United States Environmental Protection Agency

Identification of Bioactive Contaminants Associated With Wastewater Using a Receptor Pulldown Assay and High Resolution Mass Spectrometry

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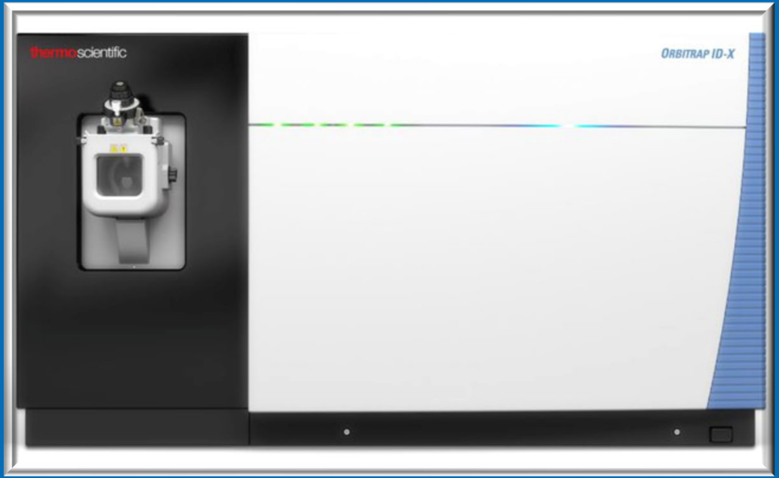

The contents of this presentation neither represent nor necessarily reflect official US EPA policy

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NPS

- Rebecca Weissinger

USGS

- B. Battaglin, P. Bradley, D. Winkleman

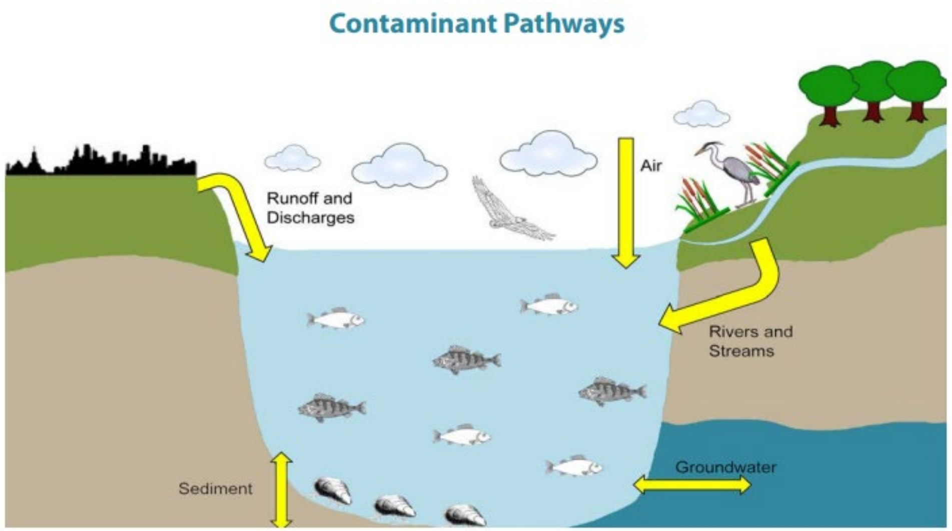
Univ. of Toronto

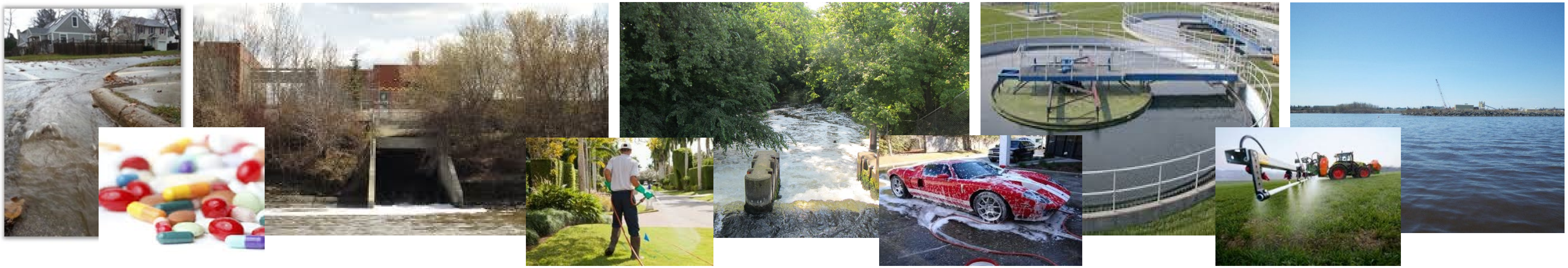
- Hui Peng

Chemicals in the Environment

- Increasing number of compounds can be detected in the environment

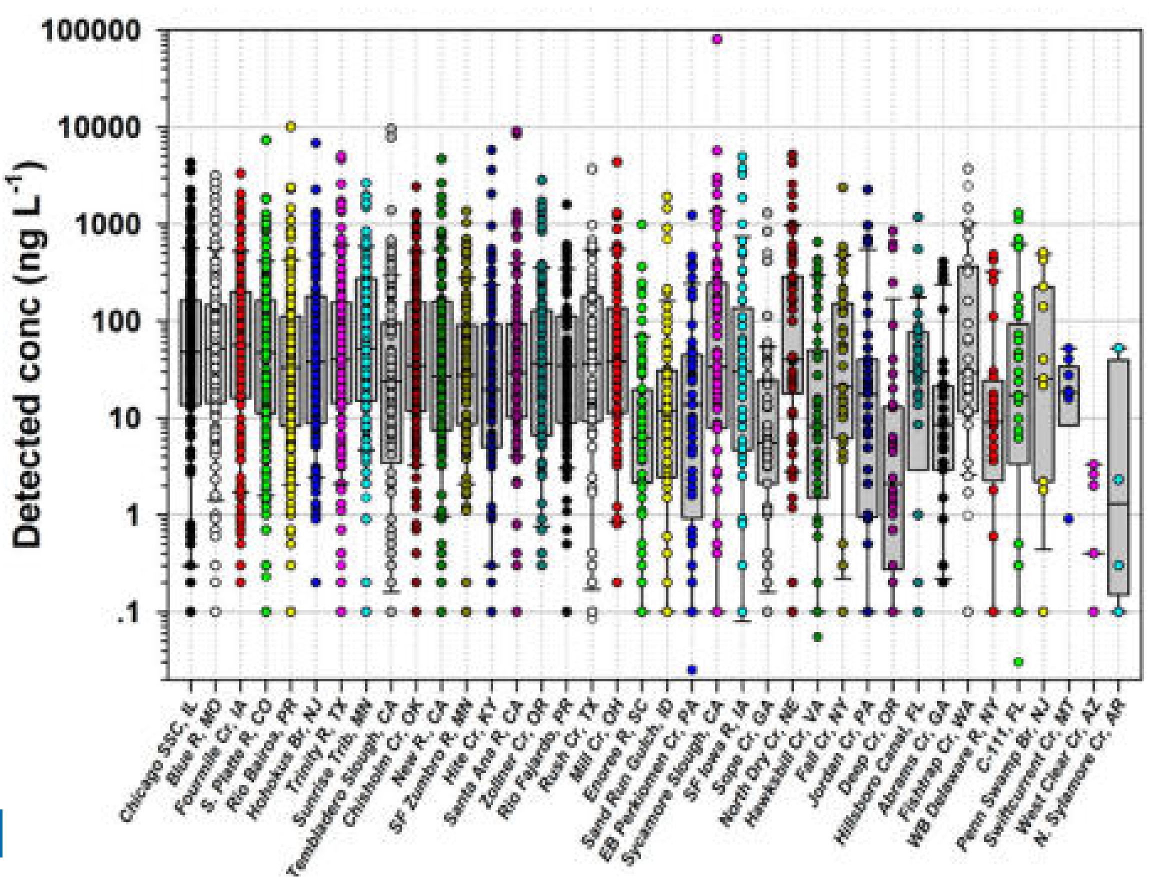
- Contaminants of emerging concern (CECs)
  - Pharmaceuticals and personal care products (PPCPs)
  - Agricultural chemicals (pesticides, growth promoters)
  - Industrial chemicals (plasticizers, flame retardants, PFAS)





Surface Water Contaminants in the US

- 406/719 organic compounds detected
- 4 – 161 compounds detected at a single site
- Cumulative concentrations ranged 8 – 103000 ng/L



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Expanded Target-Chemical Analysis Reveals Extensive Mixed-Organic-Contaminant Exposure in U.S. Streams

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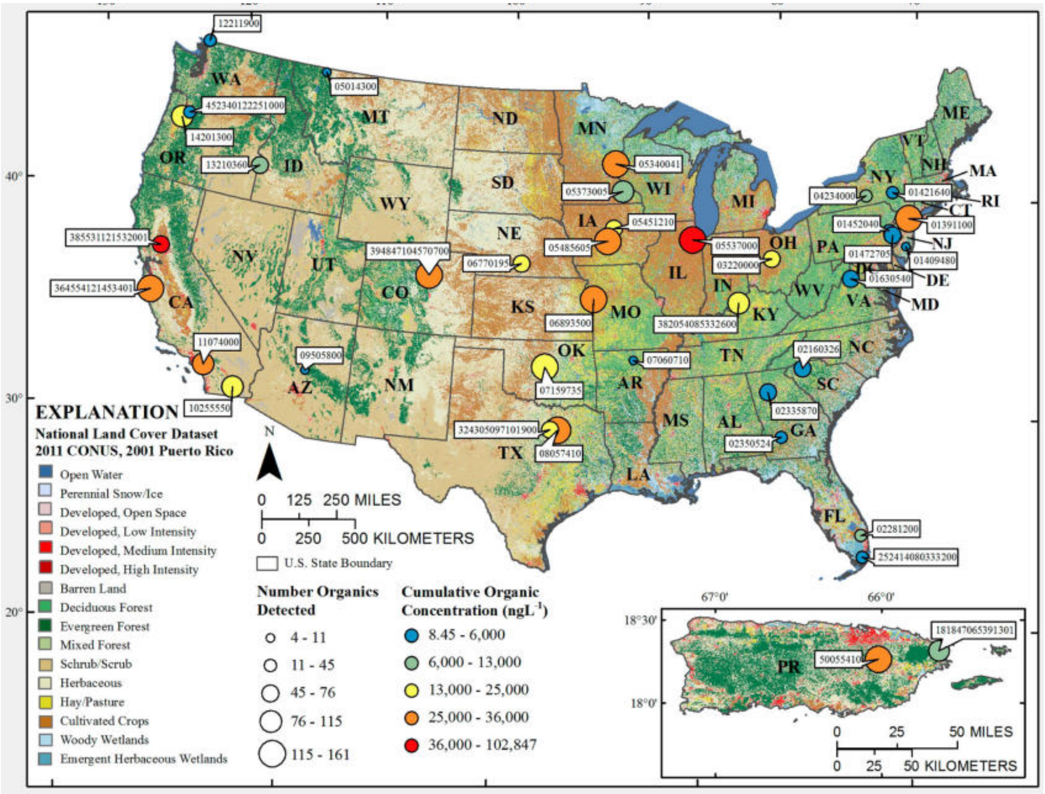
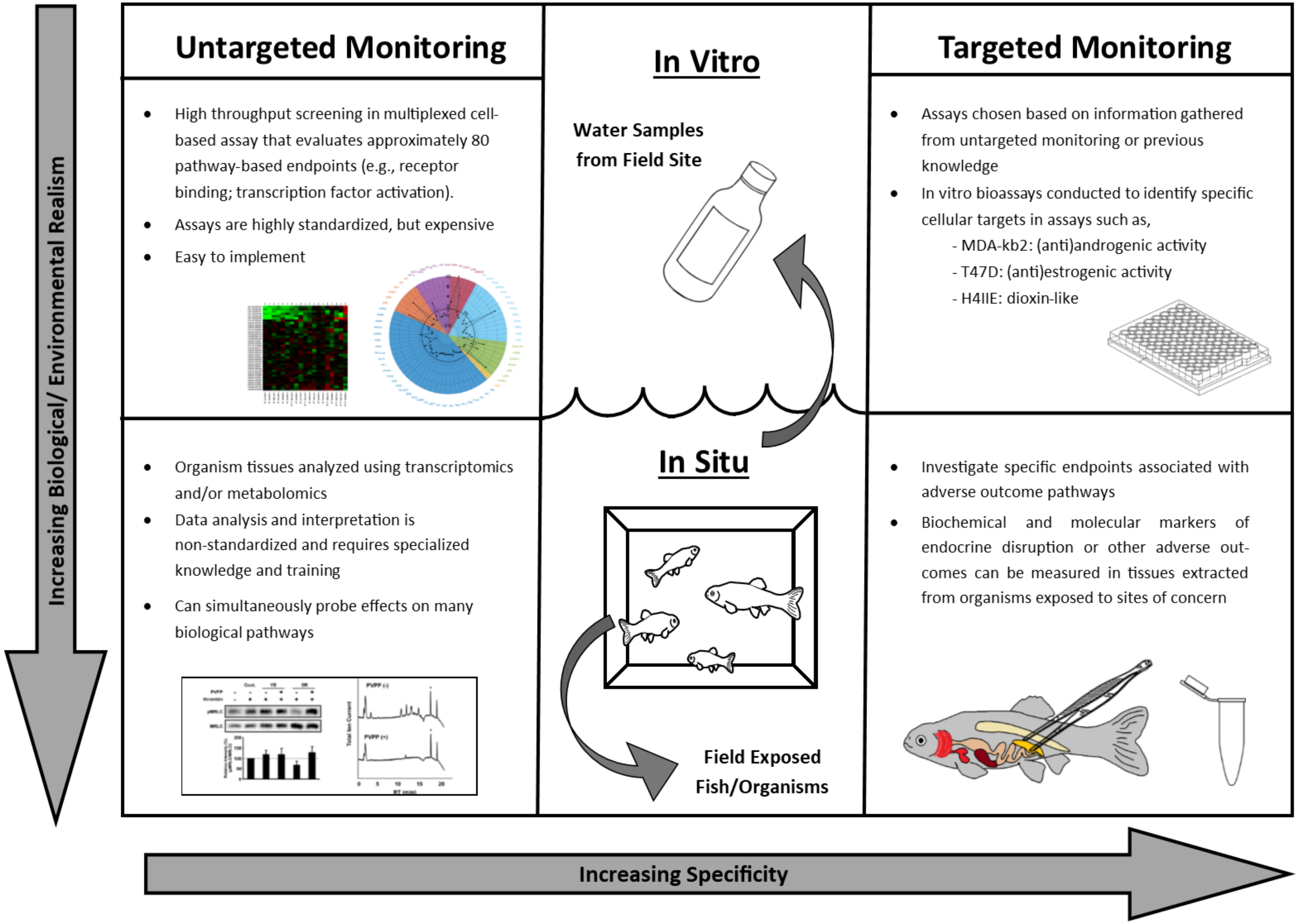


Generalized Effects-based Approach



EPA United States Environmental Protection Agency

21st Century Approach: High-Throughput Toxicology (HTT)

- EPA and other federal agencies began the Toxicity Forecaster (ToxCast)and Tox21 in 2007 as part of an effort to advance chemical toxicity assessments

- Focus on in vitro testing vs traditional whole animal testing
- Greatly reduces cost of screening chemicals; ~10,000 currently screened through 100+ assays
- Provides a new source of methods and data that can be applied to assessment of environmental data

- 1536 well HTS
- 10,000 chemicals
- 25 assays per year

EPA United States Environmental Protection Agency | NIEHS National Institute of Environmental Health Sciences | NCGC NIH Chemical Genomics Center | FDA

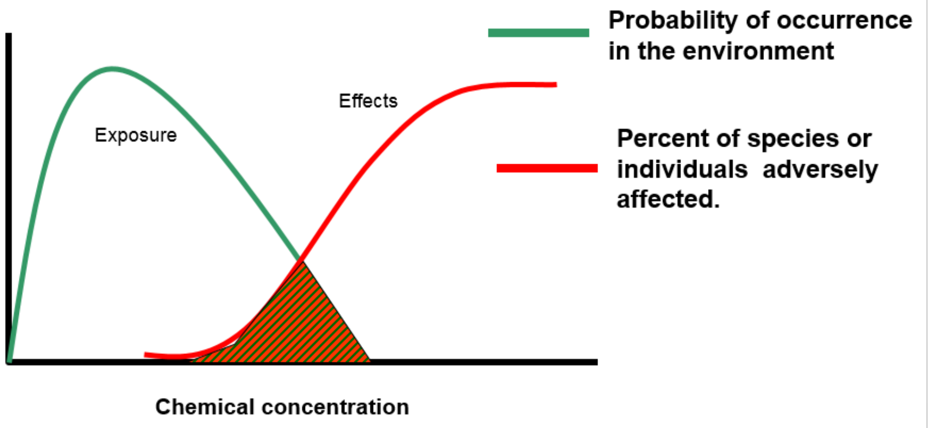
Using HTT To Predict and Prioritize Chemicals

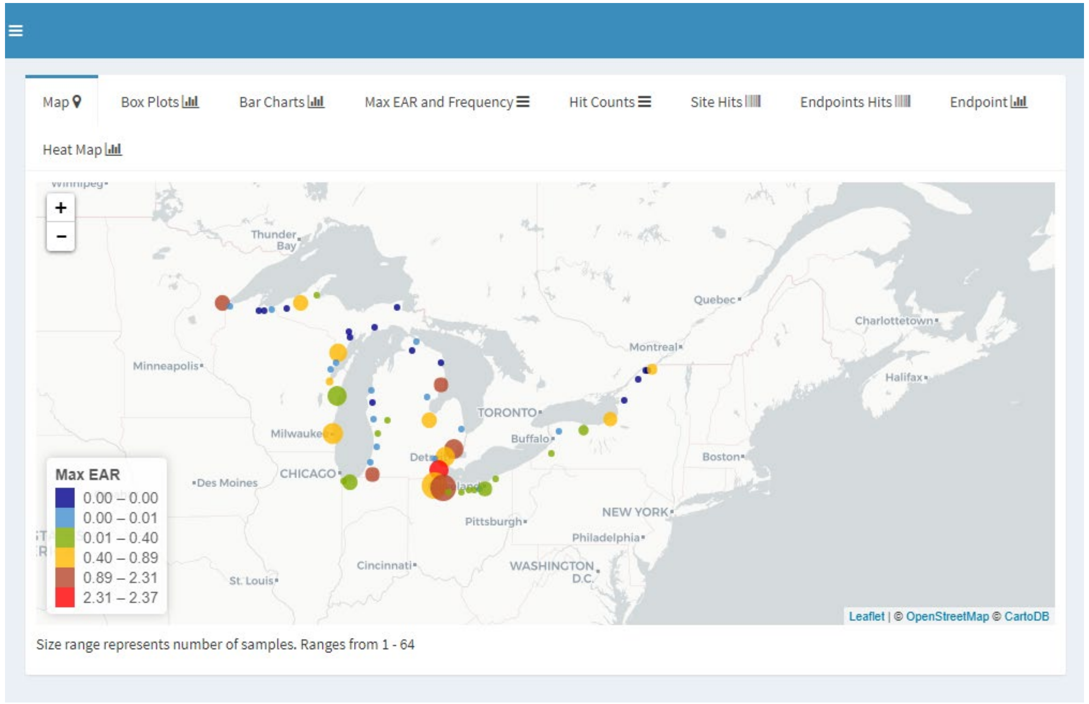
- Compare observed chemical concentrations to effects concentration in ToxCast database
  - Exposure-activity ratio (EAR)
  - Bioactivity-exposure ratio (BER)

- Simple, rapid approach to screen chemicals for potential biological effects

- Can prioritize sites or chemicals for further assessment

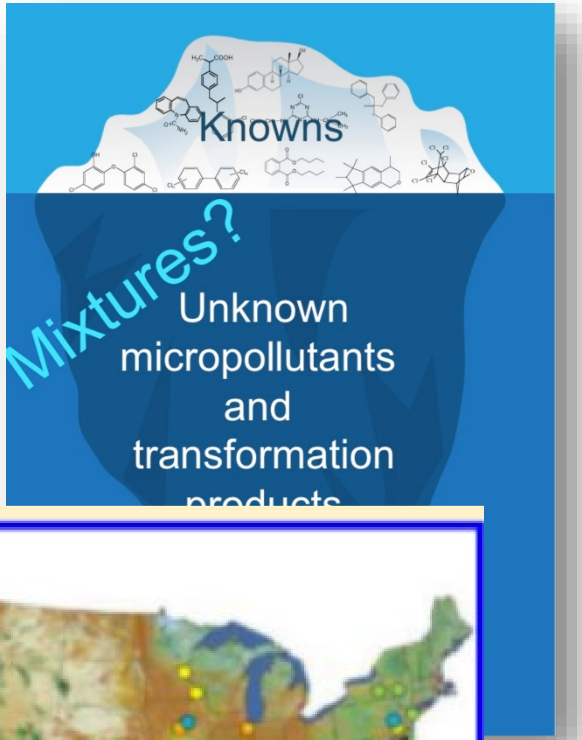
- R package “toxEval” allows for automated comparison of chemical monitoring data with ToxCast (https://github.com/USGS-R/toxEval)





HTT-based Screening of Environmental Mixtures

- HTS assays can be used as effects-based monitoring tools to directly screen environmental extracts
- Assay responses incorporate unknowns and potential interactions of mixture components



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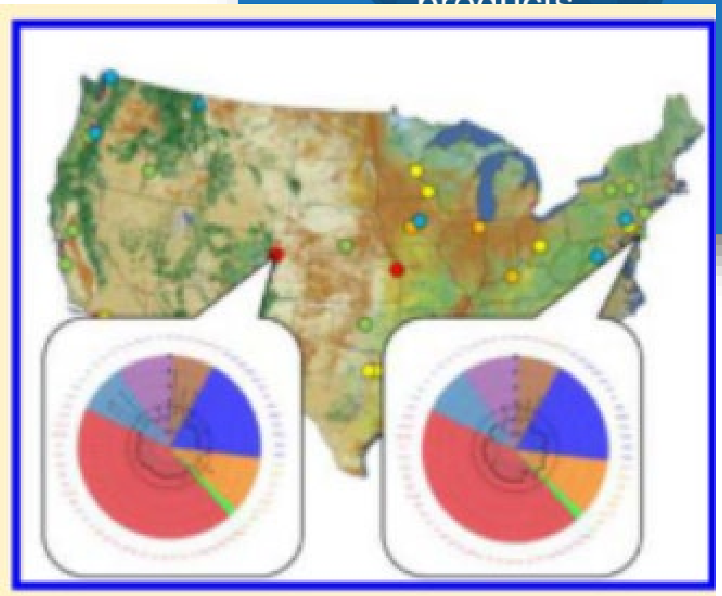
pubs.acs.org/est

Potential Toxicity of Complex Mixtures in Surface Waters from a Nationwide Survey of United States Streams: Identifying in Vitro Bioactivities and Causative Chemicals

Brett R. Blackwell,[*,†] Gerald T. Ankley,[†] Paul M. Bradley,[‡] Keith A. Houck,[§] Sergei S. Makarov,[∥] Alexander V. Medvedev,[∥] Joe Swintek,[⊥] and Daniel L. Villeneuve[†]



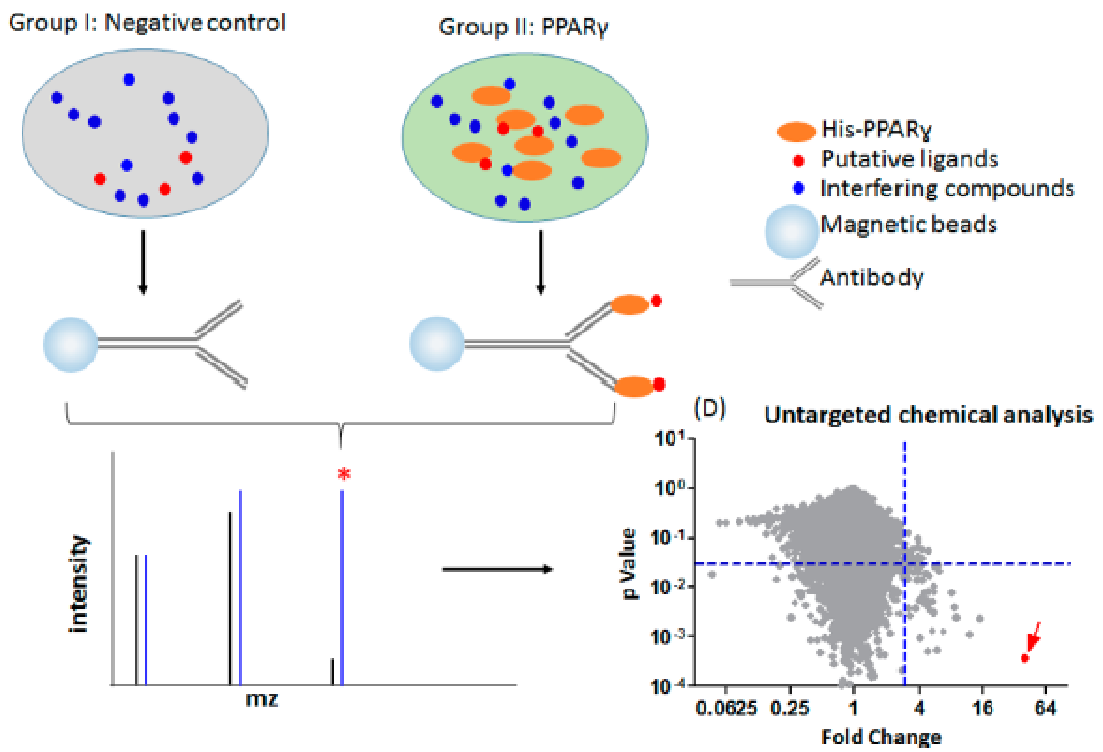
- Only one of 11 biological endpoint (estrogenic activity) explained by measured chemicals

NTA for Identification of Bioactive Chemicals

- NTA methods can help uncover the “unknowns” of chemical exposure
- Bioactivity can prioritize samples/features for NTA analysis
- Fractionation and subsequent bioassay/NTA analysis can further define chemical space of interest (effects-directed analysis, EDA)

Receptor Pulldown Assay and HR-MS

- Similar to bioassays, mass spectrometry techniques can utilize proteins to help elucidate bioactive compounds
- Effects-based monitoring data can guide selection of biological targets and environmental sites of interest
- Employing these methods in a case study of wastewater associated contaminants in the Colorado River



Peng et al. 2016, Environ. Sci. Technol. 50:7816-7824

Colorado River Case Study

- 2013 National Park Service measured CECs along Colorado River between Arches NP and Canyonlands NP
- Detected CECs far downstream of Moab WWTP
  - Designed 1950s for 5000 – millions of visitors per year
  - Treatment plant upgraded in 2018

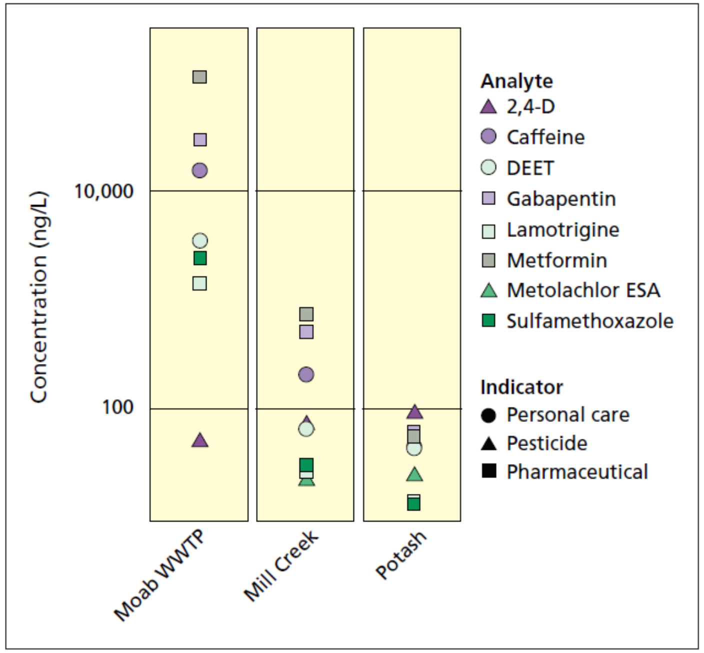
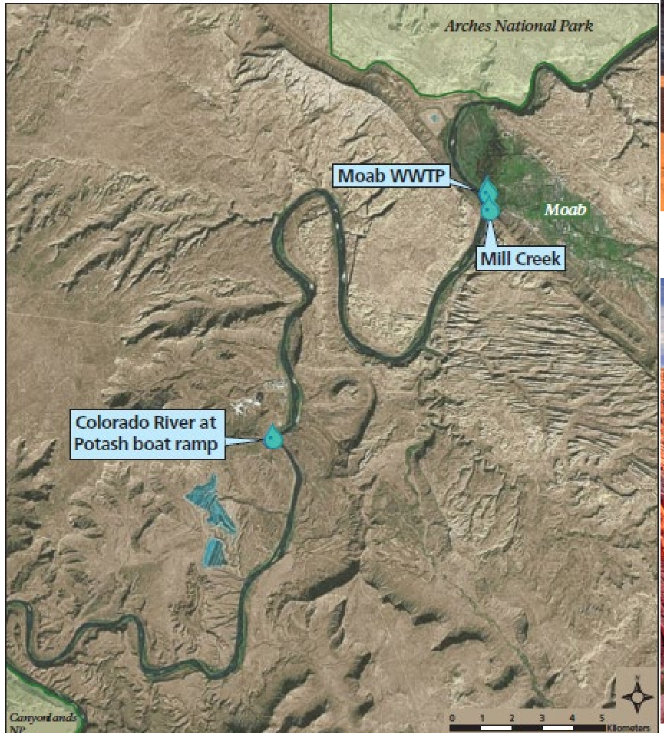
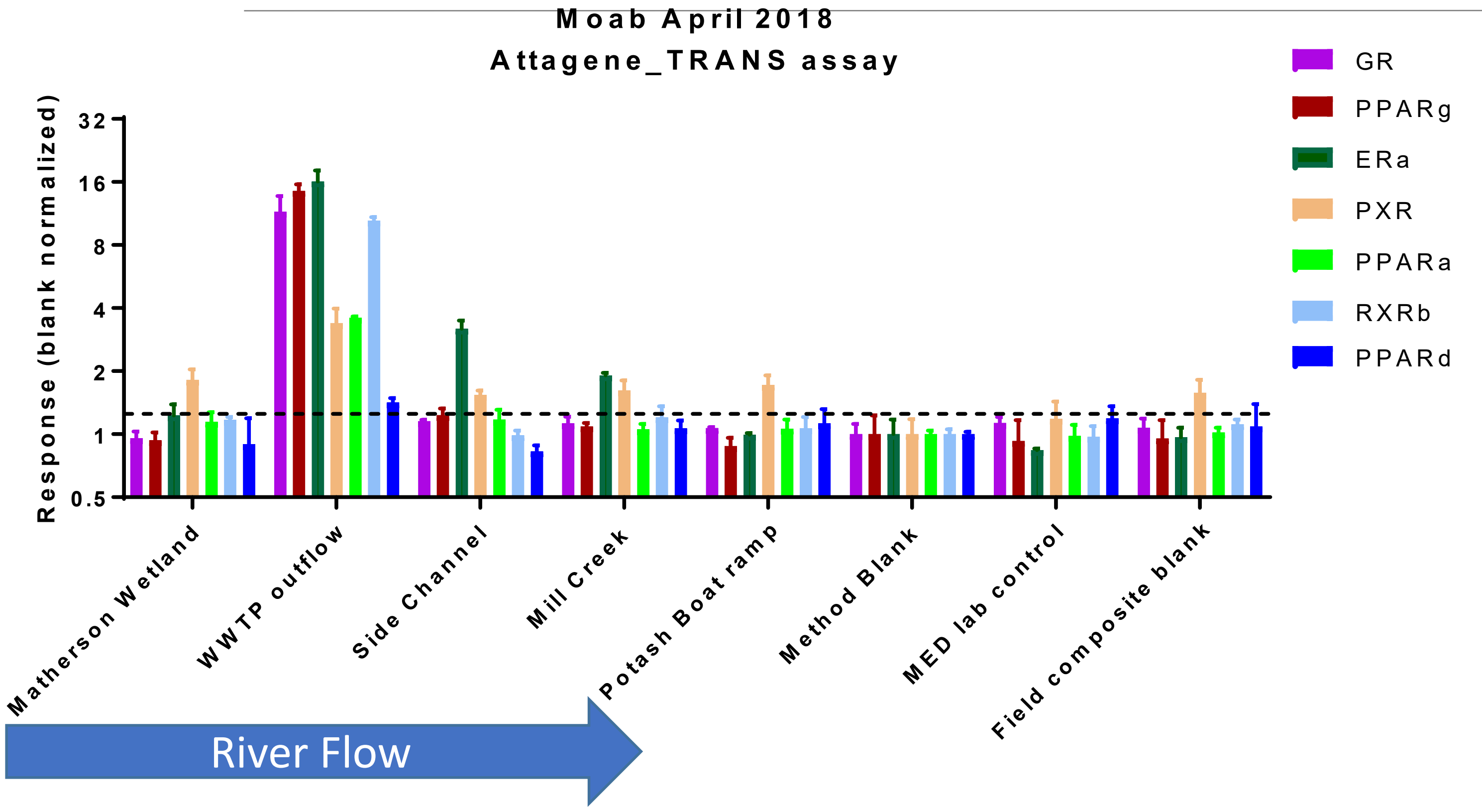


Figure 3-2. Concentrations of pharmaceuticals and personal care products decreased by orders of magnitude as samples moved downstream from the wastewater treatment plant outflow to the Colorado River at Mill Creek and to the Colorado River at the Potash boat ramp. Note the logarithmic scale on the y-axis.



Colorado River Bioactivity

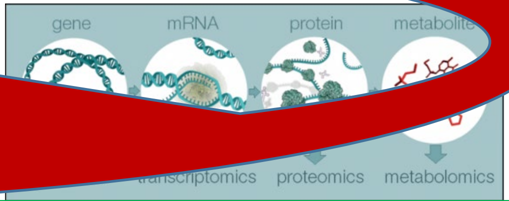


Target	Hazard Considerations
ER	• Well established hazard to aquatic vertebrates
GR	• Hazards to aquatic life not well defined
PPARg	• Hazards to aquatic life not well defined

Field Collection Methods

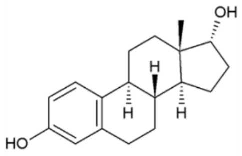
- Caged fathead minnows deployed at each site
- Autosampler deployed to collect composited water samples
  - Water samples align with duration of fish exposure
  - Provide representative samples for chemical or bioassay analysis
- Bimonthly grab water samples collected

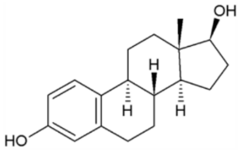
General Approach	Supervised	Unsupervised
Caged fat...	Endpoints associated with established AOPs ...iochemical and molecular markers of end... ...tradiol (E2), vitellogenin (VTG)	...ics • Metabolomics
Surface Waters; Extracts	In vitro bioassays • ER activity • GR activity • PPAR activity	Attagene Assays • In vitro assays • 70 endpoints

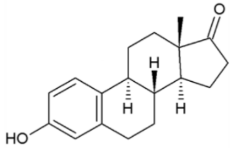


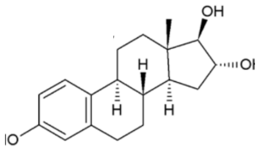
Bioassay Workflow

Targeted Steroidal Estrogens


17α-estradiol


17β-estradiol


estrone


estriol

Ambient Water Sample

Filtered

Extracted 200mg HLB

"Unknown" Chemical Mixture

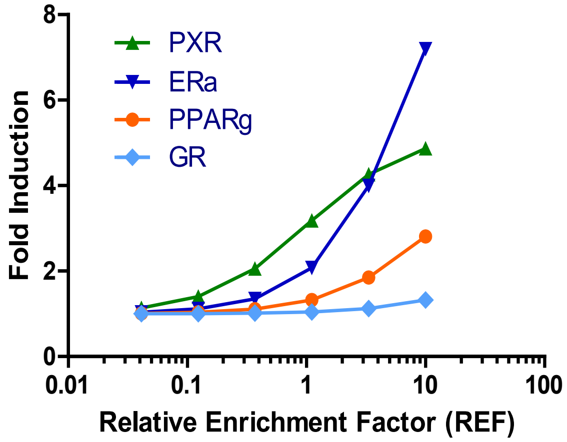
MeOH

2000x conc.

DMSO

1000x conc.

In vitro bioassays

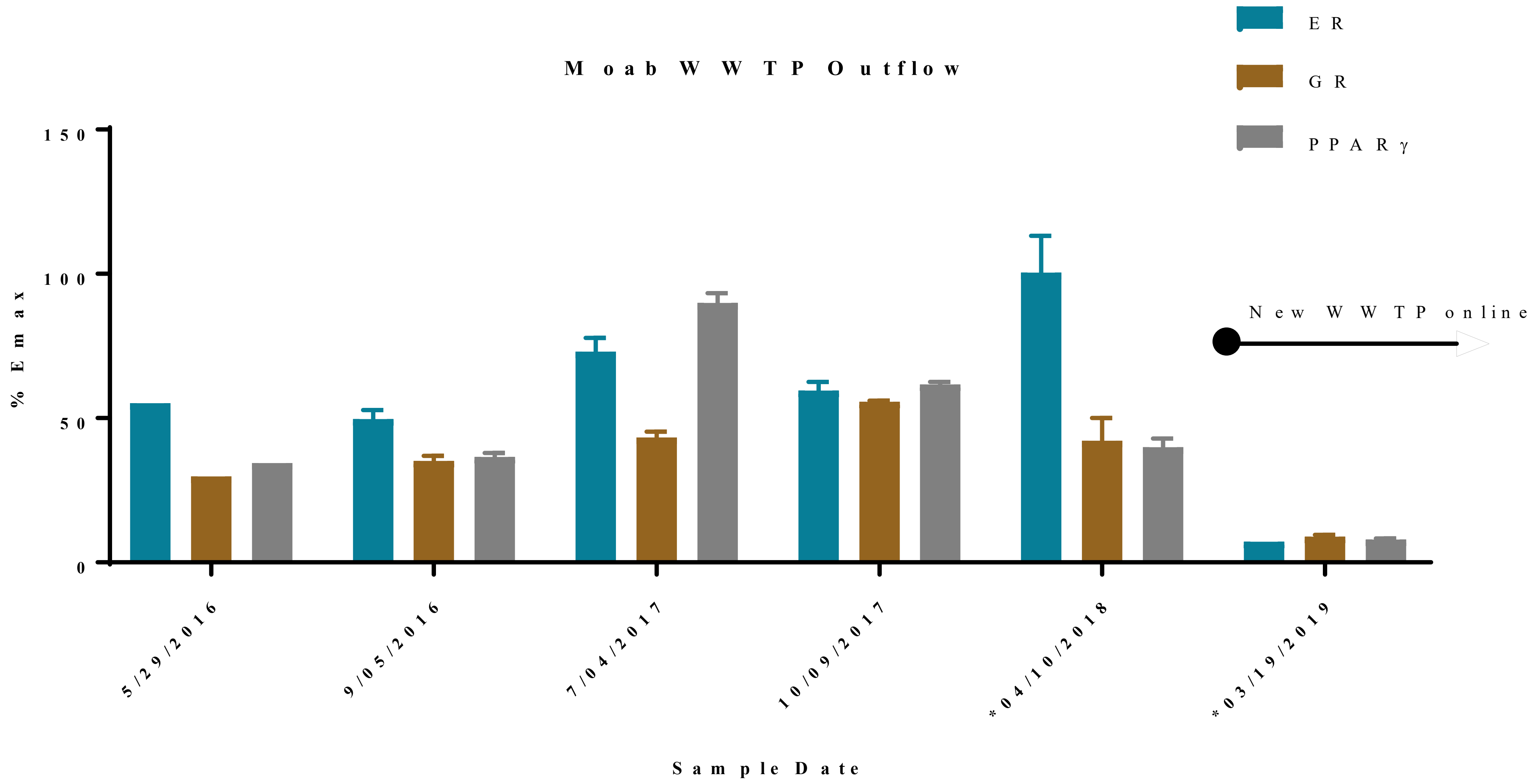


Glucocorticoid Suspect Screening

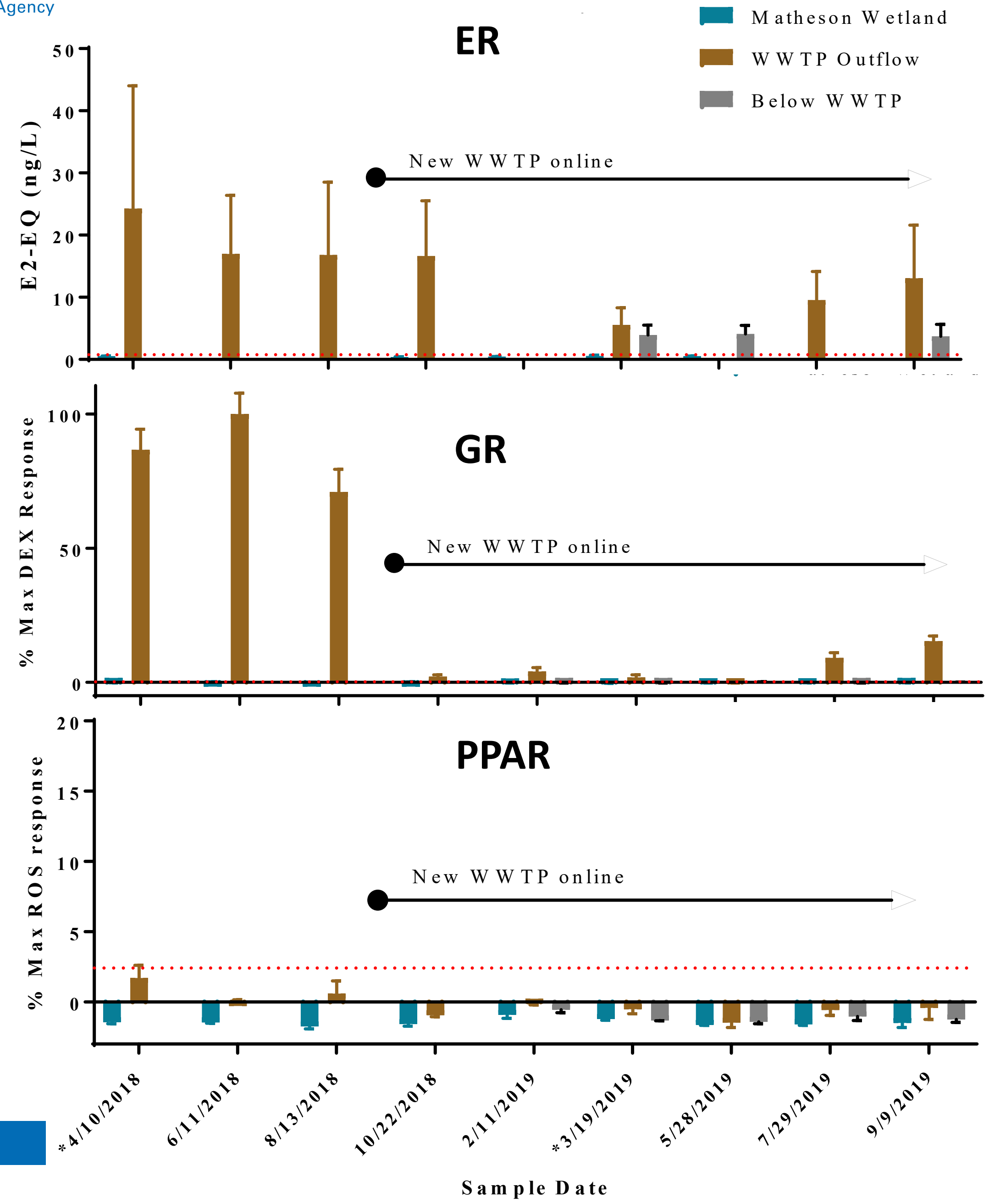
Orbitrap ID-X System

PPARγ Pulldown Assay

Attagene Trans-Factorial Bioassay Results



Targeted Bioassays



- ER and GR activity decrease greatly following WWTP upgrade
- PPARγ assay not sensitive enough to detect activity above baseline

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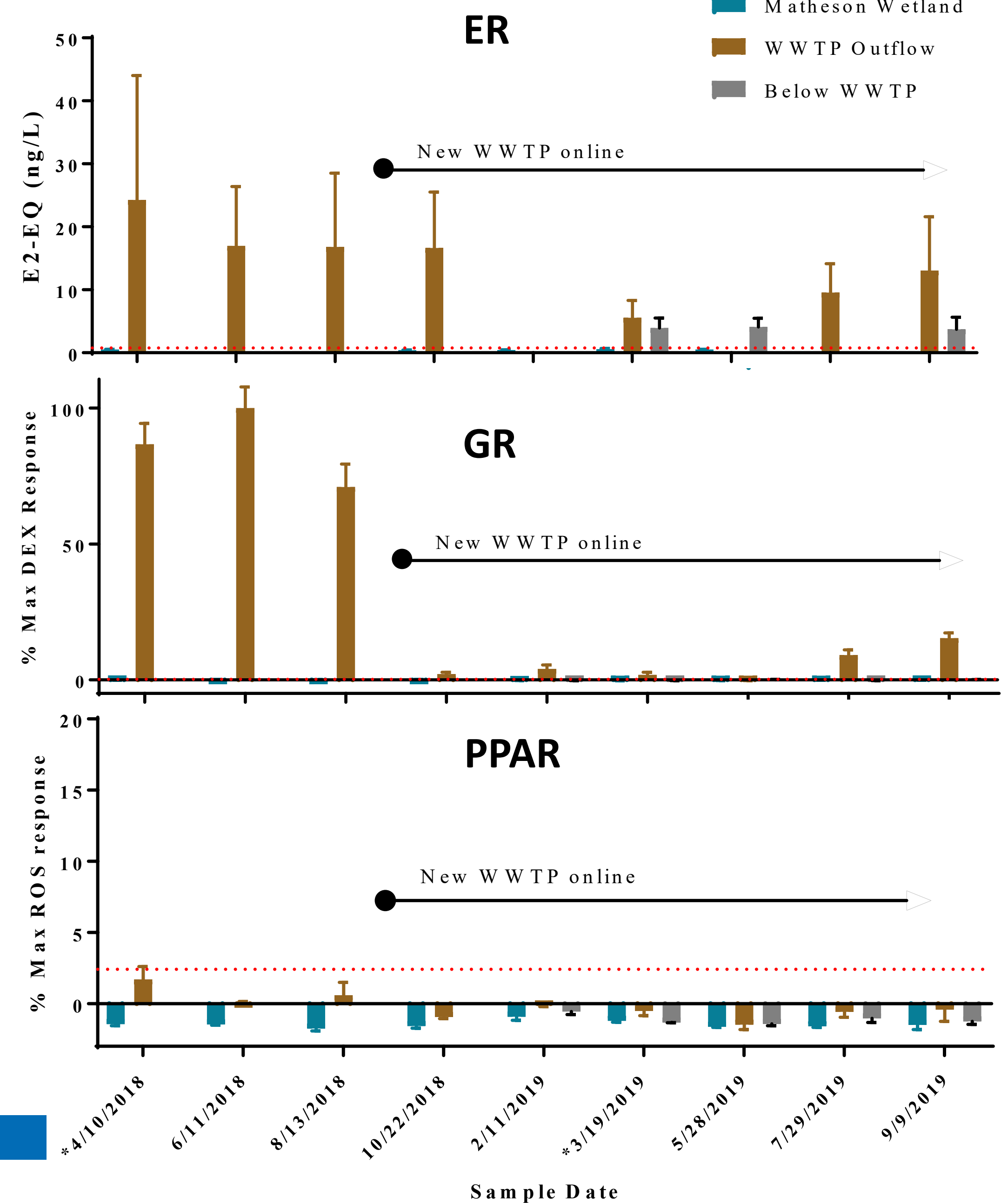
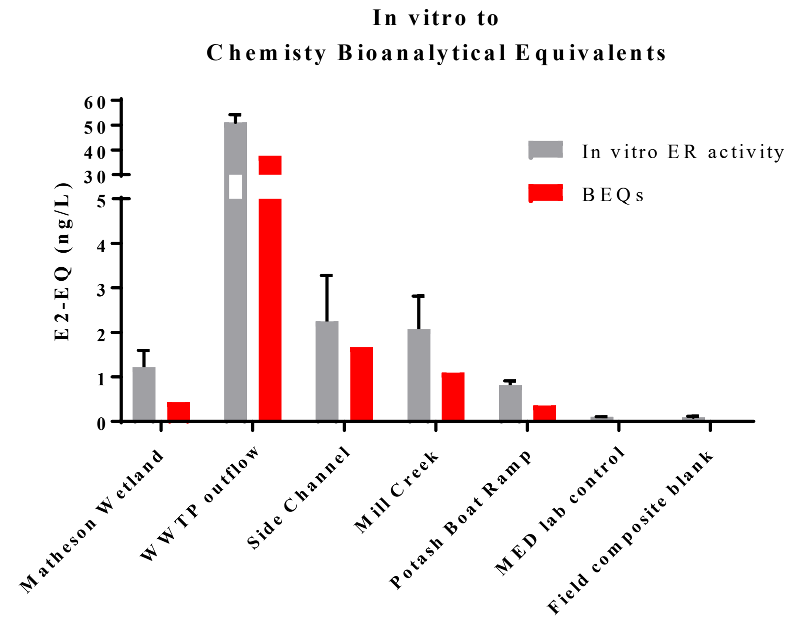
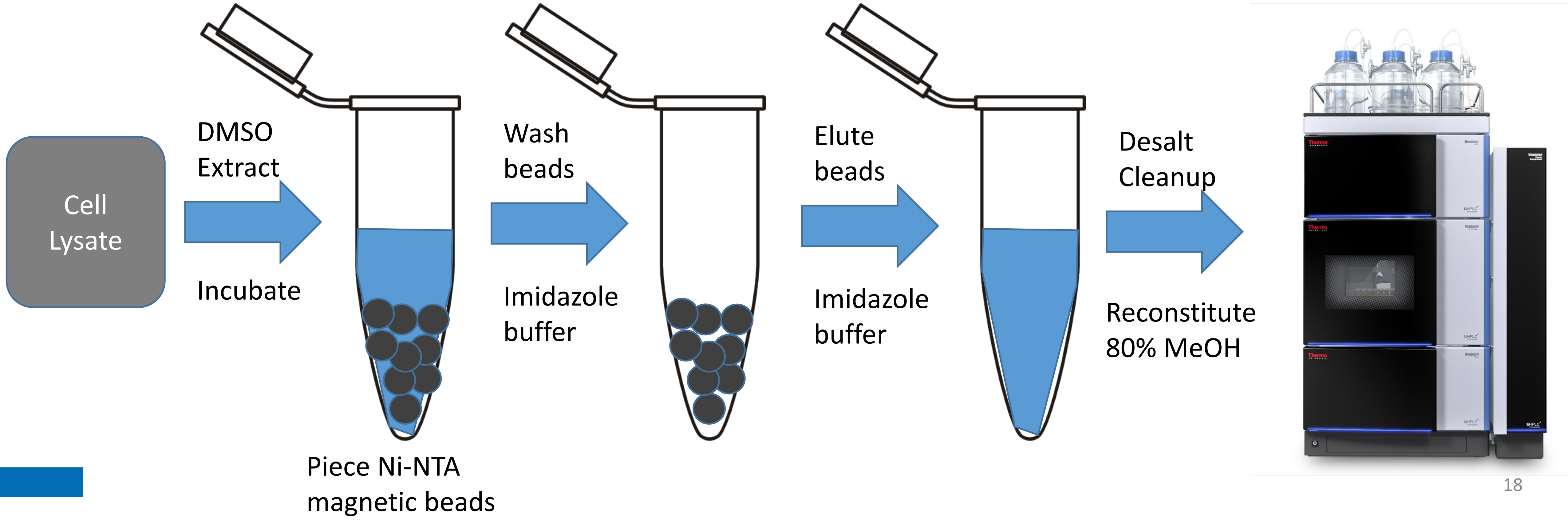




No observed chemicals can explain GR or PPAR related activity

PPARγ Receptor Pulldown Assay

- HIS-tagged human PPARγ – Ligand Binding Domain (LBD) only
- Receptor overexpressed in E.Coli; cell lysate used for assay



Pulldown Assay: LC-MS Analysis

- Vanquish Horizon LC System
- ID-X Orbitrap

Instrument Parameter	Value
Injection Volume	2 uL
Flow rate	300 uL/min
Column	C18, 1.7um, 2.1 x 150mm
Column Compartment	40°C
Polarity	Pos/Neg

Ion Source Properties

Ion Source Type	H-ESI
Spray Voltage	Static
Positive Ion (V)	3500
Negative Ion (V)	2500
Sheath Gas (Arb)	50
Aux Gas (Arb)	10
Sweep Gas (Arb)	1
Ion Transfer Tube Temp (°C)	325
Vaporizer Temp (°C)	350
APPI Lamp	Not in Use
Use Ion Source Settings from Tune	☐
FAIMS Mode	Not Installed

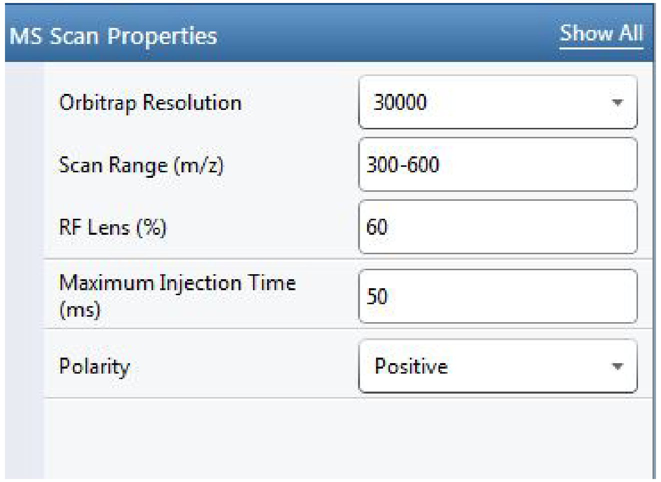
GR-Agonist Suspect Screening

- GR receptor not readily available for pulldown assay
- GR agonist much better defined – A number have been reported in wastewater and surface waters
- Suspect screening list of 45 previously identified compounds
  - Natural corticosteroids – cortisol, cortisone, corticosterone, etc
  - Synthetic corticosteroids – dexamethasone, triamcinolone, prednisone

Suspect Screening Analysis

- Vanquish Horizon LC System
- ID-X Orbitrap

Instrument Parameter	Value
Injection Volume	2 uL
Flow rate	300 uL/min
Column	C18, 1.7um, 2.1 x 150mm
Column Compartment	40°C
Polarity	Pos



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Suspect Screening Preliminary Results

- Processing HR-MS data using Compound Discoverer v3.1
- Automated workflow: Environmental Unknown ID w Online and Local Database Searches
    - Searches against ChemSpider, MZCloud, user defined suspect list
- Data analysis very preliminary to date

- Gladly taking suggestions/recommendations for successful data analysis!

Suspect Screening Preliminary Results

- Only WWTP site and process blanks analyzed
- 31 compounds with MS2 spectra

Compounds | Compounds per File | Merged Features | Features | mzCloud Results | ChemSpider Results | Mass List Search Results | Input Files | Specialized Traces

	Checked	Name	Formula	Annotation Sc	FISh Coverage	Molecular Weight	RT [min]	Area (Max.)
1			C13 H27 F N2 O7			342.18040	10.025	189060658
2			C29 H64 F N7 O17			801.43503	5.571	3000970
3		3,20-Dioxopregna-4,9(11),16-trien-21-yl acetate	C23 H28 O4			368.19894	8.226	6057044
4			C16 H26 F2 N4 O			328.20730	12.571	18729871
5			C42 H68 N3 O10 P			805.46484	5.774	45612841
6			C44 H86 F2 N8 O8			892.65360	14.326	11642104
7		Methylprednisolone	C22 H30 O5		34.57	374.20906	7.367	3358786
8		17α-Hydroxyprogesterone	C21 H30 O3		37.61	330.21851	7.733	34441552
9		Hydrocortisone Valerate	C26 H38 O6		21.74	446.26693	10.345	15144596
10			C18 H28 F N4 O3 P S			430.15993	7.646	10228015
11		2-piperidinophenyl N-[4-(benzyloxy)phenyl]carbamate	C25 H26 N2 O3			402.20213	5.774	12923567
12		N-{[(2R,4S,5R)-5-(3-Cyclopentyl-1-methyl-1H-pyrazol-5	C22 H30 N4 O2			364.22515	12.760	4844295
13			C24 H49 F O2 P2			450.31949	11.577	37465016
14		Corticosterone	C21 H30 O4		33.62	346.21401	8.791	3391338
15		Nor-9-carboxy-δ9-THC	C21 H28 O4			344.19823	14.175	3087155
16		Cortisol	C21 H30 O5		41.18	362.20897	7.534	5883962
17		2-Octyl-3,6,9,12-tetraoxatetradecane-1,14-diol	C18 H38 O6			350.26582	12.180	68752768
18		(2S,3R,4S,5S,6R)-2-[4-hydroxy-2-(3-methylbut-2-en-1-y	C17 H24 O7			362.13423	10.239	3808722
19		NP-020634	C21 H30 O6			400.18627	10.077	3117029
20		Cortisone	C21 H28 O5		52.25	360.19383	7.619	3294584

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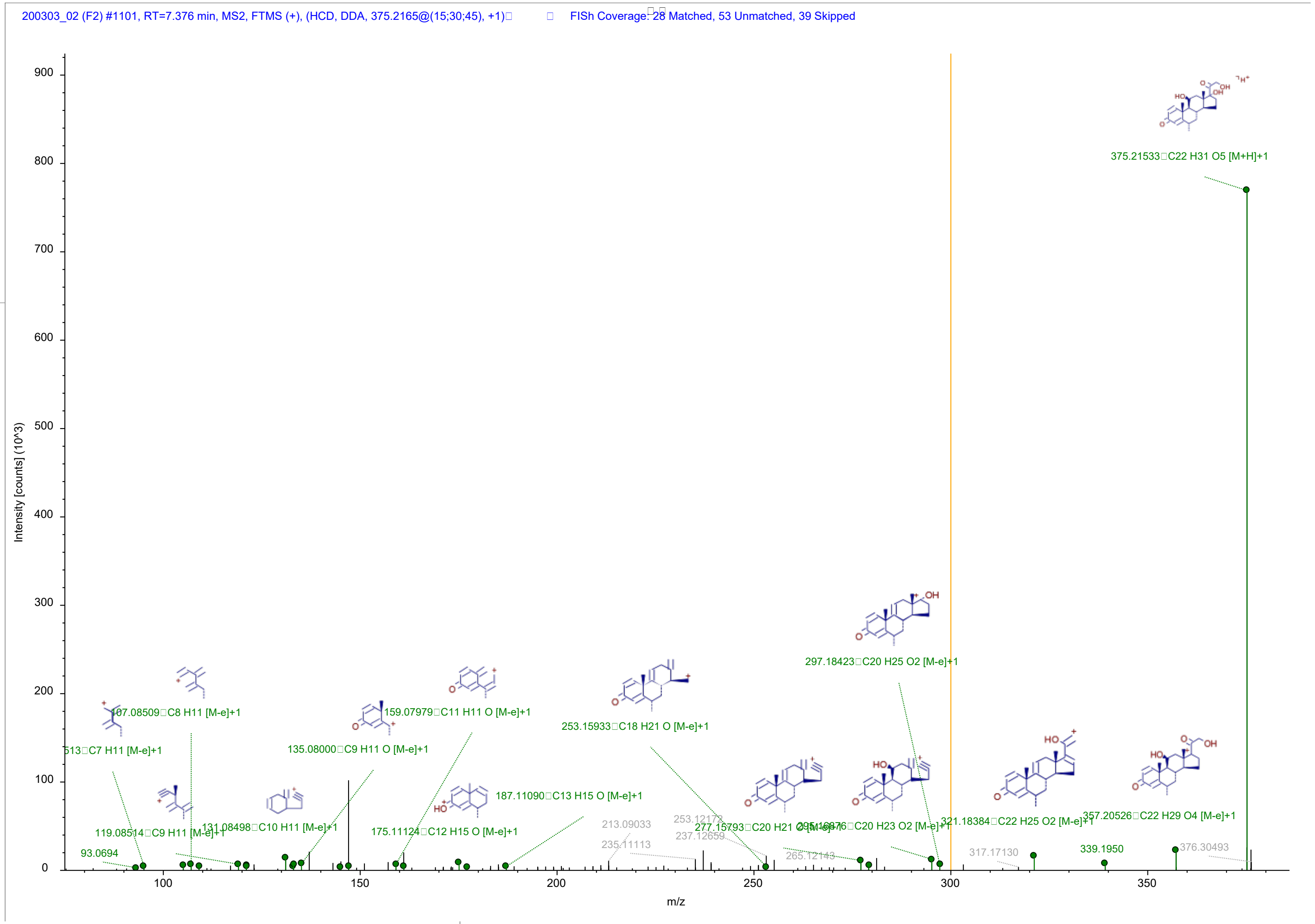

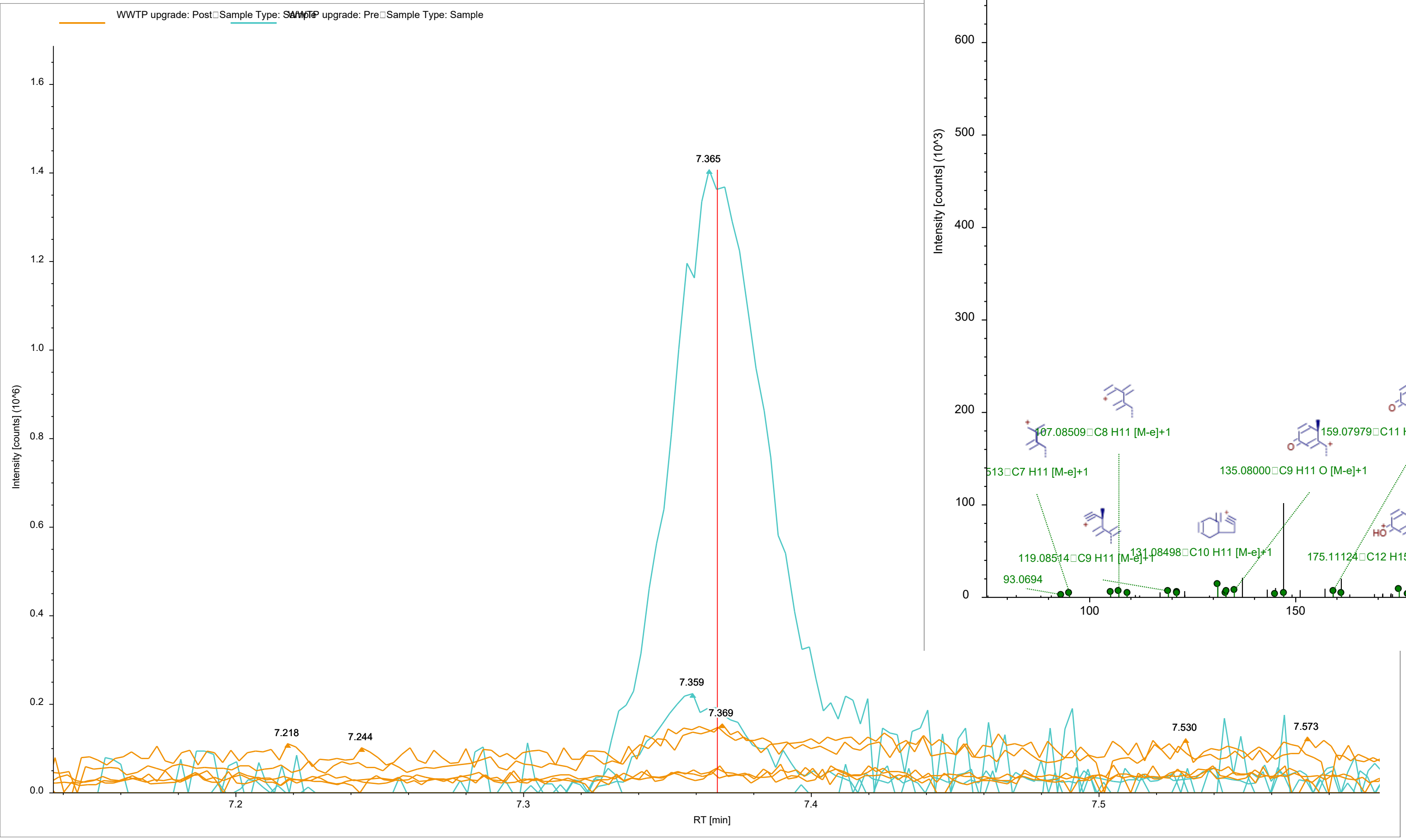

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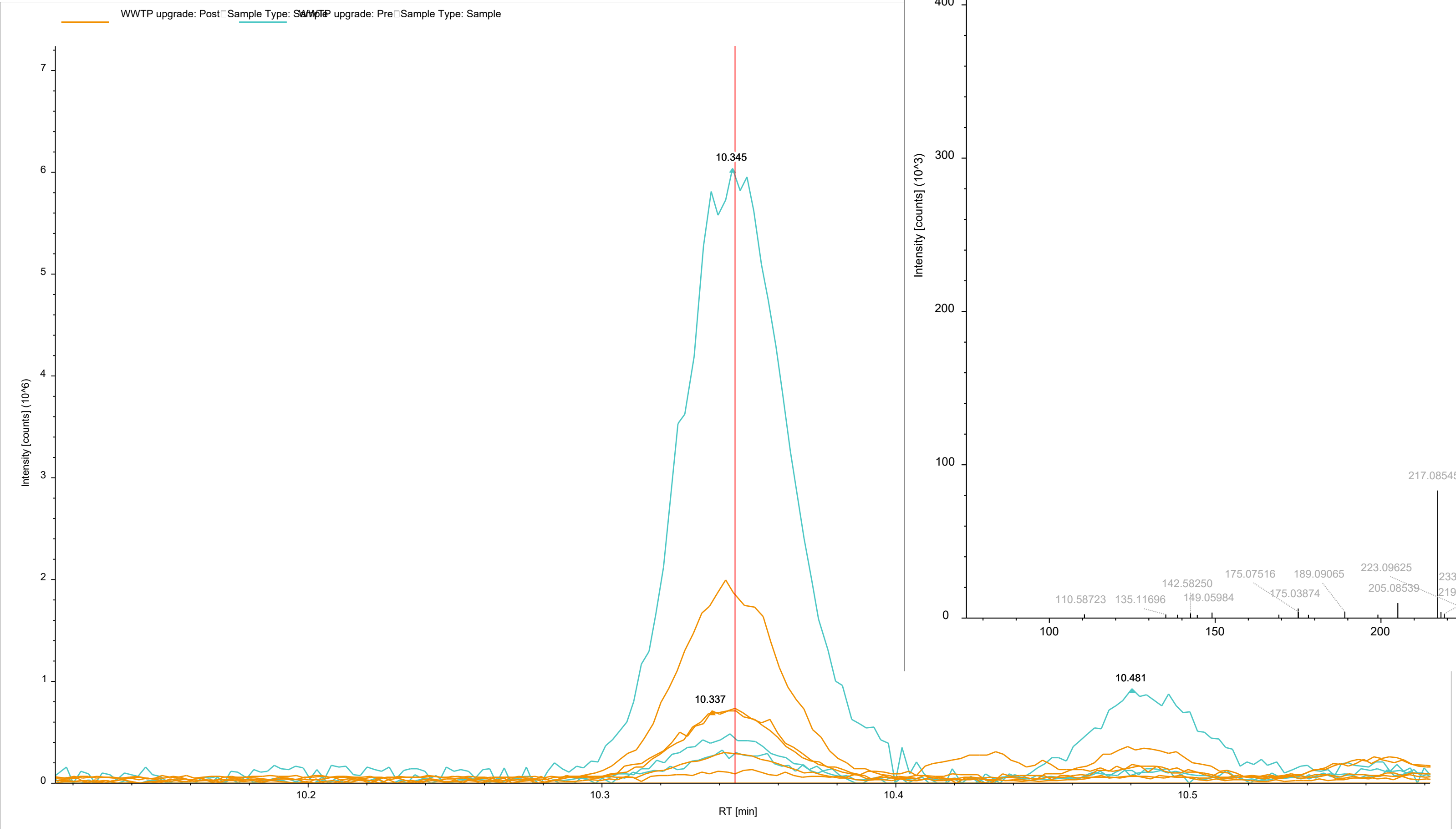

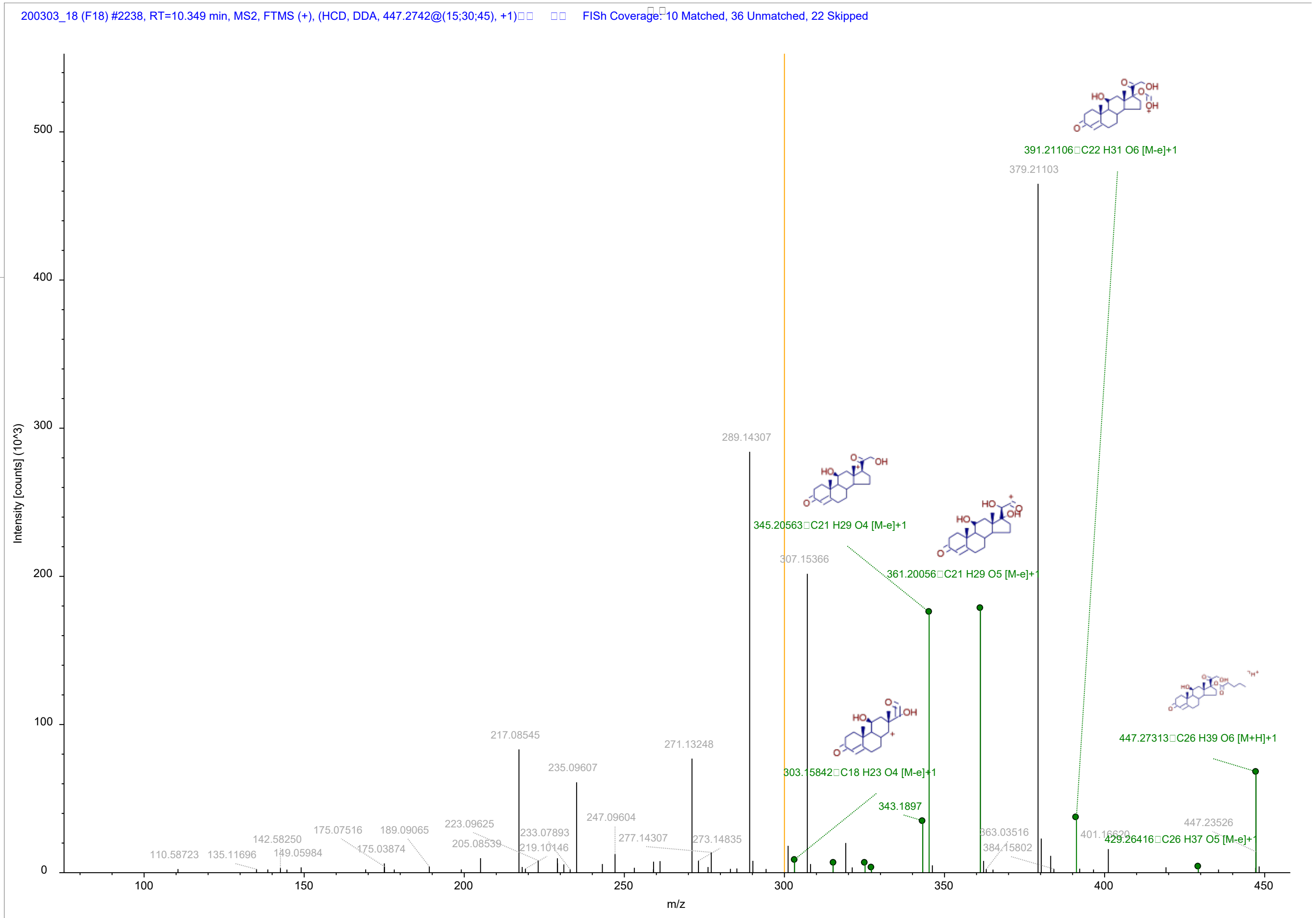

Summary

- Effects-based monitoring a powerful complement to traditional chemical monitoring
- Effects-based methods can support and guide unknowns identification
- If specific targets are known, receptor pulldown assays can be a specific, targeted method to identify bioactive components of mixtures
- Suspect screening viable if receptor agonists are well defined
- Access to lab is back – Continuing pulldown assay and suspect screeing work. Targeting mid-2021 for project completion

Questions?

Brett Blackwell, PhD

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Center for Computational Toxicology and Exposure

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