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Organic contaminants in Great Lakes tributaries: Prevalence and potential aquatic toxicity

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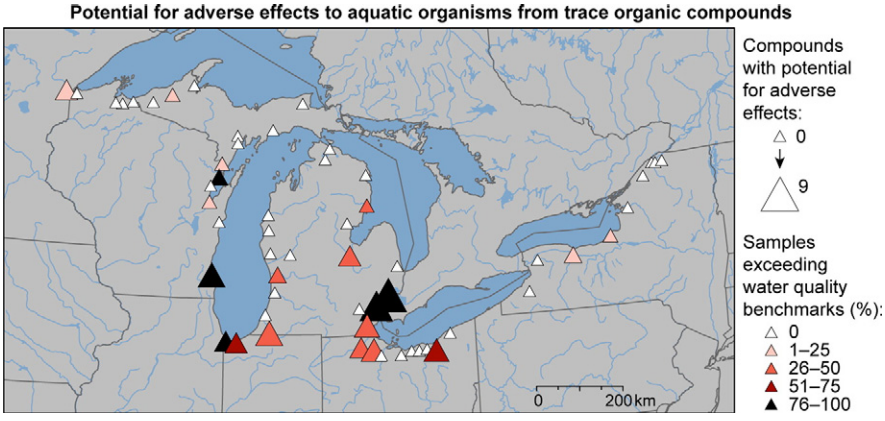
HIGHLIGHTS

- 709 samples were collected from 57 tributaries and analyzed for 69 compounds.
- Compounds commonly occurred as complex mixtures of 10 or more.
- Water-quality benchmarks were exceeded at 35% of the sampled sites.
- Estrogenic effects from nonsteroidal compounds alone were estimated at 18% of sites.
- Urban-related land use characteristics were important predictors of concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic compounds used in agriculture, industry, and households make their way into surface waters through runoff, leaking septic-conveyance systems, regulated and unregulated discharges, and combined sewer overflows, among other sources. Concentrations of these organic waste compounds (OWCs) in some Great Lakes tributaries indicate a high potential for adverse impacts on aquatic organisms. During 2010–13, 709 water samples were collected at 57 tributaries, together representing approximately 41% of the total inflow to the lakes. Samples were collected during runoff and low-flow conditions and analyzed for 69 OWCs, including herbicides, insecticides, polycyclic aromatic hydrocarbons, plasticizers, antioxidants, detergent metabolites, fire retardants, nonprescription human drugs, flavors/fragrances, and dyes. Urban-related land cover characteristics were the most important explanatory variables of concentrations of many OWCs. Compared to samples from nonurban watersheds (<15% urban land cover) samples from urban watersheds (>15% urban land cover) had nearly four times the number of detected compounds and four times the total sample concentration, on average. Concentration differences between runoff and low-flow conditions were not observed, but seasonal differences were observed in atrazine, metolachlor, DEET, and HHCB concentrations. Water quality benchmarks for individual OWCs were exceeded at 20 sites, and at 7 sites benchmarks were exceeded by a factor of 10 or more. The compounds with the most frequent water quality benchmark exceedances were the PAHs benzo[*a*]pyrene, pyrene, fluoranthene, and anthracene, the detergent metabolite 4-nonylphenol, and the herbicide atrazine. Computed estradiol equivalency quotients (EEQs) using only nonsteroidal endocrine-active compounds indicated medium

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to high risk of estrogenic effects (intersex or vitellogenin induction) at 10 sites. EEQs at 3 sites were comparable to values reported in effluent. This multifaceted study is the largest, most comprehensive assessment of the occurrence and potential effects of OWCs in the Great Lakes Basin to date.

1. Introduction

Anthropogenic activities related to industrial, agricultural, domestic, and urban water uses introduce an untold number of organic compounds into the Great Lakes and their tributaries on a daily basis (Bennie et al., 1997; Blair et al., 2013; Venier et al., 2014). Flame retardants, drugs, herbicides, plasticizers, polycyclic aromatic hydrocarbons (PAHs), and other types of compounds enter waterways through wastewater treatment plant (WWTP) discharges, combined sewer overflows, leaking septic and municipal sewer systems, urban and agricultural runoff, industrial discharges, and atmospheric deposition, among others (Barber et al., 2015; Kolpin et al., 2002).

Many of these compounds are associated with endocrine disruption or toxicity in aquatic organisms, resulting in tumors and other deformities, reproductive problems, and declines or collapses in populations (Collier et al., 2013; Ingersoll et al., 2002; Johnson et al., 2013). The ability of these compounds to bioaccumulate (Ismail et al., 2014; Jenkins et al., 2014) creates a risk to organisms higher up the food chain including mink, river otter, bald eagles, osprey, and humans (Hinck et al., 2009; Nilsen et al., 2014). Most drinking water treatment plants do not currently remove many of these compounds from the water supply, creating another exposure route for humans (Kingsbury et al., 2008; Stackelberg et al., 2004).

Studies on OWCs in environmental waters in the United States have generally reported concentrations of individual compounds at concentrations in the nanogram or microgram per liter range, often below water quality benchmarks and drinking water standards (Kolpin et al., 2013; Lee and Rasmussen, 2006; Thomas, 2009). However, low concentrations may still pose a risk to aquatic organisms, as well as organisms at higher trophic levels, because of low-dose effects (Hayes et al., 2003; Oehlmann et al., 2006; Vom Saal and Welshons, 2006), nonmonotonic dose-response curves (Vandenberg et al., 2012), additive and synergistic mixture effects (Brian et al., 2005; Sobolewski et al., 2014; Vandenberg et al., 2012), transgenerational effects (Bhandari et al., 2015; Daughton and Ternes, 1999), and a lack of established water quality benchmarks for many compounds (Stackelberg et al., 2004).

A number of factors may influence the occurrence of OWCs in environmental waters. Among them, land use may be the most important. Streams with upstream urban and (or) agricultural uses have been shown to have more frequent detections and higher concentrations of many organic compounds, compared to streams draining dominantly undeveloped areas (Bryant and Goodbred, 2009; Kingsbury et al., 2008; Nowell et al., 2013). Streamflow may be another important factor. Compounds associated with runoff, such as PAHs, may be found at higher concentrations during higher flow conditions (Baldwin et al., 2013; Thomas et al., 2007). Conversely, compounds with a constant source such as those contributed by wastewater effluent or groundwater may be diluted during high flow conditions and therefore show an inverse relation with streamflow (Kingsbury et al., 2008; Kolpin et al., 2004). For some compounds, concentrations may vary by season. Herbicide concentrations have shown a distinct seasonal pattern in some Midwestern agricultural watersheds, with summertime concentrations one to two orders of magnitude greater than wintertime (Gilliom et al., 2006; Thomas et al., 2007).

The Great Lakes represent 84% of the fresh surface water in North America (US EPA, 2015). Understanding the types of compounds entering the lakes, their spatial distribution, their sources, and the potential biological effects to aquatic communities is crucial to watershed management. Such information helps identify at-risk watersheds and serves as a benchmark for future contaminant reduction strategies and remediation efforts.

During 2010–13, the U.S. Geological Survey (USGS) conducted a study of organic compounds in Great Lakes tributaries across six states in the U.S. The goal of the study was to assess the occurrence and possible adverse biological effects of these compounds in the aquatic environment, and how they vary by land cover, flow regime, and season. A total of 709 water samples were collected from 57 tributaries, representing approximately 41% of the total inflow to the lakes (based on an average inflow of 209,500 cubic feet per second (cfs), Neff and Nicholas, 2005). Each sample was analyzed for 69 organic waste compounds (OWCs), making this the largest study of OWCs in the Great Lakes Basin to date.

2. Methods

2.1. Sampling design

Great Lakes tributary and harbor sites were sampled and samples of surface water were analyzed for OWCs between September 2010 and September 2013. Sampling sites were in Minnesota, Wisconsin, Michigan, Indiana, Ohio, and New York, collocated with existing National Monitoring Network for U.S. Coastal Waters sites (Table 1; Fig. 1). Watershed characteristics for the sites are in Table *SI*-1. Drainage areas ranged from 39 to 6330 mile^2 (mi^2), with mean annual flows from 91 to 7751 cfs (October 2010–September 2013). Watershed land cover varied from dominantly urban (up to 92% of watershed) to agricultural (84%) to forest and wetland (93%). Watershed population densities ranged from as few as 3.3 up to 2498 people/mi^2. Each watershed had at least one wastewater treatment plant (WWTP), and as many as 192. The portion of river flow from WWTP effluent ranged from <1% up to 47%.

OWCs were sampled at 57 tributary and harbor sites. In total, 709 environmental samples were collected. Thirty-eight sites were sampled 1–2 times each, generally during low-flow and medium-flow periods. Though samples at these sites were few, the large number of sites and broad geographic extent provide valuable background information on typical concentrations in Great Lakes tributaries, while also identifying tributaries of potential concern for future studies. The remaining 19 sites were sampled more frequently, with 7–64 samples each, during both runoff and low-flow conditions. The more intense sampling at these sites enabled evaluation of the effects of different streamflow conditions and seasons, and better characterization of concentration ranges.

2.2. Sample-collection

In accordance with USGS protocols (Shelton, 1994), samples were collected and processed in a manner consistent with minimal contamination of organic compounds. Glass or Teflon equipment was used during sample collection and processing, whenever possible. Samples were chilled at 4 °C and shipped overnight to the USGS National Water Quality Lab (NWQL) for analysis.

Sampling methods for OWCs varied by site type. All but eight sites were sampled manually, with whole-water samples were collected using the equal-width-increment (EWI) method (Edwards and Glysson, 1999). Subsamples were composited in a 14-L Teflon churn, homogenized, and churned into a 1-L baked amber-glass bottle. When

Table 1
Sampling locations, dominant land cover, and number of samples collected (n), 2010–2013. [A, water samples collected using an autosampler; ID, identification, HC, Harbor Canal; AgMix, agricultural mix of pasture/hay and crops].

Site name	Map ID	Dominant land cover	n	Site name	Map ID	Dominant land cover	n
St Louis, MN	S1	Wetland	31	Cheboygan, MI	H2	Wetland	2
Nemadji, WI	S2	Wetland	1	Thunder Bay, MI	H3	Wetland	2
Bad, WI	S3	Forest	1	Au Sable, MI	H4	Forest	26
White, WI	S4	Forest	1	Rifle, MI	H5	Forest	2
Montreal, WI	S5	Wetland	1	Saginaw, MI	H6	Crops	31
Presque Isle, MI	S6	Wetland	1	Black, MI	E1	Crops	2
Ontonagon, MI	S7	Forest	30	Clinton, MI [A]	E2	Urban	43
Sturgeon, MI	S8	Forest	1	Rouge, MI [A]	E3	Urban	43
Tahquamenon, MI	S9	Wetland	1	Huron, MI	E4	Urban	2
Manistique, MI	M1	Wetland	1	Raisin, MI [A]	E5	AgMix	44
Escanaba, MI	M2	Wetland	2	Maumee, OH [A]	E6	Crops	64
Ford, MI	M3	Wetland	2	Portage, OH [A]	E7	Crops	64
Menominee, WI [A]	M4	Wetland	40	Sandusky, OH	E8	Crops	2
Peshtigo, WI	M5	Wetland	1	Huron, OH	E9	Crops	2
Oconto, WI	M6	Crops	1	Vermilion, OH	E10	Crops	2
Fox, WI	M7	Crops	7	Black, OH	E11	AgMix	2
Manitowoc, WI [A]	M8	AgMix	43	Rocky, OH	E12	Urban	2
Milwaukee, WI [A]	M9	Urban	45	Cuyahoga, OH	E13	Urban	28
Indiana HC, IN	M10	Urban	2	Grand, OH	E14	Crops	2
Burns, IN	M11	Urban	31	Cattaraugus, NY	E15	AgMix	1
St Joseph, MI	M12	Crops	25	Tonawanda, NY	O1	AgMix	1
Paw Paw, MI	M13	Crops	1	Genesee, NY	O2	AgMix	14
Kalamazoo, MI	M14	AgMix	1	Oswego, NY	O3	AgMix	26
Grand, MI	M15	AgMix	2	Black, NY	O4	Forest	1
Muskegon, MI	M16	Forest	2	Oswegatchie, NY	L1	Forest	1
White, MI	M17	Crops	2	Grass, NY	L2	Forest	1
Pere Marquette, MI	M18	Forest	2	Raquette, NY	L3	Forest	1
Manistee, MI	M19	Forest	2	St Regis, NY	L4	Forest	16
Indian, MI	H1	Forest	2				

time or access prevented using the EWI method, a grab sample from centroid of flow was collected in the 1-L baked amber-glass bottle.

Permanently installed automated samplers (autosamplers) were used to collect samples at 8 of the 19 frequently-sampled sites (Table 1). The autosamplers collected either whole-water flow-weighted low-flow (approximately 40, 100-ml subsamples, collected over 24 h) or runoff event (40–95, 100-millilter subsamples collected over the duration of the event hydrograph, usually between 24 and

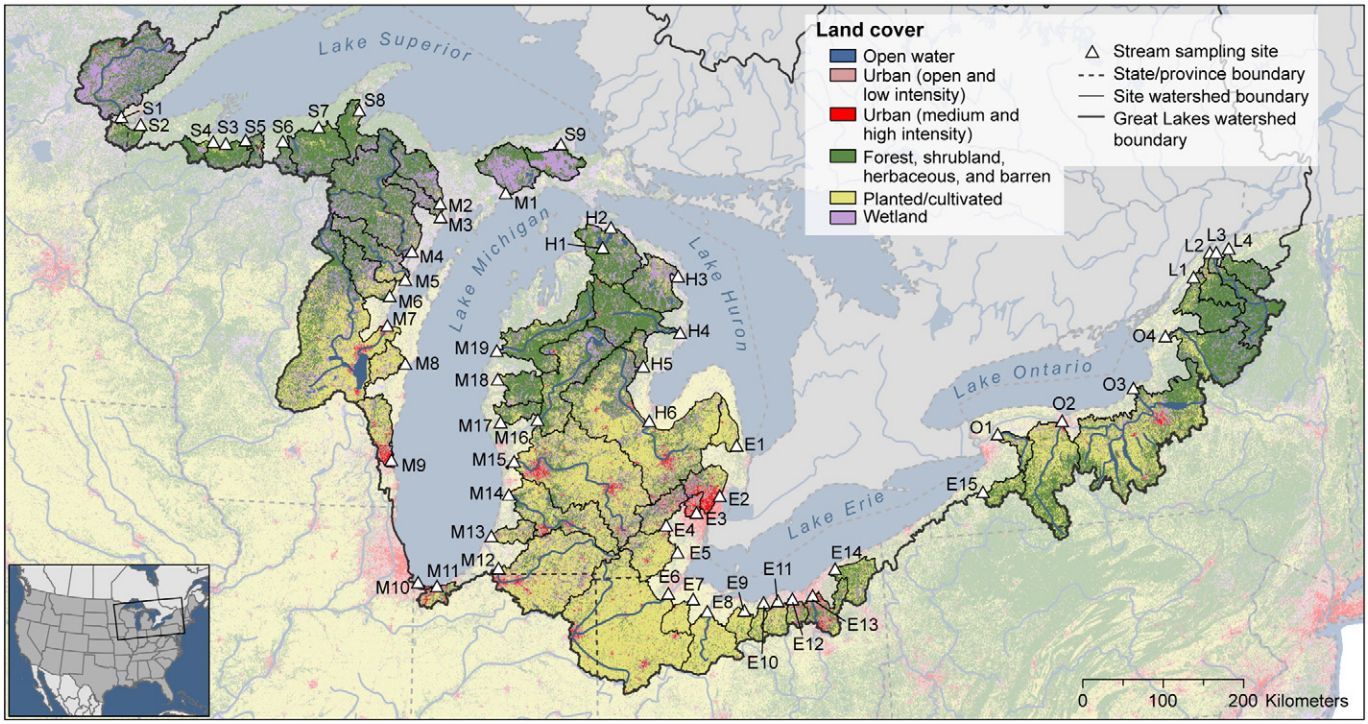

Fig. 1. Location of sampling locations, watershed boundaries, and watershed land-uses. Map IDs are defined in Table 1.

72 h) composite samples. The composited sample was retrieved within 24 h, poured into a 14-L Teflon churn for homogenization, and dispensed into a 1-L baked amber-glass bottle.

Forty-five field blanks and forty-four field replicates were collected along with the environmental samples. Field blanks from the 8 autosampler sites showed higher concentrations for some compounds, indicating potential contamination from the autosamplers. As a result, four compounds were completely omitted (phenol, bis(2-ethylhexyl) phthalate (DEHP), acetophenone, and triethyl citrate), and reporting levels for five other compounds (isophorone, *p*-dichlorobenzene, naphthalene, DEET, and bisphenol A) were artificially raised to the highest reported blank concentrations. In field replicates, the median relative percent different in concentrations was 14%. Detailed results of field blanks and replicates, and laboratory blanks and reagent spikes, are provided in SI.

2.3. Analytical methods

2.3.1. Sample analysis

Samples were analyzed at the USGS NWQL in Denver, Colorado. Whole water samples were analyzed for 69 organic waste compounds (NWQL schedule 4433; Table SI-2). At least 28 of the compounds are associated with human or aquatic toxicity (Kolpin et al., 2002; Zaugg et al., 2006) and 36 are known or suspected endocrine disrupting compounds (EDCs; The Endocrine Disruption Exchange, Inc., 2012). Compounds were extracted using continuous liquid-liquid extraction and methylene chloride solvent, then determined by capillary-column gas chromatography/mass spectrometry (GC/MS; (Zaugg et al., 2006).

2.3.2. Data analysis

Reported concentrations between ½ the RL and the RL were used. Reported concentrations less than ½ the RL were classified as below the detection limit. Dismissing reported detections less than ½ the RL biases the detection frequencies on the low side, but minimizes the risk of reporting false positives. Detection frequencies may also be biased by the different RLs for different compounds; detection frequencies of compounds with higher RLs are likely biased low.

Total sample concentrations were computed by summing all detected concentrations, using zeros for compounds without detections. OWC compounds were aggregated into 15 classes: antioxidants, dyes and pigments, fire retardants, PAHs, plasticizers, fuels, solvents, herbicides, insecticides, antimicrobial disinfectants, detergent metabolites, flavors and fragrances, nonprescription drugs, sterols, and miscellaneous (Table SI-2). The classes are consistent with those used in previous studies (Baldwin et al., 2013; Sullivan et al., 2005; Thomas et al., 2007).

The number of samples from each site varied from 1 to 64. To avoid potentially biasing some analyses by giving greater influence to sites with more samples, mean concentrations and detection frequencies were computed for each site and used for comparisons among different land cover characteristics or different flow conditions. The mean concentrations for individual compounds with at least 30% detection at a given site were estimated using "robust" regression on order statistics (ROS) to account for left-censored values (Helsel, 2012). The choice of 30% detection was based on simulations of different detection frequency by using compounds with >90% detection. At 30% detection frequency, the robust ROS method provided estimates within 5% of the actual mean. For compounds that had <30% detection at a given site, a value of ½ of the lowest mean from sites with >30% detection was substituted. This value was chosen as a simplified way to estimate the mean for compounds given that there are often variable reporting levels. This allowed for estimation of the means for the individual classes of compounds by summing the estimated means for all compounds within the class. For classes where all compounds had <30% detection for a given site, the sum of the means was computed as described above and designated as a left-censored value. These means are only used in the regression analysis to determine relations with watershed attributes.

Relations between streamflow condition (low-flow versus runoff) and OWCs were evaluated for compound class concentrations and for total sample concentrations, using zeros for nondetections. Seasonal differences in compound concentrations were assessed by comparing spring (March–April, $n = 209$), summer (June–August, $n = 208$), autumn (September–November, $n = 173$), and winter (December–February, $n = 119$) samples using the Kruskal–Wallis test with pairwise comparisons ($p < 0.05$; done using the kruskalmc function in the R package pgirmess (Giraudoux, 2015)).

To identify OWC contributions primarily from domestic human wastewater sources (for example, from leaking sanitary sewers and septic systems, or combined sewer overflows), a subset of 20 compounds considered to be likely indicators of those sources (Baldwin et al., 2013) were analyzed. These compounds include all of the fire retardants and detergent metabolites, most of the flavors/fragrances, and the antimicrobial disinfectant triclosan (Table SI-2). Total concentrations of these 20 domestic-wastewater indicator compounds were computed by summing the detected concentrations in each sample, using zeros for nondetections.

Water quality benchmarks for acute and chronic exposure to aquatic life were compiled from a variety of government agencies from the U.S. and Canada, including the U.S. Environmental Protection Agency (EPA; US EPA, 1996, 2012, 2014a, 2014b), the National Oceanic and Atmospheric Administration (NOAA; Buchman, 2008), Oak Ridge National Laboratory (Suter and Tsao, 1996), and the Canadian Council of Ministers of the Environment (CCME; Canadian Council of Ministers of the Environment, 2015; Table SI-3). Benchmarks from these different sources represent different degrees of protectiveness, though most were designed to be protective of sensitive species. Here we use the benchmarks as a general screening tool to assess potential adverse effects. Water quality benchmarks were not known for over half of the compounds analyzed.

Toxicity quotients (TQs) were computed for each site using the available water quality benchmarks, by dividing the greatest measured concentration of a compound at a particular site by the lowest known water quality benchmark for that compound. A TQ greater than 1.0 indicates the compound's toxicity criterion was exceeded in one or more samples from that site, indicating potential for adverse biological effects. Even at TQs less than 1.0 there is potential for adverse effects, though, because of low-dose effects and mixture effects with other compounds present (Brian et al., 2005; Sobolewski et al., 2014; Vandenberg et al., 2012). Therefore compounds with TQs greater than 0.5 were identified here. TQs are likely biased low at sites with fewer samples because the samples may not represent the actual concentration variability in the river.

Potential for endocrine disruption, as measured by intersex and vitellogenin (VTG) induction, was determined by computing 17β-estradiol equivalency quotients (EEQ) for each sample. EEQ's were computed by multiplying measured concentrations of eight estrogenic EDCs (bisphenol A, *p*-dichlorobenzene, 4-nonylphenol, 4-nonylphenol monoethoxylate, 4-nonylphenol diethoxylate, 4-*tert*-octylphenol, 4-*tert*-octylphenol monoethoxylate, and 4-*tert*-octylphenol diethoxylate) by their respective estradiol equivalency factors (EEF) and summing for each sample (Vajda et al., 2008; Table SI-4). EEFs used were the maximum in vitro or in vivo values from literature sources summarized in Vajda et al. (Vajda et al., 2008). The resulting EEQ's were compared to the no observable effect concentrations (NOEC; 0.005 μg/L VTG, 0.001 μg/L intersex) and the lowest observable effect concentrations (LOEC; 0.025 μg/L VTG, 0.01 μg/L intersex) for each endpoint in cyprinid fish (Brion et al., 2004; Jobling et al., 2006; Metcalfe et al., 2001). Using these benchmarks, samples were classified as low-potential (< NOEC), medium-potential (between NOEC and LOEC), or high-potential (>LOEC) for endocrine disruption from each endpoint (Jobling et al., 2006).

Linear regression analysis was used to explore how land cover, population, and wastewater discharge attributes within a watershed may

be related to contaminant concentrations. Mean compound class concentration at each site was the response variable and a suite of watershed attributes were explanatory variables (Table SI-5). Classes of compounds included in the regression analysis were limited to those that had at least 18 sites with detectable concentrations used to compute the mean. This included six compound classes: dyes and pigments, PAHs, herbicides, insecticides, flavors and fragrances, and nonprescription drugs. Land cover and population attributes used as potential explanatory variables included: urban, two categories of agriculture (pasture/hay, and crops), forest, water and wetland, impervious area, and population density. Variables used to describe wastewater effluent influence on the watersheds included: annual effluent as a fraction of streamflow, population served, annual effluent as a fraction of streamflow excluding effluent that was land applied, these three variables weighted by the inverse of the distance to the sampling point, and these three variables weighted by the inverse of mean annual streamflow. Mean annual streamflow was also used as an explanatory variable. To minimize possible spurious relations, a subset of these explanatory variables that made logical sense for each compound class were chosen for use in the stepwise regressions as defined in the supplemental information (Table SI-5). Linear regression models were estimated using stepwise left-censored regression based on the standard Tobit model using maximum likelihood estimation (Tobin, 1958). Variable selection within the stepwise regression was based upon minimization of the Bayesian Information Criterion. Data analyses were done using the R project for statistical computing with core functionality, the censReg package, and the smwrQW package as adapted from the USGS S-PLUS library (Henningsen, 2013; Lorenz et al., 2011; R Core Team, 2015).

GIS methods and quality assurance/quality control, including field blank and replicate samples and laboratory blank and recovery samples, are described in supplemental information.

3. Results

One or more compounds were detected in 92.5% of the 709 samples (Table SI-10). Mixtures of 10 or more compounds were detected in 34% of samples and at 35% of sites, with a maximum of 53 compounds detected in a single sample. The most frequently detected class of compounds was the insecticides, with an overall occurrence rate of 60%. The majority of the insecticide class detections were for two compounds, DEET and carbazole. Other frequently detected classes include the PAHs (43%), herbicides (37%, primarily metolachlor and atrazine), and flavors/fragrances (31%, primarily HHCB and benzophenone). All other compound classes were detected in <25% of samples. The solvents, miscellaneous, and antimicrobial disinfectant classes were the least frequently detected, with occurrence rates of <5%.

3.1. Land cover

Watershed land cover was related to occurrence and concentration for many of the compound classes. A pattern of relatively low concentrations in forest- and wetland-dominated watersheds, moderate concentrations in agriculture-dominated watersheds, and higher concentrations in urban-dominated watersheds was observed for the classes insecticides, PAHs, plasticizers, antioxidants, detergent metabolites, fire retardants, nonprescription drugs, sterols, flavors/fragrances, and dyes/pigments (Fig. 2). The only class with frequent detections which did not follow this pattern was the herbicides, with concentrations in agriculture-dominated watersheds comparable to or greater than those in urban-dominated watersheds.

Samples from urban-dominated watersheds (>15% urban land cover, 8 sites) had a mean of 12.7 detected compounds per sample and a mean total sample concentration of 3.15 μg/L ($n = 196$), whereas samples from nonurban watersheds (49 sites) had a mean of 3.3 detected compounds per sample and a mean total sample concentration of 0.77 μg/L ($n = 513$).

While comparing land cover categories is useful for identifying general trends in concentrations, land cover is actually a gradient. Thus, to better understand how gradients of land cover, population, and wastewater discharge attributes may relate to contaminant concentrations, multiple linear regression analysis was used. Results from this effort indicated that urban-related attributes were the most important explanatory variables for five of the six compound classes analyzed, and an agricultural attribute was most important for herbicides. However, different urban-related attributes were better for estimating different compound classes (Table SI-11). Insecticides were best estimated using the basin's percent urban land cover. PAHs, typically associated with urban runoff, were best estimated using the basin's percent impervious surface. Flavors/fragrances were best estimated using percent urban land cover and the fraction of streamflow from WWTP effluent. Dye/pigment, and nonprescription drugs were best estimated using the basin's population density. The percent crop agriculture land cover was the most important explanatory variable for herbicides, with high population density as a secondary explanatory variable, indicating the importance of agricultural as well as urban settings on herbicide presence in these watersheds.

3.2. Compounds

The most frequently detected compounds in urban watershed samples, occurring in 56–85% of samples, were pyrene, fluoranthene, phenanthrene, benzo(a)pyrene, anthracene (all PAHs), HHCB (also known as galaxolide, a fragrance used in perfumes, soaps, and detergents), 9,10-anthraquinone (dye/pigment and bird repellant), caffeine (human drug), and DEET (insecticide; Table 2). Compounds with the highest concentrations in urban watersheds include 4-nonylphenol and 4-nonylphenol diethoxylate (detergent metabolites), tris(2-butoxyethyl) phosphate (also known as TBEP, a fire retardant), cholesterol and beta-sitosterol (sterols), and 5-Methyl-1H-benzotriazole (antioxidant and component of aircraft deicing fluid).

In nonurban watersheds only DEET had an occurrence rate >50%; metolachlor (herbicide) and fluoranthene had occurrence rates >25%. Most compounds in nonurban watershed samples had occurrence rates less than one percent. Compounds with the highest concentrations in nonurban watersheds include 4-nonylphenol, 4-nonylphenol diethoxylate, cholesterol, beta-sitosterol, metolachlor, and atrazine (Table 2).

Domestic wastewater indicator compounds that are likely to enter the environment through leaking sanitary sewers and septic systems, and combined sewer overflows, had higher detection frequencies and concentrations in samples from urban watersheds. The mean total sample concentration of 20 domestic wastewater indicator compounds was 0.4 μg/L at nonurban watersheds, compared to 1.22 μg/L at urban watersheds.

3.3. Streamflow and seasonality

Streamflow condition had little effect on compound occurrences or concentrations (Fig. SI-1). Samples collected during low-flow and runoff-event periods had a median of 8.2 and 8.5 compounds, respectively (analysis limited to samples from the fourteen sites with at least eight low-flow and eight runoff-event period samples). The median total sample concentration (sum of all compounds) for low-flow samples was 2.33 μg/L, compared to 2.13 μg/L for runoff-event samples.

Seasonal differences in compound concentrations were observed for four compounds, all of which had relatively high detection frequencies. Atrazine and DEET had significantly higher concentrations in summer than in spring, autumn, or winter (Fig. 3). HHCB concentrations were significantly higher in winter. Metolachlor concentrations were significantly higher in spring and summer than in autumn. For some

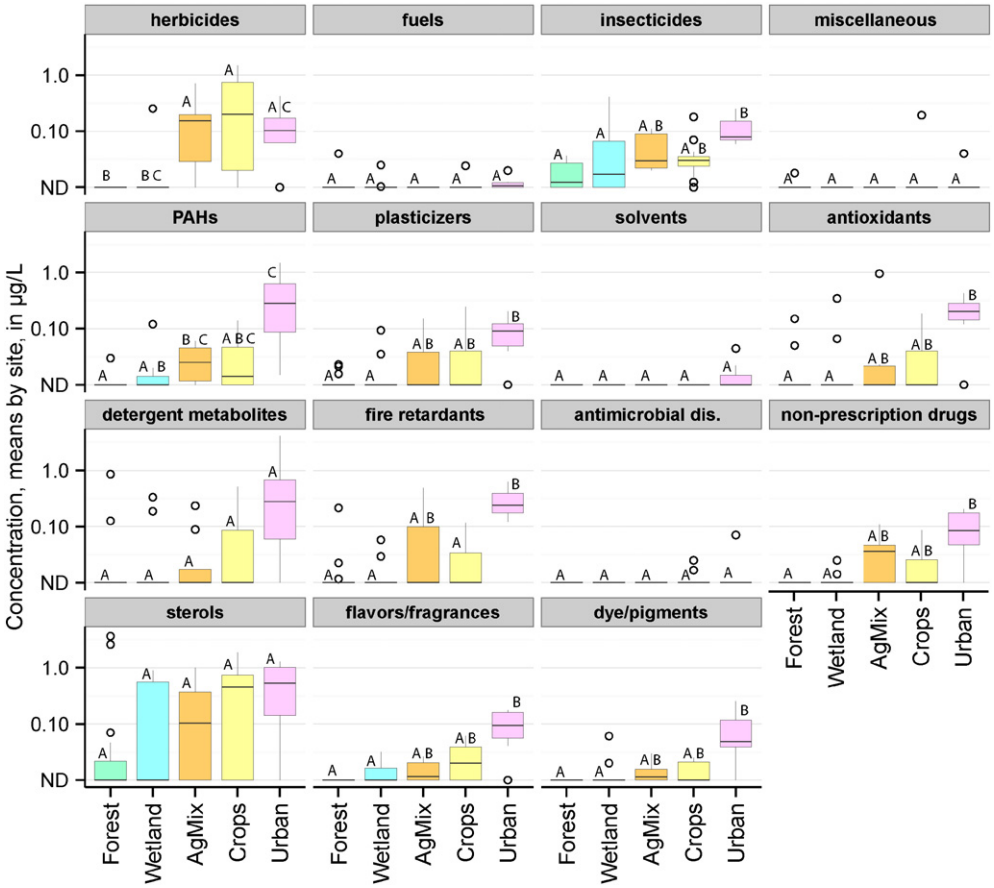

Fig. 2. Dominant land cover and site mean concentrations of compound classes. Number of sites per land use category: forest, 15; wetland, 12; AgMix, 9; Crops, 13; Urban, 8. Boxplot labels A, B, and C indicate which groups of samples are statistically similar (those sharing a common letter) and statistically different (those not sharing a common letter) using the Kruskal-Wallis multiple comparisons test (p-values < 0.05) [ND, not detected; µg/L, micrograms per liter; antimicrobial dis., antimicrobial disinfectants; AgMix, agricultural mix of pasture/hay and crops].

compounds seasonal differences were more apparent at certain sites. Most notably there was a clear seasonal pattern in atrazine and metolachlor in samples from the highly agricultural Maumee and Portage Rivers in Ohio, with summertime concentrations 1–2 orders of magnitude greater than wintertime concentrations, reflecting the application timing of these herbicides (Fig. SI-2).

3.4. Potential toxicity

One or more water quality benchmarks were exceeded in 35% of all water samples. Exceedances were limited to samples from 20 sites; at 37 sites, no benchmarks were exceeded (Fig. 4; Table SI-12). Many of the sites with regular exceedances were those dominated by urban land cover, including Burns, Clinton, Cuyahoga, Rouge, and Milwaukee. Over 90% of samples from Clinton, Rouge, and Milwaukee had one or more exceedance. Water quality benchmarks were exceeded by a factor of 10 (TQ > 10) at seven sites: Clinton, Cuyahoga, Indiana Harbor Canal, Milwaukee, Portage, Rouge, and St. Joseph. The Clinton River had the most compounds with exceedances (9), followed by the Rouge (8), St. Joseph (7), and Milwaukee (6) rivers. An additional 8 sites had compounds of potential concern with TQ_{max} between 0.5 and 1.0.

The compounds with the most frequent water quality benchmark exceedances were the PAHs benzo[a]pyrene, pyrene, fluoranthene, and anthracene, the detergent metabolite 4-nonylphenol, and the herbicide atrazine. Water quality benchmarks for these six compounds were exceeded in 6–15 of the 57 sites (Fig. 5). Water quality benchmarks were exceeded by a factor of 10 or more (up to a factor of 117) for six compounds: pyrene, benzo[a]pyrene, fluoranthene, dichlorvos, atrazine, and anthracene. A disproportionate number of toxicity exceedances occurred in the 10 urban-dominated watersheds. For example, exceedances of pyrene and benzo[a]pyrene water quality benchmarks occurred in 7/10 urban watersheds but in only 8/47 nonurban watersheds.

3.5. Potential endocrine disruption

Sixty-eight percent of sites had detections of endocrine disrupting compounds (EDCs). Mixtures of EDCs (detectable concentrations of two or more) were observed in samples from 61% of sites, and 28% of sites had a median of 5 or more EDCs per sample (up to a median of 10.5 per sample; Table SI-13). Twenty-three percent of sites had ten or more EDCs in a single sample, with a maximum of 21 EDCs in a sample from the Rouge River (Fig. 6).

Using the maximum in vitro or in vivo EEF's from Vajda (Vajda et al., 2008), 47 samples from 9 sites showed medium potential (EEQs between NOEC and LOEC) for intersex induction and 5 samples from 4 sites showed high potential (EEQs > LOEC). 22 samples from 8 sites showed medium potential for vitellogenin induction. No samples showed high potential for vitellogenin induction. The Au Sable River had the most samples with potential estrogenic activity (31%), primarily because of numerous relatively high concentrations of 4-nonylphenol. Other sites with potential estrogenic activity in over 10% of samples included the St. Joseph, Rouge, Saginaw, and Raisin Rivers (28, 28, 23, and 14% of samples, respectively).

4. Discussion

4.1. The influence of urban land cover

This study highlights the complexity of compound mixtures in streams, especially streams with urban influences such as WWTP

Table 2
Occurrence and concentration of organic waste compounds in water samples [Urban, samples from basins with >15% urban land cover, $n = 196$; nonurban, samples from basins with <15% urban land cover, $n = 513$; %, percent; μg/L, micrograms per liter; conc., concentration; ND, not detected; <RL, too few detections to compute; * means computed using left-censored data methods].

Class	Compound	Occurrence (%)		Mean conc. (μg/L)*		Max conc. (μg/L)	
		Urban	Nonurban	Urban	Nonurban	Urban	Nonurban
Antimicrobial disinfectant	*p*-Cresol	2.5	0.5	<RL	<RL	0.05	0.08
	Triclosan	3.7	0.4	<RL	<RL	0.45	0.75
Antioxidant	3-*tert*-Butyl-4-hydroxyanisole	0	0.1	ND	<RL	ND	0.17
	5-Methyl-1H-benzotriazole	38.2	0.7	0.288	0.128	1.02	0.6
	Bisphenol A	32.7	16.1	0.071	0.051	0.98	2.9
Detergent metabolites	4-Cumylphenol	0.8	0.1	<RL	<RL	0.03	0.05
	4-Nonylphenol	4.5	2.2	0.842	0.86	3.4	6.6
	4-Nonylphenol diethoxylate	28.4	1.4	1.16	0.685	5.1	2.8
	4-Nonylphenol monoethoxylate	0	0	ND	ND	ND	ND
	4-*n*-Octylphenol	0	0.1	ND	<RL	ND	0.12
	4-*tert*-Octylphenol	2.9	0	< RL	ND	0.3	ND
	4-*tert*-Octylphenol diethoxylate	24.9	1	0.163	<RL	0.3	0.2
	4-*tert*-Octylphenol monoethoxylate	0	0	ND	ND	ND	ND
Dye/pigment	9,10-Anthraquinone	72.3	14.4	0.101	0.016	0.99	0.37
Fire retardant	2,2′,4,4′-Tetrabromodiphenylether (PBDE 47)	0	0	ND	ND	ND	ND
	Tributyl phosphate	35.1	5.6	0.029	0.025	0.14	0.58
	Tris(2-butoxyethyl) phosphate (TBEP)	48.3	4.9	0.387	0.113	2.53	1.4
	Tris(dichloroisopropyl) phosphate	18.2	0.1	0.127	0.112	0.29	0.18
Flavor/ fragrance	3-Methyl-1H-indole	0	0.1	ND	<RL	ND	0.03
	Acetyl hexamethyl tetrahydro naphthalene	16.7	0.1	0.012	0.01	0.02	0.02
	Benzophenone	29.2	3.7	0.043	0.024	0.2	0.28
	Camphor	3.5	3.4	0.025	0.026	0.08	0.11
	d-Limonene	1	0.1	<RL	<RL	0.28	0.08
	Hexahydrohexamethyl cyclopentabenzopyran (HHCB)	81.2	15.3	0.077	0.014	0.23	0.12
	Indole	1	0.1	<RL	<RL	0.02	0.05
	Isoborneol	0	0.1	ND	<RL	ND	0.09
	Isoquinoline	0.6	0.1	<RL	<RL	0.2	0.1
Fuel	1-Methylnaphthalene	4.9	2.7	0.01	0.011	0.07	0.05
	2,6-Dimethylnaphthalene	2.1	0.4	<RL	<RL	0.02	0.04
	2-Methylnaphthalene	20.8	4.1	0.018	0.012	0.17	0.09
	Isopropylbenzene	0	0.1	ND	<RL	ND	0.07
Herbicide	3,4-Dichlorophenyl isocyanate	8.8	0.5	0.146	0.13	1.1	0.48
	Atrazine	25.9	18.9	0.086	0.143	9.4	40.2
	Bromacil	5.8	2.2	0.062	0.064	0.2	0.26
	Metalaxyl	6.2	0.3	0.051	0.045	0.09	0.18
	Metolachlor	34.6	29.6	0.062	0.131	1.48	6
	Pentachlorophenol	0	0.1	ND	<RL	ND	7.3
	Prometon	3.1	0.3	<RL	<RL	0.32	0.1
Nonprescription drugs	Caffeine	69.9	16	0.109	0.03	0.63	0.55
	Cotinine	10.9	0.5	0.028	0.025	0.08	0.13
	Menthol	1	0	<RL	ND	0.3	ND
Insecticide	Carbaryl	15	0.2	0.044	0.033	0.46	0.26
	Carbazole	42.8	0.7	0.031	0.009	0.34	0.16
	Chlorpyrifos	0	0	ND	ND	ND	ND
	N,*N*-diethyl-meta-toluamide (DEET)	57.1	53.8	0.105	0.064	0.9	2.66
	Diazinon	0	0	ND	ND	ND	ND
	Dichlorvos	7.7	0	0.041	ND	0.29	ND
Miscellaneous	*p*-Dichlorobenzene	1.8	0.2	<RL	<RL	0.13	0.1
	Methyl salicylate	0.4	0.7	<RL	<RL	0.04	0.12
	Tribromomethane (bromoform)	6.2	1.5	0.045	0.044	0.08	0.51
PAH	Anthracene	55.6	3.1	0.016	0.005	0.14	0.03
	Benzo[*a*]pyrene	71.1	10.9	0.077	0.007	1.35	0.19
	Fluoranthene	85.1	27.9	0.188	0.011	3.95	0.53
	Naphthalene	23.7	4.9	0.016	0.008	0.14	0.08
	Phenanthrene	76.3	14.9	0.076	0.007	1.45	0.28
	Pyrene	85.3	22.9	0.157	0.018	2.92	0.37
Plasticizer	Diethyl phthalate	9.3	3	0.15	0.158	0.9	0.7
	Triphenyl phosphate	6	1.9	0.023	0.025	0.07	0.12
	Tris(2-chloroethyl) phosphate	38.3	4.2	0.096	0.044	0.58	0.74
Solvent	Isophorone	2.4	0.3	<RL	< RL	0.88	0.11
	Tetrachloroethene	2.7	0	<RL	ND	0.44	ND
Sterols	3-Beta-Coprostanol	5.5	0.6	<RL	<RL	1.9	7.9
	Cholesterol	26	16.9	0.754	0.488	2.2	4.7
	Beta-Sitosterol	11.7	11.5	0.597	0.547	3.8	4.8
	Beta-Stigmastanol	1.8	0.4	<RL	<RL	1.5	1.8

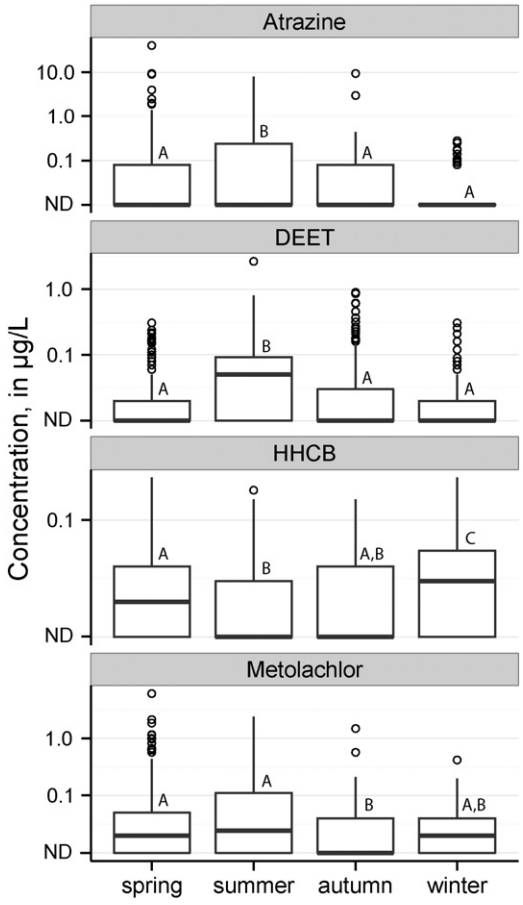

Fig. 3. Seasonal concentrations of atrazine, DEET, HHCB, and metolachlor. Boxplot labels A, B, and C indicate which groups of samples are statistically similar (those sharing a common letter) and statistically different (those not sharing a common letter) using the Kruskal-Wallis multiple comparisons test (p-values <0.05). Spring: March–May, n = 209; summer: June–August, n = 208; autumn: September–November, n = 173; winter: December–February, n = 119 [µg/l, micrograms per liter; DEET, *N*,*N*-diethyl-meta-toluamide; HHCB, Hexahydrohexamethyl cyclopentabenzopyran].

effluent, leaking sanitary sewers and septic systems, combined sewer overflows, industrial discharges, and urban runoff. A relatively low threshold of urban land cover, as low as 15%, had a large impact on the occurrence and concentration of OWCs in this study. There was an approximately fourfold difference in the mean number of detected

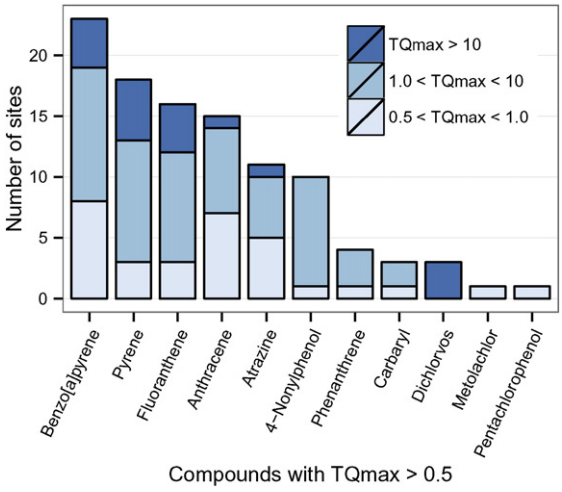

Fig. 5. Compounds with potential for adverse biological effects because of sample concentrations approaching or exceeding water quality benchmarks [TQ_{max}, maximum toxicity quotient].

compounds per sample and the mean total sample concentration between sites with >15% urban land cover and those with <15% urban land cover. Along with other urban-associated factors such as increased stream flashiness, OWCs stress stream ecosystems and contribute to degraded populations of fish, invertebrates, and other organisms (Bell et al., 2012). Numerous studies have shown that stream biotic assemblages begin to degrade where urban land cover percentages exceed 10–15% (Harris et al., 2005; Richards et al., 2010; Roy et al., 2006), similar to the threshold at which a marked increase in OWCs was observed here.

Urban-related land cover variables were important explanatory variables for several compound classes (insecticides, PAHs, herbicides, flavors/fragrances, dyes/pigments, and nonprescription drugs), but for herbicides agricultural land cover was also an important explanatory variable (Table *SI*-11). The seasonal use of herbicides atrazine and metolachlor was clearly visible in samples from the Maumee and Portage Rivers, both of which have heavily agricultural watersheds. Previous studies have reported similar seasonal patterns of herbicides in Midwestern streams (Gilliom et al., 2006; Thomas et al., 2007).

4.2. Aquatic toxicity

The majority of concentrations reported in this study were below established water quality benchmarks. However, in all likelihood the

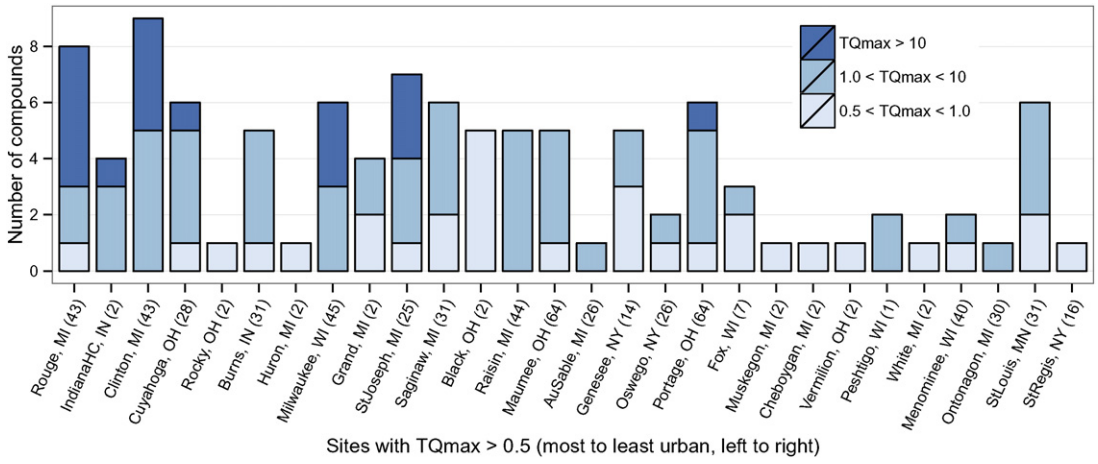

Fig. 4. Sites with compound concentrations approaching (0.5 < TQmax < 1.0) or exceeding (TQmax > 1.0) water quality benchmarks. Total number of samples at each site shown in parentheses [TQ_{max}, maximum toxicity quotient].

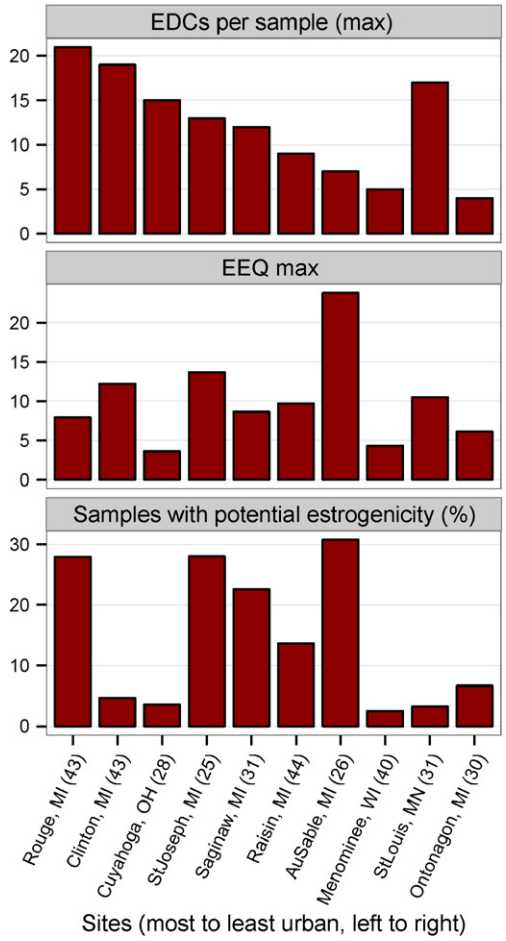

Fig. 6. Detected endocrine disrupting compounds (EDCs) per sample (of 36 EDCs measured), maximum computed 17β-estradiol equivalency quotients (EEQs), and potential estrogenicity via intersex or vitellogenin induction. Total number of samples at each site shown in parentheses. EEQs computed using bisphenol A, *p*-dichlorobenzene, 4-nonylphenol, 4-nonylphenol monoethoxylate, 4-nonylphenol diethoxylate, 4-*tert*-octylphenol, 4-*tert*-octylphenol monoethoxylate, and 4-*tert*-octylphenol diethoxylate. EDCs were detected at 29 additional sites but concentrations were not high enough to induce intersex or vitellogenin.

measured concentrations do not capture the actual range of concentrations at each site, especially at sites with few samples (Crawford, 2004). Also, water quality benchmarks were known for fewer than half of the sampled compounds. So while comparisons to water quality benchmarks are useful for identifying streams where adverse biological effects are likely, these comparisons likely underestimate the potential for adverse effects at streams with few samples or at streams impacted by compounds which lack water quality benchmarks. A number of factors, described below, contribute to the potential for adverse biological effects at low, sub-benchmark concentrations.

Effects from low-dose exposures – doses lower than those tested in toxicology assessments – are commonly observed in studies of endocrine-disrupting compounds (Vandenberg et al., 2012; Vom Saal and Welshons, 2006). For example, doses as low as 0.1 μg/L of atrazine—well below the aquatic toxicity concentration of 1.8 μg/L used in this study (Canadian Council of Ministers of the Environment, 2015)—have been shown to produce gonadal malformations in frogs, including hermaphroditism, multiple gonads, testicular dysgenesis, and testicular resorption (Hayes et al., 2003, 2002; Tavera-Mendoza et al., 2002). Low doses of BPA—for which no water quality benchmark was used in this study—have been shown to induce superfeminization in snails (0.139 μg/L; (Oehlmann et al., 2006)) and mouthpart deformities in the midge *Chironomus riparius* (0.01 μg/L; (Watts et al., 2003)).

Nonmonotonic dose-response curves, which have been reported for many of the compounds included in this study including BPA, nonylphenol, octylphenol, phenanthrene, naphthalene, and atrazine (Vandenberg et al., 2012), add to the likelihood of effects at concentrations below water quality benchmarks. Unlike a monotonic dose-response curve which maintains a consistently positive or negative slope, a nonmonotonic dose-response curve may be shaped like a U or an inverted U, potentially resulting in greater effects at low, untested concentrations. Despite hundreds of studies demonstrating nonmonotonicity in cellular and animal experiments, water quality benchmarks developed by many agencies around the world are based on assumptions of monotonicity (Vandenberg et al., 2012).

Additive or synergistic effects of compound mixtures also increase potency to aquatic organisms. Samples from 35% of sites in the present study had mixtures of 10 or more compounds. Water quality benchmarks are generally developed for individual compounds, but there is increasing evidence of additive or synergistic effects among compounds that act via the same mechanism, and even among those acting on different mechanisms. This can result in adverse biological effects despite low concentrations of individual compounds. Examples of this phenomena include estrogenic compounds (Brian et al., 2005; Jobling et al., 2006; Thorpe et al., 2001; Vajda et al., 2008), PAHs (Chaloupka et al., 1993; White, 2002), and phthalates (U.S. Consumer Safety Commission, 2014). While some of the detected compounds may be antagonists, and thus reduce toxicity, such interactions are less common than additive or synergistic interactions (Jackson et al., 2016).

Transgenerational effects not accounted for in water quality benchmarks may also result in biological effects at low concentrations. Bhandari and others (Bhandari et al., 2015) recently showed that exposing medaka fish to BPA during embryonic development caused no apparent phenotype abnormalities until two and three generations later, when they observed reduced fertilization rates and reduced embryo survival. Other compounds in this study with reported transgenerational effects include benzo[*a*]pyrene (Corrales et al., 2014; White et al., 1999), phenanthrene (Sun et al., 2015), and PAH mixtures (Vignet et al., 2015).

4.3. Estrogenicity

This study identified 52 water samples from 10 sites with a medium to high potential for estrogenic effects. This was based on the combined estrogenicity (EEQ) of eight nonsteroidal endocrine-active compounds and did not account for the estrogen contributions of steroidal estrogens such as 17α-ethynylestradiol, 17β-estradiol, and estrone, which were not sampled in this study. Other studies have found that up to 80% of the EEQ in effluent-affected streams comes from steroidal estrogens (Barber et al., 2011; Körner et al., 2001; Vajda et al., 2008), indicating that our assessment considerably underestimates the actual estrogenic potential.

EEQs reported in this study are in the range of findings from similar studies of effluent-affected streams (Barber et al., 2015; Mitchelmore and Rice, 2006; Vajda et al., 2008). Fifteen of the sites in this study had maximum EEQs within or greater than the range of concentrations seen in WWTP effluent by Vajda et al. (2008; when computed using the same compounds). At three sites, Raisin, Burns Ditch, and Indiana Harbor Canal, the median EEQs were in the effluent range reported by Vajda et al. (Vajda et al., 2008).

With its broad geographic scale, diverse sampling locations, and large number of samples and analytes, this study provides the largest assessment to date of organic contaminants and their potential effects on aquatic organisms in the Great Lakes Basin. Federal, state, and local watershed managers will use this information to help guide management decisions such as prioritizing restoration and implementing best management practices. This information will also be used as a baseline for assessing improvements and other studies in the future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.02.137.

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