Leveraging New Approach Methodologies to Complement Aquatic Life Criteria Derivation

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Project Background

- Aquatic Life Criteria (ALC) are "concentrations of pollutants in ambient water that—if not exceeded—are expected to protect fish, invertebrates, and other aquatic life from unacceptable adverse effects associated with short-term (acute) or long-term (chronic) exposure."

- The process traditionally used by the USEPA's Office of Water (OW; 1985) to derive ALC, while thorough, is:
  - time- and resource-intensive
  - requires extensive literature review
  - often limited by data availability
  - *in vivo*-focused
  - based on apical outcomes

- Required set of toxicity tests is exhaustive enough that for most chemicals the requisite information is not available

- ALC have been established by USEPA for <50 chemicals

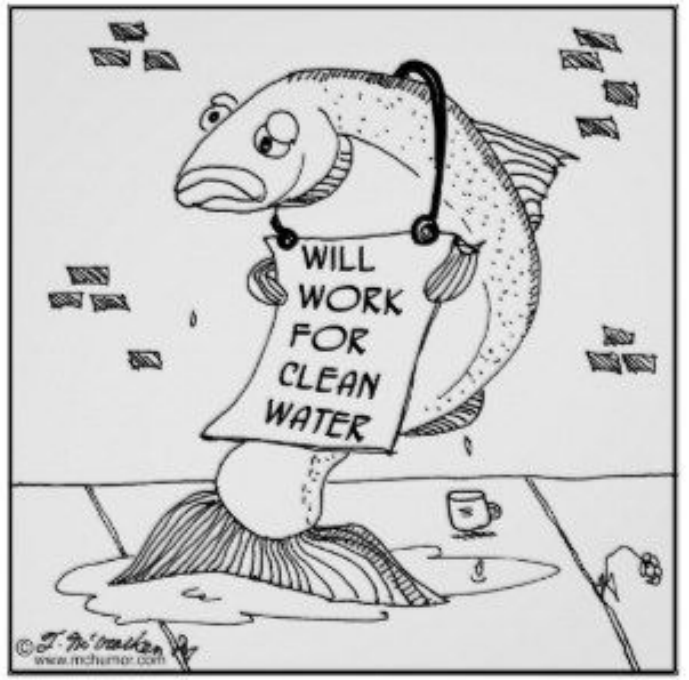
Background on NAMs

- New approach methodologies (NAMs) can provide data to fill gaps in hazard assessment and exposure characterization
- Thousands of datapoints from a single toxicity experiment and/or rapid and cost-effective screening using batteries of high throughput *in vitro* assays
- EPA's Toxicity Forecaster (ToxCast)
  - 10,000 chemicals across more than 1,000 *in silico* and *in vitro* assays have been screened
- NAM-based data can be used to calculate point-of-departure (POD) estimates that may serve as lower bound, protective, estimates of *in vivo* effect

Purpose

GOAL: Leverage NAMs data to derive* in vitro-*based benchmarks as supplement to traditional ALC process

- develop provisional prioritization and screening levels
  - guide resource prioritization (e.g., data generation and collection) for stakeholders (tribes, states, etc.)
- infer molecular mechanisms of action
- develop a process which is scalable and accessible



Theresa McCraken

Objectives

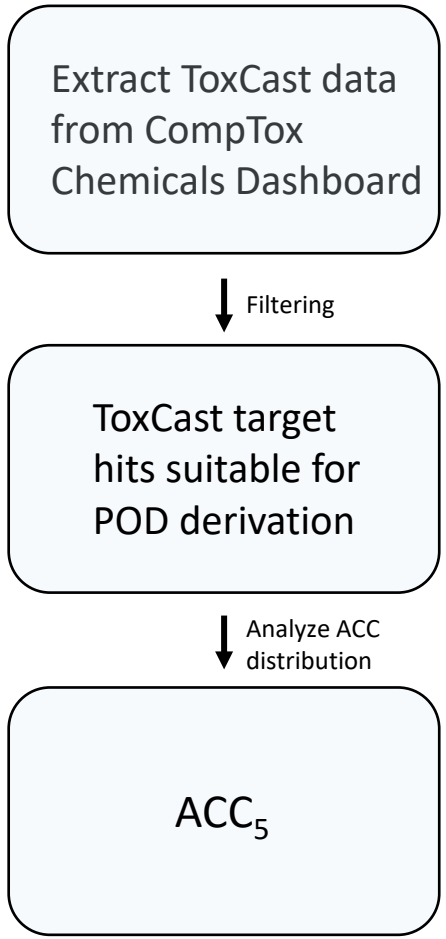
1. ***Derive chemical potency estimates (PODs) from ToxCast data***
   - 4-nonylphenol
   - Pentachlorophenol
   - PFOA
   - PFOS
   - Compare to available aquatic benchmarks

2. ***Use NAM data to investigate potential mechanisms of toxicity***
   - Three model chemicals with well-defined MoA as proof of concept
     - Celecoxib
     - Pioglitazone
     - TCDD
   - Four aforementioned industrial chemicals with published aquatic benchmarks

3. ***Define the taxonomic domain of applicability for putative mechanisms of toxicity***
   - Evaluate across species meeting OW minimum data requirements (MDR)

Objective 1: Approach

Derive chemical potency estimates (PODs) from ToxCast data



I. Filtered by hit call status: "ACTIVE"

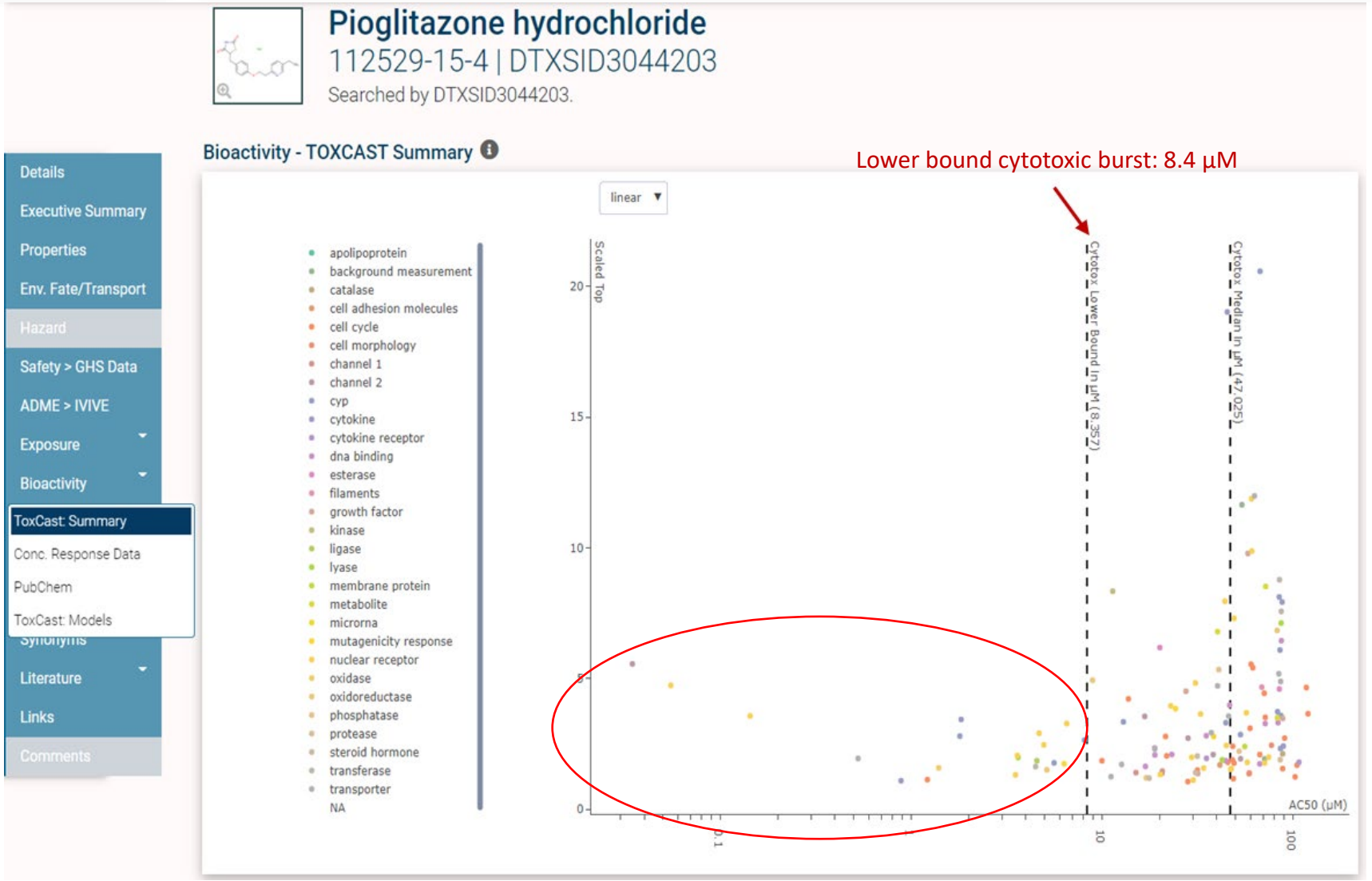
II. Only used hits active at concentrations below lower bound "cytotoxic burst"

III. Hits flagged with "only highest conc above baseline, active" and "only one conc above baseline, active" were removed for the POD calculation to minimize false positive hits

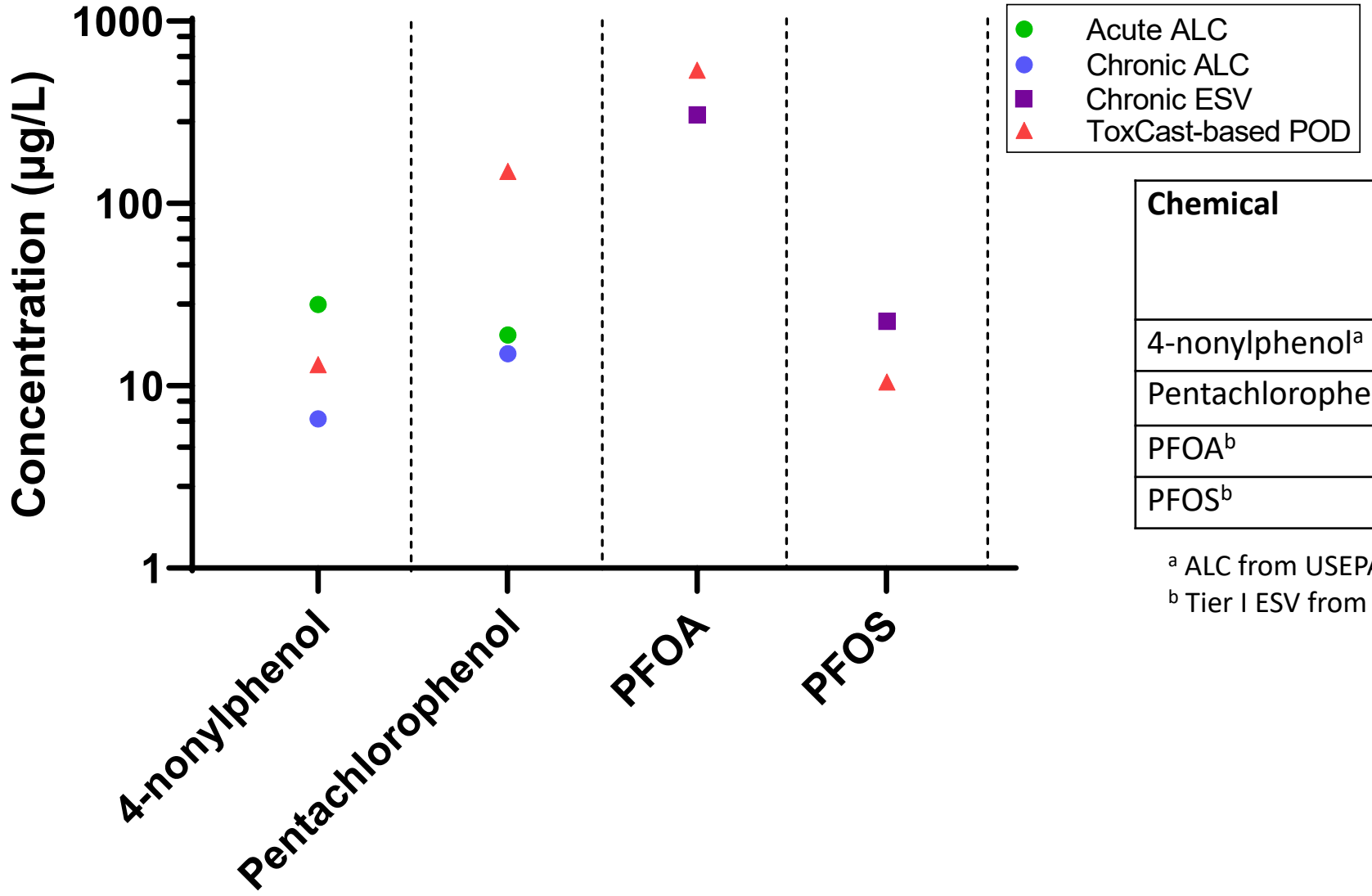
IV. Used activity concentrations at cut-off (ACC) as basis for benchmarks

V. From consequent ACC distribution, took 5^{th} percentile as protective estimate of *in vivo* effect concentration; resulting "ACC_5" used as POD

Example ToxCast Summary



Objective 1: Results (Benchmark Comparisons)



Chemical	Freshwater Benchmark		ToxCast
	Acute (µg/L)	*Chronic (µg/L)*	*ACC_5 (µg/L)*
4-nonylphenol[a]	28	6.6	13.0
Pentachlorophenol[a]	19	15	162
PFOA[b]	-	307	538
PFOS[b]	-	22.6	10.5

[a] ALC from USEPA

[b] Tier I ESV from DOE/Argonne National Laboratory, 2021

Objective 2: Approach

Probe potential mechanisms of toxicity using NAM data

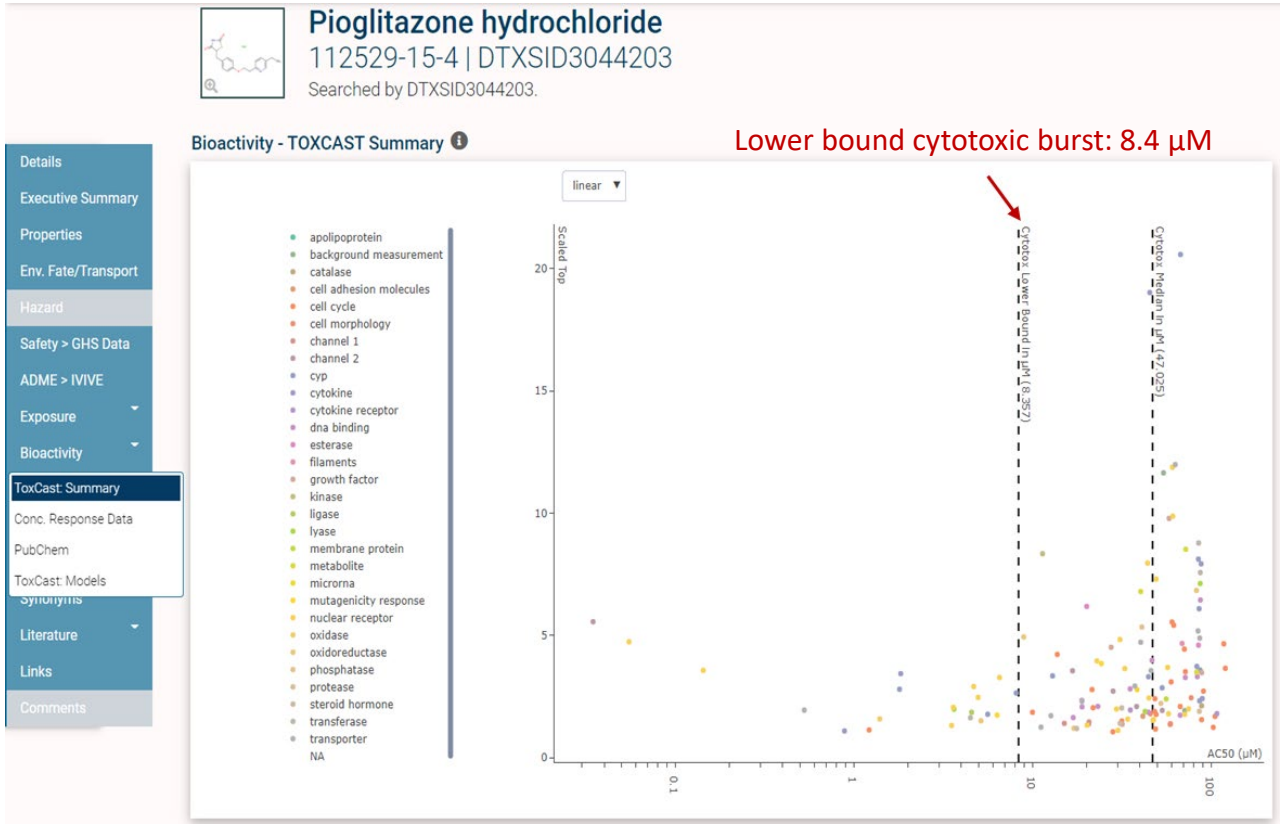
- The further below the cytotoxic concentration a response is observed, the more likely the response is reflective of a chemical-specific mode-of-action
  - Thus, target hits with ACC ≤ 0.333 of lower-bound cytotoxic burst were used for mechanistic inference

- First, evaluated three chemicals with clear mechanisms of action/toxicity to confirm viability of approach

- Next, used approach to parse molecular targets of interest for nonylphenol, pentachlorophenol, PFOA, and PFOS

Suitable Target Hits

Target hits with ACC ≤ 0.333 of cytotoxic burst were evaluated to probe potential molecular pathways of toxicity

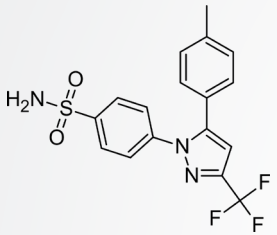


ToxCast target changes for pioglitazone at or below 2.8 µM:

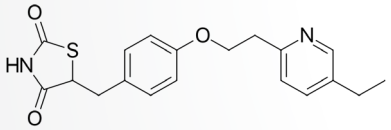
Chemical	Target	Full Name	Protein Accession	Assay ID	ACC (µM)
Pioglitazone	-	-	-	TOX21_PPARg_BLA_Agonist_ch2	0.00431
	PPARG	peroxisome proliferator-activated receptor gamma	NP_056953.2	TOX21_PPARg_BLA_Agonist_ratio	0.00951
	PPARG	peroxisome proliferator-activated receptor gamma	NP_056953.2	ATG_PPARg_TRANS_up	0.0444
	LPL	lipoprotein lipase	NP_000228.1	LTEA_HepaRG_LPL_up	0.315
	FABP1	fatty acid binding protein 1, liver	NP_001434.1	LTEA_HepaRG_FABP1_up	0.549
	PDK4	pyruvate dehydrogenase kinase, isozyme 4	NP_002603.1	LTEA_HepaRG_PDK4_up	0.552
	CYP4A11	cytochrome P450, family 4, subfamily A, polypeptide 11	NP_000769.2	LTEA_HepaRG_CYP4A11_up	0.656
	CYP4A22	cytochrome P450, family 4, subfamily A, polypeptide 22	NP_001010969.2	LTEA_HepaRG_CYP4A22_up	0.913
	CYP2C8	cytochrome P450, family 2, subfamily C, polypeptide 8	NP_000761.3	LTEA_HepaRG_CYP2C8_up	1.20
	PPARG	peroxisome proliferator-activated receptor gamma	NP_056953.2	NVS_NR_hPPARg	1.78
	Maob	monoamine oxidase B	NP_037330.1	NVS_ENZ_rMAOBC	2.11
	GADD45G	growth arrest and DNA-damage-inducible, gamma	NP_006696.1	LTEA_HepaRG_GADD45G_up	2.76

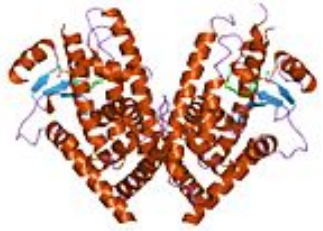
Putative Targets from ToxCast Data

Celecoxib
COX-2

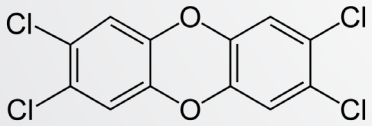


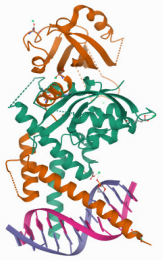
Pioglitazone
PPARγ



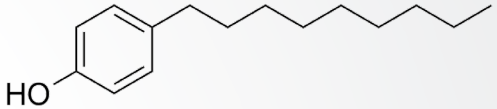


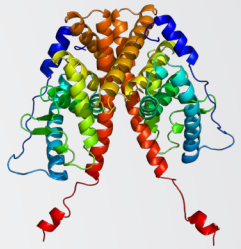
TCDD
AHR



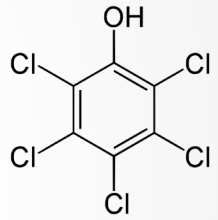


4-nonylphenol
ER

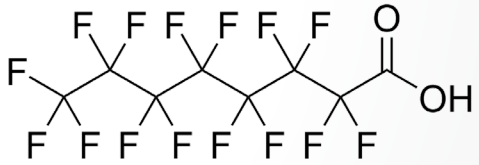


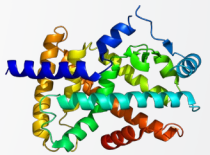


Pentachlorophenol
none identified

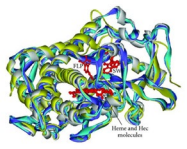


PFOA
PPARα
PPARγ



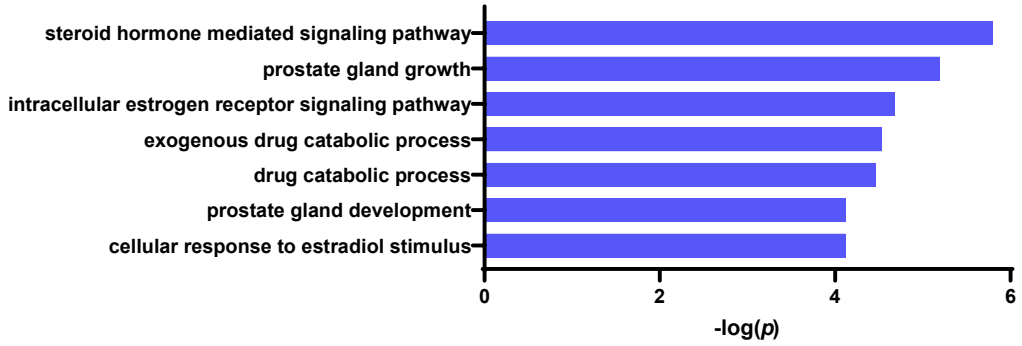


PFOS
CYP2C9
CYP2C11
CYP2C19

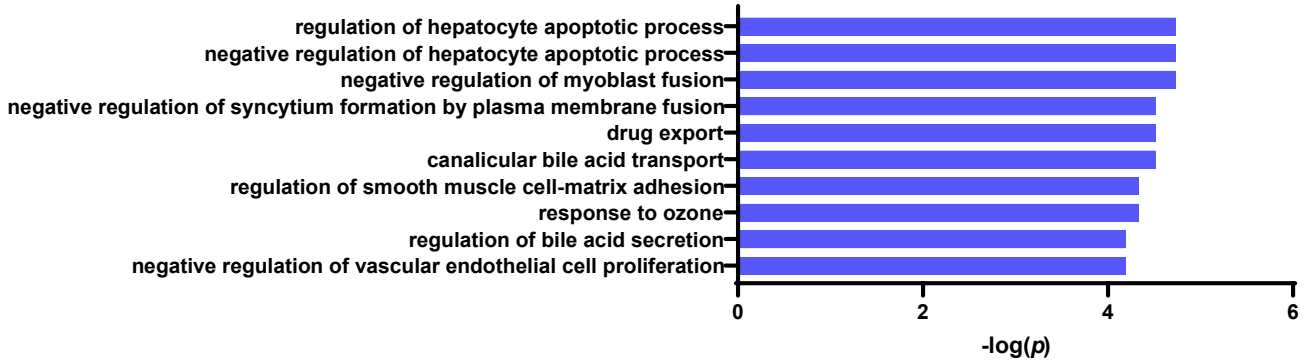


Gene Ontology Pathway Enrichment

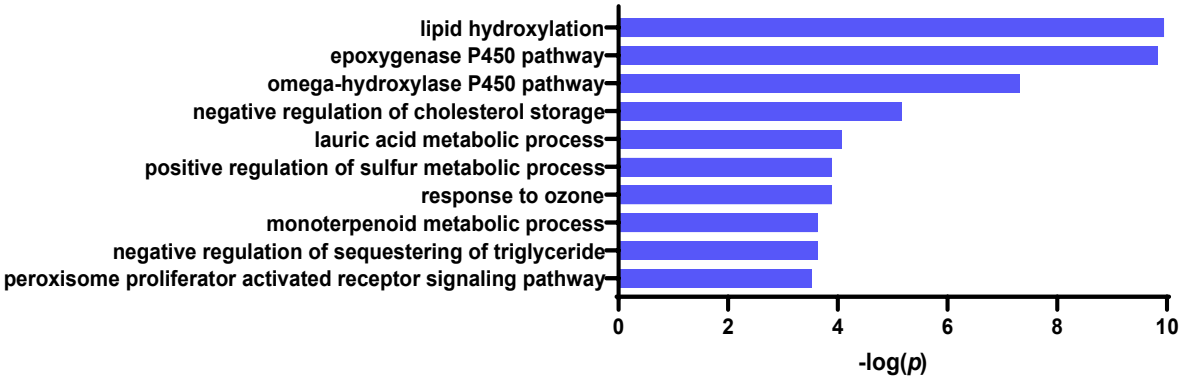
4-nonylphenol



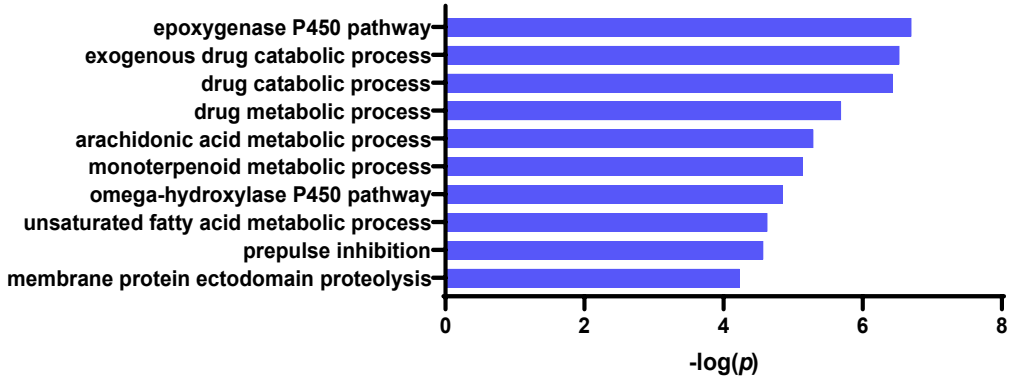
Pentachlorophenol



PFOA



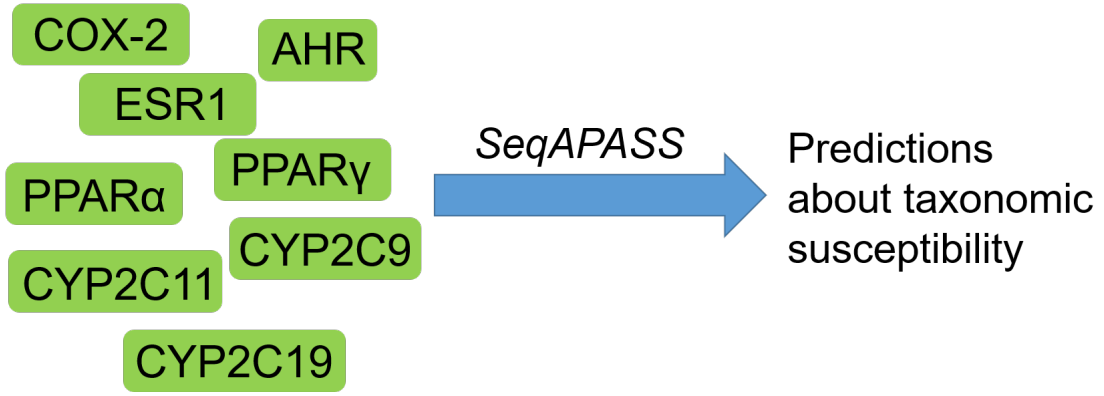
PFOS



Objective 3

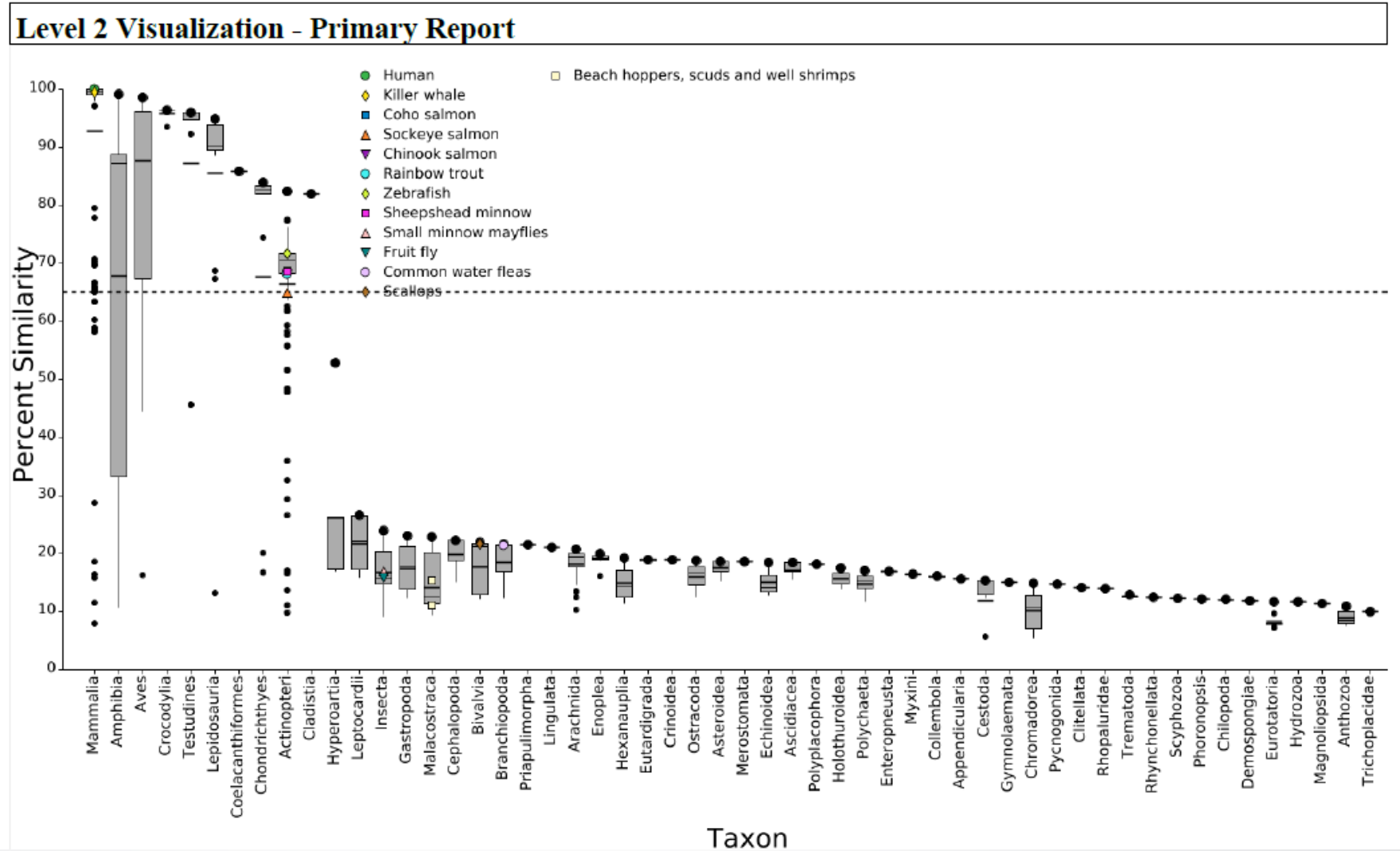
Define the taxonomic domain of applicability for putative mechanisms of toxicity

- Use EPA's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) to determine whether chemical-specific mechanisms of toxicity identified through our approach are applicable across taxa
- Analysis incorporated at least one representative species within each MDR grouping (phylogenetically different taxa) required by OW for ALC derivation
  - Can inform which species might drive assessment and help focus data generation/collection



Example SeqAPASS Evaluation: PPARγ

Sequence similarity in ligand binding domain (LBD)



Objective 3: Results

Taxonomic susceptibility predictions from SeqAPASS

Chemical	Target	Taxon					
		Mammalia	Actinopteri	Insecta	Bivalvia	Branchipodia	Malacostraca
Celecoxib	COX-2	●	●	○	●	●	●
Pioglitazone	PPARγ	●	◑	○	○	○	○
TCDD	AHR	●	●	○	○	○	○
4-nonylphenol	ESR1	●	●	○	○	○	○
Pentachlorophenol	-	-	-	-	-	-	-
PFOA	PPARα	●	●	○	○	○	○
	PPARγ	●	◑	○	○	○	○
PFOS	CYP2C9	●	◑	○	○	○	○
	CYP2C11	●	●	○	○	○	○
	CYP2C19	●	◑	○	○	○	○

Conclusions

- Overall, this work supports the use of NAM-based data to support/supplement formal ALC derivation, especially for data-poor chemicals
  - Accessible, scalable
- ToxCast-derived PODs generally aligned well with ALC/ESVs for chemicals examined without application of uncertainty or modifying factors
- NAM data can be used to infer prospective mechanisms of toxicity
  - Proof of principle with more targeted chemicals
  - Inferences about MOAs for industrial chemicals may be difficult given these compounds are generally not designed to exert effects on specific molecular pathways (e.g., pentachlorophenol)
- Use of bioinformatic tools such as SeqAPASS can be used as a line of evidence for predicting taxonomic susceptibility to environmentally-relevant chemicals
- Future directions include expanding analysis to larger set of compounds and incorporating data from other NAM approaches (e.g., high-throughput transcriptomics)

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