Pathway-Based Approaches for Assessing Biological Hazards of Complex Mixtures of Contaminants in the Great Lakes*

Gerald Ankley, USEPA, Great Lakes Toxicology and Ecology Division, Duluth MN

*Content does not necessarily reflect USEPA position or policy.

ankley.gerald@epa.gov

We are very good—and ever improving—at detecting lots of chemicals in environmental samples, but despite advances in targeted/nontargeted analytical techniques, major uncertainties remain as to assessing their risk

What are the biological implications of the measurements?

Are all chemicals responsible for biological effects being detected?

Integration of multidisciplinary biological and analytical techniques needed to address these questions

In 2007, the IJC established a Workgroup to address "Chemicals of Emerging Concern":

"…..those chemicals used by society that are unregulated or inadequately regulated, and for which there is growing concern over the risk they may pose to the health of humans and ecosystems."*

- pharmaceuticals
- organic wastewater contaminants
- hormones/steroids
- some current use pesticides
- alkylphenol ethoxylates
- synthetic musks
- perfluorinated surfactants
- polybrominated diphenyl ethers
- other flame retardants
- chlorinated paraffins

*Klecka et al. 2010

Since 2010 the EPA GLNPO has supported a multi-organizational effort through the Great Lakes Restoration Initiative to help assess CECs

-Develop integrated biological/analytical methods to detect CEC effects

-Demonstrate practical utility of the tools at different sites (incl. AOCs)

-Provide insights as to priority CECs associated with varied land uses

USGS science for a changing world

National Oceanic and Atmospheric Administration NOAA U.S. Department of Commerce

U.S. Fish & Wildlife Service Department of the Interior

US Army Corps of Engineers

United States Environmental Protection Agency

St. Cloud State University

University of St. Thomas Minnesota

Clarkson University

GLTED GLRI Research Efforts

- Development of novel approaches for sample generation/collection
- Utilization of unique data sources for prediction of biological effects
- Advancement of in vitro and in vivo methods for assessing bioactivities of complex environmental samples
- Application of pathway-based approaches to predicting hazard of chemical mixtures
- Demonstration of practical utility in the field
  - St. Louis River/Harbor, MN; Maumee River, OH; Milwaukee River/Tributaries, WI; Detroit River, MI; Lower Fox River/Green Bay, WI

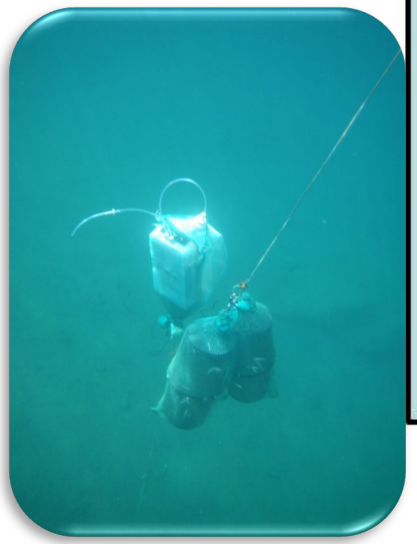
Generating Linked Biological and Analytical Samples

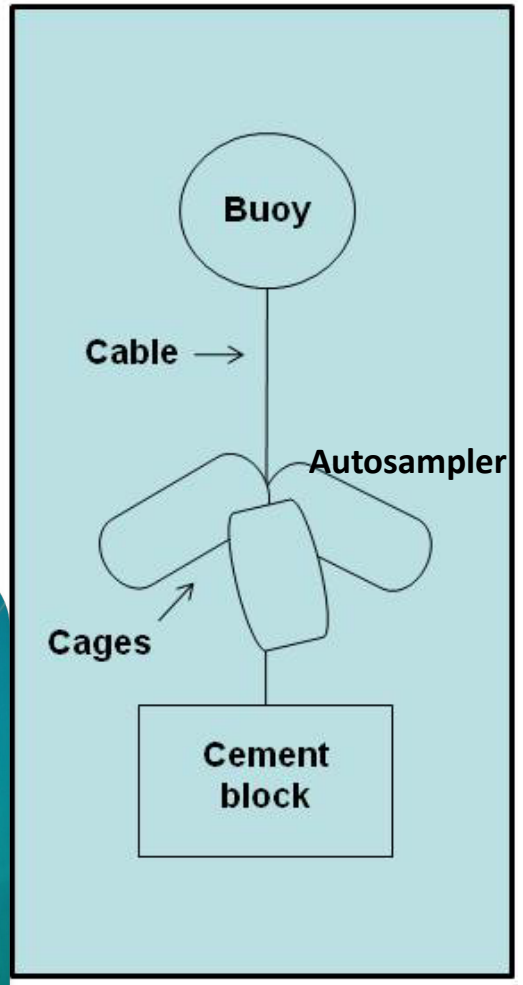
Challenge: Controlled lab exposures with field samples (water, sediment) unrealistic, but collection of wild organisms (fish, invertebrates) unreliable

Solution: In situ (caged) exposures of lab organisms

Challenge: "Grab" samples of environmental matrices (e.g., water) may not reflect temporally accurate exposure of animals in field

Solution: Employ "real time" composite samplers with simplified auto-sampling technology





Kahl MD et al. 2014. An inexpensive, temporally integrated system for monitoring occurrence and biological effects of aquatic contaminants in the field. Environ Toxicol Chem. 33:1584-95. doi: 10.1002/etc.2591.

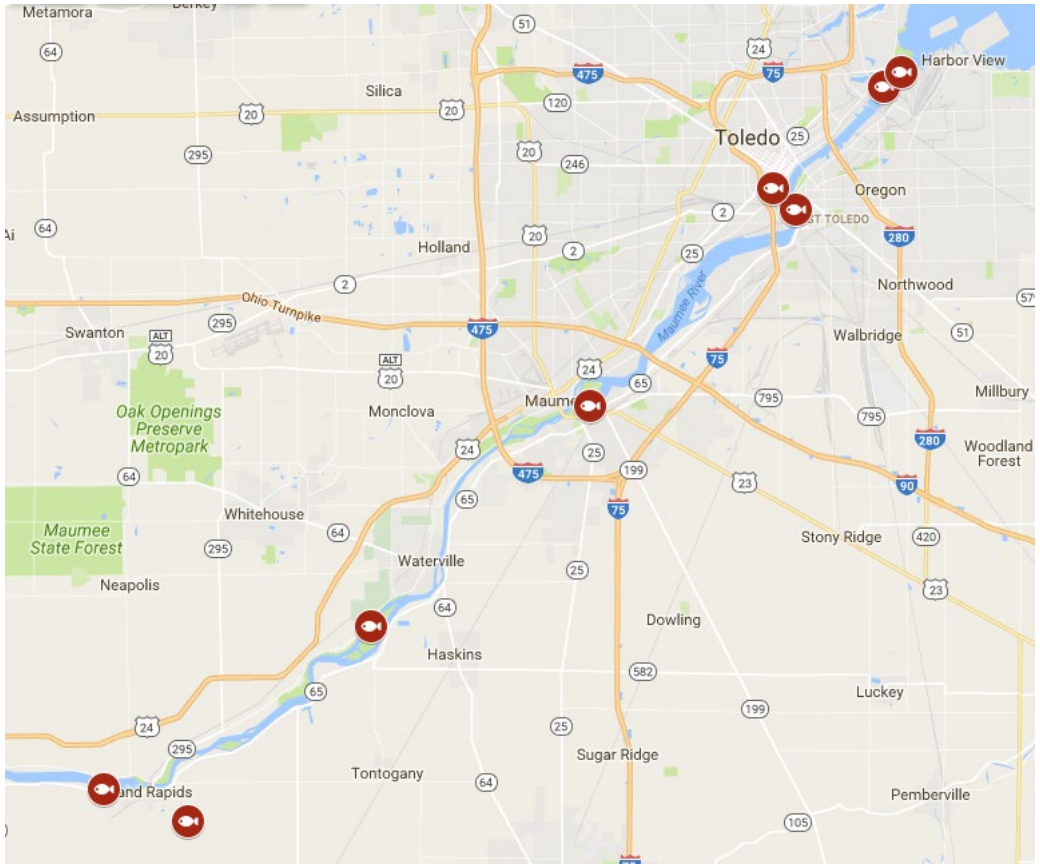
Practical Demonstration: Evaluation of Estrogens in the Maumee River AOC

Large number of estrogenic contaminants from domestic and agricultural sources

Associated with feminization of fish at sites worldwide , so common endpoint of concern

Maumee watershed impacted by variety of inputs

Gradient of sites evaluated in 2012 & 2016 using 4-d caged fish exposures with corresponding composite water sample collection



Ankley GT, et al. 2021. Pathway-based approaches for assessing biological hazards of complex mixtures of contaminants: A case study in the Maumee River. Environ Toxicol Chem. 40:1098-1122. doi: 10.1002/etc.4949.

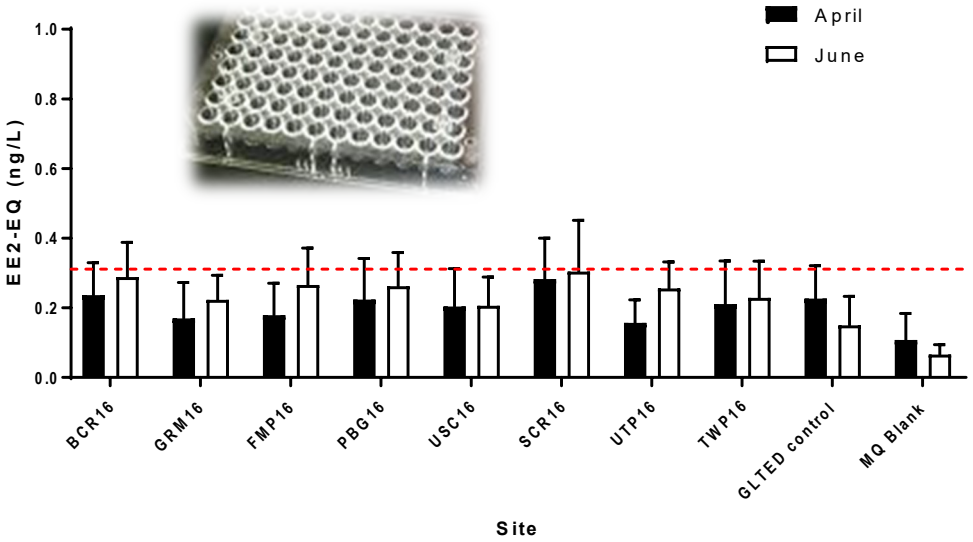
Evaluation of Estrogens in the Maumee, cont'

Detected ca. 140 of 350 wastewater indicators, drugs, and pesticides monitored in composite water samples

Some suspect estrogens present at µg/L concentrations (e.g., Bisphenol A, metformin, β-sitosterol)

Low/no estrogenic activity in sensitive T47D in vitro assay run using the composite samples

Low/no induction of vitellogenin in caged fish



Multiple complementary lines of evidence indicate estrogens not an issue

Ankley GT, et al. 2021. Pathway-based approaches for assessing biological hazards of complex mixtures of contaminants: A case study in the Maumee River. Environ Toxicol Chem. 40:1098-1122. doi: 10.1002/etc.4949.

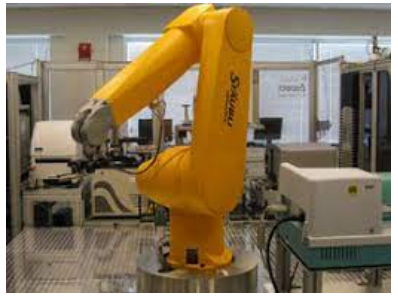
Uncovering Unknown Biological Impacts

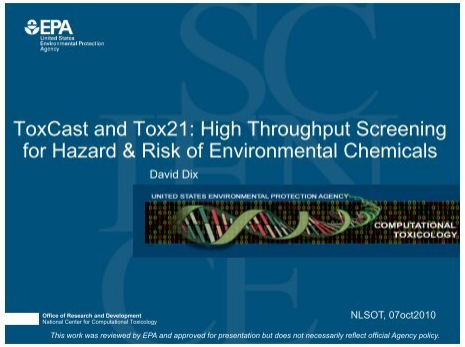
Challenge: In vivo toxicity data exist for only a fraction of detected chemicals

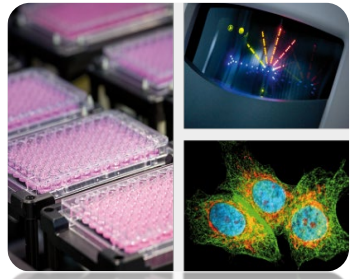
Solution: Utilize extensive HTT in vitro data to estimate hazard of untested chemicals based on hazard quotients (EAR)

Challenge: Unmeasured chemicals may be causing biological effects

Solution: Employ multi-pathway HTT platforms to “probe” environmental samples for relevant bioactivities







Schroeder AL, et al. 2016. Environmental surveillance and monitoring--The next frontiers for high-throughput toxicology. Environ Toxicol Chem. 35:513-25. doi: 10.1002/etc.3309.

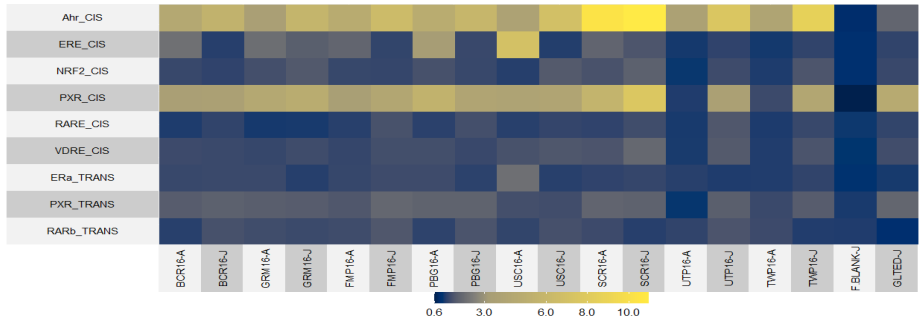
Defining Unknown Biological Impacts in the Maumee

In vivo toxicity data unavailable for less than a quarter of 140+ compounds detected, but ToxCast data available for >100

HTT-based EAR values suggest impacts on multiple biological pathways, notably several involving cytochrome P450 (CYP) isozymes

Direct measure of composite water samples with HTT showed up-regulation of CYP-related bioactivities

Hepatic CYPs involved in xenobiotic metabolism induced in caged fathead minnows (as well as in Tree swallows from nearby nest boxes)



Ankley GT, et al. 2021. Pathway-based approaches for assessing biological hazards of complex mixtures of contaminants: A case study in the Maumee River. Environ Toxicol Chem. 40:1098-1122. doi: 10.1002/etc.4949.

Unknown Biological Impacts in the Maumee cont'

Combined weight-of-evidence from integrated analytical and biological measures indicates **CECs in the Maumee system capable of affecting range of vertebrate CYPs**

Inhibition of some CYPs can affect biological systems involved in reproduction and development (e.g., sex steroid synthesis)

Induction of other CYPs can "enhance" ability to metabolize xenobiotics such as pharmaceuticals, pesticides and PAHs

- Adaptive/compensatory response?
- Adverse effects (e.g., oxidative stress, liver damage)?

Provides basis for more focused monitoring in terms of chemicals and biological endpoints

Ankley GT, et al. 2021. Pathway-based approaches for assessing biological hazards of complex mixtures of contaminants: A case study in the Maumee River. Environ Toxicol Chem. 40:1098-1122. doi: 10.1002/etc.4949.

“Take Home” Messages

- Assessing potential ecological risks of complex environmental mixtures has been a significant uncertainty for decades
- Ability to measure increasing number of contaminants of limited value unless biological significance can also be assessed
- Even the most complete analytical approaches available incapable of measuring all biologically significant chemicals
- Multidisciplinary approaches integrating chemical and biological information provide basis to address these challenges
  - Collection methods enabling direct comparison of biology/chemistry
  - “Alternative” data to predict bioeffects of detected, but untested chemicals
  - In vitro/in vivo methods to rapidly measure bioactivity of mixtures
  - Integrated approaches to extrapolate biochemical responses to effects in fish and wildlife populations

GLRI CEC Project Team

EPA – Great Lakes National Program Office

- Beth Murphy
- Todd Nettesheim
- Ted Smith
- Derek Ager

US Fish and Wildlife Service

- JoAnn Banda
- Dan Gefell
- Zach Jorgenson
- Annette Trowbridge
- Steph Hummel

USGS

- Austin Baldwin
- Mark Brigham
- Steve Corsi
- Christine Custer
- Tom Custer
- Eric Dantoin
- Laura DeCicco
- Joe Duris
- Brett Hayhurst
- Josh Larson
- Stephanie Kula
- Cheryl Silcox

EPA – ORD

- Gary Ankley
- Jason Berninger
- Brett Blackwell
- Jenna Cavallin
- Tim Collette
- John Davis
- Drew Ekman
- Kelly Fay
- Kathy Jensen
- Mike Kahl
- Carlie LaLone
- Erin Maloney
- David Miller
- Marc Mills
- Jonathan Mosley
- Shibin Li
- Eric Randolph
- Travis Saari
- Quincy Teng
- Dan Villeneuve

National Oceanic Atmospheric Administration

- Ed Johnson
- Kimani Kimbrough
- Annie Jacob

University of St. Thomas

- Dalma Martinović-Weigelt

University of St. Cloud

- Satomi Kohno
- Heiko Schoenfuss

US Army Corps of Engineers

- Lyle Burgoon
- Ed Perkins
- Natalia Vinas

Clarkson University

- Bernie Crimmins

WLSSD

- Joe Mayasich
- A. Parella
- T. Tuominen