Evaluation of Complex Mixture Toxicity: An Effects-Driven Analysis in the Milwaukee Estuary Area of Concern (Milwaukee, WI)

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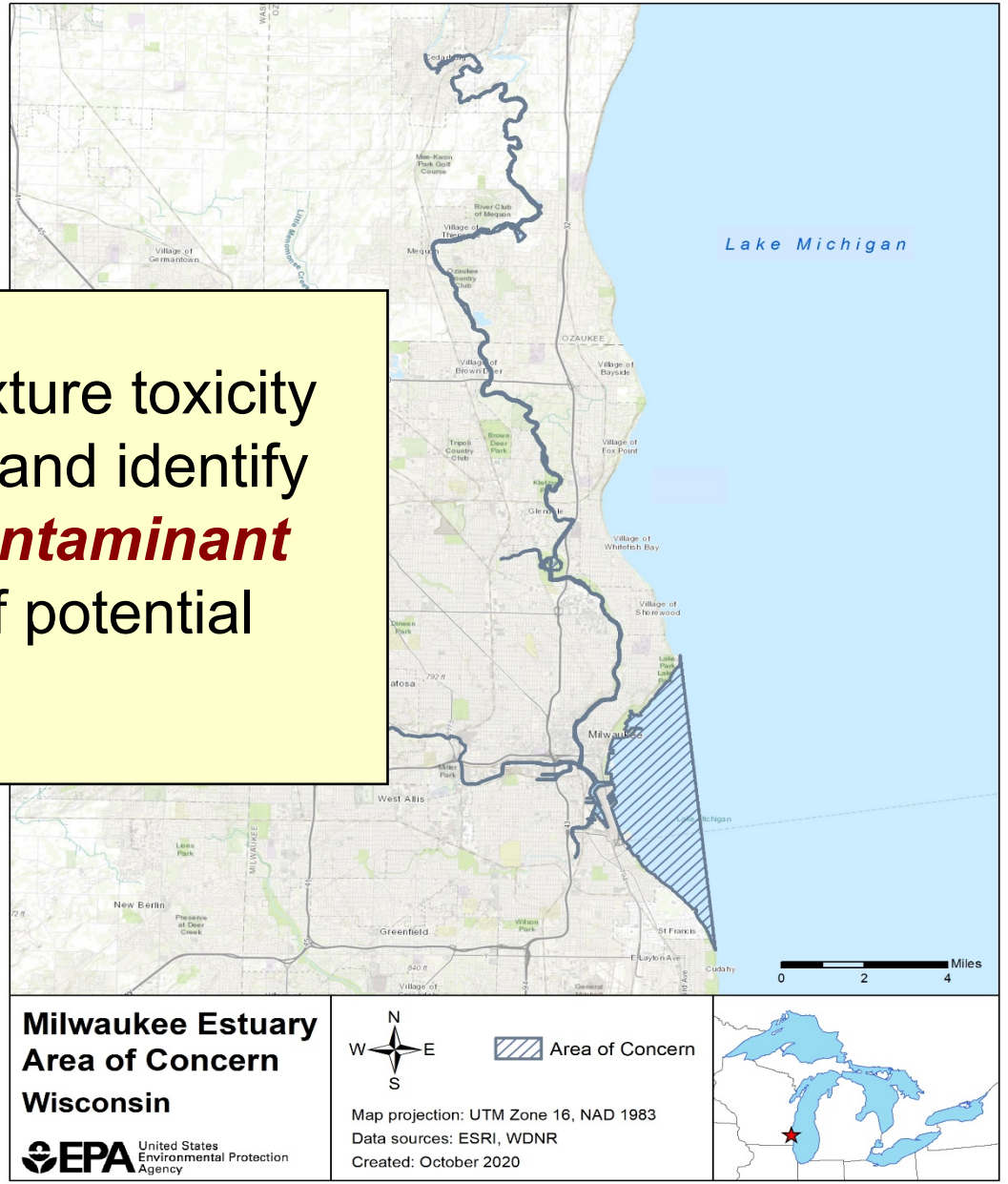
*Content does not necessarily reflect EPA position or policy.

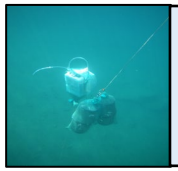
Background

- The **Milwaukee Estuary Area of Concern** is at the confluence of Milwaukee, Menomonee, Kinnickinnic Rivers and Lake Michigan (Milwaukee, WI).
- Anthropogenic a
  introduction of c
  through:
  - Historical and c
  - Wastewater tre
  - Combined sew
  - Agricultural an
- Aquatic biota inhabiting the AOC are exposed to **complex mixtures** of contaminants from varying chemical classes.

Aim: Evaluate complex mixture toxicity within the Milwaukee AOC and identify ***mixture constituents***, ***contaminant groups***, and ***mixtures*** of potential concern.

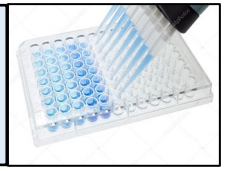


Complex Mixture Analysis



in vivo effects

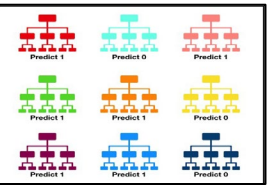
in vitro effects



in vivo effect concentrations *(ECOTOX Knowledgebase)*

Whole Mixture Effects

Endocrine-related Effects



toxEval

in vitro activities *(ToxCast Database)*

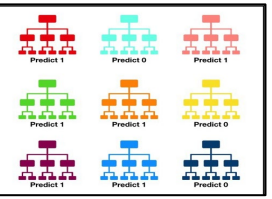
Constituent-Based Effects

Predictive Analysis

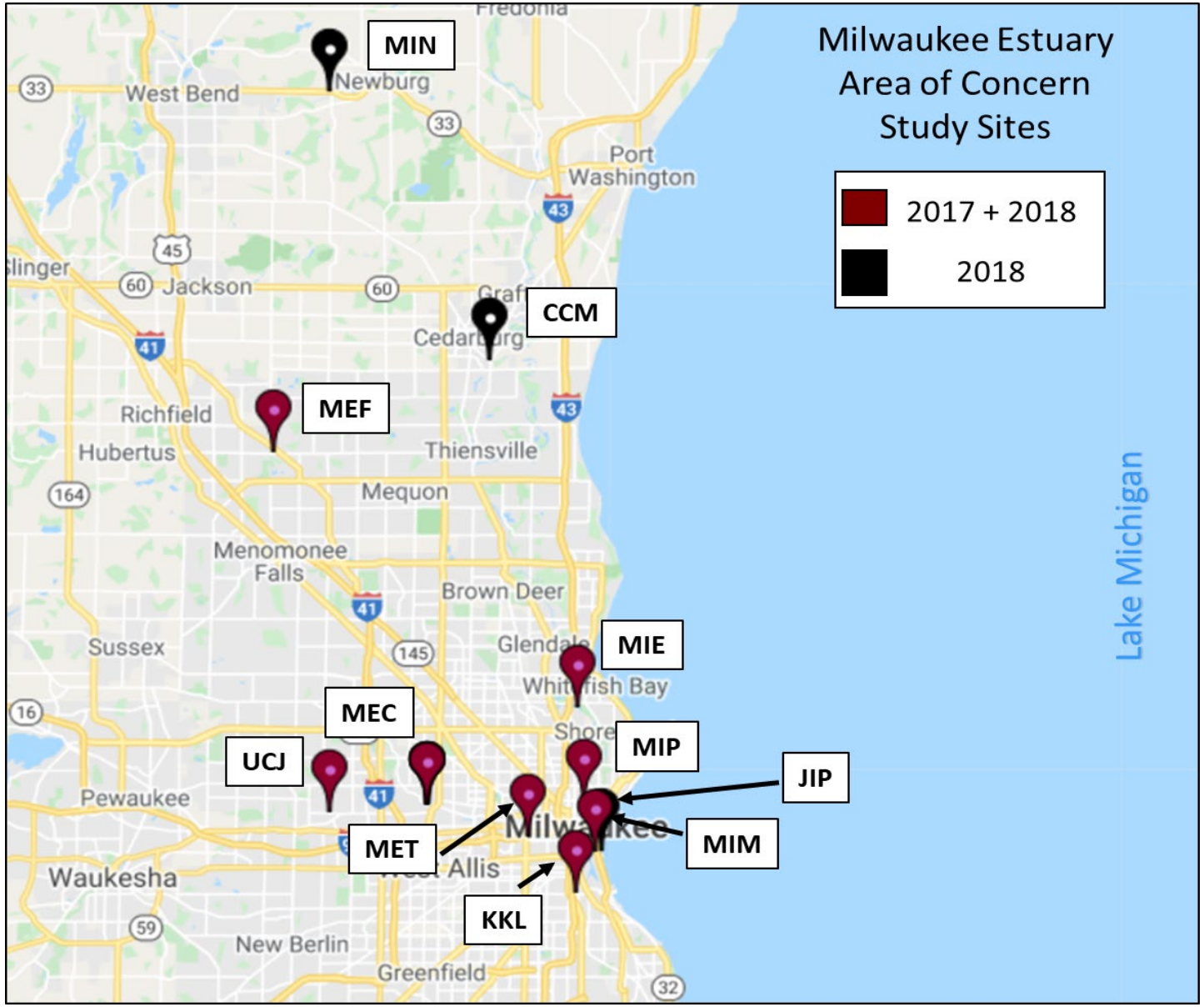
Mixture Assessment

Pharmaceutical potential *(DrugBank, MaPPFAST Database)*

Xenobiotic Metabolism-related Effects



Caged Fish 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

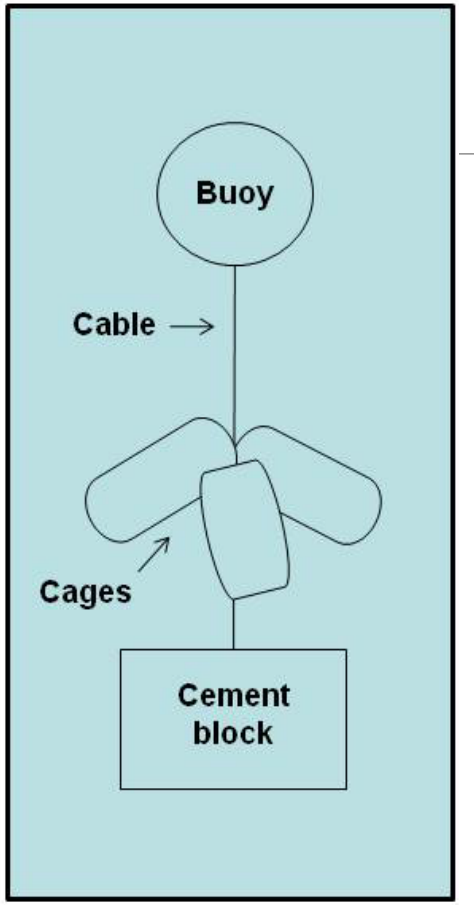
- Jones Island STP Plume (JIP)
- Cedar Creek at Green Bay Rd at Cedarburg (CCM)
- Milwaukee River at Cnty Trnk Hwy (MIN)

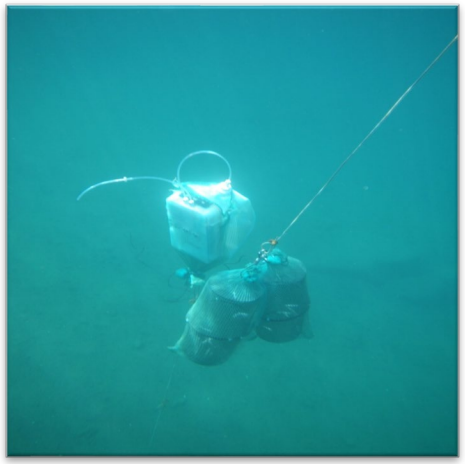
Control

- Great Lakes Ecotoxicology Division Laboratory (MED; Duluth, MN)

Caged Fish Studies

- Caged fathead minnows deployed for 96-h and ***in vivo*** effects measured:
  - Plasma (steroid hormones; E2, T)
  - Liver (gene expression; CYP1A1, CYP3A, UGT1A1)
  - Intestine (gene expression – *2017 only;* CYP1A1, CYP3A, CYP2N13, CYP2AD6, UGT1A1)
- Autosampler co-located to collect 96-h composite samples for:
  - Chemistry (nutrients, wastewater indicators (69 analytes), pharmaceuticals (110 analytes)).
  - *In vitro* bioassays (Attagene™, T47-D Kbluc (EE2-EQ)).



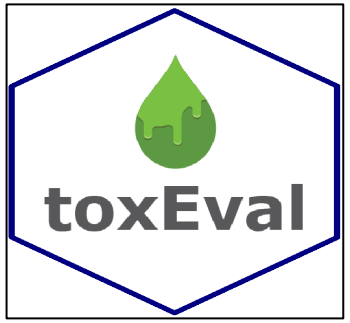


Constituent-Based Effects

in vivo Ecotoxicological Potential

Data: 96-h LC50 values for *P. promelas* derived from the ECOTOX Knowledgebase (US EPA, 2021).*
**Data-gaps filled using read-across, interspecies-extrapolation (WEB-ICE; US EPA, 2016), and quantitative structure-activity relationship (QSAR) acute toxicity estimates (VEGA, TEST, ECOSAR).*
Groupin QSAR estimated mechanism of action (Kienzler et al., 2016), structural similarities (ToxPrint fingerprin

in vitro Ecotoxicologic



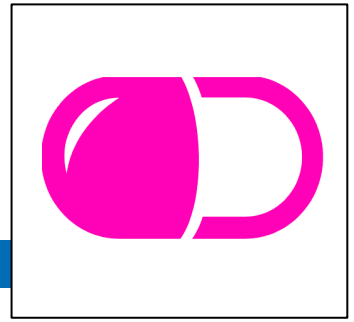
Data: Mi -assay combinations in the ToxC
Groupin ing toxEval (De Cicco et al., 2020)

Constituent- and group effects within mixtures evaluated using **Maximum Cumulative Ratios (MCR)**:

$$MCR_i = \frac{TQ_i}{TQ_{mixture}}$$

Chemicals/groups with MCR > 0.1 flagged as potentially important contributors to overall mixture effect.

Pharmaceutical Potential:

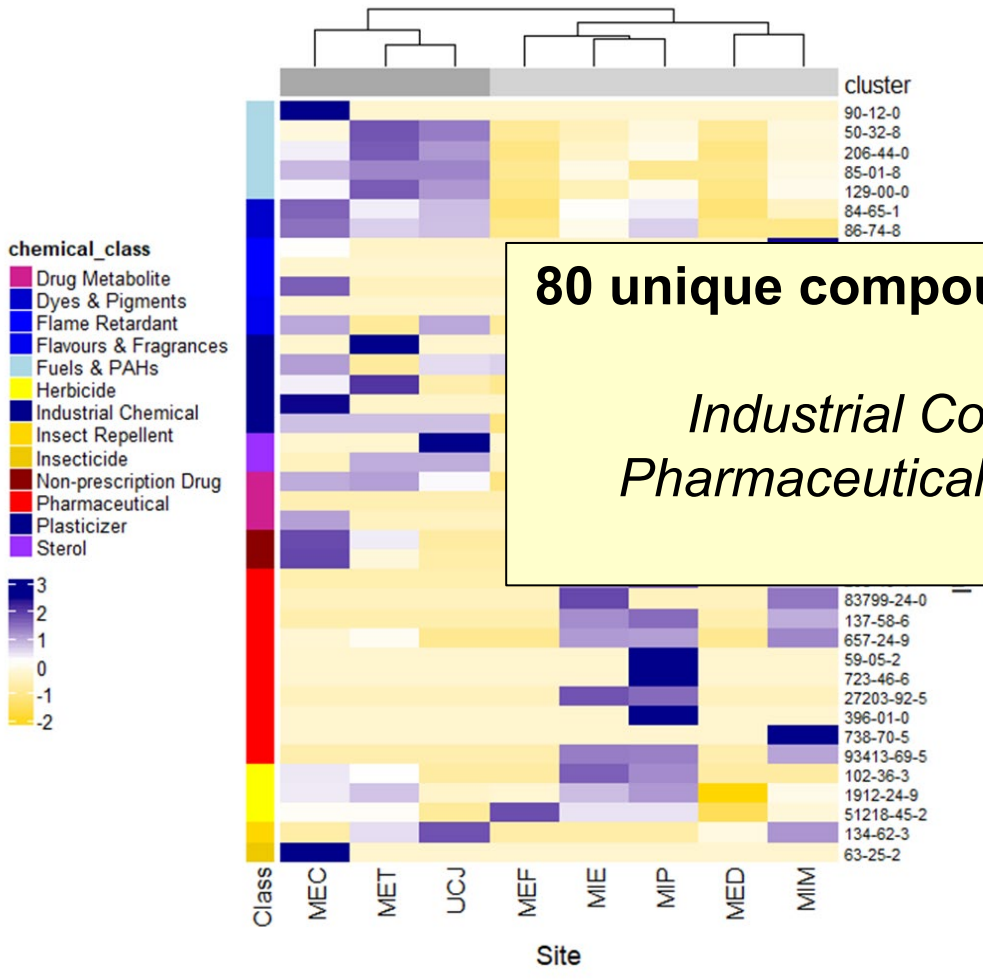


Data: Peak serum concentrations following dosing (Cmax) derived from the MaPPFAST database (Berninger et al., 2016).

Grouping: Based on pharmaceutical target and direction, derived from the DrugBank database, grouped based on DAVID GO ontologies (OMx Personal Health Analytics, Inc. 2021; Jiao et al., 2012).*
**Presence of targets in fathead minnows confirmed using SeqAPASS (v. 6.0; US EPA, 2021).*

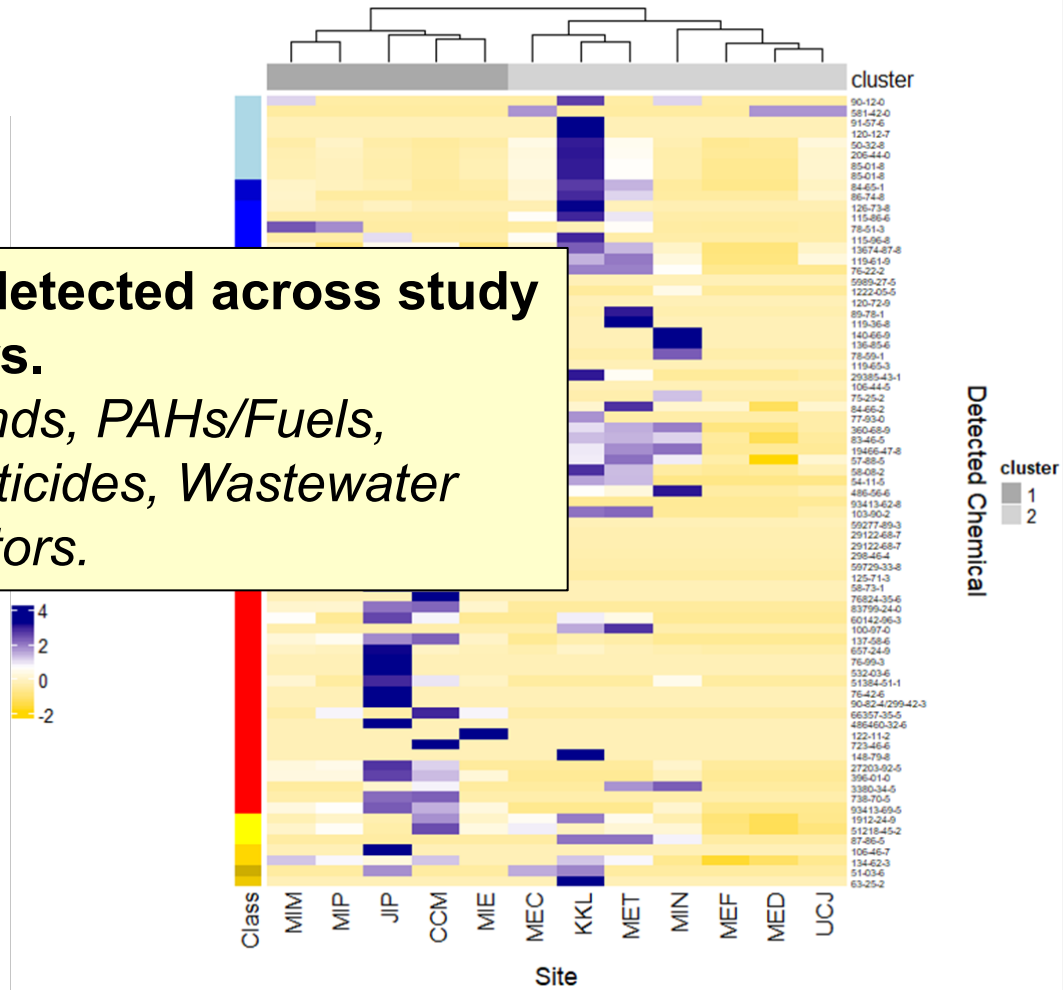
Detected Chemicals

2017



- **41**/179 chemicals detected at ≥ 1 site(s)

2018

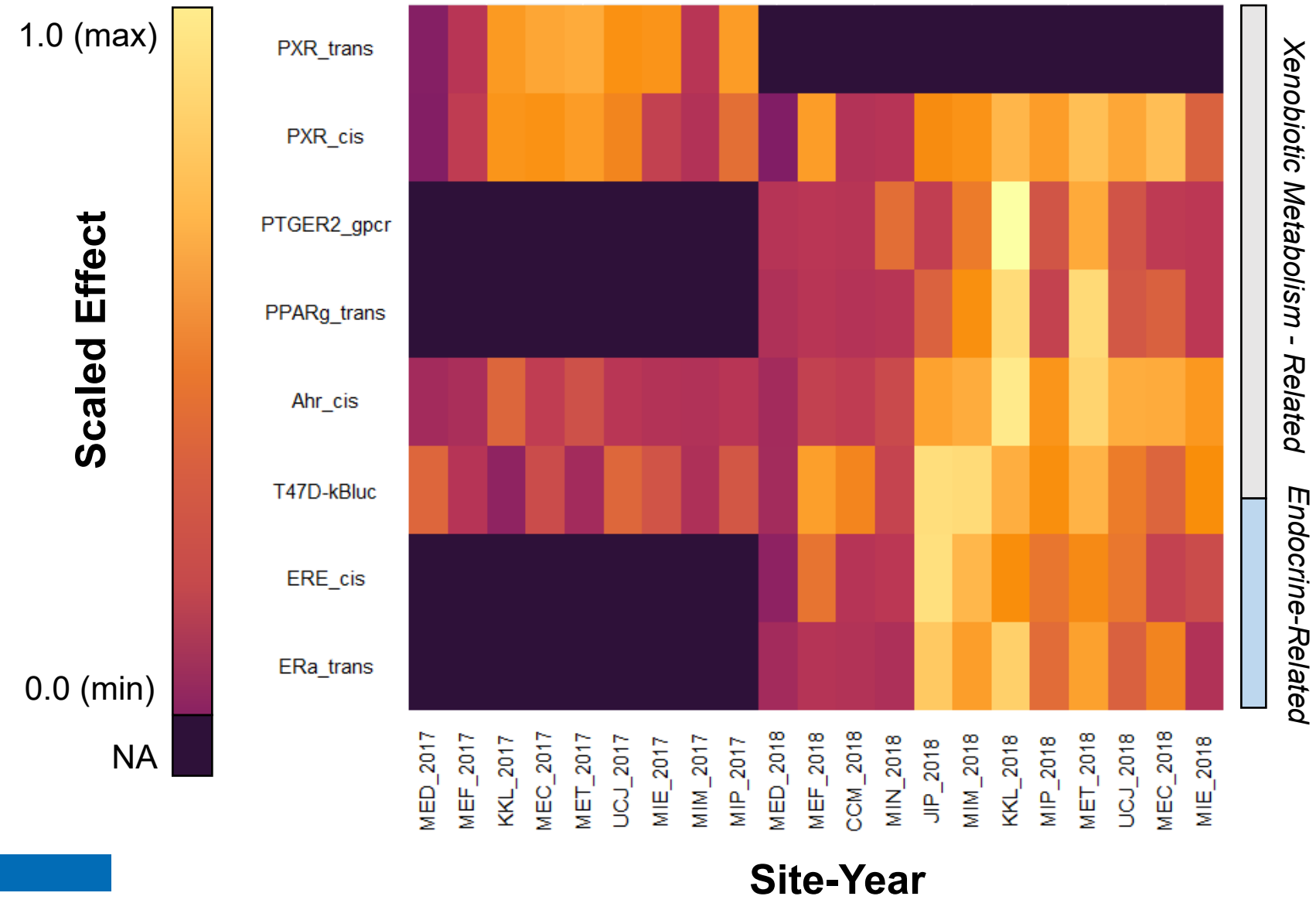


- **76**/179 chemicals detected at ≥ 1 site(s)

80 unique compounds detected across study years.

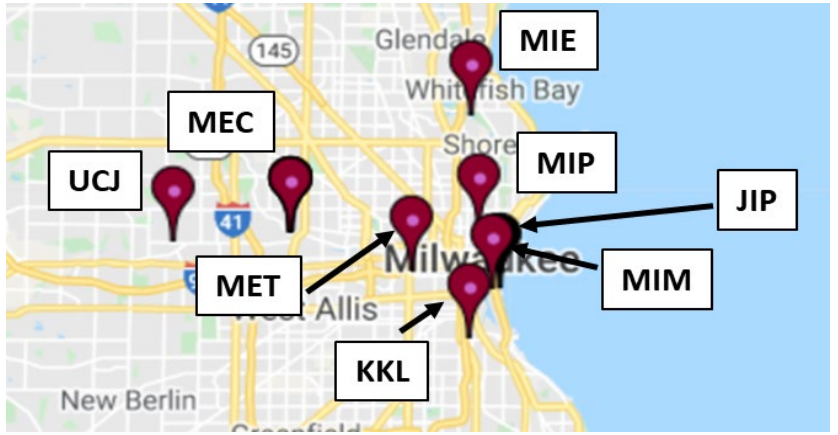
Industrial Compounds, PAHs/Fuels, Pharmaceuticals, Pesticides, Wastewater Indicators.

Whole-Mixture *in vitro* Effects

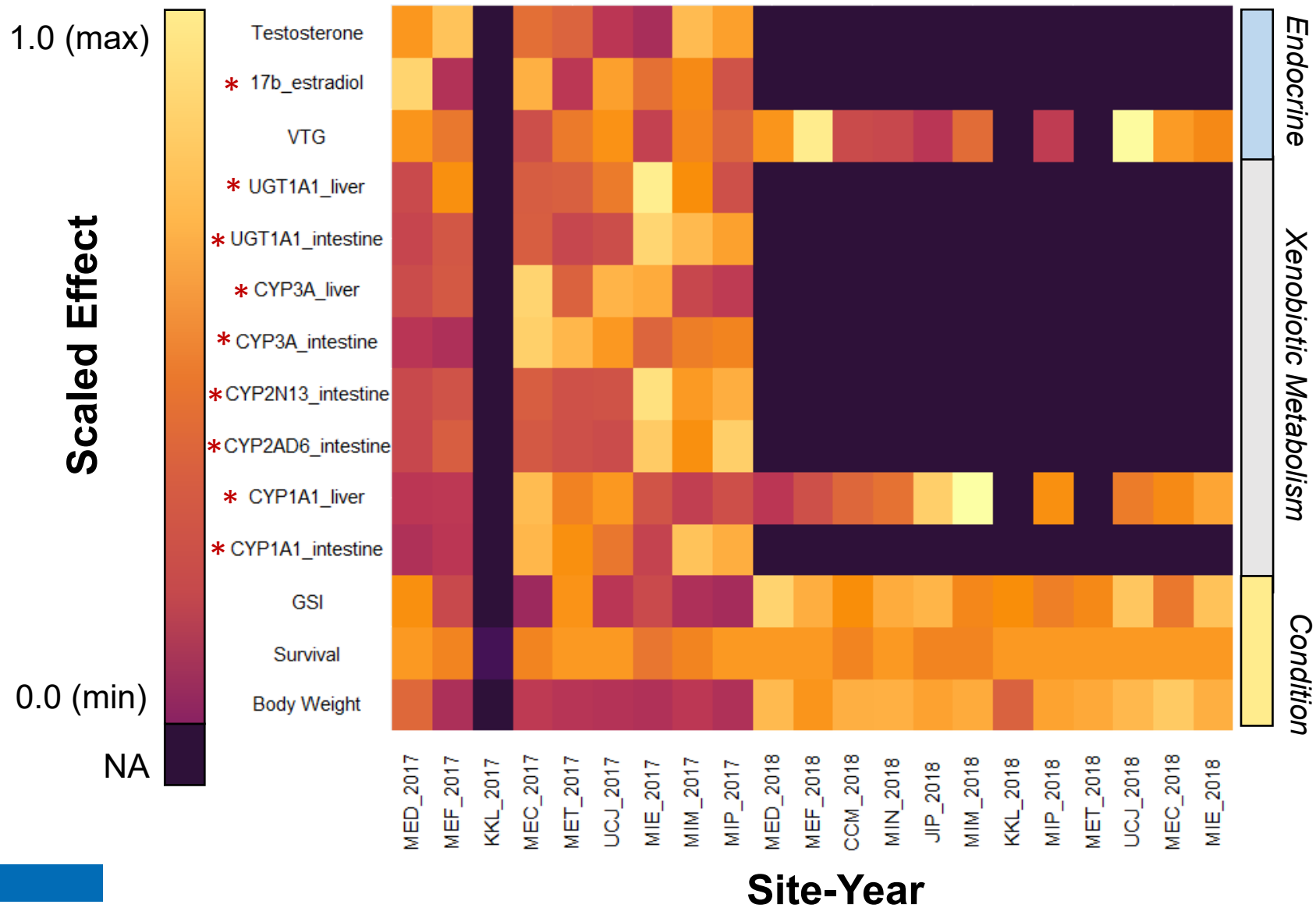


Elevated xenobiotic metabolism-related bioactivity in **4/8** sites in 2017 (MET, MEC, KKL, UCJ) & **3/11** sites in 2018 (KKL, MET, MIM).

Elevated endocrine-related bioactivity in **9/11** sites in 2018 (JIP, KKL, MET, MIM).



Whole-Mixture *in vivo* Effects

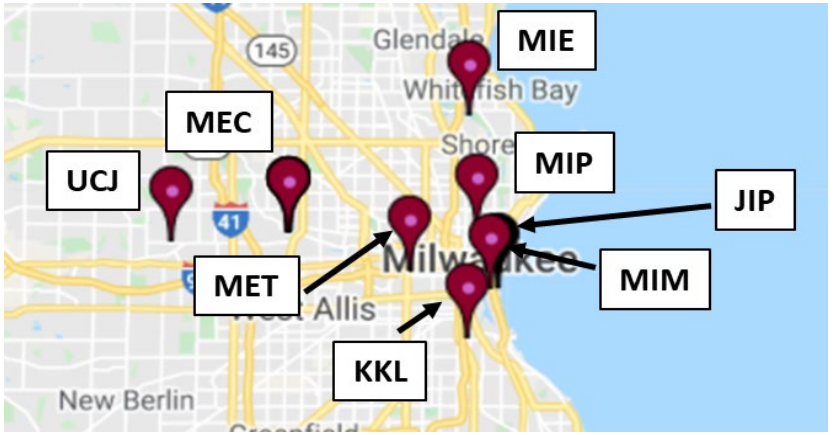


Limited *in vivo* endocrine-related effects.

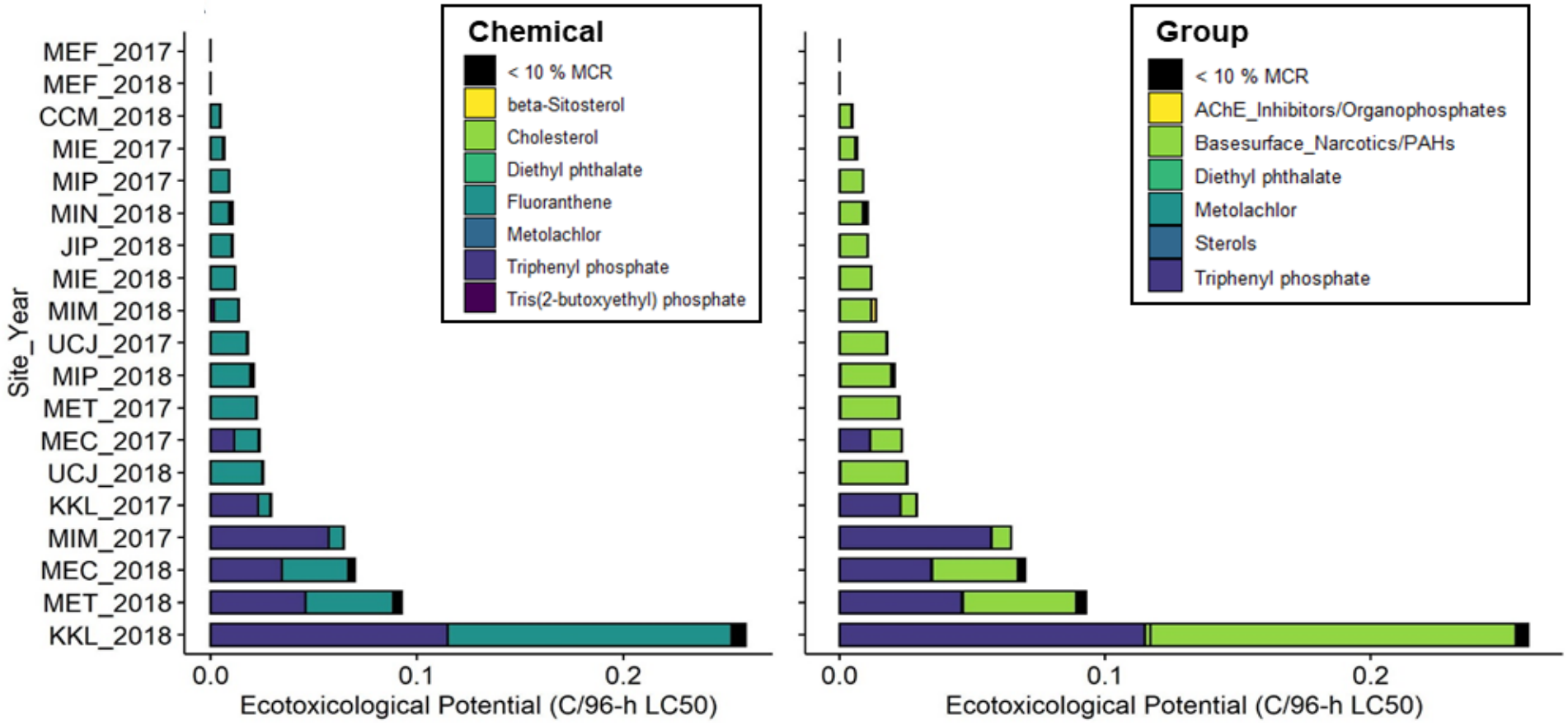
Elevated CYP1A1/CYP3A activity in **3/8** sites in 2017 (UCJ, MET, MEC) and **2/11** sites in 2018 (JIP, MIN).

Elevated intestinal CYP activity in **3/8** sites in 2017 (MIM, MIE, MIP).

No effects on body condition.

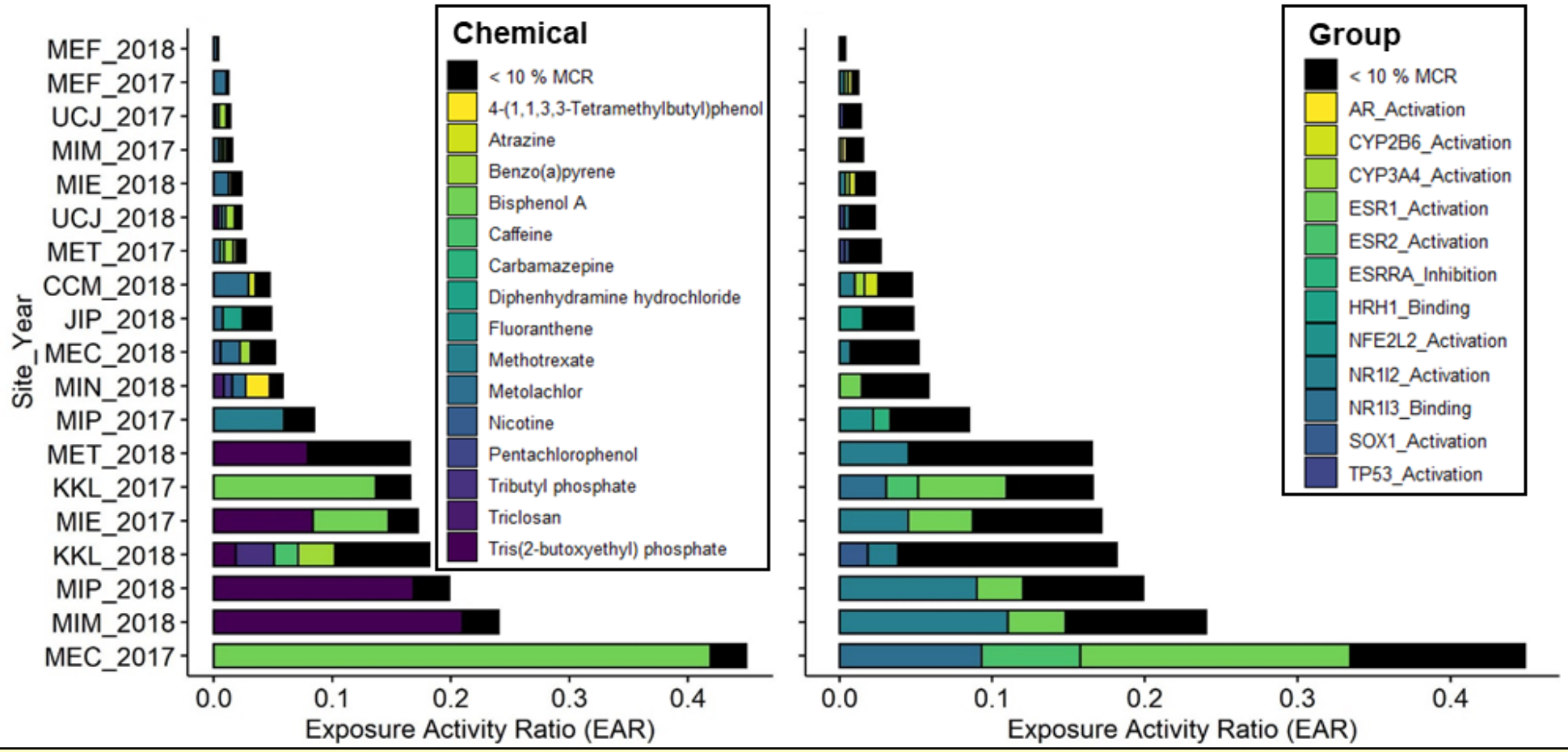


in vivo Ecotoxicological Potential



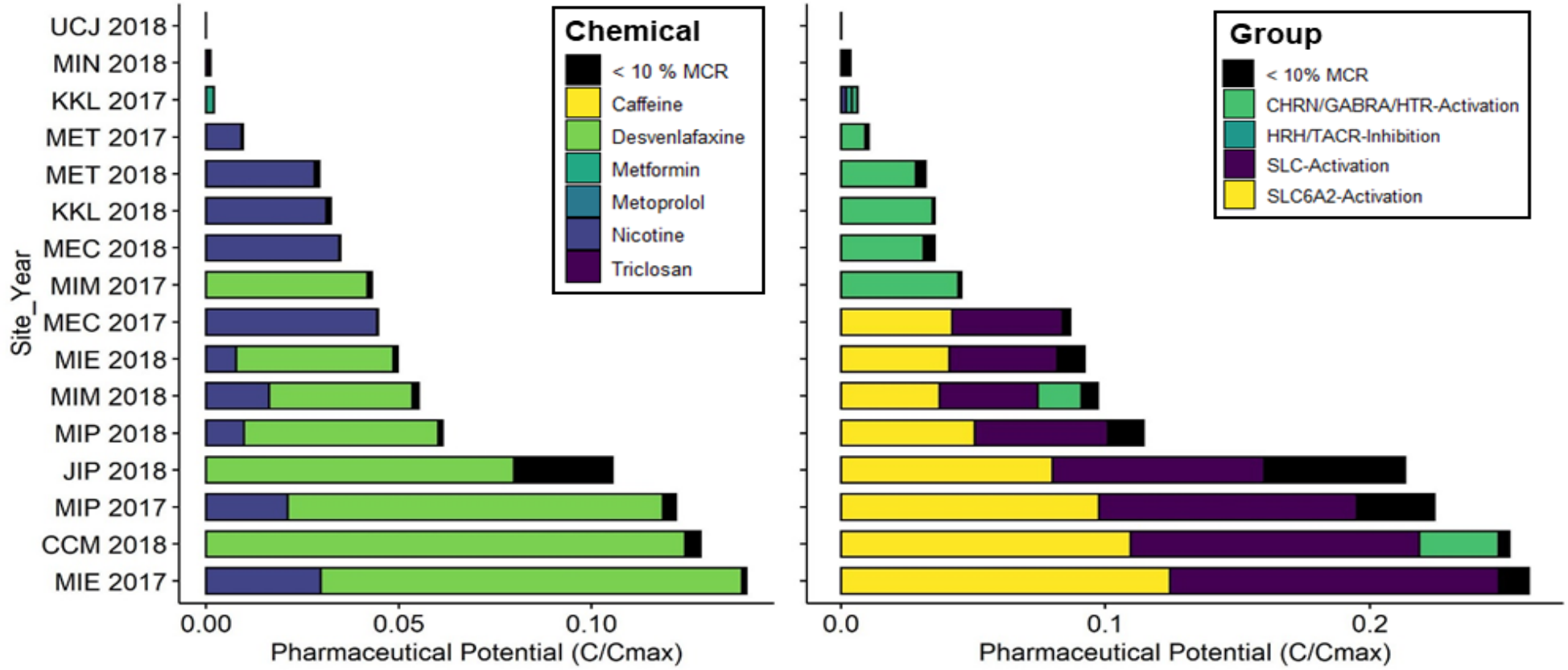
- Most prevalent drivers of *in vivo* ecotoxicological potential are **Fluoranthene, Triphenyl Phosphate,** and **Base-surface Narcotics/PAHs.**

in vitro Ecotoxicological Potential



- Most prevalent drivers of *in vitro* ecotoxicological potential are **Bisphenol A, Metolachlor, Tris-2(butoxyethyl)phosphate, estrogen receptor agonists (ESR1, ESR2), and Pregnane-X-Receptor (PXR) activators.**

Pharmaceutical Potential

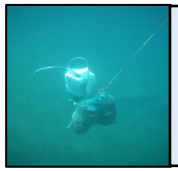


- Most prevalent drivers of pharmaceutical potential are **Desvenlafaxine, Nicotine,** **Sodium ion channel activators,** **and** **Neuroactive agonists.**

Current Findings

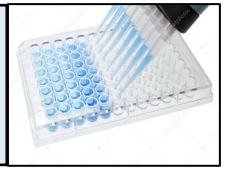
- *In vivo* and *in vitro* effects analyses indicate that adverse effects in the Milwaukee Estuary are largely related to intestinal and hepatic xenobiotic metabolism.
  - Highly impacted sites for hepatic enzyme expression tend to have higher concentrations of PAHs and other mixed-use industrial chemicals.
  - Highly impacted sites for intestinal enzyme expression tend to have higher concentrations of PPCPs.
- Evaluation of mixture potential under different effect-types highlights diverse putative effect drivers:
  - in vivo: PAHs + OP Flame Retardants; [Fluoranthene, Triphenyl Phosphate]
  - in vitro: ER agonists + PXR activators; [Metolachlor, Bisphenol A, Tris-2(butoxyethyl)phosphate]
  - Pharmacological: Sodium channel activators + neuroactive agonists [Desvenlafaxine, Nicotine]
- Identified groups & individual effect drivers = candidates for further mixture assessment*.

Future Work



in vivo effects

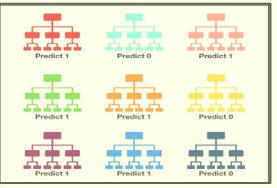
in vitro effects



in vivo effect concentrations *(ECOTOX Knowledgebase)*

Whole Mixture Effects

Endocrine-related Effects



Predictive Analysis

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in vitro activities *(ToxCast Database)*

Mixture Assessment

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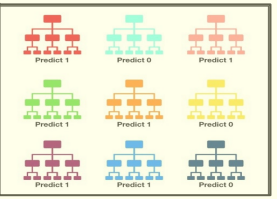
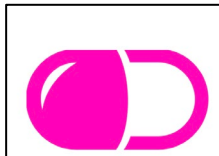
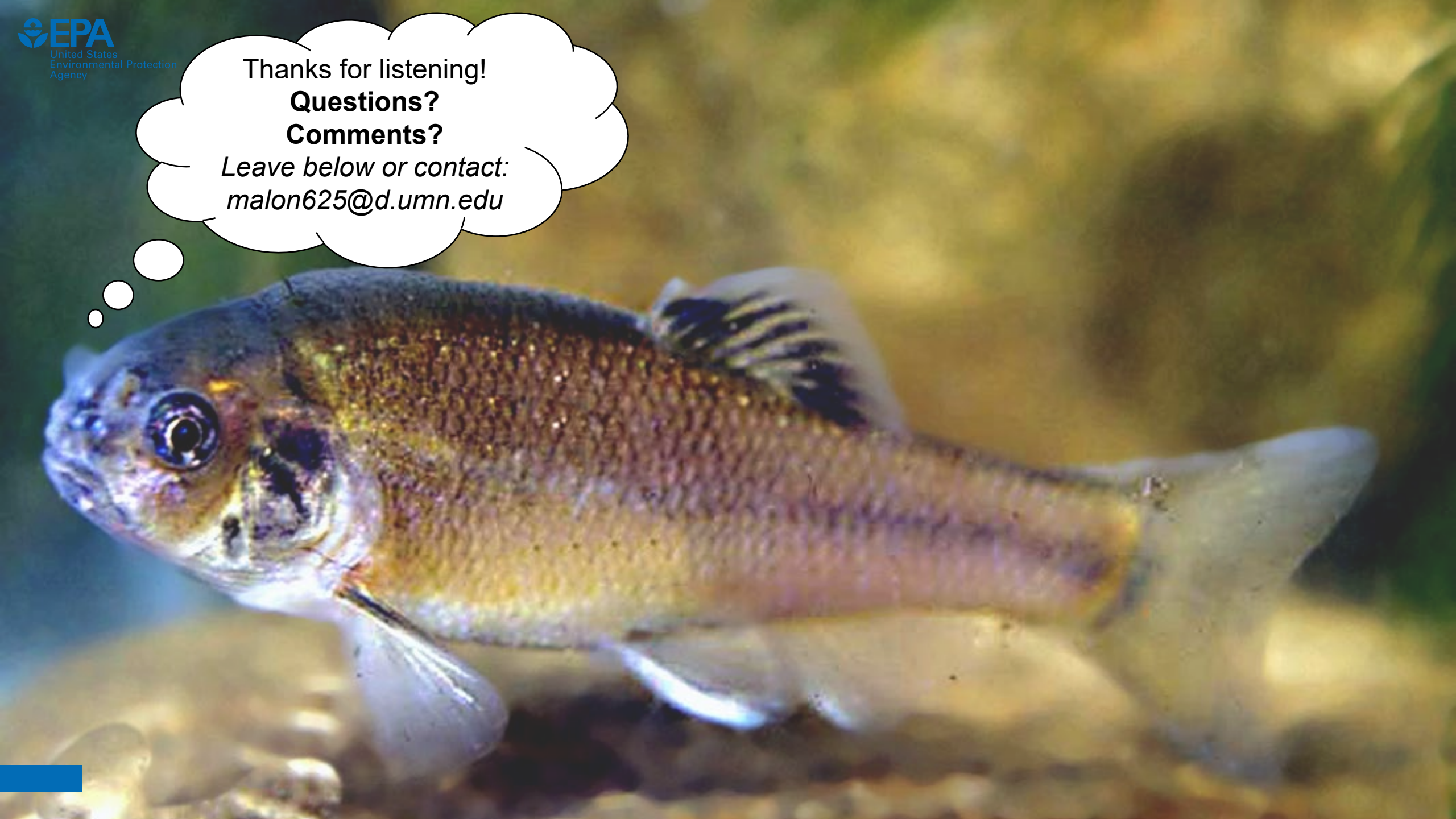




Pharmaceutical potential *(DrugBank, MaPPFAST Database)*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Comments?
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