



# **EPA Tools and Resources Webinar: Prioritizing Contaminants for Monitoring and Management**

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October 26, 2016

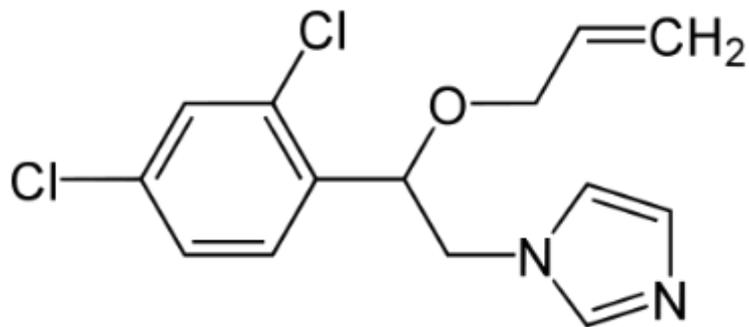
# Problem



- An ever increasing range of chemical contaminants are being detected in the environment.
- For example pharmaceuticals, personal care products, current generation pesticides, perfluorinated compounds, flame retardants, etc.



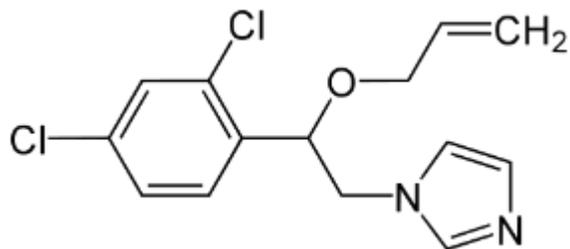
# Story Problem



- You just detected this chemical in 30% of surveyed surface waters in your state.
- Local citizen action committees and several of your state legislators want to know if this is a concern.



# Common Problem



- There are no existing water quality criteria or standards for this compound.
- There is little or no toxicity data available and no legal authority to collect those data.



Why?

- Traditional whole organism-based toxicity testing is costly & time-consuming

# Problem

## The Great Chemical Unknown

[Scientific American October 28, 2010]

- Lack of safety/hazard characterization for most chemicals acknowledged in the President's remarks during signing of 2016 TSCA reform legislation.



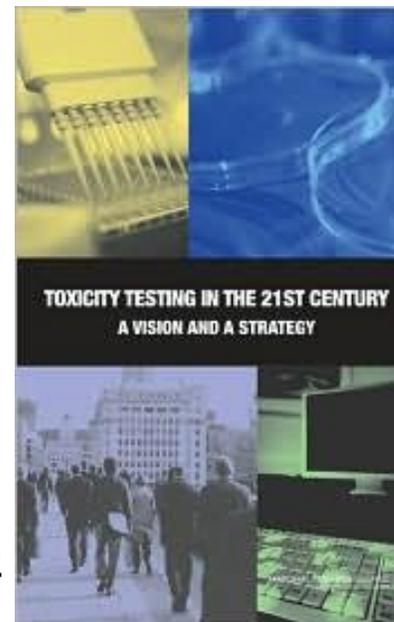
# Action – 21<sup>st</sup> Century Toxicology



“Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin”

“The vision emphasizes the development of suites of predictive, high-throughput assays .....”

“The mix of tests in the vision include tests that assess critical mechanistic endpoints involved in the induction of overt toxic effects rather than the effects themselves.”



# Action – 21<sup>st</sup> Century Toxicology



## ToxCast

> 600 assays, >2000 chemicals,

- Per chemical cost  $\approx$  20K (less than a single Fish Early Life Stage test)

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



### ToxCast HTS Assays

~500 Total Endpoints

#### Cellular Assays

#### Biochemical Assays

##### • Protein families

- GPCR
- NR
- Kinase
- Phosphatase
- Protease
- Other enzyme
- Ion channel
- Transporter

##### • Assay formats

- Radioligand binding
- Enzyme activity
- Co-activator recruitment

##### • Cell lines

- HepG2 human hepatoblastoma
- A549 human lung carcinoma
- HEK 293 human embryonic kidney

##### • Primary cells

- Human endothelial cells
- Human monocytes
- Human keratinocytes
- Human fibroblasts
- Human proximal tubule kidney cells
- Human small airway epithelial cells
- Rat hepatocytes
- Mouse embryonic stem cells (Sid Hunter)

##### • Biotransformation competent cells

- Primary rat hepatocytes
- Primary human hepatocytes

##### • Assay formats

- Cytotoxicity
- Reporter gene
- Gene expression
- Biomarker production
- High-content imaging for cellular phenotype

Primarily Human / Rat

Exception: Zebrafish development (Stephanie Padilla)

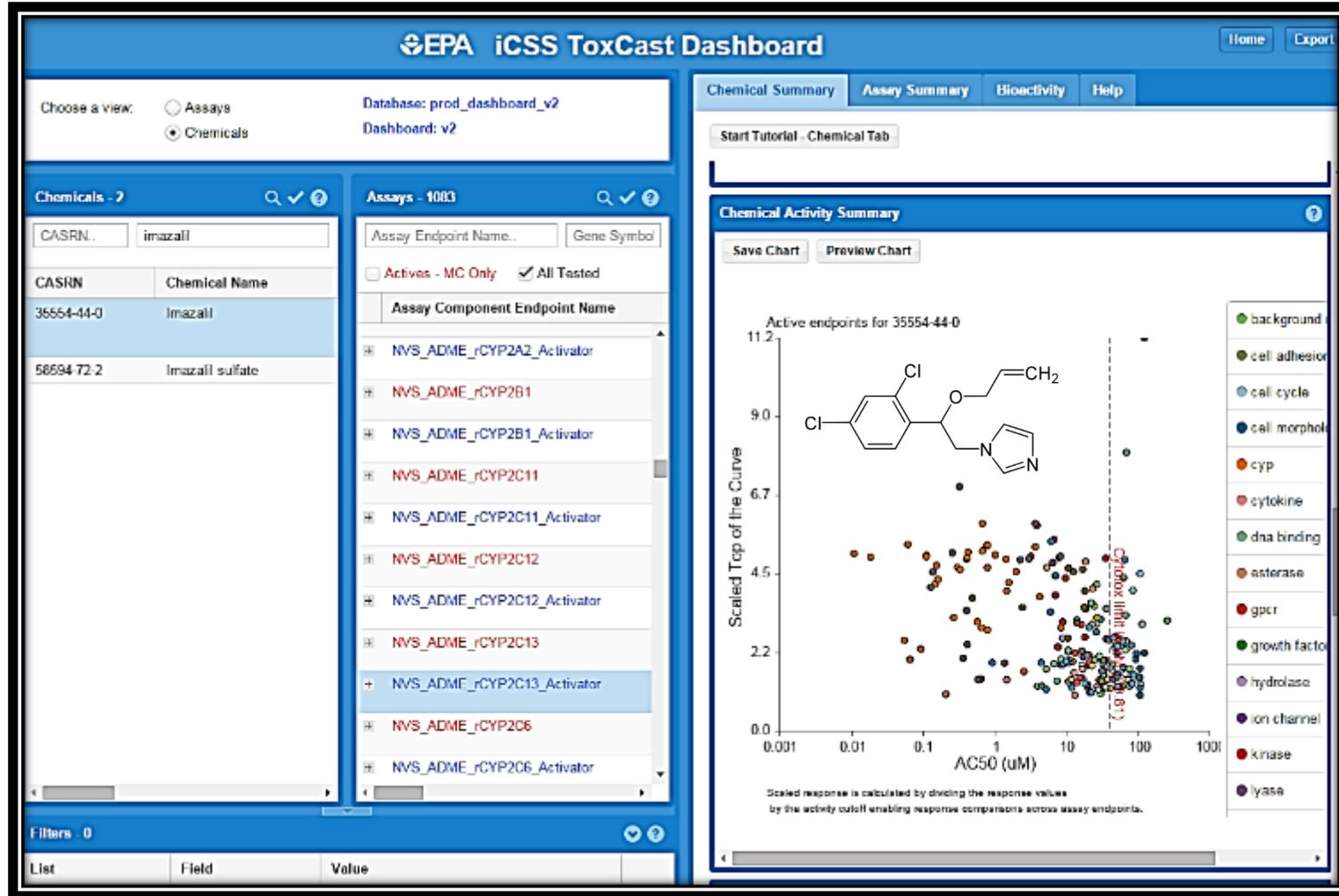
Office of Research and Development  
Computational Toxicology Research Program

13

- Rapidly, cost-effectively screen chemicals for:
  1. The kinds of biological pathways they can perturb
  2. The relative concentrations at which they perturb them

HTS = high throughput screening

# Results – Risk-based screening & prioritization tools



<http://actor.epa.gov/dashboard/>

Specific activities well below “baseline” cytotoxic concentration

Multiple lines of evidence for activity as an aromatase inhibitor

Inhibitor of hepatic cytochrome P450s (phase 1 metabolism)

Publicly accessible data and tools you can use today.

# Problem II



We don't regulate enzyme activities?

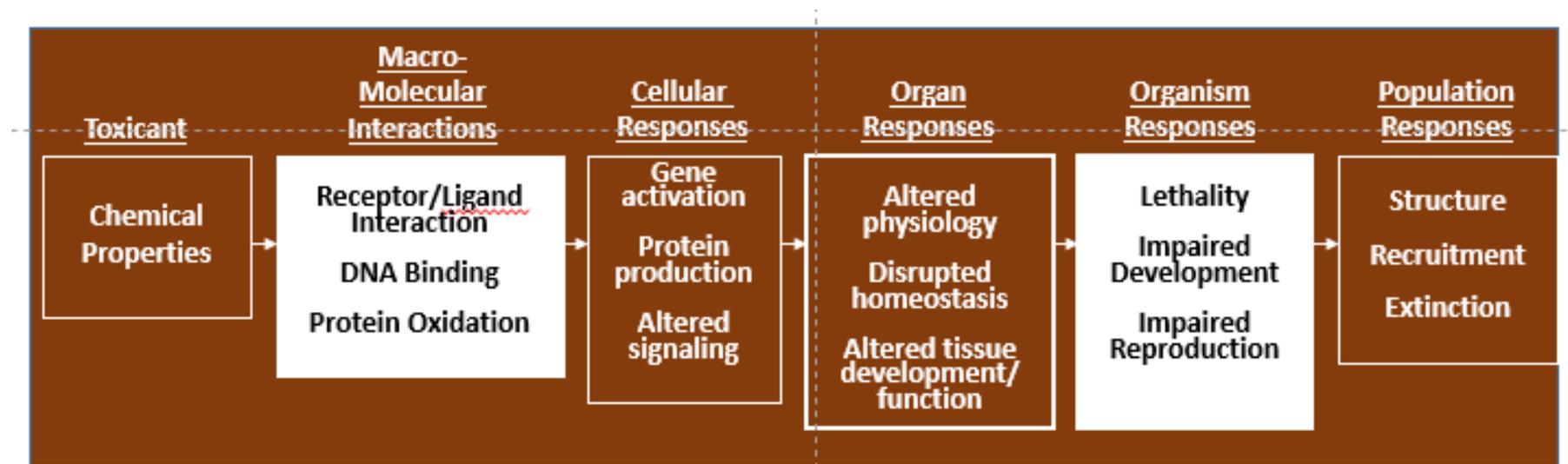
Citizens don't care about receptor binding.

What do these results mean in terms of human health or ecosystem functions and services (e.g., fish populations)?

# Action – Adverse Outcome Pathway Framework

An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment.

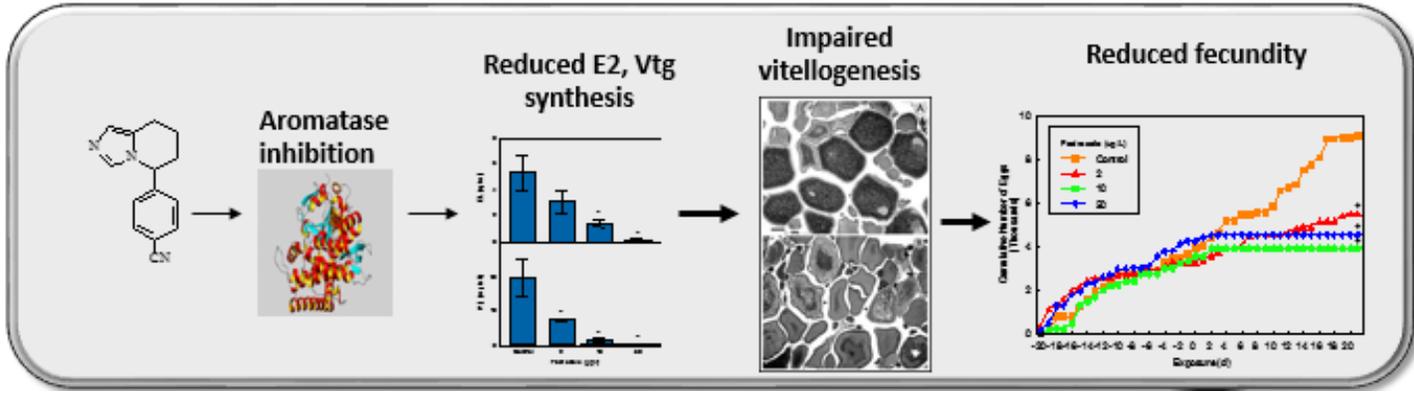
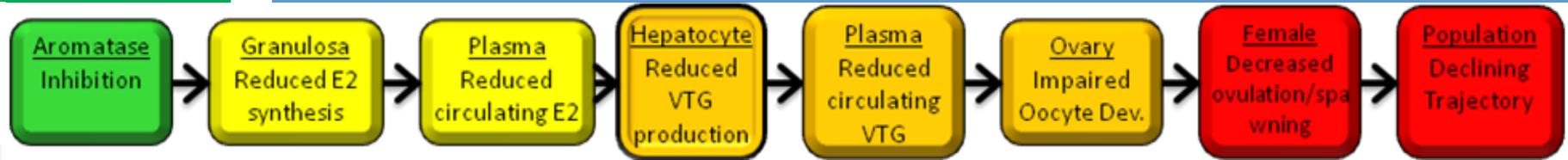
(Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)



- Helps us organize what we know
- And make more effective use of pathway-based data in risk-based decision making

# Results – Adverse Outcome Pathway Knowledgebase

Adverse Outcome Pathway  
WIKI



[aopkb.org](http://aopkb.org); Aop 25

- **Plausibility:** Based on current biological understanding inhibition of this enzyme activity can plausibly lead to reproductive impairment in oviparous vertebrates (e.g., fish).
- **Evidence:** The anticipate pattern of response has been observed:
  - Multiple species
  - Multiple chemicals
  - Transparent presentation of scientific support (e.g., literature citations)
- **Weight of evidence:** Technical experts have reviewed the support for this association, level of confidence and relevant uncertainties identified.



# Results – AOP-KB linked to ToxCast Dashboard



EPA iCSS ToxCast Dashboard

Choose a view:  Assays  Chemicals Database: prod\_dashboard\_v2 Dashboard: v2

Assays - 2

Assay Component Endpoint Name
NVS_ADME_hCYP19A1

Chemicals - 1876

CASRN	Chemical Name
93-92-5	(+/-)-Alpha-Methylbenzyl

Chemical Summary Assay Summary Bioactivity Help

product/pmol CYP19/min

<https://Aopkb.org/aopwiki>

AOP Wiki

	Gene Symbol	Wiki URL
1	CYP19A1	<a href="https://aopkb.org/aopwiki/index.php/Aop:25">https://aopkb.org/aopwiki/index.php/Aop:25</a>

- Translation of pathway perturbation to potential hazard (in vivo)
- Steadily growing resource
- Internationally harmonized

Adverse Outcome Pathway Wiki

Page Discussion

Aop:25

Main Page > AOP List > Aop:25

AOP Title

**Aromatase inhibition leading to reproductive dysfunction (in fish)**

Short name: Aromatase inhibition leading to reproductive dysfunction (in fish)

Authors

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Status

# Problem III



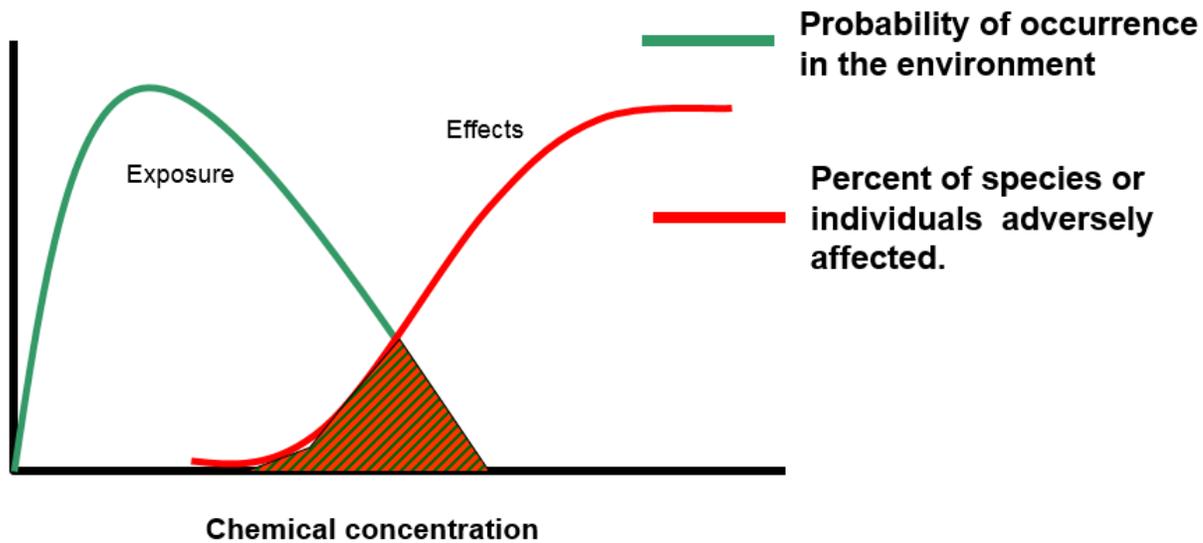
We're detecting a laundry list of chemicals.

Limited resources for monitoring and assessment.

- What are the highest priorities?
  - Chemicals
  - Sites
  - Effects

Chemical (µg/L)	Erie Pier	WLSSD Proximal	WLSSD Distal	Rice's Point
1,4-Dichlorobenzene	<0.08	0.02	<0.08	<0.08
1-Methylnaphthalene	0.01	0.02	0.02	0.02
2,6-Dimethylnaphthalene	0.01	<0.04	<0.04	<0.04
2-Methylnaphthalene	0.01	0.03	0.03	0.03
3,4-Dichlorophenyl isocyanate	0.02	0.04	0.02	<0.32
4-Nonylphenol diethoxylate (NP2EO)	0.2	1.1	1.7	<1.6
4-Nonylphenol monoethoxylate (NP1EO)	<1.6	0.28	<1.6	<1.6
4-tert-Octylphenol	<0.4	0.1	<0.4	<0.4
4-tert-Octylphenol diethoxylate (OP1EO)	<0.2	0.2	0.1	<0.2
4-tert-Octylphenol monoethoxylate (OP2EO)	<0.6	0.1	<0.6	<0.6
Acetophenone	<0.4	0.3	0.3	<0.4
Anthracene	0.0032	<0.02	<0.02	<0.02
Anthraquinone	<0.04	0.04	<0.04	<0.04
Benzophenone	0.06	0.19	0.11	<0.08
beta-Sitosterol	0.3	<4.8	0.9	0.6
bis(2-ethylhexyl) Phthalate	<2	<2	<2	1
Bisphenol A	0.07	0.62	2.75	0.03
Caffeine	0.03	0.62	0.25	0.04
Camphor	0.03	<0.08	<0.08	<0.08
Cholesterol	0.3	0.4	0.6	0.6
Cotinine	0.03	0.18	<0.08	<0.08
Diethyl phthalate	0.7	0.7	<0.4	<0.4
Fluoranthene	0.01	<0.02	<0.02	<0.02
Isophorone	0.008	0.068	0.059	<0.050
Menthol	<0.32	0.23	<0.32	<0.32
N,N-Diethyl-meta-toluamide (DEET)	0.13	0.26	0.23	0.07
Naphthalene	<0.02	0.02	0.02	0.02
4-Nonylphenol (NP, branched)	<1.6	0.4	<1.6	1.2
p-Cresol	0.02	0.04	<0.08	<0.08
Pentachlorophenol	<1.6	0.2	0.2	<1.6
Phenanthrene	0.01	<0.02	0.01	<0.02
Phenol	<0.16	<0.16	0.05	<0.16
Pyrene	0.0042	<0.02	<0.02	<0.02
Tetrachloroethene	<0.16	0.05	<0.16	<0.16
Tributyl phosphate	0.044	0.281	0.096	0.04
Triethyl citrate (ethyl citrate)	0.01	0.11	0.05	<0.04
Triphenyl phosphate	<0.08	0.01	0.01	0.03
Tris(2-butoxyethyl) phosphate	<0.64	<0.64	<0.64	0.3
Tris(2-chloroethyl) phosphate	<0.16	0.06	<0.16	<0.16
Tris(dichloroisopropyl) phosphate	<0.32	0.07	0.04	<0.32
Chloroxlenol	<0.080	0.052	<0.080	<0.080

# Results – Risk-based screening & prioritization tools



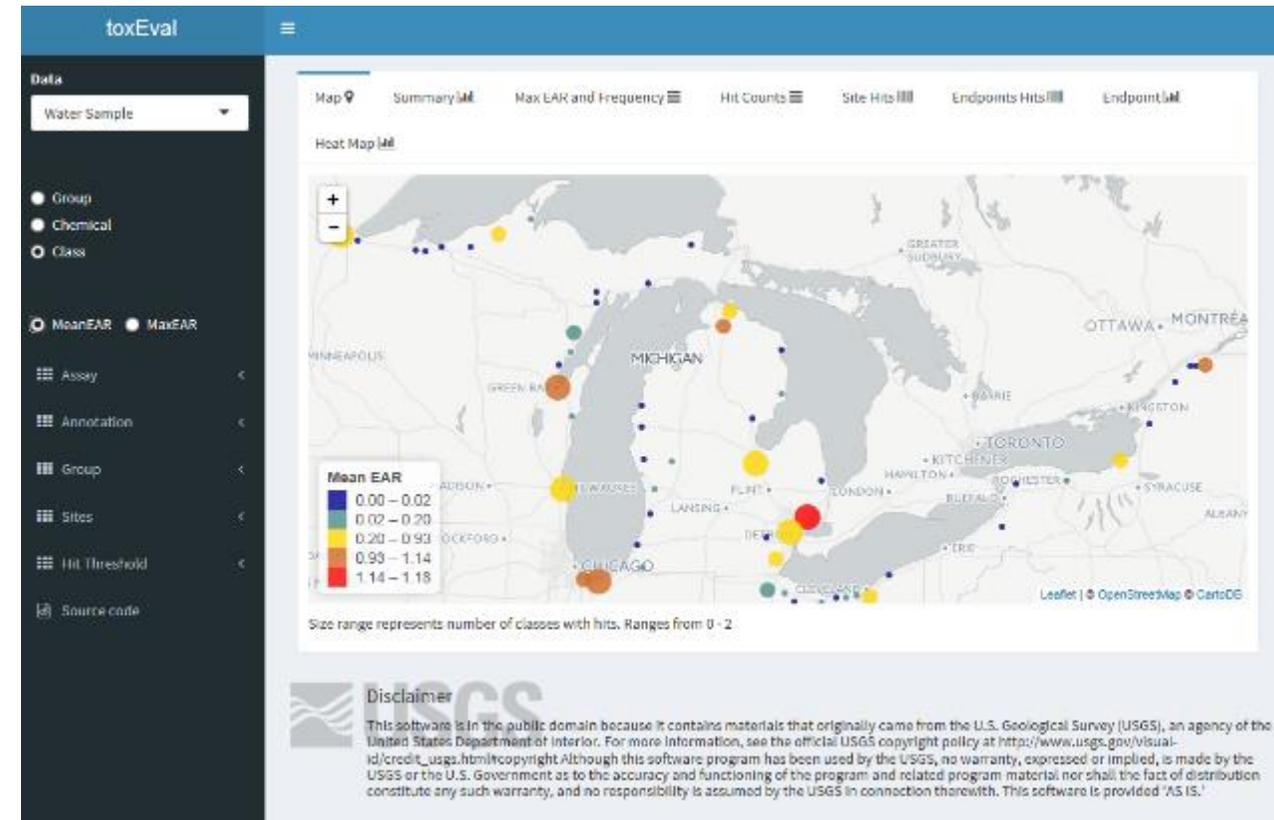
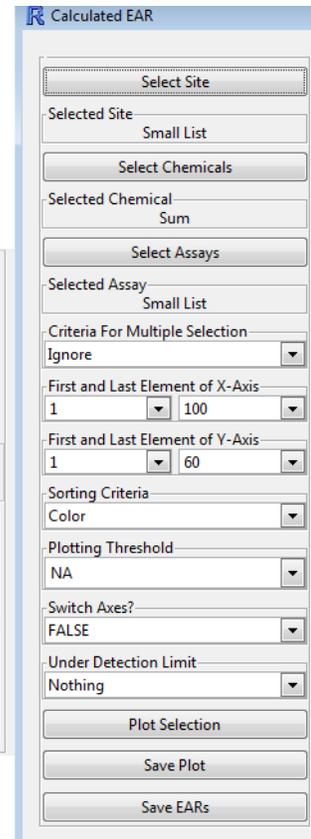
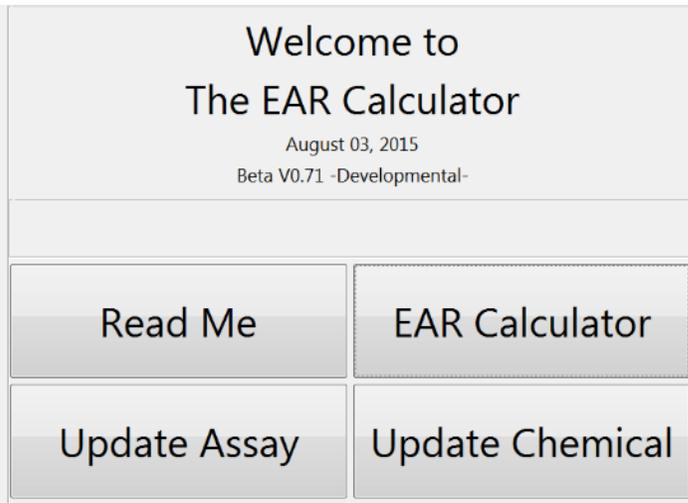
Which chemical(s) are present at high enough concentrations to elicit effects?

$$\text{Exposure: Activity Ratio (EAR; unitless)} = \frac{\text{Exposure (concentration } \mu\text{M)}}{\text{Pathway – based Activity (AC50 } \mu\text{M)}}$$

- Simple concept, simple calculation
- Not as simple for a matrix of 300 chemicals x 650 assay endpoints: 195,000 calculations

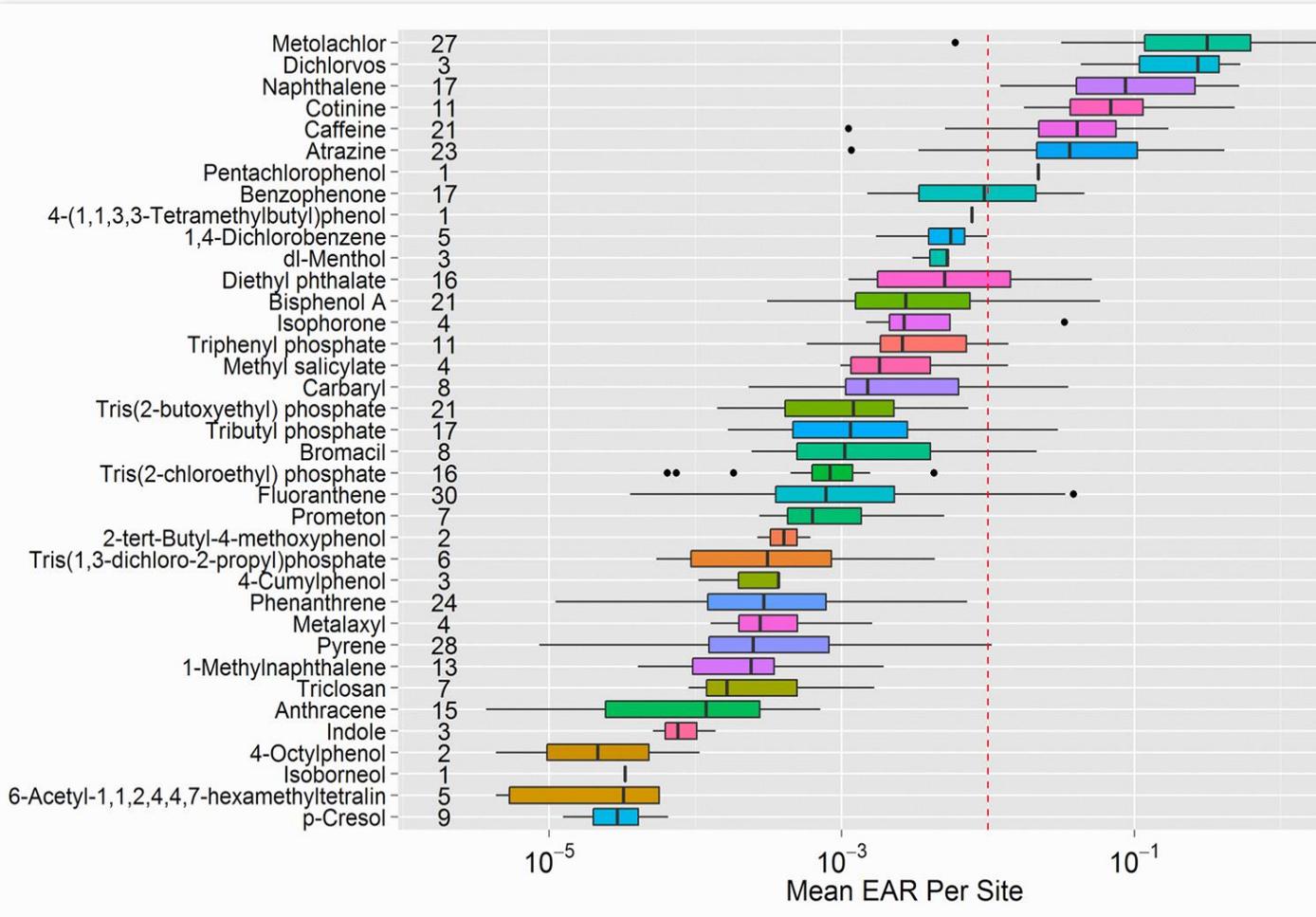
# Results – Risk-based screening & prioritization tools

- EARs rapidly calculated and visualized using EAR Calculator/ToxEval
  - Tool developed in R
  - GUI, user friendly



- Intended to be publicly accessible tools
- Conducting case studies to develop guidance on appropriate use

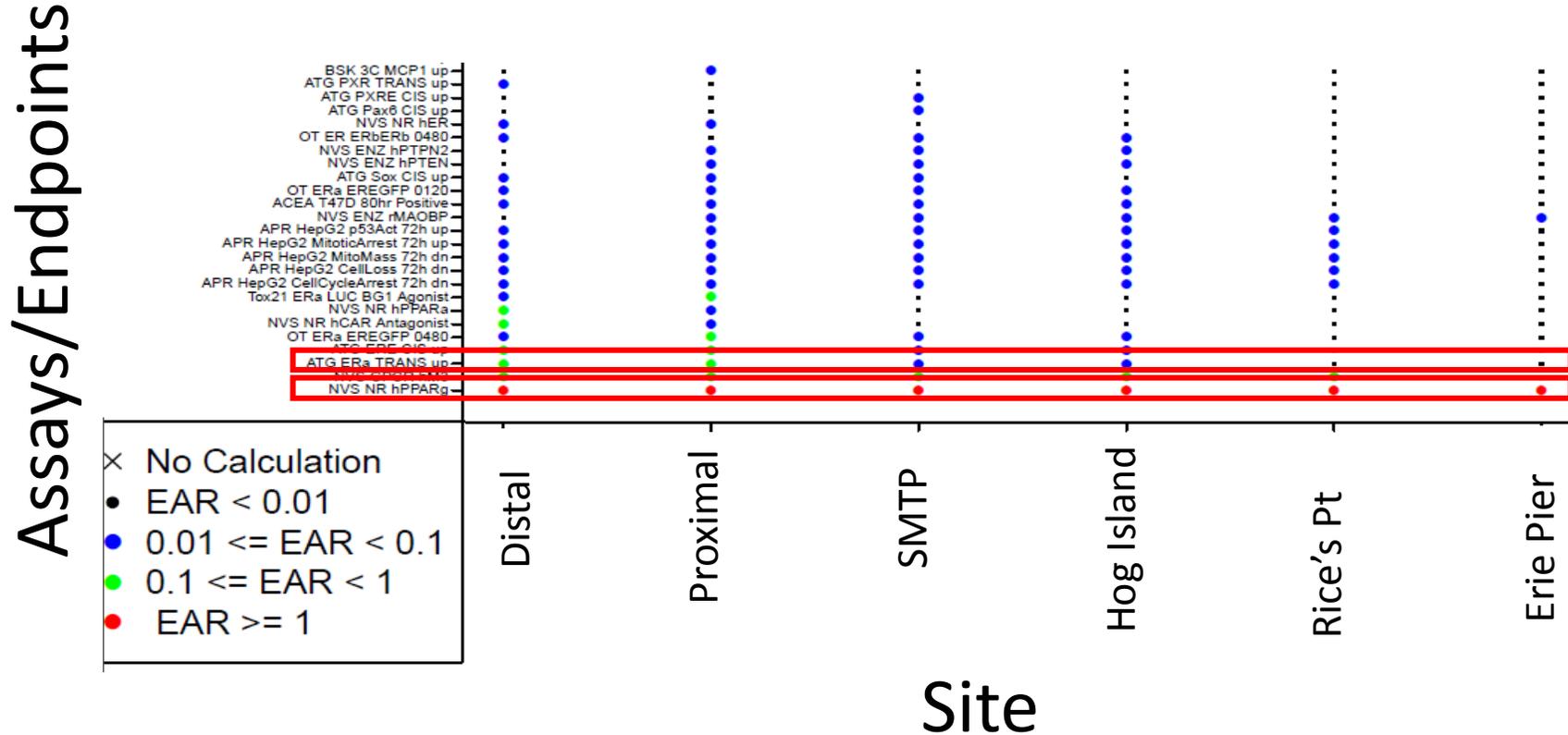
# Results – Risk-based screening & prioritization tools



Identifying chemicals present at or near bioactive concentrations at the greatest number of sites.

# Results – Risk-based screening & prioritization tools

EAR Results for the Chemical: Sum



Can sum the EARs for all chemicals acting on a particular assay target.

Identify most relevant bioactivities/hazards at a site considering the mixture of chemicals detected.

$$EAR \text{ sum (unitless)} = \sum \frac{Exposure \text{ (dose } \mu\text{M)}}{Activity \text{ (AC50 } \mu\text{M)}}$$

# Problem IV

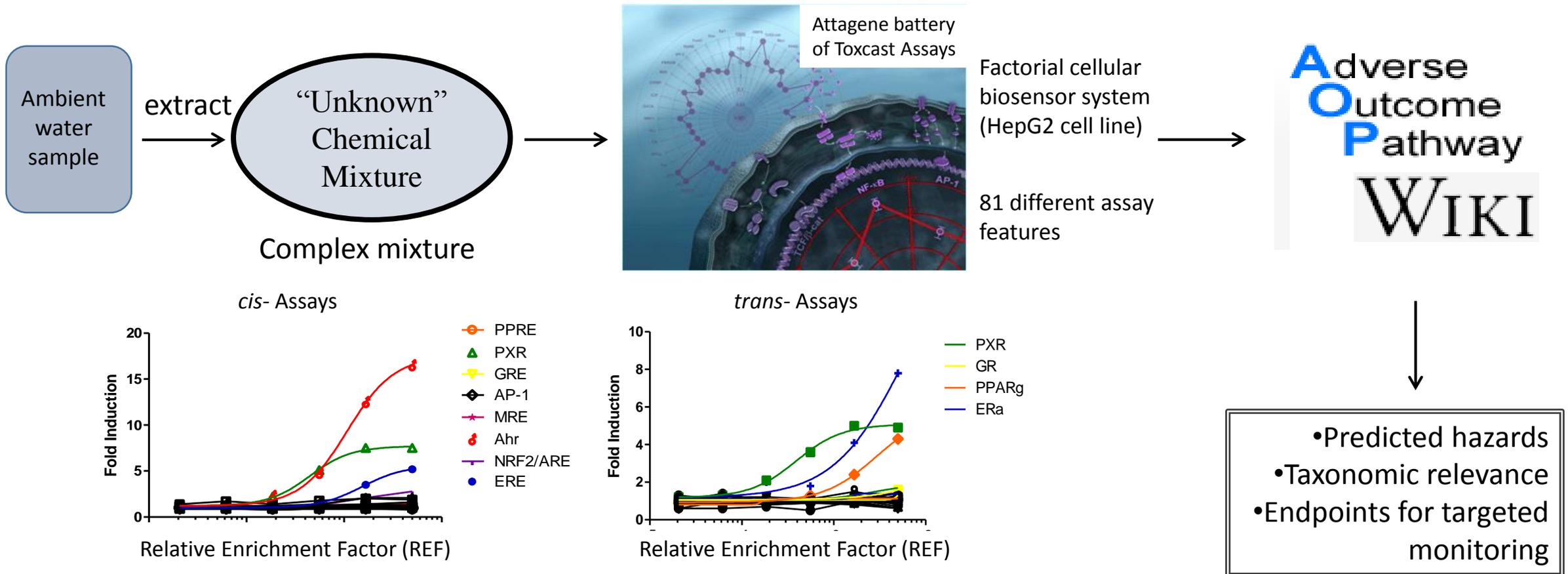


- Real-world exposures are to mixtures, not single chemicals
- Still only measuring/detecting a small fraction of the chemicals that occur in the environment
- Accounting for unknowns

# Action – 21<sup>st</sup> Century Toxicology



## High throughput screening tools can be applied to environmental mixtures



# Results – Risk-based screening & prioritization tools



## High throughput screening –based Bio-activities

Gene Transcription Factors	Genes	Ext. Blank	Erie Pier	Proximal	Distal	Rice's Point
Aryl hydrocarbon receptor (AhR) / Xenobiotic Response	AHR	2.95	1.94	0.91	1.07	2.45
Pregnane X receptor (PXR), Xenobiotic Pathway	PXRE		1.61	0.41	0.78	1.84
Pregnane X receptor	PXR		0.46	0.35	0.72	3.28
Estrogen Receptor (ER) pathway	ERE			2.13	3.11	4.29
Estrogen receptor- $\alpha$	ER $\alpha$			2.70	2.99	
Estrogen receptor- $\beta$	Er $\beta$			3.18*	4.00#	
Vitamin D receptor (VDR) / vitamin D pathway	VDRE		1.67	1.45	1.11	
Antioxidant Response Pathway	NRF2			2.62	2.60	
Hypoxia-inducible factor-1a (HIF1a) / hypoxia pathway	HIF1a			0.38	0.47*	
Peroxisome proliferator-activated receptor-d	PPAR $\gamma$			3.39	3.16	
Metal Response Pathway (MTF-1)	MRE				3.94	
Phenobarbital responsive enhancer module /constitutive androstane receptor (CAR) pathway	PBREM				1.79	
Retinoic acid receptor -related orphan receptor proteins (ROR) a,b,g	RORE				3.15	

*AC50 expressed as Relative enrichment factor (REF) with regard to ambient concentrations (e.g., REF of 2 = water has to be concentrated 2-fold to elicit 50% activity in the assay)*

St. Louis River, MN case study

Differences in overall activity among sites

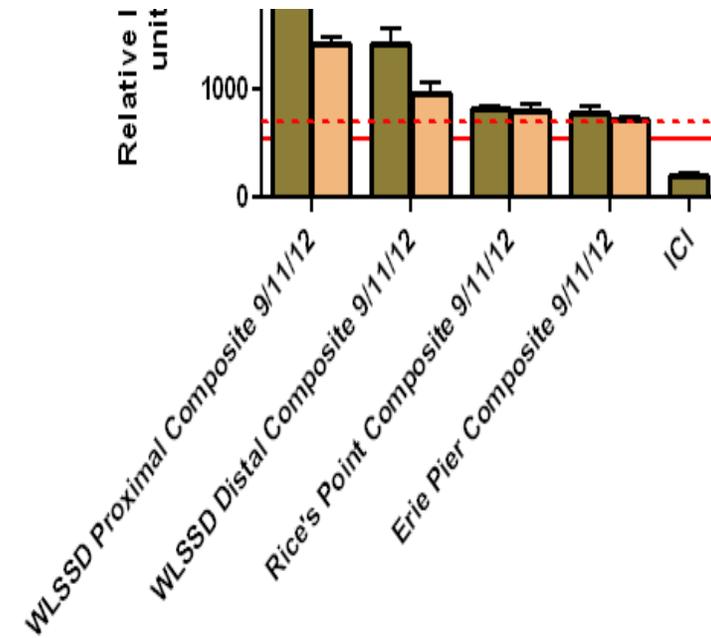
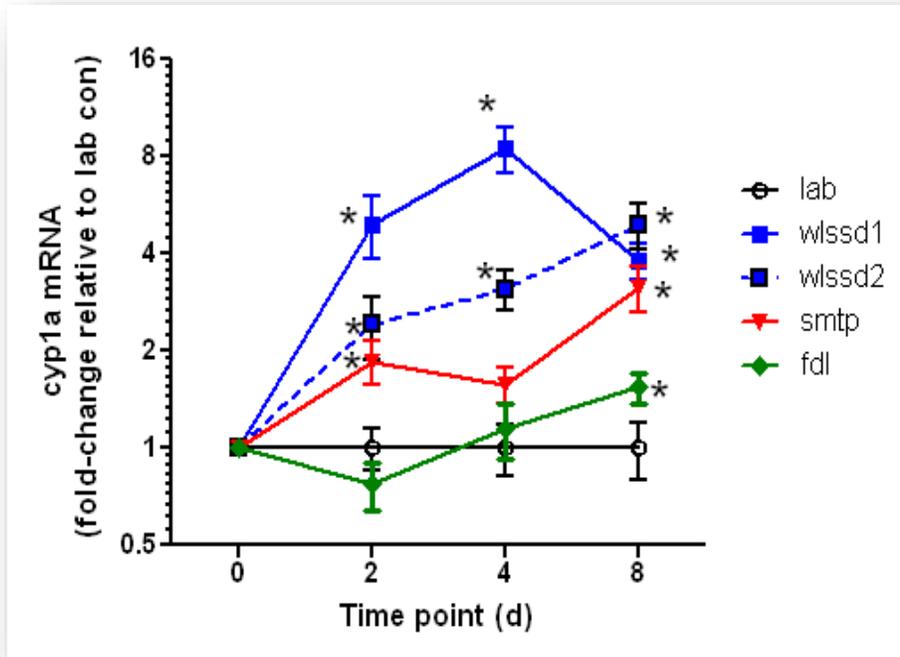
Most activity near Waste Water Treatment Plant

- Aryl hydrocarbon receptor activation
- Estrogen receptor activation

# Results – Risk-based screening & prioritization tools



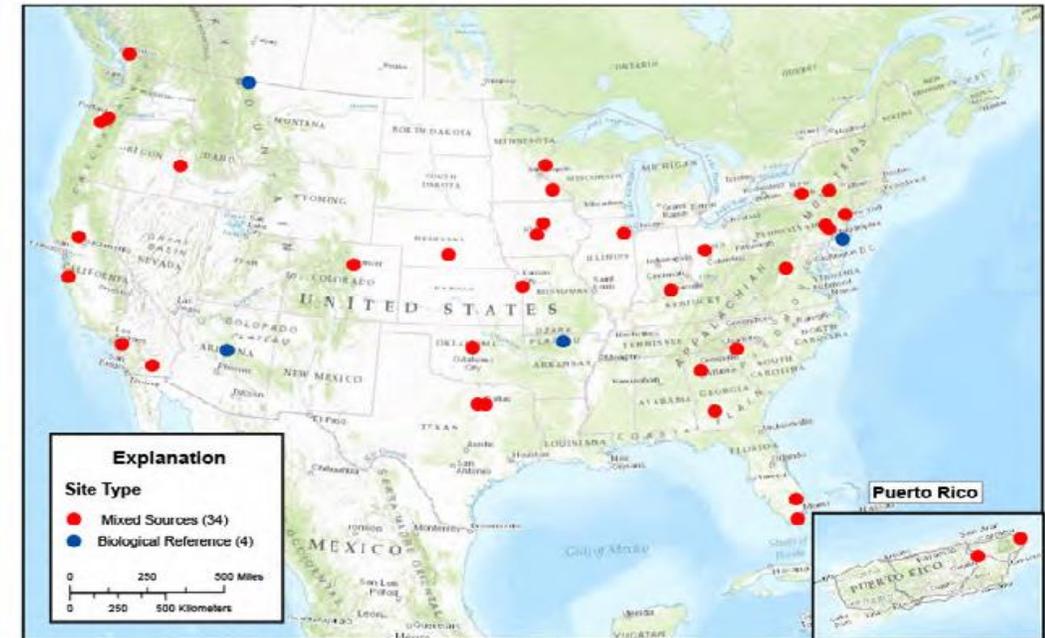
## HTS-based activities confirmed in follow-up monitoring assays



- AhR-mediated cyp1a induction in vivo
- ER activity declines with distance from WWTP discharge
- AOP linking to developmental toxicity
- AOP linking to reprod & develop tox

# Application Case Studies

- Great Lakes tributaries and near-shore areas
- USGS nation-wide mixture study (38 streams)
- South Platte River, CO (effluent dominated)
- Colorado River basin, UT
- St. Croix River basin, MN
- Shenandoah River, VA
- Zumbro and Crow Rivers, MN
- Lake Shagawa, MN
- Concord River, MA



Demonstrated application across all 10 EPA Regions – wide range of streams and ecotypes.

# Take-Home Messages



- Pathway-based bioeffects data are being generated at a rapid pace.
  - Legislative drivers are in place for that to continue
  - Those data are available today for use by the states and public
- AOPs offer a formal framework for linking pathway-based bioeffects to hazards of concern for ecological and/or human health risk assessment.
  - Organize knowledge and weight of evidence disseminated via internationally harmonized knowledge-base
  - Accessible, transparent and scientifically credible
- Pathway-based data + AOPs can provide information regarding hazard(s) associated with chemicals for which traditional toxicity data are lacking.

# Take-Home Messages



- Using modern computational tools, simple concepts like Exposure: Activity Ratios (EARs) can be applied to large data matrices (chemical x assay).
- EARs can be used to prioritize:
  - Sites at which **management** actions may be needed
  - Hazards/effects that may need to be **monitored** in resident populations
  - Chemicals for which **standards/criteria** should be developed
- EARs can be summed to consider integrated impact(s) of site-specific mixtures.
- High throughput screening can be applied to environmental samples for early warning of potential effects, even for chemicals that are not measured.



# Take-Home Messages

When faced with the challenge of detecting chemicals of unknown toxicity or trying to assess impacts of mixtures, states can use these tools and approaches to:

- Make effective use of new pathway-based data streams in decision making.
- Identify relevant hazards associated with individual chemicals or mixtures.
- Rank and prioritize chemicals, sites and hazards to optimize resource investment.



# Contact

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