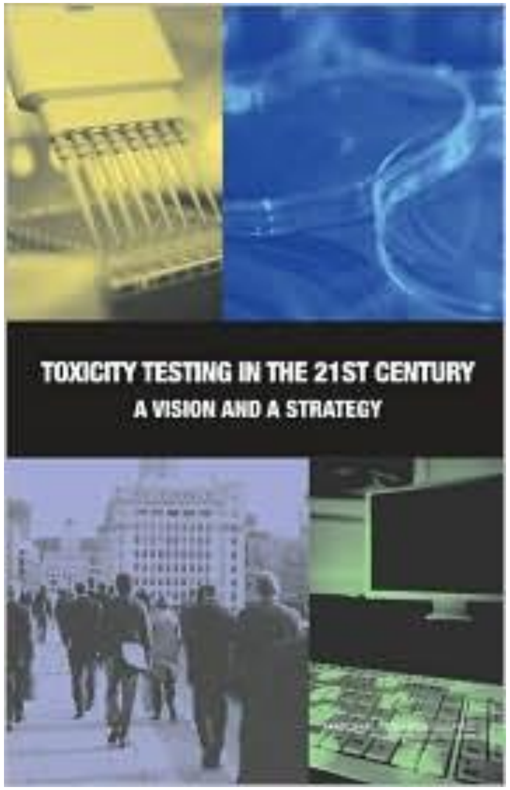
Exploring the Potential Utility of High-throughput Bioassays Associated with U.S. EPA ToxCast Program for Effects-based Monitoring and Surveillance

Anthony Schroeder
Postdoctoral Research Associate
EPA's Computational Toxicology Communities of Practice
January 22, 2014

Toxicity Testing in the 21st Century



- Greater use of computational tools for chemical characterization.
- Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods
- Apply our growing knowledge of biological systems
  - Identify Toxicity pathways: Cellular response pathways that, *when sufficiently perturbed*, are expected to result in adverse health effects.
  - Develop appropriate high-throughput assays
  - Scientifically-defensible extrapolation via biologically based computational/predictive models.

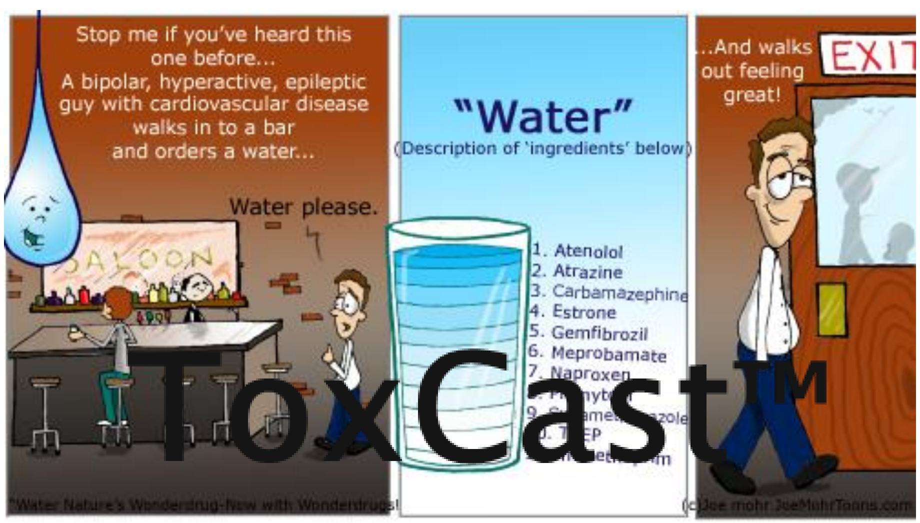
In vitro and computational methods → Conservation, animal welfare

Toxicity pathways → Scientifically credible foundation

Comprehensive array of in vitro tests (high through-put) → Depth, breadth

Next Frontier in 21st Century Toxicity Testing

- EPA's ToxCast Program
  - Screened over 2,000 individual chemicals through ~700 assays
- Tox21 Collaboration
  - Screened ~10,000 individual chemicals through ~50 assays
- Potential to screen the majority of chemicals currently in production or use in the next few years
- Application for these 21st century tools could be useful for biological monitoring and surveillance of complex environmental mixtures



Bio-effects Monitoring

- Biological effects monitoring can be a powerful complement to chemical monitoring
  - Detect exposure to chemicals for which analytical methods are unavailable or impractical.
  - Integrate the impacts of complex mixtures and multiple stressors.
  - Provide “early warning” of potential hazards before they manifest in an obvious way.

Spectrum of Monitoring Challenges

Surveillance

Status and trends
BUIs, AOCs

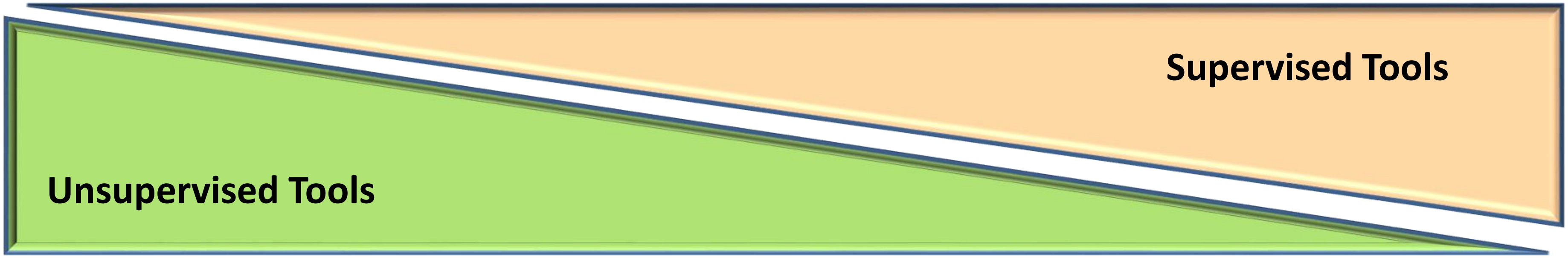
Point source
discharge

Remediation
/ restoration

Spill response

Increasing knowledge of problem(s)

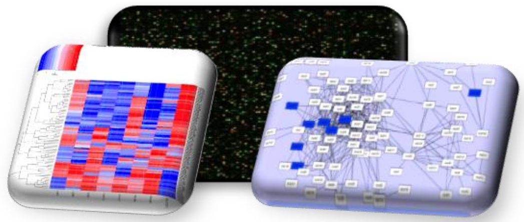
Increasing specificity of effect(s)
Increasing specificity of chemical(s)



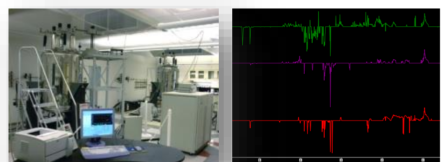
General Approach	Unsupervised	Supervised

Fish exposed in situ

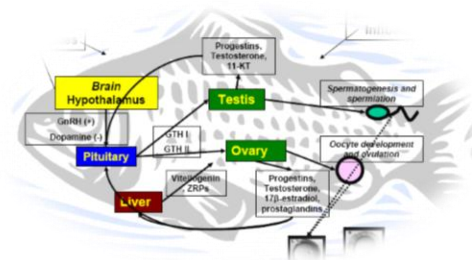
DNA-microarray
Transcriptomics



Metabolomics



- Endpoints associated with established adverse outcome pathways:

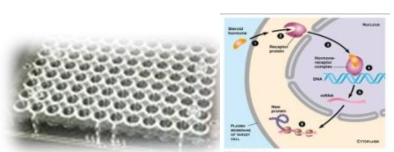


- E.g., Biochemical and molecular markers of endocrine disruption and adverse reproductive outcomes.

Surface water samples/extracts

High throughput in vitro screening

In vitro bioassays



- MDA-kb2: (anti)androgenic activity
- T47D: (anti)estrogenic activity
- H4IIE: dioxin-like contaminants
- H295R: steroidogenesis inhibitors
- Others ……

Desired Characteristics for Environmental Surveillance and Monitoring

- **Efficient and cost effective** → **In vitro; rapid, predictive responses in vivo**
- **Provides early warning** → **Focus on pathway perturbation and initiating events rather than apical toxicity outcomes**
- **Integrates effects of mixtures, multiple stressors** → **Effects-based monitoring, in vitro, in vivo – esp. in situ exposures**
- **Casts a broad net – cover wide range of chemical and biological space** → **Unsupervised approaches**

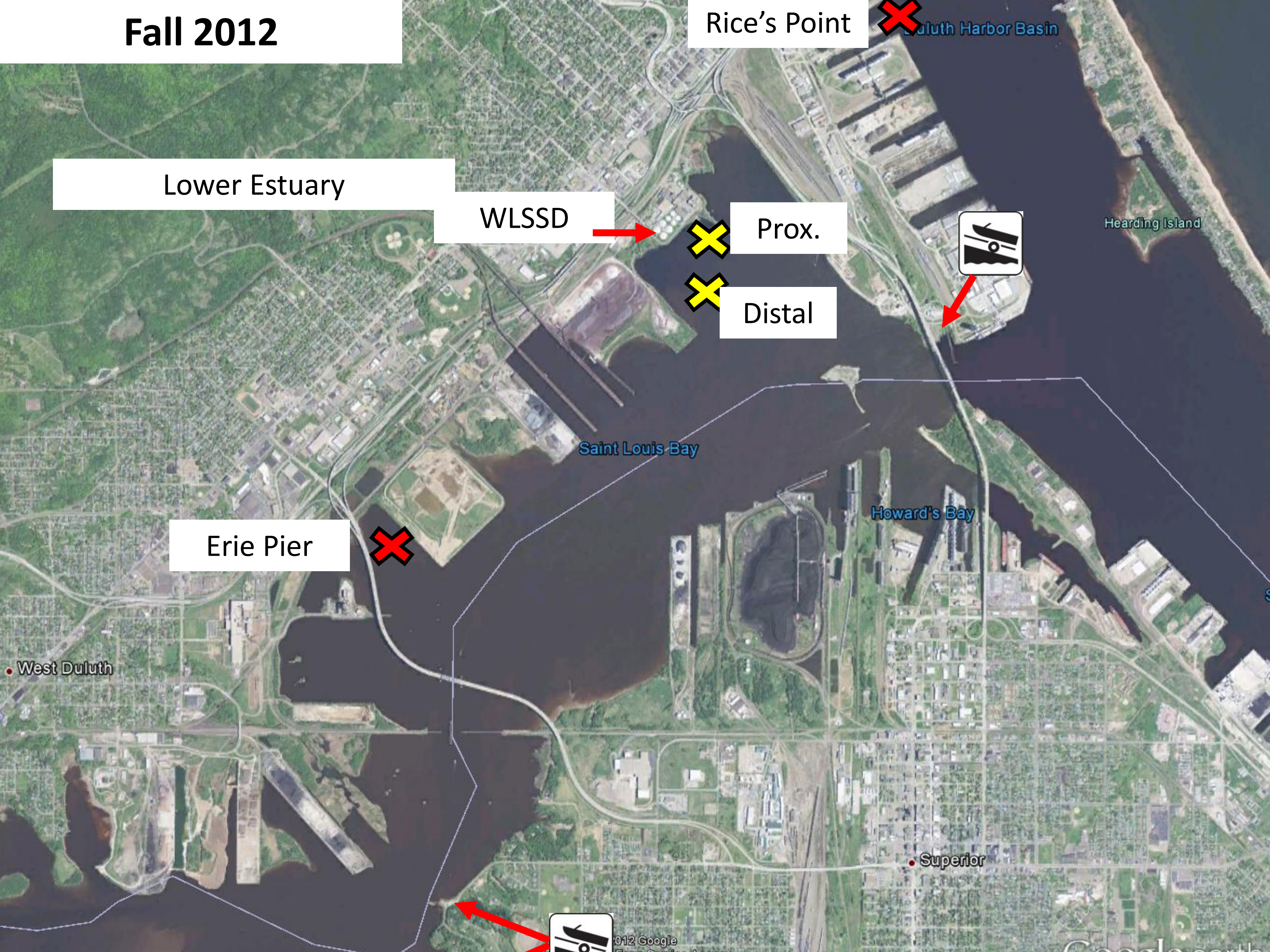
  'omics' in vivo, HTP in vitro
- **Diagnostic – can help identify or eliminate potential causes** → **Bioassay integrated with chemical analysis**

Applicability of HTS Assays For Environmental Surveillance and Monitoring

- Can high-throughput screening assays be used as a diagnostic tool?
  - Presence of analytical data – Can HTS assays allow for associations between chemicals detected and their biological effects?
  - Little or no analytical data – Can HTS assays help to identify chemicals that may be causing biological effects?
- Can we hypothesize adverse outcomes based on high-throughput screening results?

Attagene (Attagene Inc., Morrisville, NC) Assay Battery

- Factorial cellular biosensor system (HepG2 cell line)
- Combined libraries of cis- and trans-regulated transcription factor reporter constructs
- Multiplexed for simultaneous evaluation of TF activities
- 81 different assay features evaluated with 160 + measurement endpoints
- Identify transcription factors induced by a chemical



Fall 2012
Rice's Point
Duluth Harbor Basin
Lower Estuary
WLSSD
Prox.
Distal
Hearding Island
Saint Louis Bay
Howard's Bay
Erie Pier
West Duluth
Superior

Chemicals Detected - Fall 2012

Chemical	EriePr	WLSSD proximal	WLSSD distal	RicesPt		Chemical	EriePr	WLSSD proximal	WLSSD distal	RicesPt
1,4-Dichlorobenzene, µg/L	<0.08	0.02	<0.08	<0.08		Pentachlorophenol, µg/L	<1.6	0.2	0.2	<1.6
1-Methylnaphthalene, µg/L	0.01	0.02	0.02	0.01		Phenanthrene, µg/L	0.01	<0.02	0.01	0.01
2,6-Dimethylnaphthalene, µg/L	0.01	<0.04	<0.04	0.01		Phenol, µg/L	<0.16	<0.16	0.05	<0.16
2-Methylnaphthalene, µg/L	0.01	0.03	0.03	0.02		Pyrene, µg/L	0.0042	<0.02	<0.02	<0.02
3,4-Dichlorophenyl isocyanate, µg/L	0.02	0.04	0.02	0.02		Tetrachloroethene, µg/L	<0.16	0.05	<0.16	<0.16
4-Nonylphenol diethoxylate, µg/L	0.2	1.1	1.7	<1.6		Tributyl phosphate, µg/L	0.044	0.281	0.096	0.020
4-Nonylphenol monoethoxylate , µg/L	<1.6	0.28	<1.6	<1.6		Triethyl citrate, µg/L	0.01	0.11	0.05	<0.04
4-tert-Octylphenol, µg/L	<0.4	0.1	<0.4	<0.4		Triphenyl phosphate, µg/L	<0.08	0.01	0.01	<0.08
4-tert-Octylphenol diethoxylate, µg/L	<0.2	0.2	0.1	<0.2		Tris(2-chloroethyl) phosphate, µg/L	<0.16	0.06	<0.16	<0.16
4-tert-Octylphenol monoethoxylate, µg/L	<0.6	0.1	<0.6	<0.6		Tris(dichloroisopropyl) phosphate, µg/L	<0.32	0.07	0.04	<0.32
Acetophenone, µg/L	<0.4	0.3	0.3	<0.4		Chloroxylenol, µg/L	<0.080	0.052	<0.080	<0.080
Anthracene, µg/L	0.0032	<0.02	<0.02	<0.02		Oxycodone, µg/L	0.638	0.122	0.398	0.108
9,10-Anthraquinone, µg/L	<0.04	0.04	<0.04	<0.04		Carbamazepine, µg/L	<0.160	0.041	<0.160	<0.160
Atrazine, µg/L	<0.16	<0.16	<0.16	0.03		Celecoxib, µg/L	0.127	<0.640	<0.640	<0.640
Benzophenone, µg/L	0.06	0.19	0.11	0.14		Citalopram, µg/L	<0.080	0.013	<0.080	<0.080
beta-Sitosterol,µg/L	0.3	<4.8	0.9	<4.8		Diphenhydramine, µg/L	<0.080	0.065	<0.080	<0.080
Bisphenol A, µg/L	0.07	0.62	2.75	0.10		Ibuprofen, µg/L	<0.640	0.93	0.392	<0.640
Caffeine, µg/L	0.03	0.62	0.25	0.05		Iminostilbene, µg/L	<0.080	0.066	<0.080	<0.080
Camphor, µg/L	0.03	<0.08	<0.08	0.02		Lidocaine, µg/L	<0.080	0.022	0.017	<0.080
Cholesterol, µg/L	0.3	0.4	0.6	<1.6		Phenytoin, µg/L	<0.160	0.048	<0.160	<0.160
Cotinine, µg/L	0.03	0.18	<0.08	<0.08		Piperonyl butoxide, µg/L	<0.080	0.019	<0.080	<0.080
Diethyl phthalate, µg/L	0.70	0.70	<0.4	0.2		Tramadol, water, µg/L	<0.040	0.086	0.052	<0.040
Fluoranthene, µg/L	0.01	<0.02	<0.02	<0.02		Venlafaxine, µg/L	<0.040	0.084	<0.040	<0.040
Isophorone, µg/L	0.008	0.068	0.059	0.007		Methocarbamol, µg/L	<0.640	0.18	<0.640	<0.640
N,N-Diethyl-m-toluamide (DEET), µg/L	0.13	0.26	0.23	0.2		3-beta-Coprostanol, ng/L	<200	1,620	454	<200
Naphthalene, µg/L	<0.02	0.02	0.02	0.01		Bisphenol A, ng/L	<100	612	4330	<100
4-Nonylphenol (sum of all isomers), µg/L	<1.6	0.4	<1.6	<1.6		Cholesterol, ng/L	1,460	3,440	4,390	1,090
p-Cresol, µg/L	0.02	0.04	<0.08	<0.08		Estrone, ng/L	<0.80	2.07	<0.80	<0.80

Erie Pier – ToxCast Results

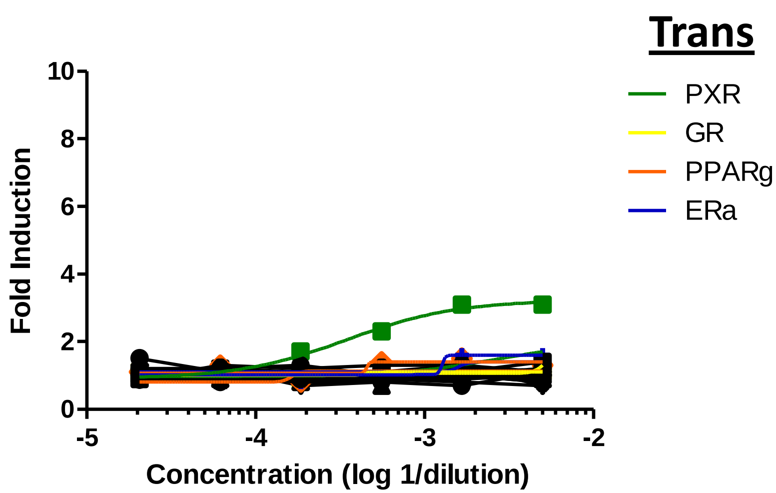
Response Profile

ATG_Ahr_CIS

ATG_PXR_TRANS

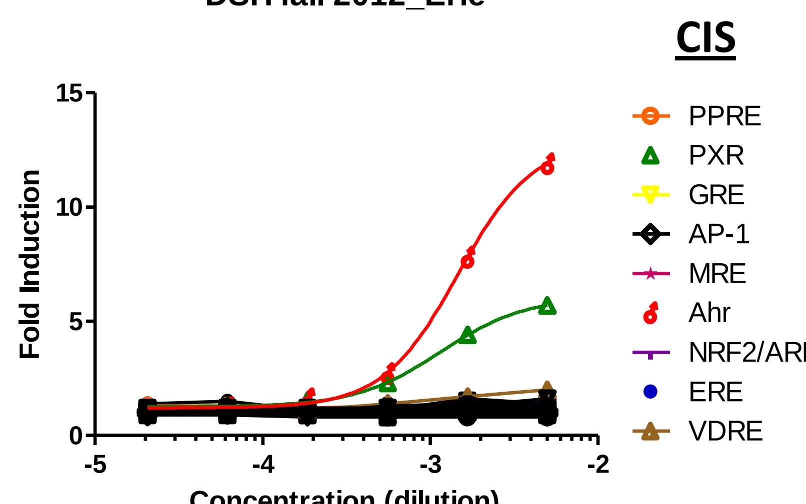
ATG_PXRE_CIS

DSH fall 2012_Erie



	GR	PPARg	ERa	PXR
EC50	~ 0.0004253	~ 0.0004548	0.001270	0.0003512

DSH fall 2012_Erie

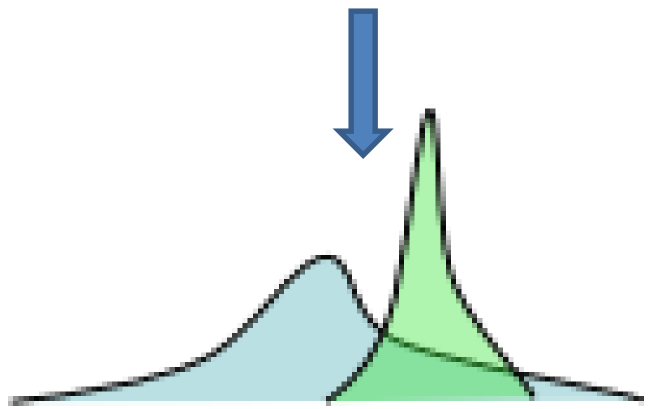


	PXR	Ahr	ERE
EC50	0.001572	0.003779	0.002013

Risk-based Chemical Prioritization

Analytes measured in environmental water sample

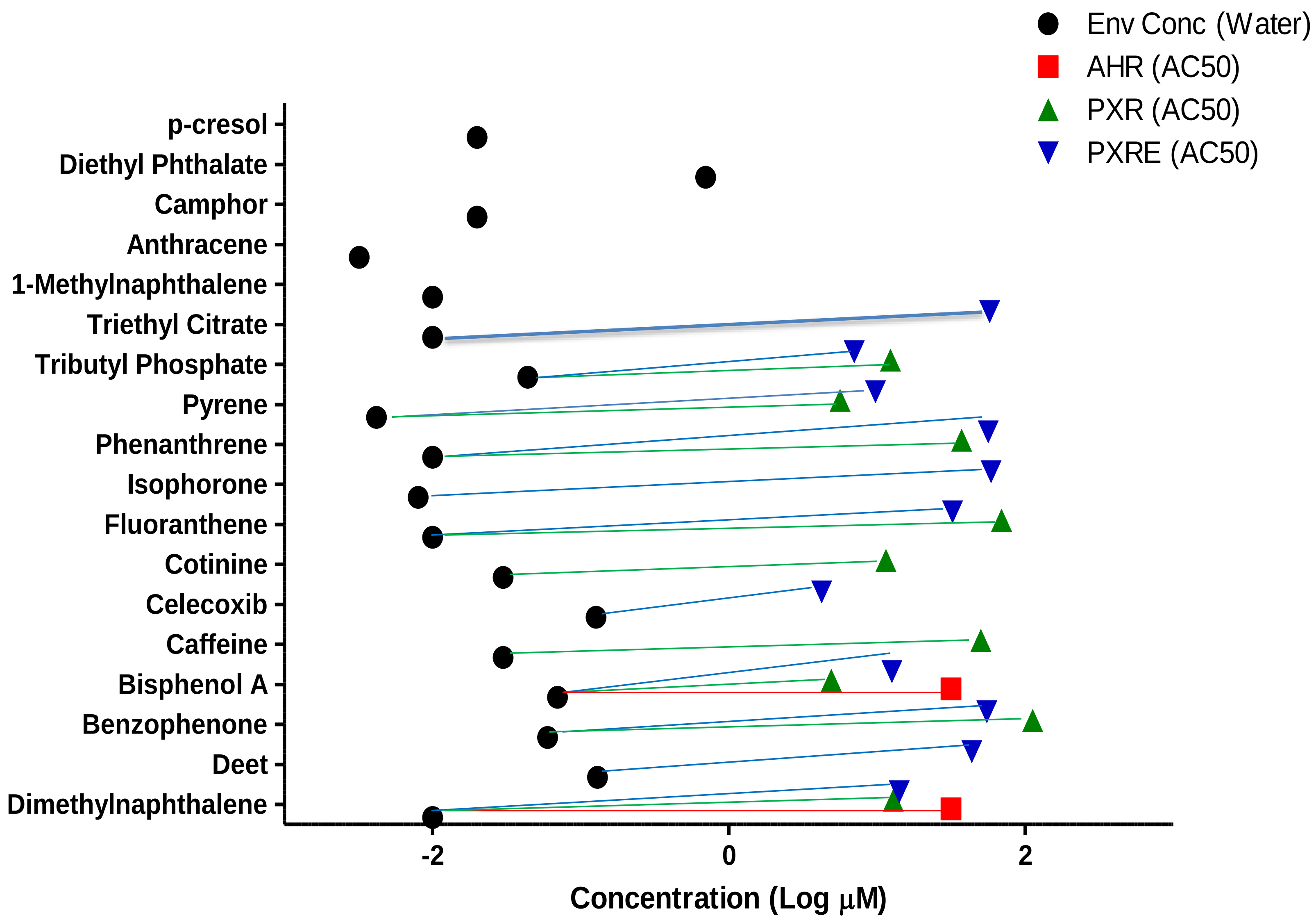
Concentration needed to activate ToxCast target



Analyte concentration overlaps the concentration needed to activate target

Prioritize chemicals associated with response

Erie Pier – ToxCast Results



Assumptions and Limitations

- Bioavailability
- Metabolism
- Bioconcentration
- Serves as a “first-cut” prioritization approach

Rice's Point – ToxCast Results

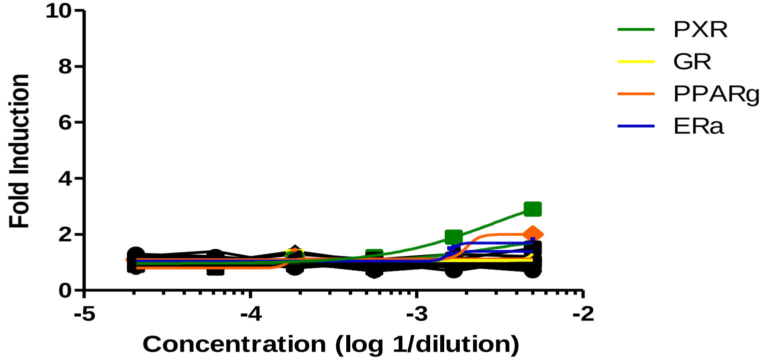
Response Profile

ATG_Ahr_CIS

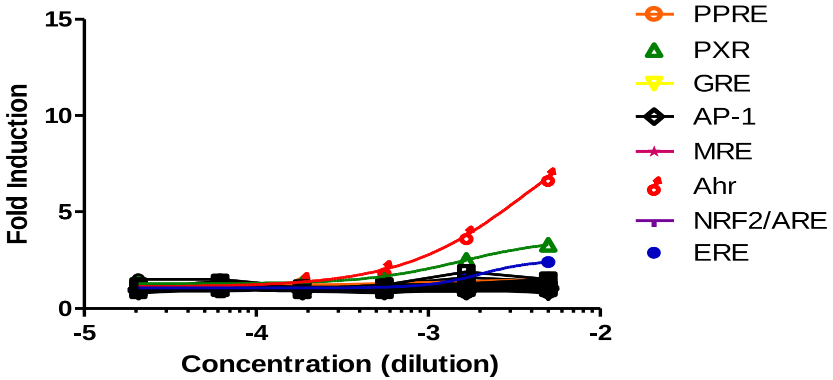
ATG_ERE_CIS

ATG_PXR_TRANS

ATG_PXRE_CIS

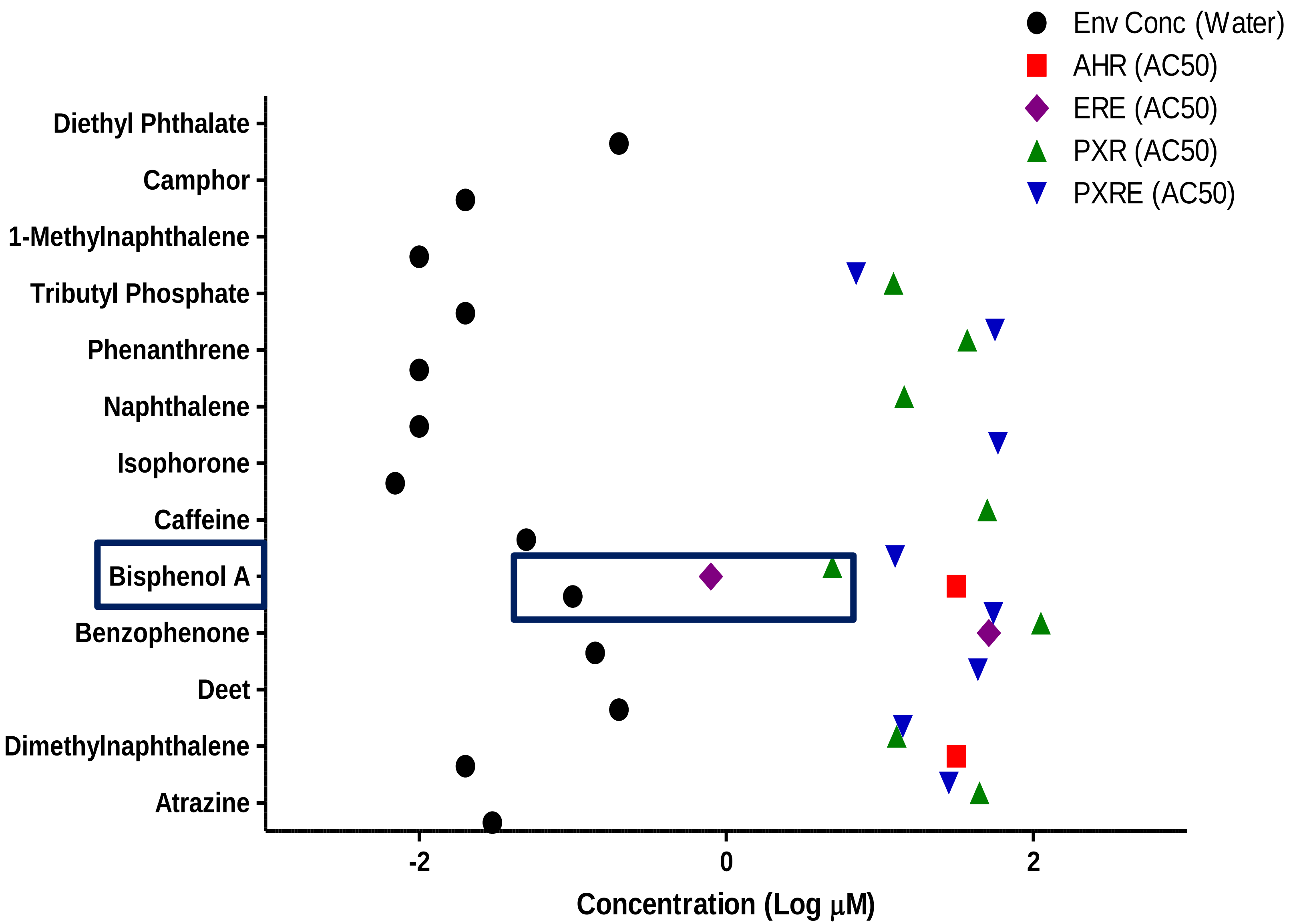


	GR	PPARg	ERa	PXR
EC50	~ 8.358e-005	~ 0.001997	~ 0.001588	0.002916



	PXR	Ahr	ERE
EC50	0.001572	0.003779	0.002013

Rice's Point – ToxCast Results

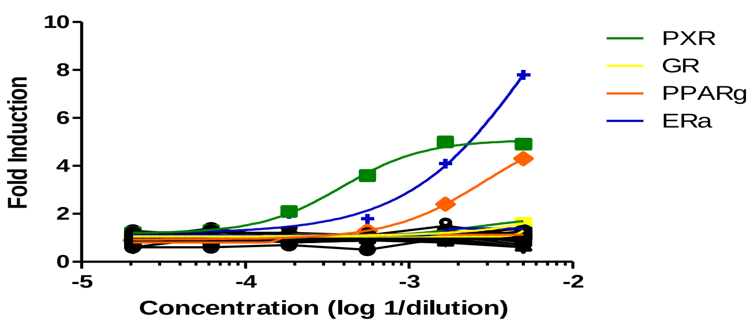


WLSSD Proximal – ToxCast Results

Response Profile

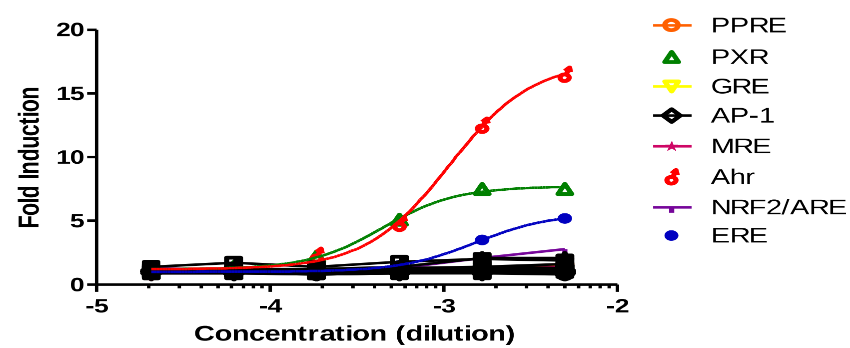
ATG_Ahr_CIS
ATG_ERa_TRANS
ATG_ERE_CIS
ATG_NRF2_ARE_CIS
ATG_PPARg_TRANS
ATG_PXR_TRANS
ATG_PXRE_CIS
ATG_VDRE_CIS

DSH fall 2012_Prox



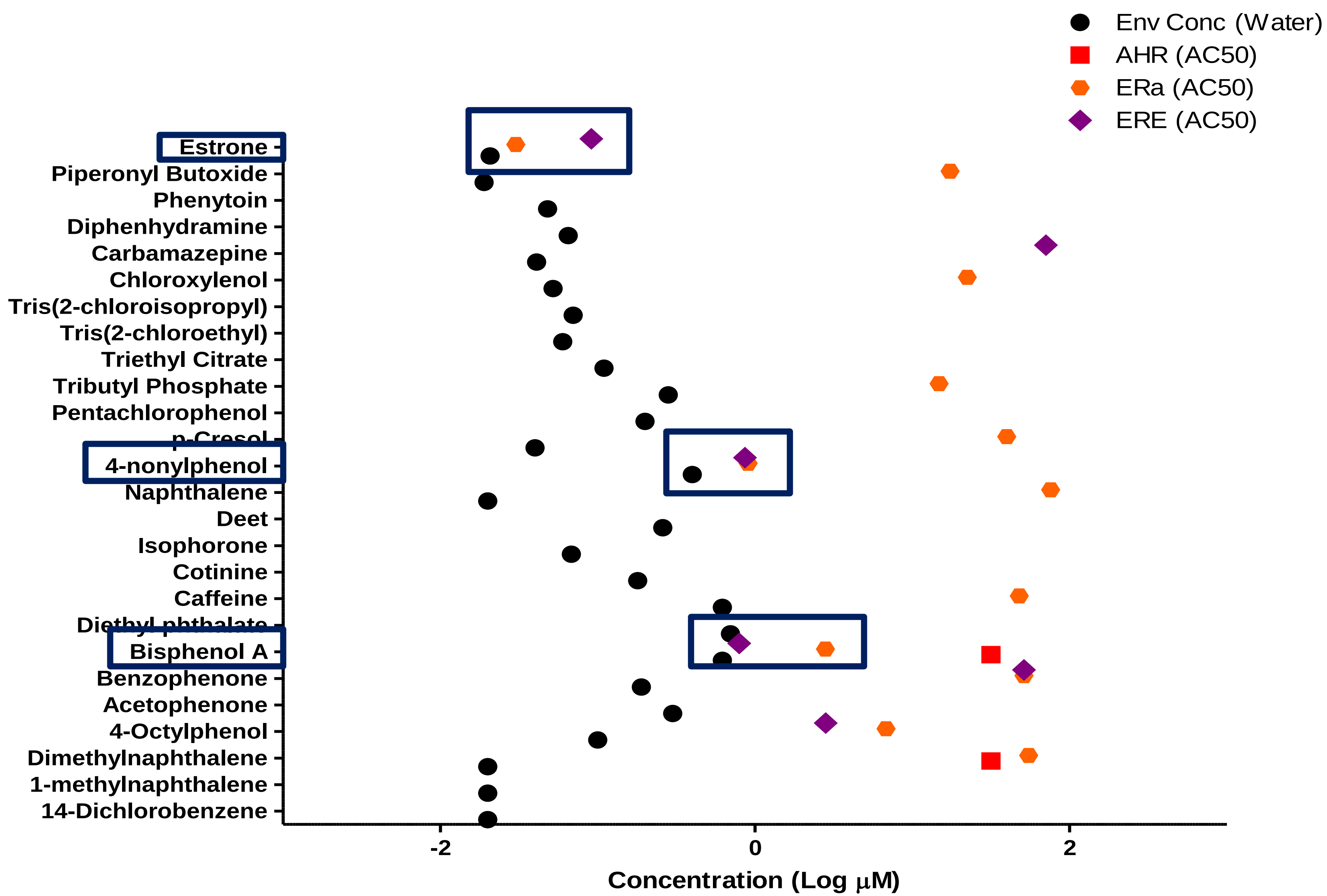
	GR	PPARg	ERa	PXR
EC50	~ 1.995	0.002864	0.005989	0.0003913

DSH fall 2012_Prox



	PXR	Ahr	ERE
EC50	0.0004439	0.001077	0.001534

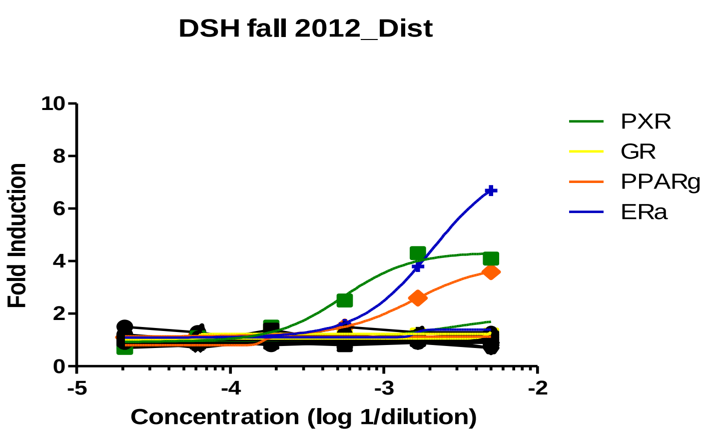
WLSSD Proximal – ToxCast Results



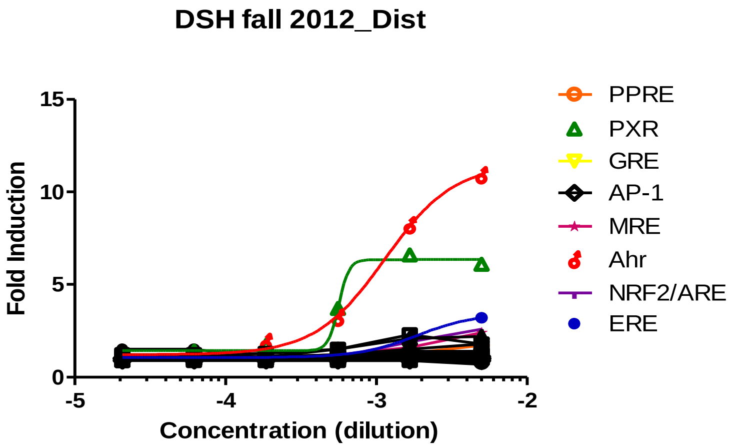
WLSSD Distal – ToxCast Results

Response Profile

ATG_Ahr_CIS
ATG_ERa_TRANS
ATG_ERE_CIS
ATG_HIF1a_CIS
ATG_MRE_CIS
ATG_NRF2_ARE_CIS
ATG_PPARg_TRANS
ATG_PXR_TRANS
ATG_PXRE_CIS
ATG_VDRE_CIS

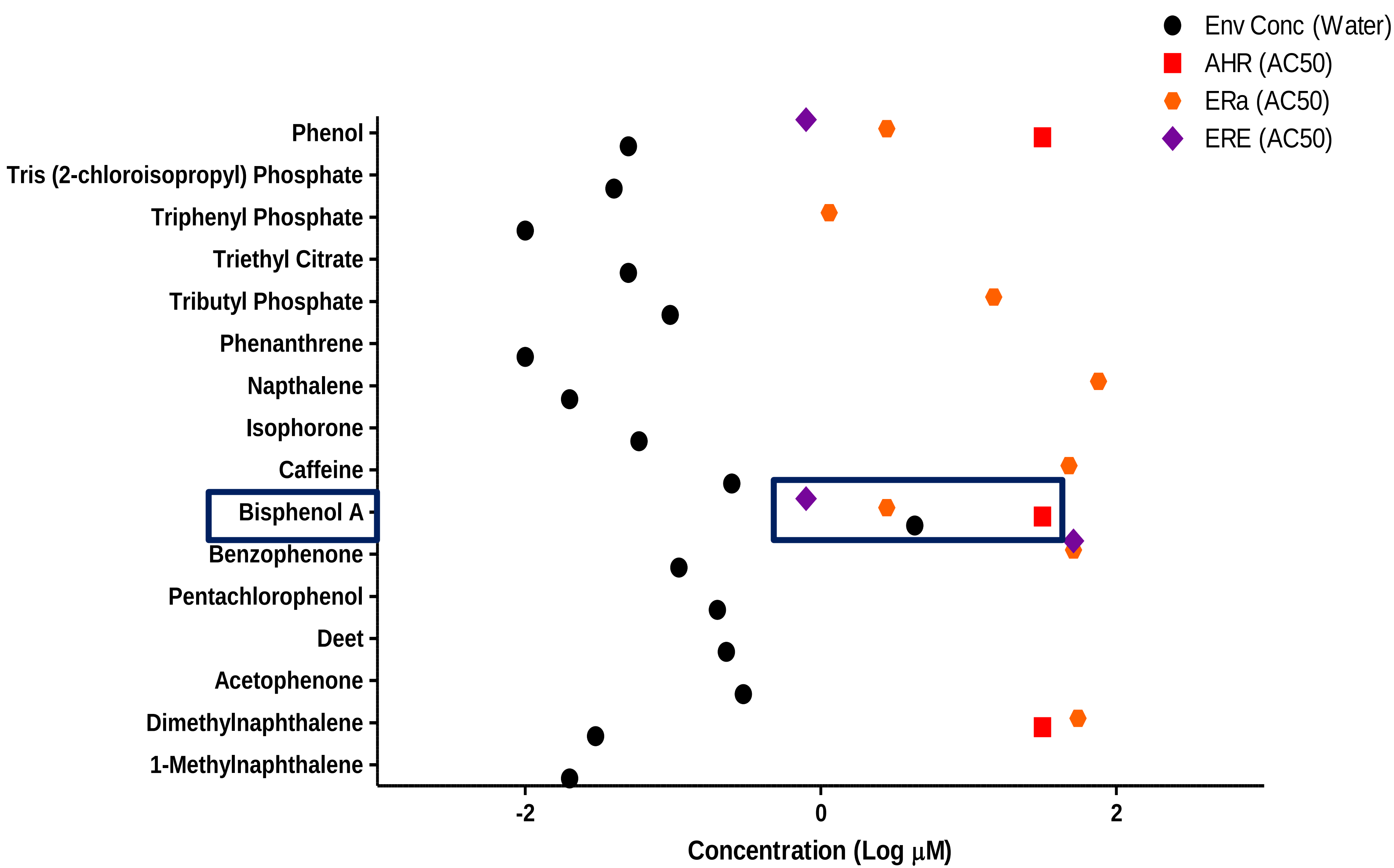


	GR	PPARg	ERa	PXR
EC50	~ 6.185e-005	0.001564	0.002067	0.0005430

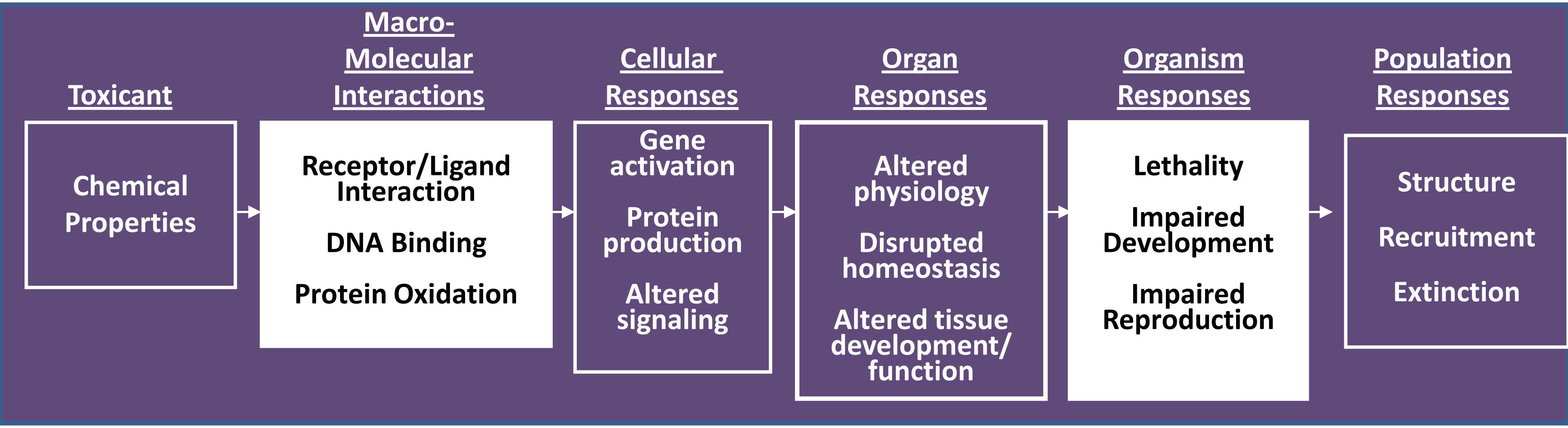


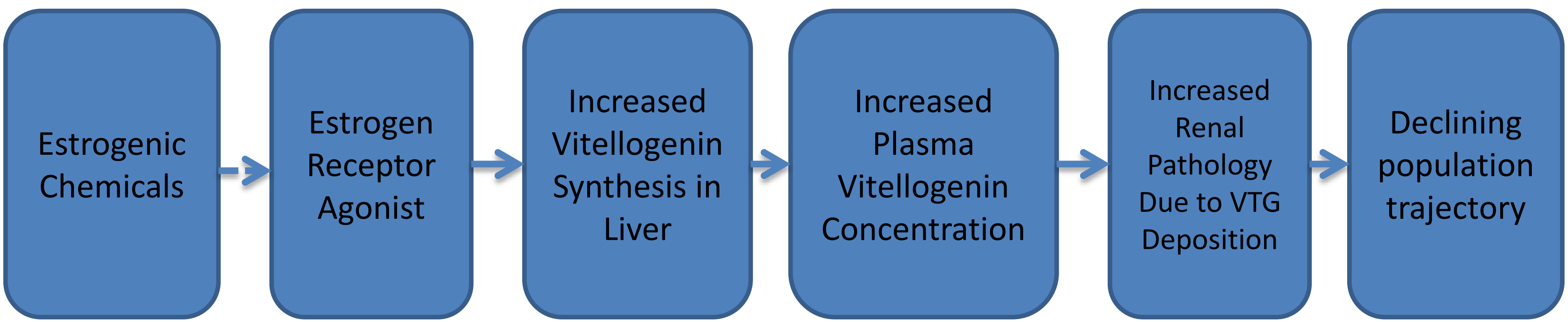
	PXR	Ahr	ERE
EC50	~ 0.0005630	0.001138	0.001861

WLSSD Distal – ToxCast Results



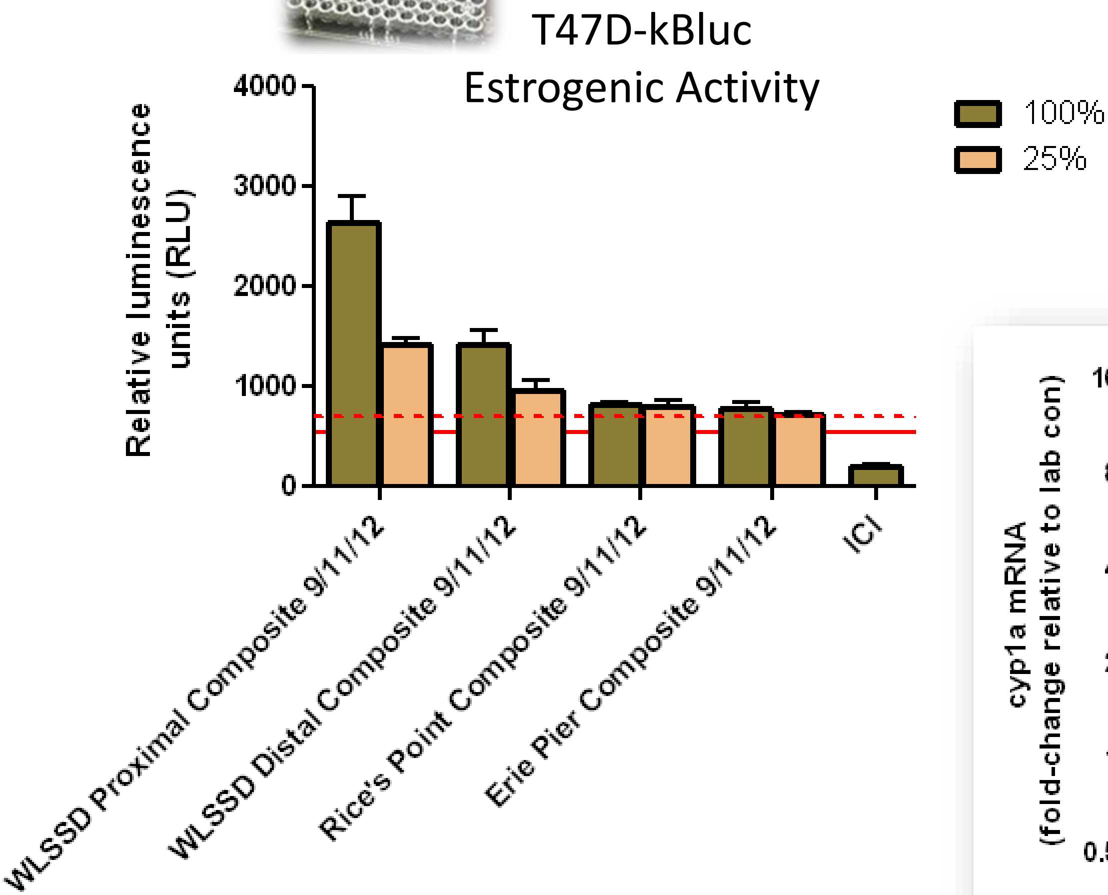
Adverse Outcome Pathways to Support HTS Results

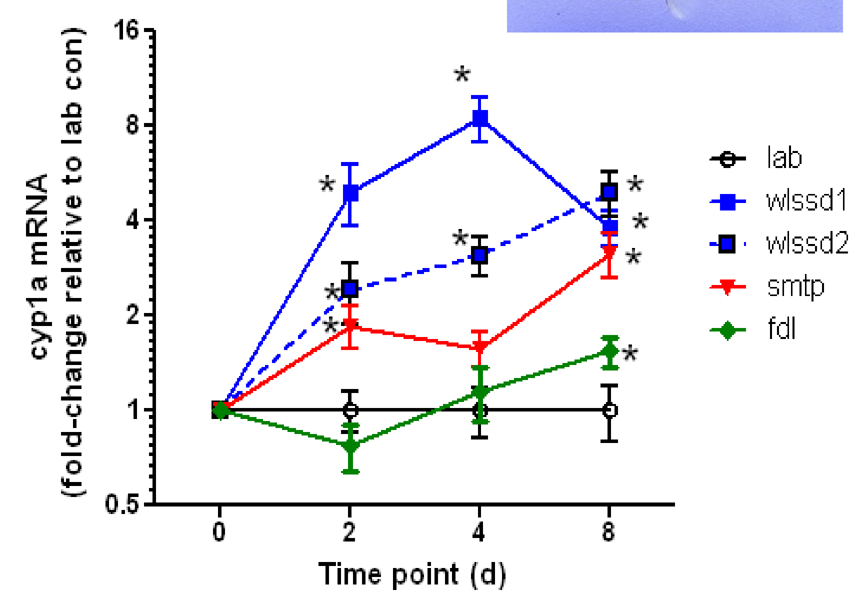


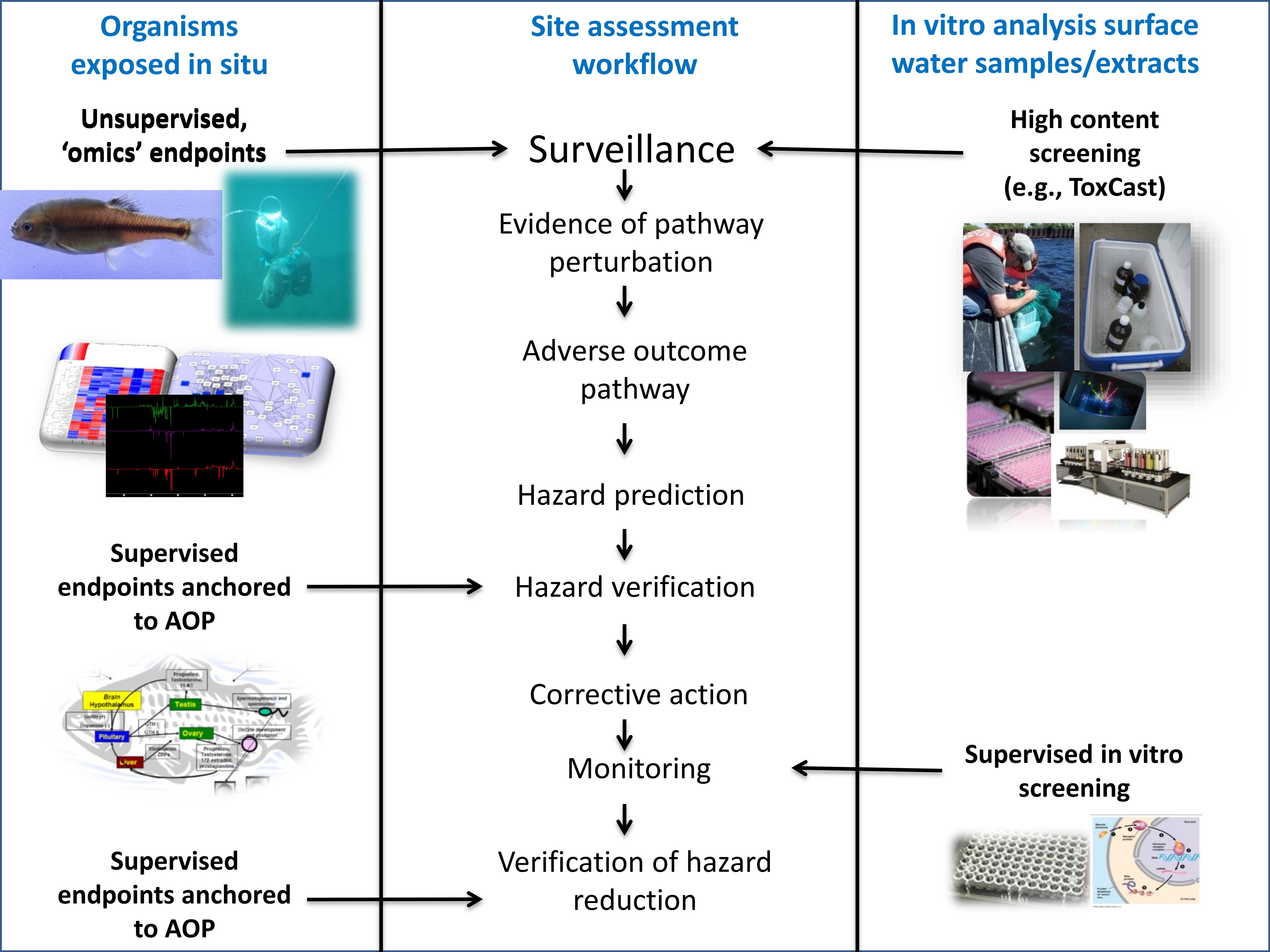


https://aopkb.org/aopwiki/index.php/Aop:29

“Validation” of ToxCast Results With Supervised Results

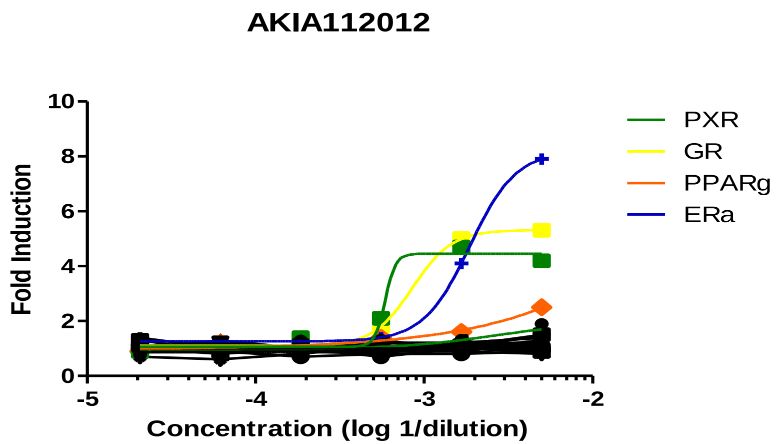




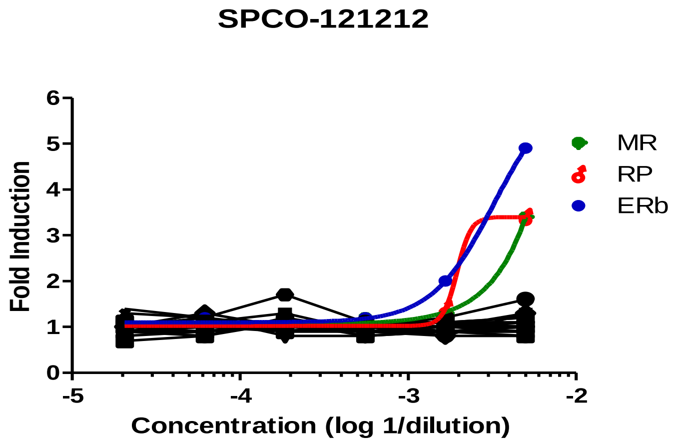

Organisms exposed in situ
Site assessment workflow
In vitro analysis surface water samples/extracts
Unsupervised, 'omics' endpoints
Surveillance
High content screening (e.g., ToxCast)
Evidence of pathway perturbation
Adverse outcome pathway
Hazard prediction
Supervised endpoints anchored to AOP
Hazard verification
Brain Hypothalamus
Testis
Pituitary
Ovary
Liver
Corrective action
Monitoring
Supervised in vitro screening
Supervised endpoints anchored to AOP
Verification of hazard reduction

Other Environmental Samples Screened

- 24 samples screened through Attagene battery
- 8 samples collected from various locations throughout United States as a part of a USGS mixtures study
- 6 samples collected over large spatial gradient along Maumee River, Ohio
- Remaining samples were collected from locations around Duluth-Superior Harbor
- Observed a wide variety of response profiles



	GR	PPARg	ERa	PXR
EC50	0.0008525	~ 128.0	0.001873	~ 0.0005895



	MR	RP	ERb
EC50	~ 0.1213	~ 0.001955	0.003241

Future Directions

- Very promising initial results of the capabilities of HTS to identify biological effects and function as a diagnostic tool
- Use predesigned mixtures to test concentration addition within the assay
- Develop putative AOPs for each target in ToxCast

Acknowledgements

RESEARCH TEAM

- US EPA – MED
  - G. Ankley, J. Berninger, B. Blackwell, J. Cavallin, E. Durhan, E. Eid, K. Jensen, M. Kahl, C. LaLone, E. Makynen, D. Miller, K. Nelson, R. Milsk, A. Schroeder, M. Severson, K. Stevens, D. Villeneuve
- **US EPA-NCCT: Keith Houck, Richard Judson**
- US EPA – ERD
  - T. Collette, J. Davis, D. Ekman, D. Skelton, Q. Teng
- US Army – ERDC
  - E. Perkins
- Mississippi State University
  - N. Garcia-Reyero
- U of St. Thomas
  - D. Martinović-Weigelt

Funding: Great Lakes Restoration Initiative (GLRI)

- Grant to US FWS, USGS
- Edwin Smith (GLNPO)

OTHER CONTRIBUTORS

- WLSSD: K. Hamel, J. Macor, J. Mayasich, A. Parella, T. Tuominen
- SMTP: D. Nelson
- US EPA-MED: A. Cotter, M. Knuth, S. Miller, J. VanAlstine, R. Kreiss, J. Filkins, *M. Weberg,* C. Elonen
- Wilson Labs: J. Jenson, K. Lott
- USGS: V. Blazer, K. Lee, L. Iwanowicz
- US FWS: A. Trowbridge, D. DeVault, Z. Jorgenson, J. Banda, L. Thomas
- GBMSD: J. Kennedy
- GLWI – UW-Milwaukee: R. Klaper, B. Foreman

	Study	Sample ID	ToxCast:	Concentration factor	Solvent	Volume	Site Location:	EPA Region	Notes
1	USGS National Water Collection	AKIA112012	MeOH to DMSO	1000X	DMSO	250 ul	Fourmile Creek below Ankeny WWTP outfall	7	High estrogenic activity in T47D assay
2	USGS National Water Collection	ZRMN-120612	yes	1000X	DMSO	250 ul	South Fork Zumbro River below WWTP near Rochester, MN	5	Alignment with Rochester study sites
3	USGS National Water Collection	SPCO-121212	yes	1000X	DMSO	250 ul	South Platte at Sand Creek, CO	8	Region 8 interest
4	USGS National Water Collection	SCGA-041713	yes	1000X	DMSO	250 ul	Sope Creek nr Merietta, GA	4	Proximity to Athens
5	USGS National Water Collection	RFPR-032713	yes	1000X	DMSO	250 ul	Rio Fajardo bl. Fajardo WWTP outfall, PR	2	EE2 manufacturer discharges to WWTP
6	USGS National Water Collection	C111HS-041013	yes	1000X	DMSO	250 ul	C-111 Canal 100' ab S-177 nr Homestead	4	Pure crop Ag site, lots of pesticides
7	USGS National Water Collection	PSNJ-121112	MeOH to DMSO	1000X	DMSO	250 ul	Penn Swamp Par. M Batsto, NJ	2	Low contamination site
8	USGS National Water Collection	IRIA-052113	yes	1000X	DMSO	250 ul	South Fork of Iowa River near New Providence	7	Ag/CAFO influenced site

	Study	Sample ID	Extraction	Concentration factor	Solvent	Volume
1	Maumee Fall 2012	FAR-UP	Steroid hormones	1000X	DMSO	250ul
2	Maumee Fall 2012	US-WWTP	Steroid hormones	1000X	DMSO	250ul
3	Maumee Fall 2012	MX-WWTP	Steroid hormones	1000X	DMSO	250ul
4	Maumee Fall 2012	DS-WWTP	Steroid hormones	1000X	DMSO	250ul
5	Maumee Fall 2012	EF-WWTP	Steroid hormones	1000X	DMSO	250ul
6	Maumee Fall 2012	Extraction blank	Steroid hormones	1000X	DMSO	250ul

	Study	Sample ID	Mill Status	Conc	Solvent	Volume	Collected
1	Sappi 2013	WLSSD-EF	Std operations	1000X	DMSO	250 ul	May
2	Sappi 2013	Proximal	Std operations	1000X	DMSO	250 ul	May
3	DSH fall 2012	Prox	Std operations	1000X	DMSO	250 ul	Sep
4	DSH fall 2012	Dist	Std operations	1000X	DMSO	250 ul	Sep
5	DSH fall 2012	Rice	Std operations	1000X	DMSO	250 ul	Sep
6	DSH fall 2012	Erie	Std operations	1000X	DMSO	250 ul	Sep
7	DSH spr 2012	Prox	Std operations	1000X	DMSO	250 ul	May
8	DSH spr 2012	LSW	Composite con	1000X	DMSO	**215 ul**	May
9	DSH spr 2012	SMTP	Std operations	1000X	DMSO	250 ul	May
10	DSH spr 2012	Knife	Std operations	1000X	DMSO	250 ul	May

Analyte Measured	Environmental Concentration (µM)	AC10 For Attagene Target (µM)			
		AHR	ERE	PXR	PXRE
1-Methylnaphthalene	0.01		24.40018		
Atrazine	0.03			5.53998	16.19899
Benzophenone	0.14		14.64951	52.19814	28.25752
Bisphenol A	0.1	10.29691	0.027731	0.289647	0.66764
Deet	0.2				10.08277
Dimethylnaphthalene	0.02	0.167466	31.09038	0.4421	3.6907
Isophorone	0.007				19.54236
Naphthalene	0.01				74.07301
Phenanthrene	0.01		27.32858	28.31575	42.08448
Tributyl Phosphate	0.02		1.895407	0.274448	0.839176
Camphor	0.02				
Diethyl Phthalate	0.2				
Caffeine	0.05				

Analyte Measured	Environmental Concentration (µM)	AC10 For Attagene Target (µM)		
		AHR	ERa	ERE
4-nonylphenol	0.4		0.094985	0.125015
4-Octylphenol	0.1		0.390271	1.382882
Bisphenol A	0.62	10.29691	0.012086	0.027731
Caffeine	0.62		19.94302	
Carbamazepine	0.041		18.03479	8.657079
Dimethylnaphthalene	0.02	0.167466	11.13664	31.09038
Estrone	0.0207		0.000558	0.000614
Isophorone	0.068			
Piperonyl Butoxide	0.019	21.49978	3.57621	3.231781
Tributyl Phosphate	0.281		9.317208	1.895407
Tris(2-chloroisopropyl)	0.07		1.582723	0.882853
1,4-Dichlorobenzene	0.02			
Acetophenone	0.3			

Analyte Measured	Environmental Concentration (µM)	AC10 For Attagene Target (µM)		
		AHR	ERa	ERE
Dimethylnaphthalene	0.03	0.167466	11.13664	31.09038
1-Methylnaphthalene	0.02			24.40018
Pentachlorophenol	0.2	4.338555		1.687677
Benzophenone	0.11		4.809068	14.64951
Bisphenol A	4.33	10.29691	0.012086	0.027731
Caffeine	0.25		19.94302	
Napthalene	0.02		24.81304	
Phenanthrene	0.01			27.32858
Tributyl Phosphate	0.096		9.317208	1.895407
Triphenyl Phosphate	0.01		1.139987	
Tris (2-chloroisopropyl) Phosphate	0.04		1.582723	0.882853
Phenol	0.05			
Acetophenone	0.3			
Deet	0.23			
Isophorone	0.059			
Triethyl Citrate	0.05			

Other Studies Related to HTS and ToxCast

- 11 pre-designed mixtures were ran through Attagene battery of assays
  - Results will provide more information about the nature of environmental mixtures and their responses in ToxCast
- All individual chemicals used in the mixtures were also ran
- Potential to run environmental samples for GLRI and predesigned mixtures to further challenge the system

Mixture 1 - 7 Chemical Mixture
(Equal and Environmentally relevant concentrations)

Chemical

Bisphenol A

1,4-dichlorobenzene

Naphthalene

Benzophenone

Triclosan

Triethyl Citrate

Tris(2-butoxyethyl) phosphate

Mixture 2 - In Vivo EDC Chemical mixture (Equal and in vivo effect concentrations)

Chemical

Prochloraz

EE2

Spironolactone

Cyp Acetate

Mixture 3 - Environmentally relevant EDC mixture (Equal and environmentally relevant concentrations)

Chemical

Propiconazole

Bisphenol A

Fipronil

Prometon

Trenbalone

DES

Spironolactone

EE2

Vinclozolin

Mixture 4 - Estrogen Antagonist Mixture (Equal Concentration Only)

Chemical

Benzo(b)fluoranthene

Fenarimol

Raloxifene Hydrochloride

Tamoxifen

Mixture 5 - Estrogen Agonist Mixture (Equal Concentration Only)

Chemical

EE2

Methoxychlor

17b estradiol

4-octylphenol

Mixture 6 - Estrogen Agonist/Antagonist Mixture (Equal Concentration Only)

Chemical

Benzo(b)fluoranthene

Fenarimol

Raloxifene Hydrochloride

Tamoxifen

EE2

Methoxychlor

17b estradiol

4-octylphenol

Mixture 7 - 5 Chemical mixture with single targets (Equal and environmentally relevant concentrations)

Chemical

1,4 dichlorobenzene

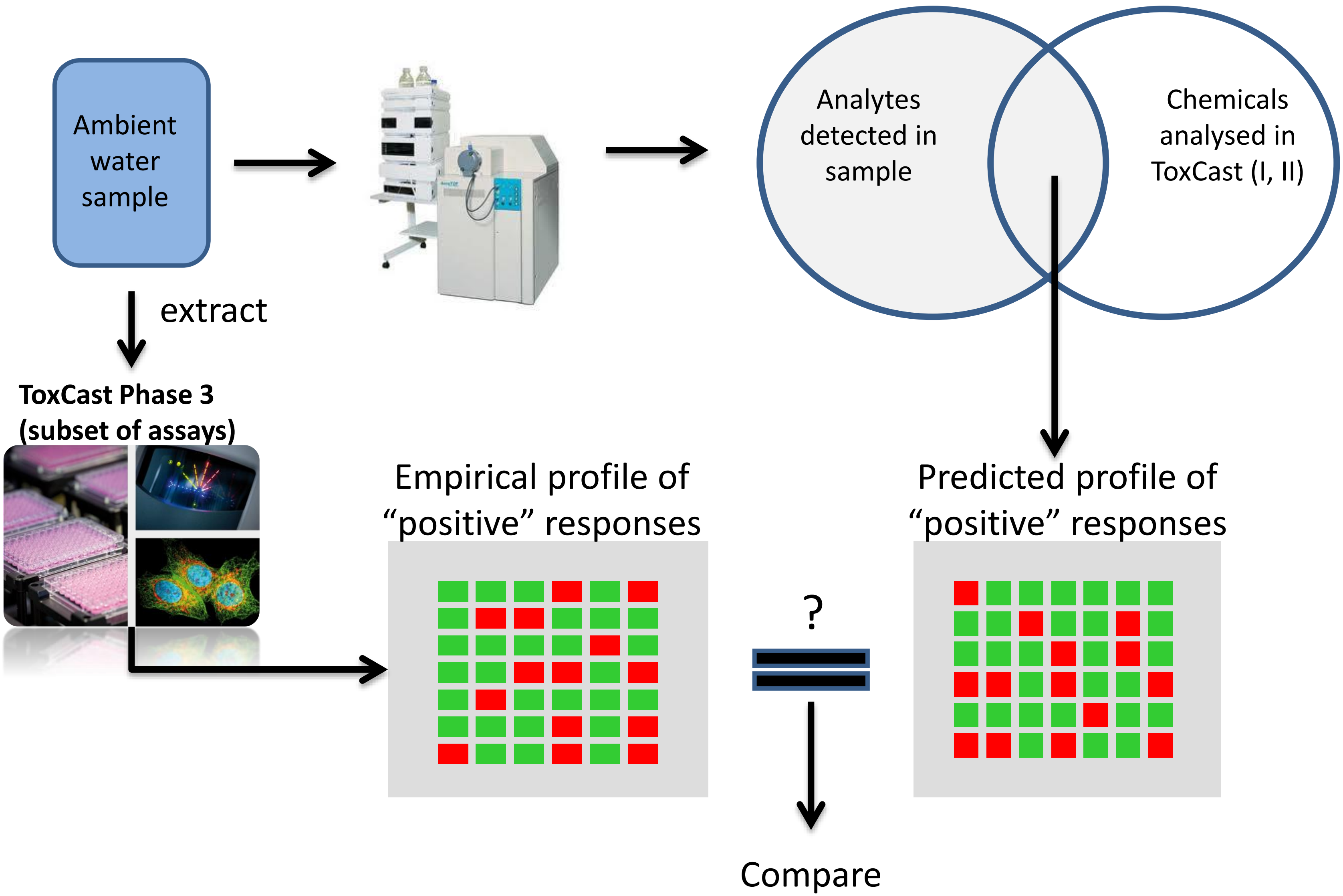
1-methylnaphthalene

estriol

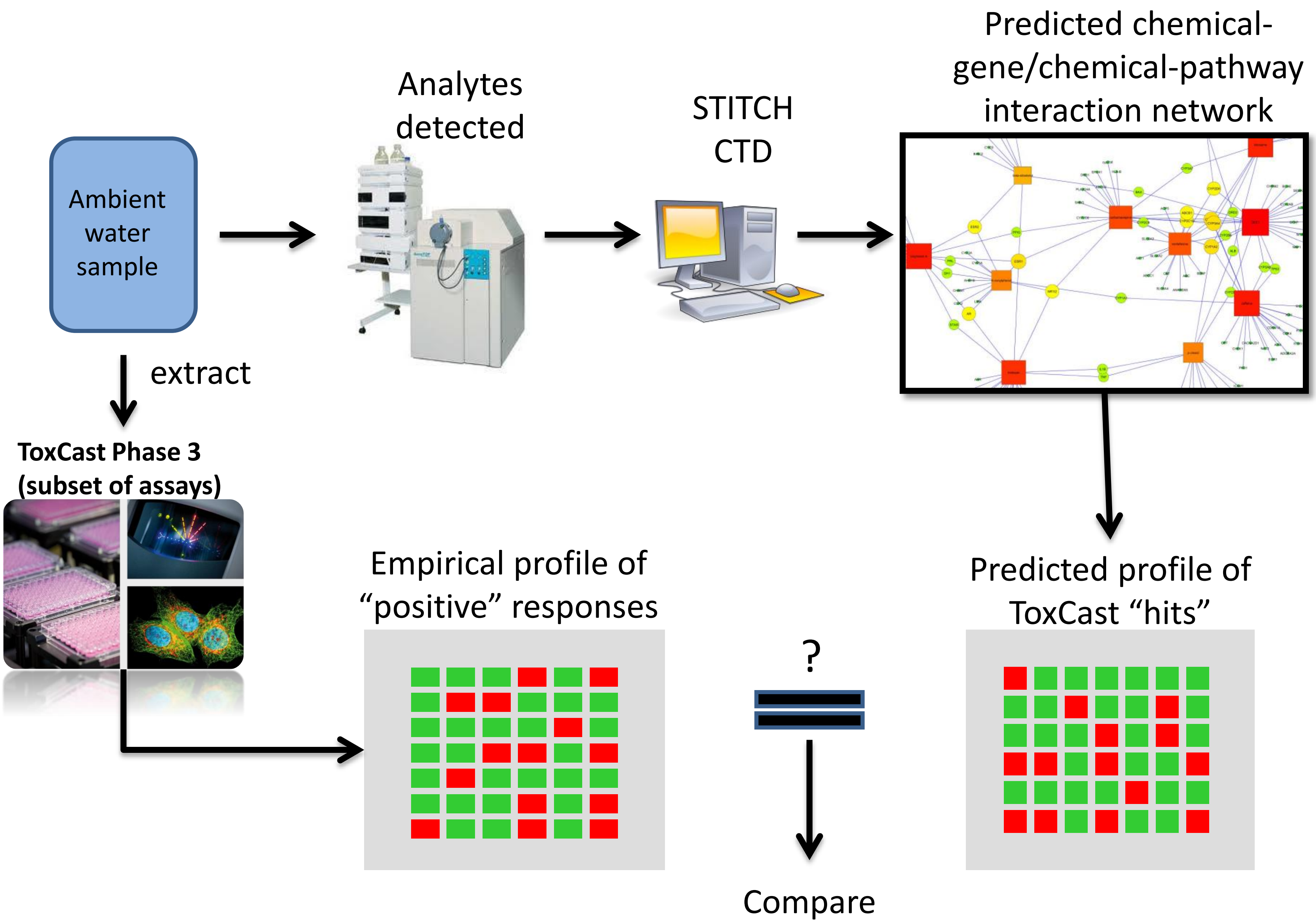
cotinine

Deet (N,N-Diethyl-3-methylbenzamide)

Predicting effects of mixtures



Another approach



Need for Expanded AOP Knowledge-base

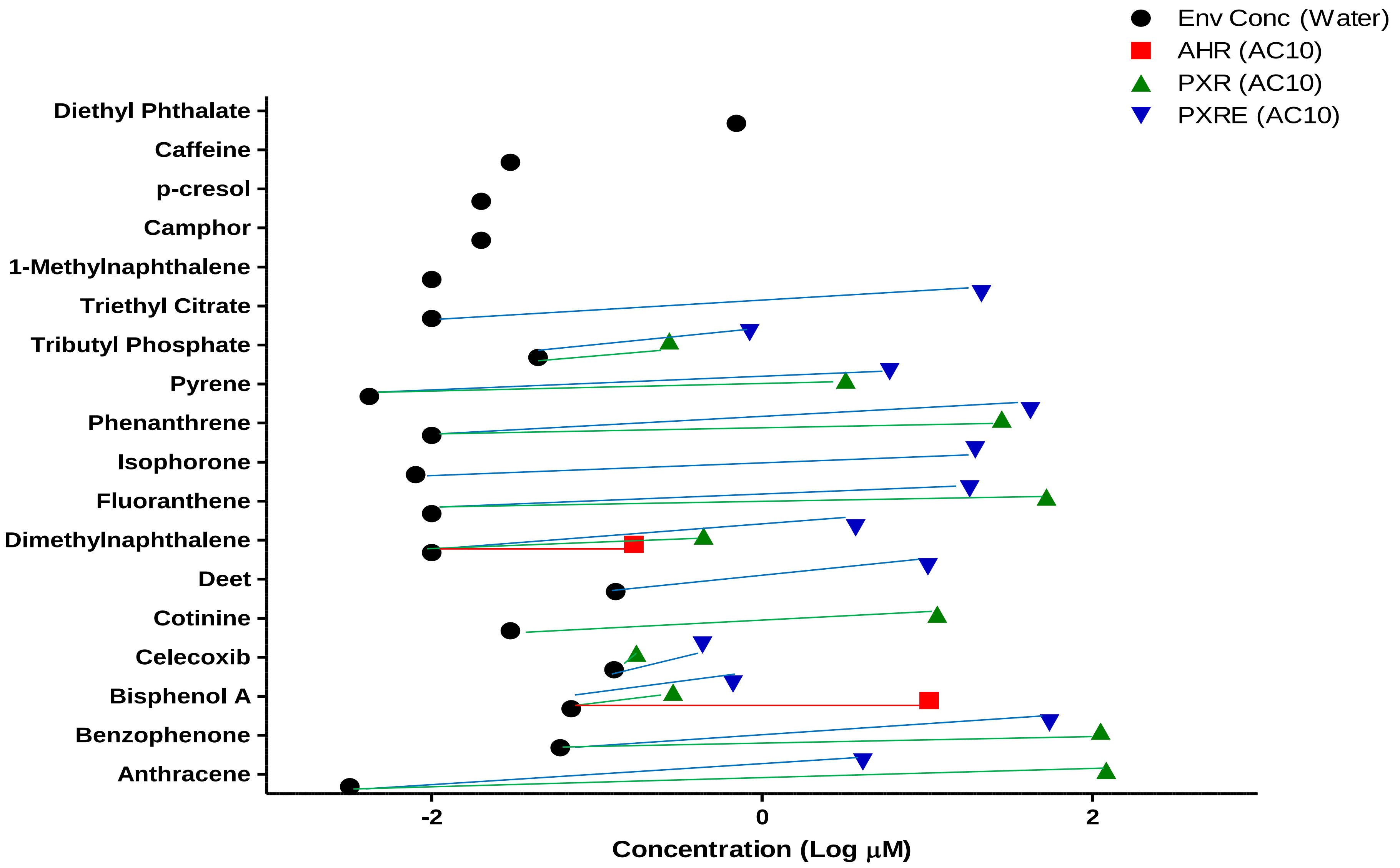
GR Activation →

MR Activation →

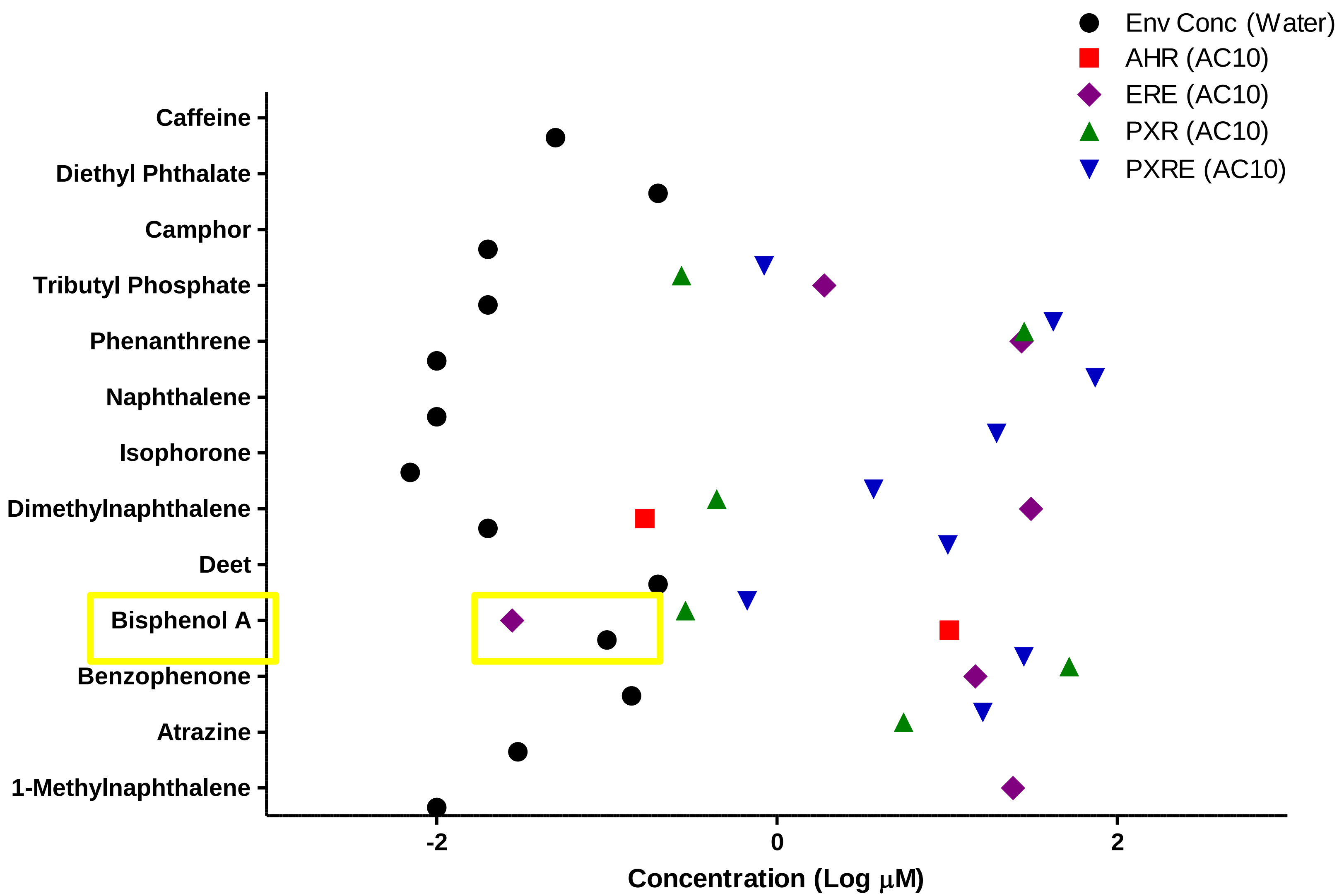
Which assays and endpoints?

- ORD initiative to develop putative AOPs for all the targets covered in ToxCast.

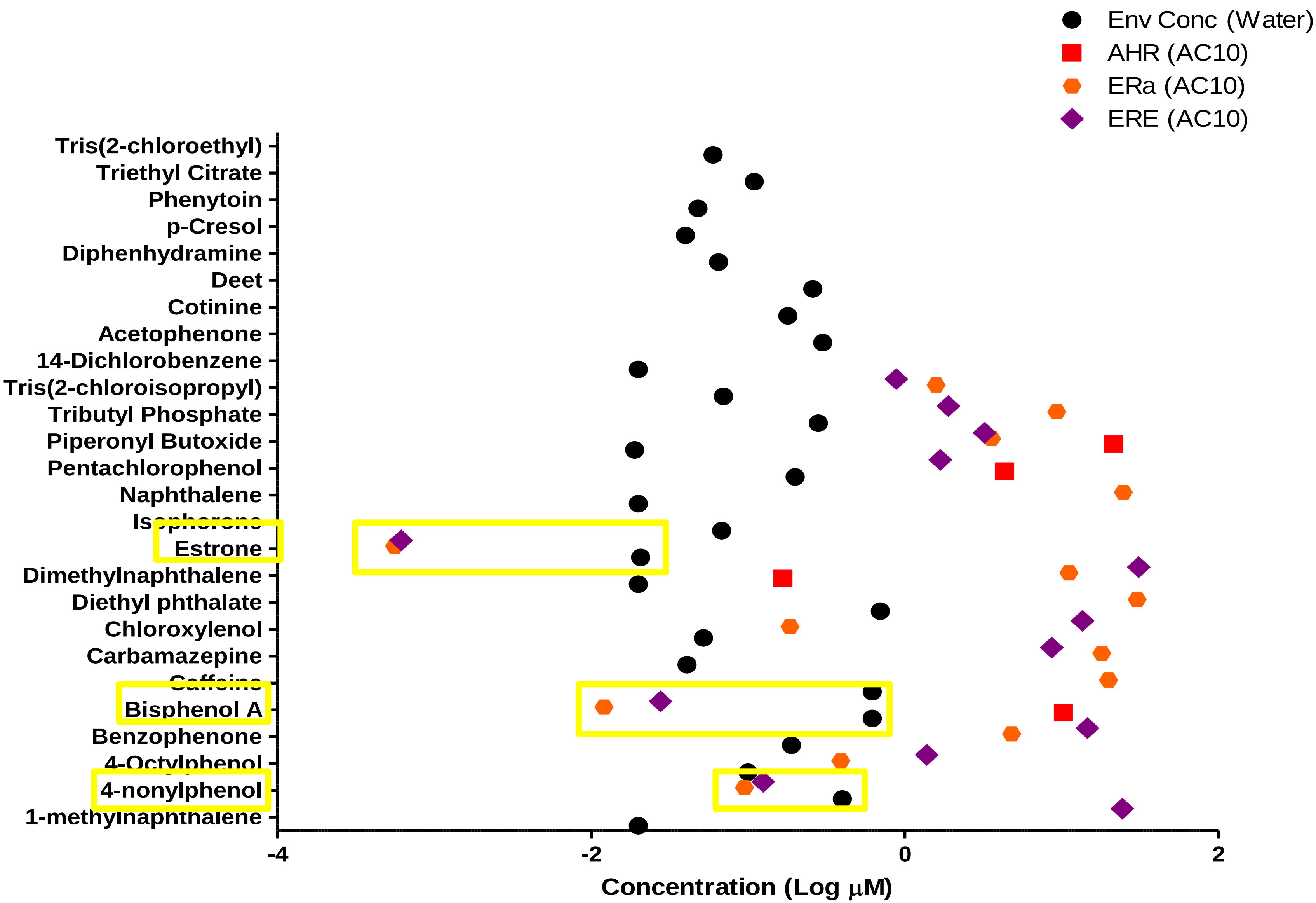
Erie Pier – ToxCast Results



Rice’s Point – ToxCast Results



WLSSD Proximal – ToxCast Results



WLSSD Distal – ToxCast Results