EPA
United States
Environmental Protection
Agency

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

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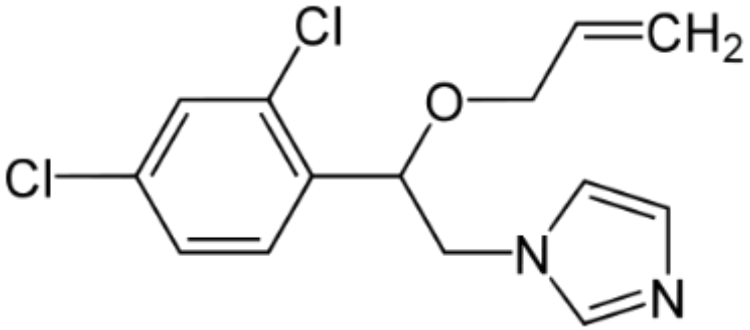
October 26, 2016

Problem

- An ever increasing range of chemical contaminants are being detected in the environment.

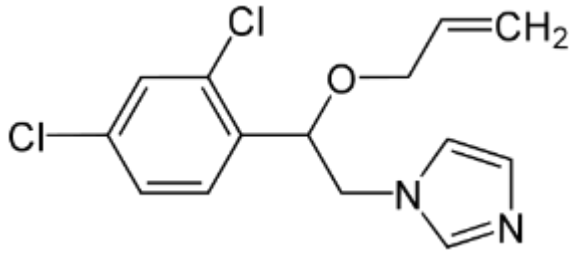
- For example pharmaceuticals, personal care products, current generation pesticides, perfluorinated compounds, flame retardants, etc.


Story Problem

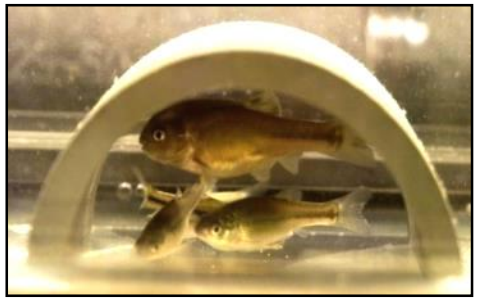


- You just detected this chemical in 30% of surveyed surface waters in your state.
- Local citizen action committees and several of your state legislators want to know if this is a concern.

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]

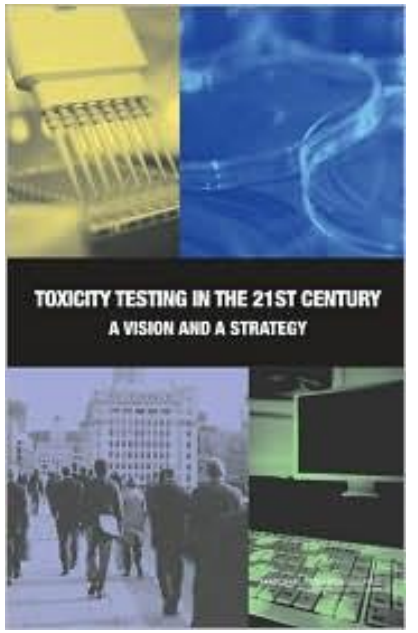
- Lack of safety/hazard characterization for most chemicals acknowledged in the President's remarks during signing of 2016 TSCA reform legislation.

Action – 21st 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."

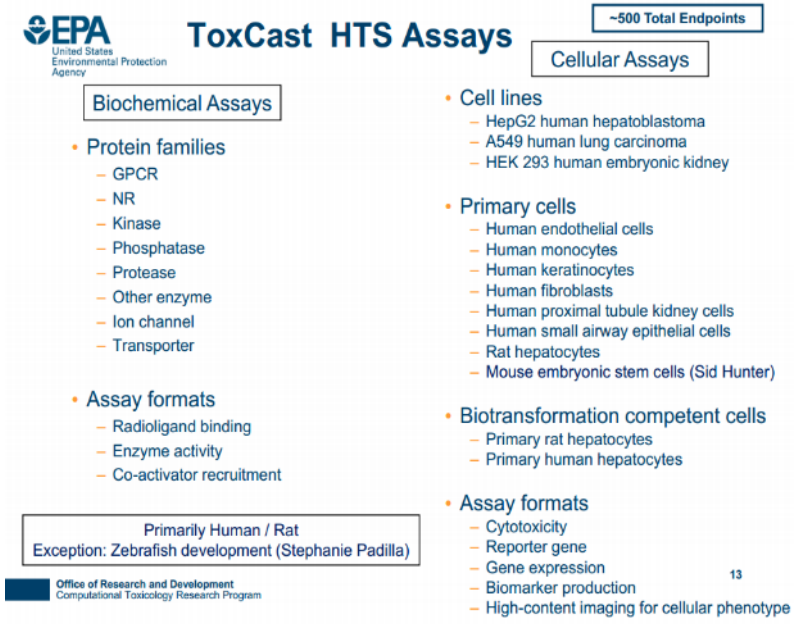


Action – 21st Century Toxicology

ToxCast

> 600 assays, >2000 chemicals,

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



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

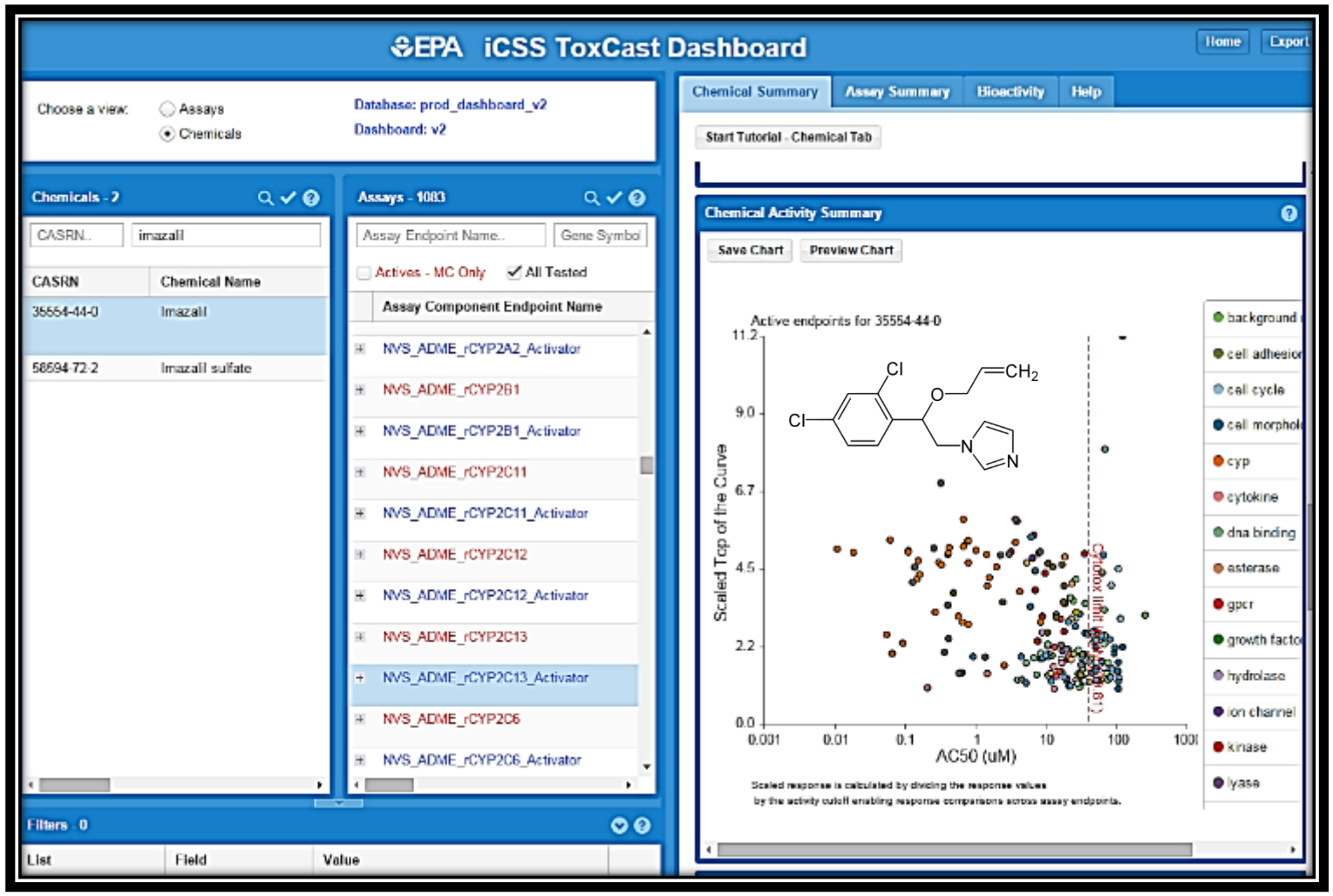
Tox21

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

- 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?

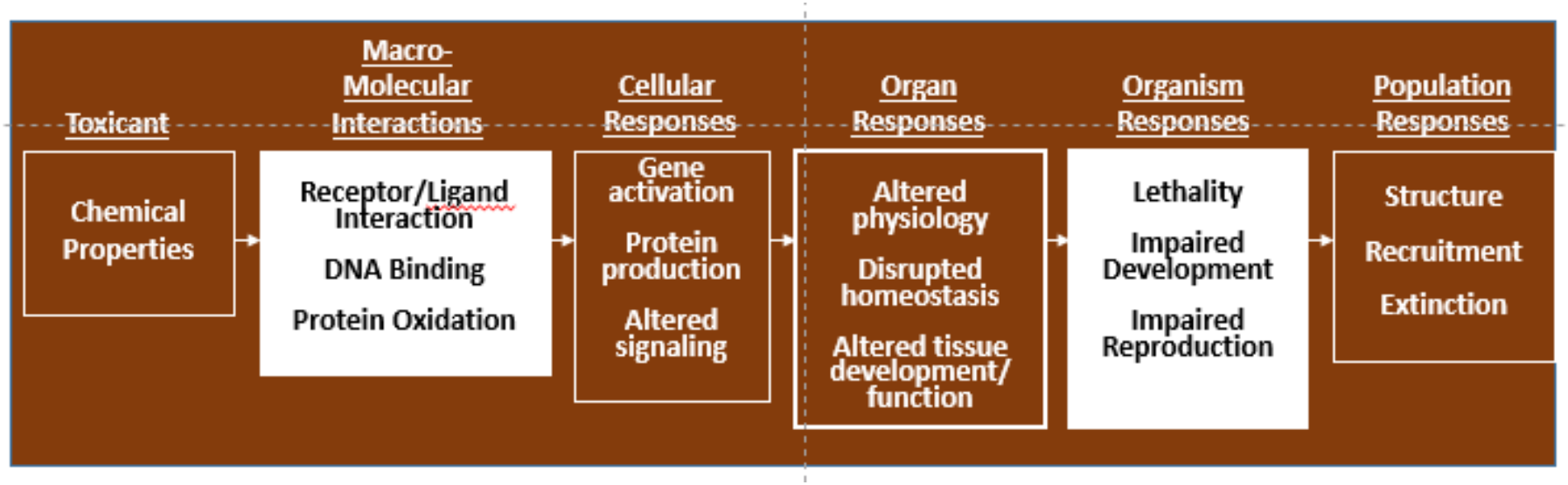
Citizens don’t care about receptor binding.

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.

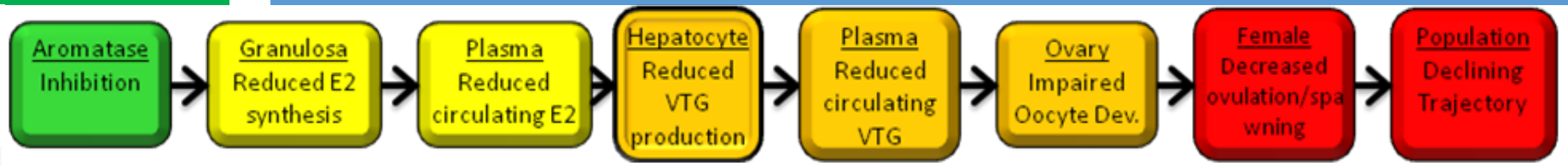
(Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)

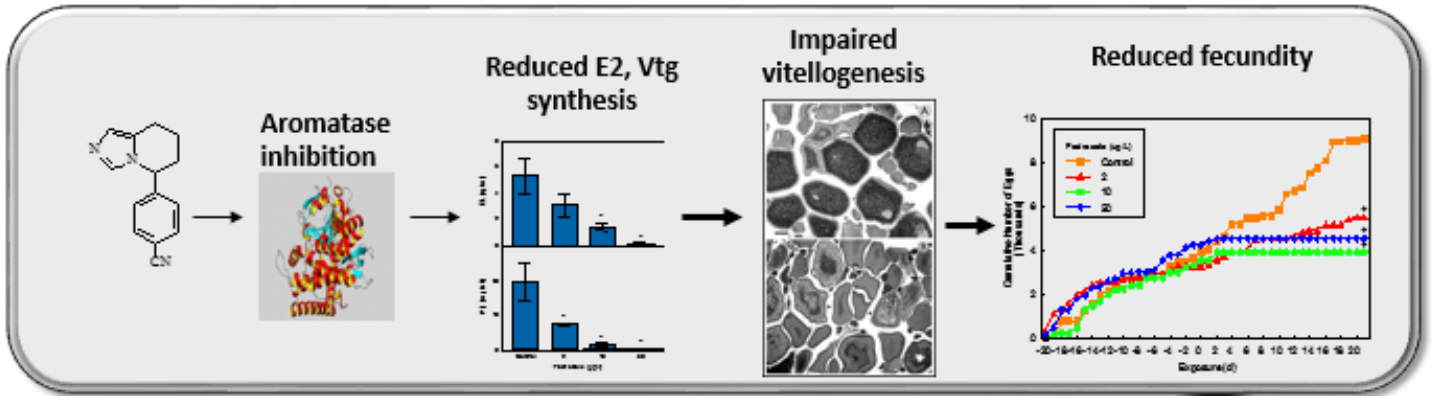


- Helps us organize what we know
- And make more effective use of pathway-based data in risk-based decision making

Results – Adverse Outcome Pathway Knowledgebase

Adverse Outcome Pathway WIKI

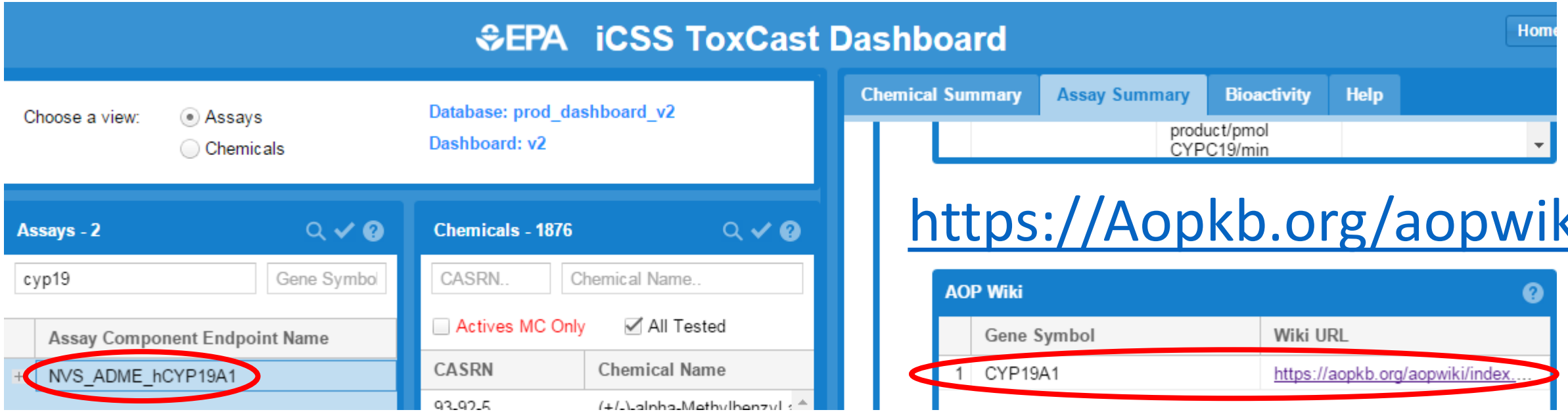




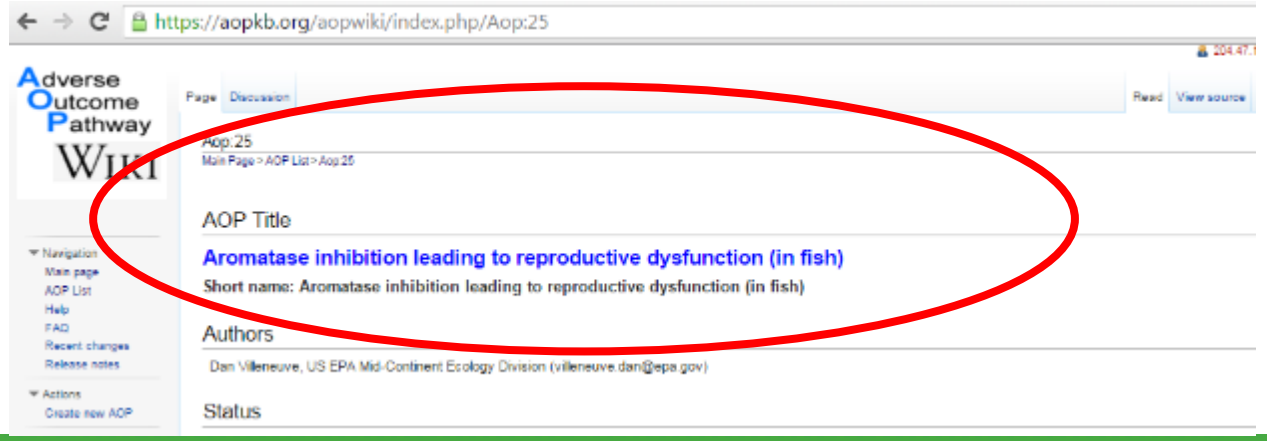
aopkb.org; Aop 25

- **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



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



Problem III

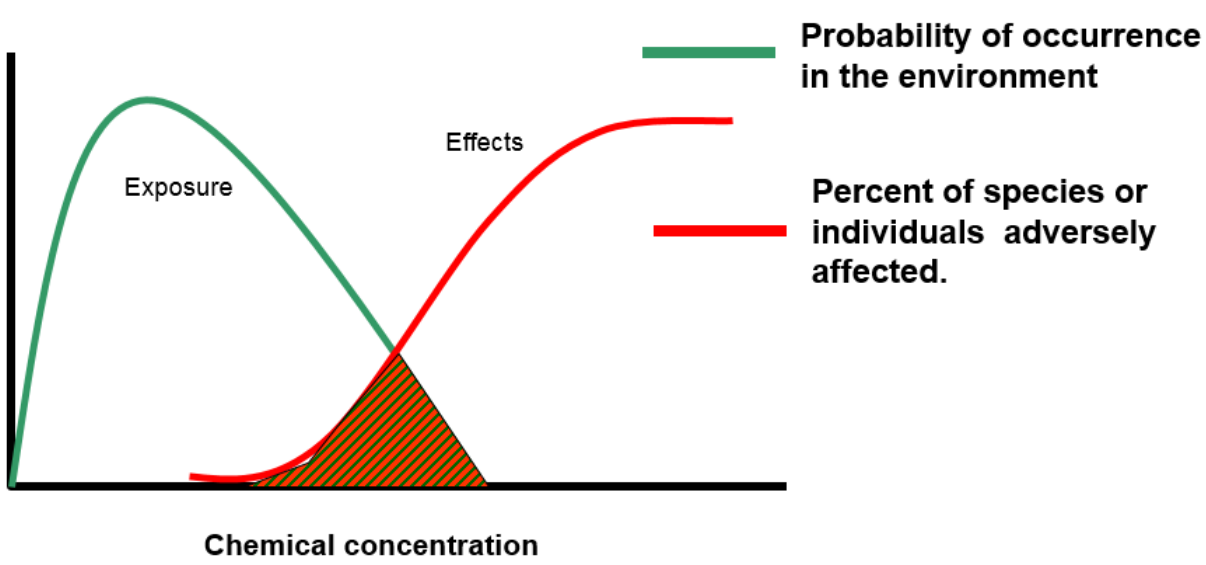
We're detecting a laundry list of chemicals.

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
Chloroxylenol	<0.080	0.052	<0.080	<0.080

Results – Risk-based screening & prioritization tools



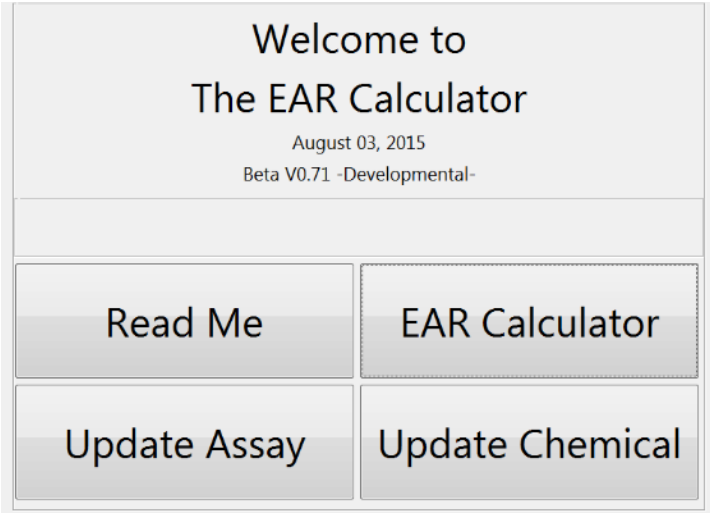
Which chemical(s) are present at high enough concentrations to elicit effects?

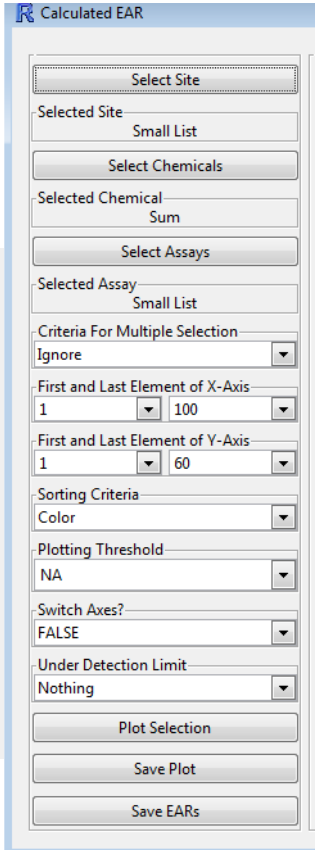
$$Exposure: Activity\ Ratio\ (EAR; unitless) = \frac{Exposure\ (concentration\ uM)}{Pathway - based\ Activity\ (AC50\ uM)}$$

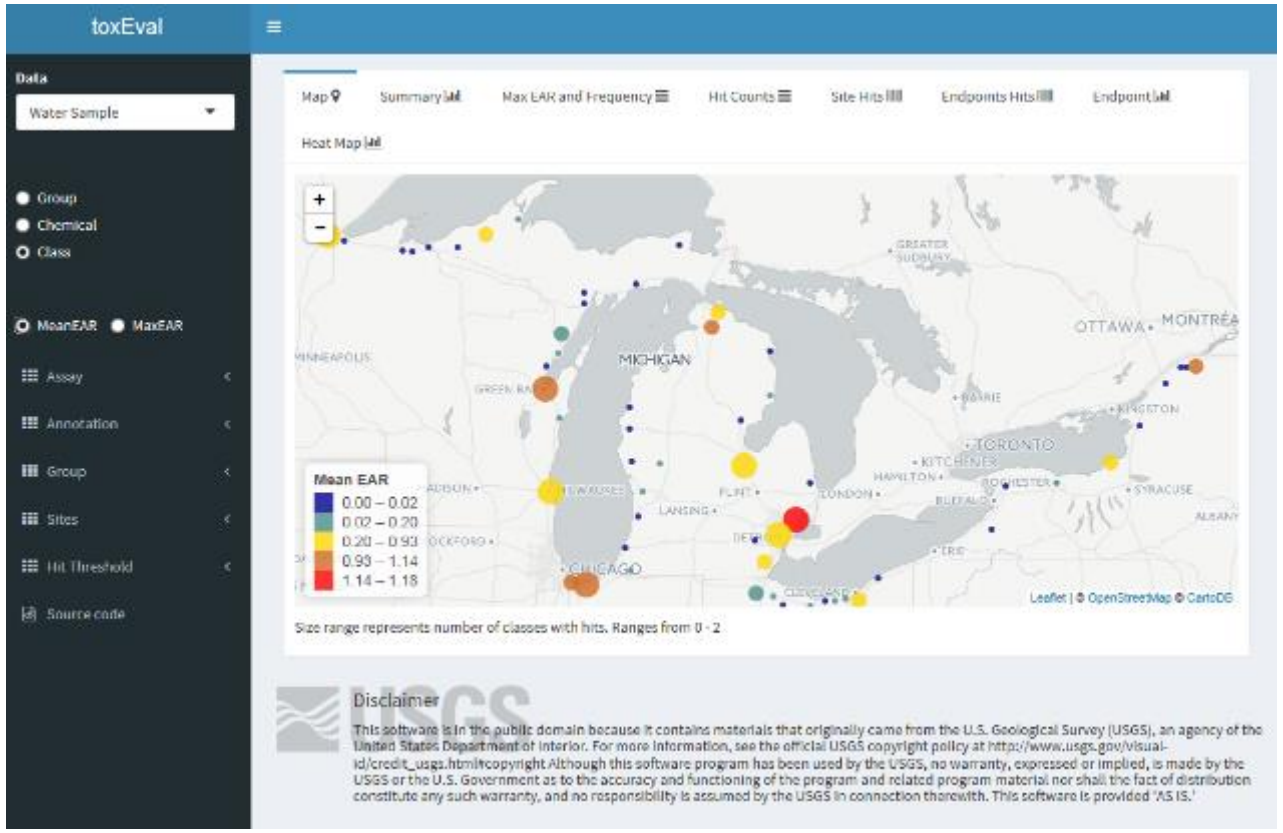
- Simple concept, simple calculation
- Not as simple for a matrix of 300 chemicals x 650 assay endpoints: 195,000 calculations

Results – Risk-based screening & prioritization tools

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

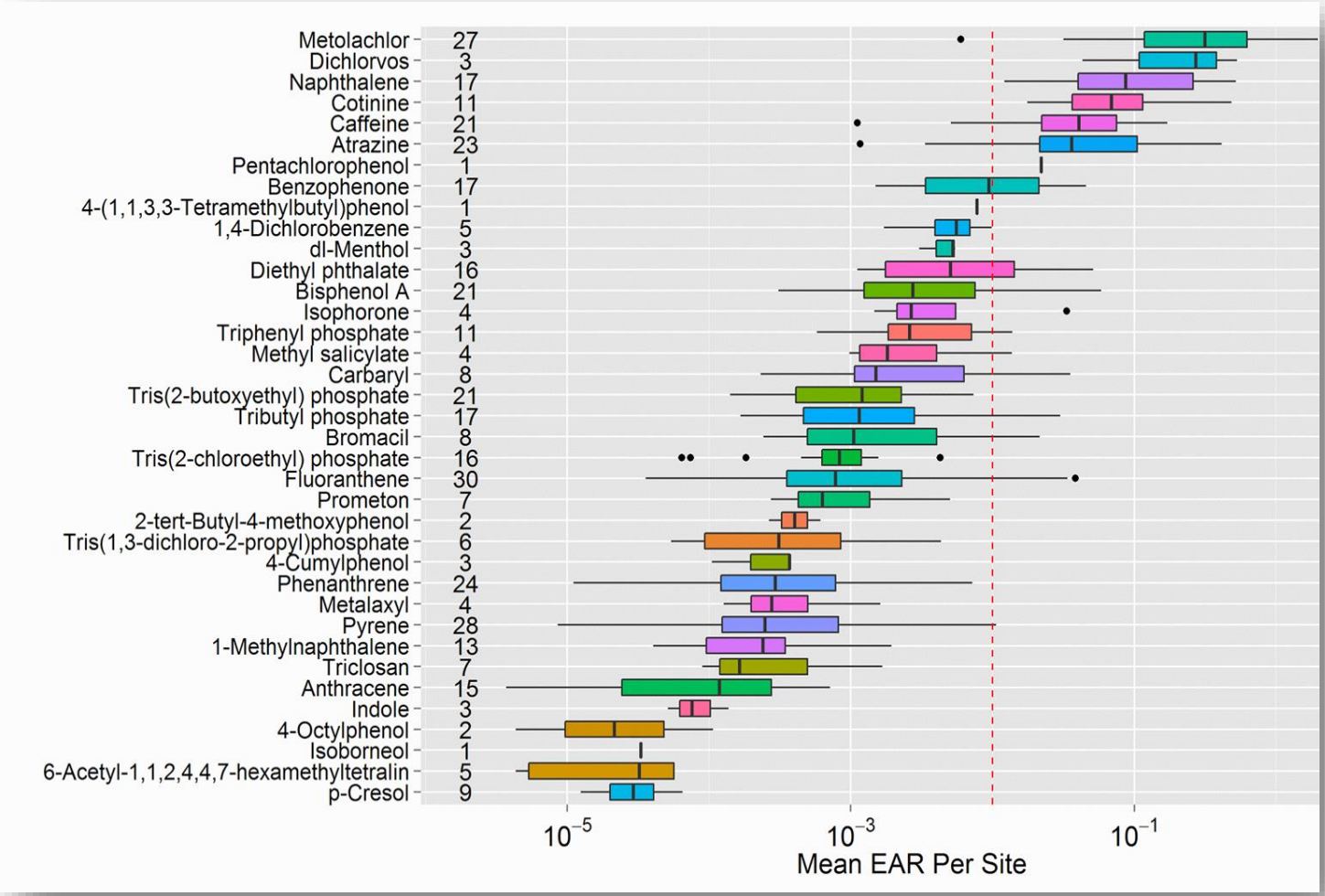






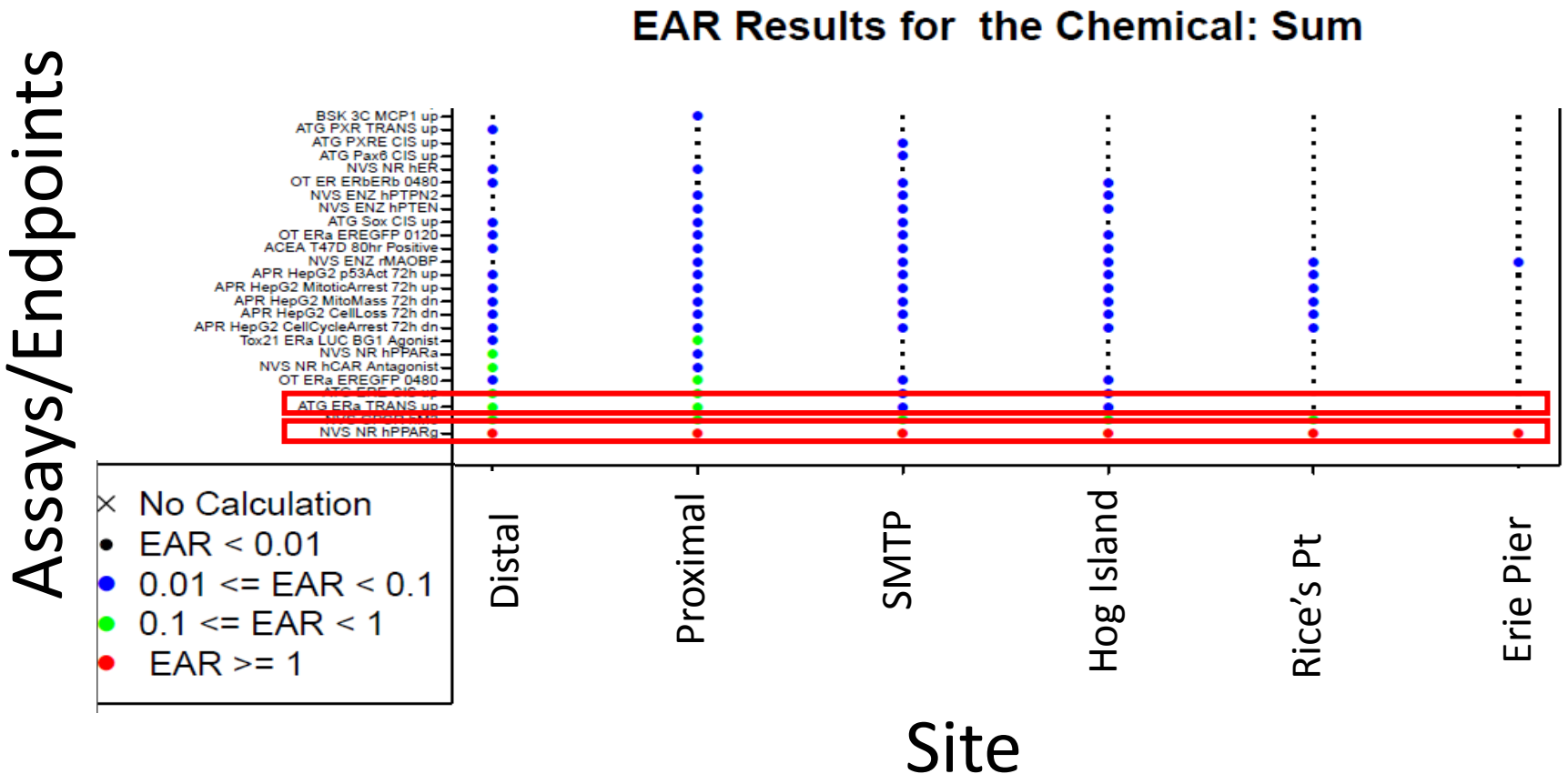
- Intended to be publicly accessible tools
- Conducting case studies to develop guidance on appropriate use

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



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.

$$EAR\ sum\ (unitless) = \sum \frac{Exposure\ (dose\ uM)}{Activity\ (AC50\ uM)}$$

Problem IV

Escher et al. 2013. Environ. Sci. Technol. 47: 7002-7011

- 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 – 21st Century Toxicology

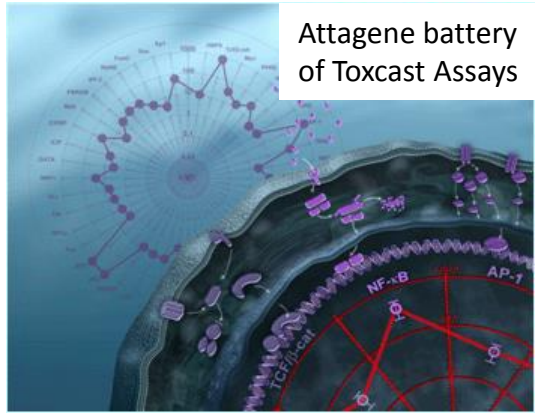
High throughput screening tools can be applied to environmental mixtures

Ambient water sample

extract

"Unknown" Chemical Mixture

Complex mixture

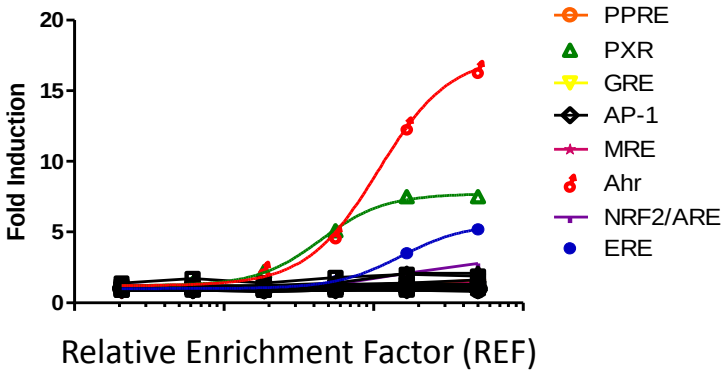


Factorial cellular biosensor system (HepG2 cell line)

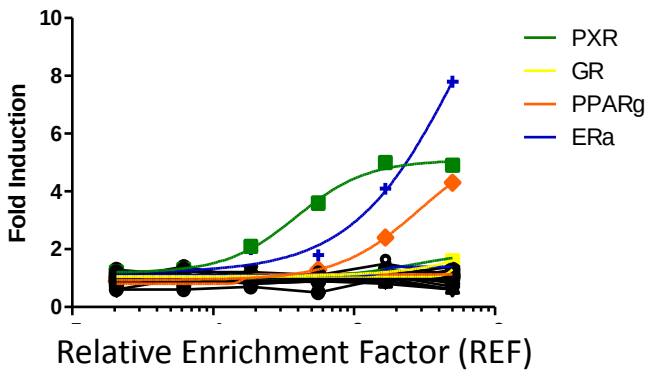
81 different assay features

Adverse Outcome Pathway WIKI

cis- Assays



trans- Assays



- Predicted hazards
- Taxonomic relevance
- Endpoints for targeted monitoring

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-α	ERα			2.70	2.99	
Estrogen receptor-β	Erβ			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	PPARg			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

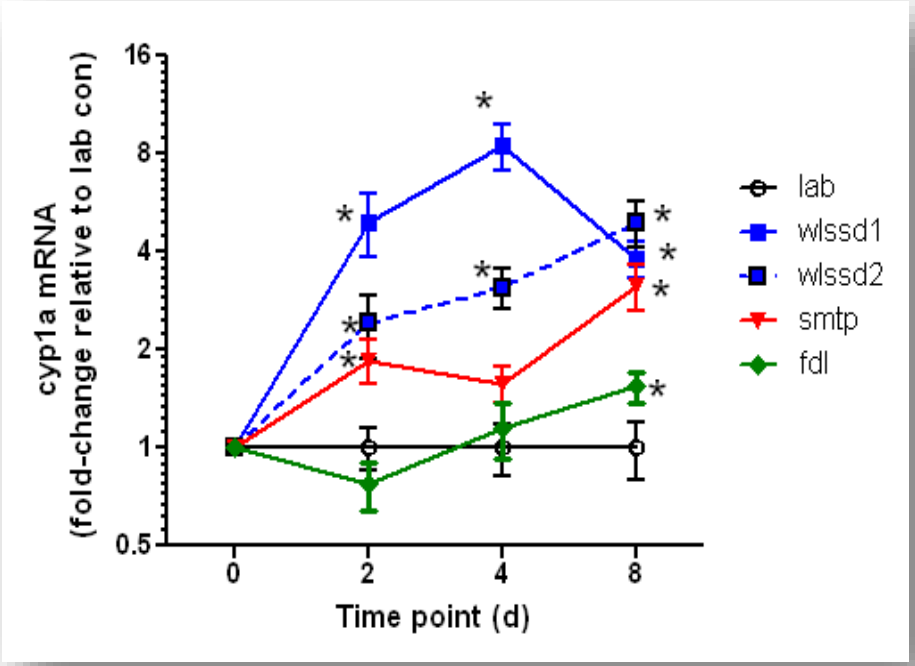
Differences in overall activity among sites

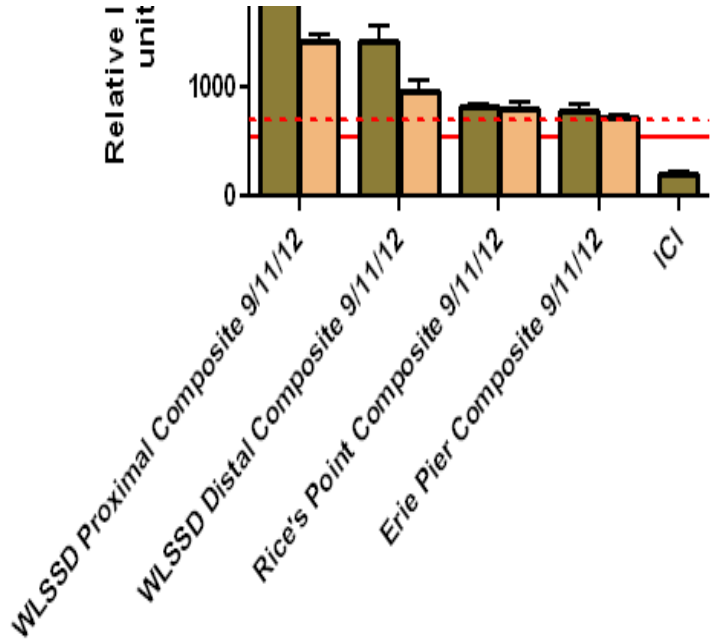
Most activity near Waste Water Treatment Plant

- Aryl hydrocarbon receptor activation
- Estrogen receptor activation

Results – Risk-based screening & prioritization tools

HTS-based activities confirmed in follow-up monitoring assays

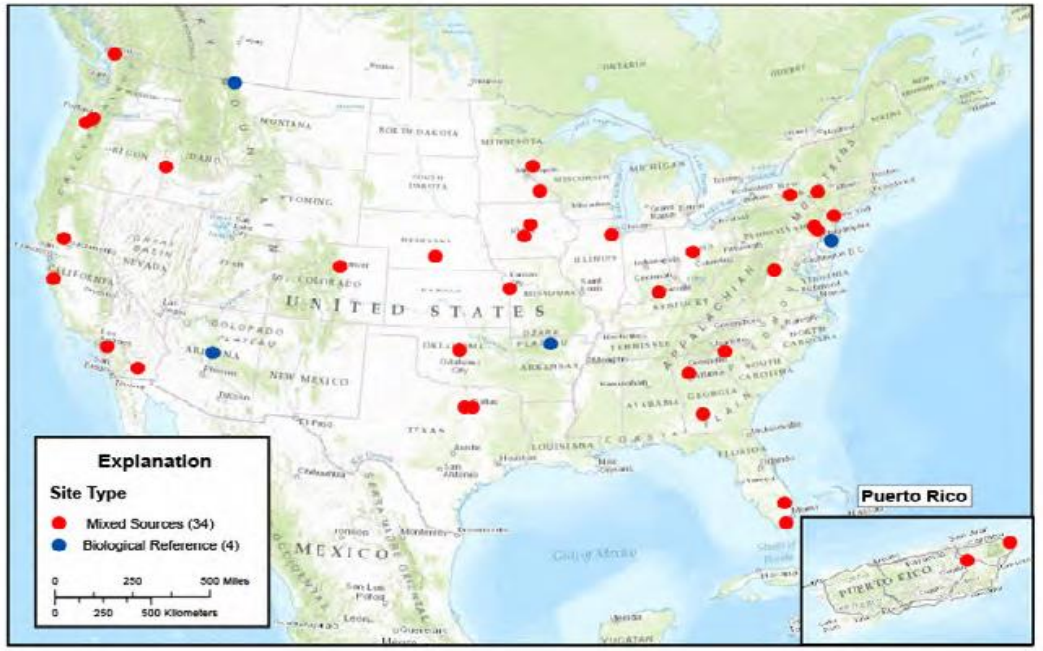




- AhR-mediated cyp1a induction in vivo
- AOP linking to developmental toxicity
- ER activity declines with distance from WWTP discharge
- AOP linking to reprod & develop tox

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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