Effects–Based Monitoring Under the Great Lakes Restoration Initiative: Evaluation of Spatial and Temporal Patterns in the Milwaukee Estuary

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Introduction

- Contaminants of emerging concern (CECs) (e.g. pharmaceuticals, pesticides, and industrial chemicals) have been increasingly detected in the Great Lakes[1,2]
- Under the "Toxic Substances and Areas of Concern" Focus Area, the Great Lakes Restoration Initiative (GLRI) aims to identify CECs and evaluate their potential impacts on Great Lakes ecosystems.
- This study aimed to employ effects-based approaches to help identify priority CECs/CEC mixtures in the Milwaukee Estuary area of concern (AOC) (Milwaukee, WI).
- To characterize the significance of biologically-active CECs, caged fish studies with adult fathead minnows (*Pimephales promelas*) were carried out at a range of sites (**Fig. 1**) across the Milwaukee AOC.
- To evaluate CEC spatiotemporal distribution and identify potential alternative ecotoxicological effects, 4 d composite water samples were concurrently collected, analyzed for a suite of pharmaceuticals and wastewater indicators, and screened for *in vitro* biological activity.

Objective:

- Utilize a weight-of-evidence, effect-driven approach to prioritize CECs and mixtures detected in the Milwaukee AOC for future ecotoxicological investigation and/or regulatory action.

Methods

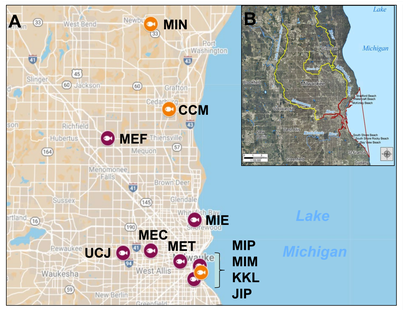

Figure 1. Map of field sites (A) within the Milwaukee Estuary AOC (B; denoted in red). Field sites deployed in 2017 - 2018 highlighted in purple; field sites deployed in only 2018 highlighted in orange.

Study Details

	2017	2018
Study year	2017	2018
Number of sites	8	11
Exposure scheme	4 d *in situ* exposures of caged *Pimephales promelas*; 2 fish/cage; 6M, 6F	
Controls	4-d flow-through tests; GLTED EPA Laboratory (MED; Duluth, MN), University of Wisconsin-Milwaukee (UWM; Milwaukee, WI).	
Water samples	4 d composite/site	
Analytical schedule	LCM56 (Pharmaceutical); GCM99 (Wastewater Indicator) **180 Screened Analytes**	
Tissue samples	Plasma (steroids); Liver (qPCR); Intestine (qPCR)	Liver (qPCR)
In vitro analyses	Trans-FACTORIAL™ bioassay (Attagene, RTP, NC) Estrogen Receptor Agonist Assay (T47/D-Kbluc)[3]	Trans-FACTORIAL™ bioassay (Attagene, RTP, NC) Estrogen Receptor Agonist Assay (T47/D-Kbluc)[3]
In vivo analyses	Gene Expression (qPCR; Cytochrome P450 (CYP) 1A1, 2AD6, 2N13, 3A; UDP glucuronosyltransferase (UGT) 1A, vitellogenin (VTG)); Steroid Analysis (Radioimmunoassay (RIA); Estrogen, Testosterone)	Gene Expression (qPCR; CYP1A1, VTG)

Data Analysis

Experimental Data

***In vivo* data** (analyzed using GraphPad Prism)
- One-way ANOVA and/or Kruskall-Wallis tests followed by Dunn/Tukey post-hoc analyses (α = 0.05).

***In vitro* data** (analyzed using GraphPad Prism)
- Non-linear regression (log-agonist vs. response)
- Significant response threshold = Mean + 3(SD) of the control media response

Chemical Detection
- **Frequency/Concentration:**
  - Evaluated frequency and concentration (mean, max) of detected chemicals.
- **Spatiotemporal trends:**
  - Cluster analysis (pvclust, R) used to identify spatiotemporal trends in CEC detection.

Environmental Fate
- Derived aquatic half-life ($t_{1/2}$) and bioconcentration factor (BCF) ranges using QSAR (OPERA[4], Epi Suite™) and experimental values.
- Characterized persistence and bioaccumulation (ECHA classification)[5]

Toxicity Benchmarks
- **Water Quality Benchmarks:**
  - Compared CEC concentrations to WQ benchmarks (U.S. EPA) using ToxCast (toxEval, R).[6].

Effect-Based Prioritization
- **High-throughput Screening**
  - Applied ToxCast (toxeval, R) to identify CECs driving elevated Exposure-Activity Ratios (EARs)[6] across sites.
- **Effect-Driver Prioritization**
  - Utilized Random Forest Regression[7] (randomForest, R) to identify significant chemical predictors of *in vivo* and *in vitro* effects.

CEC Prioritization Strategy

Characterize Chemical Detects
Flag high frequency (detected in >50% sites) and high concentration (upper quartile) CECs.

Evaluate Environmental Fate Properties
Flag CECs designated as P and/or B.

Compare to Toxicity Benchmarks
Flag chemicals exceeding water quality benchmarks and/or toxicity quotients.

Distinguish Chemical Drivers of Effect
Flag chemicals identified as key drivers of elevated EAR values (ToxCast).
Flag chemicals identified as significant predictors of in vitro and in vivo effects (Random Forest)

Identify Priority Mixtures
Identify mixture effects using analytical data (ToxCast) and in vivo/in vitro responses at study sites.
Utilize adverse outcome pathways (AOPs) to identify potential adverse outcomes and prioritize CEC mixtures.

Weight of Evidence Categorization of Priority CECs

CECs/mixtures requiring regulatory action	CECs/mixtures requiring further study	Non-priority CECs/mixtures

Priority Categories

Results

Site-Specific Effects

- No significant impacts on *P. promelas* survival or body condition.
- No significant estrogenic effects at Milwaukee AOC sites:
  - Estrogenicity of water samples (T47/D-Kbluc assay; $p > 0.05$) .
  - Relative abundance of vitellogenin mRNA in male livers (qPCR; $p > 0.05$).
  - Serum concentrations of estrogen (E2) and testosterone (RIA; $p > 0.05$).

Data not presented

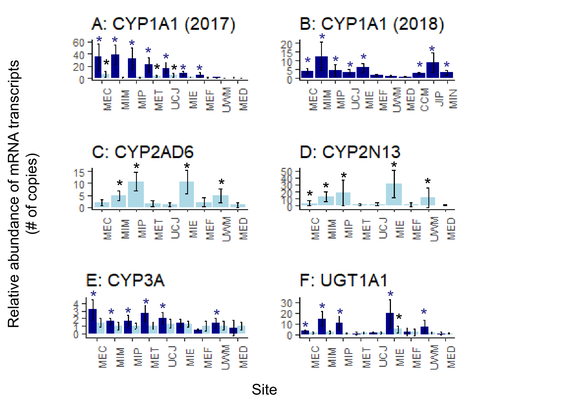

Figure 2. Relative abundances of transcripts encoding for (A-B) *CYP1A1*, (C) *CYP2N13*, (D) *CYP2AD6*, (E) *CYP3A* and (F) *UGT1A1* in livers (dark blue) intestines (light blue) of caged *P. promelas* within Milwaukee AOC test sites and lab controls (UWM, MED). Significant differences from control denoted with asterix (*).

Analytical Chemistry

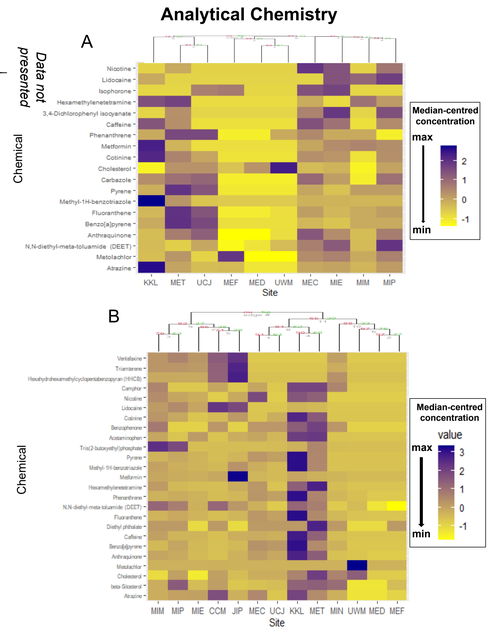

Figure 3. CECs detected at high frequency (>50% sites) across the Milwaukee Estuary AOC in (A) 2017 and (B) 2018.
Data ratio-normalized to mean concentration within each chemical group; dark blue = higher relative concentrations, yellow = lower relative concentrations.

Environmental Fate Properties

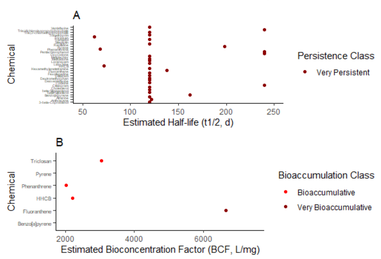

Figure 4. CECs detected in the Milwaukee AOC classified as persistent (A) or bioaccumulative (B) based on ECHA classification scheme[5].

Water Quality Benchmark Analysis

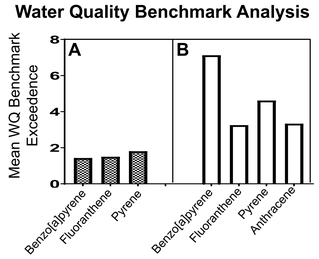

Figure 5. Median exceedences of CECs flagged due to water quality benchmark exceedence in (A) 2017 and (B) 2018.
**An additional 64 detected CECs could not be compared to WQ benchmarks.*

Chemical Effect Drivers

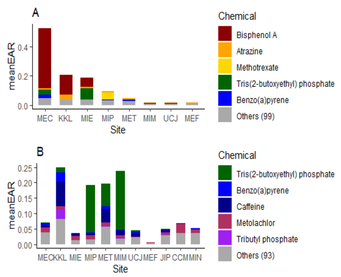

Figure 6. CECs flagged as key drivers of elevated exposure activity ratios (EARs) in (A) 2017 and (B) 2018.
** An additional 27 detected CECs could not be evaluated with ToxCast.*

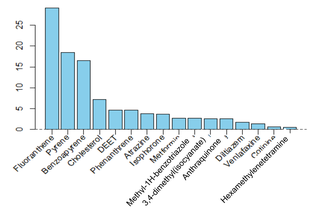

Figure 7. Random forest regression analysis highlighting significant chemical drivers of elevated intestinal *CYP 1A1* transcript abundance in 2017 study samples.

Key Findings

- *Site-specific effects:*
  - Significant elevation of antioxidant enzymes in several sites in Milwaukee AOC.
  - Notable differences between transcript expression in intestines and livers.
- *Chemical detects:*
  - 32 CECs detected in high frequency (>50 % of sites) across study years.
  - Tris(2-butoxyethyl)phosphate CEC with highest max concentrations in both study years.
- *Environmental fate and toxicity benchmark analysis:*
  - 26 CECs flagged as vP ($t_{1/2}$ > 120 d); 3 as vPB ($t_{1/2}$ > 120 d; BCF > 2000); and 3 as vPvB ($t_{1/2}$ > 120 d; BCF > 5000).
  - 4 PAHs flagged due to exceedence of WQ benchmarks; 64 detected CECs flagged due to lack of WQ benchmarks.
- *Chemical effect drivers:*
  - 8 CECs determined to be key drivers of elevated EAR values.
  - 16 CECs defined as important predictors of elevated intestinal CYP1A1 transcripts.

30/180 CECs currently flagged as priority compounds (regulatory) and 21/180 CECs currently flagged as requiring further study.

Future Work

- Derive toxicity quotients and evaluate exceedences in Milwaukee AOC.
- Prioritize CECs driving other *in vivo* and *in vitro* endpoints (not intestinal *CYP1A1* expression) using Random Forest Regression
- Utilize adverse outcome pathways to identify priority CEC mixtures defined based on common mechanisms of action and/or through ToxCast analyses.

Acknowledgements and References

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Mention of products or trade names does not indicate endorsement by the U.S. federal government. The contents of this poster do not necessarily reflect U.S. EPA policy.

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