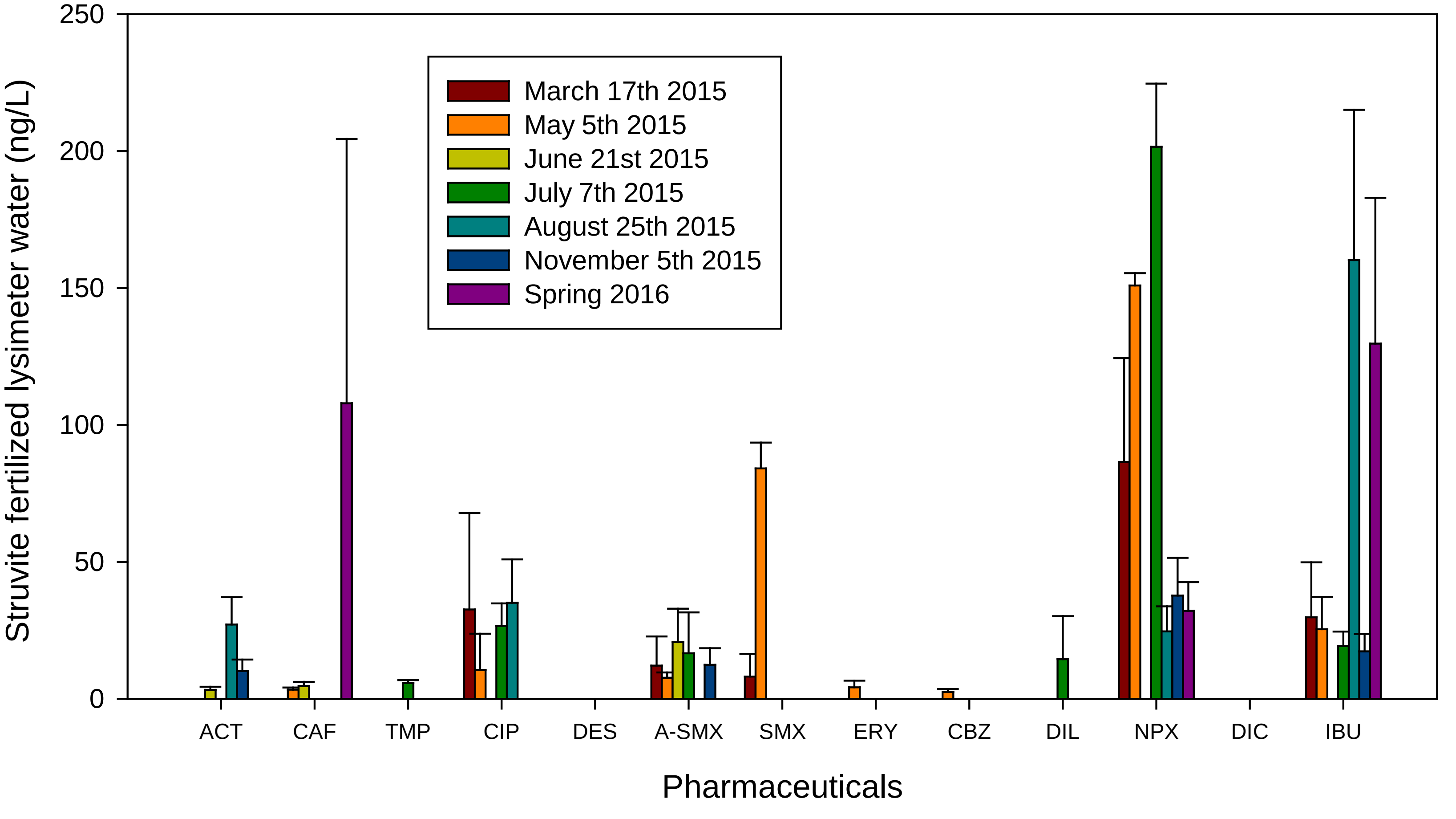
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

USDA NIFA
United States Department of Agriculture
National Institute of Food and Agriculture

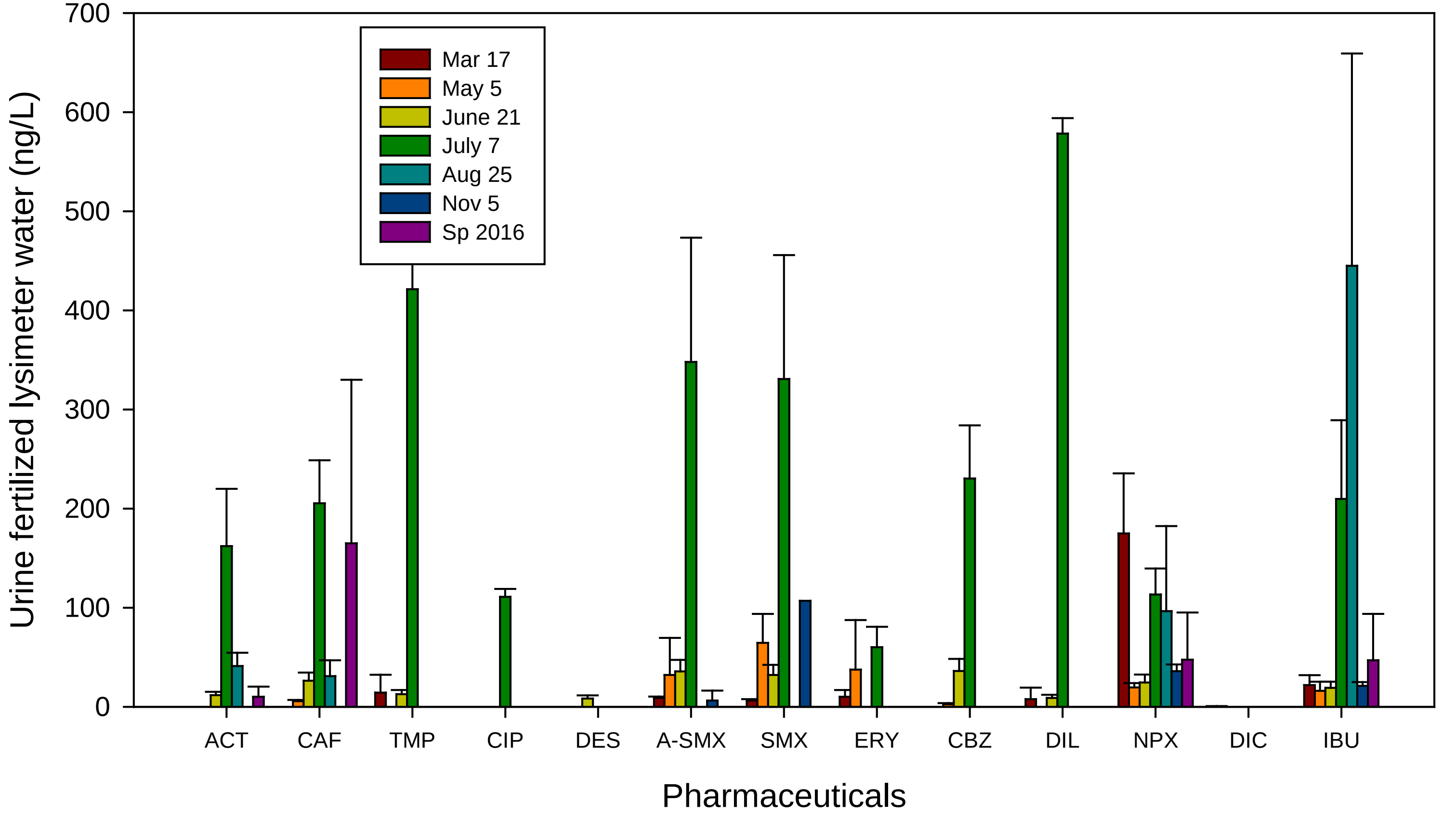
SINCE 1971
Sea Grant
New York
Bringing Science to the Shore

WERF
Water Environment Research Foundation
Collaboration. Innovation. Results.

Lysimeter Water: Struvite



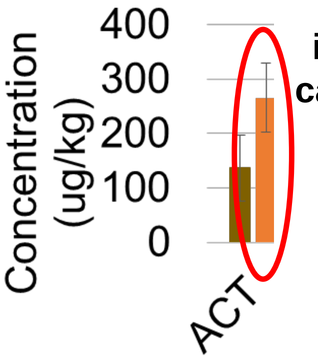
Lysimeter Water: Urine



Pharmaceutical levels in crops vs. tablets

265.4 ug/kg → **26.54 ug/kg**

Freeze dried: Concentration is 10x because carrots are >90% water



According to USDA avg carrot weighs ~ 122 g

0.122 kg x 26.54ug/kg = **3.24 ug ACT per carrot** → **Tylenol tablet ~500 mg ACT**

Must consume…

150,400 carrots =