EPA United States Environmental Protection Agency

Weight-of-Evidence Prioritization of Organic Contaminants Detected in the Milwaukee Estuary Area of Concern (Milwaukee, WI)

Maloney EM, Ankley GT, Vitense K, Blackwell BR, Cavallin JE, Feifarek DJ, Jensen KM, Kahl KM, Poole ST, Randolph EC, and Villeneuve DL.

ECE21 2021

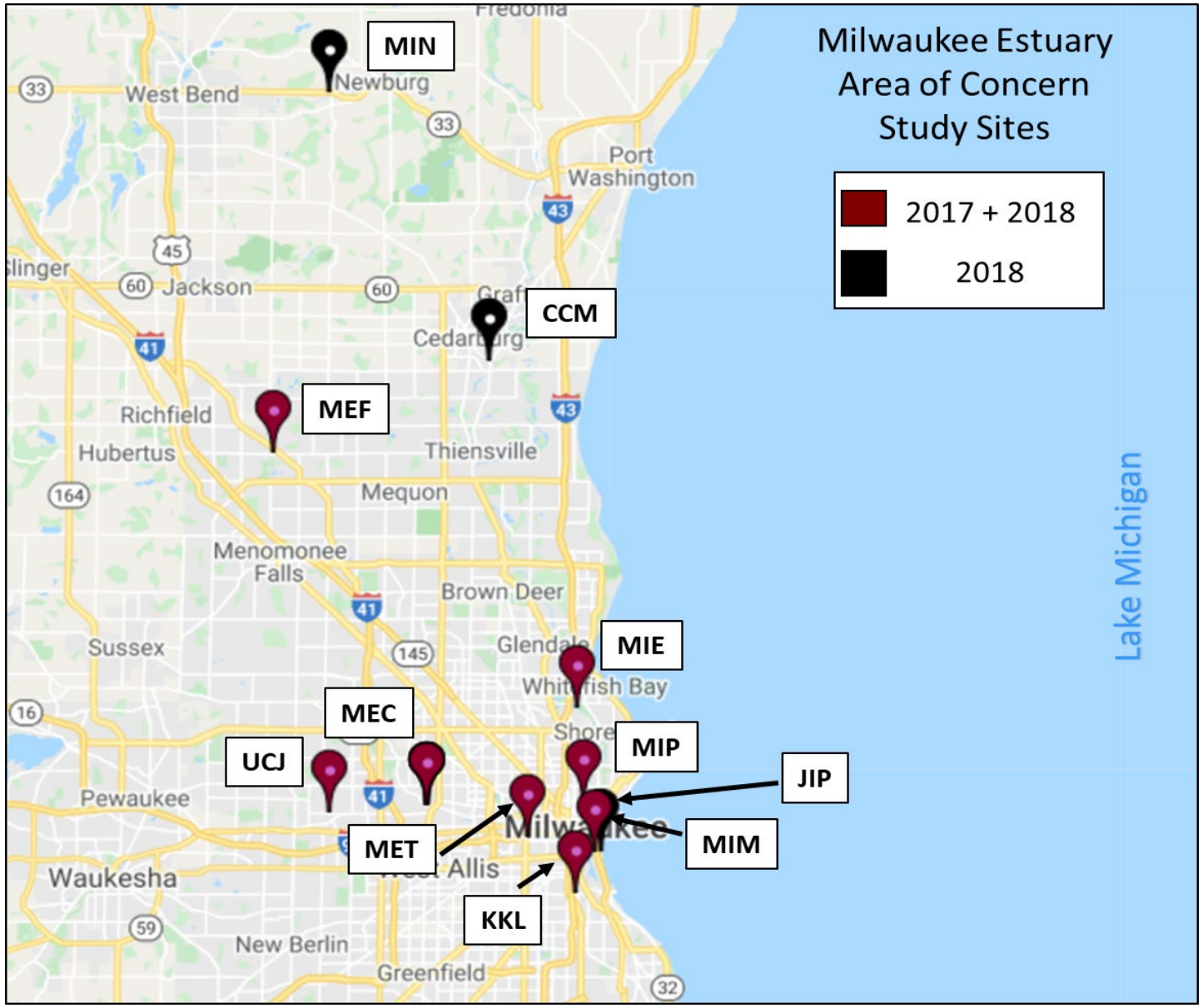
April 27 – 28, 2021

Background

- The Milwaukee Estuary watershed is at the confluence of Milwaukee, Menomonee, Kinnickinnic Rivers and Lake Michigan.
- Milwaukee Estuary Area of Concern (AOC) established in 1987.
- AOC receives discharges from wastewater treatment plants, industrial operations, combined sewer overflows, and agricultural and urban run-off.
  - **Aquatic biota likely subjected to repeated/continuous exposure to multiple environmental contaminants.**

Study Objective: Identify chemicals of potential high and low ecotoxicological concern within the Milwaukee Estuary AOC.

Study Sites



2017-18 Sites

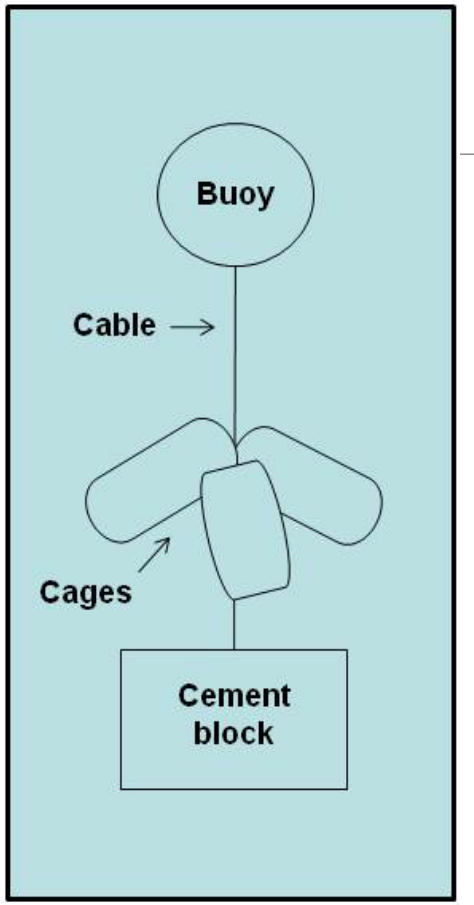
- Kinnickinnic River at Lincoln (KKL)
- Menomonee River at 25th St (MET)
- Menomonee River at Freistadt Road (MEF)
- Milwaukee at Estabrook (MIE)
- Milwaukee at Mouth (MIM)
- Milwaukee River at Walnut St. (MIP)
- Menomonee near Church St. at Wauwatosa (MEC)
- Underwood Creek at Juneau Blvd (UCJ)

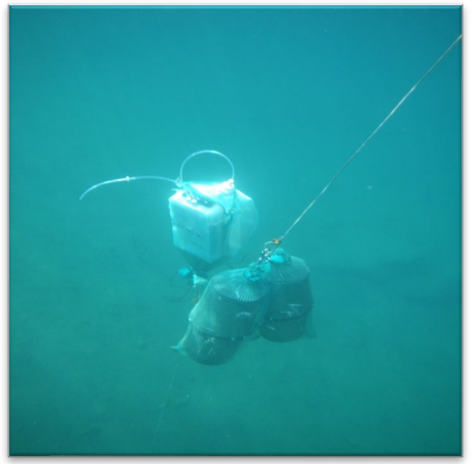
2018 (only) Sites

- Jones Island STP Plume (JIP)
- Cedar Creek at Green Bay Rd at Cedarburg (CCM)
- Milwaukee River at Cnty Trnk Hwy (MIN)

Experimental Methods

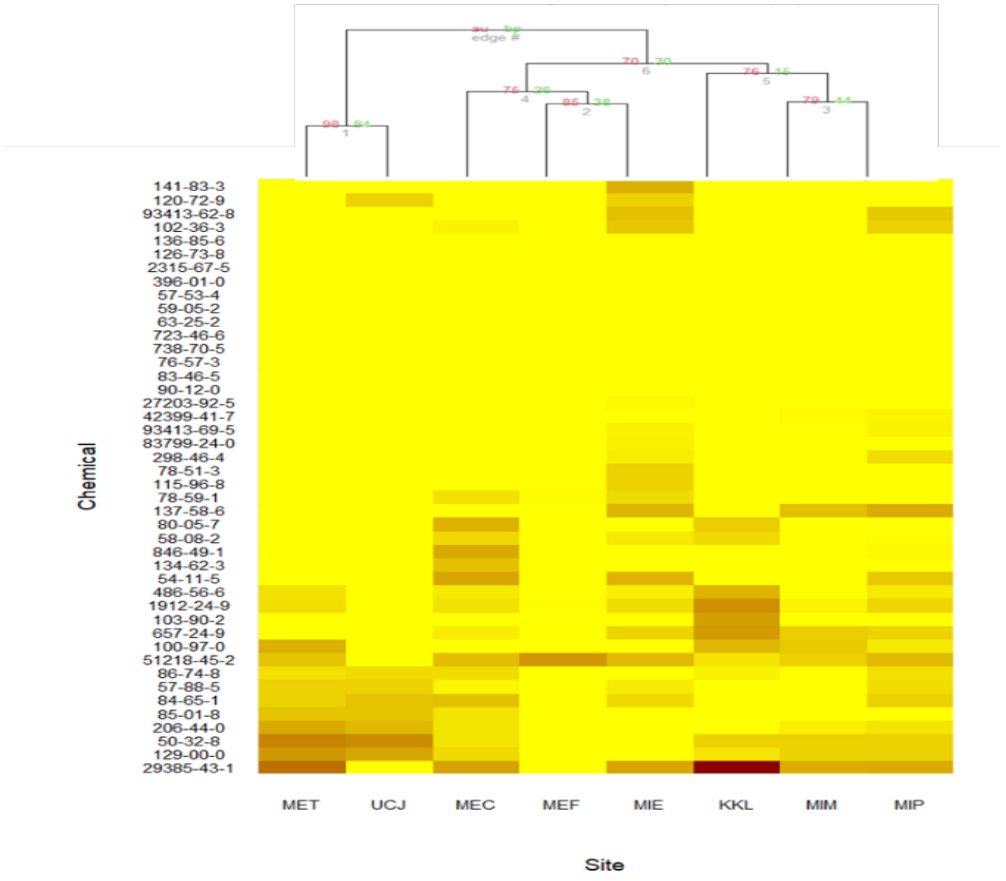
- Caged fish deployed at study sites for a 96-h exposure period.
  - Mucous (metabolomics)
  - Plasma (steroid hormones, vitellogenin)
  - Liver (gene expression, omics)
  - Intestine (gene expression – *2017 only*)
- Autosampler co-located to collect 96-h composite samples.
  - Chemistry (nutrients, wastewater indicators (69 analytes), pharmaceuticals (110 analytes)).
  - *In vitro* bioassays (Attagene™, T47-D KBluc, cell-based metabolomics).





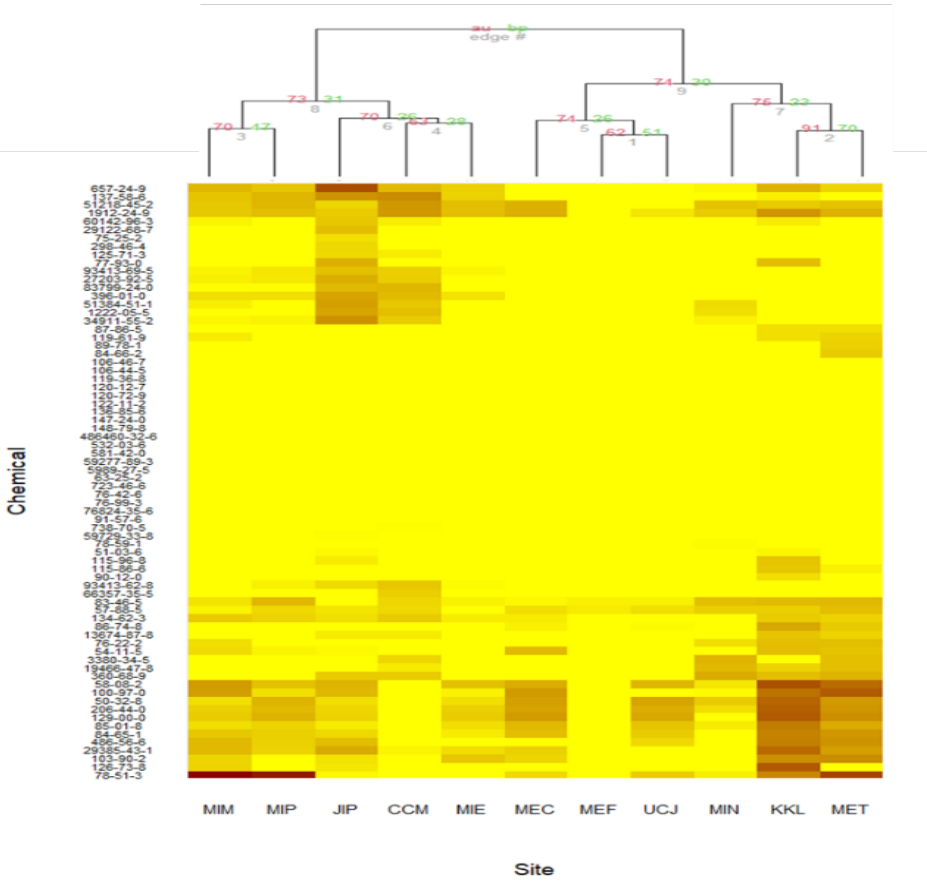
Detected Chemicals

2017: Year of the PAHs



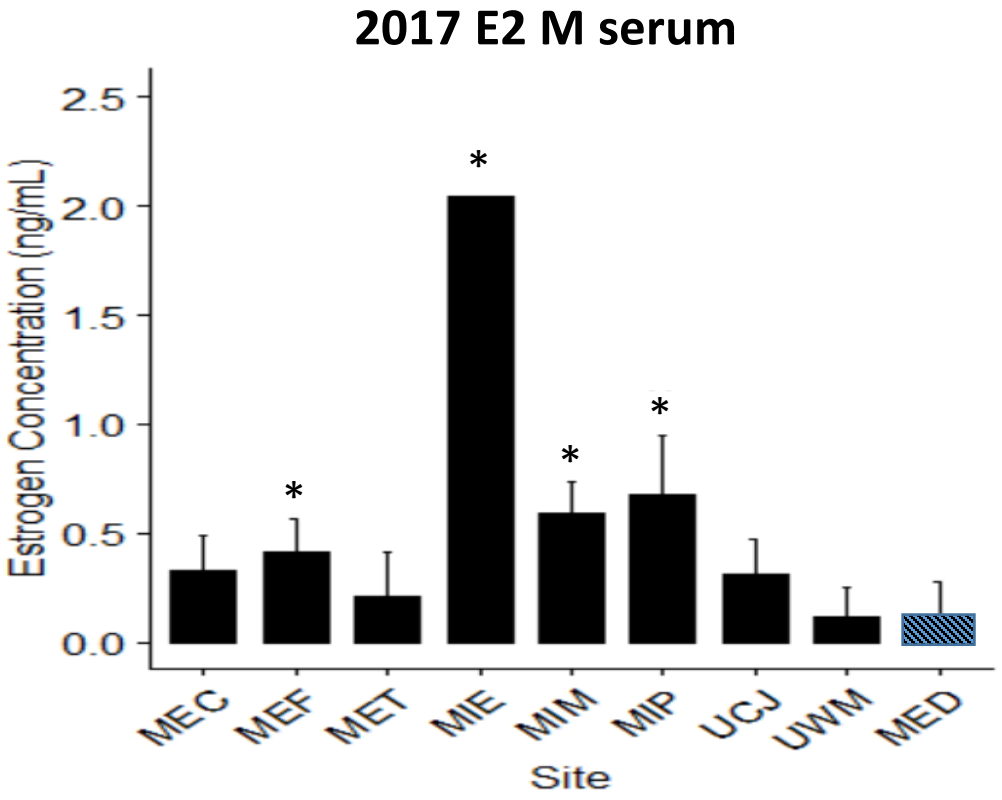
- 46/178 chemicals detected at ≥ 1 site(s)
- KKL, MIE, MIP = greatest contaminant loads.
  - **PAHs detected across all sites.**

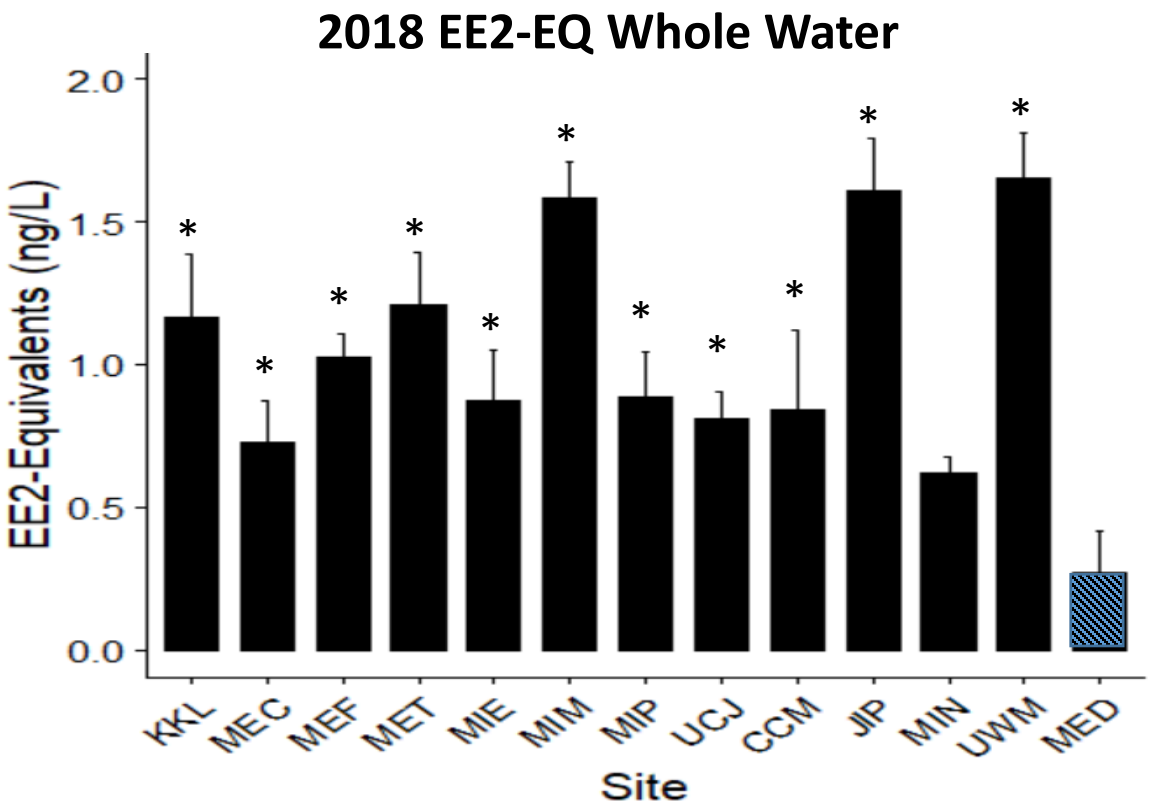
2018: Year of the Pharmaceuticals



- 77/178 chemicals detected at ≥ 1 site(s)
- **JIP, MIE, CCM = greatest pharmaceutical contaminant loads.**

Endocrine-Related Activity



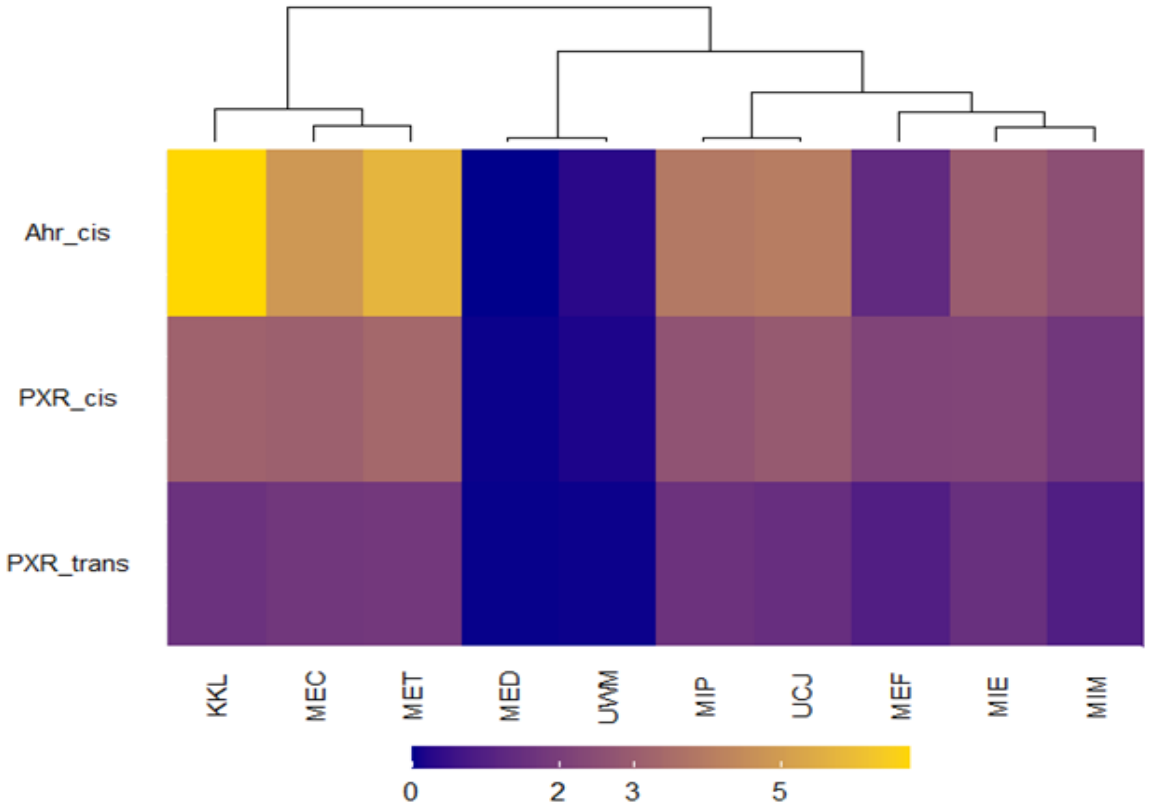


* Indicates statistical significance compared to GLTED laboratory control (MED, blue).

Elevated endocrine-related activity in 2017 serum samples (MEF, MIE, MIM, MIP); elevated endocrine-related activity at most study sites in 2018.

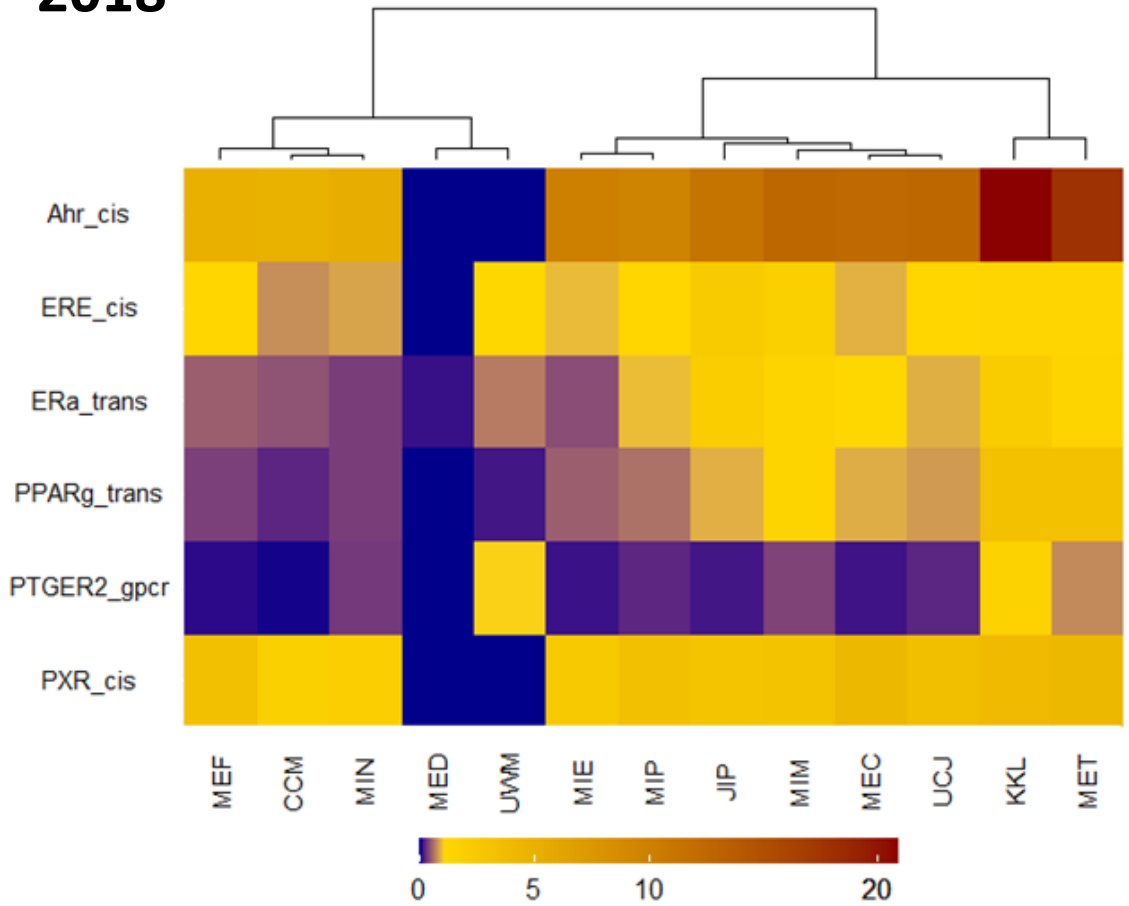
Bioactivity

2017



- AhR elevated at all sites but MEC & UWM.
- PXR_cis elevated at all sites but UWM.
- PXR_trans elevated at MEC, MET & UCJ.

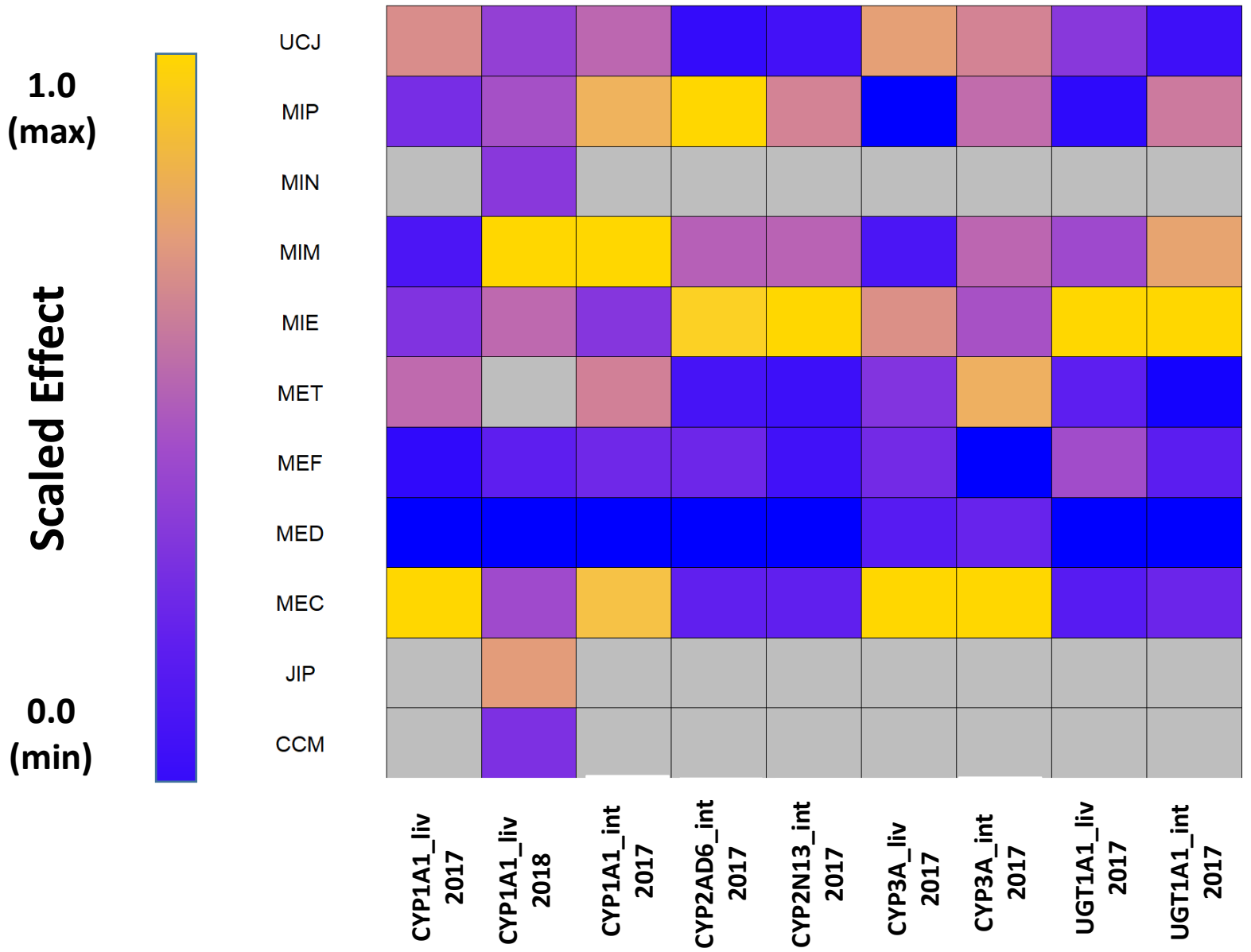
2018



- ERa elevated at KKL and JIP.
- ERE elevated at MIM and JIP.

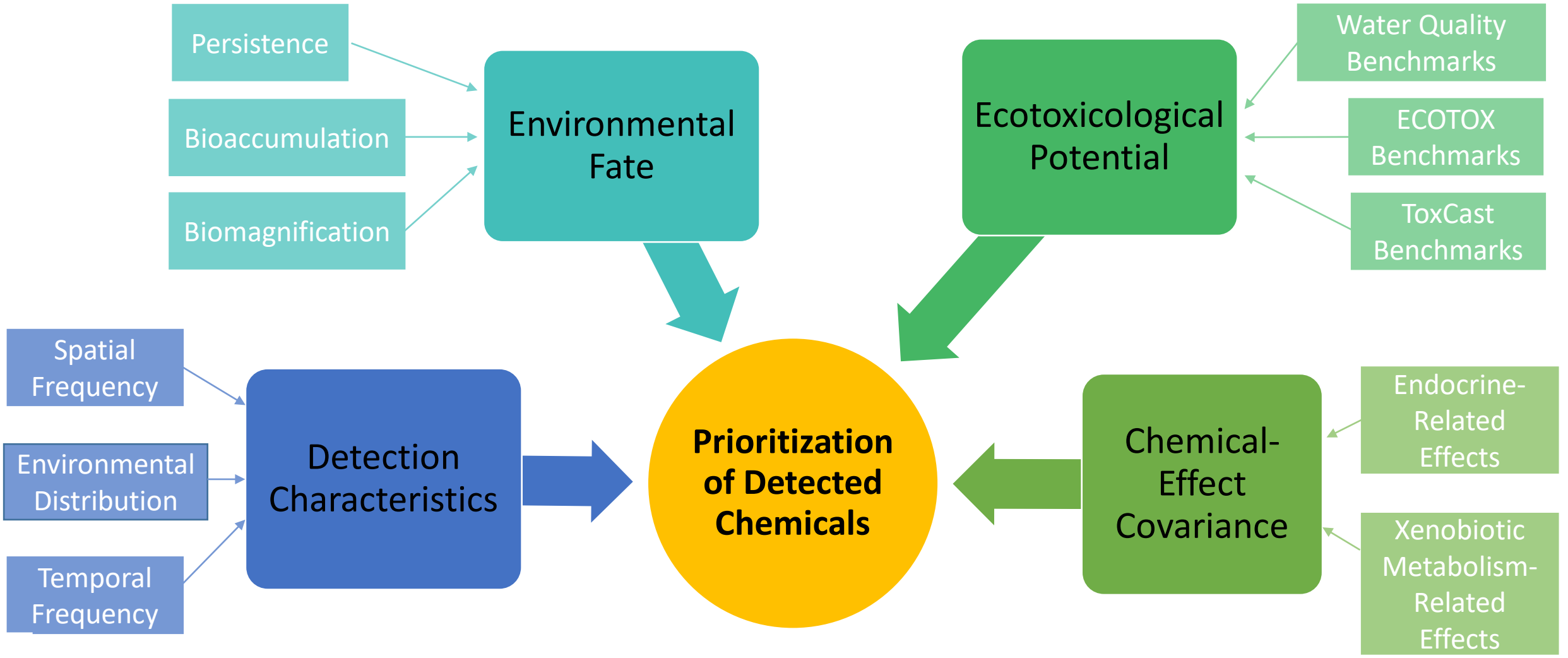
- AhR elevated at all sites but UWM.
- PXR elevated at all sites but UWM.
- PPARg elevated at MET.
- PTGER2 partially elevated at KKL.

Xenobiotic Metabolism Related Activity



- Elevated intestinal metabolic enzyme activity at 7/8 sites in 2017.
- Elevated hepatic metabolic enzyme activity at 3/8 sites in 2017.
- Elevated hepatic metabolic enzyme activity at 8/11 sites in 2018.
- **Overall xenobiotic-metabolism related effects elevated at MIE, MIM, MEC.**

Weight-of-Evidence Prioritization Framework



Chemical Prioritization

	Detection Characteristics			Environmental Fate			Ecotoxicological Potential (Benchmarks)						Chemical-Effect Covariance				
Chemical	*Spatial Frequency*	*Temporal Frequency*	*Environmental Distribution*	*Persistence*	*Bioaccumulation*	*Biomagnification*	*Water Quality*		*ECOTOX*		*ToxCast*		*Endocrine Related*	*Xenobiotic Related*	***CPS***	***PS_{chem} (%)***	**Priority Rank**
Highest Priority	10	10	10	10	10	10	10	10	5	5	2.5	2.5	2.5	2.5	100	**100**	**1**
	10	10	10	10	10	10							2.5	2.5	65	**100**	**1**
	10	10	0	10	10	10	5	5	5	5	2.5	2.5	2.5	2.5	80	**80**	**2**
	10	5	5	10	5	5	5	10	5	5	2.5	2.5	2.5	2.5	74	**74**	**3**
	10	5	5	10	5	5			5	5	2.5	2.5	2.5	2.5	59	**73.8**	**4**
	10	5	5	10	5	5	5	5	5	2.5	2.5	2.5	2.5	2.5	66.5	**66.5**	**5**
	5	5	5	5	5	5	5	5	2.5	2.5	1.25	1.25	1.25	1.25	60	**60**	**6**
	5	5	5	10	5	5			2.5	2.5			1.25	0	42	**56**	**7**
	5	5	5	5	5	5							2.5	2.5	35	**53.8**	**8**
	5	0	10	0	5	5	0	0	2.5	0	2.5	2.5	0	2.5	38	**38**	**9**
	5	5	5	0	0	0					0	0	0	2.5	18	**25.7**	**10**
	5	0	5	0	0	0	0	0	0	2.5	0	2.5	0	0	15	**15**	**11**
	0	5	5	0	0	0	0	0	0	0	0	0	1.25	1.25	12.5	**12.5**	**12**
	0	0	0	0	0	0	0	0	0	0	0	0	0	2.5	3	**3**	**13**
	0	0	0	0	0	0							0	0	0.5	**0.8**	**14**
Lowest Priority	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**15**

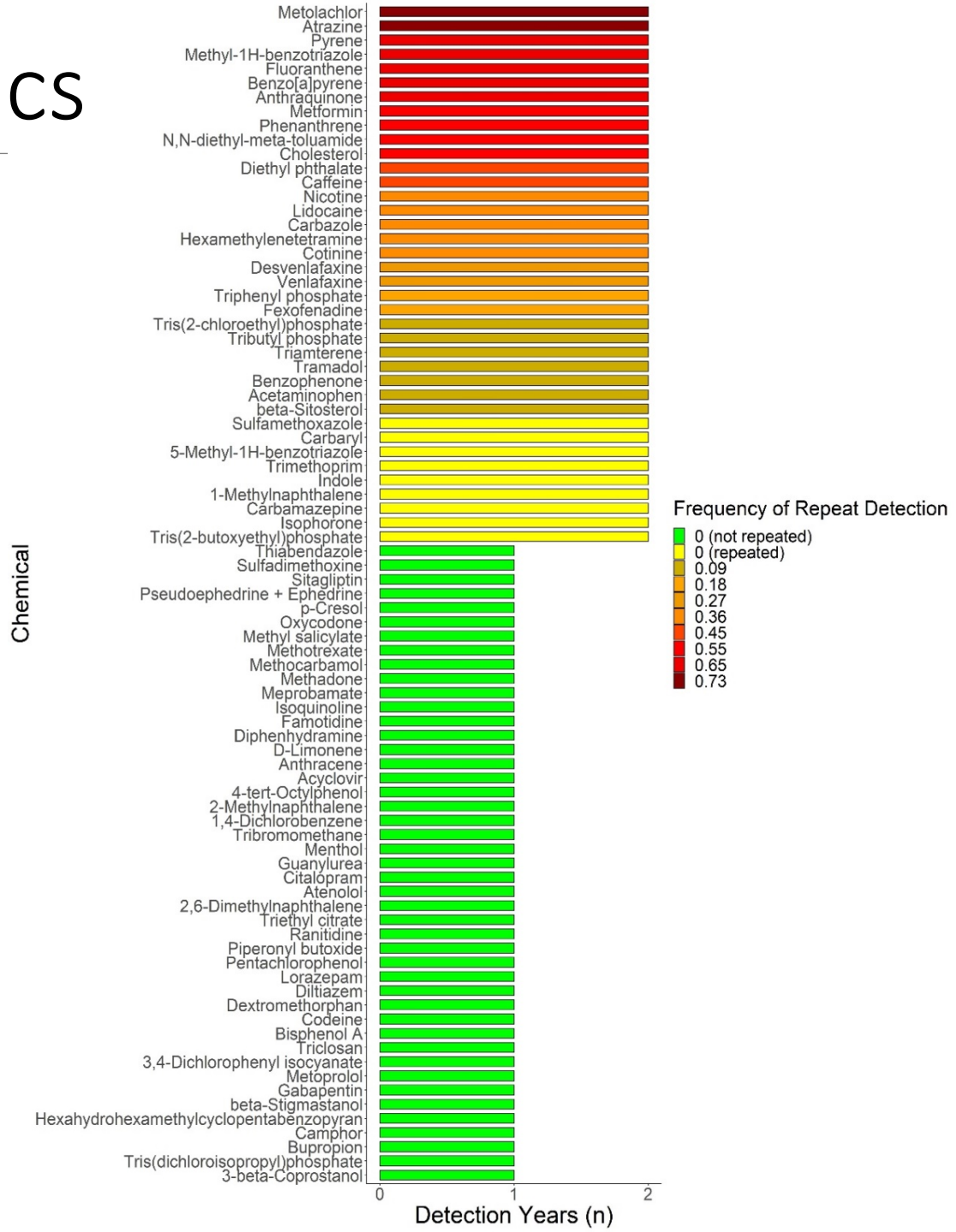
Prioritization Score: High | Medium | Low | Data Limited

$$PS_{chemical}\ (\%) = \frac{\Sigma PS_{detect} + \sum PS_{fate} + \sum PS_{benchmark} + \sum PS_{effect}}{CPS_{max}}\ x\ 100$$

Detection Characteristics

- **Spatial Frequency**: Detection across study sites.
- **Temporal Frequency:** Detection across study years.
- **Environmental Distribution:** Detection across compartments (tissue, sediment, water).

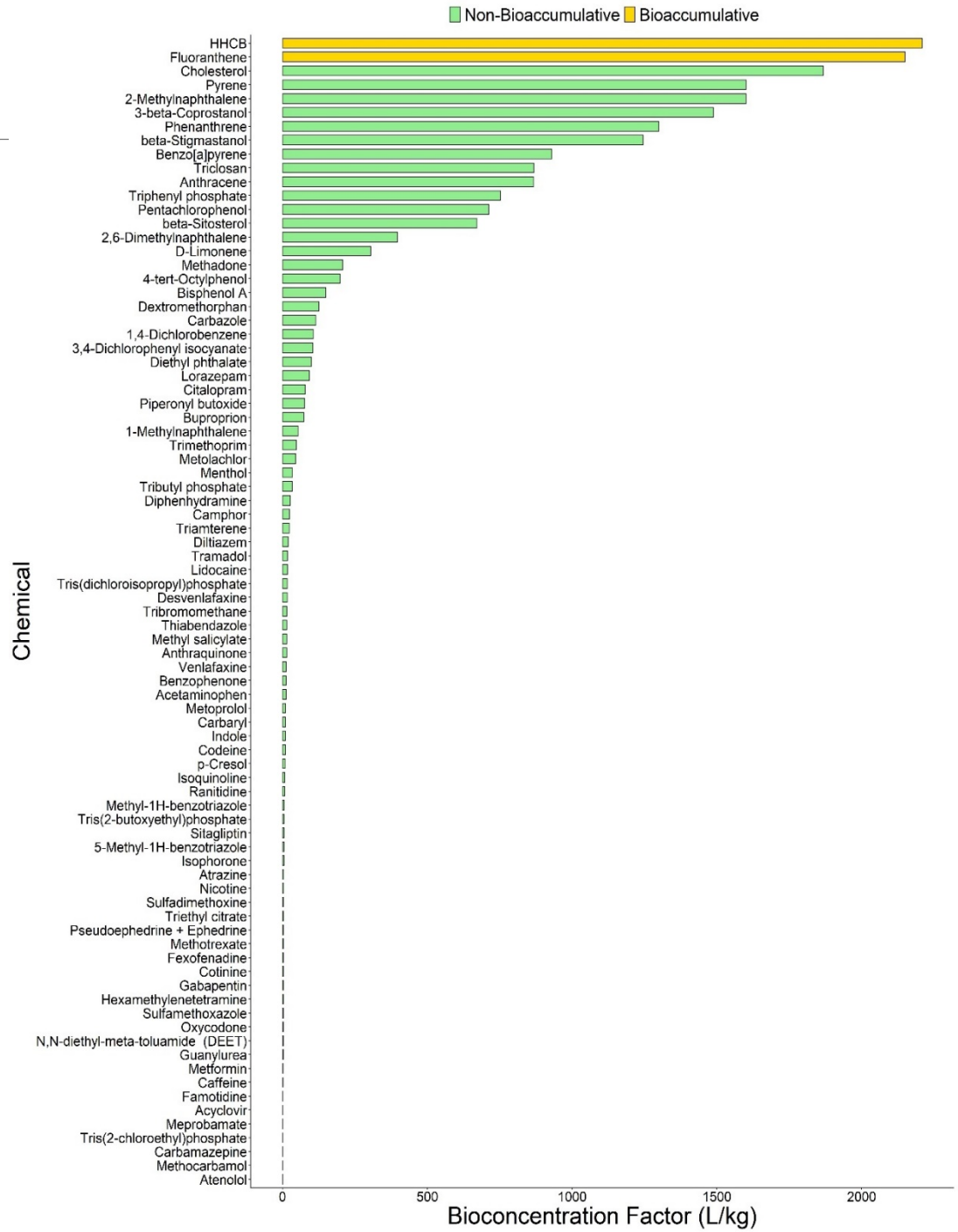
Prioritization Subcategory	Detection Characteristics	Score
Spatial Frequency	≥ 50% of sites.	10
	20 ≤ x < 50% of sites.	5
	< 20 % of sites.	0
Temporal Frequency	$n_{year} = 2, n_{unique\_sites} > 0$	10
	$n_{year} = 2, n_{unique\_sites} = 0$	5
	$n_{year} = 1$	0
Environmental Distribution	Tissues	10
	Sediment + Water	5
	Water or Sediment	0



Environmental Fate

- Characterized using QSAR estimates (EPISuite, OPERA, BMFpred) or experimental data.
- **Persistence** ($t_{1/2}$, d) and **bioaccumulation** (BCF, L/kg) evaluated using ECHA PBT/vPvB classifications.
- **Biomagnification** characterized as described by Gobas et al. (2009).

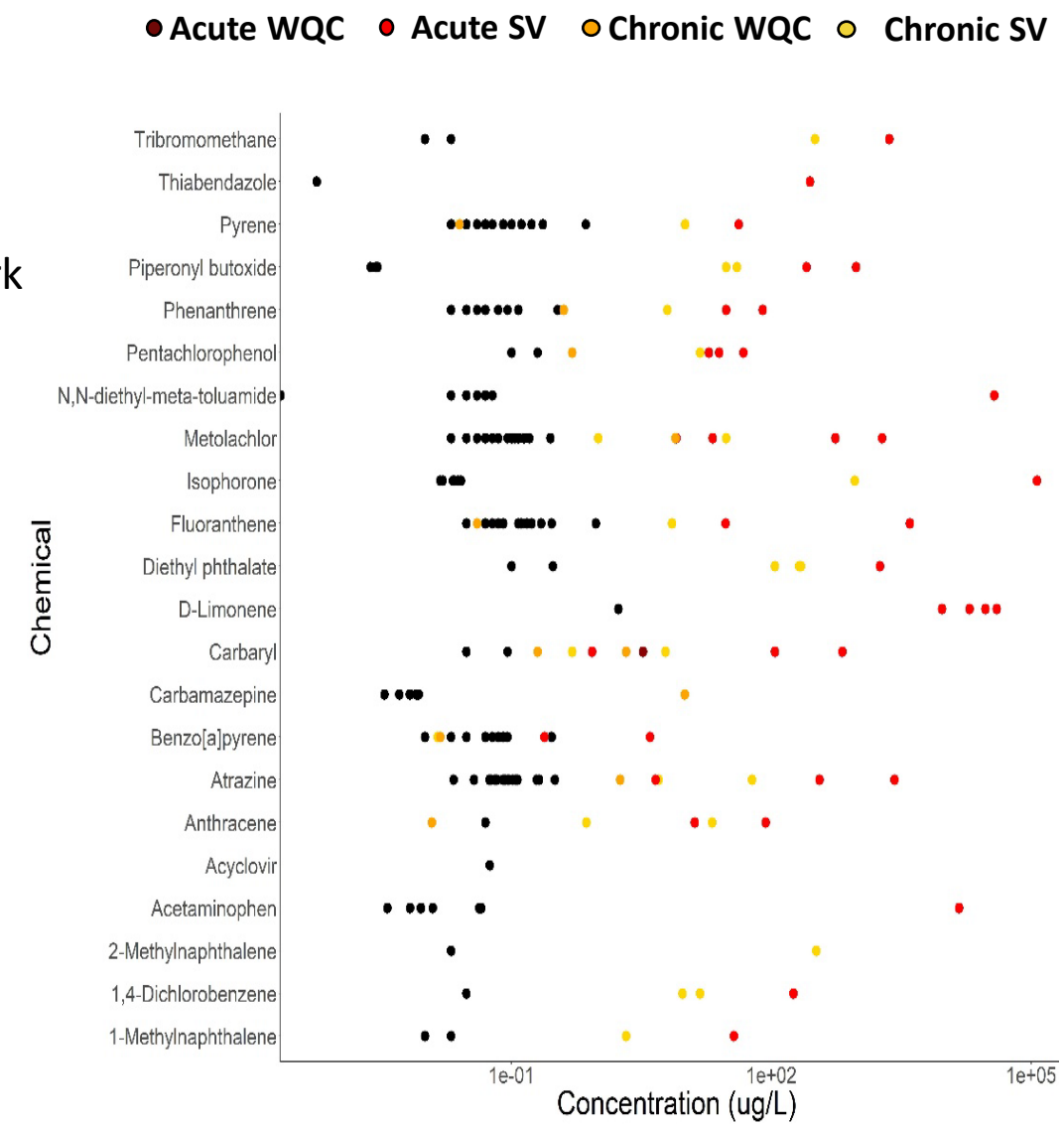
Prioritization Subcategory	Environmental Fate Characteristic	Score
Persistence	$t_{1/2,\ freshwater} < 40$ d	0
	$40 \leq t_{1/2,\ freshwater} < 60$ d	5
	$t_{1/2} \geq 60$ d	10
Bioaccumulation	BCF < 2000	0
	2000 ≤ BCF < 5000	5
	BCF ≥ 5000	10
Biomagnification	BMF < 1	0
	BMF > 1	10



Water Quality Benchmarks

- Compared detect concentrations to Canadian & US water quality benchmarks:
  - **Water Quality Criteria (WQC):**
    - CCME Water Quality Guidelines; USEPA Water Quality Criteria
  - **Screening Values (SV):**
    - Ecotoxicity thresholds (USEPA), aquatic life benchmark (USEPA), screening concentrations (USEPA, NOAA).

Prioritization Subcategory	Benchmark Exceedence Characteristics	Score
Exceedence Frequency	≥ 50% detects exceed benchmark.	10
	0 < x < 50 % detects exceed benchmark.	5
	No benchmark exceedence.	0
Benchmark Type	No available benchmark.	DL
	Acute water quality criteria.	10
	Acute screening value.	7.5
	Chronic water quality criteria.	5
	Chronic screening value.	2.5
	No benchmark exceedence.	0
	No available benchmark.	DL

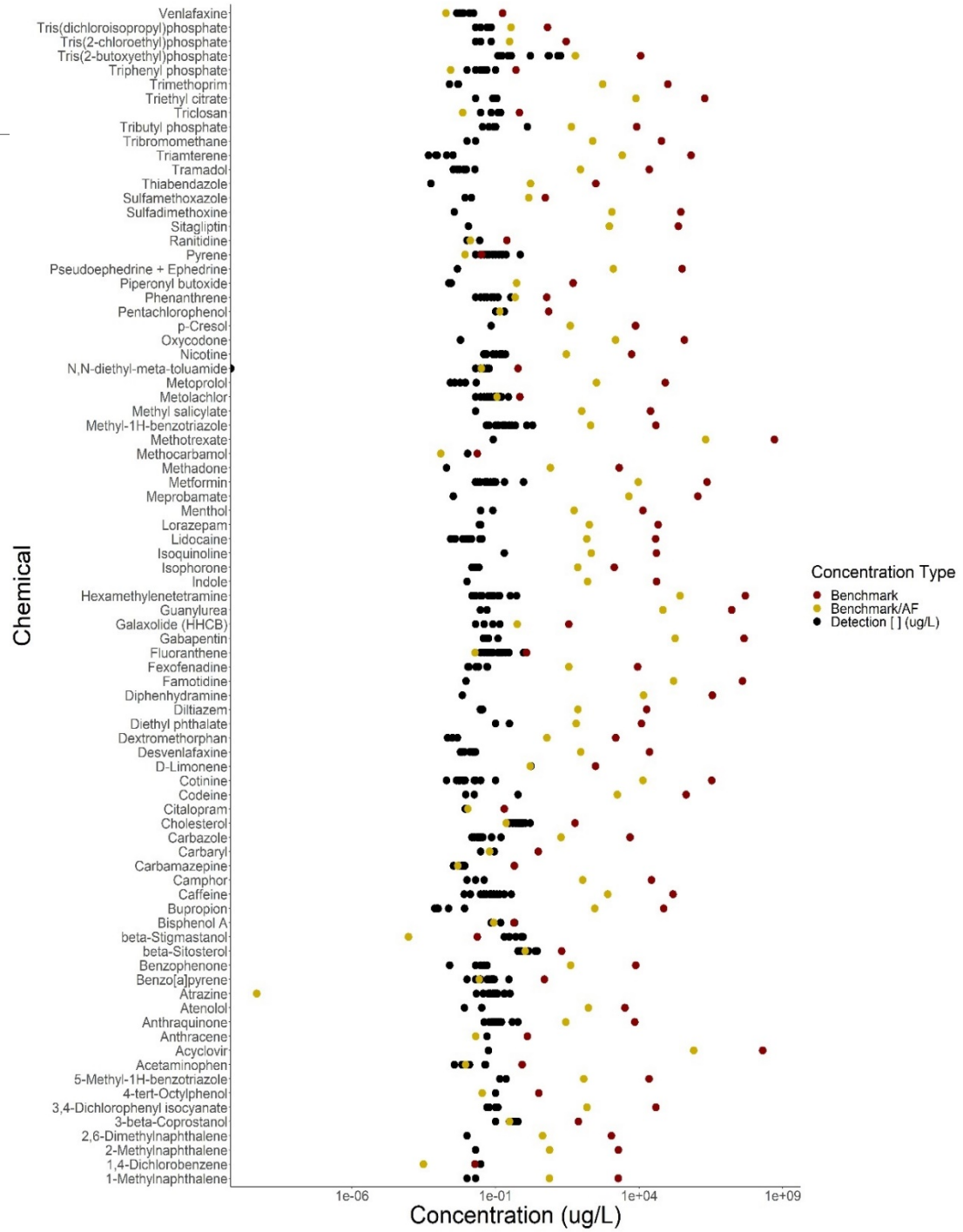


ECOTOX Benchmarks

- Compared detect concentrations to *in vivo* effect concentrations from the ECOTOX Knowledgebase.
- QSAR (TEST, ECOSAR) used to generate ecotoxicity estimates for data-limited compounds.
- ECOTOX Benchmark Types:
  - **Unadjusted:** minimum *in vivo* effect concentration.
  - **Uncertainty-adjusted:**

$$\frac{\min(Effect\ Concentration)}{AF_{endpoint}\ x\ AF_{length}\ x\ AF_{richness}}$$

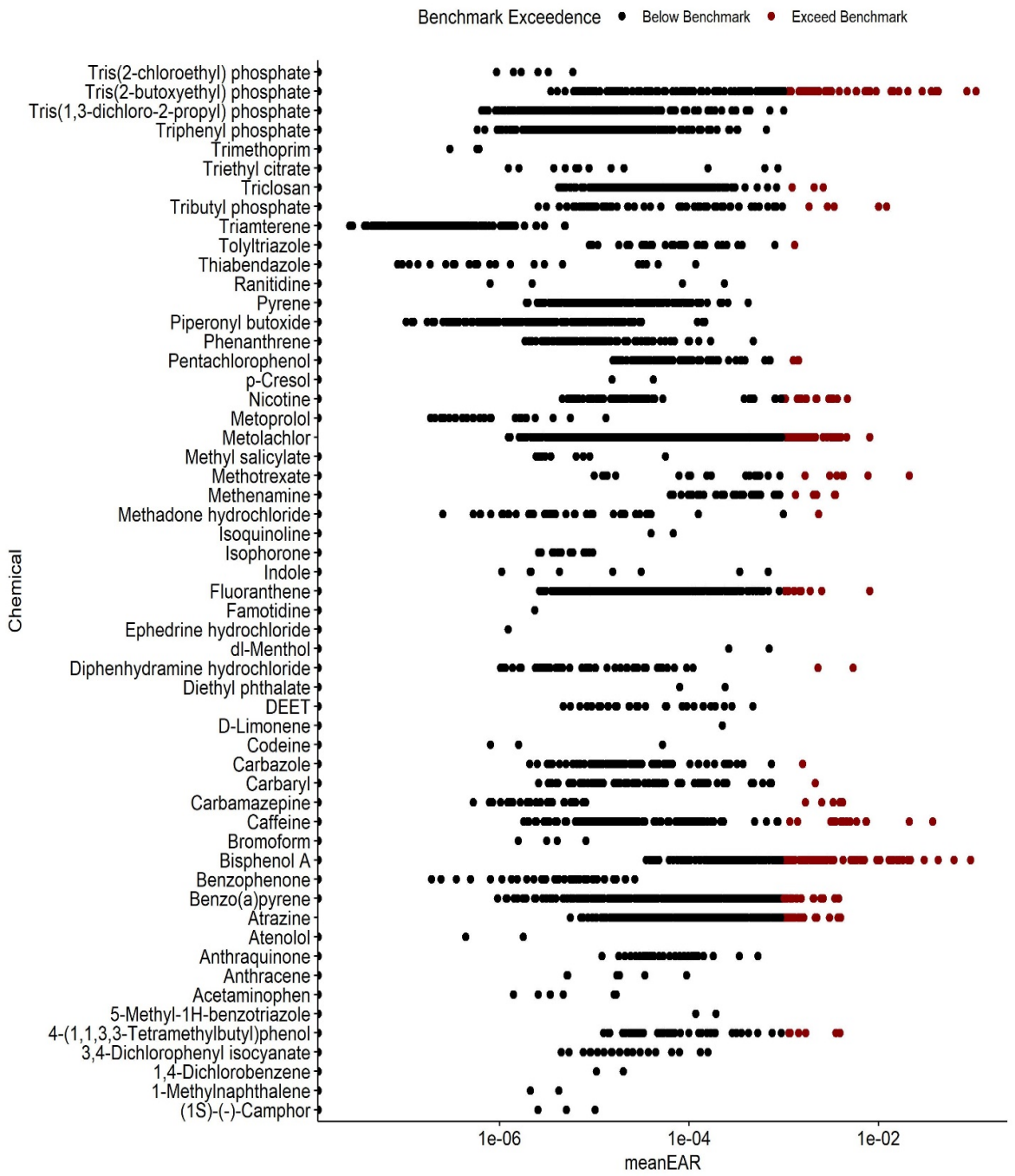
Prioritization Subcategory	Benchmark Exceedence Characteristics	Score
Exceedence Frequency	≥ 50% detects exceeded.	5
	0 < x < 50 % detects exceeded.	2.5
	No benchmark exceedence.	0
Benchmark Type	Unadjusted benchmark.	5
	Uncertainty- adjusted benchmark.	2.5
	No benchmark exceedence.	0
	QSAR-generated benchmark.	DL



ToxCast Benchmarks

- Compared detect concentrations to *in vitro* effect concentrations (ACC) derived from the ToxCast database.
- EAR threshold of 10^{-3} used as ToxCast benchmark cut-off for exceedence frequency.
- Number of chemical-assay combinations exceeding ToxCast benchmarks evaluated to characterize potential affected pathways.

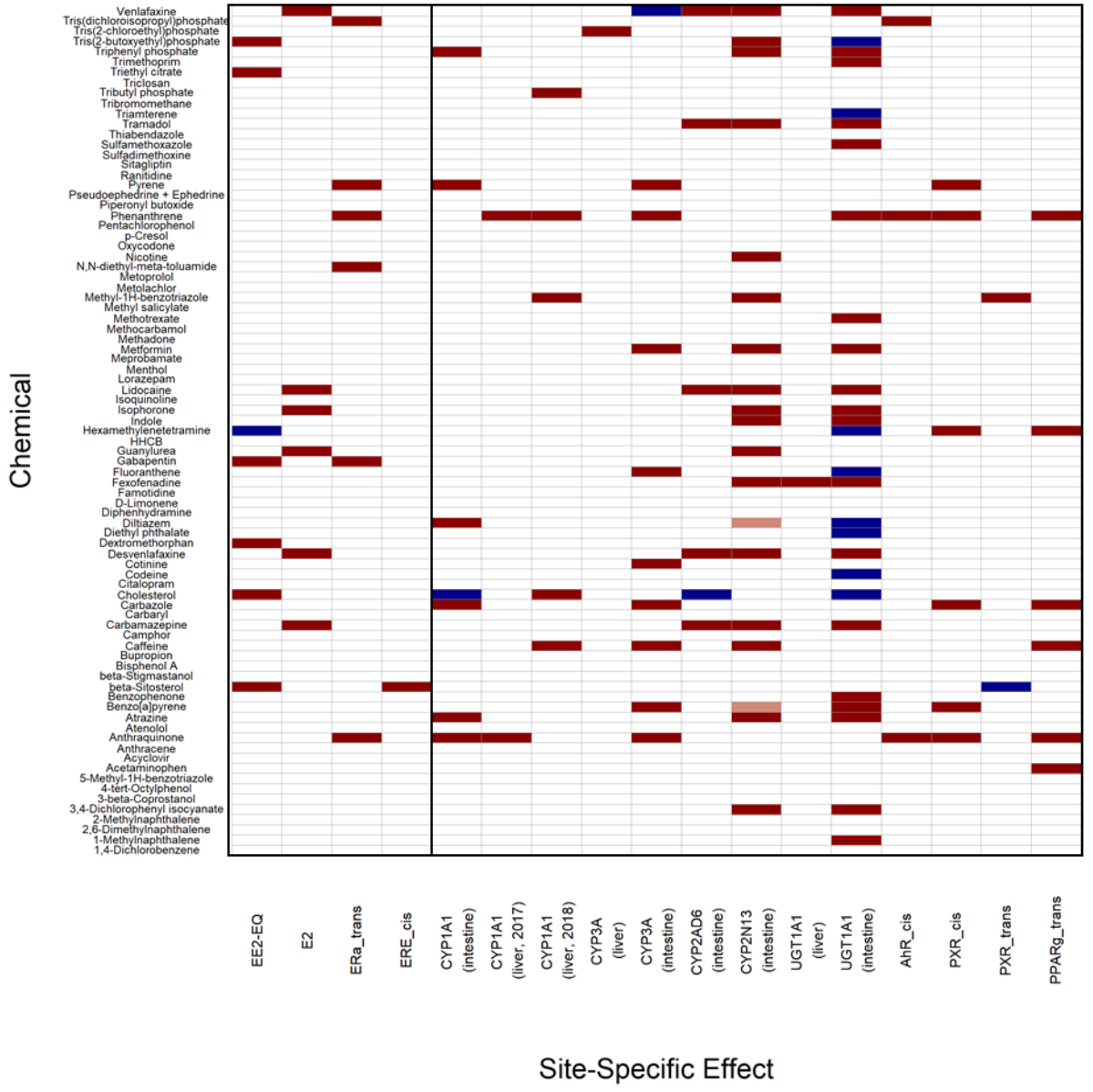
Prioritization Subcategory	Benchmark Exceedence Characteristics	Score
Exceedence Frequency	≥ 50% detect exceedence.	2.5
	0 < x < 50 % detect exceedence.	1.25
	No benchmark exceedence.	0
Assay Exceedence	Exceedence in ≥ 5 assays.	2.5
	Exceedence in 0 < x <5 assays.	1.25
	Did not exceed benchmark.	0
	No available ToxCast assays.	DL



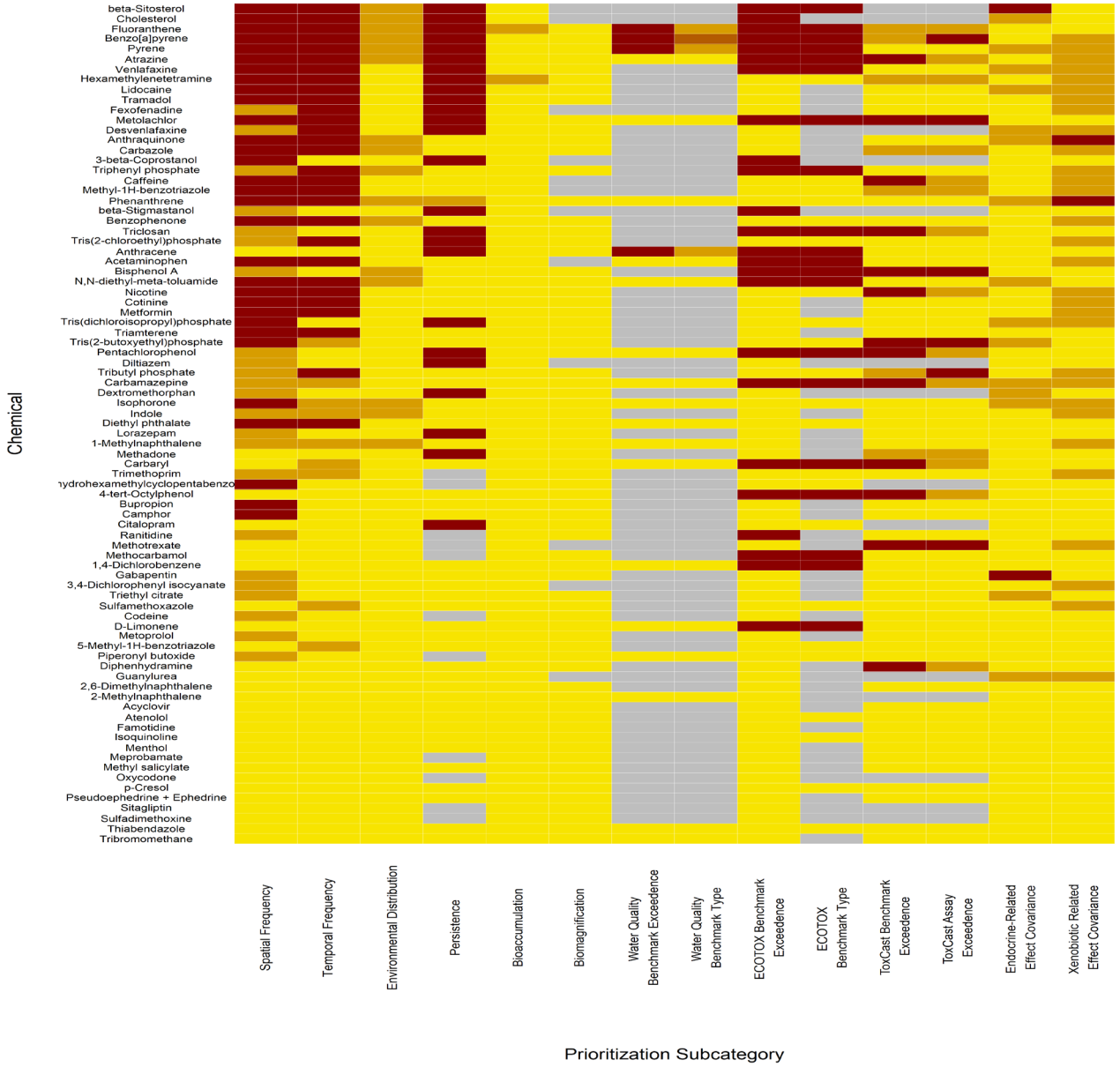
Effect Covariance

- Chemicals prioritized based on covariance with endocrine related and xenobiotic metabolism related effects.
- Random forest regression used to generate predictive models – 'important' chemical predictors identified for each effect.
- Weight-of-evidence covariance prioritization of chemicals across effect categories.

Prioritization Subcategory	Effect Covariance Characteristics	Score
Chemical-Effect Covariance [c]	Net-positive covariance in ≥ 50% of effect subgroups.	2.5
	Net-positive covariance in 1 < x < 50 % of effect subgroups.	1.25
	Insignificant/negative covariance across effect subgroups.	0



Milwaukee AOC Prioritization: Key Findings



Potential High Priority Compounds:

- β-sitosterol
- *Cholesterol*
- Fluoranthene
- Benzo[a]pyrene
- Pyrene
- Atrazine
- Venlafaxine

Potential Low Priority Compounds:

- 2,6-dimethylnaphthalene
- 2-methylnaphthalene
- Acyclovir
- Atenolol
- Famotidine
- Isoquinolone
- Menthol
- Meprobamate
- Methyl salicylate
- *Oxycodone*
- p-Cresol
- *Pseudo/ephedrine*
- Sitagliptin
- Sulfadimethoxine
- Thiabendazole
- Tribromomethane

Data-Limited Compounds:

- *3-β-coprostanol*
- *β-stigmastanol*
- *Desvenlafaxine*
- *Diltiazem*
- *Dextromethorphan*
- *Guanylurea*

Conclusion Highlights

- Prioritized of 83 chemicals detected in the Milwaukee Estuary AOC based on *detection characteristics*, *environmental fate* & *ecotoxicological potential*.
  - **6 High Priority Compounds** highlighted for point-source evaluation/regulatory consideration.
  - **14 Low Priority Compounds** highlighted that likely pose low risks to the Milwaukee Estuary AOC watershed.
  - **9 Data-Limited Compounds** that require further ecotoxicological/environmental fate investigation before they can be adequately prioritized.

Developed an alternative prioritization framework that can be employed or adapted to transparently prioritize contaminants within freshwater watersheds.

Thank you for your attention!

Questions or Comments?