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REPRODUCTIVE HEALTH OF BASS IN THE POTOMAC, USA, DRAINAGE: PART 2. SEASONAL OCCURRENCE OF PERSISTENT AND EMERGING ORGANIC CONTAMINANTS

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Abstract—The seasonal occurrence of organic contaminants, many of which are potential endocrine disruptors, entering the Potomac River, USA, watershed was investigated using a two-pronged approach during the fall of 2005 and spring of 2006. Passive samplers (semipermeable membrane device and polar organic chemical integrative sampler [POCIS]) were deployed in tandem at sites above and below wastewater treatment plant discharges within the watershed. Analysis of the samplers resulted in detection of 84 of 138 targeted chemicals. The agricultural pesticides atrazine and metolachlor had the greatest seasonal changes in water concentrations, with a 3.1- to 91-fold increase in the spring compared with the level in the previous fall. Coinciding with the elevated concentrations of atrazine in the spring were increasing concentrations of the atrazine degradation products desethylatrazine and desisopropylatrazine in the fall following spring and summer application of the parent compound. Other targeted chemicals (organochlorine pesticides, polycyclic aromatic hydrocarbons, and organic wastewater chemicals) did not indicate seasonal changes in occurrence or concentration; however, the overall concentrations and number of chemicals present were greater at the sites downstream of wastewater treatment plant discharges. Several fragrances and flame retardants were identified in these downstream sites, which are characteristic of wastewater effluent and human activities. The bioluminescent yeast estrogen screen in vitro assay of the POCIS extracts indicated the presence of chemicals that were capable of producing an estrogenic response at all sampling sites.

Keywords—Potomac River Passive sampling Emerging contaminants Wastewater

INTRODUCTION

The Potomac River (USA) watershed is an important spawning and nursery ground for both migratory and resident fish species. Recent studies of fish health in the Potomac watershed have found sites with alarming numbers of the fish that exhibit external lesions as well as incidences of intersex, specifically testicular oocytes, in male smallmouth bass (*Micropterus dolomieu*) from areas receiving surface runoff and direct inputs from agricultural, industrial, and other human activities [1,2].

Throughout the Potomac River watershed, multiple point and nonpoint sources exist, consisting largely of rural communities and agriculture in the upper regions and of industry and municipal wastewater treatment plant (WWTP) discharges in the lower regions [3]. According to the Maryland Department of the Environment, of the 747 surface-water discharges permitted within the Maryland (USA) portion of the Potomac River watershed, 117 are WWTPs. Wastewater treatment plants are widely recognized as a source of endocrine-disrupting compounds, which cover a wide range of chemical classes, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, phthalates, alkylphenol surfactants, heavy metals, and natural and synthetic hormones [4–6].

Common practices of taking a discrete or grab sample of 1 to 2 L of water for chemical analysis often are insufficient at providing information on the trace, but potentially significant toxicologically, concentrations of anthropogenic organic contaminants. Passive samplers extract contaminants from volumes of water (often tens to hundreds of liters over a typical 30-d deployment) much greater than is possible with discrete samples, allowing chemical concentrations in the parts-per-trillion to parts-per-quintillion (ng/L to fg/L) range to be detected. Discrete water samples only represent conditions present at the instant of sampling and, as such, can miss episodic events (i.e., spills, surface runoff, and meteorological events). Repetitive sampling schemes, which would be necessary to detect episodic changes in chemical concentrations, can be logistically challenging and expensive, particularly in remote locations or areas that experience frequent hydrological changes. Passive samplers provide data as a time-weighted average concentration over the deployment period (weeks to months), which is a fundamental part of the ecological risk assessment processes for chemical stressors.

Two of the most widely used and studied passive samplers are the semipermeable membrane device (SPMD) and the polar organic chemical integrative sampler (POCIS). The SPMD consists of a nonporous, lay-flat, polyethylene membrane tube

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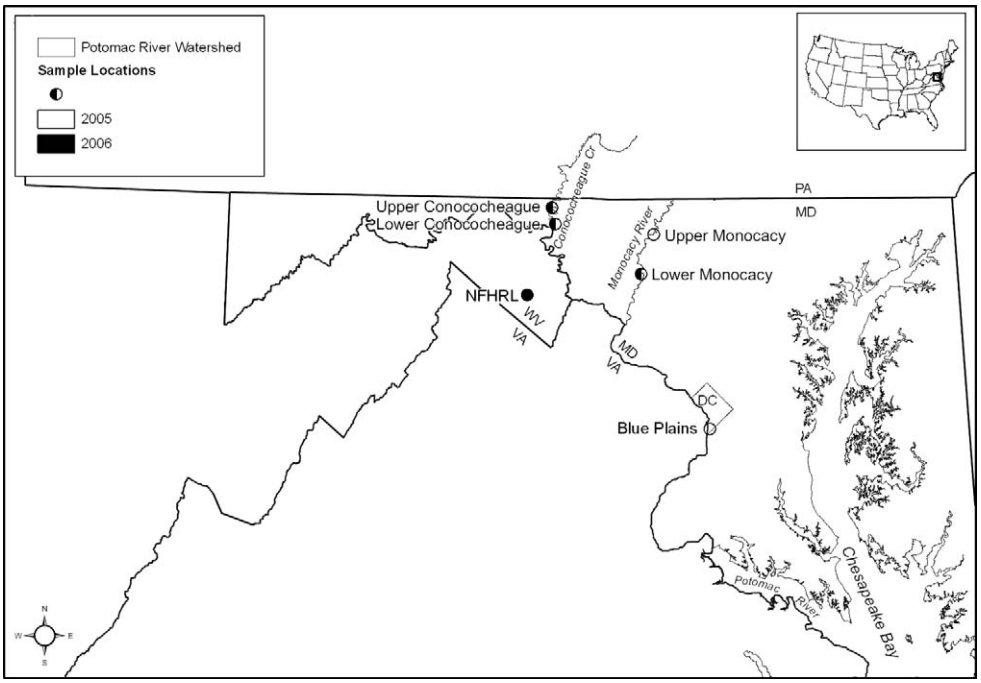

Fig. 1. Map of Potomac River (USA) watershed indicating the 2005/2006 sampling locations. NFHRL = National Fish Health Research Laboratory, Kearneysville, West Virginia, USA (reference site).

containing a neutral lipid (triolein). These devices are designed to mimic key aspects of the bioconcentration process, which results in elevated contaminant concentrations after exposure to trace hydrophobic organic contaminants in aquatic environments. Sampling of compounds with moderate to high octanol–water partition coefficients ($K_{OW} > 3$) is integrative (i.e., extracted residues are constantly accumulated without significant losses back into the environment), and analyte concentrations are reported as time-weighted average values [7]. The POCIS is designed to mimic an organism's exposure to hydrophilic organic contaminants with low to moderate K_{OW} (i.e., <3). The POCIS consists of a solid-phase sorbent or mixture of sorbents contained between two sheets of a microporous polyethersulfone membrane. Sampling of compounds by the POCIS is integrative, and analyte concentrations are reported as time-weighted average values [8,9]. By using SPMDs and POCIS in concert, it is possible to monitor for large numbers of organic contaminants possessing a wide range of chemical and physical properties.

The versatility of passive sampling devices allows chemical analyses to be performed, but the contaminants these devices sample also can be coupled with in vitro reporter system assays. By doing so, the net biological effect of the complex mixtures captured by these devices can be quantified relative to a target standard [10,11]. In the instance of chemicals that may affect reproduction, a handful of assays have been developed that report the binding of chemicals to sex hormone receptors. Many of these assay platforms involve the use of estrogen-sensitive mammalian cell lines that have been genetically modified to produce specific enzymes that can be quantified following exposure to estrogen [12–15]. Although these assay platforms are sensitive, mammalian cells tend to be affected by the inherent toxicity of many chemicals and can be cumbersome to perform. Recently, a bioluminescent yeast estrogen screen (BLYES) has been developed that is sensitive ($\sim 4 \times 10^{-11}$ M) and less susceptible to toxic chemicals compared with mammalian cell reporter systems [16].

Here, we complement the passive sampler technology with a series of chemical analyses and an estrogen reporter assay to assess the contaminant profiles of water receiving input from different land-use practices within the Potomac River watershed. The combination of chemical analyses and in vitro assays along with physical observations of fish health and biological reproductive endpoints [2] will be used to help bridge the gap in understanding the potential causes of intersex and instances of endocrine disruption.

MATERIALS AND METHODS

Sampling sites

The mainstem of the Potomac River and two of its tributaries, Conococheague Creek and the Monocacy River, which can receive a significant portion of their flow from the effluent of WWTPs, were selected based on their proximity to WWTP discharges and availability of largemouth bass (*Micropterus salmoides*) and smallmouth bass (*M. dolomieu*) for collection for biological measurements (Fig. 1) [2]. In the fall of 2005, passive samplers were placed for 31 d during the months of September and October at two sites on the Monocacy River, two in the Conococheague Creek, and one in the Potomac River at the Blue Plains WWTP outfall in Washington, DC. The Monocacy River and Conococheague Creek each had sites upstream and downstream of known WWTP discharges. In the spring of 2006, a second set of passive samplers were deployed for 49 d during April and May in the Monocacy River (downstream) and in the Conococheague Creek (upstream and downstream). A reference site at the U.S. Geological Survey (USGS) National Fish Health Research Laboratory (NFHRL; Kearneysville, WV, USA) was added to the spring sampling, replacing the Blue Plains site. The upstream sites were located at least 15 km upstream of the nearest major WWTP input; a

major WWTP was designated as having a discharge of greater than 1 million gallons per day (mgd; 3.8 million liters per day [mld]). A small WWTP, however, was known to discharge 0.003 mgd (0.011 mld) approximately 3 km upstream of the upstream Conococheague Creek site. The downstream sites were located immediately downstream of the WWTP discharges.

Conococheague Creek (Fig. 1) originates in Pennsylvania (USA) and flows south into the Potomac River at Williamsport (MD, USA). Land use of the 911-km^2 watershed is largely agricultural (61%) and forested (34%), with minor urban influence (5%). Effluent from the Conococheague WWTP comprised 3.2 and 1.6% of the estimated mean flow at the downstream site during the fall and spring sampling periods, respectively (USGS stream flow-gage 1614500). The Monocacy River (Fig. 1), with a drainage area of 1,927 km^2, forms near the Maryland and Pennsylvania (USA) border and flows south through the City of Frederick (MD, USA) and into the Potomac River. Land use of the Monocacy watershed is similar to the Conococheague with 60% agricultural, 33% forested, and 7% urban. Two WWTPs are suspected of influencing the downstream Monocacy site, with an estimated 3.7 and 2.3% of the mean flow during the fall and spring sampling periods, respectively (USGS stream flow-gage 1643000). In Washington, DC, the Blue Plains WWTP is the largest plant in the Potomac River watershed, using a combination of nitrification/denitrification, filtration, chlorination/dechlorination, and postaeration. It serves the District of Columbia, Montgomery and Prince Georges counties in Maryland, and Fairfax and Loudon counties in Virginia (USA), and it has an average output of 370 mgd (1,400 mld) of treated wastewater. The percentage effluent during baseflow conditions could not be estimated, because the area is in the tidal region of the Potomac River and no USGS stream gauges are located nearby. The NFHRL reference site is a research pond with no WWTP input. This pond receives surface water from other research ponds at the facility and may be susceptible to chemical input from surface runoff and transport from nearby farms.

Passive sampler construction

The SPMDs and POCIS were fabricated according to established procedures [7–9]. For each site, one deployment canister containing eight POCIS and one deployment canister with four SPMDs were prepared. This provided sufficient samplers to allow replicate analyses at each site. Field blanks for both sampler types at each site also were prepared.

The POCIS used in the present study contained the triphasic admixture of 80:20 (w/w) Isolute ENV+ and S-X3 dispersed Ambersorb 1500 enclosed between two polyethersulfone membranes [8]. Each POCIS unit had an effective sampling surface area of 41 cm^2 and a membrane surface area to sorbent mass ratio of approximately 180 cm^2/g, conforming to the specification of a standard POCIS [8].

The SPMDs used in this project consisted of 97 cm long (86 cm between the lipid-containment seals) by 2.5 cm wide, lay-flat, low-density polyethylene tubing containing 1.0 ml of purified triolein [17]. The membrane surface area to total SPMD volume ratio of SPMDs used in the present study was approximately 86 cm^2/ml, and triolein represented approximately 20% of the mass of the SPMDs, conforming to a standard SPMD as defined by Huckins et al. [7]. Two of the four SPMDs deployed and one of the two field blank SPMDs at each site were fortified with 1 μg of each of the five perdeuterated PAHs selected as performance reference compounds (PRCs; acenaphthylene-d_{10}, acenaphthene-d_{10}, fluorene-d_{10}, phenanthrene-d_{10}, and pyrene-d_{10}). A PRC is an analytically noninterfering organic compound with moderate to high SPMD fugacity that is added to the lipid before membrane enclosure and field deployment [7]. By comparing the rate of PRC loss during field exposures with that of laboratory studies, adjustments to the sampling rates of targeted chemicals can be made to reflect the site-specific sampling rates more accurately. The amount of loss will depend on environmental factors, such as exposure time, facial flow/velocity at the sampler's surface, temperature, and biofouling. Because of the strong sorptive properties of the adsorbents used in the POCIS, initial attempts to incorporate PRCs into the POCIS to date have failed [9].

Sample processing and chemical analysis

Each SPMD and POCIS was extracted individually before designating extracts for specific processing and analysis procedures. A list of the targeted chemicals is presented in Table 1. Neat chemical standards and custom chemical mixtures were obtained from AccuStandard, ChemService, Sigma, and LGC Promochem. All solvents were Optima grade from Fisher Scientific. The SPMDs were processed and analyzed for PAHs, organochlorine (OC) pesticides, and total PCBs. Select organic wastewater chemicals (OWCs), agricultural pesticides, and hormones were measured in the POCIS. The extracts from a single deployed POCIS and blank from each site were screened for the potential estrogenic activity of sequestered chemicals using the BLYES assay.

Semipermeable membrane devices

The procedures used for preparing SPMD samples for analysis were similar to published approaches [18,19]. Briefly, the target analytes were recovered by dialysis with hexane, and then the dialysates were fractionated by size-exclusion chromatography (SEC) before class-specific cleanup and analysis.

Polycyclic aromatic hydrocarbons. Samples designated for the analysis of PRCs and PAHs were processed using a triad-sorbent column consisting of phosphoric acid silica gel, potassium hydroxide–impregnated silica gel, and silica gel [19]. Analysis of selected PAHs and PRCs was performed using an Agilent 6890 gas chromatograph (GC; Agilent Technologies) coupled to a 5973N mass-selective detector (MSD; Agilent Technologies) with a HP-5MS capillary column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm; Agilent Technologies) as reported by Alvarez et al. [18]. Quantitation was achieved using a seven-point calibration curve ranging from 10 to 4,000 ng/ml with 2-methylnaphthalene-d_{10} and benzo[*e*]pyrene-d_{12} as internal standards.

Organochlorine pesticides and polychlorinated biphenyls. The OC/PCB SPMD samples were further enriched using a Florisil column followed by fractionation on silica gel [19]. The first silica gel fraction contained more than 95% of the total PCBs, hexachlorobenzene, heptachlor, and mirex and 40 to 80% of the *p,p′*-dichlorodiphenyldichloroethylene when present in extracts. The second fraction contained the remaining 28 target OC pesticides and 5% or less of the total PCBs (largely mono- and dichlorobiphenyl congeners). Analysis of the SPMD samples for PCBs and OCs were conducted using a Hewlett-Packard Model 5890 series-II GC equipped with an electron-capture detector (ECD; Hewlett-Packard) and a DB-35MS capillary column (length, 30 m; inner diameter, 0.25

Table 1. Selected chemicals targeted for analysis in passive samplers deployed in the Potomac, USA, watershed during the fall of 2005 and spring of 2006 samplings[a]

Organochlorine pesticides and PCBs[b]	Polycyclic aromatic hydrocarbons[b]	Organic wastewater chemicals[c]	Agricultural pesticides[c]	Hormones[c]
α-Benzenehexachloride	Acenaphthene	1,4-Dichlorobenzene	Acetochlor	17β-Estradiol
β-Benzenehexachloride	Acenaphthylene	4-*n*-Octylphenol	Alachlor	17α-Ethinylestradiol
δ-Benzenehexachloride	Anthracene	4-*tert*-Octylphenol	Ametryn	Estriol
cis-Chlordane	Benzo[*a*]anthracene	Acetophenone	Atraton	Estrone
trans-Chlordane	Benzo[*a*]pyrene	Anthraquinone	Atrazine	
Chlorpyrifos	Benzo[*b*]fluoranthene	Atrazine	Chlorpyrifos	
Dacthal	Benzo[*ghi*]perylene	Benzophenone	Dacthal	
Diazinon	Benzo[*k*]fluoranthene	Bromacil	Desethylatrazine	
Dieldrin	Chrysene	Bromoform	Desisopropylatrazine	
o,p′-DDE	Dibenz[*a,h*]anthracene	Caffeine	Diazinon	
p,p′-DDE	Fluoranthene	Camphor	Fipronil	
o,p′-DDD	Fluorene	Carbaryl	Fonofos	
p,p′-DDD	Indeno[1,2,3-*cd*]pyrene	Carbazole	Malathion	
o,p′-DDT	Naphthalene	Celestolide (ADBI)	Methyl parathion	
p,p′-DDT	Phenanthrene	Chlorpyrifos	Metolachlor	
Endrin	Pyrene	Cholesterol	Metribuzin	
Endosulfan	1,2-Dimethylnaphthalene	Cotinine	Pendimethalin	
Endosulfan II	1-Ethylnaphthalene	Diazinon	Prometon	
Endosulfan sulfate	1-Methylfluorene	Dichlorvos	Prometryn	
Heptachlor	1-Methylnaphthalene	Diethyl phthalate	Propazine	
Heptachlor epoxide	2,3,5-Trimethylnaphthalene	Diethylhexylphthalate	*S*-ethyl dipropyl carbamothioate	
Hexachlorobenzene	2-Methylfluoranthene	D-Limonene	Simazine	
Lindane	2-Methylnaphthalene	Ethyl citrate	Simetryn	
p,p′-Methoxychlor	2-Methylphenanthrene	Galaxolide (HHCB)	Terbuthylazine	
Mirex	3,6-Dimethylphenanthrene	Indole	Terbutryn	
cis-Nonachlor	4-Methylbiphenyl	Isophorone	Trifluralin	
trans-Nonachlor	9-Methylanthracene	Isopropylbenzene (cumene)		
Oxychlordane	Benzo[*b*]naphtho[2,1-*d*]thiophene	Isoquinoline		
Pentachloroanisole	Benzo[*b*]thiophene	Menthol		
cis-Permethrin	Benzo[*e*]pyrene	Metalaxyl		
trans-Permethrin	Biphenyl	Methyl salicylate		
Trifluralin	Dibenzothiophene	*N,N*-diethyltoluamide (DEET)		
	Perylene	*para*-Cresol		
Total PCBs		Phantolide (AHMI)		
		Phenol		
		Prometon		
		Tetrachloroethylene		
		Tonalide (AHTN)		
		Traseolide (ATII)		
		Tri(2-chloroethyl) phosphate		
		Tri(butoxyethyl) phosphate		
		Tri(dichloroisopropyl) phosphate		
		Tributyl phosphate		
		Triphenyl phosphate		

[a] DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; PCB = polychlorinated biphenyl.
[b] Chemicals in this category were analyzed for in semipermeable membrane device extracts.
[c] Chemicals in this category were analyzed for in polar organic chemical integrative sampler extracts.

mm; film thickness, 0.25 μm; J&W Scientific) [18]. Quantitation of OCs and PCBs were accomplished using a six-point internal standard calibration curve with PCB congeners I-30 and I-207 as internal standards. The concentrations of the OC standards ranged from 1.0 to 80 ng/ml. The PCB calibration standards were composed of a 1:1:1:1 (w/w/w/w) mixture of Aroclors 1242, 1248, 1254, and 1260, covering the range from 200 to 4,000 ng/ml.

Polar organic chemical integrative sampler

The procedures used for preparing the POCIS samples for analysis in the present study are similar to published approaches [8,9,18]. Chemicals of interest were recovered from the POCIS sorbent using 50 ml of 1:1:8 (v/v/v) methanol: toluene:dichloromethane, followed by 20 ml of ethyl acetate. The extracts were reduced in volume by rotary evaporation, filtered, and composited into 2-POCIS equivalent samples, thereby increasing the amount of chemical present in each sample to aid in detection. It often is desirable to combine POCIS extracts, because sampling rates often are low as a result of their small surface area.

Organic wastewater chemicals. Analysis of the waste indicator chemicals was performed on raw POCIS extracts because of the difficulty in adequately cleaning up a sample while maintaining the integrity of such a diverse set of chemicals. Analyses were performed on the GC-MSD system described previously using a temperature program of injection at 40°C, which was held for 3 min, then ramped at 9°C/min to 320°C and held for 3 min. Identification of the targeted chemicals was performed using positive-ion electron-impact ionization full-scan mass spectrometry. Quantitation was performed by comparison of unique ions for each chemical to a four-point calibration curve from 100 to 5,000 ng/ml with *p*-terphenyl-d_{14} as the internal standard.

Agricultural pesticides. Details regarding the processing and analysis of POCIS for agricultural pesticides have been reported previously [18]. Briefly, the extracts were fractionated using SEC, followed by sample cleanup and enrichment using Florisil adsorption chromatography. Analysis was performed using the GC-MSD system described previously [18]. A six-point calibration curve ranging from 10 to 2,000 ng/ml with *p*-terphenyl-d_{14} as the internal standard was used for quantification.

Hormones. Processing methods for selected hormones from POCIS have been reported previously [20]. Briefly, the extracts were fractionated by SEC, with the collect window initiated at 5% of the time between the apexes of the chromatographic reference peaks diethylhexylphthalate and biphenyl [19]. The post-SEC samples were enriched and fractionated by adsorption chromatography using potassium hydroxide–impregnated silica gel. Half of each extract was taken to near dryness under high-purity N_2, redissolved in 0.5 ml of 1:1 (v/v) water:acetonitrile, and analyzed by high-performance liquid chromatography (HPLC). These underivatized extracts were analyzed with a Hewlett-Packard 1090 Series II liquid chromatograph with a diode-array detector and a Supelco Discovery® C_8 analytical column (length, 150 mm; inner diameter, 4.6 mm; film thickness, 5 μm particle diameter). The remaining extract halves were derivatized for GC-MSD analysis. Quantitation of the HPLC analyses was performed using external calibration of an eight-point calibration curve ranging from 10 to 500 ng of each hormone injected on-column. A separate raw extract (no processing) from each site also was derivatized and analyzed by GC-MSD to rule out any unexpected procedural recovery problems.

Derivatization of extracts and calibration standards for GC-MSD analysis was initiated by the addition of 2% methoxyamine-HCL in pyridine followed by heating at 70°C for 2 h. Then, a mixture of bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane and triethylamine was added to the samples, with an additional 18 h on the heating block at 70°C. Next, the derivatized samples were solvent-exchanged into hexane, then run through silica gel (300-mg) minicolumns to remove color and any precipitate. The derivatized hormones were recovered from the silica gel with hexane before analysis. Analysis of the derivatized extracts was performed using the GC-MSD system described previously, with the temperature program of injection at 90°C, which was ramped at 25°C/min to 200°C, at 4°C/min to 255°C, and at 10°C/min to 310°C and then held for 3 min. A five-point calibration curve ranging from 50 to 5,000 ng/ml with *p*-terphenyl-d_{14} as the internal standard was derivatized concurrently with the field samples and blanks.

In vitro bioluminescent yeast estrogen screen

The BLYES was employed to estimate estrogenic potential of compounds accumulated by the POCIS during the duration of the deployment. Strain BLYES was kindly supplied by the Sayler Laboratory. The assay was performed in accordance with the published methods of Sanseverino et al. [16] with slight modifications. In short, strain BLYES was grown in modified minimal medium without leucine and uracil (YMM [leu^-, ura^-]) at 30°C and shaking at 150 rpm to an approximate optical density at 600 nm of 1.0. One hundred microliters were transferred to each well of a black, 96-well Costar microtiter plate preloaded with 100 μl of POCIS sample diluted 10% in YMM (leu^-, ura^-). All samples were assayed in triplicate per plate, and each plate contained a series of 17β-estradiol (E_2) standards ranging from 8.2×10^{-14} to 8.0×10^{-7} M. Samples were assayed on four separate occasions to assess repeatability. Stock E_2 and POCIS samples were solubilized in methanol. Control wells contained YMM (leu^-, ura^-) and the appropriate concentration of methanol to assess baseline bioluminescence of strain BLYES. Plates were incubated at 30°C in a humidified chamber at 100 rpm on an orbital shaker for 3 h and then loaded into SPECTRAFluor Plus plate reader (Tecan) for kinetic bioluminescence measurements. The measurements of the test plates were taken every 30 min for 6 h, and induced bioluminescence was determined using an integration time of 2 s/well and a gain value of 150. Estrogenicity was measured as the fold-induction of bioluminescence relative to the E_2 control. Relative estrogenicity also was determined for each site by subtracting the measured relative light units of deployed POCIS values from the corresponding site-specific POCIS control. All relative light unit data were assigned a relative estrogenicity via interpolation from the standard curve using a four-parameter logistic equation using Prism 4 for Windows® (GraphPad Software).

Statistical analyses were performed with SyStat 11 at $\alpha = 0.05$. One-way analysis of variances (ANOVAs) examined differences in bioluminescence between sites and rivers. The Tukey–Kramer post hoc test was executed if the general ANOVA model was significant.

Quality control

The method detection limit (MDL) and method quantitation limit (MQL) were estimated from the average signal to noise

ratio of the response of targeted chemicals from the instrumental analysis of the laboratory and field matrix blanks (SPMD or POCIS). A detailed discussion of the types of blanks used has been given elsewhere [7,9,18]. The MDLs were determined as the mean plus three standard deviations of the response of a coincident peak present in the blanks [21]. The MQLs were determined as the mean plus 10 standard deviations of the target chemicals [21]. In cases when no coincident peak was present, the MQL was set at the low-level calibration standard, and the MDL was estimated to be 20% of the MQL. This process of determining MDL/MQL values from the blanks accounts for any bias resulting from the sampler's materials, handling, shipping, storage, and processing.

Throughout the passive sampler processing and procedural steps, matrix spikes and instrumental verification checks were employed to monitor for potential problems. Radiolabeled surrogates of model compounds were added to select quality-control samples and immediately measured using a liquid scintillation counter (model LS6500; Beckman Coulter) at specific steps in the processing scheme to rapidly determine processing recoveries and identify potential problems. Select SPMDs from each study period were fortified with [^{14}C]phenanthrene (a common PAH), with recoveries of 91 and 89% for the fall and spring, respectively. Select POCIS were spiked with [^{3}H]ethinylestradiol (a widely used synthetic hormone) in both the fall and spring, with recoveries of 94 and 84%, respectively. In spring, a POCIS was spiked with [^{14}C]diazinon (a common organophosphate insecticide), resulting in a recovery of 66%. Recovery of chemicals throughout the SEC system, monitored using [^{14}C]phenanthrene, averaged 97%, with a relative standard deviation ($n = 4$) of 3.7%.

Matrix (i.e., fabrication and field) blanks for the passive samplers were processed and analyzed concurrently with the field-deployed samplers. Overall, the blanks did not indicate any problems of sample contamination resulting from the materials and/or processing and handling of the samplers in the laboratory or field. The fall SPMDs did show a slightly elevated background of OC pesticides during the GC-ECD analysis, which contributed to somewhat higher MDLs and MQLs for that sample set. The interfering peaks were determined not to be the chemicals of interest but, rather, to be coeluting materials originating from the polyethylene membrane of the SPMDs, because these peaks were present at a similar intensity and retention time in SPMD matrix blanks run concurrently.

For reporting purposes, the MDLs and MQLs for each sample set were calculated as the approximate ambient water concentrations based on the average PRC data across the sites for each sampling period. When sampling rate information was not available, the MDLs and MQLs were expressed as the mass of chemical sequestered by a single sampler (i.e., ng/POCIS or ng/SPMD).

Estimation of ambient water concentrations

Using previously developed models [7–9], PRC loss data, chemical sampling rates (when available), and amounts of chemicals sampled, the average water concentrations of selected chemicals can be estimated. Uptake of chemicals into passive samplers generally follows linear, curvilinear, and equilibrium phases of sampling. Integrative (or linear) sampling is the predominant phase for compounds with log $K_{OW} \geq 5.0$ and exposure periods of up to one month in SPMDs and for most of the chemicals tested in the POCIS. During the linear uptake phase, the ambient chemical concentration (C_w) is determined by

$$C_w = N/R_s t \quad (1)$$

where N is the amount of the chemical accumulated by the sampler (typically ng), R_s is the sampling rate (L/d), and t is the exposure time (d). Previous data indicate that many chemicals of interest sampled by the POCIS remain in the linear phase of sampling for at least 56 d [8,9]; therefore, the use of a linear uptake model (Eqn. 1) for the calculation of ambient water concentrations was justified.

For SPMDs, regression models have been created that estimate a chemical's site-specific R_s and its C_w based on the log K_{OW} of the chemical, the PRC's release rate constant (k_e), and the SPMD–water partition coefficient (K_{sw}) [7]. A PRC's k_e is determined from the amount of PRC initially added to the SPMD (N_0) and the amount remaining (N), as shown in Equation 2. The log K_{sw} is determined from a regression model of the PRC's log K_{OW}, as shown in Equation 3, where a_0 is the intercept (determined to be −2.61 for PCBs, PAHs, and nonpolar pesticides and −3.20 for polar pesticides). The $R_{s,PRC}$ can then be calculated as shown in Equation 4, where V_s is the volume of the SPMD:

$$k_e = -[\ln(N/N_0)]/t \quad (2)$$

$$\log K_{sw} = a_0 + 2.321 \log K_{OW} - 0.1618(\log K_{OW})^2 \quad (3)$$

$$R_{s,PRC} = V_s K_{sw} k_e \quad (4)$$

The extrapolation of C_w from measured values of N requires knowledge of a chemical's site-specific sampling rate ($R_{s,i}$), which is determined from a third-order polynomial (Eqn. 5), where $\alpha_{(i/PRC)}$ is the compound-specific effect on the sampling rate and the relationship between the $R_{s,PRC}$ and $R_{s,i}$ (Eqn. 6):

$$\log \alpha_{(i/PRC)} = 0.0130(\log K_{OW})^3 - 0.3173(\log K_{OW})^2 + 2.244 \log K_{OW} \quad (5)$$

$$R_{s,i} = R_{s,PRC}(\alpha_i/\alpha_{PRC}) \quad (6)$$

The C_w of a chemical in the water can then be calculated by

$$C_w = N/\{V_s K_{sw}[1 - \exp(-R_s t/V_s K_{sw})]\} \quad (7)$$

RESULTS

Chemical analyses

In the present study, 138 individual chemicals (not including the ~120 individual PCB congeners used to estimate total PCBs) were selected as representative anthropogenic organic chemicals that may be present from agricultural, industrial, and municipal inputs (Table 1). Analysis of the passive samplers resulted in the detection of 84 of these targeted chemicals. Chemicals that were detected in a passive sampler from at least one site are shown as the mean of replicate samples in Tables 2 to 5. In cases when the value of one replicate was less than the MDL, the value of the other replicate was given representing the maximum observed value. In general, the replication was quite good, with an average relative percentage difference of 17% ($n = 458$). Based on the availability of chemical sampling rates and the PRC data, water concentrations were estimated from the chemical residues sampled by the SPMDs and POCIS [7–9]. If the sampling rate for a chemical was unknown, the result was given as mass of chemical per sampler to be used for comparing the relative loading between sites.

Table 2. Estimated water concentrations of detected organochlorine pesticides in semipermeable membrane devices from the 2005/2006 sampling periods in the Potomac, USA, watershed[a]

	Site identification[b] and sampling year											
	UP C Creek (pg/L)		DS C Creek (pg/L)		UP Mon River (pg/L)		DS Mon River (pg/L)		Blue Plains (pg/L)		NFHRL (pg/L)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
α-Benzenehexachloride	<130[c]	<210	*250*[d]	<210	*220*	—[e]	*180*	<210	*230*	—	—	<210
β-Benzenehexachloride	<4.6	<140	<4.6	<140	<4.6	—	*5.5*	*170*	*9.3*	—	—	<140
δ-Benzenehexachloride	89[f]	<2.5	94	29	<49	—	93	29	220	—	—	<2.5
cis-Chlordane	21	24	72	52	17	—	38	35	330	—	—	*7.1*
trans-Chlordane	22	20	64	67	18	—	46	30	240	—	—	*10*
Chlorpyrifos	<11	120	120	180	*19*	—	48	160	480	—	—	280
Dacthal	<9.5	<150	21	<150	*15*	—	*16*	<150	<9.5	—	—	<150
Dieldrin	180	130	300	200	100	—	200	150	550	—	—	19
o,p′-DDE[g]	<12	13	<12	8.9	<12	—	<12	11	*15*	—	—	*4.7*
p,p′-DDE	78	80	83	88	*44*	—	70	57	87	—	—	*34*
o,p′-DDD[h]	29	*37*	30	*46*	<8.8	—	110	*40*	61	—	—	<19
p,p′-DDD	*22*	22	*41*	*36*	<18	—	*33*	*26*	*47*	—	—	9.1
o,p′-DDT	*41*	<8.4	62	*15*	<38	—	480	98	180	—	—	<8.4
p,p′-DDT	<74	*110*	*170*	*110*	<74	—	*100*	<90	*160*	—	—	<90
Endrin	*48*	54	81	88	55	—	70	59	*51*	—	—	21
Endosulfan	*85*	*270*	*74*	550	*80*	—	*96*	*300*	1,100	—	—	*420*
Endosulfan II	550	<900	1,400	2,900	830	—	1,200	*1,000*	5,000	—	—	<900
Heptachlor	<0.8	<1.9	<0.8	54	<0.8	—	<0.8	6.9	25	—	—	<1.9
Heptachlor epoxide	*69*	*44*	170	64	*68*	—	150	*37*	410	—	—	*35*
Hexachlorobenzene	83	38	*54*	41	<22	—	<22	*18*	*55*	—	—	<14
Lindane	*440*	<540	620	<540	*460*	—	550	<540	*470*	—	—	<540
p,p′-Methoxychlor	<88	<20	*94*	*21*	<88	—	*97*	*28*	140	—	—	<20
Mirex	26	5.3	*6*	<0.8	19	—	<1.3	*3.8*	<1.3	—	—	<0.8
cis-Nonachlor	*7.1*	<10	9.8	*14*	*6.8*	—	11	<10	35	—	—	<10
trans-Nonachlor	35	*47*	52	*58*	<25	—	49	*45*	110	—	—	<37
Oxychlordane	<2.2	*1.6*	7.2	9.4	*3*	—	7.4	3	60	—	—	*1.4*
Pentachloroanisole	56	<120	230	<120	110	—	190	<120	310	—	—	<120
cis-Permethrin	<240	*8.5*	<240	<7.0	<240	—	*270*	<7.0	<240	—	—	<7.0
Trifluralin	*120*	3.4	180	<0.6	<110	—	200	<0.6	230	—	—	<0.6
Total PCBs[i]	<210	3,900	*220*	580	<210	—	410	790	2,600	—	—	<210

[a] Only compounds detected in at least one sample are listed. A full list of compounds analyzed for is given in Table 1. Reported values are the mean of replicate samples.
[b] UP C Creek = upstream Conococheague Creek; DS C Creek = downstream Conococheague Creek; UP Mon River = upstream Monocacy River; DS Mon River = downstream Monocacy River; Blue Plains = Potomac River at Blue Plains WWTP, Washington, DC; NFHRL = National Fish Health Research Laboratory, Kearneysville, West Virginia.
[c] Less than (<) values are below the method detection limit (MDL).
[d] Italic values are estimates greater than the MDL but less than the method quantitation limit (MQL) and are shown for informational purposes only.
[e] — = site was not sampled during this study year.
[f] Values in roman type are reportable values greater than the MQL.
[g] DDD = dichlorodiphenyldichlorethane.
[h] DDE = dichlorodiphenyldichloroethylene.
[i] Total polychlorinated biphenyls (PCBs) determined from a 1:1:1:1 (w/w/w/w) mixture of Aroclors 1242, 1248, 1254, and 1260.

The number and relative water concentrations of the OC pesticides were similar between the fall and spring samplings (Table 2). Pentachloroanisole (a degradation product of pentachlorophenol), chlorpyrifos, *cis/trans*-chlordane, dieldrin, and endrin were commonly measured across the sampling sites and study periods. Endosulfan and its degradation product, endosulfan II, were present at the greatest concentrations (up to 5 ng/L) at the Blue Plains site. As expected, the highest concentrations for most of the targeted chemicals were found at the Blue Plains site, which is heavily influenced by urbanization. Up to 80% of the targeted PAHs, including the priority pollutant PAHs, were identified in SPMDs from the fall and spring samplings (Table 3). In the fall, the downstream Monocacy River and Blue Plains sites were the most heavily contaminated with PAHs, with concentrations of up to 4.7 ng/L (phenanthrene). The downstream Monocacy River site continued to be the most contaminated with PAHs in the spring, with fluoranthene having the maximum concentration of 5.4 ng/L.

A screen for chemicals potentially originating from wastewater inputs identified several OWCs, such as fragrances, plasticizers, and flame retardants (Table 4). The Blue Plains site had the greatest number of detections and the highest concentrations of OWCs from the fall sampling. Surprisingly, the upstream Conococheague Creek samples also had detectable levels of fragrances and flame retardants, indicating a potential wastewater input. Atrazine, also identified at all sites in the agricultural pesticides screen, was confirmed by the OWC screen. In the spring sampling, the downstream Monocacy River site had the greatest number of OWCs, which was consistent to the chemical data from OC pesticide and PAH analyses.

Several chemicals associated with agricultural practices were found during both the fall and spring samplings (Table 5). Atrazine, metolachlor, and the atrazine metabolites desisopropylatrazine (DIA) and desethylatrazine (DEA) were the most commonly identified. In the fall, atrazine concentrations

Table 3. Estimated water concentrations of detected polycyclic aromatic hydrocarbons in semipermeable membrane devices from the 2005/2006 sampling periods in the Potomac, USA, watershed[a]

	Site identification[b] and sampling year											
	UP C Creek (pg/L)		DS C Creek (pg/L)		UP Mon River (pg/L)		DS Mon River (pg/L)		Blue Plains (pg/L)		NFHRL (pg/L)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Acenaphthene	210[c]	370	170	340	220	—[d]	360	480	410	—	—	320
Anthracene	*52*[e]	130	*60*	99	*55*	—	250	230	160	—	—	*40*
Benzo[*a*]anthracene	37	<1.8[f]	39	140	23	—	140	230	370	—	—	<1.8
Benzo[*a*]pyrene	*25*	*29*	*16*	*24*	<9.5	—	*29*	130	78	—	—	<6.0
Benzo[*b*]fluoranthene	74	<5.2	77	<5.2	30	—	210	<5.2	260	—	—	<5.2
Benzo[*ghi*]perylene	*49*	<7.9	*52*	<7.9	*13*	—	65	92	130	—	—	<7.9
Benzo[*k*]fluoranthene	54	120	53	96	23	—	130	750	130	—	—	<5.7
Chrysene	230	240	230	160	130	—	880	1,700	1,200	—	—	*13*
Dibenz[*a,h*]anthracene	<10	<6.4	<10	<6.4	<10	—	<10	<6.4	*13*	—	—	<6.4
Fluoranthene	950	890	730	810	980	—	4,400	5,400	4,000	—	—	100
Fluorene	200	160	170	130	190	—	420	300	570	—	—	101
Indeno[1,2,3-*cd*]pyrene	*41*	*34*	*35*	*21*	<12	—	*37*	76	*40*	—	—	<7.2
Naphthalene	*730*	<140	*910*	<140	*760*	—	*760*	<140	1,200	—	—	<140
Phenanthrene	1,200	1,200	950	980	1,400	—	4,700	3,300	2,400	—	—	510
Pyrene	620	500	770	2,800	540	—	2,600	3,500	4,000	—	—	<21
1,2-Dimethylnaphthalene	*46*	<18	*60*	<18	*40*	—	*78*	*61*	120	—	—	<18
1-Ethylnaphthalene	<17	<15	*38*	<15	*19*	—	*59*	<15	85	—	—	<15
1-Methylfluorene	150	51	300	<6.9	*96*	—	390	230	1,000	—	—	<6.9
1-Methylnaphthalene	2,500	*300*	260	*260*	*210*	—	300	*190*	540	—	—	<180
2,3,5-Trimethylnaphthalene	87	<7.4	100	<7.4	*42*	—	220	<7.4	410	—	—	<7.4
2-Methylfluoranthene	37	34	40	36	25	—	110	220	220	—	—	<5.4
2-Methylnaphthalene	*240*	<270	*310*	<270	<230	—	*330*	<270	*530*	—	—	<270
2-Methylphenanthrene	120	180	120	160	150	—	580	660	560	—	—	<7.4
3,6-Dimethylphenanthrene	*34*	<5.4	42	<5.4	40	—	160	<5.4	420	—	—	<5.4
4-Methylbiphenyl	<130	<9.2	<130	600	<130	—	<130	260	<130	—	—	360
9-Methylanthracene	<8.6	<6.1	<8.6	<6.1	<8.6	—	<8.6	*29*	<8.6	—	—	<6.1
Benzo[*b*]naphtho[2,1-*d*]thiophene	21	*25*	20	31	15	—	140	290	180	—	—	<5.6
Benzo[*e*]pyrene	95	86	100	74	32	—	170	390	330	—	—	<6.1
Biphenyl	*60*	<42	*98*	<42	*75*	—	*83*	<42	*180*	—	—	<42
Dibenzothiophene	*68*	75	*56*	*57*	*71*	—	220	210	220	—	—	<15
Perylene	64	55	97	46	61	—	56	45	240	—	—	<5.5

[a] Only compounds detected in at least one sample are listed. A full list of compounds analyzed for is given in Table 1. Reported values are the mean of replicate samples.

[b] UP C Creek = upstream Conococheague Creek; DS C Creek = downstream Conococheague Creek; UP Mon River = upstream Monocacy River; DS Mon River = downstream Monocacy River; Blue Plains = Potomac River at Blue Plains WWTP, Washington, DC; NFHRL = National Fish Health Research Laboratory, Kearneysville, West Virginia.

[c] Values in roman type are reportable values greater than the method quantitation limit (MQL).

[d] — = site was not sampled during this study year.

[e] Italic values are estimates greater than the method detection limit (MDL) but less than the MQL and are shown for informational purposes only.

[f] Less than (<) values are below the MDL.

ranged from 23 ng/L (downstream Monocacy River) to 110 ng/L (downstream Conococheague Creek). Concentrations of DEA in the fall peaked at 59 ng/L in the upstream Conococheague Creek site. In the spring, atrazine concentrations were greatest, with a maximum concentration of 2,100 ng/L at the downstream Monocacy River site.

Initial analyses of the hormones in the POCIS extracts using HPLC were inconclusive; therefore, a portion of the extracts were reanalyzed by GC-MSD after derivatization to gain sensitivity and selectivity. No hormones were identified using either method. Because it was suspected that natural and/or synthetic hormones may have been present at the sites, a raw extract from a separate POCIS from each site was derivatized and analyzed by GC-MSD. As with the previous analyses, none of the targeted hormones was identified above the estimated MQL of 2.5 ng/L. Concentrations of E_2 in the fall at downstream Conococheague Creek and in the spring at upstream Conococheague Creek POCIS, and concentrations of E_2 and 17α-ethinylestradiol in the spring at downstream Monocacy River POCIS, were at the MDL.

In vitro bioluminescent yeast estrogen screen

Analysis of POCIS extracts with strain BLYES indicated that all sites surveyed contained chemicals with measurable estrogenicity (Fig. 2). Extracts collected during the fall sampling (corrected to their respective field blank) induced 2.50- to 6.22-fold more bioluminescence than with estrogen-free growth medium alone. Statistically significant differences were observed between the study sites (one-way ANOVA, $f = 55.99$, $p < 0.001$). Sampling sites upstream and downstream of targeted WWTPs within the same river did not statistically differ (Fig. 2a). Induction at the Blue Plains sampling site was nearly twice the amount observed at the other sites in the fall (Fig. 2a). In the spring, induction was lowest at the NFHRL reference site, whereas induction was greatest in the upstream Conococheague and downstream Monocacy (Fig. 2b). Extracts

Table 4. Amounts of waste indicator chemicals detected in polar organic chemical integrative sampler (POCIS) from the 2005/2006 sampling periods in the Potomac, USA, watershed[a]

	Site identification[b] and sampling year											
	UP C Creek (ng/POCIS)		DS C Creek (ng/POCIS)		UP Mon River (ng/POCIS)		DS Mon River (ng/POCIS)		Blue Plains (ng/POCIS)		NFHRL (ng/POCIS)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Atrazine	350[c]	4,450	400	5,100	690	—[d]	170	25,000	400	—	—	1,400
Benzophenone	*30*[e]	*30*	*30*	<20[f]	<20	—	*30*	*45*	*40*	—	—	<20
Carbazole	<20	<20	<20	<20	<20	—	<20	200	200	—	—	<20
Celestolide (ADBI)	<20	<20	130	<20	<20	—	<20	130	130	—	—	<20
Diethylhexylphthalate	*320*	*360*	*300*	610	400	—	<280	*340*	3,500	—	—	570
Ethyl citrate	100	110	250	130	<20	—	120	330	330	—	—	100
Galaxolide (HHCB)	<20	<20	340	30	<20	—	210	1,900	960	—	—	<20
Metalaxyl	*40*	<20	<20	<20	*40*	—	<20	<20	<20	—	—	<20
N,N-diethyltoluamide (DEET)	*50*	*55*	*55*	*65*	*50*	—	*50*	120	110	—	—	*40*
Phantolide (AHMI)	<20	*70*	*70*	*70*	<20	—	<20	*80*	*80*	—	—	<20
Prometon	*95*	*95*	120	110	100	—	<20	120	150	—	—	<20
Tonalide (AHTN)	<20	<20	110	<20	<20	—	*30*	230	520	—	—	<20
Traseolide (ATII)	<20	<20	<20	<20	<20	—	<20	150	<20	—	—	<20
Tri(2-chloroethyl) phosphate	*75*	*60*	170	*80*	*85*	—	*95*	160	360	—	—	*60*
Tri(dichloroisopropyl) phosphate	<20	250	300	260	260	—	280	500	500	—	—	220
Tributyl phosphate	<20	<20	210	200	<20	—	200	220	290	—	—	<20
Triphenyl phosphate	*60*	<52	<52	<52	<52	—	*60*	*70*	*70*	—	—	<52

[a] Only compounds detected in at least one sample are listed. A full list of compounds analyzed for is given in Table 1. Reported values are the mean of replicate samples.
[b] UP C Creek = upstream Conococheague Creek; DS C Creek = downstream Conococheague Creek; UP Mon River = upstream Monocacy River; DS Mon River = downstream Monocacy River; Blue Plains = Potomac River at Blue Plains WWTP, Washington, DC; NFHRL = National Fish Health Research Laboratory, Kearneysville, West Virginia.
[c] Values in normal type are reportable values greater than the method quantitation limit (MQL).
[d] — = site was not sampled during this study year.
[e] Italic values are estimates greater than the method detection limit (MDL) but less than the MQL and are shown for informational purposes only.
[f] Less than (<) values are below the MDL.

from all sites during both sample years induced statistically elevated bioluminescence relative to responses to the estrogen-free controls ($p < 0.001$). Estimated estrogenicity relative to E_2 for all sites was in the nanomolar range. Estrogenic activity was detected in the field blanks, because bioluminescence was induced 1.1- to 3.2-fold higher than that in estrogen-free controls during the fall season and 1.0- to 2.9-fold during the spring. In all cases, induction by extracts from deployed POCIS devices were statistically greater than their corresponding field blanks.

Table 5. Estimated water concentration of detected agricultural pesticides in polar organic chemical integrative sampler (POCIS) from the 2005/2006 sampling periods in the Potomac, USA, watershed[a]

	Site identification[b] and sampling year											
	UP C Creek (ng/L)		DS C Creek (ng/L)		UP Mon River (ng/L)		DS Mon River (ng/L)		Blue Plains (ng/L)		NFHRL (ng/L)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Atraton	<0.13[c]	<0.08	<0.13	<0.08	1.9[d]	—[e]	<0.13	<0.08	<0.13	—	—	<0.08
Atrazine	47	380	110	430	92	—	23	2100	54	—	—	120
Desethylatrazine	59	18	18	20	52	—	8.3	11	10	—	—	66
Desisopropylatrazine	18	*2.8*[f]	18	*2.8*	19	—	18	*2.8*	18	—	—	15
Metolachlor	0.73	7.5	1.1	9	12	—	11	97	1.9	—	—	<0.90
Prometon	1.1	*1.2*	3.2	*1.4*	2.1	—	1.4	1.8	6.1	—	—	<0.45
Simazine	8.1	17	<0.29	18	12	—	<0.29	38	<0.29	—	—	7.4
Terbuthylazine	<0.23	<0.72	<0.23	<0.72	<0.23	—	<0.23	<0.72	9.1	—	—	<0.72
DAR[g] values	1.4	0.05	0.2	0.05	0.6	—	0.4	0.01	0.2	—	—	0.63

[a] Only compounds detected in at least one sample are listed. A full list of compounds analyzed for is given in Table 1. Reported values are the mean of replicate samples.
[b] UP C Creek = upstream Conococheague Creek; DS C Creek = downstream Conococheague Creek; UP Mon River = upstream Monocacy River; DS Mon River = downstream Monocacy River; Blue Plains = Potomac River at Blue Plains WWTP, Washington, DC; NFHRL = National Fish Health Research Laboratory, Kearneysville, West Virginia.
[c] Less than (<) values are below the method detection limit (MDL).
[d] Values in roman type are reportable values greater than the method quantitation limit (MQL).
[e] — = site was not sampled during this study year.
[f] Italic values are estimates greater than the MDL but less than the MQL and are shown for informational purposes only.
[g] DAR = desethyatrazine (mol/L) to atrazine (mol/L) ratio used as an indicator of pesticide transport.

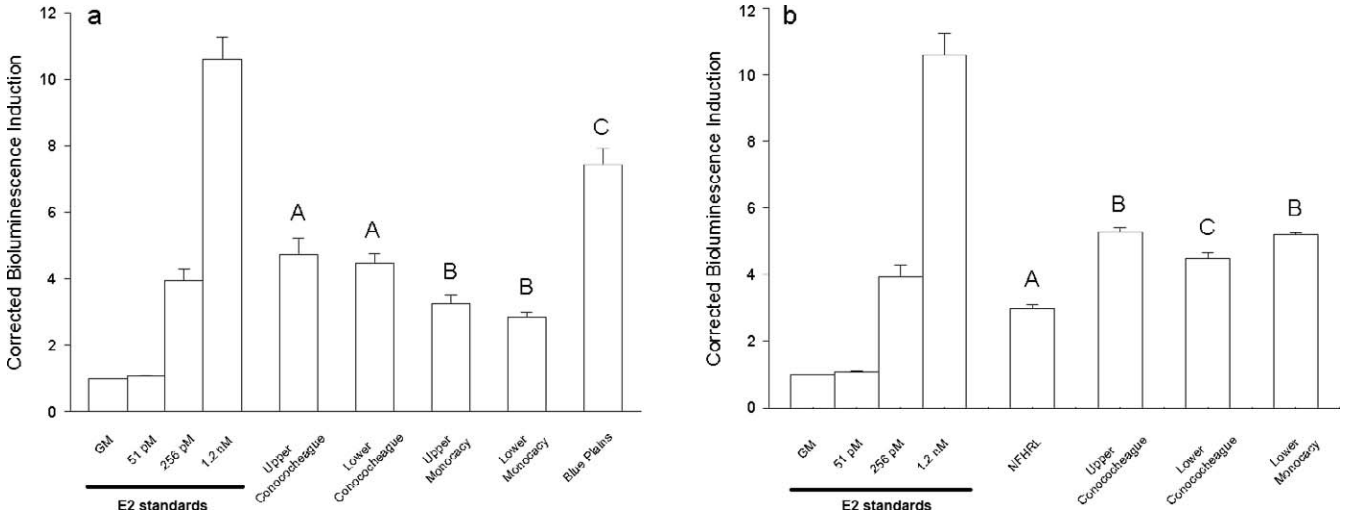

Fig. 2. Response of the bioluminescence yeast estrogen screen (BLYES) to polar organic chemical integrative sampler (POCIS) from 2005 (**a**) and 2006 (**b**). Induction of bioluminescence, relative to the 17β-estradiol control (E_2), is depicted as the difference of field-deployed POCIS versus the site-specific field blanks. Data were compared via one-way analysis of variance (Tukey–Kramer post hoc test). Sites denoted with different uppercase letters are statistically different ($p < 0.05$), whereas those with the same letters are not. GM = modified minimal growth medium without leucine and uracil (YMM [leu$^-$, ura$^-$]); NFHRL = 2006 reference site located at the U.S. Geological Survey's National Fish Health Research Laboratory, Kearneysville, West Virginia, USA.

DISCUSSION

Evaluation of chemical occurrence and relative concentrations were used to determine seasonal patterns, degradation of chemicals, and differences between sampling sites in common waterways (upstream vs downstream sites). Comparison of the data from the fall and spring samplings revealed no substantial differences between the occurrence or concentrations of OC pesticides, PAHs, or other OWCs. The BLYES indicated that the only significant difference in the total estrogenicity of sampled chemicals between the fall and spring samplings was at the downstream Monocacy River site (two-sample t test, $p = 0.001$). Kolpin et al. [22] reported decreasing concentrations of OWCs as stream flow increased, largely as a result of dilution. This effect was not observed in the present study, however, because the ratio of WWTP effluent to mean stream flow was largely unchanged between sampling periods.

The greatest changes in concentration between the sampling periods were for the agricultural pesticides atrazine and metolachlor. For both chemicals, the concentrations were 3.1- to 91-fold greater in the spring sampling, which was expected because of increased pesticide application corresponding to spring crop planting in the largely agricultural reaches of the watershed. Considering that the mean stream flow only increased twofold between the fall and spring (flow was measured at the downstream sites only), any variation in the POCIS R_s was considered to be negligible. The estimated water concentrations were similar to those reported by Alvarez et al. [20] from a sampling on the nearby North Fork of the Shenandoah River in northern Virginia during the spring and early summer of 2007.

Corresponding to the differences in atrazine concentrations are the changes in the occurrence of two of atrazine's main degradation products, DEA and DIA. At the three sites with both fall and spring samplings (upstream Conococheague Creek, downstream Conococheague Creek, and downstream Monocacy River), DIA concentrations were below the MQL in the spring but at quantifiable levels in the fall. Quantifiable concentrations of DEA were present at all three sites in both the spring and fall, with a threefold increase in concentration in the fall upstream Conococheague Creek sample. Greater concentrations of DIA and DEA in the fall can be attributed to degradation of the parent compound (atrazine) following spring and summer application.

A relative measure of residence time and mode of transport of agricultural chemicals in the system was determined using the deethylatrazine to atrazine ratio (DAR). The DAR is calculated by dividing the concentration of DEA by that of atrazine [23,24]. A DAR value of greater of 1.0 indicates primarily groundwater transport to the river, where atrazine is converted to DEA via metabolic activity of soil bacteria and fungi [23]. A DAR value of less than 1.0 is an indicator of point-source contamination, because transport to the river is mainly through surface runoff. Calculation of DAR ratios for the study sites shows that only upstream Conococheague Creek (i.e., 1.4) during the fall had a value indicative of a nonpoint-source contamination. A substantial decrease in the DAR was observed at all sites between the fall and spring sampling (upstream Conococheague Creek, 1.4 to 0.05; downstream Conococheague Creek, 0.2 to 0.05; downstream Monocacy River, 0.4 to 0.01), which clearly shows the fresh application of atrazine and subsequent runoff during the spring planting season (Table 5). The NFHRL reference pond had a DAR of 0.63, which likely resulted from overspraying and surface runoff from adjacent farms.

Generally, concentrations and numbers of chemicals detected were greater in water collected from sites downstream of WWTP discharges. In particular, OWCs had the greatest occurrence and concentrations in the downstream sites influenced by WWTP discharges. Similarly, the downstream Monocacy River site had much greater PAH concentrations than the corresponding upstream site, indicating that the WWTPs may have been a major source of PAHs in the Monocacy River. In contrast to these findings, the levels of PAHs and OWCs were relatively constant between the upstream and downstream Conococheague Creek sites. At both Monocacy River and Conococheague Creek, no substantial differences were found for

the agricultural pesticides between the upstream and downstream sites. The BLYES assay also showed elevated estrogenicity in samples from the upstream Conococheague Creek site, suggesting the presence of a WWTP or other waste discharge. A combination of a WWTP approximately 3 to 5 km upstream of the upstream site and leachate from septic tanks in this largely rural region of the watershed may have contributed to the elevated concentrations. A previous study showed that water concentrations of many OWCs remain largely unchanged over distances of 3 km [25].

The BLYES assay indicated that chemicals were present at each site that were capable of promoting an estrogenic effect at a level statistically greater than the background response observed in the blanks. It is not clearly understood which chemicals associated with the sampler matrix or sample processing may have been responsible for the observed response in the field blanks; however, it has been reported that the estrogenic response likely results from impurities in the POCIS membrane [18]. Chemical analysis of select natural and synthetic steroidal hormones found levels to be at or less than the MDL. However, because of the strong responses observed in the BLYES, one or more estrogens or estrogen mimicking chemicals likely contributed to the response. A definitive identification of the estrogen mimics would involve a combination of analytical chemistry methods and in vivo or in vitro estrogenic assays in a manner similar to toxicity identification and evaluation tests. Such methods were beyond the scope of the present study.

Iwanowicz et al. [2] found that intersex had occurred in 82 to 100% of the male smallmouth bass collected at both the upstream and downstream sites during the fall sampling. This suggests that multiple chemical stressors that are not solely associated with agriculture or WWTP effluent may be responsible for reproductive impairment in fish. Little is known about the long-term chronic effects resulting from exposure to trace concentrations of OWCs [26]. Atrazine is a likely suspect because of its widespread use in the region and elevated concentrations at the study sites; however, direct effects on the reproductive health of various fish species have not been found [27–29]. Although a direct link between intersex and organic contaminants has not been identified, the present study provides important information about the types and relative concentrations of chemicals that were present in areas where intersex in fish occurs.

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