Predictive Toxicology to Prioritize Contaminants for Monitoring and Management

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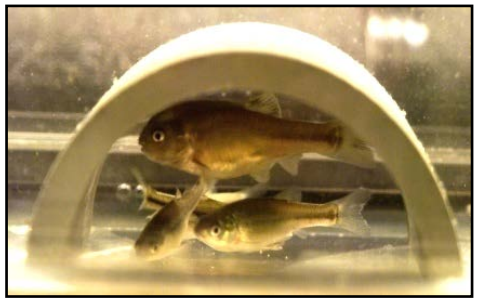
**The contents of this presentation neither represent nor necessarily reflect official US EPA policy.*

Growing lists of detected contaminants

- Improvements in analytical methods and instrumentation
- Example pharmaceuticals, personal care products, current generation pesticides, perfluorinated compounds, flame retardants, etc. (targeted analytes)
- A variety of transformation, breakdown, degradation-related products (NTA)

Contaminants of emerging/unknown concern

- Detecting compounds for which there are no existing water quality criteria or standards.
- Often there is little or no toxicity data available
- Can take years to generate new toxicity data for novel compounds and often no legal authority to collect those data.

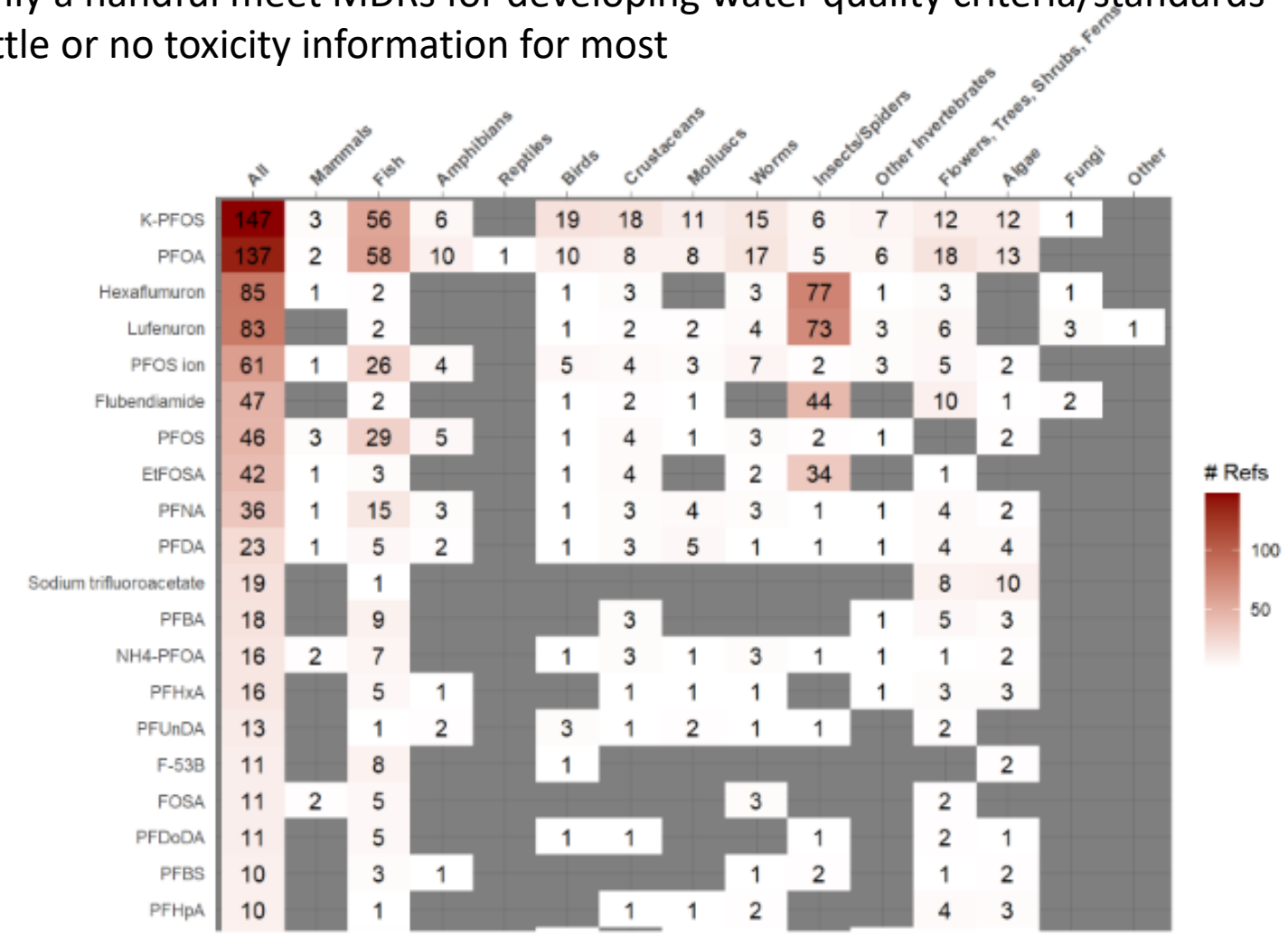


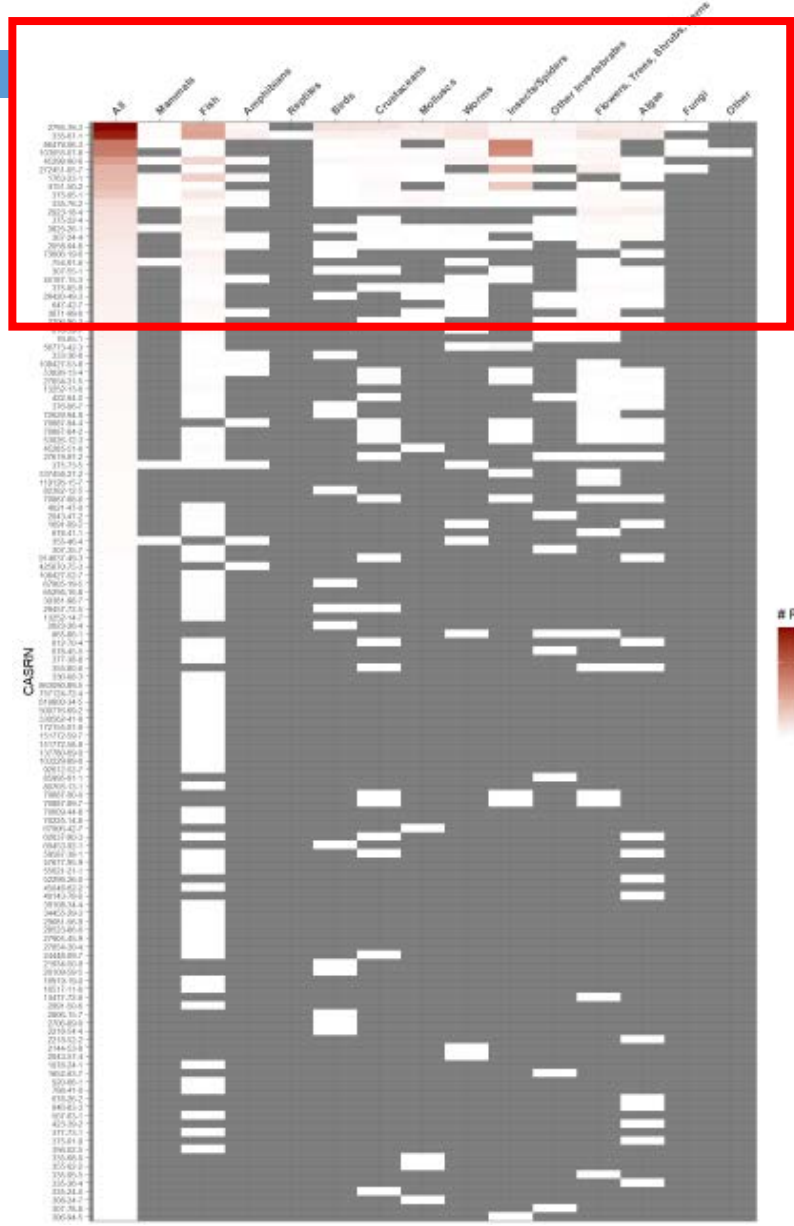
- Traditional whole organism-based toxicity testing is costly & time-consuming

Example: The data landscape for PFAS

- 3000-5000 structures in commerce
- Only a handful meet MDRs for developing water quality criteria/standards
- Little or no toxicity information for most

	All	Mammals	Fish	Amphibians	Reptiles	Birds	Crustaceans	Molluscs	Worms	Insects/Spiders	Other Invertebrates	Flowers, Trees, Shrubs, Ferns	Algae	Fungi	Other
K-PFOS	147	3	56	6		19	18	11	15	6	7	12	12	1	
PFOA	137	2	58	10	1	10	8	8	17	5	6	18	13		
Hexaflumuron	85	1	2			1	3		3	77	1	3		1	
Lufenuron	83		2			1	2	2	4	73	3	6		3	1
PFOS ion	61	1	26	4		5	4	3	7	2	3	5	2		
Flubendiamide	47		2			1	2	1		44		10	1	2	
PFOS	46	3	29	5		1	4	1	3	2	1		2		
EtFOSA	42	1	3			1	4		2	34		1			
PFNA	36	1	15	3		1	3	4	3	1	1	4	2		
PFDA	23	1	5	2		1	3	5	1	1	1	4	4		
Sodium trifluoroacetate	19		1									8	10		
PFBA	18		9				3				1	5	3		
NH4-PFOA	16	2	7			1	3	1	3	1	1	1	2		
PFHxA	16		5	1			1	1	1		1	3	3		
PFUnDA	13		1	2		3	1	2	1	1		2			
F-53B	11		8			1							2		
FOSA	11	2	5						3			2			
PFDoDA	11		5			1	1			1		2	1		
PFBS	10		3	1					1	2		1	2		
PFHpA	10		1				1	1	2			4	3		



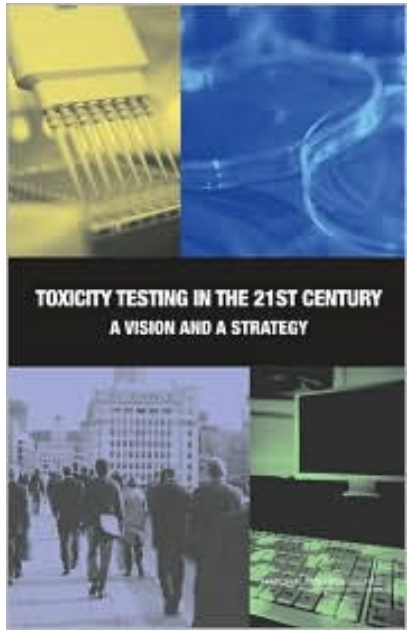


21st Century Toxicology

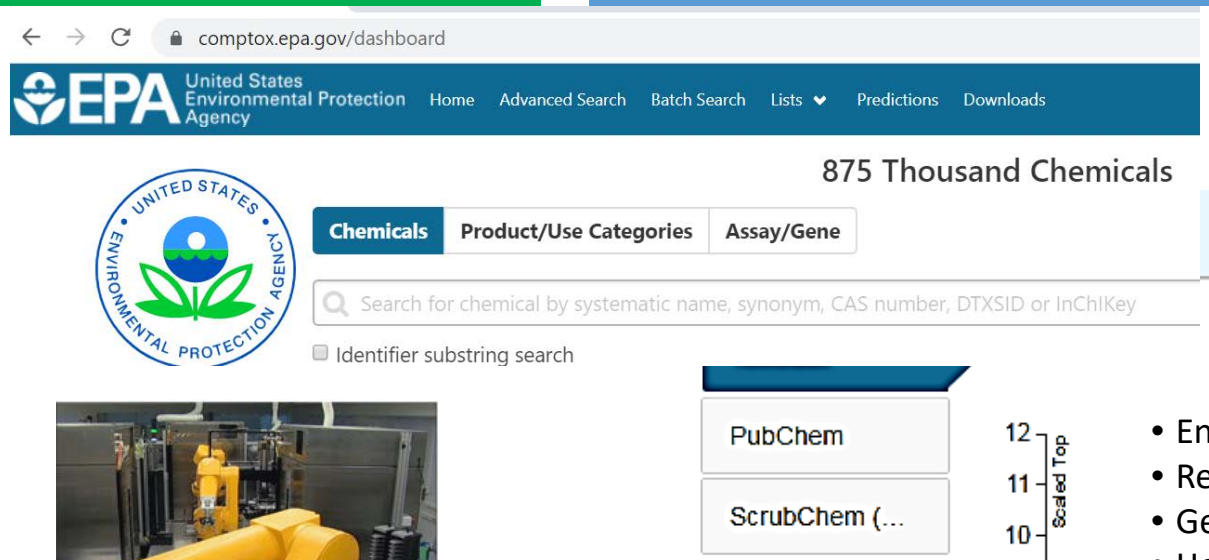
"Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin"

"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."



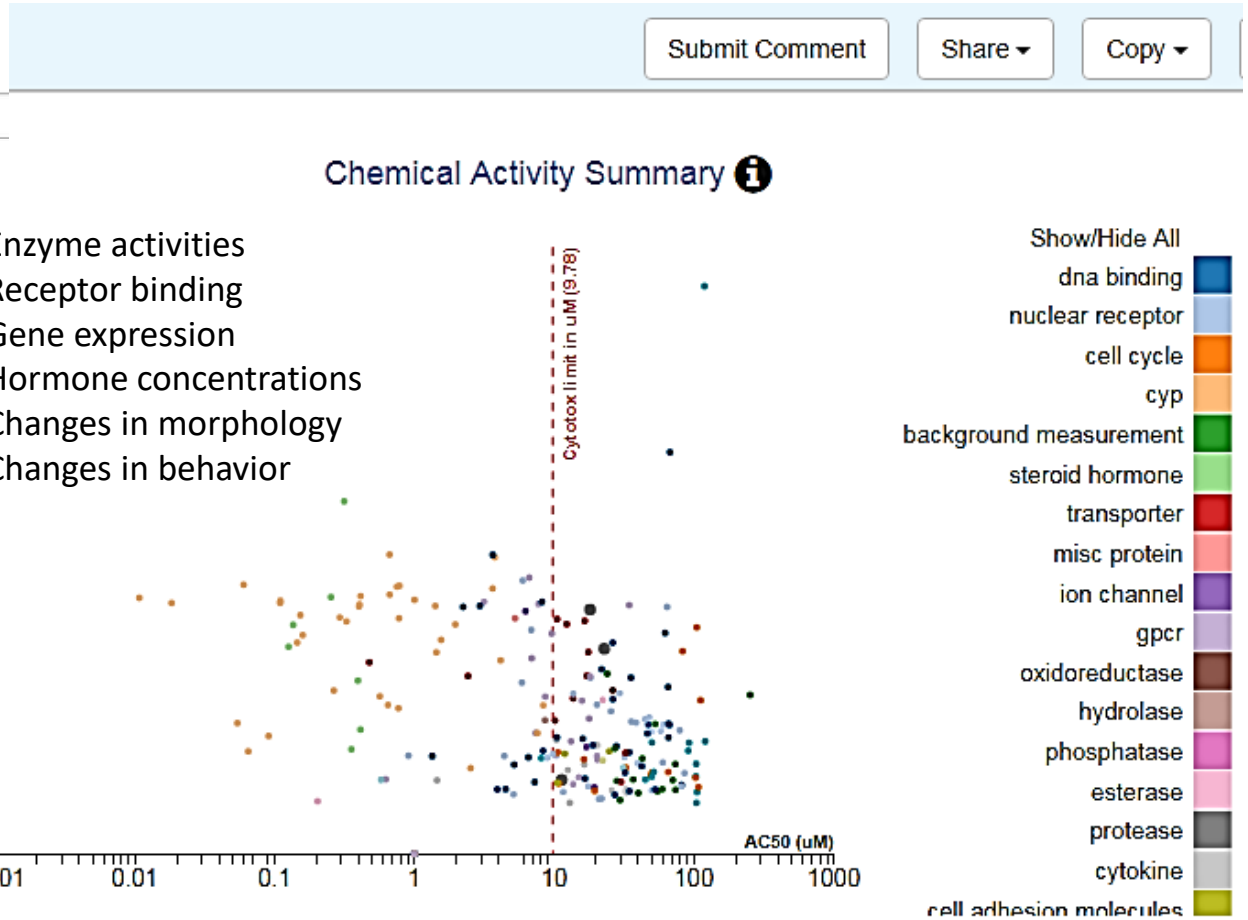
Predictive Toxicology Data



Comptox.epa.gov/dashboard

EPA CompTox chemistry dashboard

- What chemicals can do biologically
- At what concentrations



Predictive Toxicology Data

- Don’t have comprehensive coverage for all contaminants
- Rapidly growing database
- New data can be generated relatively rapidly and cost-effectively compared to traditional animal testing

Example – monitoring and surveillance in the Great Lakes basin

- 65 chemicals detected
- Traditional benchmarks available for 34/65 (52%)
- ToxCast data for 54/65 (83%)

Corsi SR, De Cicco LA, Villeneuve DL, Blackwell BR, Fay KA, Ankley GT, Baldwin AK. Prioritizing chemicals of ecological concern in Great Lakes tributaries using high-throughput screening data and adverse outcome pathways. Sci Total Environ. 2019 Oct 10;686:995-1009. doi: 10.1016/j.scitotenv.2019.05.457.

Challenge

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)?

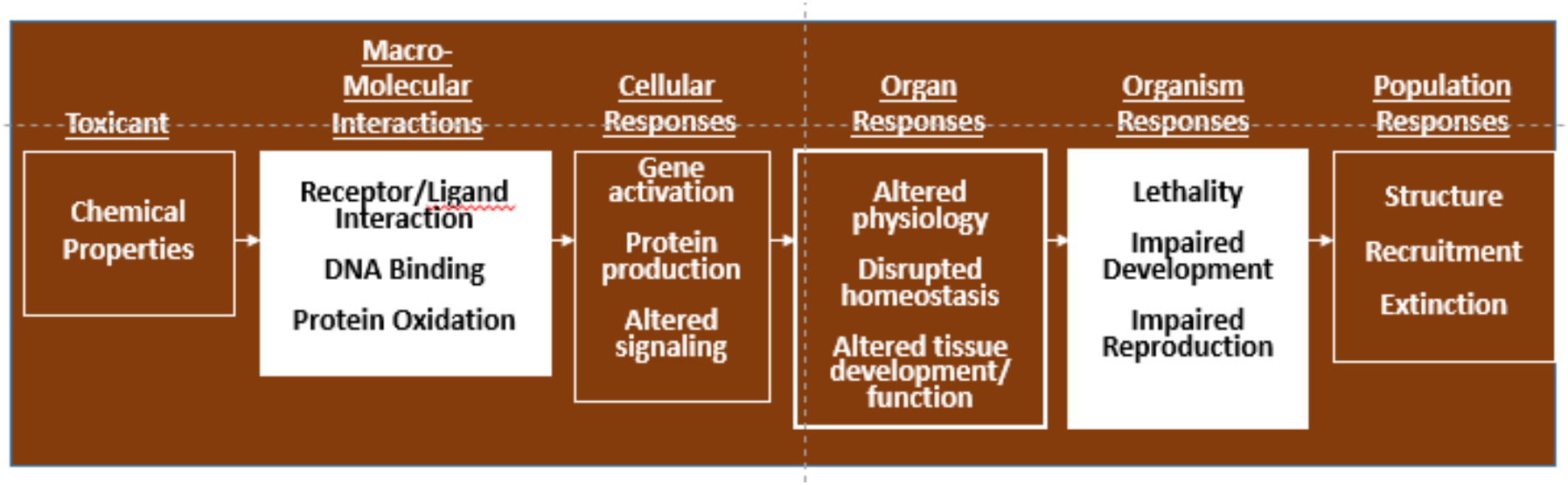
Data translation

Need access to specialized knowledge to understand what these results mean

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.

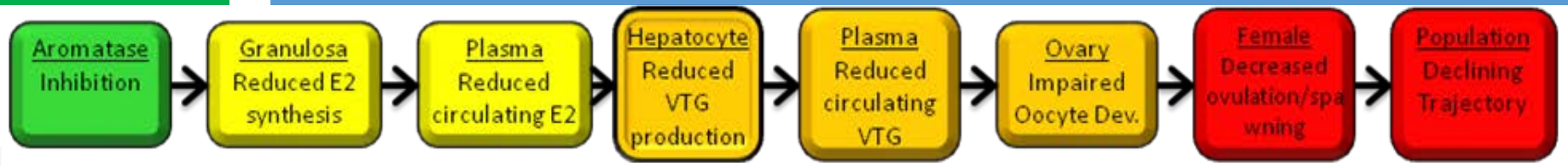
(Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)

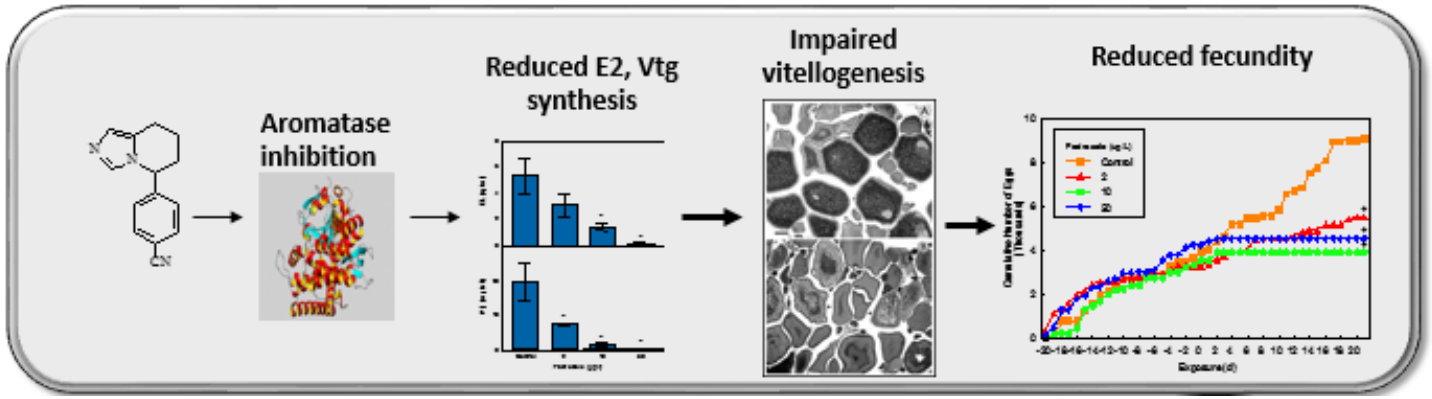


- Synthesizes information and evidence from relevant sources
- Presents it in a simple, graphical and narrative format

Adverse Outcome Pathway Knowledgebase

Adverse Outcome Pathway WIKI





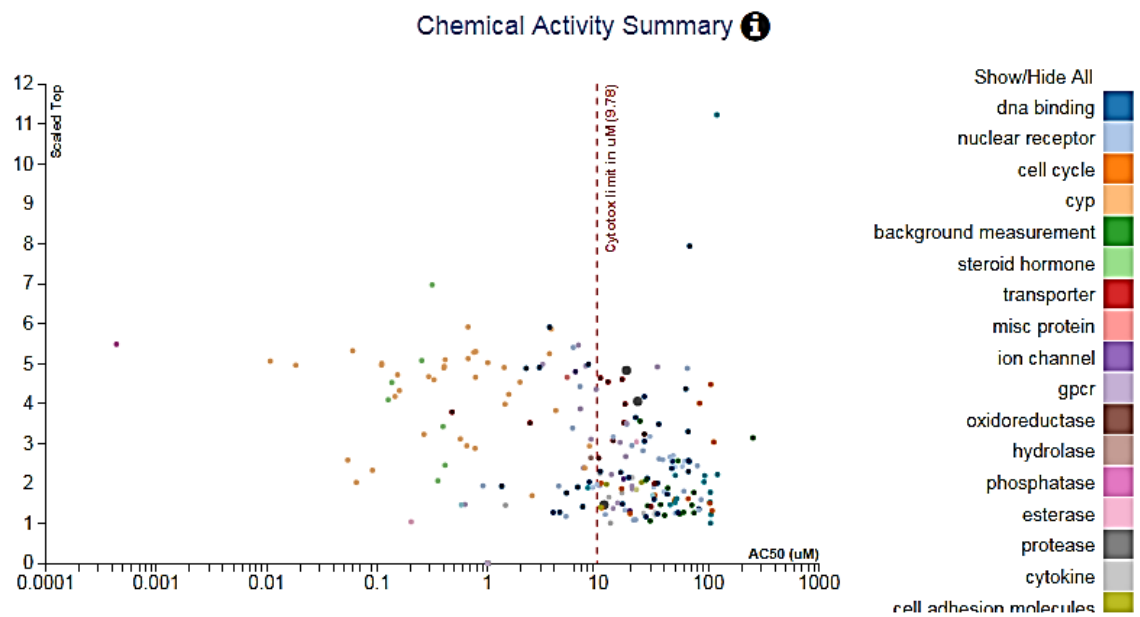
[aopkb.org; Aop 25]

- **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.

Predictive Toxicology Data + AOPs

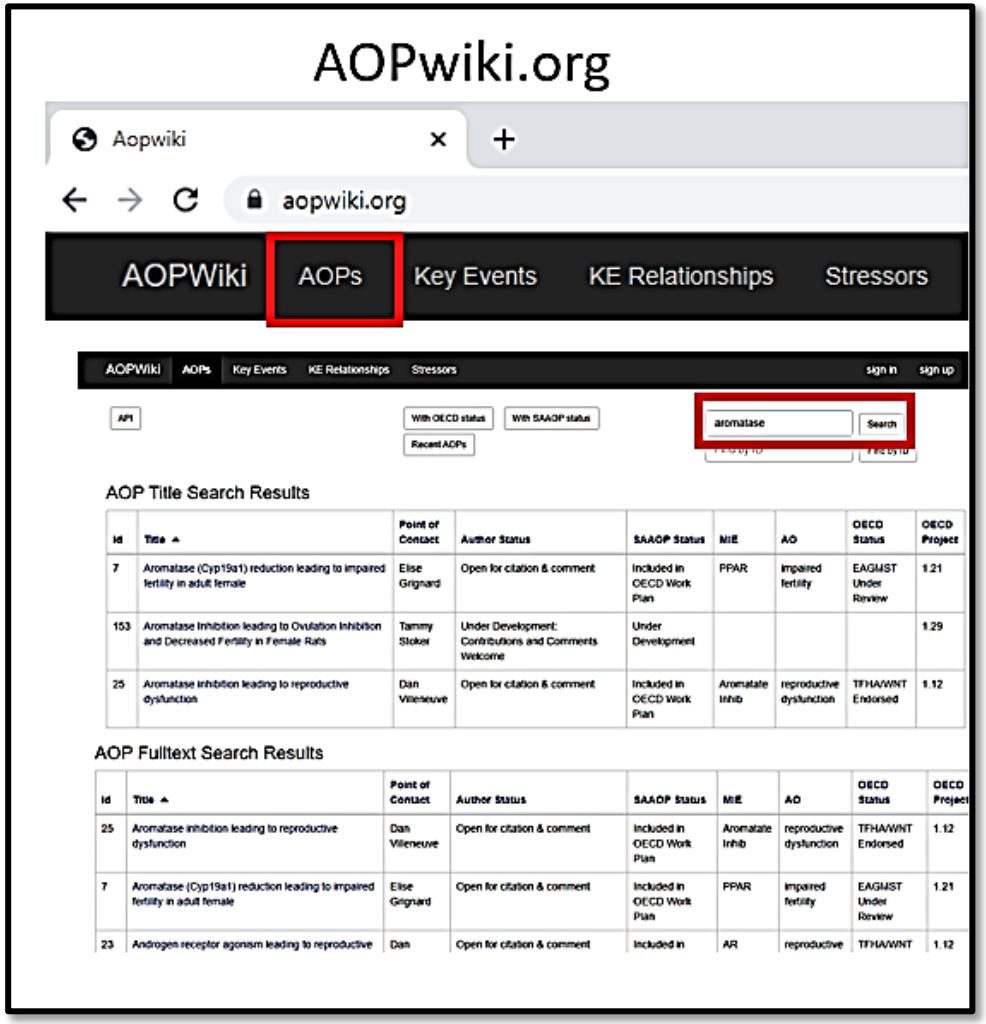
What can this chemical do biologically?

Comptox.epa.gov/dashboard



Why does that matter?

AOPwiki.org



Predictive Toxicology and Environmental Monitoring

Screening and Prioritization

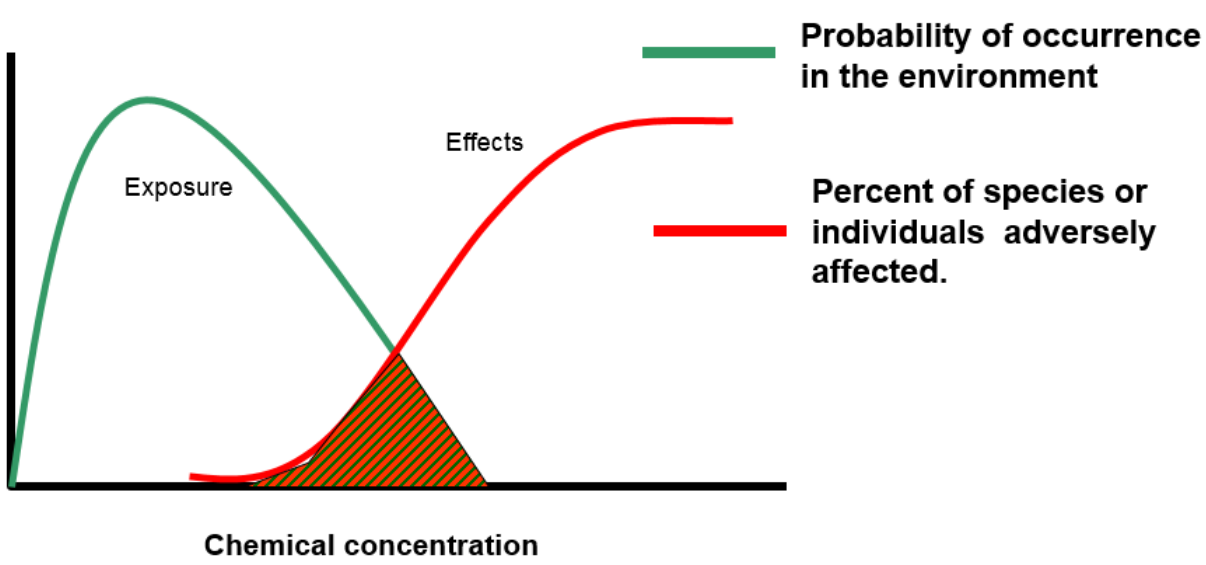
We're detecting a laundry list of chemicals.

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

Risk-based screening & prioritization tools



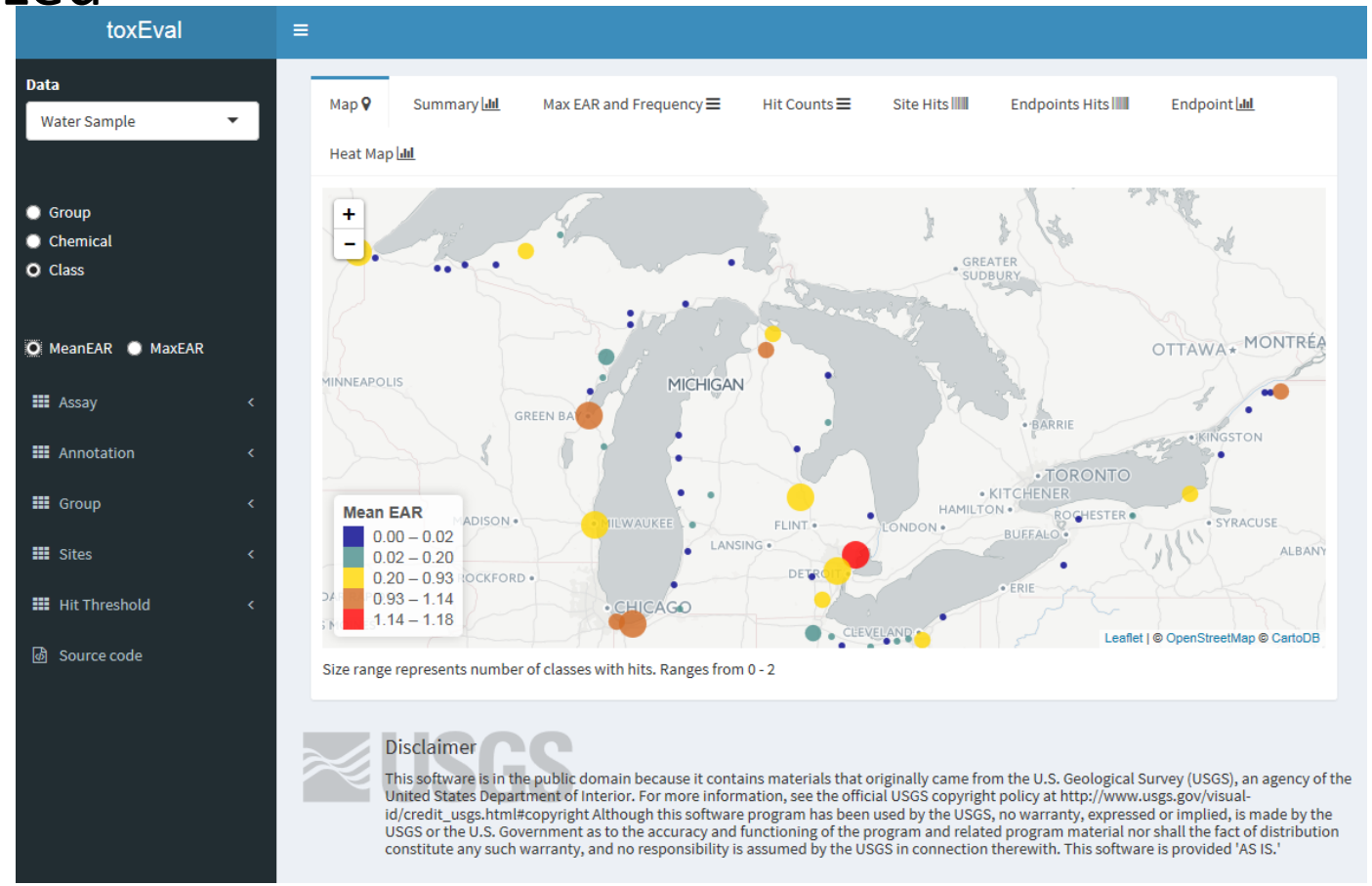
Which chemical(s) are present at high enough concentrations to elicit effects?

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

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

Risk-based screening & prioritization tools

- EARs rapidly calculated and visualized using toxEval



Intended audience:

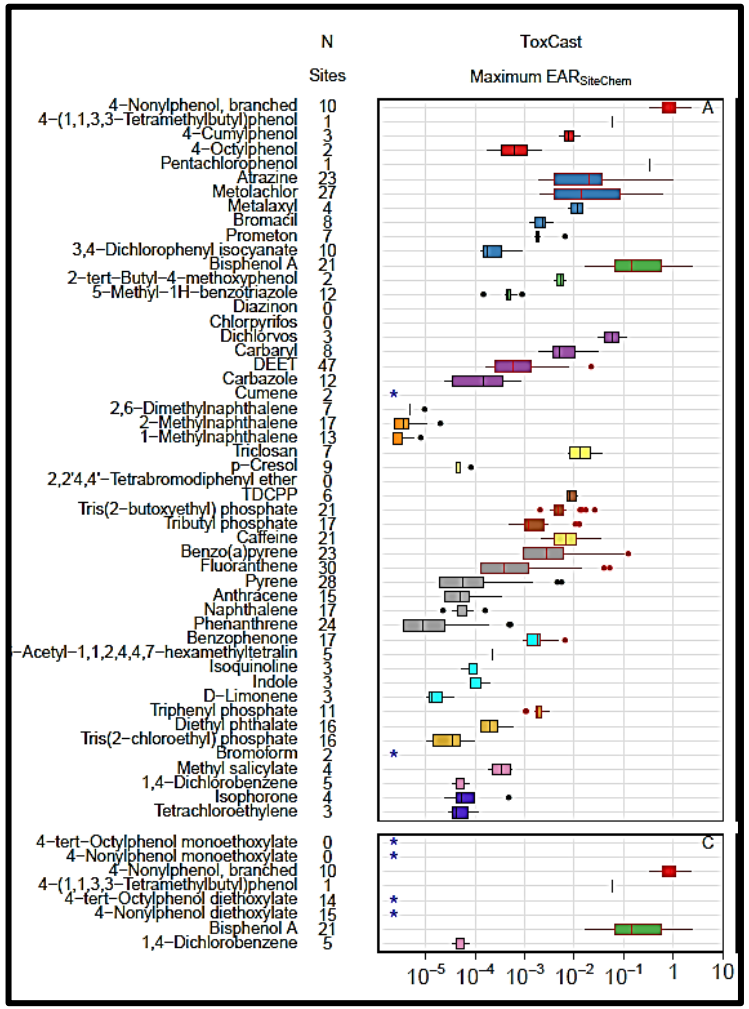
Regulators and resource managers (federal, state, local)

Researchers (government, academia, industry, NGO)

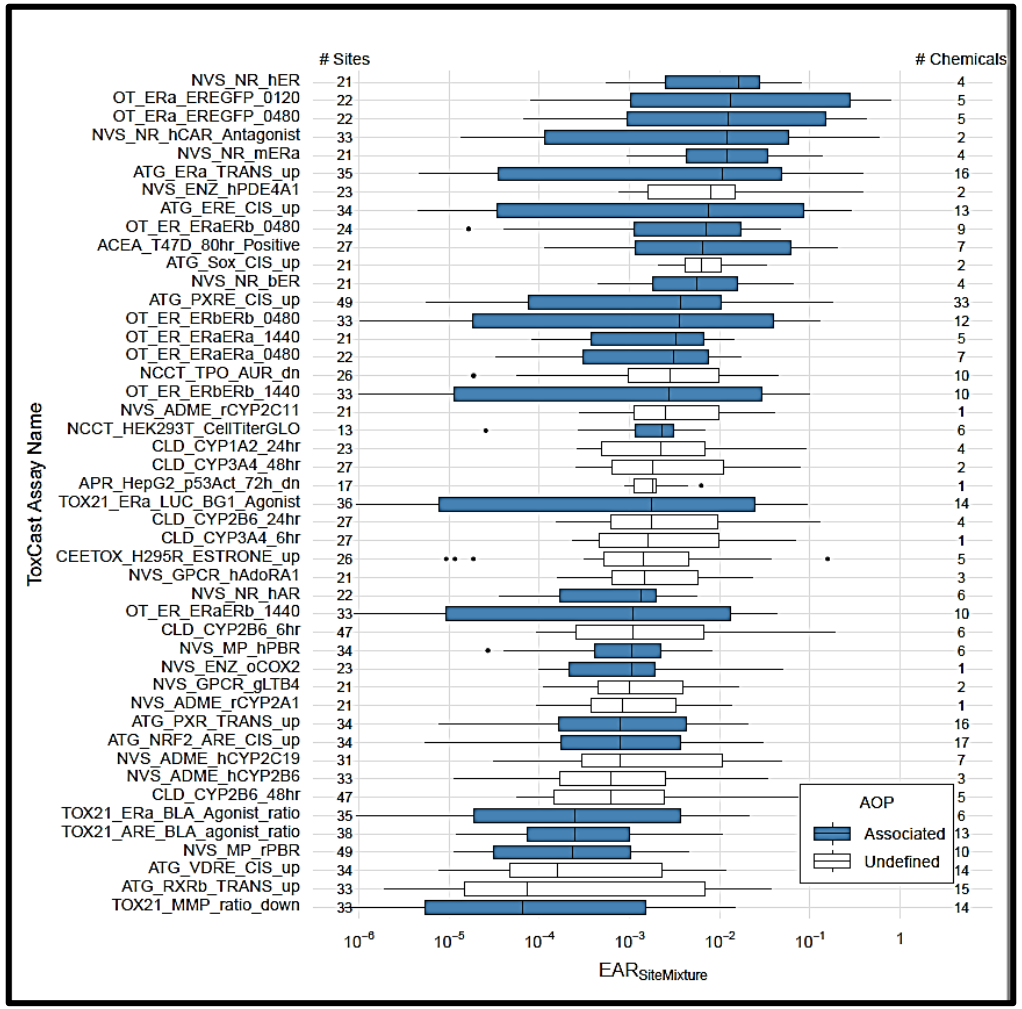
https://github.com/USGS-R/toxEval

Risk-based screening & prioritization

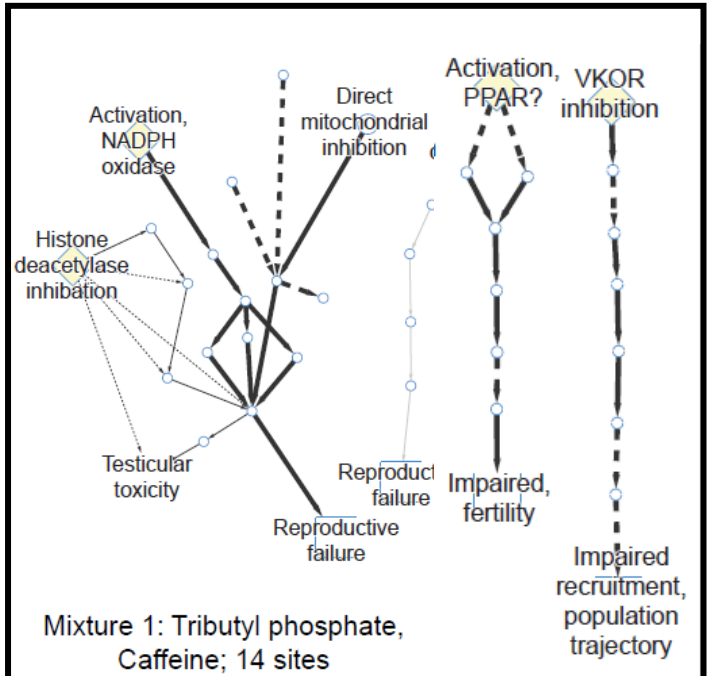
Which chemicals?



Which biological pathways?



What adverse effects might occur?



Corsi SR, et al.. Sci Total Environ. 2019 Oct 10;686:995-1009. doi: 10.1016/j.scitotenv.2019.05.457.

What about the contaminants we're not detecting?

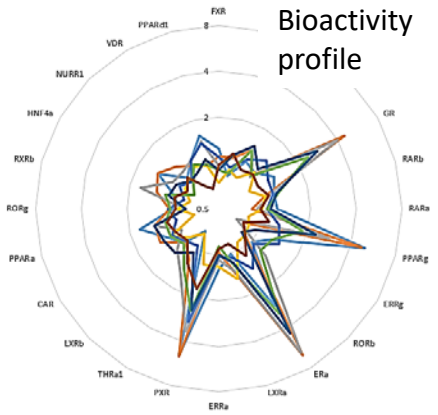
Escher et al. 2013. Environ. Sci. Technol. 47: 7002-7011

- 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

High throughput screening assays can be applied to environmental mixtures

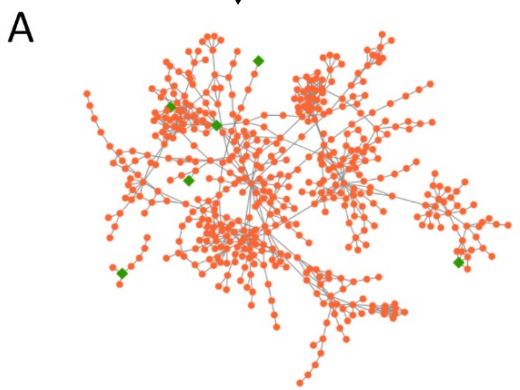
EX: BIOACTIVITY-BASED ENVIRONMENTAL MONITORING

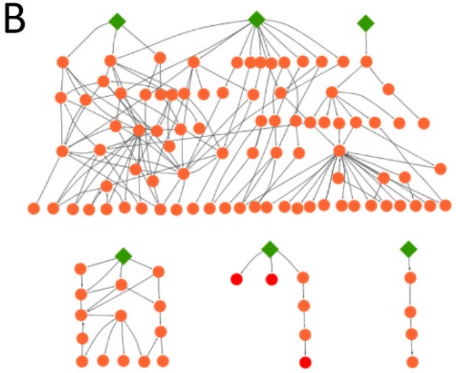
sample →

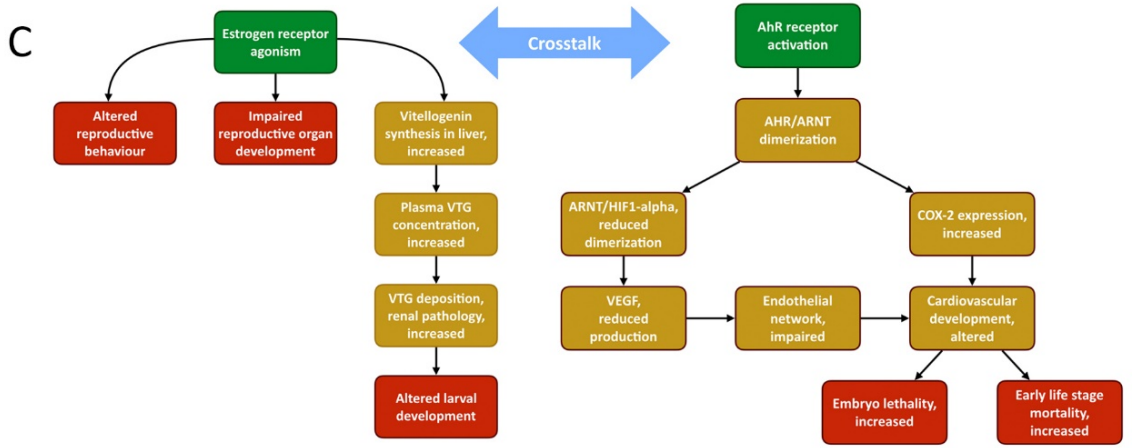


Bioactivity profile →

Adverse Outcome Pathway WIKI







A. 6 MIEs identified as hits by Attagene assay are KEs in global AOP network.

B. Network filtered to subset of AOPs directly linked to the 6 MIEs

C. Further filtered by taxonomic applicability.

Embryolethality
Reproductive hazard

Take Home

- There are a range of predictive toxicology tools with immediate application to environmental monitoring challenges.

- The science is mature but still evolving

- This is a very brief introduction to the concepts and tools

- Detailed training is available
  - EPA's chemistry dashboard / ToxCast
  - AOP-Wiki / AOP-knowledgebase
  - ToxEval

- Opportunity for case studies

Post-doctoral research opportunities

1. **Ecological Effects of Per- and Poly-Fluorinated Alkyl Substances (PFAS)**
   - Contact: Villeneuve.dan@epa.gov
   - URL: https://zintellect.com/Opportunity/Details/EPA-ORD-CCTE-GLTED-2020-08-A

2. **Analytical methods for evaluating toxicokinetics of per- and poly-fluorinated alkyl substances (PFAS) in challenging sample matrices.**
   - Contact: Blackwell.Brett@epa.gov
   - URL: https://zintellect.com/Opportunity/Details/EPA-ORD-CCTE-GLTED-2020-11-A

3. **University of Wisconsin-Madison – U.S. Environmental Protection Agency Fellowship with a focus on PFAS toxicology**
   - Contact: jennifer.hauxwell@aqua.wisc.edu