Heiko Schoenfuss ORCID iD: 0000-0001-5464-992X

Daniel L. Villeneuve ORCID iD: 0000-0003-2801-0203

Assessing Contaminants of Emerging Concern in the Great Lakes Ecosystem: A Decade of Method Development and Practical Application

Gerald T. Ankley[a,*]0000-0002-9937-615X, Steven R. Corsi[b], Christine M. Custer[c], Drew R. Ekman[d], Stephanie L. Hummel[e], Kimani L. Kimbrough[f], Heiko L. Schoenfuss[g]0000-0001-5464-992X, and Daniel L. Villeneuve[a]0000-0003-2801-0203

[a]Great Lakes Toxicology and Ecology Division, US Environmental Protection Agency, Duluth, Minnesota

[b]Upper Midwest Water Science Center, US Geological Survey, Madison, Wisconsin

[c]Upper Midwest Environmental Sciences Center, US Geological Survey, La Crosse, Wisconsin

[d]Ecosystem Processes Division, US Environmental Protection Agency, Athens, Georgia

[e]Great Lakes Regional Office, US Fish and Wildlife Service, Bloomington, Minnesota

[f]National Oceanic and Atmospheric Administration, Silver Spring, Maryland, USA

[g]Aquatic Toxicology Laboratory, St. Cloud State University, St. Cloud, Minnesota, USA

[*]**Correspondence**: ankley.gerald@epa.gov

07/06/2023; 07/24/2023; 08/27/2023

ABSTRACT

Assessing the ecological risk of contaminants in the field typically involves consideration of a complex mixture of compounds which may or may not be detected via instrumental analyses. Further, there are insufficient data to predict the potential biological effects of many detected compounds, leading to their being characterized as contaminants of emerging concern (CECs). Over the past several years, advances in chemistry, toxicology, and bioinformatics have resulted in a variety of concepts and tools that can enhance the pragmatic assessment of the ecological risk of CECs. The present Focus article describes a 10+- year multiagency effort supported through the U.S. Great Lakes Restoration Initiative to assess the occurrence and implications of CECs in the North American Great Lakes. State-of-the-science methods and models were used to evaluate more than 700 sites in about approximately 200 tributaries across lakes Ontario, Erie, Huron, Michigan, and Superior, sometimes on multiple occasions. Studies featured measurement of up to 500 different target analytes in different environmental matrices, coupled with evaluation of biological effects in resident species, animals from in situ and laboratory exposures, and in vitro systems. Experimental taxa included birds, fish, and a variety of invertebrates, and measured endpoints ranged from molecular to apical responses. Data were integrated and evaluated using a diversity of curated knowledgebases and models with the goal of producing actionable insights for risk assessors and managers charged with evaluating and mitigating the effects of CECs in the Great Lakes. This overview is based on research and data captured in approximately about 90 peer-reviewed journal articles and reports, including approximately about 30 appearing in a virtual issue comprised of highlighted papers published in *Environmental Toxicology and Chemistry* or *Integrated Environmental Assessment and Management*.

KEYWORDS:

Contaminants; Mixtures; Ecological risk; Great Lakes; Review

supplement

Supporting material.Copy_of_GLRI_Focus_Article_SI_Tables_Final_Revised.xlsx

BACKGROUND

Evaluating the threats chemical contaminants pose to aquatic ecosystems is challenging and complex. The number and variety of anthropogenic chemicals known to be present in surface waters increase as new chemicals are introduced and as analytical methods of detection improve. This adds to an already growing pool of unknown (and hence unmeasured) chemicals (e.g., biotic and abiotic transformation products). Further contributing to this complexity, biological effects data exist only for a limited number of individual chemicals (Judson et al., 2009), with little to no information as to how these chemicals interact in complex mixtures. In addition, toxicity tests are commonly conducted using species amenable to laboratory assessments, making it necessary to extrapolate results from these surrogates to the diversity of organisms present in the environment. Indeed, the threat a contaminant poses to an ecosystem must be evaluated relative to not only its concentrations and frequency of detection but also its toxicity to resident species with varying susceptibilities in the context of potential interactions in a complex milieu of co-occurring contaminants and other stressors. As a result, effective prioritization of possibly impacted locations, potential effects, and contaminants on which to focus resources and management actions is an ongoing and critical need.

To address this, the scientific community has developed tools and strategies for integrating chemical and biological data and temporal–spatial metadata to assist the process of prioritization. Modern analytical instruments measure contaminants at vanishingly low concentrations, while providing the resolution and accuracy needed to facilitate identification. This has produced improvements in monitoring large numbers of known contaminants (i.e., targeted analysis) while expanding characterization of complex contaminant mixtures, including unknown contaminants (i.e., nontargeted analysis) that may present risks to biota. Environmental sample collection systems have also improved, allowing the assessment of time-integrated samples that capture changing conditions in ecosystems, and can be matched to in situ tests with organisms (e.g., caged fish) to detect meaningful relationships between contaminant exposure and biological responses. The use of new approach methodologies (NAMs) has facilitated rapid screening of data-poor chemicals, as well as field-collected water and sediment samples for biological activity using cell-based (in vitro) assays capable of detecting chemical impacts on a variety of biological pathways (Kavlock et al., 2018; Villeneuve et al., 2019). Simultaneously, methods for extracting and analyzing high-content data from in vivo experiments employing ‘omics tools (e.g., transcriptomics, proteomics, and metabolomics) have greatly expanded the number of endpoints that can be measured in a single assay. In this way, ‘omics offers a platform for collecting unbiased, hypothesis-generating data sets; discovering novel biomarkers of exposure/effect; and surveilling biota for adverse effects when a priori toxicity information is absent. To ensure that responses measured by these various biological assays are anchored to adverse effects relevant to regulators (e.g., impaired reproduction), the adverse outcome pathway (AOP) concept (Ankley et al., 2010) has become central to modern toxicology. The AOP framework organizes existing information that provides scientific support for causal relationships between measurable effects spanning levels of biological organization, thereby providing the scientific basis for inference and extrapolation of expected hazards based on responses measured at lower levels of organization (e.g., molecular, biochemical, cellular). Finally, predictive models, curated knowledgebases, and bioinformatic tools are being leveraged to integrate chemical and biological data sets for better predicting risks and possible mitigation options, with the ultimate goal of supporting management decision-making concerning complex contaminant mixtures.

While there are many ecosystems threatened by human activities worldwide, the Laurentian Great Lakes basin is prominent as a critical hub of social, economic, and recreational activities, which receives a steady input of anthropogenic chemicals. Decades of industrial, domestic, agricultural, and nonpoint contamination, as well as heavy urban land use in many regions, have introduced complex mixtures of contaminants throughout the basin, raising concerns over the potential impacts to Great Lakes ecosystems and the services they provide. So-called legacy contaminants, which include metals (e.g., lead, arsenic, cadmium, and mercury) and some persistent organic chemicals such as polychlorinated biphenyls (PCBs), remain a priority based on years of monitoring and established biological effects. However, other contaminant classes are garnering increased attention as their widespread presence and potential risks to resident biota are being recognized. Such contaminants include pharmaceuticals, personal care products, steroidal hormones, synthetic musks, perfluorinated chemicals, flame retardants, and numerous others. Labeled contaminants of emerging concern (CECs), these chemicals encompass an enormous diversity of structural and functional properties; and perhaps not surprisingly, there is a general lack of understanding of their risks to aquatic ecosystems, including the Great Lakes. Uncertainties surrounding the nature and risks of these contaminants is reflected in the nondescriptive term CECs, which serves as a catch-all for contaminants that are largely unregulated yet have the potential to produce adverse effects (Diamond & Burton, 2021).

In a binational commitment to protect the Great Lakes, the United States and Canada created the Great Lakes Water Quality Agreement (GLWQA) to provide a framework for identifying priorities and critical actions needed to improve water quality (GLWQA, 2012). The GLWQA includes 10 topical annexes, each identifying specific issues of concern. Annex 3, "Chemicals of Mutual Concern," addresses negative impacts of anthropogenic chemicals on "habitats and biodiversity throughout the Great Lakes." Moreover, Annex 3 identifies some legacy contaminants and CECs as chemicals of mutual concern and incorporates a binational process for nongovernmental stakeholders to nominate chemicals for inclusion. The United States instituted the Great Lakes Restoration Initiative (GLRI) in 2010, "as a non-regulatory program to accelerate efforts to protect and restore the largest system of fresh surface water in the world, and to provide additional resources to make progress toward the most critical long-term goals for this important ecosystem" (GLRI, 2019). Many activities have been initiated in response to the GLRI, one of which involves CECs. Over the past decade, multiple US federal agencies combined efforts to develop and deploy state-of-the-science chemical detection and biological effects-based tools germane to the assessment of environmental contaminants (Ekman et al., 2013; GLRI, 2019). The present Focus article provides a high-level summary of an interagency collaborative research effort undertaken to address objectives under GLRI Focus Area 1 (Toxic Substances and Areas of Concern). Collectively, the GLRI-CEC effort integrated leading-edge approaches in environmental surveillance and monitoring across multiple model organisms, locations, and environmental media to generate insights to aid long-term protection of the Great Lakes. Importantly, the tools and concepts developed and evaluated through the GLRI-CEC program also provide a basis for assessing effects of complex mixtures of CECs in aquatic systems throughout the world to aid in management actions, decision-making, and implementation of best practices.

PROJECT GOALS AND APPROACHES

This collaborative effort featured three primary goals for improving assessment of the impacts of CECs on Great Lakes ecosystems: (1) characterize and evaluate the extent to which CECs threaten fish and wildlife populations in the Great Lakes, (2) develop and pilot state-of-the-art surveillance techniques for biological effects from CECs in the Great Lakes basin, and (3) develop information and tools for resource managers to address CEC threats to fish and wildlife populations. These goals reflect the multidisciplinary nature of Great Lakes surveillance and monitoring and the need for contributions from federal agencies and academic institutions that possess expertise in a range of relevant scientific fields (a full list of participants is provided in Supplemental Information, Table S1). Resources for the GLRI-CEC effort were provided through bipartisan congressional action and administered by the Great Lakes National Program Office of the US Environmental Protection Agency (USEPA).

In 2010, scientists and managers from the Great Lakes National Program Office met with federal and academic colleagues to strategize how to address CEC contamination and its effects in the Great Lakes. These meetings laid the groundwork for GLRI projects conducted under Phase 1 of the effort (2010–2014), where partners commenced investigation of the presence and distribution of CECs in the Great Lakes. For GLRI Phase 2 (2015–2019), activities were designed to refine and expand upon information gathered during Phase 1 efforts, including better definition of potential routes of exposure and impacts on fish and wildlife.

The combined outputs of Phases 1 and 2 can be broadly organized into four categories (Supporting Information, Tables S2, S3): (1) Developing and Refining Methods and Models, (2) Prioritization Approaches and Their Application, (3) Occurrence of Chemical Mixtures and Integrated Biological Assessments, and (4) Hypothesis-Driven Research Addressing Specific Questions. Work carried out under the Developing and Refining Methods and Models category includes advances in methods for measuring and/or predicting biological responses to CECs at the individual and population levels. The Prioritization Approaches and Their Application category was designed to provide screening-level information on chemicals across multiple use classes and identify contaminants with the greatest potential to adversely affect Great Lakes ecosystems. Efforts associated with the third category, Occurrence of Chemical Mixtures and Integrated Biological Assessments, included chemical and biological effects-based monitoring of resident organisms through evaluation of extant populations of invertebrate, fish, and avian species; in situ exposures of test organisms; and laboratory-based studies with chemical mixtures informed by monitoring data. Finally, Hypothesis-Driven Research Addressing Specific Questions included field- and laboratory-based studies designed to determine likely sources and biological effects of chemicals that, despite being routinely detected, had considerable knowledge gaps regarding their ecological risks.

Study site selection was critical to the success of work conducted through the interagency GLRI-CEC collaboration. Study sites were chosen to capture the diversity of land uses, contaminant inputs, and biota characteristic of the Great Lakes basin (Figure 1; Supporting Information, Table S4). The team monitored a consistent (or core) set of sites on multiple occasions (e.g., annually), while also conducting more intensive assessment studies at sites considered especially relevant for specific classes of contaminants (e.g., wastewater-impacted sites for pharmaceuticals) that typically lasted one field season. Hypothesis-driven investigations included in situ studies at sites with specific land cover characteristics and laboratory-based exposures designed to focus on specific contaminants.

To date, the collaborative GLRI-CEC effort has resulted in several agency reports, two software packages, and almost 90 journal articles (Supporting Information, Tables S2 and S3). Many of the associated articles are compiled in a virtual issue comprised of papers appearing in *Environmental Toxicology and Chemistry* or *Integrated Environmental Assessment and Management* (https://setac.onlinelibrary.wiley.com/doi/toc/10.1002/(ISSN)1552-8618.great-lakes; Supporting Information, Table S2). For the purposes of the present Focus article, the publications have been associated with the broad categorical areas noted above; however, many span more than one category. In addition, further analyses of the data generated through this GLRI effort, not addressed in the present study, will continue to be added to the virtual issue.

STUDY CATEGORY 1: DEVELOPING AND REFINING METHODS AND MODELS

A critical aspect of the GLRI-CEC work featured development of methods and adaptation of existing techniques to better understand the occurrence and effects of CECs and their mixtures in the environment. The following illustrations focus on work concerning sample collection, use of NAMs, and forecasting effects of CECs on fish populations.

Sampling techniques to enhance exposure assessment

Advances in the ability to detect large numbers of chemicals at remarkably low (nanograms per liter) concentrations have enabled a better understanding of CECs present in aquatic environments. However, results of instrumental analyses alone do not suffice to predict potential risks of chemicals in aquatic ecosystems. For example, there often are insufficient toxicological data to predict potential adverse effects of detected chemicals. An equally important challenge is defining exposure of organisms in the environment to complex mixtures from a temporal perspective. Most analytical determinations are made using samples that reflect only a “snapshot” of the chemical profile present at a given point of time, which may or may not reflect longer-term exposures relevant to an organism in the environment. Accordingly, an aspect of the work conducted through the GLRI-CEC effort focused on development and application of sampling methods that better represent the temporal relationship between contaminant detections and relevant biological responses.

Kahl et al. (2014) describe an inexpensive, field-deployable, automated system that enables the collection of time-averaged composite samples of surface water. When co-located with in situ deployment of fish (e.g., fathead minnows [*Pimephales promelas*]) or potentially other organisms, this provides a basis for connecting a range of biological responses in the organisms (e.g., changes in gene, protein, or metabolite expression; altered sex steroid concentrations) to the time-averaged concentrations of tens to hundreds of contaminants present in the same water over the same time. This linked caged fish–composite sampler approach was employed at many of the GLRI study sites (e.g., Ankley et al., 2021; Davis et al., 2016; Garcia-Reyero et al., 2022; Li et al., 2017; Maloney et al., 2023a, 2023b), thereby enhancing the ability to associate exposures with effects.

In addition to automated composite sampling, the project made effective use of passive sampling technologies to estimate time-weighted average contaminant concentrations in the water column. Extended-duration sample collection can increase relevance for biological impact assessment over grab sampling. Alvarez et al. (2021) employed passive samplers—semipermeable membrane devices and polar organic integrative samplers—at 69 Great Lakes tributaries during 2010 and 2014. In all, approximately 140 contaminants were detected. Estimated concentrations were then used as a basis for hazard predictions based on in vitro and in vivo bioeffects data from open-access knowledgebases (Alvarez et al., 2021).

NAMs to address data limitations

Over the past several years, a variety of computational and biological techniques collectively referred to as NAMs have been developed to help efficiently address hazard-assessment needs for chemicals with little or no existing toxicity data (Kavlock et al., 2018; Villeneuve et al., 2019). As largely unregulated contaminants, many CECs have only limited available toxicity data, necessitating consideration of NAMs as a basis for generating information to infer the potential toxicity of individual contaminants or complex mixtures. For example, Schroeder et al. (2016) describe two different ways in which high-throughput (HTP) in vitro systems can support ecological hazard assessments using examples from the GLRI-CEC studies. One approach involves mining the large amounts of publicly accessible information generated from single-chemical testing with suites of HTP assays (e.g., the ToxCast database; Judson et al., 2009) to provide bioactivity estimates for data-poor contaminants detected in environmental samples. The second approach involves direct analysis of complex environmental mixtures (e.g., surface water extracts) on HTP platforms that evaluate effects on dozens to hundreds of components of biological pathways. One insight gleaned from the GLRI-CEC HTP data was the frequent detection of bioactivities indicating induction of cytochrome P450 (CYP) isozymes associated with xenobiotic metabolism, as well as the occurrence of a range of chemicals likely to elicit this response (e.g., polycyclic aromatic hydrocarbons [PAHs] and some pharmaceuticals; Blackwell et al., 2017). To support application of some of these NAMs, a variety of methodological adaptations to simplify sample preparation for in vitro testing were required. An example of this from the GLRI-CEC work involved measurement of the in vitro estrogenicity of minimally modified whole-effluent samples (Wehmas et al., 2011).

Responses measured in various in vitro assays can, in many instances, align with key events of existing AOPs (e.g., activation of receptors, inhibition of enzymes). This provides a basis for translation of the HTP information into potential apical effects in organisms in the field. Insights derived from HTP-derived single-chemical data and/or direct HTP assays have been integral to interpretation of many of the field studies conducted through the GLRI-CEC work, several of which used their results to inform AOP-based predictions of effects (Alvarez et al., 2021; Ankley et al., 2021; Baldwin et al., 2022; Blackwell et al., 2017; Corsi et al., 2019; Li et al., 2017; Loken et al., 2023; Maloney et al., 2023a, 2023b; Oliver et al., 2023; Pronschinske et al., 2022;).

Mosley et al. (2018) describe another application of a NAM to better understand exposure and effects of complex mixtures in fish. High-resolution mass spectrometry was used for a comprehensive evaluation of the epidermal mucous metabolome of fathead minnows exposed to effluent from the Duluth (MN, USA) wastewater-treatment plant (WWTP), which discharges into the St. Louis River, a Lake Superior tributary (Cavallin et al., 2016). The results indicated that the mucous metabolite profiles reflected physiological effects of the effluent (e.g., estrogenicity) and enabled identification of chemicals possibly responsible for the effects (e.g., bisphenol A [BPA]).

Novel approaches to predicting population status

Ecological risk assessments typically focus on impacts of chemicals on populations. Consequently, there is a need to translate biological monitoring data, including the types of molecular and biochemical measurements made using NAMs, into population-level metrics. Miller et al. (2013, 2015) used the AOP concept to link biochemical measures of the effects of a pulp and paper mill effluent on white sucker (*Catostomus commersonii*) to changes in population status. The modeling effort employed data from long-term monitoring of white suckers from Jackfish Bay (Lake Superior, Canada) during different phases of mill operation and provided the type of quantitative population forecasts required by risk managers examining risk-mitigation strategies.

Vaugeois et al. (2022) developed a model for predicting the effects of chemicals with differing toxic modes of action on populations of lake sturgeon (*Acipenser fulvescens*), an ecologically important species in the Great Lakes. Based on the life history of the fish, the researchers concluded that chemicals affecting early life stages (e.g., eggs) would have a greater impact on population status than chemicals with modes of action that adversely affect juveniles or adults. This type of mechanism-based insight enables consideration of population-level responses associated with exposure to different types of environmental contaminants, again providing risk assessors/mangers with insights needed to make informed risk-mitigation decisions that could help population recovery efforts.

STUDY CATEGORY 2: PRIORITIZATION APPROACHES AND THEIR APPLICATION

Over the course of the GLRI-CEC project, concentrations of hundreds of organic chemicals were documented in various matrices collected from more than 700 locations within more than 200 Great Lakes tributaries. While this information is valuable, the kinds of questions stakeholders typically want to address include the following: (1) Which CECs should we be most concerned about? (2) How can staff time and resources allocated to contaminant issues make the greatest impact? (3) Will investments in projects like habitat restoration have their intended benefits, or will contaminants continue to cause unacceptable effects? (4) Which contaminants most require additional knowledge to credibly assess risk to Great Lakes ecosystem function? These questions all reflect the need to focus energy and attention on the most important contaminants (i.e., prioritization) in the Great Lakes, a critical consideration given the realities associated with staff and funding limitations.

An area of emphasis over the duration of this GLRI-CEC project has been prioritization to aid in management decisions and risk assessment. For example, LaLone et al. (2014) proposed a framework to leverage existing sources of data to prioritize human and veterinary pharmaceuticals based on potential for unintended effects on nontarget animals. This framework led to construction of a knowledgebase (Berninger et al., 2016) compiling data pertinent to the persistence, fate, and potency of pharmaceuticals. Likewise, it stimulated development of the USEPA’s Sequence Alignment to Predict Across-Species Susceptibility tool (LaLone et al., 2016). Like other GLRI-CEC prioritization strategies that followed, an important philosophy was that, while concentrations in the environment matter, they need to be contextualized relative to concentrations of the chemicals known to (or plausibly able to) produce biological effects.

Broadly speaking, contaminant data were consistent with expected patterns of distribution based on land use, even when assessing potential exposure in unsampled tributaries (Kiesling et al., 2022). Higher contaminant concentrations in water and sediment were generally located in more urbanized areas. There were anticipated associations between sources like WWTP discharges and pharmaceuticals, personal care products, and other wastewater indicators, as well as land-use characteristics. Agricultural-use pesticides were more prominent in watersheds with greater proportions of agricultural land use and at a time of the year when crop-protection products were being applied (Ankley et al., 2021; Baldwin et al., 2016; Loken et al., 2023; Oliver et al., 2023). The basin-wide occurrence tool developed in Kiesling et al. (2022), which incorporates these land-use associations, can be used to help managers prioritize whether to monitor for CECs and their impacts in areas where concentrations are currently unknown.

Prioritization approaches evolved considerably over the span of the project. For example, evaluation of data collected during GLRI Phase 1 (2010–2014) identified 20 contaminants at some sites that approached or exceeded concentrations that caused toxicity in laboratory experiments. These included multiple PAHs, the herbicide atrazine, the insecticide dichlorvos, and the pharmaceuticals ibuprofen and venlafaxine (USEPA, 2022). However, these prioritizations were limited by their reliance on the availability of traditional guideline animal toxicity data (Gefell et al., 2019). Over the course of GLRI Phase 2, prioritization efforts increasingly incorporated other computationally accessible lines of hazard information. For example, bioactivity data from the USEPA's Toxicity Forecaster (ToxCast program; Judson et al., 2009) are available for several thousands of chemicals. Effect concentrations reported in various ToxCast assays do not translate to a bright-line cutoff for potential adverse effects in fish or wildlife. However, relative potency of contaminants in standardized bioactivity assays is meaningful for prioritization. Calculation of the ratio of detected concentrations in the environment and a chemical's potency in a ToxCast assay (an exposure-to-activity ratio) was identified as a useful relative prioritization tool (Blackwell et al., 2017; Corsi et al., 2019; Maloney et al., 2023a). In addition, exposure-to-activity ratios can be summed across multiple chemicals to provide a relative ranking of complex mixtures found in different sites or samples. Consequently, a computational tool for calculating exposure-to-activity ratios (ToxEval; De Cicco et al., 2018) was developed and has been applied in numerous prioritizations (see Baldwin et al., 2022; Oliver et al., 2023; Pronschinske et al., 2022). More recently, curated chemical effects information from the ECOTOX knowledgebase and predictions from a variety of quantitative structure–activity relationships for predicting toxicity, persistence, and bioaccumulation have been incorporated into risk-based prioritization approaches (Maloney et al., 2023a). An updated risk-based prioritization currently is being applied to over 550 chemicals detected in surface water samples, as well as additional compounds detected in mussel and fish tissues and in sediment (E. Maloney, Shell Global, The Hague, Netherlands, personal communication, August 16, 2023).

The GLRI-CEC effort also featured several relatively novel examples of chemical and/or site prioritization. Davis et al. (2016) narrowed a list of 86 detected chemicals to those found to covary with changes in metabolite profiles of fathead minnows caged at the same sites and for the same durations over which the contaminant data were collected. While covariance does not demonstrate causality, lack of covariance provides a sound basis for deprioritizing chemicals as potential causative agents. Elliott et al. (2021) evaluated multiple lines of evidence at specific sites to aid in prioritizing management activities to minimize hazards to fish (Figure 2). Finally, Woolnough et al. (2020) found that CEC concentrations in fish tissue did not correlate with water and sediment detections, highlighting the need for careful understanding of the media type, exposure routes, and chemical-specific adsorption, distribution, metabolism, and elimination processes when prioritizing sampling to support decision-making associated with human health or ecological risks.

Additional efforts are presently underway with completion of active field sampling in 2019 and compilation of the data to integrate all lines of available evidence into an overarching prioritization. A critical feature of this effort is consideration of areas of uncertainty for individual contaminants. By organizing the prioritization into a stepwise process that considers each available line of evidence, the framework will not only parse chemicals into high, medium, or low priority but also suggest action categories based on the extent and quality of available information. Where data quality and confidence are high, priority contaminants may immediately warrant definitive risk assessment and/or management actions. In contrast, where the data availability and/or confidence are low, the priority action may be to collect data critical to moving the contaminant to a higher-confidence category. It is expected that this overarching prioritization will serve as a synthesis of the overall effort, allow questions such as those framed above to be addressed by stakeholders, and provide a strong foundation and rationale for targeted CEC management actions and research that will protect the Great Lakes in the years to come.

STUDY CATEGORY 3: OCCURRENCE OF CHEMICAL MIXTURES AND INTEGRATED BIOLOGICAL ASSESSMENTS

Organisms are exposed to complex mixtures of chemical pollutants that differ spatially and temporally in complexity and concentration (Elliott et al., 2017, 2018). Understanding biological consequences in such a complex system is challenging for many reasons, including data limitations and lack of knowledge concerning potential interactions among contaminants (Nilsen et al., 2019). One approach to assessing mixture effects explored by scientists involved in the GLRI-CEC project focused on the use of various types of available effects/bioactivity data in conjunction with models to forecast possible interactive risks of chemicals detected in environmental samples (Maloney et al., 2023b). However, not every contaminant present will or can be detected, and for many compounds that are detected, no biological effects data exist. Accordingly, integration of biological effects-based approaches along a gradient of environmental control and relevance was employed to help ascertain the ecological consequences of CEC mixture exposures.

Exposing aquatic organisms to CECs, singly or in mixtures, in a laboratory setting can effectively control many factors known to affect toxicity. However, the large number of possible chemical combinations can quickly become unwieldy using component-based test designs (Elliott et al., 2018). In addition, the types of animals amenable for laboratory testing are limited. Despite these challenges, important information about the biological effects of CECs has been assembled through laboratory exposures of fathead minnows, largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), and plain pocketbook mussels (*Lampsilis cardium*) found in Great Lakes tributaries (Blackwell et al., 2022; Cipoletti et al., 2019; Gill et al., 2022). For example, the microbiota of the largemouth bass gut was unaltered by exposure to a mixture of agricultural CECs commonly found in Great Lakes tributaries (Gill et al., 2022). In contrast, co-exposures of freshwater mussels to the same mixture resulted in an increase in pathogenic gut microbiota (Gill et al., 2022). A similar agricultural mixture of CECs at environmental concentrations caused a decline in fecundity of fathead minnows in the second exposure generation (Cipoletti et al., 2018). In addition, adult male minnows exposed to either the agricultural or an urban CEC mixture that included estrogenic chemicals induced plasma vitellogenin (VTG; egg yolk protein), indicating a potentially improper utilization of energy (Cipoletti et al., 2018; Schoenfuss et al., 2021). These examples of laboratory exposure studies facilitate identifying specific pathways/endpoints affected by mixtures and discerning possible cause-and-effect relationships that are difficult to ascertain in field studies. However, they also suffer the limits of ecological relevance with a lack of consideration of multiple routes of exposure and little consideration of dynamics of chemicals in the environment such as fluctuating exposures.

Cause-and-effect relationships in an environmental setting may be clarified through in situ exposures. Organisms can be selected for known exposure history, age, and sex; and reference controls can be used (Kahl et al., 2014). This approach was deployed in multiple Great Lakes tributaries with concurrent collection of water quality, temperature, and contaminant data. On-site laboratory exposures yielded information about altered second-generation fecundity in minnows at field locations with greater agricultural exposure (Cipoletti et al., 2019). Using the same-site waters for freshwater mussel (*Lampsilis* sp.) exposures did not cause changes in gonadal histology but resulted in minor differences in sperm density (Rzodkiewicz et al., 2022). Fathead minnows exposed in cages for 96 h in the Maumee River (OH, USA) allowed for the detection of perturbations in multiple biological pathways, as suggested from prior single-chemical exposures (Ankley et al., 2021). These studies were augmented with 14-day caged sunfish deployments at nearby sites that corroborated physiological changes at agricultural and wastewater-dominated sites (Cipoletti et al., 2020). A relatively novel in situ approach was employed by Cavallin et al. (2016), who conducted fathead minnow reproduction tests on-site at a WWTP using a real-time flow of finished effluent. Although these types of in situ approaches can be logistically intense and subject to unanticipated environmental perturbations (e.g., storms), they nonetheless can yield crucial information about biological pathway disruption while maintaining good environmental control to reduce the number of confounding variables (e.g., organismal homogeneity, exposure history).

Finally, while mixtures to which the organisms are exposed in situ are realistic, even when as many contaminants are measured as possible (e.g., Custer et al., 2016, 2017a), there remains the possibility of unmeasured/unknown contaminants or chemicals whose occurrence preceded sampling. The GLRI-CEC project employed several approaches focused on assessment of resident populations of exposed species. For example, monitoring of tissues of dreissenid mussels in the Great Lakes basin supported the notion that PAHs are mostly land-derived (Kimbrough et al., 2021). Similarly, blood samples taken from lake sturgeon provided information about the occurrence and possible origins of select CECs that might contribute to understanding why sturgeon populations are struggling to recover in the Great Lakes (Banda et al., 2020). For example, studies on juvenile lake sturgeon from a streamside hatchery indicated that although exposure to CECs was occurring, this did not impact thyroid hormone titers enough to affect imprinting in the fish (Hummel et al., 2022). In other studies, collection of resident sunfish found a prevalence of adverse physiological effects at agriculturally dominated sites that would not necessarily have been predicted based solely on contaminant measurements (Cipoletti et al., 2020; Jorgenson et al., 2018; Thomas et al., 2017).

Tree swallows (*Tachycineta bicolor*) have been a particularly important field model for the GLRI-CEC project, in part because of their willingness to inhabit constructed nest boxes at sites of interest. This enables direct assessment of reproductive success of the birds (Custer et al., 2018, 2019). Samples also can be collected from the tree swallows to help determine the possible occurrence of unmeasured or unknown contaminants through use of a variety of biomarkers that respond to different chemical classes (Custer et al., 2017b). For example, induction of hepatic CYP activity has been used to indicate exposure to PCBs, dioxins/furans, and/or PAHs, as well as unknown contaminants that interact with the aryl hydrocarbon receptor. Genetic damage in red blood cells has been used to determine whether there might be exposure to clastogenic chemicals, such as some metals. In addition to measurement of targeted biomarkers, the GLRI-CEC tree swallow work has employed nontargeted ‘omics measurements of perturbations in genes and pathways that may allow association with specific chemical exposures (Tseng et al., 2023). An example of the insights afforded through use of biomarkers in tree swallows occurred in nest box studies along the Maumee River (OH, USA), where birds nesting at sites lower in the drainage had elevated CYP activity at sites with elevated PAHs (Custer et al., 2020), suggesting a possible causal link. Alterations in apical endpoints related to reproduction also have provided a basis for identifying problematic chemicals. At two sites along the Maumee River there were observations of reduced hatching success in tree swallows that were correlated with higher exposure to polybrominated diphenyl ethers (Custer et al., 2020), suggesting the need for additional studies focused on potential adverse effects of these poorly understood CECs in Great Lakes systems. These types of field studies, while labor-intensive, are invaluable for directly measuring possible effects, as well as generating testable hypotheses for controlled laboratory or modeling studies. Furthermore, studies with resident species directly relate to the actual environmental conditions natural resource managers encounter when managing populations and implementing restoration efforts.

STUDY CATEGORY 4: HYPOTHESIS-DRIVEN RESEARCH ADDRESSING SPECIFIC QUESTIONS

Observations of unusual/unexpected contaminant profiles or biological responses in complex field settings often are not readily interpretable relative to identifying causes or consequences. For example, high concentrations of pharmaceuticals in the vicinity of a

WWTP discharge often occur and could be of ecological concern, but unexpected observations of elevated levels of a contaminant (or class of contaminants) with no definable source can be of as much concern. Analogously, an abnormal biological response in caged or field-collected fish may be relatively easy to explain; for example, elevated plasma VTG (normally only found in females) in male fish is an unambiguous indicator of exposure to estrogenic chemicals. Conversely, some biological changes can be much less interpretable. Virtually any multicomponent monitoring study will yield results requiring further research. Chemical and biological data from the GLRI-CEC studies have provided many opportunities to design and conduct hypothesis-driven research to examine the cause(s) and/or significance of field observations. Below are four illustrative examples.

Reproductive consequences of an estrogenic effluent

Studies worldwide have shown that municipal WWTP effluents can cause estrogenic responses such as feminization of male fish. Various GLRI-CEC studies documented in vitro and/or in vivo estrogenicity at several locations around the Great Lakes, including near the Duluth WWTP. However, the observation of biological effects does not necessarily identify the chemicals causing estrogenicity or whether this may translate to adverse apical effects, for example, on reproduction. The Duluth WWTP offered a unique opportunity to directly address these questions using a 3-week reproduction study with fathead minnows continually exposed to the treated effluent just prior to discharge (Cavallin et al., 2016). Several known estrogenic chemicals were monitored over the course of the study, but only two were consistently detected, the plasticizer BPA and the naturally occurring steroid estrone (E1). In vitro measures of estrogenicity of the effluent revealed an order-of-magnitude variation in activity over time, and the effluent induced VTG in male fish, confirming that it is estrogenic in vivo. Finally, although 100% effluent caused reproductive effects in the fish, this appeared not to be due to its estrogenic properties. Further—and perhaps of most practical importance to the site—dilution of the effluent to concentrations simulating those in the immediate environment of the discharge completely obviated the reproductive effects.

An unexpected environmental estrogen

A second example of the GLRI-CEC fieldwork contributing to hypothesis-based research also involved fish reproductive endocrinology. Prior studies with caged fathead minnows from effluent-impacted GLRI sites (and other locations around the United States) had noted elevated concentrations of the highly potent endogenous steroid 17β-estradiol (E2) in plasma of male fish, which under normal conditions do not have detectable E2 (Ankley et al., 2017). However, E2 was not detected in the vicinity of the caged fish, indicating that it was not accumulated from the water. Also, the less potent E1 usually was present, leading to the hypothesis that elevated plasma E2 might be derived from E1 via a reaction catalyzed by 17β-hydroxysteroid dehydrogenases, a family of enzymes involved in steroidogenesis in vertebrates, including most fish species (Tapper et al., 2021).

Laboratory studies confirmed the ability of male fathead minnows to convert exogenous E1 into endogenous E2, resulting in levels of plasma E2 sufficient to cause estrogenic responses such as induction of VTG (Ankley et al., 2017; Tapper et al., 2021). Previous studies had largely discounted E1 as a contributor to in vivo effects, compared to more potent estrogens such as E2 and the synthetic estrogen 17α-ethinylestradiol. These experimental insights suggest that E1 is an important contaminant to consider not only in the Great Lakes but at sites around the world where feminized fish have been observed.

Targeting a priority pharmaceutical

A defining characteristic of many CECs detected in the environment are insufficient effects data to assess potential hazard or risk. With the extensive number of chemicals monitored/detected in water, sediment, and biological tissues in the GLRI studies, it was not surprising that many data-poor contaminants were identified. One prominent example was metformin, a drug widely used to treat diabetes, and its primary metabolite guanylurea. Not only were both frequently present at elevated concentrations, but some reports in the literature suggested that metformin may cause reproductive endocrine disruption in fish (Niemuth & Klaper, 2015). To address the dearth of high-quality effects data, comprehensive studies were undertaken to assess possible endocrine and reproductive toxicity of the two chemicals (Blackwell et al., 2022). Neither metformin nor guanylurea appeared to be endocrine-active or to cause reproductive dysfunction, even at concentrations far exceeding those observed in the GLRI samples. This suggests that metformin may not be a priority chemical for routine monitoring.

Identifying a widespread source of PAHs

A long history of industrial, agricultural, and other anthropogenic activities has resulted in a legacy of contaminated sediments in many Great Lakes tributaries and estuaries. Some of these contaminants have been significantly reduced or eliminated through regulation and remediation. Other contaminants have substantial modern sources and thus continue to accumulate in aquatic sediments. Polycyclic aromatic hydrocarbons are one such class of contaminants. With a combination of modern and legacy sources of contamination, PAHs have been identified as CECs at 61% of Great Lakes areas of concern, and initial monitoring for the GLRI-CEC identified PAHs as a class of contaminants likely to have adverse effects in many Great Lakes tributaries (USEPA, 2022). Accordingly, a multiple-lines-of-evidence approach was used to identify likely sources of PAHs in surficial streambed sediment samples from 71 Great Lakes tributary locations in 26 watersheds (Baldwin et al., 2020). Based on chemical profile correlations, principal component analysis, positive matrix factorization source-receptor modeling, mass fractions analysis, and land-use correlations, coal tar–sealed pavement was identified as the likely source of PAHs in most sampling locations. Common PAH sediment toxicity thresholds were exceeded at 41% to 62% of sites. With PAH sources continuing to contribute contamination and the tendency of PAHs to deposit into the bed sediments of streams, estuaries, and lakes, these results have consequential implications for the health of aquatic life in the Great Lakes basin.

THE GLRI-CEC PROJECT: INSIGHTS AND LESSONS

Evaluating the potential effects of complex mixtures of contaminants long has been one of the greatest challenges facing ecological risk assessors. Although environmental chemists have made advances in detecting ever larger numbers of chemicals in the environment, this has not necessarily reduced risk-assessment uncertainties. There still is no guarantee that all chemicals of biological significance are measured, and there often are insufficient data to estimate the biological effects of chemicals that are detected. Consequently, it is difficult to establish a chemical(s) of greatest potential concern. Further, understanding the interactions of chemical mixtures in terms of producing effects remains an inexact science. A critical avenue to addressing these various challenges has been integration of biological effects-based measures (or predictions) with chemical monitoring. Over the past 10+ years there have been notable advances in methods, models, and curated knowledgebases that can be applied to effects-based analysis of complex environmental mixtures. The GLRI-supported work captured in this virtual issue describes the development and practical application of an array of effects-based assessment approaches used in conjunction with extensive chemical monitoring data to provide insights as to the occurrence and management of CECs in the Great Lakes.

As the types of tools and approaches described in this paper have been applied to assessing CEC issues in the Great Lakes, several themes have emerged that both guide further investigation and support decision-making by stakeholders. As a simple illustrative example, multiple lines of evidence indicate that while estrogenic chemicals and/or activity occur at many Great Lakes sites, the potential for widespread adverse effects in aquatic life appears to be low. Similarly, we found very few situations where there was substantial potential for short-term adverse effects of chemicals (e.g., lethality) in aquatic species, a marked improvement in conditions occurring at many Great Lakes locations in the past. Nonetheless, both direct evidence and indirect evidence from the laboratory and field in the context of the suite of assays and endpoints used for this work indicate the potential for longer-term sublethal alterations in organisms in the Great Lakes ecosystem via pathways associated with basic metabolic function. In addition, at several agriculturally impacted sites, such as the Maumee River, data suggest the potential for impacts associated with herbicides, which in the past have often received little attention in terms of ecological effects. Prioritization work conducted through the GLRI effort identified several CECs that require additional toxicity and monitoring data to adequately address potential risk. Just as importantly, the work identified contaminants that seem not to be associated with biological effects and could be deemphasized by risk assessors/managers. Significantly, some of the prioritized chemicals—prominently PAHs—could be perceived as legacy contaminants yet, because of new or continued inputs, could be of increasing biological concern. For additional insights and details concerning practical implications resulting from the GLRI-CEC work, the reader is encouraged to consult the various publications in Supporting Information, Tables S2 and S3, including summary reports for the project (USEPA, 2022).

Looking forward, while significant progress has been made, the effectiveness of approaches for assessing CECs present in complex aquatic systems such as the Great Lakes would benefit from the refinement of existing methods, as well as modification of strategies for their practical application (Table 1). For example, to augment exhaustive targeted analytical techniques such as those used for this GLRI work, an increased focus on the development of nontargeted methods for broadening assessments of complex contaminant mixtures would be beneficial. The field also would benefit from the development of NAMs tailored specifically to ecological as opposed to human health effects of CECs, such as HTP assays for invertebrates and plants. There also is a need to explore approaches that enable a more seamless integration of monitoring data for complex chemical mixtures with prediction of biological effects; the eco-exposome is an example of a recently described concept potentially useful for this purpose (Scholz et al., 2022). Also beneficial would be expanded awareness and attention given to the risks of CEC exposures in the presence of other stressors.

Improvements to Great Lakes health could benefit from advances in these important areas alongside continued pursuit of those highlighted in this virtual issue. Without a doubt, the long-term health of the North American Great Lakes will depend on binational scientific and political support for the implementation of these and other next-generation tools and approaches.

AUTHOR CONTRIBUTION

Gerald T. Ankley, Steven R. Corsi, Christine M. Custer, Drew R. Ekman, Stephanie L. Hummel, Kimani L. Kimbrough, Heiko L. Schoenfuss, Daniel L. Villeneuve: Conceptualization; Writing–original draft; Writing–review & editing.

ACKNOWLEDGMENTS

We thank our many colleagues (Supporting Information, Table S1) who have contributed to aspects of the work described in the present study and the associated publications listed in Supporting Information, Tables S2 and S3, with special thanks to S. Elliott, J. Cavallin, and K. Jensen for helping assemble materials for this paper. We thank E. Smith, T. Nettesheim, M. Tuchman, E. Murphy, and D. Ager from the USEPA’s Great Lakes National Program Office for their support over the course of the GLRI-CEC effort. D. Tillitt and S. Streets provided helpful comments on an earlier version of the manuscript. Funding was provided through the GLRI.

DISCLAIMER

This paper has been reviewed in accordance with USEPA guidelines, but the findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service, the USEPA, or the National Oceanic and Atmospheric Administration. Information products released to the public contain no statements that suggest that the products do not meet US Geological Survey standards of scientific excellence, integrity, and objectivity. Use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US government.

DATA AVAILABILITY STATEMENT

This Focus article includes no original data.

REFERENCES

Alvarez, D. A., Corsi, S. R., De Cicco, L. A., Villeneuve, D, L., & Baldwin, A. K. (2021). Identifying chemicals and mixtures of potential biological concern detected in passive samplers from Great Lakes tributaries using high-throughput data and biological pathways. *Environmental Toxicology and Chemistry*, 40(8), 2165–2182. https://doi.org/10.1002/etc.5118

⃠Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., Mount, D. R., Nichols, J. W., Russom, C. L., Schmieder, P. K., Serrano, J. A., Tietge, J. E., & Villeneuve, D. L. (2010). Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry*, *29*, 730–741. https://doi.org/10.1002/etc.34

Ankley, G. T., Feifarek, D., Blackwell, B., Cavallin, J. E., Jensen, K. M., Kahl, M. D., Poole, S., Randolph, E., Saari, T., & Villeneuve, D. L. (2017). Re-evaluating the significance of estrone as an environmental estrogen. *Environmental Science & Technology*, 51(8), 4705−4713. 10.1021/acs.est.7b00606.

Ankley, G. T., Berninger, J. P., Blackwell, B. R., Cavallin, J. E., Collette, T. W., Ekman, D. R., Fay, K. A., Feifarek, D. J., Jensen, K. M., Kahl, M. D., Mosley, J. D., Poole, S. T., Randolph, E. C., Rearick, D., Schroeder, A. L., Swintek, J., & Villeneuve, D. L. (2021). Pathway-based approaches for assessing biological hazards of complex mixtures of contaminants: A case study in the Maumee River. *Environmental Toxicology and Chemistry*, 40(4), 1098–1122. https://doi.org/10.1002/etc.4949

Baldwin, A. K., Corsi, S. R., De Cicco, L. A., Lenaker, P. L., Lutz, M. A., Sullivan, D. J., & Richards, K. D. (2016). Organic contaminants in Great Lakes tributaries: Prevalence and potential aquatic toxicity. *Science of The Total Environment*, 554–555, 42–52. https://doi.org/10.1016/j.scitotenv.2016.02.137

Baldwin, A. K., Corsi, S. R., Oliver, S. K., Lenaker, P. L., Nott, M. A., Mills, M. A., Norris, G. A., and Paatero, P. (2020). Primary sources of polycyclic aromatic hydrocarbons to streambed sediment in Great Lakes tributaries using multiple lines of evidence. *Environmental Toxicology and Chemistry*, 39(7), 1392–1408. https://doi.org/10.1002/etc.4727

Baldwin, A. K., Corsi, S. R., Stefaniak, O. M., Loken, L. C., Villeneuve, D. L., Ankley, G. T, Blackwell, B. R., Lenaker, P. L., Nott, M. A., & Mills, M. A. (2022). Risk-based prioritization of organic chemicals and locations of ecological concern in sediment from Great Lakes tributaries. *Environmental Toxicology and Chemistry*, 41(4), 1016–1041. https://doi.org/10.1002/etc.5286

Banda, J. A., Gefell, D., An, V., Bellamy, A., Biesinger, Z., Boase, J., Chiotti, J., Gorsky, D., Robinson, T., Schlueter, S., Withers, J., & Hummel, S. L. (2020). Contaminants of emerging concern: Body burden characterization in lake sturgeon. *Environmental Pollution*, 266(1), 11501 https://doi.org/10.1016/j.envpol.2020.115051

Berninger, J. P., LaLone, C. A., Villeneuve, D. L., Ankley, G. T. (2016). Prioritization of pharmaceuticals for potential environmental hazard through leveraging a large-scale mammalian pharmacological dataset. Environmental Toxicology and Chemistry. 40(4):Pages 1098-1122. https://doi.org/10.1002/etc.4949.

Blackwell, B. R., Ankley, G. T., Corsi, S. R., DeCicco, L. A., Houck, M. K., Judson, R. S., Li, S., Martin, M. T., Murphy, E., Schroeder, A. L., Smith, E. R., Swintek, J., & Villeneuve, D. L. (2017). An “EAR” on environmental surveillance and monitoring: A case study on the use of Exposure–Activity Ratios (EARs) to prioritize sites, chemicals, and bioactivities of concern in Great Lakes waters. *Environmental Science & Technology*, 51(15), 8713–8724. https://doi.org/10.1021/acs.est.7b01613

Blackwell, B. R., Ankley, G. T., Biales, A. D., Cavallin, J. E., Cole, A. R., Collette, T. W., Ekman, D, R., Hofer, R. N., Huang, W., Jensen, K. M., Kahl, M. D., Kittelson, A. R., Romano, S. N., See, M. J., Teng. Q., Tilton, C. B., & Villeneuve, D. L. (2022). Effects of Metformin and its metabolite Guanylurea on Fathead Minnow (*Pimephales promelas*) Reproduction. *Environmental Toxicology and Chemistry*, 41, 2708–2720. https://doi.org/10.1002/etc.5450

Cavallin, J. E., Jensen, K. M., Kahl, M. D., Villeneuve, D. L., Lee, K. E., Schroeder, A. L.,Mayasich, M., Eid, E. P., Nelson, K. R., Milsk, R. Y., Blackwell, B. R., Berninger, J. P., LaLone, C. A., Blanksma, C., Jicha, T., Elonen, C., Johnson, R., & Ankley, G. T. (2016). Pathway-based approaches for assessment of real-time exposure to an estrogenic wastewater treatment plant effluent on fathead minnow reproduction. *Environmental Toxicology and Chemistry*, 35(3), 702–716. https://doi.org/10.1002/etc.3228

Cipoletti, N., Jorgenson, Z. G., Banda, J. A., Kohno, S., Hummel, S. L., & Schoenfuss, H. L. (2020). Biological consequences of agricultural and urban land-use along the Maumee River, a major tributary of the Laurential Great Lakes watershed. *Journal of Great Lakes Research*, 48, 1001–1014. https://doi.org/10.1016/j.jglr.2020.04.013

Cipoletti, N., Jorgenson, Z. G., Banda, J. A., Hummel, S. L., Kohno, S., & Schoenfuss. H. L. (2019). Land use contributions to adverse biological effects in a complex agricultural and urban watershed: A case study of the Maumee River. *Environmental Toxicology and Chemistry*, 38(5), 1035–1051. https://doi.org/10.1002/etc.4409

Corsi, S. R., De Cicco, L. A., Villeneuve, D. L., Blackwell, B. R., Fay, K. A., Ankley, G. T., & Baldwin, A. K. (2019). Prioritizing chemicals of ecological concern in Great Lakes tributaries using high-throughput screening data and adverse outcome pathways. *Science of The Total Environment*, 686, 995–1009. https://doi.org/10.1016/j.scitotenv.2019.05.457

Custer, C. M., Custer, T. W., Dummer, P. M., Goldberg, D., & Franson, J. C. (2016). Concentrations and spatial patterns of organic contaminants in tree swallow (*Tachycineta bicolor*) eggs at United States and binational Great Lakes Areas of Concern, 2010–2015. *Environmental Toxicology and Chemistry*, 35(12), 3071–3092. https://doi.org/10.1002/etc.3496

Custer, T. W., Custer, C. M., Dummer, P. M., Goldberg, D., Franson, J. C., & Erickson, R. A. (2017a). Organic contamination in tree swallow (*Tachycineta bicolor*) nestlings at United States and binational Great Lakes Areas of Concern. *Environmental Toxicology and Chemistry*, 36(3), 735–748. https://doi.org/10.1002/etc.3598

Custer, T. W., Custer, C, M., Dummer, P. M., Bigorgne. E., Oziolor, E. M., Karouna-Renier, N., Schultz, S., Erickson, R. A., Aagaard, K., Matson, C.W. (2017b). EROD activity, chromosomal damage, and oxidative stress in response to contaminants exposure in tree swallow (Tachycineta bicolor) nestlings from Great Lakes Areas of Concern. Ecotoxicology. 26(10):1392-1407. doi: 10.1007/s10646-017-1863-7

Custer, C. M., Custer. T. W., Dummer, P. M., Goldberg, D., & Franson, J. C. (2018). Annual variation in polychlorinated biphenyl (PCB) exposure in tree swallow (*Tachycineta bicolor*) eggs and nestlings at Great Lakes Restoration Initiative (GLRI) study sites. *Environmental Monitoring and Assessment*, 190(4), 227. doi: 10.1007/s10661-018-6617-3

Custer, C. M., Custer, T. W., Delaney, R., Dummer, P. M., Schultz, S., & Karouna-Renier N. (2019). Perfluoroalkyl contaminant exposure and effects in tree swallows nesting at Clarks Marsh, Oscoda, Michigan, USA. *Archives of Environmental Contamination and Toxicology*, 77(1), 1–13. 10.1007/s00244-019-00620-1

Custer, C. M., Custer, T. W., Dummer, P. M., Schultz, S., Tseng, C. Y., Karouna-Renier, N., & Matson, C. W. (2020). Legacy and contaminants of emerging concern in tree swallows along an agricultural to industrial radient: Maumee River, Ohio. *Environmental Toxicology and Chemistry*, 39(10), 1936–1952. https://doi.org/10.1002/etc.4792

Davis, J. M., Ekman, D. R., Teng, Q., Ankley, G.T., Berninger, J.P., Cavallin, J. E., Jensen. K, M., Kahl, M. D., Schroeder, L., Villeneuve, D. L., Jorgenson, Z. G., Lee, K. E., & Collette, T. W. (2016). Linking field-based metabolomics and chemical analyses to prioritize contaminants of emerging concern in the Great Lakes basin. *Environmental Toxicology and Chemistry*, 35(10), 2493–2502. https://doi.org/10.1002/etc.3409

De Cicco, L. A., Corsi, S. R. Villeneuve, D. L., Blackwell, B. R., & Ankley, G. T. (2018). ToxEval: Evaluation of measured concentration data using the ToxCast high-throughput screening database or a user-defined set of concentration benchmarks. R Package Version 1.0.0, 2018. https://code.usgs.gov/water/toxEval, doi:10.5066/P906UQ5I

⃠Diamond, J., & Burton, G. A. (2021). Moving beyond the term “contaminants of emerging concern.” Environmental Toxicology and Chemistry, 40, 1527–1529. https://doi.org/10.1002/etc.5022

⃠Ekman, D. R., Ankley, G. T., Blazer, V. S., Collette, T. W., Garcia-Reyero, N., Iwanowicz, L. R., Jorgenson, Z. G., Lee, K. E., Mazik, P. M., Miller, D. H., & Perkins, E.

J. (2013). Biological effects–based tools for monitoring impacted surface waters in the Great Lakes: A multiagency program in support of the Great Lakes Restoration Initiative. *Environmental Practice*, *15*, 409–426.

Elliott, S M., Brigham, M. E., Lee, K. E., Banda, J. A., Choy, S. J., Gefell, D. J., Minarik, T. A., Moore, J. N., & Jorgenson, Z. J. (2017). Contaminants of emerging concern in tributaries to the Laurentian Great Lakes: I. Patterns of occurrence. *Plos One* 12(9):e0182868.

Elliott, S. M., Brigham, M. E., Kiesling, R. L., & Schoenfuss, H. L. (2018). Environmentally relevant chemical mixtures of concern in waters of the United States tributaries to the Great Lakes. *Integrated Environmental Assessment and Management*, 14, 509–518. https://doi.org/10.1002/ieam.4041

Elliott, S, M., Gefell, D. J., Kiesling, R. L., Hummel, S. L., King, C. K., Christen, C. H., Kohno, S., & Schoenfuss. H. L. (2021). Multiple lines of evidence for identifying potential hazards to fish from contaminants of emerging concern in Great Lakes tributaries. *Integrated Environmental Assessment and Management*, 18, 1246–1259. https://doi.org/10.1002/ieam.4561

Garcia-Reyero, N., Arick, M. A. 2nd, Woolard, E A., Wilbanks, M., Mylroie, J. E., Jensen, K, Kahl, M., Feifarek, D., Poole, S., Randolph, E., Cavallin, J., Blackwell, B. R., Villeneuve, D., Ankley, G. T., & Perkins, E. J. (2022). Male fathead minnow transcriptomes and associated chemical analytes in the Milwaukee estuary system. *Scientic Data*, 9(1), 476. doi: 10.1038/s41597-022-01553-6

Gefell, D. J., Banda, J. A., Moore, J. N., Secord, A. L., & Tucker. W. A. (2019). Ecological hazard assessment contaminants of emerging concern in the U.S. Great Lakes Basin. Part B supplement document: Technical resources for ecological hazard assessments of contaminants of emerging concern in freshwater fish. (BTPR3018-2019). U.S. Department of the Interior; U.S. Fish and Wildlife Service. https://digitalmedia.fws.gov/digital/collection/document/id/2250/rec/2

Gill, S. P., Learman, D. R., Annis, M. L., & Woolnough, D. A. (2022). Freshwater mussels and host fish gut microbe community composition shifts after agricultural contaminant exposure. *Journal of Applied Microbiology*, 133, 3645–3658. https://doi.org/10.1111/jam.15801

Great Lakes Restoration Initiative. (2019). Great Lakes Res*toration Initiative action plan III (fiscal year 2020–fiscal year 2024)*. Great Lakes Interagency Task Force. https://www.epa.gov/sites/default/files/2019-10/documents/glri-action-plan-3-201910-30pp.pdf

Great Lakes Water Quality Agreement. (2012). Protocol amending the agreement between the United States of America on Great Lakes water quality, 1978, as amended on October 16, 1983, and November 18, 1987. Signed September 7, 2012. Entered into force February 12, 2013. https://binational.net/agreement/full-text-the-2012-great-lakes-water-quality-agreement/

Hummel, S.L., Bellamy, A., Tucker, W. A., & Eckes, O. T. (2022). Exposure of juvenile lake sturgeon to contaminants of emerging concern (CECs), including polybrominated diphenyl ethers

(PBDEs): Location differences and effects on thyroid hormones. *North American Journal of Fisheries Management*, 42, 123–139. 10.1002/nafm.10732

Jorgenson Z. G., Thomas, L., Elliott, S. M., Cavallin, J. E., Randolph, E. C., Choy, S. J., Alvarez, D. A., Banda, J. A., Gefell, D. J., Lee, K. E., Furlong, E. T., & Schoenfuss, H. L. (2018). Contaminants of emerging concern presence and adverse effects in fish: A case study in the Laurentian Great Lakes. *Environmental Pollution*, 236, 718–733. 10.1016/j.envpol.2018.01.070.

⃠Judson, R., Richard, A., Dix, D. J., Houck, K., Martin, M., Kavlock, R., Dellarco, V., Henry, T., Holderman, T., Sayre, P., Tan, S., Carpenter, T., & Smith, E. (2009). The toxicity data landscape for environmental chemicals. *Environmental Health Perspectives*, *117*, 685–695. https://doi.org/10.1289/ehp.0800168

Kahl, M. D., Villeneuve, D. L., Stevens, K., Schroeder, A., Makynen, E. A., LaLone, C. A., Jensen, K. M., Hughes, M., Holmen, B. A., Eid, E., Durhan, E. J., Cavallin, J. E., Berninger, J., & Ankley, G. T. (2014). An inexpensive, temporally integrated system for monitoring occurrence and biological effects of aquatic contaminants in the field. *Environmental Toxicology and Chemistry*, 33(7), 1584–1595. https://doi.org/10.1002/etc.2591

☑Kavlock, R. J., Bahadori, T., Barton-Maclaren, T. S., Gwinn, M. R., Rasenberg, M., & Thomas, R. S. (2018). Accelerating the pace of chemical risk assessment. *Chemical Research in Toxicology*, *31*, 287–290. https://doi.org/10.1021/acs.chemrestox.7b00339

Kiesling, R. L., Elliott, S. M., Kennedy, J. L., & Hummel, S. L. (2022). Validation of a vulnerability index of exposure to chemicals of emerging concern in surface water and sediment of Great Lakes tributaries of the United States. *Science of the Total Environment*, 830:154618 https://doi.org/10.1016/j.scitotenv.2022.154618

Kimbrough, K., Jacob, A., Regan, S., Davenport, E., Edwards, M., Leight, A. K., Freitag, A., Rider, M., & Johnson, W. E. (2021). Characterization of polycyclic aromatic hydrocarbons in the Great Lakes Basin using dreissenid mussels. *Environmental Monitoring and Assessment*, 193(12), 833. 10.1007/s10661-021-09401-7

LaLone, C., Berninger, J., Villeneuve, D., & Ankley, G. T. (2014). Leveraging existing data for prioritization of the ecological risks of human and veterinary pharmaceuticals to aquatic organisms. *Philosophical Transactions of the Royal Society B Biological Sciences*, 369(1656), 1–10.

LaLone, C.A., D.L. Villeneuve, D. Lyons, H.W. Helgen, S.L. Robinson, J.A. Swintek, Saari T. & Ankley, G.T. (2016). Sequence alignment to predict across species susceptibility (SeqAPASS): A web-based tool for addressing the challenges of species extrapolation of chemical toxicity. *Toxicological Sciences*, 153, 228-245.

Li, S., Villeneuve, D., Berninger, J., Blackwell, B., Cavallin, J., Hughes, M., Jensen, K., Jorgenson, Z., Kahl, M., Schroeder, A., Stevens, K., Thomas, L., Weberg, M., & Ankley, G. (2017). 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.

Science of the Total Environment, 579, 825–837. http://dx.doi.org/10.1016/j.scitotenv.2016.11.021

Loken, L. C., Corsi, S. R., Alvarez, D. A., Ankley, G. T., Baldwin, A. K., Blackwell, B. R., De Cicco, L. A., Nott, M. A., Oliver, S. K., & Villeneuve, D. L. (2023). Prioritizing pesticides of potential concern and identifying potential mixture effects in Great Lakes tributaries using passive samplers. *Environmental Toxicology and Chemistry*, 42(2), 340–366. https://doi.org/10.1002/etc.5491

Maloney, E. M., Villeneuve, D. L, Blackwell. B. R., Vitense, K., Corsi, S. R., Pronschinske, M. A., Jensen, K. M., & Ankley, G. T. (2023a). A framework for prioritizing contaminants in retrospective ecological assessments: Application in the Milwaukee Estuary (Milwaukee, WI). *Integrated Environmental Assessment and Managagement*, 19(5), 1276–1296 https://doi.org/10.1002/ieam.4725

Maloney, E. M., Villeneuve, D. L., Jensen, K. M., Blackwell, B. R., Kahl, M. D., Poole, S. T., Vitense, K., Feifarek, D. J., Patlewicz, G., Dean, K., Tilton, C., Randolph, E. C., Cavallin, J. E., LaLone, C. A., Blatz, D., Schaupp, C. M., & Ankley, G. T. (2023b). Evaluation of complex mixture toxicity in the Milwaukee Estuary (WI, USA) using whole mixture and component-based evaluation methods. *Environmental Toxicology and Chemistry*, 42(6), 1229–1256. https://doi.org/10.1002/etc.5571

Miller, D. H., Tietge, J. E., McMaster, M. E., Munkittrick, K. R., Xia, X., Griesmer, D. A., & Ankley, G. T. (2013). Assessment of status of white sucker (*Catostomus commersoni*) populations exposed to bleached kraft pulp mill effluent. *Environmental Toxicology and Chemistry*, 32(7), 1592–1603. https://doi.org/10.1002/etc.2218

Miller, D. H., Tietge, J. E., McMaster, M. E., Munkittrick, K. R., Xia, X., Griesmer, D. A., & Ankley, G. T. (2015). Linking mechanistic toxicology to population models in forecasting recovery from chemical stress: A case study from Jackfish Bay, Ontario, Canada. *Environmental Toxicology and Chemistry*, 34(7), 1623–1633. https://doi.org/10.1002/etc.2972

Mosley, J. D., Ekman, D. R., Cavallin, J. E., Villeneuve, D. L., Ankley, G. T., & Collette, T. W. (2018). High-resolution mass spectrometry of skin mucus for monitoring physiological impacts and contaminant biotransformation products in fathead minnows exposed to wastewater effluent. *Environmental Toxicology and Chemistry*, 37(3), 788–796. https://doi.org/10.1002/etc.4003

⃠Niemuth, N. J., & Klaper, R. D. (2015). Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. *Chemosphere*, *135*, 38–45.

Nilsen, E., Smalling, K. L., Ahrens, L., Gros, M., Miglioranza, K. S. B., Picó, Y., & Schoenfuss, H. L. (2019). Critical review: Grand challenges in assessing the adverse effects of contaminants of emerging concern on aquatic food webs. *Environmental Toxicology and Chemistry*, 38, 46–60. https://doi.org/10.1002/etc.4290

Oliver, S. K., Corsi, S. R., Baldwin, A. K., Lott, M. A., Ankley, G. T., Blackwell, B. R., Villeneuve, D. L., Hladik, M. L., Kolpin, D. W., Loken, L., De Cicco, L. A., Meyer, M. T., &

Loftin, K. A. (2023). Pesticide prioritization by biological effects in tributaries of the Laurentian Great Lakes. *Environmental Toxicology and Chemistry*, 42, 367–438. https://doi.org/10.1002/etc.5522

Pronschinske, M. A., Corsi, S. R., DeCicco, L. A., Furlong, E. T., Ankley, G. T., Blackwell, B. R., Villeneuve, D. L., Lenaker, P. L., & Nott, M. A. (2022). Prioritizing pharmaceutical contaminants in Great Lakes tributaries using risk-based screening techniques. *Environmental Toxicology and Chemistry*, 41(9), 2221–2239. https://doi.org/10.1002/etc.5403

Rzodkiewicz, L. D., Annis, M. L., & Woolnough. D. A. (2021). Contaminants of emerging concern may poze prezygotic barriers to freshwater mussel recruitment. *Journal of Great Lakes Research*, 48(3), 768–781. https://doi.org/10.1016/j.jglr.2022.04.002

Schoenfuss, H. L., (2021). The effects of contaminants of emerging concern on water quality. In Roy K., Ed: *Chemometrics and cheminformatics in aquatic toxicology*, Chapter 2, pp 23–44. John Wiley & Sons. https://doi.org/10.1002/9781119681397.ch2

⃠Scholz, S., Nichols, J. W., Escher, B. L., Ankley, G. T., Altenburger, R., Blackwell, B., Brack, W., Burkhard, L., Collette, T. W., Doering, J. A., Ekman, D. R., Fay, K., Fischer, F., Hackermuller, J. W., Hoffman, J., Lai, C., Leuthold, D., Martinovic-Weigelt, D., Pollesch, N.,... van Bergen, M. (2022). The eco-exposome concept: An integrated assessment of mixtures of chemicals. *Environmental Toxicology and Chemistry*, *41*, 30–45.

Schroeder, A. L., Ankley, G. T., Houck, K. A., & Villeneuve, D. L. (2016). Environmental surveillance and monitoring: The next frontiers for high-throughput toxicology. *Environmental Toxicology and Chemistry*, 35(3), 513–525. https://doi.org/10.1002/etc.3309

Tapper, M. A., Kolanczyk, R. C., LaLone, C. A., Denny, J. S., and Ankley, G. T. (2021). Conversion of estrone to 17β-estradiol: A potential confounding factor in assessing risks of environmental estrogens to fish. *Environmental Toxicology and Chemistry*, 39, 2028-2040. https://doi.org/10.1002/etc.4828

Thomas L. M., Jorgenson, Z. G., Brigham, M. E., Choy, S. J., Moore, J. N., Banda, J. A., Gefell, D. J., Minarik, T. A., & Schoenfuss, H.L. (2017). Contaminants of emerging concern in tributaries to the Laurentian Great Lakes: II. Biological consequences of exposure. *PLoS ONE* 12(9): e0184725. 10.1371/journal.pone.0184725

Tseng, C. Y., Custer, C. M., T..W. Custer, P. M. Dummer, N. Karouna-Renier, & C. W. Matson. (2023). Multi-omics responses in tree swallow (*Tachycineta bicolor*) nestlings from the Maumee Area of Concern, Maumee River, Ohio. *Science of the Total Environment*, 856:159130. http://dx.doi.org/10.1016/j.scitotenv.2022.159130

US Environmental Protection Agency. (2022). Contaminants of Emerging Concern in the Great Lakes. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-22/057, 2022. https://doi.org/10.23645/epacomptox.21044455

Vaugeois, M., Venturelli, P. A., Hummel, S. L., & Forbes, V. E. (2022). Population modeling to inform management and recovery efforts for lake sturgeon, *Acipenser fulvescens*. *Integrated Environmental Assessment and Management*, 18, 1597–1608. https://doi.org/10.1002/ieam.4578

☑Villeneuve, D. L., Coady, K., Escher, B. I., Mihaich, E., Murphy, C. A., Schlekat, T., & Garcia-Reyero, N. (2019). High-throughput screening and environmental risk assessment: State of the science and emerging applications. *Environmental Toxicology and Chemistry*, *38*, 12–26.

Wehmas, L. C., Cavallin, J. E., Durhan, E. J., Kahl, M. D., Martinovic, D., Mayasich, J., Tuominen, T., Villeneuve, D. L., & Ankley, G. T. (2011). Screening complex effluents for estrogenic activity with the T47D-KBluc cell bioassay: Assay optimization and comparison with in vivo responses in fish. *Environmental Toxicology and Chemistry*, 30(2), 439–445. https://doi.org/10.1002/etc.388

Woolnough, D.A., Bellamy, A., Hummel, S. L., & Annis, M. (2020). Environmental exposure of freshwater mussels to contaminants of emerging concern: Implications for species conservation. *Journal of Great Lakes Research*, 46, 1625–1638. https://doi.org/10.1016/j.jglr.2020.10.001

FIGURE 1 Map of sampling locations for the Great Lakes Restoration Initiative—Contaminants of Emerging Concern collaborative project. Further detail concerning the sampling sites can be found in Supporting Information, Table S4.

FIGURE 2 Predicted vulnerability and hazard and measured biological response at sites where streamside exposure experiments were conducted between 2016 and 2019. Adapted from Elliott et al. (2021; Supporting Information, Table S2).

TABLE 1 Methods and strategies needed to further improve ecological evaluation of contaminants of emerging concern

Needed methods/strategies	**Benefits**
Increased use of nontargeted methods for contaminant monitoring	Greater appreciation for fraction of contaminants not captured by current targeted monitoring methods Identify unknown transformation/degradation products that may prove biologically relevant

Needed methods/strategies	Benefits
	More informed hypothesis generation
Greater development of eco-focused NAMs	Improved relevance Reduced animal use Increased number of assessments Lowered costs
Improved strategies for identifying relationships between stressor measurements and biological effects	Better prioritization of contaminants/sites More efficient resource allocation
Expanded awareness of interactive effects	Greater focus on assessing risks from combined exposures to CECs and Habitat degradation High nutrient loads Low dissolved oxygen Increased water temperatures Elevated chloride, metals, and so on

Abbreviations: NAMs, new approach methodologies; CECs, contaminants of emerging concern.

Figure 1. Map of sampling locations for the Great Lakes Restoration Initiative-Contaminants of Emerging Concern collaborative project. Further detail concerning the sampling sites can be found in SI Table S4.

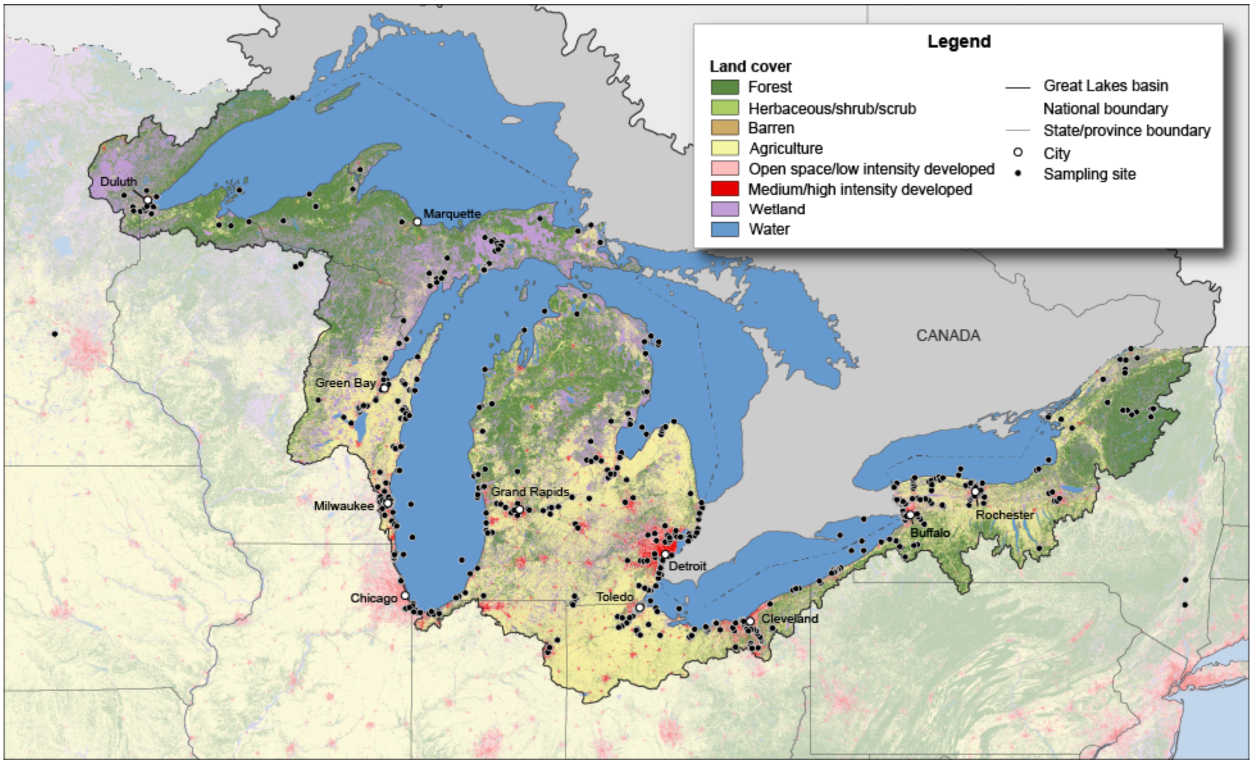

Figure 2. Predicted vulnerability, hazard, and measured biological response at sites where streamside exposure experiments were conducted between 2016 and 2019. Adapted from Elliott et al. (2021; SI Table S2).