



## Endocrine Disrupter Emerging Pollutants

Dr. David Klein

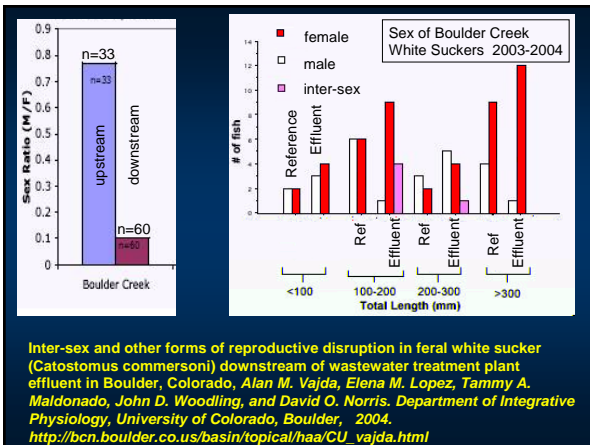


## Endocrine Disrupters

Prior to 1999 there was no nation-wide program to test for pharmaceuticals in water in the USA.

Currently the USGS is leading the way on testing for these compounds in surface, ground and drinking water.

<http://toxics.usgs.gov/pubs/FS-027-02/>

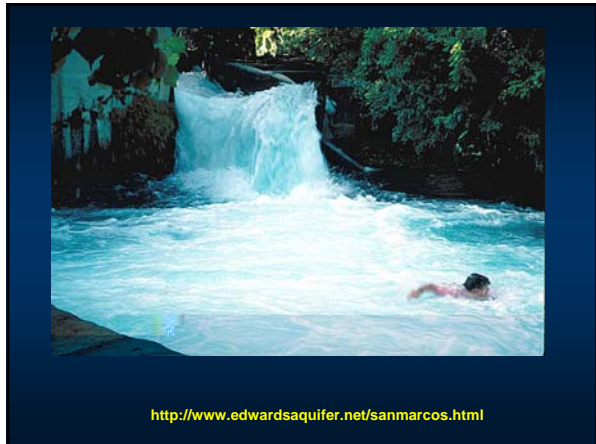


## Spring Lake in San Marcos, Texas



<http://www.edwardsaquifer.net/sanmarcos.html>

- Pouring forth millions of gallons of clear, 72° water daily, the springs are the headwater of the San Marcos River
- Archaeologists believe this may be the oldest continually inhabited site in North America with over 12,000 years worth of archaeological artifacts sealed underneath Spring Lake which was created by a dam in 1854 by German settlers.



### 27 Compounds of Interest

Compound; CAS	Use/Source
4-Nonylphenol; 104-40-5	Detergent Metabolite
4-Octylphenol; 1806-26-4	Detergent Metabolite
6-acetyl-1,1,2,4,4,7-hexamethyltetraline (AHTN), 21145-77-7	Musk Fragrance
Acetaminophen; 103-90-2	Antipyretic
Benzophenone; 119-61-9	Fragrance Fixative
Bisphenol A; 80-05-7	Polymers
Caffeine; 58-08-2	Stimulant

### Compounds of Interest Cont.

Compound; CAS	Use/Source
Estradiol; 50-28-2	Reproductive Hormone
Estriol; 50-27-1	Reproductive Hormone
Ethinylestradiol; 57-63-6	Synthetic Estrogen
Fluoranthene; 206-44-0	PAH
Fluoxetine; 54910-89-3	Anti-Depressant
DEET; 134-62-3	Insecticide
Sitosterol; 83-46-5	Plant Sterol

### Compounds of Interest Cont.

Compound; CAS	Use/Source
Carbamazepine; 298-46-4	Anti-Epileptic
Cholesterol; 57-88-5	Plant/Animal Steroid
Codeine; 76-57-3	Analgesic
Coprostan-3beta-ol; 360-68-9	Fecal Steroid
Cotinine; 486-56-6	Nicotine Metabolite
Diazinon; 333-41-5	Pesticide
Diltiazem; 42399-41-7	Blood Pressure Stabilizer

### Compounds of Interest Cont.

Compound; CAS	Use/Source
Sulfamethoxazol; 723-46-6	Antibiotic
Tributyl Phosphate; 126-73-8	Fire Retardant
Triclosan; 3380-34-5	Anti-Microbial
Triethyl Citrate; 77-93-0	Cosmetics
Trimethoprim; 738-70-5	Antibiotic
Tris(2-chloroethyl) phosphate; 115-96-8	Fire Retardant

### Target compounds

#### GC-MS-MS

- 4-Nonylphenol
- 4-Octylphenol
- Triclosan
- Coprostan-3β-ol
- Benzophenone
- Fluoranthene
- Cholesterol
- Bisphenol A

Note: some compounds can be analyzed by both LC and GC

## Target compounds LC-MS-MS

Ibuprofen	Tributyl Phosphate
Codeine	TCEP
Sulfamethoxazole	DEET
Trimethoprim	Cotinine
Fluoxetine	Diazinon
Carbamazepine	Estradiol
Acetaminophen	Estriol
Diltiazem hydrochloride	Caffeine
Ethyl Citrate	Ethinylestradiol

## Sample Extraction For LC-MS

1 Liter water sample (pH adjusted to 3 with H<sub>2</sub>SO<sub>4</sub>)

↓

Surrogate added (acetaminophen-d14 for 50 ng/L final concentration)

↓

SPE cartridge (Oasis HLB 200mg 6 cc)  
Preconditioned with ultra pure water and methanol

↓

Sample passed through cartridge using a vacuum extraction manifold

↓

Elute with methanol (5 mL)  
Extract is reduced under nitrogen flow to 1 mL final volume

Internal Standard added (Caffeine- 13C3)

## Sample Extraction for GC-MS

1 Liter water sample (pH adjusted to 3 with H<sub>2</sub>SO<sub>4</sub>)

↓

Surrogate added (fluoranthene-d10 for 50 ng/L final concentration)

↓

SPE cartridge  
Preconditioned with dichloromethane, methanol

↓

Sample passed through cartridge using a vacuum extraction manifold

↓

Elute with acetone followed by dichloromethane  
Extract is reduced under nitrogen flow to 1 mL final volume

Internal Standard added (Chrysene-d12)

## Method Validation for LC-MS

Seven replicate San Marcos River samples we spiked  
With the following target compounds at 500 ng/L.

Sulfamethoxazole	Tributyl Phosphate
Trimethoprim	DEET
Fluoxetine	Cotinine
Acetaminophen	Diazinon
Diltiazem hydrochloride	Caffeine
Ethyl Citrate	

## Instrument Settings ESI+ LC-MS-MS

Reverse phase column: Restek Allure<sup>®</sup> C18 5µm particle, 150X 4.6mm

Mobil phase: 0.4mL/minute, 20 µL injection,  
5% methanol, 95% Water(0.1% formic acid) binary gradient  
22 minutes : 95% methanol, 5% water(0.1% formic acid)

LCQ Advantage Ion Trap: ESI +

## LC-MS-MS parameters:

Compound	Retention		LC-MS	
	MW	Time (min)	Precurs	Product Ions (RA.%)
Cotinine	176	5.00	177	146.1 (80), 98.1 (55), 80.1 (56)
Codeine	299	10.13	300	215.1 (100), 243.0 (70), 282.2 (20)
Acetaminophen	151	10.86	152	110.1 (100), 134 (5)
Trimethoprim	290	10.94	291	123.1 (50), 181.1 (10), 230.2 (60), 258 (55), 276.1 (34)
Caffeine	194	12.13	195	138.1
Sulfamethoxazole	253	12.50	254	147.2 (10), 156.0 (20), 188.0 (30)
Diltiazem HCl	415	14.53	415	178.1 (100), 369.9 (88), 415 (85)
Friedhyl Citrate	276	15.35	277	202.9 (60), 230.5 (59)
Fluoxetine	309	15.58	310	147.7 (100)
Carbamazepine	236	15.88	237	219.9
DEET	191	17.47	192	119 (100), 100 (5)
Diazinon	304	19.88	305	153.1 (52), 169.1 (100)
Tributyl Phosphate	266	20.43	267	210.9 (100), 154.8 (26)
Caffeine-13C3	197	12.33	198	140.1
Acetaminophen-d4	155	12.47	156	114.2 (100), 137.1 (5), 156.2 (25)

## LC-MS-MS parameters:

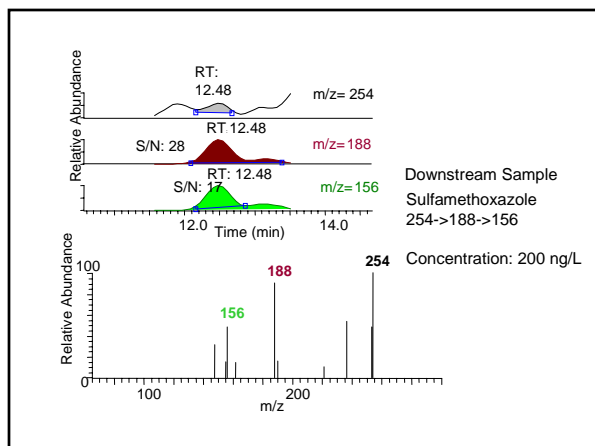
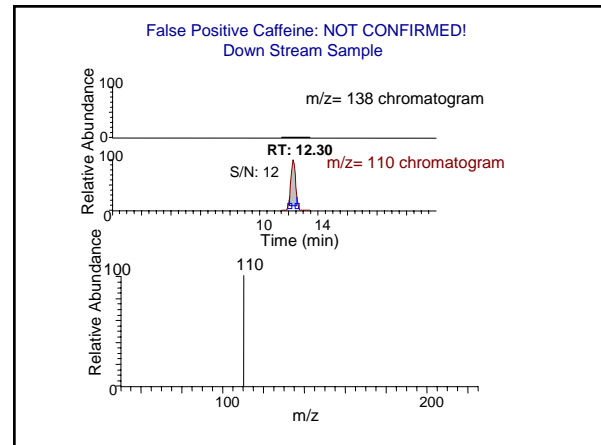
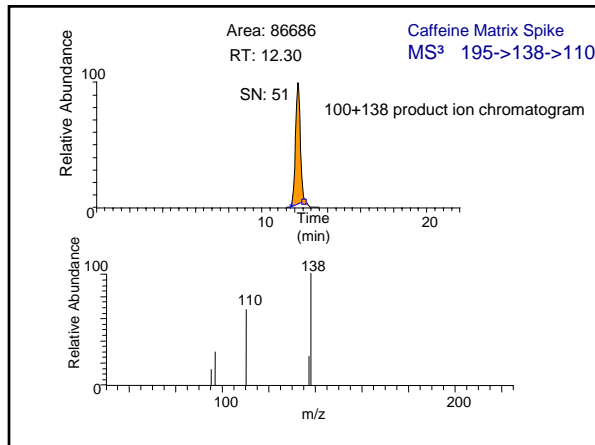
Compound	MW	Retention		LC-MS <sup>2</sup>		Collision energy (eV)	Product Ions (RA,%)
		Time (min)	Precursor	Product Ion->			
Caffeine	194	12.13	195	138		35	110.0 (85), 69.1 (6)
Carbamazepine	236	15.88	237	220		30	194.3 (100)
Caffeine-13C3	197	12.33	198	140		35	111.9 (100), 70.1 (6)

## GC-MS-MS

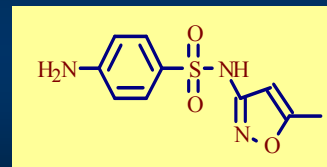
Thermo Polaris Q GC-MS Ion Trap

Cool on-column injection, 1µL injection,

RTX-XLB 30 meter, 0.25mmID X 0.25µm



Sulfamethoxazole M.W 253



## Pit Falls

- Insect repellent
- Caffeine
- Cigarettes
- Detergents
- Fragrances
- Common anti-biotics
- Common steroidal anti-itch formulations

## Acknowledgements

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