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An integrated approach for identifying priority contaminant in the Great Lakes Basin – Investigations in the Lower Green Bay/Fox River and Milwaukee Estuary areas of concern

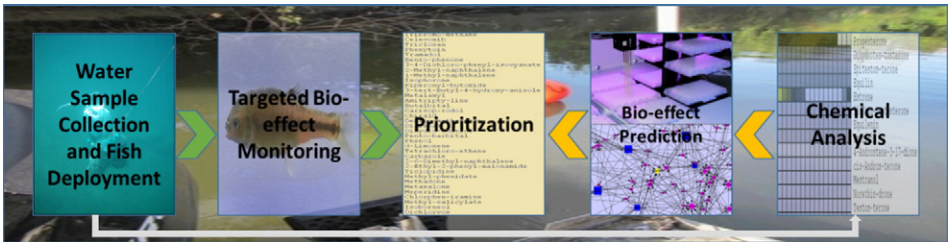
Shibin Li [a,b,*], Daniel L. Villeneuve [a], Jason P. Berninger [a,b,1], Brett R. Blackwell [a], Jenna E. Cavallin [a], Megan N. Hughes [a], Kathleen M. Jensen [a], Zachary Jorgenson [c], Michael D. Kahl [a], Anthony L. Schroeder [d], Kyle E. Stevens [a], Linnea M. Thomas [a], Matthew A. Weberg [a], Gerald T. Ankley [a]

[a] U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, MN, USA, 55804
[b] National Research Council, U.S. Environmental Protection Agency, 6201 Congdon Boulevard, Duluth, MN 55804, USA
[c] US Fish and Wildlife Service, Twin Cities Ecological Field Services Field Office, 4101 American Blvd East, Bloomington, MN 55425, USA
[d] University of Minnesota Crookston, Math, Science and Technology Department, 2900 University Ave., Crookston, MN 56716, USA

HIGHLIGHTS

- Prioritization of chemicals was performed on two Areas of Concerns in the Great Lakes.
- An integrated risk-based surveillance and monitoring approach was applied.
- Bio-effect prediction methodologies were used to identify additional biological pathways.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental assessment of complex mixtures typically requires integration of chemical and biological measurements. This study demonstrates the use of a combination of instrumental chemical analyses, effects-based monitoring, and bio-effects prediction approaches to help identify potential hazards and priority contaminants in two Great Lakes Areas of Concern (AOCs), the Lower Green Bay/Fox River located near Green Bay, WI, USA and the Milwaukee Estuary, located near Milwaukee, WI, USA. Fathead minnows were caged at four sites within each AOC (eight sites total). Following 4 d of in situ exposure, tissues and biofluids were sampled and used for targeted biological effects analyses. Additionally, 4 d composite water samples were collected concurrently at each caged fish site and analyzed for 132 analytes as well as evaluated for total estrogenic and androgenic activity using cell-based bioassays. Of the analytes examined, 75 were detected in composite samples from at least one site. Based on multiple analyses, one site in the East River and another site near a paper mill discharge in the Lower Green Bay/Fox River AOC, were prioritized due to their estrogenic and androgenic activity, respectively. The water samples from other sites generally did not exhibit significant estrogenic or androgenic activity, nor was there evidence for endocrine disruption in the fish exposed at these sites as indicated by the lack of alterations in ex vivo steroid production, circulating steroid concentrations, or vitellogenin mRNA expression in males. Induction of hepatic *cyp1a* mRNA expression was detected at several sites, suggesting the presence of chemicals that activate the aryl hydrocarbon receptor. To expand the scope beyond targeted investigation of

* Corresponding author at: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, MN 55804, USA.
E-mail address: lishibin1116@gmail.com (S. Li).
[1] Current address: U.S. Geological Survey Columbia Environmental Research Center, 4200 E New Haven Rd, Columbia, MO, USA, 65201.

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endpoints selected a priori, several bio-effects prediction approaches were employed to identify other potentially disturbed biological pathways and related chemical constituents that may warrant future monitoring at these sites. For example, several chemicals such as diethylphthalate and naphthalene, and genes and related pathways, such as cholinergic receptor muscarinic 3 (CHRM3), estrogen receptor alpha1 (esr1), chemokine ligand 10 protein (CXCL10), tumor protein p53 (p53), and monoamine oxidase B (Maob), were identified as candidates for future assessments at these AOCs. Overall, this study demonstrates that a better prioritization of contaminants and associated hazards can be achieved through integrated evaluation of multiple lines of evidence. Such prioritization can guide more comprehensive follow-up risk assessment efforts.

1. Introduction

The Great Lakes Restoration Initiative (GLRI) was initiated in 2010 to accelerate efforts to protect and restore the largest system of fresh surface water in the world – the North American Great Lakes. The GLRI is addressing a variety of threats to the Great Lakes including nutrient runoff, introduction of invasive species, habitat depletion, and potential effects of contaminants, including chemicals of emerging concern (CECs). The present work is part of a multiagency GLRI effort aimed at development and application of novel methods for assessing complex mixtures of aquatic contaminants in Great Lakes tributaries, including multiple Areas of Concern (AOCs) (Ekman et al., 2013).

Assessing the potential for adverse biological effects of complex mixtures of contaminants in aquatic environments is challenging, and often best achieved through the integration of multiple lines of evidence. Traditionally, instrumental analysis has been the most widely used approach for surveillance and monitoring of mixtures of chemical contaminants in aquatic environments (e.g., Wagner et al., 1998; Stackelberg et al., 2001; Foster et al., 2005; Kolpin et al., 2002). While instrumental analysis can effectively characterize the occurrence of many contaminants, this approach alone has shortcomings relative to complex mixture assessment (Escher et al., 2011; Escher et al., 2013; Leusch et al., 2014). Some chemicals are not detected at biologically relevant concentrations using standard extraction and instrumental analyses, while in other instances the identity of chemicals present in a sample may not be known. When chemicals are successfully detected, there often are insufficient toxicological data available to estimate possible hazards of detected chemicals. Lastly, there is often a limited understanding of chemical interactions among the complex chemical mixtures that exist in the environment.

In response to these recognized limitations, complementary biological effects-based monitoring approaches have been employed by a number of regulatory programs in the United States, such as the National Pollution Discharge Elimination System (NPDES) program for effluents (USEPA, 1984). Effects-based methods provide a direct measure of the integrated biological response to a complex mixture. Responses measured may be highly integrative, as in the case for testing associated with the NPDES program which utilizes apical endpoints in whole animals, or they may be specific to certain chemical classes and/or biological activities, as in the case of in vitro assays focused on a single signaling pathway (Macova et al., 2011; Tang et al., 2013).

Under the umbrella of effects-based monitoring, methods seek to balance the degree of control with environmental realism. For example, assays with fish may be conducted in the lab with field-collected water samples, which provides control of confounding environmental factors and low logistic cost, but fails to consider uncertainties due to fluctuating chemical exposures or the possible degradation of contaminants in collected/stored samples. At the other end of the spectrum, direct evaluation of feral fish considers chemical impacts in a realistic exposure scenario, but is only occasionally used due to high collection costs. Exposing fish in situ with caging systems can offer a cost-effective middle-ground between controlled laboratory exposure and field monitoring. Although the exposure duration of caged fish will typically be less than wild fish, in the short-term both can experience similar fluctuating chemical exposures. Further, since fish used in caging studies typically are from laboratory cultures, they have a known chemical exposure history and health status, which is not the case with field-collected animals.

Ideally, in caged fish studies chemical characterization of the exposure would closely match what the animal's experience. Grab samples may not reflect an exposure over the course of the study, as both chemical concentration and composition can fluctuate. Different types of passive samplers offer the potential to capture integrated contaminant samples (Miege et al., 2012; Vrana et al., 2001), but back-calculation to actual water concentrations is challenging (Yılmaz et al., 2014). As an alternative, Kahl et al. (2014) described a composite sampling system that provides a time-integrated sample of water directly matched to organism deployment.

Samples collected from the field can be assessed using targeted (or supervised) or untargeted (unsupervised) measurements (Ekman et al., 2013). Targeted assays/endpoints are employed when there are specific biological activities/pathways of concern. For example, a common in vivo measurement for detecting exogenous estrogens is vitellogenin (egg yolk precursor; vtg) protein or mRNA in male fish (Hutchinson et al., 2006; Sumpter and Jobling, 1995). Analogously water samples can also be assessed using targeted in vitro systems such as the estrogen-responsive T47D-KBluc cell bioassay (Cavallin et al., 2015; Wehmas et al., 2011; Wilson et al., 2004).

Untargeted approaches such as 'omics' measurements in fish tissues (e.g., Berninger et al., 2014; Garcia Reyero et al., 2011; Martinovic-Weigelt et al., 2014; Skelton et al., 2014) and analysis of water samples or extracts using batteries of pathway-based in vitro assays (Escher et al., 2014; Schroeder et al., 2016) are an important complement to supervised measurements when, as typically is the case with environmental samples, unknown/unmeasured contaminants are of concern. Untargeted approaches ideally assess activities of contaminants without bias for any specific biological pathways, which is especially important for surveillance efforts (Ekman et al., 2013; Escher et al., 2014; Schroeder et al., 2016).

A significant challenge in utilizing the sometimes extensive amount of analytical chemistry data collected in conjunction with monitoring/surveillance efforts is identification of potential biological hazard. Comprehensive biological effects data typically are available only for a small number of the measured chemicals, and what is available often is widely distributed across the peer-reviewed literature. However, critical progress has been made to capture and centralize information concerning chemical effects on molecular/biochemical endpoints in both in vitro and in vivo systems. For example, the Comparative Toxicogenomics Database (CTD; ctdbase.org) includes >24 million gene-chemical, chemical-disease and gene-disease interactions collected from the open literature (Davis et al., 2015). Another increasingly important source of bioeffects data for a large number of chemicals is being generated through high-throughput toxicity testing (HTT). For example, the US Environmental Protection Agency (USEPA) Toxicity ForeCaster (ToxCast™) and associated Tox21 programs have generated dose-response data for >8500 unique substances using up to 821 assay endpoints (Dix et al., 2007; Houck et al., 2013; Judson et al., 2010). These data are publically available in a variety of formats