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Characterization of Missouri surface waters near point sources of pollution reveals potential novel atmospheric route of exposure for bisphenol A and wastewater hormonal activity pattern ☆

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HIGHLIGHTS

- Assessed contaminants in surface water near chemical release sites.
- Anti-hormonal receptor activities were predictive of wastewater inputs.
- Bisphenol A concentrations were greater in surface water near airborne releases.
- Airborne release of bisphenol A may represent underestimated human exposure source.

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ABSTRACT

Surface water contamination by chemical pollutants increasingly threatens water quality around the world. Among the many contaminants found in surface water, there is growing concern regarding endocrine disrupting chemicals, based on their ability to interfere with some aspect of hormone action in exposed organisms, including humans. This study assessed water quality at several sites across Missouri (near wastewater treatment plants and airborne release sites of bisphenol A) based on hormone receptor activation potencies and chemical concentrations present in the surface water. We hypothesized that bisphenol A and ethinylestradiol would be greater in water near permitted airborne release sites and wastewater treatment plant inputs, respectively, and that these two compounds would be responsible for the majority of activities in receptor-based assays conducted with water collected near these sites. Concentrations of bisphenol A and ethinylestradiol were compared to observed receptor activities using authentic standards to assess contribution to total activities, and quantitation of a comprehensive set of wastewater compounds was performed to better characterize each site. Bisphenol A concentrations were found to be elevated in surface water near permitted airborne release sites, raising questions that airborne releases of BPA may influence nearby surface water contamination and may represent a previously underestimated source to the environment and potential for human exposure. Estrogen and androgen receptor activities of surface water samples were predictive of wastewater input, although the lower sensitivity of the ethinylestradiol ELISA relative to the very high sensitivity of the bioassay approaches did not allow a direct comparison. Wastewater-influenced sites also had elevated anti-estrogenic and anti-androgenic equivalence, while sites without wastewater discharges exhibited no antagonist activities.

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1. Introduction

The contamination of surface water sources is nearly ubiquitous, with surface water investigations in the United States and European Union revealing organic wastewater pollutants in 80% and 97% of samples, respectively (Kolpin et al., 2002; Loos et al., 2009). Frequent among these contaminants are endocrine disrupting chemicals (EDCs), which are able to interfere with some aspect of hormone action (Zoeller et al., 2012). As of 2013, approximately 1000 synthetic and naturally occurring chemicals with EDC activity had been identified (The Endocrine Disruption Exchange, n.d.), with exposure linked to a number of adverse health effects (Guillette et al., 1994; Ottinger et al., 2008; Vandenberg et al., 2012). EDCs are capable of acting at much lower concentrations than chemicals without EDC activity that are tested at very high doses in traditional toxicological risk assessments. In addition, EDCs can exhibit non-monotonic dose response curves (resulting in quantitatively and qualitatively different outcomes at low versus high concentrations), and may exert greater effects during critical windows of development, when exposure can derail normal development and lead to adult disease (Vandenberg et al., 2012; Welshons et al., 2003; Myers et al., 2009; vom Saal et al., 2007).

EDCs that interact with the estrogen and androgen receptors are common in surface water around the globe (Kolpin et al., 2002; Loos et al., 2009; Van der Linden et al., 2008). Major sources for these compounds include wastewater effluents (Furuichi et al., 2004; Murk et al., 2002; Belfroid et al., 1999; Pawlowski et al., 2004; Kuch and Ballschmiter, 2001; Williams et al., 2003; Cargouet et al., 2004), industrial discharges (Calafat et al., 2008; Vandenberg et al., 2010; Kassotis et al., 2014), agricultural operations (Hayes et al., 2011; Hayes, 2002; Hayes et al., 2010; Loos et al., 2010), and natural sources (Kolodziej et al., 2004; Kolpin et al., 2014; Sychrová et al., 2012). Two hormonally active contaminants that are routinely found in surface water are ethinylestradiol (EE2), an estrogen receptor agonist (Folmar et al., 2002; Thorpe et al., 2003) used in many oral contraceptives, and bisphenol A (BPA; 4,4′-isopropylidenediphenol, Chemical Abstract Number 80-05-7), a selective estrogen receptor modulator with agonist/antagonist activity as well as antagonist activities for the androgen receptor and other receptor activities (Vandenberg et al., 2012; vom Saal et al., 2007; Reif et al., 2010; Richter et al., 2007; Zoeller et al., 2005), that is used in the production of plastic and numerous other consumer products (Calafat et al., 2008; Latini, 2005; Pak et al., 2007). Both chemicals contribute to estrogenicity in surface water (Johnson and Williams, 2004; Johnson et al., 2000), in part due to the failure of routine wastewater treatment plant (WWTP) processes to adequately remove these and similar compounds from wastewater (Kuch and Ballschmiter, 2001; Westerhoff et al., 2005; Campbell et al., 2006; Braga et al., 2005). Some researchers have found BPA and EE2 to be significant contributions to estrogenicity in surface water, accounting for up to 50% of activity (Cargouet et al., 2004; Sun et al., 2008). However, many others have not found these two chemicals to contribute significant activities and instead report estrone, estradiol, nonylphenol, and/or octylphenol as major contributors (Furuichi et al., 2004; Hashimoto et al., 2005; Cespedes et al., 2005), while others have been unsuccessful in accounting for the majority of observed activity (Murk et al., 2002; Wang et al., 2011). Additionally, another significant source of BPA release into the aquatic environment includes groundwater leachates from common landfills. Indeed, nearly all landfill leachates tested in a recent national survey across the United States contained BPA (95%), with concentrations as great as mg/L (Masoner et al., 2014). BPA contributes up to 84% of the estrogenicity of these leachates, suggesting that the relative contributions in water are likely source dependent (Kawagoshi et al., 2003). Surface water concentrations are reported for these two estrogenic EDCs at between 0.1–1.5 ng/L for EE2 and 3–30 ng/L for BPA (Murk et al., 2002; Belfroid et al., 1999; Kuch and Ballschmiter, 2001; Cargouet et al., 2004; Bhandari et al., in press). Moreover, EE2 and BPA have been reported in surface water at concentrations known to cause adverse effects in wildlife (Petrovic et al., 2004; Papoulias et al., 1999; Colborn, 1995, 2003).

Reporter gene assays have been utilized to assess total receptor activities of complex mixtures of chemicals in the environment (Pawlowski et al., 2004; Zhao et al., 2011; Soto et al., 2003). Reporter gene assays do not provide quantification of any particular chemical or analyte; however, they provide a highly sensitive detection system for multiple chemicals with a specific receptor-mediated mechanism of action and can be used to target hormonally active chemicals for subsequent analytical identification. This is particularly important as the types and concentrations of contaminants in water vary widely (Kolpin et al., 2002; Loos et al., 2009, 2010; Barnes et al., 2008; Lapworth et al., 2012). As estrogenic or androgenic disrupting chemicals can act additively (Silva et al., 2002; Rajapakse et al., 2002; Christiansen et al., 2008; Kortenkamp and Faust, 2010; Ermler et al., 2011), there is a particular cause for concern over mixtures of hormonally active contaminants with a similar mode of action in water. Chemical mixtures in water can also include receptor antagonists coming from wastewater (Ihara et al., 2014; Liscio et al., 2014; Bellet et al., 2012), agricultural operations (Orton et al., 2011), and other sources (Chen et al., 2014). Receptor-based assay systems provide an integrated assessment of hormonal and anti-hormonal activities within a particular receptor group. Receptor-based cell assays do not offer the level of complexity known to regulate endocrine function of vertebrates in vivo; however, they do offer an initial estimate of receptor-mediated transcriptional response of an intact cell.

The goals of this study were to assess the water quality at potentially threatened sites across Missouri and to ascertain whether the type of point source contamination to surface water could be determined based on receptor activities and chemical concentrations present in the water sampled from each location. Six sites were selected for investigation, including two nearby permitted atmospheric release sites of BPA, and four downstream of current or historical WWTP effluent discharge sites (Fig. 1, Table 1). At each site, grab water samples were collected to ascertain chemicals present at a given point in time, and passive samplers were deployed to measure chemicals present in the water at the specific location over approximately 35 days. We hypothesized that 1) concentrations of BPA and EE2 would be greater near permitted airborne release sites and WWTP effluent inputs, respectively, and 2) that BPA and EE2 would be responsible for the majority of estrogenic and BPA for the majority of anti-androgenic receptor activities observed in water samples collected near the respective site types. Concentrations of BPA and EE2 were compared to observed receptor activities of individual chemical standards to assess the contribution to total receptor-based activities. Further, quantitation of a comprehensive set of wastewater compounds was performed to help characterize each site. Altogether, receptor activities and individual chemical concentrations were analyzed to determine point source pollution potential and contamination signatures.

2. Materials and methods

2.1. Selection of sampling sites

Water sampling sites were selected based on their proximity to: 1) Superfund National Priorities List (NPL) locations with reported atmospheric discharges of BPA; or 2) downstream from current or historical municipal wastewater treatment plant discharges. NPL sites are defined under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) as sites warranting further investigation. Missouri NPL discharges of BPA were identified from the U.S. Environmental Protection Agency (US EPA) Toxic Release Inventory (http://www2.epa.gov/toxics-release-inventory-tri-program) with watersheds within the immediate area (<16 km) of the NPL site. Two NPL sites with BPA discharges were selected and water-sampling locations identified for collection of water grab samples and passive water

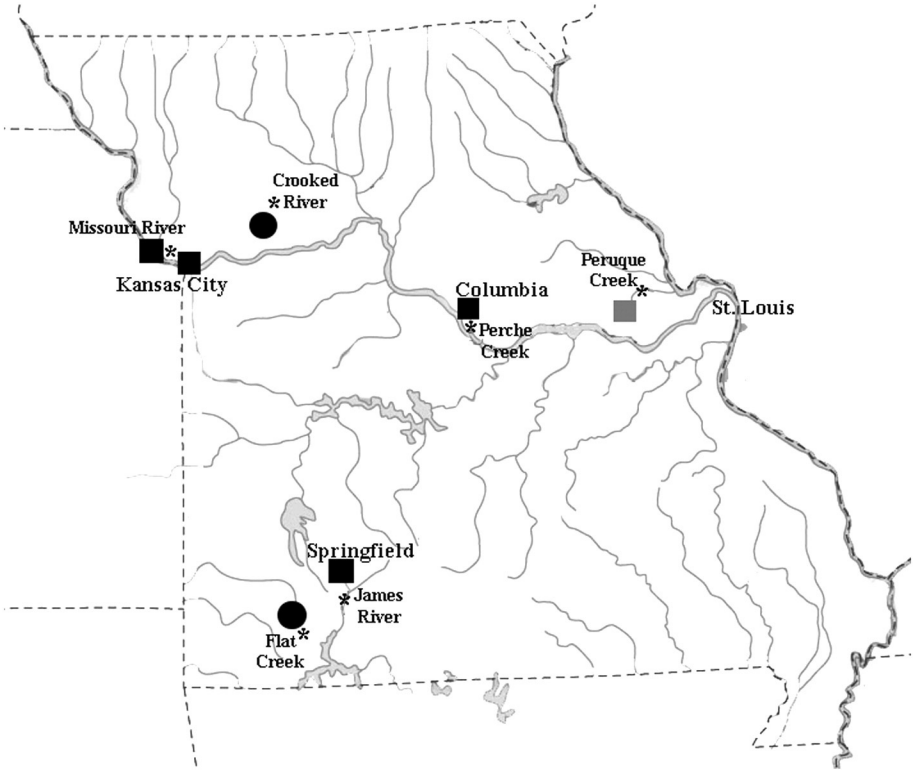

Fig. 1. Map of Missouri sample collection sites and pollution sources. Pictoral representation of the sample collection area throughout Missouri. Asterisks denote sampling sites, with names of sites provided on the map, squares denote nearby WWTPs (black are current facilities and gray is historical), and circles denote permitted BPA airborne release sites for 2012, available via the EPA TOXMAP Geographic Information System at: http://toxmap-classic.nlm.nih.gov/toxmap/main/.

sampling using the polar organic chemical integrative sampler (POCIS; see below). Alternatively, four locations were selected for evaluation of 17α-ethinylestradiol (EE2) in surface waters downstream of current or historical wastewater treatment discharges (Table 1).

2.2. Collection of grab and POCIS water samples

Whole water samples (4 L, n = 1 per site) were collected at the time of deployment of the POCIS. These water samples were transported to the laboratory in coolers and split into aliquots for each type of analysis. Each aliquot was processed separately for measurement of BPA (250 mL/replicate), EE2 (1.0 L/replicate), or steroid receptor reporter assay (SRRA) activity (1.0 L/replicate). At each study site, POCIS were deployed in a protective canister for a period of 35 to 38 days (Supplemental Table 1). The POCIS were constructed according to standard procedures by placing 200 mg of Oasis HLB between two microporous polyethersulfone (0.1 μm pore diameter) membranes (Alvarez, 2010). The membranes were sealed using two metal rings to prevent loss of the sorbent. At the field sites, the samplers were secured to the shore using woven steel cables, taking care to position the samplers in areas with flow but somewhat secluded to protect against vandalism. Flow rates for the rivers at the time of grab sampling and throughout the passive sampling window were assessed using the USGS National Water Information System, available at http://waterdata.usgs.gov/nwis/uv/?referred_module=sw. Station numbers included Crooked River: 06895000, Flat Creek: 07052820, Missouri River (Kansas City): 06893000, and James River: 07050700. While stream flow data was not available for the Peruque Creek or Perche Creek, stations in nearby rivers or creeks were available to gauge rainfall during the POCIS sampling period. These stations included Cuivre River (near Peruque

Table 1
Water sample collection locations and site descriptions.

Site number & name	Near NPL[1]	WWTP[2]	GPS[3]	Blanks[4]	Sample date[5]	Approx. flow[6]	Temp[7]
Crooked River near Richmond, MO	X		39°17.645' N, 93°53.588' W		7/18/2013	1.0	
Flat Creek near Jenkins, MO	X		36°47.463' N, 93°43.332' W		7/18/2013	63	24.3
Missouri River at Riverside Park, Kansas City, MO		X	39°08.307' N, 94°32.446' W	FB #2	7/18/2013	37,500	
James River Delaware Access at Nixa		X	37°03.140' N, 93°23.530' W		7/18/2013	27	23.6
Perche Creek, McBaine, MO		X	38°52'30.47" N, 93°26'14.11" W	FB #1	7/16/2013	8.0[8]	24
Peruque Creek near O'Fallon, MO		X	38°53.028' N, 90°39.510' W		7/19/2013	40[8]	

N = 1 4-liter sample collected from each site.
1) Site location was within 16 km of NPL site with reported BPA discharge; 2) downstream of a current or historical WWTP discharge; 3) geographic positioning system coordinates of the collection/deployment location; 4) POCIS field blank evaluated from these locations; 5) sample collection date for grab samples and deployment date for the POCIS passive samplers; 6) reported water flow at the time of sample collection or POCIS deployment in cubic feet per second as recorded by the U.S. Geological Survey National Water Information System available at: http://waterdata.usgs.gov/mo/nwis/rt; 7) measured water temperature (C) at the time of grab sampling or POCIS deployment; and 8) estimated flow rates based on historical data, no recent measurements taken. Gray highlighting was used to help distinguish BPA release sites from other sites.

Creek): 05514500 and Petitie Saline Creek (near Perche Creek): 06909950.

Upon collection, the Peruque Creek POCIS sampler was found to be out of the water due to decreased flow rate. This may have compromised the data from this sampler, as it was not fully submerged at the site throughout the entire collection period. This has been noted in results and tables that these extracts may represent an underestimation of wastewater chemicals present in water at these sites during the period of sampling.

The POCIS and field blanks (not exposed to site water) were transported to and from the study sites on ice in sealed containers to prevent contamination. At the laboratory, the POCIS were extracted with solvents appropriate for the designated analysis, according to standard procedures (Alvarez et al., 2008). For POCIS to be analyzed for a suite of waste indicator chemicals, 25 mL of 80:20 (v:v) dichloromethane:methyl-tert-butyl ether was used for the extraction. The remaining POCIS designated for the analysis of BPA and EE2, and the bioindicator tests were extracted using 25 mL of methanol (Alvarez et al., 2008, 2014). The final extracts were sealed in amber glass ampoules for transfer to partner laboratories. Limits of detection and limits of quantitation were determined for each of the analytical procedures (Keith, 1991).

2.3. Chemicals

17β-Estradiol (E2; estrogen agonist, 98% pure), ICI 182,780 (ICI; estrogen antagonist, 98% pure), 4,5α-dihydrotestosterone (DHT; androgen agonist, ≥97.5% pure), and flutamide (androgen antagonist, 100% pure) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Stock solutions of all chemicals were prepared in HPLC-grade methanol (Fisher cat # A452-1) and stored at −20 °C. Organic solvents used in the preparation and extraction of the POCIS were all Fisher Optima grade (Fisher Scientific). Analytical standards for the determination of the waste indicator chemicals were purchased from ChemService (West Chester, PA), Sigma-Aldrich (St. Louis, MO), and LCG Standards (Teddington, Middlesex, UK).

2.4. Cell culture

MCF-7 cells (ATCC # HTB-22) were maintained in Gibco Minimum Essential Medium (MEM) supplemented with 5% newborn calf serum, 2 mM glutamax, 0.1 mM non-essential amino acids, and 6 ng/mL bovine insulin. Chemical dilutions were performed in media as described above with the following exceptions: medium used was phenol red-free and serum was charcoal-stripped to remove endogenous steroids. Cell lines were transferred to this modified medium two days prior to the start of assays.

2.5. Hormone receptor reporter gene assays

Hormone receptor activities were measured using reporter gene assays utilizing hormone response elements linked to the luciferase gene. Each test concentration for all samples and controls was performed in quadruplicate within each assay and each assay was repeated three times. Cells were co-transfected with the respective vectors for the receptor to be tested using MEM with reduced serum as described previously (Kassotis et al., 2014). Briefly, cells were transfected in T25 or T75 flasks using Lipofectamine LTX and Plus Reagent and then seeded into 96-well tissue culture plates. Cells were induced with a dilution series of the positive/negative controls or of the water samples at several concentrations using a 1% methanol vehicle. Estrogen assays used a dose response of E2 as a positive control (EC50 ~ 150 pM, concentration required to exhibit half of maximal activity) and ICI as a negative control (100 nM; IC50 ~ 250 pM, concentration required to suppress half the positive control activity). Androgen assays used a dose response of DHT as a positive control (EC50 ~ 250 pM) and flutamide as a negative control (10 μM; IC50 ~ 1.66 μM). After induction for 18–24 h, cell lysate was used for luciferase reporter gene and beta-galactosidase assays as described previously (Kassotis et al., 2014; Brasier et al., 1989). Limit of detection (LOD) and limit of quantitation (LOQ) were determined as three and ten-times the lowest concentration exhibiting a significant elevation in baseline luciferase expression. Inter-assay and intra-assay coefficients of variation (CVs) were consistently below 5% and 10%, respectively.

2.5.1. Calculation of estrogen/androgen receptor equivalent concentrations

Agonist activities were calculated as a percent activity relative to the maximal positive control responses. Percent activities were then compared to the positive control dose response curves (expressed in percent activity), using authentic standards, to calculate the equivalent concentrations of positive controls for each experimental sample. Antagonist activities were calculated as a percent suppression of the response to authentic standards at their EC50 concentrations: 150 pM E2 or 250 pM DHT for estrogen and androgen receptor assays. Percent activities were then compared to negative control dose response curves (expressed as percent inhibition from positive control EC50s), using authentic standards, to calculate the equivalent concentrations to positive controls for each experimental sample. In cases where an EC50 was not reached by experimental samples, the nearest comparable activity was utilized to determine equivalence.

2.6. Measurement of EE2 in water samples

Analysis of EE2 in water sample extracts was conducted by enzyme-linked immunosorbent assay (ELISA) as previously described (Schneider et al., 2004). Briefly, 100 mL aliquots of the water samples were adjusted to pH 7.0 prior to filtering through a glass fiber filter (Gelman types A–E) to remove suspended solids. Each sample was then extracted on an SPE cartridge (Phenomenex Strata X SPE-6 mL, 500 mg reversed-phase), rinsed, and eluted with 10 mL of methylene chloride. The methylene chloride eluate was evaporated to dryness under nitrogen (at 40 °C), samples were reconstituted in 100 μL of methanol, and each sample was then adjusted up to 1.00 mL with 18.2 MΩ water (Synergy, Millipore). Extracts were added to a 96-well microtiter plate that had been coated with polyclonal rabbit anti-EE2 antibodies (Abraxis, Warminster, PA). Following incubation, a tracer conjugated with horseradish peroxidase was applied. After tracer incubation the plate was washed and a color substrate added (TMB). After color development, a stop solution (sulfuric acid) was added before reading the absorbance at 450 nm. The EE2 concentrations were determined by quantifying the absorbance values in relation to the known values of the standard curve, assayed in the same manner. The LOD for this procedure was 0.5 ng EE2/L water (Schneider et al., 2004).

2.7. Measurement of BPA in water samples

BPA was measured in river water samples by isotope dilution liquid chromatography with tandem mass spectrometry (LC/MS/MS) following SPE. Recoveries were determined from parallel-processed blank samples (HPLC-grade water from Fisher Scientific, Waltham, MA) spiked with known amounts of authentic BPA (Sigma, St. Louis, MO). BPA was measured in the POCIS samples directly. Assays were conducted after the addition of C13-BPA (Cambridge Isotope Laboratories Inc., Andover, MA) and d6-BPA (C/D/N Isotopes, Quebec, Canada) as internal standards to monitor for recovery. All solvents were HPLC grade.

Water samples were filtered using GF/D filters (Whatman) into acetone-rinsed glass bottles. Filtered water was acidified with formic acid and then run through preconditioned Oasis HLB cartridges (Waters, Milford, MA). The cartridges were eluted with methanol. The eluate was evaporated to dryness under nitrogen and reconstituted in

1 mL methanol. BPA was quantified using a Thermo TSQ Quantum Access Max (Thermo Fisher Scientific, Waltham, MA) connected to an integrated Thermo-Accela LC system. Analytes were detected using electrospray ionization with negative polarity, and conditions (tube lens setting, collision energy) were optimized for each analyte using the instrument software. Separations were performed on a 100 × 4.6 mm 3 micron Hyperclone HPLC column (Phenomenex, Torrance, CA), at a flow rate of 350 μL/min. A gradient mobile phase was employed using 10:90 acetonitrile:water and acetonitrile with 0.01% ammonia, ranging from 40–96% acetonitrile. Thermo LCQuan software was used to autotune, acquire, and process the data. BPA was detected using selected reaction monitoring for m/z 227 > 212 and quantitation was made against standard curves of the analytes at concentrations ranging from 1–200 ng/mL. The LOQ for BPA, accounting for sample concentration in the laboratory, was 8.0 ng/L. This method is an NIH-validated BPA assay and is free of contamination.

2.8. Measurement of wastewater chemicals present in POCIS water samples

For the analysis of waste indicator chemicals (Supplemental Table 2), the extracts were reduced in volume to 1 mL under nitrogen and analyzed using a Thermo Trace Ultra gas chromatograph with a TriPlus RSH autosampler and an ISQ mass spectrometer (Thermo Scientific) in full-scan mode. Chromatographic separation was performed using a DB-5MS (30 m, 0.25 mm ID, 0.10 μm film thickness) capillary column (Agilent Technologies, Wilmington, DE) (Alvarez et al., 2008, 2014). The estimation of time-weighted average water concentrations of chemicals sequestered by the POCIS requires knowledge of the sampling rate for each chemical along with the sampling duration. Using models previously developed (Alvarez, 2010) and experimentally-derived or theoretically-estimated sampling rates, the dissolved concentrations of chemicals in the POCIS were estimated.

2.9. Blanks and quality control measures

Quality control measures were incorporated into all aspects of the sampling, extraction, and chemical analysis procedures. These measures included the use of replicates, internal and external standards in the chemical analyses, positive and negative control samples in the bioassays, and method of addition analysis. Specific information regarding these measures is described in Section 2.5 above for the bioassays. POCIS field blanks were opened to the air during the deployment and retrieval operations at two of the sites, but were not exposed to any water (Perche Creek and Missouri River; Supplemental Table 1). These samples were processed and analyzed in the laboratory in an identical manner as all study samples to provide a reference for potential atmospheric chemical contamination during the deployment process and for matrix effects (Supplemental Table 3). In addition to field blanks, process control sample blanks were also employed to account for laboratory processing and matrix effects for all procedures: SRRA, BPA and EE2 quantitations, and wastewater compound analysis. These data are presented along with site data for each analysis performed.

Table 2
Ethinylestradiol and bisphenol A grab sample concentrations. Italicized samples are those measured below the limit of detection.

	Ethinylestradiol		Bisphenol A	
	Mean (ng/L)	SEM	Mean (ng/L)	SEM
Site/source				
Crooked River	<0.4	–	320	95 ng/L
Flat Creek	<0.4	–	100	30 ng/L
Missouri River @ KC	<0.4	–	35	16 ng/L
James River	<0.4	–	49	20 ng/L
Perche Creek	<0.4	–	50	10 ng/L
Peruque Creek	<0.4	–	49	6.0 ng/L
Blanks/controls				
Process control	<0.4	–	<18	–

N = three replicate tests for each concentration.
Concentrations for ethinylestradiol and bisphenol A in grab samples from each site as well as process control. Replicate values averaged and presented as a mean and standard error. Ethinylestradiol measurements were performed using the commercially-available ELISA test, while BPA analysis was performed using NIH-validated LC/MSMS assay that is free of any contamination. Gray highlighting was used to help distinguish BPA release sites from other sites. SEM = Standard error of the mean.

2.10. Statistical treatment of data

Activity for all water samples was determined based on the following criteria. A compound was considered to have an androgenic activity when there was a significant increase ($p < 0.05$) in the treatment over the vehicle in no less than 50% of the repeats, as determined by paired Student's T-tests. A compound was considered to have an anti-androgenic activity when there was a significant decrease ($p < 0.05$) in the treatment from the added agonist control (EC50 of positive control, as described above) in no less than 50% of the repeats. Determination of anti-androgenic activity was also contingent on the absence of toxicity, determined using the beta-galactosidase assay as described previously (Kassotis et al., 2014).

3. Results

3.1. Analytical quantitation of bisphenol A and ethinylestradiol in grab and POCIS water samples

Bisphenol A was detected in grab samples from all sites assessed, while EE2 was detected at none (Table 2). BPA concentration means at Peruque Creek, Missouri River, James River, and Perche Creek ranged from 30–50 ng/L, which is just above the 3–30 ng/L typically reported in surface water (Bhandari et al., in press). Water from Flat Creek was two to three times greater than BPA concentrations at our other sites, and Crooked River water was approximately ten times greater than other sites. Both of these sites were near NPL BPA release sites, suggesting that atmospheric releases may be a risk factor for increased BPA concentrations in nearby surface water. Despite the elevated BPA concentrations, these were not sites that exhibited an anti-androgenic activity (Table 3). While Flat Creek water did exhibit an estrogenic activity, the BPA concentration was approximately six times less than required to account for observed receptor activity (Table 4). In POCIS samples, EE2 was detected at concentrations from 6–17 pg/L at Perche Creek, James River, and Crooked River, while BPA concentrations ranged from low pg/L to 1.5 ng/L across sites (Supplemental Table 2).

3.2. Estrogen and androgen receptor activities of grab and POCIS water samples

Anti-estrogenic and anti-androgenic activities predominated in the grab water samples and were predictive of a WWTP effluent input upstream of the collection site (Table 3). Only the four sites with current or historical wastewater effluent impacts had significant anti-estrogenic and anti-androgenic activities in the water. Further, anti-estrogenic and anti-androgenic activities agreed between sites; Peruque Creek exhibited greater antagonist activities than Perche Creek, followed by the Missouri River and James River. Estrogenic activity was observed only at Flat Creek, and limited androgenic activity was observed near both BPA releases and two WWTP inputs. Concentrations of BPA at Flat Creek were insufficient to account for all of the estrogenic activity observed, with measured concentrations at less than one fifth of BPA equivalence in the water there (Table 4). EE2 was below detection limit of the ELISA assay at all of the sites and could not account for observed estrogenic activity. The concentrations of BPA were also much lower than those required to account for observed anti-androgenic activities (Table 4). Taken together, it is likely that other chemicals (natural or anthropogenic) are the cause of the antagonist activities.

Table 3
Measured equivalent hormone concentrations for grab water samples. Italicized data are those measured below the limit of detection.

	Estrogenic	Anti–estrogenic	Androgenic	Anti–androgenic
	EEQ (pg/L)	IEQ (ng/L)	DEQ (ng/L)	FEQ (μg/L)
Site/source				
Crooked River	<1.4	<1.5	0.3	<0.7
Flat Creek	6.8	<1.5	<0.2	<0.7
Missouri River @ KC	<1.4	5.3	<0.2	10
James River	<1.4	2.0	0.4	1.6
Perche Creek	<1.4	11	0.3	5.9
Peruque Creek	<1.4	19	<0.2	48
Blanks/controls				
Process control	<1.4	<1.5	<0.2	<0.7

N = three replicate assays and four quadruplicate replicates of each test concentration within each assay.
Measured equivalent concentrations for each grab water sample. Equivalent concentrations calculated based on percent activity of the water sample, corrected for sample concentration within the assay, and compared to chemical standards. EEQ = estradiol equivalent concentration, IEQ = ICI equivalent concentration (estrogen receptor antagonist ICI 182,780, see Materials and methods section), DEQ = dihydrotestosterone equivalent concentration, FEQ = flutamide equivalent concentration.
Gray highlighting was used to help distinguish BPA release sites from other sites.

Water sample extracts collected by the POCIS exhibited similar but distinct patterns of receptor-based activities as compared to those of the grab samples (Supplemental Table 4). While the four WWTP sites again exhibited both antagonist activities, Crooked River exhibited elevated antagonist equivalence relative to other sites. This is suggestive of an episodic release of anti-estrogenic and anti-androgenic contaminants that was not captured by the grab sample for this site. Both estrogenic and androgenic activities were infrequently reported above the LOQ, with a significant androgenic activity only observed in water collected at the James River site. It is likely that the high levels of antagonist activities measured at these sites may have prevented an accurate assessment of the total agonist activities present. Importantly, POCIS equivalent activities were not normalized to the amount of water sampled over the collection period. POCIS normalization requires knowledge of the partitioning rate of specific chemicals, thus normalizing for the total chemical milieu is not feasible when evaluating extracts with a bioassay approach. Consequently, there are limits to the utility of hormone receptor bioassays with a passive sampling device.

Field blanks and process controls were negative for all endpoints examined, with the exception of antagonist activities in the Missouri River POCIS field blank. This passive sampler was deployed in Kansas City, a major metropolitan area, and the noted activity may reflect greater levels of atmospheric pollutants at the site.

Table 4
Calculated BPA and EE2 equivalents for grab sample activity.

	Estrogenic		Anti-androgenic
	EeEQ (pg/L)	BEQe (μg/L)	BEQaa (μg/L)
Site/source			
Crooked River	<1.2	<0.6	<5.7
Flat Creek	20	0.6	<5.7
Missouri River @ KC	<1.2	<0.6	140
James River	<1.2	<0.6	27
Perche Creek	<1.2	<0.6	170
Peruque Creek	<1.2	<0.6	570
Blanks/controls			
Process Control	<1.2	<0.6	<5.7

Estimated BPA and EE2 equivalent concentrations for each grab water sample. Estimated equivalent concentrations calculated based on mean percent activity of the water sample relative to pure chemical standards. EeEQ = ethinylestradiol equivalent concentration, BEQe = bisphenol A estrogenic equivalent concentration, and BEQaa = bisphenol A anti-androgenic equivalent concentration. Gray highlighting was used to help distinguish BPA release sites from other sites.

3.3. Analytical quantitation of wastewater compounds present in POCIS water samples

Thirteen wastewater chemicals were detected in POCIS water samples across all sites (Table 5). These chemicals included fragrances, cosmetics, plasticizers, flame-retardants, and pesticides, many of which are known EDCs. Three sites downstream of WWTPs (Perche Creek, James River, Missouri River) and one with no known input (Crooked River) exhibited a wastewater contamination signature, with a greater diversity and greater concentrations of wastewater compounds. In contrast, Flat Creek and Peruque Creek displayed fewer wastewater chemicals with most at lower concentrations. However, the WWTP discharge into Peruque Creek ended in 1993 when a new WWTP was constructed, potentially explaining the reduced wastewater compounds detected in the POCIS extract from this site (Stephan, 2014). Also, reduced water flow had exposed this sampler by the time of collection and may have compromised complete measurement of chemicals present.

4. Discussion

BPA concentrations were found to be elevated in water at sites near permitted airborne release sites (Table 2). EE2 concentrations were below the LOD at all sites examined, likely due to an insufficiently sensitive ELISA assay with an LOD of 0.5 ng/L, as we have described previously (Bhandari et al., in press). Researchers in Germany detected EE2 at 40% of sites using an LOD of 0.05 ng/L (Kuch and Ballschmiter, 2001), while researchers in neighboring Austria detected EE2 at only 1% of sites with an LOD of 0.1 ng/L (Hohenblum et al., 2004). This suggests that sensitivity of detection is critical for evaluating this chemical, particularly due to its potency.

Observed estrogen and androgen receptor activities of grab water samples were predictive of WWTP input. WWTP sites had elevated levels of anti-estrogenic and anti-androgenic equivalence, while sites without current WWTP discharges exhibited no antagonist activities (Table 3). Wastewater chemical concentrations and variety in POCIS extracts were elevated at four sites: Perche Creek, James River, Missouri River, and Crooked River. Some wastewater chemicals were present at only WWTP sites, though there was neither a uniform pattern to the chemicals detected nor their concentrations (Table 5). For example, while Flat Creek water (non-WWTP) contained only two of 44 chemicals tested, Crooked River water (non-WWTP) contained eight and appeared similar to other WWTP-impacted sites (Missouri River: 9, James River: 7, Perche Creek: 6). In contrast, Peruque Creek water (historical WWTP) contained only three of 44 chemicals and appeared more similar to Flat Creek than WWTP sites.

Flat Creek and Crooked River were both near sites with permits for airborne BPA releases and water samples collected in these surface water sources exhibited 3–10 times the concentration of other sites in this study (Table 2) and greater than typical surface water concentrations of BPA (Bhandari et al., in press). Flat Creek and Crooked River discharge sites were permitted to release approximately 7800 and 850 kg of BPA per year, respectively (USEPA Toxic Release Inventory, http://toxmap-classic.nlm.nih.gov/toxmap/main/index.jsp; CAS# 80-05-7). Prevailing winds between the facility and nearby Flat Creek were towards the sampling site, where we observed approximately three times the BPA concentration typically reported in surface waters (Table 2), as reviewed previously (Bhandari et al., in press). Prevailing winds blew away from the Crooked River site during the sampling month, though during the previous month blew towards this site (http://www.ncdc.noaa.gov/cdo-web/datatools/findstation). The low flow rate of the Crooked River may have compounded the contamination, leading to BPA concentrations an order of magnitude above typical

Table 5
Detected waste indicator compound residue concentrations in POCIS samples.

Site identification	Crooked River	Flat Creek	MO River at KC	James River	Perche Creek	Peruque Creek[b]
Waste indicator compounds	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1,4-Dichlorobenzene	*ND*	*ND*	*3.0*[a]	*ND*	*ND*	**12**
Acetophenone	*ND*	*ND*	*ND*	*ND*	**3.1**	**11**
Methyl_salicylate	*ND*	*ND*	*ND*	*ND*	*ND*	**28**
Indole	*ND*	*ND*	**320**	*ND*	**96**	*ND*
Diethyl_phthalate	*99*[a]	*ND*	*ND*	*ND*	*ND*	*ND*
DEET	**14**	*ND*	**14**	**18**	**24**	*ND*
Ethyl_citrate	*ND*	*ND*	*ND*	**15**	*ND*	*ND*
Tris-(2-chloropropyl)phosphate (TCPP)	**270**	**370**	**52**	**270**	**140**	*ND*
TCPP_isomer	**410**	**320**	**140**	**340**	**260**	*ND*
Galaxolide	**7.2**	*ND*	**14**	**14**	**0.8**	*ND*
Tonalide	**0.9**	*ND*	*0.5*[a]	**1.1**	*ND*	*ND*
Tri(dichloroisopropyl) phosphate (TDCPP)	**61**	*ND*	**9.0**	**34**	*ND*	*ND*
Diethylhexylphthalate	*35*[a]	*ND*	*34*[a]	*ND*	*ND*	*ND*
Bisphenol A (BPA)	**1.5**	**0.2**	**0.5**	**0.4**	**1.0**	**0.2**
17α-ethinylestradiol (EE2)	*0.02*[a]	*ND*	*ND*	*0.01*[a]	*0.01*[a]	*ND*

Concentrations and identities of waste indicator compounds detected in passive sampler extracts. Results in bold type are reportable values greater than the method quantitation limit. ND denotes values below the method detection limit.

[a] Results in italic type are estimated values greater than the method detection limit but less than the method quantitation limit. These values have a greater amount on uncertainty in the absolute value.

[b] Results for this site may be an underestimation due to low water flow at the site resulting in sampler device recovered outside water at the end of sampling period.

surface water (Bhandari et al., in press). Altogether, our results suggest that airborne releases of chemicals and subsequent surface water contamination nearby may represent a potential undescribed source of human and animal exposure.

BPA is ubiquitously released into the atmosphere (Fu and Kawamura, 2010; Salapasidou et al., 2011), with annual production of more than 6.8 billion kg (Research, 2014). Atmospheric release occurs through varied sources including: permitted industrial releases, uncontrolled waste burning, and paint application (Fu and Kawamura, 2010; Sidhu et al., 2005). Global estimates vary substantially and have been estimated up to 90,000 kg in 2005 (Fu and Kawamura, 2010; Sidhu et al., 2005; Cousins et al., 2002), though these releases have likely increased substantially in recent years as the EPA reported 41,900 kg in known aerial releases in 2012 from permitted industrial sources in the United States alone (http://toxmap-classic.nlm.nih.gov/toxmap/main/), suggesting considerably higher releases from all sources. Based on the order of magnitude enhancement of BPA concentrations in water near the releases examined in this study, these data may represent a potential underrepresented source of BPA to the environment. While the contribution of airborne pollutants to water sources has long been acknowledged through wet and dry deposition (Baker and Eisenreich, 1990; Odabasi et al., 1999), the complexities of atmospheric processes related to BPA fate and transport have not been considered important sources to the environment (Cousins et al., 2002).

The proposed atmospheric half-life of BPA is estimated to be short, on the order of hours (Cousins et al., 2002). This was estimated based on a low vapor pressure and Henry's Constant of BPA in simple partitioning models and proposed photolytic degradation (Cousins et al., 2002). Therefore, these simple modeling efforts initially suggested that only a negligible amount of BPA would be found in the atmospheric compartment (Cousins et al., 2002). However, it has only been in recent years that atmospheric measurements of BPA have been made in anything more than sporadic events (Fu and Kawamura, 2010; Salapasidou et al., 2011; Huang et al., 2012). In these recent air monitoring efforts, measureable concentrations of BPA in atmospheric samples (1–17,400 pg/m^3) have been observed in urban, industrial, rural, marine, and even remote Artic or Antarctic locations (Fu and Kawamura, 2010; Salapasidou et al., 2011; Huang et al., 2012). Thus, current knowledge indicates that BPA is atmospherically transported, and even to remote locations. Our findings of elevated BPA surface water concentrations at two locations in proximity to registered BPA releases provide a preliminary indication that atmospheric releases of BPA may be related to the elevated surface water concentrations in nearby streams. However, more detailed studies are required to confirm this suspicion. A complete evaluation of this hypothesis would include measurements of the discharge air, spatial and temporal sampling of air, modeling of prevailing wind vectors, measurement of hydrographic data, and evaluation of potential alternative sources of BPA to the streams. Alternative sources of the BPA in these waters could be from direct releases (e.g. spills or discharges) into the surface waters or directional gradients of contaminated ground water flow into the surface waters. No other contributory sources were identified at these sites, and the lack of a described wastewater input, along with 3–10 fold increases in baseline water concentrations of BPA support the hypothesis that this may be due to these aerial releases. To our knowledge, this is the first time that water contamination concerns have been raised about industrial atmospheric releases of BPA.

Clear differences were noted between grab and passive sample extracts at certain sites, including Flat Creek and Crooked River, which have certified airborne releases of BPA nearby and no known WWTP inputs. While no antagonist activities and few wastewater compounds were reported for Flat Creek as expected, Crooked River exhibited significant POCIS antagonist activities and eight wastewater compounds, despite no antagonism in the grab sample (Table 3, Supplemental Table 4). This disparity between the grab sample and passive sampler activities at Crooked is potentially explained by flow rate. Despite having a flow rate of 1 cubic foot per second (ft^3/s) at the time of grab sampling, flow rate increased more than 10-fold to 11 ft^3/s and returned by the time of POCIS collection (USGS National Water Information System: http://nwis.waterdata.usgs.gov/nwis; site ID: 06895000). This elevated water flow and subsequent runoff may have liberated hormonally active chemicals from the sediment and surrounding areas and contributed to the activity observed. Similar responses were seen at Peruque Creek, a site where high antagonist receptor activities but low wastewater chemical presence were measured. This site also experienced heavy rainfall during the sampling period, and while flow rate in this creek is not monitored, the nearby Cuivre River experienced a 5-fold increased flow rate, increasing from 17 to 87 ft^3/s and back (USGS National Water Information System: site ID: 05514500). While this site used to receive discharge from the Lake St. Louis and O'Fallon WWTPs, a new activated sludge treatment plant that discharges into the Cuivre River was constructed in 1984 and all discharges into Peruque Creek ended by 1993 (Stephan, 2014). Lastly, the POCIS sampler not being submerged at time of collection may also have contributed to the reduced activity.

These examples highlight several challenges inherent in characterizing EDC activities in surface water. First and foremost, connecting total hormone receptor activities to analytical measurements requires full knowledge of all hormonally active chemicals, which is generally not known (Liscio et al., 2014; Kortenkamp et al., 2014; Rostkowski et al., 2011). This complicates both normalization of total hormone activities and any attempts to estimate water concentrations of hormonal activities from POCIS extracts, as these require knowledge of each compound to correctly account for sequestration rates. For this reason, passive samplers, in connection with bioassay approaches, provide a different type of information and need to be evaluated against the materials (chemicals) sequestered in the device (POCIS in this case), as opposed to trying to estimate water concentrations of the overall activities. This highlights the fact that the nature of the information provided by the combination of bioassays on water grab sample extracts, as opposed to bioassays on passive sampler extracts (e.g. POCIS) will not be equivalent, but useful nonetheless. Further, many details of the passive sampling technology such as the stability of chemicals on the membrane are not well characterized, so the use of passive samplers on a qualitative basis is recommended. Lastly, analytical techniques are also in many cases not as sensitive as required to quantify very potent EDCs, such as EE2 at environmental concentrations (Bhandari et al., in press). Taken together, combining bioassays with analytical results can provide a more comprehensive picture of total pollutants, though this approach has inherent challenges in implementation. Approaches such as effects-directed analysis will certainly be important to help understand the chemicals or chemical classes that contribute to the various hormonal activities measured in water or other environmental samples (Rostkowski et al., 2011).

Distance between discharge site and sampling site and volume of discharge are also critical factors in interpreting results. The James River had high wastewater compound detection, indicative of WWTP input, yet generally had low antagonist activities. However, the Springfield activated sludge WWTP discharges first into Wilson's Creek at its confluence with South Creek and meets with the James River approximately 13 km downstream (Hinkston, 2014). Wilson's Creek also experiences losing reach (Hinkston, 2014), where the water table lies below the river bed and surface water is able to migrate through the bed into the underlying aquifer as it flows downstream (Wicks et al., 2004). As our sampling site was approximately 15 km downstream from the discharge, it may receive limited or intermittent water from Wilson's Creek. This non-direct discharge into a transient surface water resource might account for the diluted activity observed in the reporter gene assays for this site. Further, this plant handles less than 5.7 million L of wastewater per day (Hinkston, 2014). Wastewater compounds were still reported via the passive sampler at concentrations equivalent to other surface water sites with wastewater inputs, suggesting that the chemicals displaying the antagonist receptor activities are likely not being measured in the analytical suite employed herein.

In contrast, Perche Creek has the Columbia WWTP plant upstream, handling approximately 64.4 million L of wastewater per day (Huebotter, 2014). As expected from a WWTP-influenced watershed, this site also had some of the greatest antagonist activities for both grab and passive samples and contained six wastewater compounds. Before the construction of the current facility, two trickling filter plants discharged wastewater into Hinkson Creek, which then joined Perche before its discharge into the Missouri River. This practice continued until after the construction of the new plant in 1994, at which time wetland treatment units were incorporated into the process. Treated effluents now pass through four of these wetland treatment units before discharge into the Eagle Bluffs Conservation area. While no direct WWTP effluent discharge currently occurs into Perche or Hinkson, storm overflow and migration of treated effluent into ground water at wetland treatment units and Conservation wetlands into Perche Creek are still potential concerns (Huebotter, 2014). The scale of treatment, migration of fluids after treatment, and small size of the creek are likely causes of the greater activity exhibited by these samples.

Lastly, the Missouri River samples exhibited expected activities. The Kansas City area discharges treated effluent from six wastewater treatment plants from a population of more than 450,000, so we would expect greater endocrine disrupting activities and the presence of numerous wastewater compounds in the effluent and receiving water sources. Both grab water samples and POCIS extracts exhibited greater antagonist activities and wastewater analysis revealed nine wastewater compounds, the greatest variety detected at any sampling site.

5. Conclusions

Our results suggest that airborne releases of BPA may potentially represent an underestimated source to the environment, affecting water concentrations and potentially resulting in human and wildlife exposure. Further work is required to confirm this connection, including a more comprehensive sampling of sites, varying distances from permitted release sites, and assessment of other potential sources of BPA to the surface water (e.g. contaminated ground water). Overall, hormone receptor activities in surface water samples and wastewater chemical concentrations were indicative of WWTP or airborne BPA release nearby. Based on the historic information at these sites, our results also suggest that water contamination may not always improve over time when the primary known discharge source is removed, potentially due to the accumulation of chemicals in sediments. Importantly, BPA and EE2 were not found to be the sole sources of the hormonal activity observed in these water samples, and further work is required to elucidate the chemicals contributing the antagonist activities we observed in surface waters. In particular, approaches such as effects-directed analysis may be useful to help determine the relative contributions of various chemicals or chemical classes to the observed hormonal activity.

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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.2015.04.013.

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