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Integrated Assessment of Wastewater Reuse, Exposure Risk, and Fish Endocrine Disruption in the Shenandoah River Watershed

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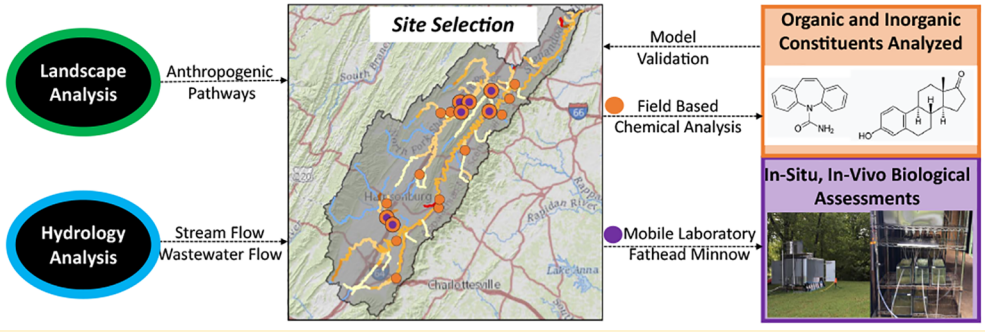
Supporting Information



ABSTRACT: Reuse of municipal and industrial wastewater treatment plant (WWTP) effluent is used to augment freshwater supplies globally. The Shenandoah River Watershed (U.S.A.) was selected to conduct on-site exposure experiments to assess endocrine disrupting characteristics of different source waters. This investigation integrates WWTP wastewater reuse modeling, hydrological and chemical characterization, and in vivo endocrine disruption bioassessment to assess contaminant sources, exposure pathways, and biological effects. The percentage of accumulated WWTP effluent in each river reach (ACCWW%) was used to predict environmental concentrations for consumer product chemicals (boron), pharmaceutical compounds (carbamazepine), and steroidal estrogens (estrone, 17-β-estradiol, estriol, and 17-α-ethinylestradiol). Fish endocrine disruption was evaluated using vitellogenin induction in adult male or larval fathead minnows. Water samples were analyzed for >500 inorganic and organic constituents to characterize the complex contaminant mixtures. Municipal ACCWW% at drinking water treatment plant surface water intakes ranged from <0.01 to 2.0% under mean-annual streamflow and up to 4.5% under mean-August streamflow. Measured and predicted environmental concentrations resulted in 17-β-estradiol equivalency quotients ranging from 0.002 to 5.0 ng L^{-1} indicating low-to-moderate risk of fish endocrine disruption. Results from the fish exposure experiments showed low (0.5- to 3.2-fold) vitellogenin induction in adult males.

INTRODUCTION

Treated wastewater is used to augment freshwater supply throughout the U.S. and globally.[1−3] A challenge in reusing wastewater treatment plant (WWTP) effluent is the presence of biologically active chemicals that are introduced by domestic, commercial, and industrial activities, and that are not completely removed during treatment.[4,5] Widespread occurrence of municipal WWTP-derived contaminants has been reported in streams across the U.S. and Europe.[6−9] Streamwater composition depends on flow regime, landscape characteristics, and municipal WWTP discharges.[10] Industrial WWTP discharges also contribute to streamflow and contaminant loading.[11] Knowing the accumulated volume of wastewater in a stream is essential to assessing chemical exposure pathways and potential risks. Rivers provide a hydrologic connection between WWTP discharges and downstream uses, and chemicals present in effluents potentially can affect ecosystems and drinking-water supplies.[12]

Exposure to endocrine disrupting chemicals (EDCs), such as steroid hormones, can interfere with the normal function of the endocrine system of wildlife and humans and is an issue of global

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concern.[13,14] Endocrine disruption attributed to exposure to EDCs has been observed in wild fish populations,[15,16] including intersex (testicular oocytes) in smallmouth bass (*Micropterus dolomieu*) in the Chesapeake Bay Watershed and Shenandoah River.[17−21] The river-reach scale, at which water and fish sampling occurs and drinking water treatment plant (DWTP) intakes are located, integrates the influence of municipal- and industrial-WWTP contributions from the upstream watershed. Realistic environmental exposure scenarios require that effects of wastewater reuse on streamwater quality and fish endocrine disruption be addressed at different spatial, temporal, and mechanistic scales.[22]

This integrated interdisciplinary study investigates wastewater reuse, water chemistry, and fish endocrine disruption, and applies "lessons from endocrine disruption"[23] and "principles of sound ecotoxicology".[24] A diversity of approaches were used from study design to completion to establish relations between landscape, hydrological, chemical, and biological characteristics of a river system with population-level fish endocrine disruption. The study applied: (1) landscape analysis to establish geologic and anthropogenic features; (2) hydrological modeling of streamflow characteristics and accumulated proportion of WWTP effluent; (3) consumer-product-chemical modeling to predict environmental concentrations of EDCs and other compounds; (4) landscape analysis and modeling to inform site selection for field investigations; (5) comprehensive inorganic and organic chemical analysis to measure environmental concentrations in streamwater; (6) endocrine disruption evaluation using modeled estrogenic biological responses and comparison to in situ/in vivo experiments using fathead minnows (*Pimephales promelas*); and (7) development of modeling tools to assess the effects of wastewater reuse on water quality and aquatic organisms at the watershed scale.

METHODS

Study Area and Hydrology. The Shenandoah River Watershed (SRW) is located in the Valley and Ridge province of northern Virginia and eastern West Virginia.[25,26] Three major tributaries (North River, Middle River, and South River) converge south of Staunton to form the South Fork Shenandoah River (a Strahler[27] 5th-order stream), which joins the North Fork Shenandoah River near Front Royal to form the Shenandoah River (6th-order stream), which joins the Potomac River (7th-order stream), which flows into the Chesapeake Bay (Figure 1A). The estimated water travel time from the South River to the Potomac River is 15 to 20 d.[28] There are 22 U.S. Geological Survey (USGS) streamgages (Figure 1B; Table S1 of the Supporting Information, SI) that provide long-term records for calculation of flow exceedance probabilities. The 25th percentile flow exceedance probability represents high-flow conditions, the 50th percentile represents median-flow conditions, the 75th percentile represents low-flow conditions, and the 90th and 95th percentiles represent drought conditions.[29]

The 2008 population of the SRW was 333 833 people, with approximately 50% living in incorporated urban areas (public water supply, centralized sewage collection, and wastewater treatment systems) and 50% living in unincorporated rural areas (household water supply wells and on-site wastewater disposal systems).[30,31] In 2014, the population of the SRW was 407 124.[32] Major cities (Figure 1A) located in the South Fork Shenandoah River drainage include Harrisonburg (population 52 258; WWTP discharges to the North River), Staunton (population 24 427; WWTP discharges to the Middle River),

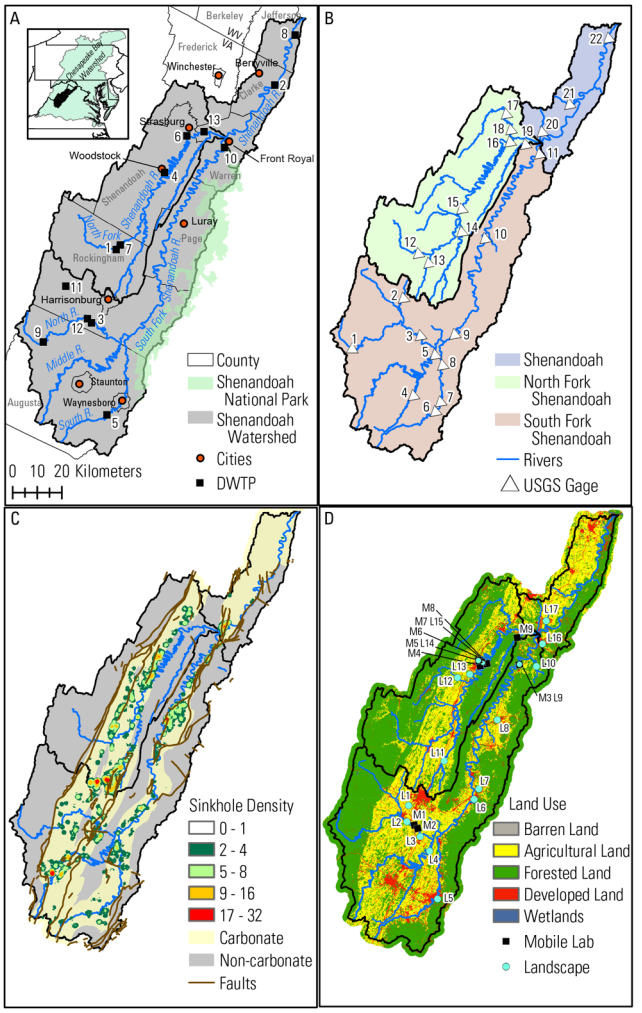

Figure 1. Maps of the Shenandoah River Watershed showing (A) rivers, counties, cities, and drinking water treatment plant (DWTP) intakes (Table S3), (B) 8-digit Hydrologic Unit Code (HUC8) boundaries and USGS streamgages (Table S1), (C) carbonate and noncarbonate geology, and (D) mobile laboratory experiment locations, landscape sampling locations, and land-use characteristics (Table S5). [Numbers correspond to locations listed in Tables S1, S3, and S5; Sinkhole density in panel C represents the number of sinkholes per 100 km^2; Map Projection Albers equal area; North American Datum 1983].

and Waynesboro (population 21 186; WWTP discharges to the South River). Communities that discharge WWTP effluent to the North Fork Shenandoah River are smaller and include Strasburg (population 6542) and Woodstock (population 5214). Front Royal (population 15 055) is located at the confluence of the North Fork Shenandoah River and South Fork Shenandoah River and the WWTP discharges to the South Fork Shenandoah River. The 2014 population density for the entire SRW was 51 people per km^2, with the North Fork Shenandoah River drainage having 30 people per km^2, the South Fork Shenandoah River drainage having 52 people per km^2, and the Shenandoah River drainage having 73 people per km^2.[32]

The SRW is underlain by igneous and metamorphic rocks interspersed with carbonate sedimentary rocks (Figure 1C).[33,34] The carbonate areas have extensive karst hydrology characterized by springs and sinkholes.[35−37] Land use in the SRW is controlled by the mountainous topography, with highland tributaries predominated by forest and river valley streams

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predominated by agriculture and developed land, including urban areas (Figure 1D).[38] Agricultural activities include row crops (primarily corn and soybean) and animal feeding operations (AFOs).[39,40]

Accumulated Wastewater. The national-scale "de facto reuse incidence in our nation's consumable supply" (DRINCS) model[41,42] was downscaled to the SRW and used to estimate the accumulated effluent discharged from all municipal and industrial WWTPs. The DRINCS model encompassed 9 834 000 km^2 with a 10^{th}-order stream (Mississippi River) and the SRW model encompassed 7920 km^2 with a 6^{th}-order stream. The downscaling included adding municipal WWTPs and DWTPs that serve communities with populations <10 000. A web-based tool was developed[43,44] to calculate accumulated municipal and industrial WWTP discharges based on the National Hydrography Data set Plus Version 2 (NHDPlus V2) stream networks with enhanced runoff method (EROM) streamflow estimates,[26] Virginia StreamStats data,[45] and USGS streamgage data. WWTP locations were obtained from the Clean Watershed Needs Survey (CWNS),[46] and the Virginia and West Virginia Pollutant Discharge Elimination Systems.[47] Information for USGS streamgages (Table S1), municipal and industrial WWTP facilities (Table S2), and DWTPs with surface water intakes (Table S3) were linked to each NHDPlus V2 stream segment through the common identifier number.

The amount of wastewater in a given river reach is represented by the percentage of accumulated wastewater (ACCWW%), which was calculated using Python scripting language,[48] NHDPlus V2 network connectivity attributes, EROM streamflows, and reported WWTP discharges (Figure S1):

$$[(\Sigma \text{ upstream and incoming WWTP discharge})/(\text{EROM streamflow} + \Sigma \text{ upstream WWTP discharge})] \times 100 \quad (1)$$

Scenarios for WWTP inputs were based on monthly and annual 2015 discharge data reported by the facilities. Scenarios for streamflow conditions were based on mean annual EROM streamflow (1971 to 2000) and mean August EROM streamflow (1971 to 2000). The ACCWW% is analogous to the cumulative dilution factor (i.e., a 5% ACCWW% equates to 20-fold in-stream dilution].[12]

Predicted Chemical Exposure Pathways. The ACCWW% was used to account for accumulated contaminant loading from upstream municipal WWTP discharges. This approach considers loading of contaminants to WWTPs and removal during treatment, followed by loading to the receiving stream, dilution with native water, and in-stream attenuation.[49−51] Predicted environmental concentrations (PECs) for select consumer product chemicals and EDCs were estimated using population served by the WWTP, per capita chemical use, per capita water use, loss associated with human metabolism, loss during WWTP treatment, in-stream dilution, and in-stream degradation (Figure S2; Table S4). PECs were not calculated for industrial WWTPs because their effluent composition is controlled by processes unique to each facility and further evaluations were outside the scope of this study.

Biological Risk Assessment. Fish endocrine disruption in the SRW was assessed using modeled PECs, the fathead minnow as the model organism, and relative 17-β-estradiol potency factors for mixtures of steroidal estrogens.[52] The reproductive biology and endocrinology of the fathead minnow have been well-characterized,[53,54] and vitellogenin induction in male fathead minnows (and other fish species) follows well-defined dose−response curves for estrone, 17-β-estradiol, and 17-α-ethinylestradiol (individually and as mixtures) that allows reliable determination of relative 17-β-estradiol potencies.[55−58]

Concentrations of individual estrogen compounds were converted to 17-β-estradiol equivalents using relative potencies for estrone, 17-β-estradiol, and 17-α-ethinylestradiol of 0.38, 1.0, and 30, respectively.[55,56] Species sensitivity distribution analysis estimated 17-β-estradiol relative potencies for estrone, 17-β-estradiol, estriol, and 17-α-ethinylestradiol of 0.3, 1.0, 0.003, and 20, respectively.[58] For each sample the 17-β-estradiol equivalents for the estrogens were summed to give a total 17-β-estradiol equivalency quotient (E2EQ). The predicted-no-effects concentrations (PNECs) for estrone, 17-β-estradiol, estriol, and 17-α-ethinylestradiol are 6, 2, 60, and 0.1 ng L^{-1}, respectively.[58] This work focuses on steroid estrogens because of the targeted estrogen-receptor mediated mode-of-action and adverse-outcome-pathways for the fathead minnow model organism.[59]

Field-Based Chemical and Biological Characterization. The landscape analysis and ACCWW% assessments were used to identify locations for intensive field investigations to characterize chemical exposure pathways and biological responses. Mobile laboratory fish exposure protocols[60,61] were used to provide an integrated assessment of endocrine disruption at 9 locations in the SRW (Figure 1D, Table S5).[62] The sites represent different land uses and included 2 municipal WWTPs. The North River WWTP served 70 900 people, treated sewage (46% domestic, 15% commercial, and 39% industrial) using advanced activated sludge processes with enhanced nutrient removal and chlorine disinfection, had a 2015 mean annual discharge of 0.53 m^3 s^{-1}, and contributed 4.3% of the downstream flow. The North Fork Shenandoah River WWTP served 5230 people, treated sewage (100% domestic and commercial) using activated sludge with membrane bioreactor nutrient removal and ultraviolet light disinfection, had a 2015 mean annual discharge of 0.067 m^3 s^{-1}, and contributed 1.6% of the downstream flow.

Twelve mobile-laboratory experiments were conducted from 2014 to 2016 (Table S5). Reproductively mature (6 mo old) male and female fathead minnows were exposed to river water (or WWTP effluent) for 21 d under continuous-flow conditions with controlled temperature, oxygenation, diet, and photoperiod.[60−62] Fish were maintained in 10-L glass aquaria at a density of 5 fish per tank, and organisms (n = 10 for each treatment) were collected on day 0, 7, and 21. Individual organisms from each time point were analyzed for a variety of biomarkers including plasma vitellogenin by enzyme-linked immunosorbent assay and hepatic vitellogenin mRNA analysis.[62]

Contemporaneous water samples were collected from the inflows to the mobile laboratories at day 0, 7, 14, and 21. Extensive chemical analyses (21 separate analytical methods) were conducted and 601 organic and inorganic constituents were measured (59 of the constituents were measured by multiple methods). Details of sampling procedures, analytical methods, and quality assurance data are presented elsewhere.[62] Measured environmental concentrations (MECs) and bioassay results from these field-based exposure experiments were used to evaluate calculated PECs and E2EQs.

The landscape analysis and ACCWW% assessments also were used to select 17 additional sites across the SRW to cover the gradient of potential WWTP effluent exposures (Figure 1D; Table S5). Water samples were collected and analyzed for a

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more limited suite of constituents (499 constituents using 17 separate analytical methods) than the mobile laboratory experiments.[62] A different approach for assessing endocrine disruption was used for the landscape sampling, and a short-term in vivo bioassay was developed using larval fathead minnows exposed for 7 d (daily water replacement) and analysis of the whole organism for vitellogenin mRNA.[62]

Statistical correlation analysis of PECs, MECs, and vitellogenin induction was performed using the nonparametric Spearman's coefficient (ρ) and associated p-value.[63] To resolve ties, tied data were randomly assigned a rank within the range of uncertainty and 10 000 permutations of the Spearman test were performed. The mean ρ and p-value of the permutation distribution are reported.

RESULTS AND DISCUSSION

Wastewater Reuse. The municipal ACCWW% for the SRW were initially calculated using data from the DRINCS model,[41,42] which included 29 municipal WWTPs having a combined design flow of 1.4 m^3 s^{-1}. Higher resolution data specific to the SRW were developed to enhance the national-scale data and included 81 municipal WWTPs having a combined design flow of 3.1 m^3 s^{-1} and a combined 2015 mean annual discharge of 1.6 m^3 s^{-1} (Figure 2A; Table S2).[43] The mean annual municipal ACCWW% at the most downstream SRW site (confluence with the Potomac River) using the watershed-scale data was 2.2 times greater than using the national-scale data. Additionally, there were 25 industrial WWTPs in the SRW with a combined design flow of 1.4 m^3 s^{-1} and a combined 2015 mean annual discharge of 0.81 m^3 s^{-1} (Figure 2B; Table S2) that were not represented in the DRINCS model.

The ACCWW% analysis indicated that 422 of the 1754 river segments in the SRW receive WWTP discharges. The municipal ACCWW% under mean annual streamflow ranged from 0 to 17% (Figure 2C). Under mean August streamflow, the municipal-plus-industrial ACCWW% ranged up to 42% (Figure 2D). At the confluence of the Shenandoah River with the Potomac River, the municipal-plus-industrial ACCWW% was 2.6% under mean annual streamflow and 5.8% under mean August streamflow.

The municipal and municipal-plus-industrial ACCWW% were evaluated using long-term flow statistics at the 22 USGS streamgages (Table S1). For streamgages located near where the mobile laboratory experiments were conducted (Figure 3A) the municipal-plus-industrial ACCWW% ranged from 0.01 to 4.7% under 25th percentile streamflow condition, 0.02 to 9.8% under 50th percentile streamflow conditions, and 0.12 to 30% under 90th percentile streamflow conditions. The median municipal-plus-industrial ACCWW% was 5.8% for third-order to sixth-order streams under 50th percentile streamflow conditions. The median municipal-plus-industrial ACCWW% was 0.09% for first-order and second-order streams because of the limited WWTP discharges.

There were 13 municipal DWTPs in the SRW that had surface water intakes (Figure 1A; Table S3) and the mean annual withdrawals ranged from 0.014 to 0.30 m^3 s^{-1} (Figure 4A). Five of the DWTPs had no upstream municipal WWTPs and thus no municipal de facto reuse. The other DWTPs had municipal ACCWW% ranging from 0.01 to 2.0% under mean annual streamflow (Figure 4B) and from 0.01 to 4.5% under August streamflow (Table S3). The municipal-plus-industrial ACCWW % at the DWTP intakes ranged from 0.01 to 3.3% under mean annual streamflow (Table S3) and from 0.01 to 7.0% under August streamflow (Figure 4C). Only 3 DWTPs had no municipal-plus-industrial de facto reuse. The national-scale DRINCS analysis of DWTPs with surface water intakes reported municipal ACCWW% values ranging from 2.3 to 16%.[42]

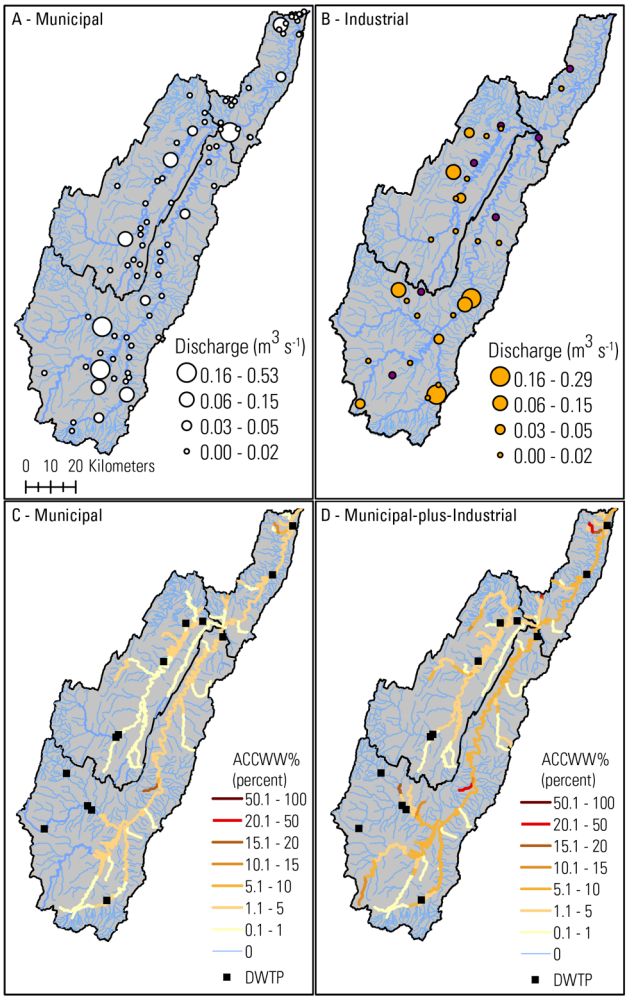

Figure 2. Maps of the Shenandoah River Watershed showing (A) locations and relative sizes of municipal wastewater treatment plants (WWTPs), (B) locations and relative sizes of industrial WWTPs, (C) municipal percentage of accumulated wastewater (ACCWW%) using National Hydrography Database Plus V2 (NHDPlus V2) mean annual streamflow and 2015 mean annual municipal WWTP flow, and (D) municipal-plus-industrial ACCWW% using NHDPlus V2 mean August streamflow and 2015 mean annual WWTP flow. [See Tables S1 and S2 for streamflow and WWTP flow information; Map Projection Albers equal area; North American Datum 1983; refs 25 and 26 are sources of base map].

Predicted and Measured Contaminant Concentrations. Stream reaches influenced by wastewater reuse have complex chemical mixtures containing bioactive chemicals, such as EDCs, pharmaceuticals, pesticides, and trace elements derived from municipal and industrial WWTP discharges, urban runoff, and agricultural activities.[6−8,64] Although this work focuses on PEC calculations (Figure S2; Table S6) for boron (consumer product wastewater tracer), carbamazepine (pharmaceutical wastewater tracer), and estrogenic hormones (specific reproduction mode-of-action and outcomes), the approach can be applied to any constituent for which MECs were determined,[62] provided reliable chemical and biological

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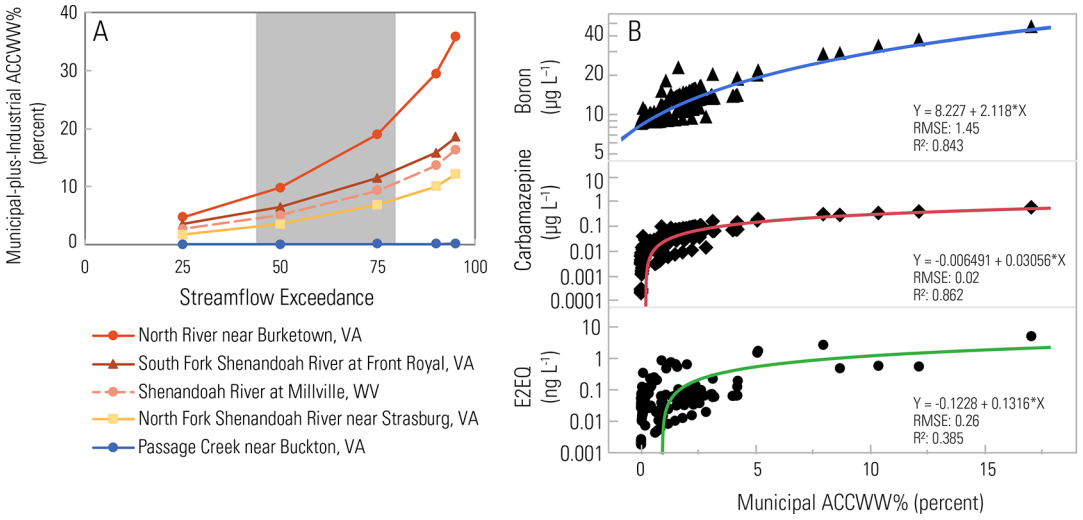

Figure 3. Relations between (A) municipal-plus-industrial percentage of accumulated wastewater (ACCWW%) at select U.S. Geological Survey streamgages in the Shenandoah River Watershed and streamflow exceedance probabilities, and (B) predicted environmental concentrations for boron, carbamazepine, and 17-β-estradiol equivalency quotient (E2EQ) and municipal ACCWW%. [See Figure 1B and Table S1 for streamgage information; gray area in panel A represents range of exceedance probabilities that occurred during the 2014, 2015, and 2016 mobile laboratory experiments; RMSE, residual mean square error].

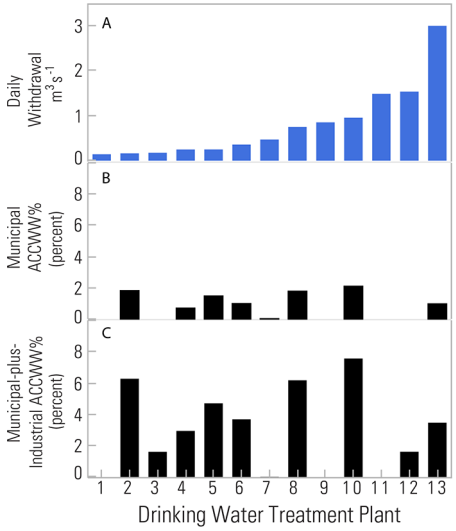

Figure 4. Percentage of accumulated wastewater (ACCWW%) at drinking water treatment plants (DWTPs) in the Shenandoah River Watershed with surface water sources: (A) 2015 mean annual DWTP withdrawals, (B) municipal ACCWW% using National Hydrography Database Plus V2 (NHDPlus V2) mean annual streamflow and 2015 mean annual municipal WWTP flow, (C) municipal-plus-industrial ACCWW% using NHDPlus V2 mean August streamflow and 2015 mean annual municipal and industrial WWTP flows. [See Figure 1A and Table S3 for DWTP information; see Figure 2A and Table S2 for WWTP information; numbers correspond to locations listed in Table S3].

model input parameters are available. Because of uncertainty in the chemical composition of industrial WWTP discharges, the "down-the-drain" chemical loading approach used for municipal WWTPs is not applicable. However, potential for significant chemical loading to the SRW from industrial WWTP discharges is illustrated by mercury contamination in the South River.[65] Although beyond the scope of this work, the data gap on industrial processes and chemical discharges could be addressed by incorporating information from additional databases.[66,67]

Chemical analyses of source-water samples (n = 56) from the mobile-laboratory experiments detected 312 (several detected by more than one method) of the 601 organic and inorganic constituents measured by all methods (Table S7). The more limited analysis of the landscape samples (n = 17) detected 124 (several detected by more than one method) of the more limited number (499) of organic and inorganic constituents measured (Table S8). Details on quality assurance data (field blanks, replicates, matrix spikes, and surrogate standards) and the complete data set are presented elsewhere.[62]

Boron is a good tracer of municipal WWTP effluents because it is used as a consumer-product chemical (the bleaching agent sodium perborate is used in laundry detergents), is not removed during WWTP treatment, and persists in the stream environment.[64,68] Boron also is naturally occurring, and loading from municipal WWTPs often only represents a fraction of background levels.[69] Boron PECs in the SRW were corrected for background concentrations based on MECs from Passage Creek (mean = 8.5 μg L^{-1}; Table 1), and are near concentrations reported for precipitation.[70] Municipal WWTP sources contributed low boron PECs (Figure 5A; Table S6) in the SRW because of the large stream dilution factors (municipal ACCWW% generally < 5%; Figure 2C).

There was a significant positive correlation (ρ = 0.67, p = 0.001; Figure S3A) between boron PECs and mobile laboratory and landscape sampling MECs (Figure 5A) despite uncertainties in the factors that go into calculating PECs (Figure S2). For example, using 2 sets of model input data (Table S4)[71,72] results in median boron PECs of 11 and 16 μg L^{-1} and maximum boron PECs of 47 and 110 μg L^{-1}. Boron MECs in the SRW WWTP effluents ranged from 50 to 158 μg L^{-1} (Table S7) and were lower than typically reported (>250 μg L^{-1}) for municipal WWTPs.[64,68−72] These low values could reflect the trend of decreasing boron consumption observed in the U.K.,[70] or other factors, such as industrial wastewater contributions. The maximum boron MEC (158 μg L^{-1}) was near the species sensitivity distribution PNEC of 340 μg L^{-1}.[73]

In contrast to boron, the synthetic pharmaceutical compound carbamazepine has no natural sources and its presence in stream

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Table 1. Predicted Environmental Concentrations (PECs) and Measured Environmental Concentrations (MECs) for Select Consumer Product Chemicals and Endocrine Disrupting Compounds in River Water and Wastewater Treatment Plant (WWTP) Effluents Where Mobile Laboratory Experiments Were Conducted during 2014, 2015, and 2016 in the Shenandoah River Watershed[a]

	SHR	PC	NRA	SFSR	NFSRA	NFSRB1	NFSRB2	NRW	NFSRW
USGS Station Number	0163363995	01635484	01621515	0163062888	01633626	01633640	01633641	382021078-554700	385310078-284900
NHDPlus V2 common identifier	8441261	8441257	5908727	5907115	8441273	8440841	8440841	5908727	8440841
Mean annual municipal ACCWW%	0.00	0.01	0.00	2.16	0.69	1.05	1.05	NA	NA
Mean August municipal ACCWW%	0.00	0.03	0.00	4.58	1.66	2.54	2.54	NA	NA
Mean annual municipal-plus-industrial ACCWW%	0.00	0.01	0.53	3.54	1.23	1.58	1.58	NA	NA
Mean August municipal-plus-industrial ACCWW%	0.00	0.03	1.53	7.53	2.94	3.82	3.82	NA	NA
Boron PEC ($\mu g\ L^{-1}$)	NA	0.20	NA	3.8	0.80	1.1	1.1	130	76
Boron PEC background corrected ($\mu g\ L^{-1}$)	NA	8.7	NA	12	9.3	9.6	9.6	138	84
Boron MEC ($\mu g\ L^{-1}$) mean ± SD	9.1 ± 1.4	8.5 ± 0.8	8.8 ± 0.8	10 ± 1.1	8.7 ± 0.8	15 ± 2.8	8.2 ± 0.3	50 ± 0.4	130 ± 16
Carbamazepine PEC ($\mu g\ L^{-1}$)	NA	0.003	NA	0.051	0.011	0.015	0.015	1.8	1.1
Carbamazepine MEC ($\mu g\ L^{-1}$) mean ± SD	<0.001 ± NA	<0.001 ± NA	0.001 ± 0.0001	0.008 ± 0.003	0.013 ± 0.03	0.014 ± 0.006	0.002 ± 0.001	0.067 ± 0.02	0.280 ± 0.08
Estrone PEC ($ng\ L^{-1}$)	NA	0.009	NA	0.032	0.009	0.010	0.010	0.70	0.40
Estrone MEC ($ng\ L^{-1}$) mean ± SD	0.31 ± 0.04	<0.05 ± NA	1.3 ± 0.44	1.1 ± 0.69	1.1 ± 0.38	<0.05 ± NA	0.76 ± 0.16	1.4 ± 0.03	0.76 ± NA
17-β-Estradiol PEC ($ng\ L^{-1}$)	NA	0.007	NA	0.014	0.004	0.005	0.005	0.200	0.100
17-β-Estradiol MEC ($ng\ L^{-1}$) mean ± SD	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA	2.4 ± NA	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA
Estriol PEC ($ng\ L^{-1}$)	NA	0.194	NA	0.299	0.098	0.105	0.105	3.80	2.20
Estriol MEC ($ng\ L^{-1}$) mean ± SD	<0.05 ± NA	4.8 ± NA	<0.05 ± NA	8.0 ± NA	3.0 ± 1.1	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA	5.1 ± NA
17-α-Ethinylestradiol PEC ($ng\ L^{-1}$)	NA	0.00049	NA	0.0019	0.00048	0.00058	0.00058	0.051	0.030
17-α-Ethinylestradiol MEC ($ng\ L^{-1}$) mean ± SD	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA	2.4 ± NA	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA	<0.05 ± NA
E2EQ_PEC ($ng\ L^{-1}$ 17-β-estradiol equivalent)	NA	0.027	NA	0.072	0.020	0.023	0.023	2.01	1.16
E2EQ_MEC ($ng\ L^{-1}$ 17-β-estradiol equivalent)	0.12 ± 0.02	0.14 ± NA	0.49 ± 0.17	16 ± 35	0.46 ± 0.14	<0.05 ± NA	0.29 ± 0.06	0.53 ± 0.01	0.22 ± 0.10
E2EQ_BLYES ($ng\ L^{-1}$ 17-β-estradiol equivalent)	1.2 ± NA	<0.31 ± NA	<0.31 ± NA	<0.31 ± NA	<0.31 ± NA	<0.31 ± NA	<0.31 ± NA	<0.31 ± NA	<0.31 ± NA
Vitellogenin induction (fold) mean ± SD	3.2 ± 5.3	1.6 ± 2.0	0.53 ± 0.49	1.2 ± 1.9	1.5 ± 2.3	1.3 ± 1.2	0.12 ± 0.12	0.43 ± 0.72	2.8 ± 2.8
Vitellogenin mRNA induction (fold) mean ± SD	0.51 ± 0.57	0.71 ± 1.5	0.20 ± 0.37	0.61 ± 1.4	0.69 ± 1.0	0.39 ± 0.37	0.11 ± 0.14	0.06 ± 0.10	0.42 ± 0.24

[a][Site locations are shown in Figure 1B and described in Table S5; USGS, U.S. Geological Survey; MEC values represent mean of detects for weekly samples for all experiments conducted at the site (n = 4 to 12); SD, standard deviation of detects; Spring Hollow Run (SHR—2016); Passage Creek (PC—2014 and 2015); North River above WWTP (NRA—2014); South Fork Shenandoah River (SFSR—2014, 2015, and 2016); North Fork Shenandoah River above WWTP (NFSRA—2015 and 2016); North Fork Shenandoah River 100 m below WWTP (NFSRB1—2016); North Fork Shenandoah River 1000 m below WWTP (NFSRB2—2015); North River WWTP (NRW—2014); North Fork Shenandoah River WWTP (NFSRW—2015 and 2016); NHDPlus V2, National Hydrography Dataset Plus Version 2; ACCWW%, percentage of accumulated wastewater; PECs calculated using protocols outlined in Figure S2; E2EQ_PEC, 17-β-estradiol equivalency quotient calculated using PECs for estrone, 17-β-estradiol, estriol, and 17-α-ethinylestradiol, and their respective 17-β-estradiol relative potency factors (0.38, 1.0, 0.003, and 30); E2EQ_MEC, 17-β-estradiol equivalency quotient calculated using MECs for estrone, 17-β-estradiol, estriol, and 17-α-ethinylestradiol, and their respective 17-β-estradiol relative potency factors (0.38, 1.0, 0.003, and 30); E2EQ_BLYES, 17-β-estradiol equivalency quotient measured using in vitro bioluminescent yeast estrogen screen; Vitellogenin induction, plasma vitellogenin fold-induction in adult male fathead minnows after 21-d exposures relative to initial controls; Vitellogenin mRNA induction, liver vitellogenin messenger RNA fold-induction in adult male fathead minnows after 21-d exposures relative to initial controls; --, not determined because no upstream WWTPs; NA, not available].

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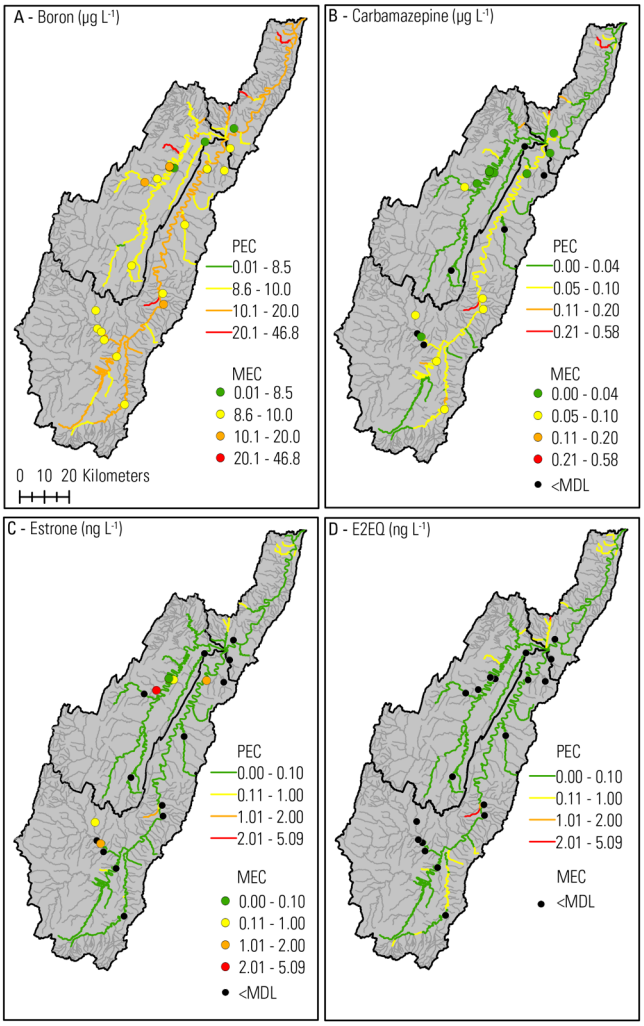

Figure 5. Maps of the Shenandoah River Watershed showing predicted environmental concentrations (PECs, shown as stream segment lines) for contaminants derived from municipal wastewater treatment plant discharges using mean August streamflow, and measured environmental concentrations (MECs, shown as circles) from the mobile laboratory and landscape samplings: (A) boron, (B) carbamazepine, (C) estrone, and (D) 17-β-estradiol equivalency quotient (E2EQ). [See Figure S2 and Table S4 for PEC calculations and model input data; see Table S6 for PEC results; see Tables S7 and S8 for MEC results; E2EQ in ng L^{-1} 17-β-estradiol equivalency calculated using PEC values and relative 17-β-estradiol potencies for estrone, 17-β-estradiol, estriol, and 17-α-ethinylestradiol of 0.38, 1, 0.003, and 30, respectively; Map Projection Albers equal area; North American Datum 1983; refs 25 and 26 are sources of base map].

waters is derived from anthropogenic sources, such as municipal WWTPs.[74] Carbamazepine PECs ranged from <0.001 to 0.58 μg L^{-1} with a median of 0.004 μg L^{-1} (Figure 5B; Table S6). Mobile laboratory and landscape sampling carbamazepine PECs and MECs (Tables S6, S7, and S8) were significantly correlated (ρ = 0.73, p = 0.0004; Figure S3B). The SRW carbamazepine results were in the range reported for U.S. waters (0.002 to 0.33 μg L^{-1}).[75] The maximum carbamazepine MEC for the SRW was 1.14 μg L^{-1} (Table S7), well below the PNEC of 232 μg L^{-1}.[75]

Natural steroid estrogens, such as estrone, have multiple environmental sources including municipal WWTPs and livestock production.[76−78] In contrast, the synthetic oral contraceptive 17-α-ethinylestradiol is consumed by humans, and discharged by municipal WWTPs.[79] Estrone was the most frequently detected steroid estrogen in the SRW with MECs in the mobile laboratory experiments ranging from <0.05 to 3.2 ng L^{-1} and PECs ranging from <0.001 to 1.7 ng L^{-1} (Figure 5C; Tables 1, S6, and S7). There was no significant correlation between estrone MECs and PECs at mobile laboratory sites (ρ = 0.32, p = 0.46; Figure S3C). Only one water sample had detectable 17-α-ethinylestradiol (2.4 ng L^{-1}, Table 1). The 17-α-ethinylestradiol PECs ranged from <0.0001 to 0.094 ng L^{-1} (median 0.0015 ng L^{-1}) under mean annual streamflow (Table S6). Although the PEC values are in the range of reported PECs for U.S. waters (median 0.0006 ng L^{-1}),[79] the single 17-α-ethinylestradiol MEC was above the PNEC of 0.35 ng L^{-1}.[80] The detection frequency for 17-β-estradiol, estriol, and 17-α-ethinylestradiol were too low for quantitative correlation analysis, but PECs were consistently below the method detection limit, indicating a qualitative agreement between MECs and PECs.

The E2EQ PECs (Figure 5D; Table S6) indicated that >98% of the stream reaches in the SRW had <0.12 ng L^{-1} 17-β-estradiol equivalents, three reaches had 1 to 2 ng L^{-1} 17-β-estradiol equivalents, and two reaches exceeded 2 ng L^{-1} 17-β-estradiol equivalents. There was no significant correlation between E2EQ PECs and MECs for all sites (ρ = 0.26, p = 0.31; Figure S3D), although both values generally were below 1 ng L^{-1} 17-β-estradiol equivalents. Uncertainties associated with calculation of E2EQs include the relative 17-β-estradiol potency values. For example, the estrogenic effects of estrone can be influenced by multiple organismal and environmental factors.[81,82] This work focuses on 4 estrogens with known estrogen-receptor mediated adverse-outcome-pathways in fathead minnows. However, many other potential EDCs with various modes-of-action were identified (Tables S7 and S8).[83] In contrast to the steroid estrogens, the lack of information on factors, such as modes-of-action and dose−response curves, for the other potential EDCs make it difficult to extend this analysis beyond the targeted compounds.

Fish Endocrine Disruption. The predicted E2EQs for the SRW (Figure 5D; Table S6) ranged from <0.05 to 5.0 ng L^{-1} 17-β-estradiol equivalents (mean = 0.11 ng L^{-1} 17-β-estradiol equivalents) and for the mobile laboratory sites ranged from 0.06 to 0.48 ng L^{-1} 17-β-estradiol equivalents, suggesting low-to-moderate risk for fish endocrine disruption.[52] Vitellogenin induction in male fathead minnows after 21 days of exposure was minimal for both plasma protein concentrations and hepatic mRNA levels. The highest vitellogenin plasma protein concentrations in adult male fathead minnows occurred at SHR and NFSRW, with fold-induction changes of 3.2 and 2.8 relative to initial controls, respectively (Table 1). Hepatic mRNA vitellogenin levels in adult males were lower at all mobile laboratory exposure sites than initial controls. The magnitudes of these fold-inductions were low compared to previous experiments involving exposure of adult male fathead minnows to estrogenic municipal WWTP effluents, where vitellogenin plasma protein increases were 100 to 1000 fold above controls.[5,61] Thus, there is little evidence for significant estrogenic endocrine disruption at these sites, consistent with their low E2EQ PECs and E2EQ MECs (Table 1). There was no correlation between E2EQ PECs and vitellogenin plasma protein induction in adult male fathead minnows at mobile laboratory sites (ρ = −0.15, p = 0.73; Figure S3E) or between E2EQ PECs and vitellogenin mRNA in larval fathead minnows at landscape sites (ρ = −0.13, p = 0.60; Figure S3F). This lack of

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correlation could be due to the low predicted and measured concentrations for the steroid estrogen compounds. Additionally, the lack of correlation between these variables could result from sources of steroid estrogens and EDCs other than municipal and industrial WWTPs, such as septic systems or agricultural operations. The results for the SRW are consistent with similar catchment-scale and national-scale EDC risk assessments that report mean E2EQ PECs ranging from 0.10 to 0.93 ng L^{-1} 17-β-estradiol equivalents.[84−87]

IMPLICATIONS OF WASTEWATER REUSE

Wastewater reuse is an important component of the anthropogenic hydrological cycle and influences water availability and water quality. Water composition and potential biological effects at a given location along a river reach are influenced by aggregated inputs and process in the upstream catchment. The on-site, continuous-flow fish exposure experiments conducted during this study captured the intrinsic variability that occurs in streamwater composition and EDC concentrations.[10,88,89] The ACCWW%, PEC, MEC, and fish exposure results suggest that SRW source waters have low-to-moderate risk for estrogenic endocrine disruption resulting from municipal WWTP discharges. However, these findings do not adequately account for the widespread endocrine disruption observed in smallmouth bass.[17−21] Because vulnerability to contaminant exposures can vary with species and critical life-history characteristics of free-living fishes, predictions of adverse effects based on short-term exposures using adult fathead minnows may not adequately reflect the risk of lifelong exposure to low concentrations, or to periodic short-term exposure to high concentrations of EDCs.

The modeling approach (Figure S2) used to determine PECs provides a tool for screening level assessment of endocrine disruption at the watershed scale. There are limitations to this approach, including uncertainties in municipal WWTP facility information (flows, population served, and treatment process), per capita consumption rates (contaminant and water), WWTP removal rates, and in-stream removal rates. Uncertainty in mass loading varies by constituent, being less for compounds, such as carbamazepine and 17-α-ethinylestradiol (per capita consumption constrained by specific pharmacological uses) than for boron (per capita consumption poorly constrained because of diverse sources). It is even more difficult to estimate per capita excretion rates for biogenic constituents, such as 17-β-estradiol and estrone, because production and elimination is unique to individual organisms and changes over time.

In-stream mixing and dilution in the model presented here is based on mean annual and mean August streamflows from long periods of record, whereas the WWTP flows were based on 2015 mean annual flows. These average values are representative of, but are different from, the instantaneous hydrological conditions represented by sampling events. Removal of EDCs by WWTPs is a function of contaminant, treatment type, and operational conditions.[5] In-stream attenuation involves complex hydrological, physical, and biogeochemical processes.[64,89] Although this work focused on estrogenic chemicals and endocrine disruption in fish, the methods apply to a broad range of contaminants and biological end points, including pharmaceutical compounds and human health.[90,91]

Flow-exceedance percentiles for the streamgages (Figure 3A, Table S1) provide insight into variability in ACCWW% that can be used to infer endocrine disruption risk using E2EQ calculations. When E2EQ values are plotted against municipal ACCWW% (Figure 3B), concentrations >1 ng L^{-1} 17-β-estradiol equivalent are associated with ACCWW% greater than 8%. During the mobile-laboratory experiments, streamflows were between the 50th and 75th percentile, and little endocrine disruption would be predicted given the low municipal ACCWW%. In comparison, during 2002 the SRW experienced a severe drought and flows were less than the 90th percentile for extended periods[29,92] during which municipal ACCWW% for the streamgages ranged from 7.0 to 26, potentially resulting in exposures of fish to E2EQ values >1 ng L^{-1}.

Landscape analysis that integrates hydrological, geochemical, and biological processes allows evaluation of a variety of contaminant sources and factors that control water quality. Although this study focused on WWTPs, the SRW has relatively limited urban development and is predominated by agricultural land, which includes intensive row-crop and animal production (Figure 1D). Previous assessment of endocrine disruption in the SRW found a strong relation between density of AFOs and in vitro estrogenic activity in the streamwater that was attributed to manure management practices.[39] Water samples in the earlier study were analyzed using the in vitro bioluminescent yeast estrogen screen (BLYES)[93] and had E2EQ values ranging from <0.31 to 7.2 ng L^{-1} 17-β-estradiol equivalents (23% of samples were below MDL).[39] Another investigation of endocrine disruption in the SRW applied a randomized and stratified sampling design, and BLYES estrogenic activity ranged from <0.31 to 1.2 ng L^{-1} 17-β-estradiol equivalents (58% of samples were below MDL).[94] In that study, predominantly agricultural watersheds BLYES estrogenic activity was strongly correlated with AFO density, but in urbanized watersheds the strongest correlation was with municipal WWTP density. In the present study, in vitro BLYES assay was applied to water samples from the SRW mobile laboratory experiments (n = 56) and values ranged from <0.31 to 1.2 ng L^{-1} 17-β-estradiol equivalents (98% of samples were below MDL).[62] The in vitro results are consistent with the in vivo adult and larval fathead minnow bioassays and chemical data.

The national-scale DRINCS model[41,42] was used to estimate endocrine disruption risk using a hazards quotient approach, and under median-streamflow conditions 32% of the receiving streams in the U.S. had hazard quotients with safety factors <10 for 17-β-estradiol and 17-α-ethinylestradiol, suggesting widespread potential risks.[12] The down-scaled application of the national model to the SRW indicated a lower risk for endocrine disruption, and <5% of the stream reaches had hazard quotients with safety factors <10. These results are consistent with the relatively low urban development and high in-stream WWTP effluent dilution factors in the SRW.

A major factor influencing endocrine disruption risk at the landscape level is the relation between population density and freshwater available for dilution. A study in England, where widespread fish endocrine disruption was observed, reported a high population density (388 people per km^2), a low water availability (3.8 m^3 d^{-1} $person^{-1}$), and a mean E2EQ value of 0.93 ng L^{-1} 17-β-estradiol equivalents.[85] In contrast, a study from Japan reported a similar population density (339 people per km^2) but greater water availability (19.6 m^3 d^{-1} $person^{-1}$), and a mean E2EQ value of 0.10 ng L^{-1} 17-β-estradiol equivalents.[87] The SRW had low population density (51 people per km^2) and high water availability (18.8 m^3 d^{-1} $person^{-1}$), and a mean E2EQ value of 0.11 ng L^{-1} 17-β-estradiol equivalents.

Aquatic risk assessments based on a finite set of contaminants and biomarkers cannot adequately address all factors occurring

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in dynamic stream ecosystems, including changing water composition and environmental conditions, species-specific vulnerability, life-history effects, trophic level, and dietary exposure. The tools developed in this study can be used to assess effects of human population growth, WWTP infrastructure upgrades, changes in land-use practices, and climate change on exposure to EDCs and potential for endocrine disruption. Many uncertainties remain, such as the loading of EDCs from industrial WWTPs and agricultural practices. There also is a need to further account for organism specific factors such as species, sex, and life history that affect contaminant exposure pathways and outcomes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b05655.

Three figures showing (1) methodology for calculating ACCWW%, (2) methodology for calculating PECs, and (3) correlation plots for select constituents (DOCX)

Eight tables describing (1) streamgages, (2) municipal and industrial WWTPs, (3) DWTPs, (4) input parameters for calculating PECs, (5) mobile laboratory and landscape sampling locations, (6) PECs for select wastewater constituents, (7) summary statistics for MECs from the mobile laboratory samples, and (8) summary statistics for MECs from the landscape samples (XLSX)

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The authors declare no competing financial interest.

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■ REFERENCES

(1) National Research Council. *Issues in Potable Reuse: The Viability of Augmenting Drinking Water Supplies with Reclaimed Water*; National Academies Press: Washington, DC, 1998.

(2) National Research Council. *Water Reuse: Potential for expanding the Nation's Water Supply through Reuse of Municipal Wastewater*; National Academies Press: Washington, DC, 2012.

(3) United Nations World Water Assessment Programme. Wastewater—The Untapped Resource. The United Nations World Water Development Report 2017, UNESCO, Paris, 2017.

(4) Andersen, H.; Siegrist, H.; Halling-Sorensen, B.; Ternes, T. A. Fate of estrogens in a municipal sewage treatment plant. *Environ. Sci. Technol.* **2003**, *37*, 4021−4026.

(5) Barber, L. B.; Vajda, A. M.; Douville, C.; Norris, D. O.; Writer, J. H. Fish endocrine disruption responses to a major wastewater treatment facility upgrade. *Environ. Sci. Technol.* **2012**, *46*, 2121−2131.

(6) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999−2000 - A national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.

(7) Focazio, M. J.; Kolpin, D. W.; Barnes, K. K.; Furlong, E. T.; Meyer, M. T.; Zaugg, S. D.; Barber, L. B.; Thurman, M. E. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States − II; Untreated drinking water sources. *Sci. Total Environ.* **2008**, *402*, 201−216.

(8) Bradley, P. M.; Journey, C. A.; Romanok, K. M.; Barber, L. B.; Buxton, H. T.; Foreman, W. T.; Furlong, E. T.; Glassmeyer, S. T.; Hladik, M. L.; Iwanowicz, L. R.; Jones, D. K.; Kolpin, D. W.; Kuivila, K. M.; Loftin, K. A.; Mills, M. A.; Meyer, M. T.; Orlando, J. L.; Reilly, T. J.; Smalling, K. L.; Villeneuve, D. L. Expanded target-chemical analysis reveals extensive mixed-organic-contaminant exposure in USA streams. *Environ. Sci. Technol.* **2017**, *51*, 4792−4802.

(9) Loos, R.; Gawlik, B. M.; Locoro, G.; Rimaviciute, E.; Contini, S.; Bidoglio, G. EU-wide survey of polar organic persistent pollutants in European river waters. *Environ. Pollut.* **2009**, *157*, 561−568.

(10) Johnson, A. C. Natural variations in flow are critical in determining concentrations of point source contaminants in rivers: an estrogen example. *Environ. Sci. Technol.* **2010**, *44*, 7865−7870.

(11) Phillips, P. J.; Smith, S. G.; Kolpin, D. W.; Zaugg, S. D.; Buxton, H. T.; Furlong, E. T.; Esposito, K.; Stinson, B. Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater treatment plant effluents. *Environ. Sci. Technol.* **2010**, *44*, 4910−4916.

(12) Rice, J.; Westerhoff, P. High levels of endocrine pollutants in US streams during low flow due to insufficient wastewater dilution. *Nat. Geosci.* **2017**, *10*, 587−591.

(13) Norris, D. O., Carr, J. A., Eds. *Endocrine Disruption: Biological Bases for Health Effects in Wildlife and Humans*; Oxford University Press: Oxford, UK, 2006.

(14) Bergman, A.; Heindel, J. J.; Jobling, S.; Kidd, K. A.; Zoeller, R. T. *State of the Science of Endocrine Disrupting Chemicals−2012*; World Health Organization: Geneva, Switzerland, 2013.

(15) Jobling, S.; Nolan, M.; Tyler, C. R.; Brighty, G.; Sumpter, J. P. Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* **1998**, *32*, 2498−2506.

(16) Vajda, A. M.; Barber, L. B.; Gray, J. L.; Lopez, E. M.; Woodling, J. D.; Norris, D. O. Reproductive disruption in fish downstream of an estrogenic wastewater effluent. *Environ. Sci. Technol.* **2008**, *42*, 3407−3414.

(17) Blazer, V. S.; Iwanowicz, L. R.; Iwanowicz, D. D.; Smith, D. R.; Young, J. A.; Hedrick, J. D.; Foster, S. W.; Reeser, S. J. Intersex (testicular oocytes) in smallmouth bass from the Potomac River and selected nearby drainages. *J. Aquat. Anim. Health* **2007**, *19*, 242−253.

(18) Blazer, V. S.; Iwanowicz, L. R.; Henderson, H.; Mazik, P. M.; Jenkins, J. A.; Alvarez, D. A.; Young, J. A. Reproductive endocrine disruption in smallmouth bass (*Micropterus dolomieu*) in the Potomac River basin: spatial and temporal comparisons of biological effects. *Environ. Monit. Assess.* **2012**, *184*, 4309−4334.

DOI: 10.1021/acs.est.8b05655
Environ. Sci. Technol. 2019, 53, 3429−3440

(19) Iwanowicz, L. R.; Blazer, V. S.; Guy, C. P.; Pinkney, A. E.; Mullican, J. E.; Alvarez, D. A. Reproductive health of bass in the Potomac, U.S.A., drainage: Part 1. Exploring the effects of proximity to wastewater treatment plant discharge. *Environ. Toxicol. Chem.* **2009**, *28*, 1072−1083.
(20) Kolpin, D. W.; Blazer, V. S.; Gray, J. L.; Focazio, M. J.; Young, J. A.; Alvarez, D. A.; Iwanowicz, L. R.; Foreman, W. T.; Furlong, E. T.; Speiran, G. K.; Zaugg, S. D.; Hubbard, L. E.; Meyer, M. T.; Sandstrom, M. W.; Barber, L. B. Chemical contaminants in water and sediment near fish nesting sites in the Potomac River Basin: Determining potential exposures to smallmouth bass (*Micropterus dolomieu*). *Sci. Total Environ.* **2013**, *443*, 700−716.
(21) Iwanowicz, L. R.; Blazer, V. S.; Pinkney, A. E.; Guy, C. P.; Major, A. M.; Munney, K.; Mierzykowski, S.; Lingenfelser, S.; Secord, A.; Patnode, K.; Kubiak, T. J.; Stern, C.; Hahn, C. M.; Iwanowicz, D. D.; Walsh, H. L.; Sperry, A. Evidence of estrogenic endocrine disruption in smallmouth and largemouth bass inhabiting Northeast U.S. national wildlife refuge waters: A reconnaissance study. *Ecotoxicol. Environ. Saf.* **2016**, *124*, 50−59.
(22) Franco, A.; Price, O. R.; Marshall, S.; Jolliet, O.; Van den Brink, P. J.; Rico, A.; Focks, A.; De Laender, F.; Ashauer, R. Toward refined environmental scenarios for ecological risk assessment of down-the-drain chemicals in freshwater environments. *Integr. Environ. Assess. Manage.* **2017**, *13*, 233−248.
(23) Sumpter, J. P.; Johnson, A. C. Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment. *Environ. Sci. Technol.* **2005**, *39*, 4321−4332.
(24) Harris, C. A.; Scott, A. P.; Johnson, A. C.; Panter, G. H.; Sheahan, D.; Roberts, M.; Sumpter, J. P. Principles of sound ecotoxicology. *Environ. Sci. Technol.* **2014**, *48*, 3100−3111.
(25) U.S. Geological Survey. National Watershed Boundary Dataset (WBD), Digital Vector Data from the National File-GDB 10. U.S. Geol. Survey: Washington, DC, 2014. [https://rockyftp.cr.usgs.gov/vdelivery/Datasets/Staged/WBD/FileGDB101/WBD_National.zip].
(26) U.S. Environmental Protection Agency. National Hydrography Dataset Plus streams - NHDPlus Version 2, Vector Digital Data Set. U.S. Environ. Protect. Agency: Washington, DC, 2012. [https://www.epa.gov/waterdata/nhdplus-national-hydrography-dataset-plus].
(27) Strahler, A. N. Quantitative analysis of watershed geomorphology. *Trans. Am. Geophys. Union* **1957**, *38*, 913.
(28) Taylor, K. R.; James, R. W., Jr.; Helinsky, B. M. Travel time and dispersion in the Shenandoah River and its tributaries, Waynesboro, Virginia to Harpers Ferry, West Virginia. U. S. Geol. Survey Water Res. Invest. Report 86−4065: U. S. Geol. Survey: Washington, DC, 1986.
(29) Krstolic, J. L.; Ramey, R. C. South Fork Shenandoah River habitat-flow modeling to determine ecological and recreational characteristics during low-flow periods. U.S. Geol. Survey Sci. Invest. Report 2012−5081: U.S. Geol. Survey: Washington, DC, 2012.
(30) Central Shenandoah Planning District Commission. Upper Shenandoah River Basin Water Supply Plan, 2011.
(31) Northern Shenandoah Valley Regional Commission. Northern Shenandoah Regional Water Supply Plan, 2011.
(32) U.S. Census Bureau. Cartographic boundary file for state, county, and urban areas of the United States, 1:500,000, 2014. Accessed July 14, 2017 at [https://www.census.gov/geo/maps-data/data/tiger-cart-boundary.html.].
(33) Southworth, S.; Aleinikoff, J. N.; Bailey, C. M.; Burton, W. C.; Crider, E. A.; Hackley, P. C.; Smoot, J. P.; Tollo, R. P. Geologic map of the Shenandoah National Park region Virginia. U.S. Geol. Survey Open-File Report 2009−1153, 1 plate, scale 1:100 000: U. S. Geol. Survey: Washington, DC, 2009.
(34) Virginia Department of Mines, Minerals, and Energy. Digital representation of the 1993 Geologic Map of Virginia. Publication 174, Virginia Div. Geol. Mineral Res.: Charlottesville, VA, 2005.
(35) Nelms, D. L.; Moberg, R. M., Jr. Hydrology and groundwater availability in Clarke County, Virginia. U. S. Geol. Survey Sci. Invest. Report 2010−5112: U.S. Geol. Survey; Washington, DC, 2010.
(36) Virginia Department of Mines, Minerals and Energy. Reconnaissance mapping of karst-related sinkholes in Virginia, Vector Digital Data, unpublished material, Virginia Div. Geol. Mineral Res.: Charlottesville, VA, 2004. Available upon request.
(37) Virginia Department of Environmental Quality. Springs−Vector Digital Data, unpublished material. Richmond, VA, 2015. Available upon request.
(38) Homer, C. G.; Dewitz, J. A.; Yang, L.; Jin, S.; Danielson, P.; Xian, G.; Coulston, J.; Herold, N. D.; Wickham, J. D.; Megown, K. Completion of the 2011 National Land Cover Database for the conterminous United States - Representing a decade of land cover change information. *Photogrammetric Engin. Remote Sensing* **2015**, No. 81, 345−354.
(39) Ciparis, S.; Iwanowicz, L. R.; Voshell, J. R. Effects of watershed densities of animal feeding operations on nutrient concentrations and estrogenic activity in agricultural streams. *Sci. Total Environ.* **2012**, *414*, 268−276.
(40) Gordon, S. E.; Jones, D. K.; Williams, B. M.; Wright, C. Potential contaminant sources and other landscape variables summarized for NHDPlus Version 2.1 catchments within the Chesapeake Bay Watershed. U.S. Geol. Survey data release: U.S. Geol. Survey: Washington, DC, 2017. [https://doi.org/10.5066/F7SQ8ZB3].
(41) Rice, J.; Wutich, A.; Westerhoff, P. Assessment of de facto wastewater reuse across the U.S.: Trends between 1980 and 2008. *Environ. Sci. Technol.* **2013**, *47*, 11099−11105.
(42) Rice, J.; Westerhoff, P. Spatial and temporal variation in de facto wastewater reuse in drinking water systems across the U.S.A. *Environ. Sci. Technol.* **2015**, *49*, 982−989.
(43) Kandel, C. M.; Rapp, J. L.; Barber, L. B. Shenandoah River accumulated wastewater ratio mapper. U.S. Geol. Survey: Washington, DC, 2017. Accessed May 2017 at [https://va.water.usgs.gov/webmap/shenmap/].
(44) Kandel, C. M.; Rapp, J. L.; Barber, L. B. Shenandoah River accumulated wastewater ratio. U.S. Geological Survey data release: U.S. Geol. Survey: Washington, DC, 2017. [https://doi.org/10.5066/F7RF5S8X].
(45) U.S. Geological Survey. The StreamStats Program for Virginia, Interactive Map. U.S. Geol. Survey: Washington, DC, 2018. [https://streamstats.usgs.gov/ss/]
(46) U.S. Environmental Protection Agency. Clean water needs survey 2008 report to congress. Rept. No. EPA-832-R-10−002; U.S. Environ. Protect. Agency: Washington, DC, 2010.
(47) Virginia Department of Environmental Quality. Virginia Pollutant Elimination Discharge System documentation, 2015. Accessed January 2016 at [http://www.deq.virginia.gov/Programs/Water/PermittingCompliance/PollutionDischargeElimination/PermitsFees.aspx#GGPs].
(48) Python Software Foundation. Python Language Reference, Version 2.7.13 2017.
(49) Rapaport, R. A. Prediction of consumer product chemical concentrations as a function of publicly owned treatment works treatment type and riverine dilution. *Environ. Toxicol. Chem.* **1988**, *7*, 107−115.
(50) Anderson, P. D.; D'Aco, V. J.; Shanahan, P.; Chapra, S. C.; Buzby, M. E.; Cunningham, V. L.; Duplessie, B. M.; Hayes, E. P.; Mastrocco, F. J.; Parke, N. J.; Rader, J. C.; Samuelian, J. H.; Schwab, B. W. Screening analysis of human pharmaceutical compounds in U.S. surface waters. *Environ. Sci. Technol.* **2004**, *38*, 838−849.
(51) Kapo, K. E.; DeLeo, P. C.; Vamshi, R.; Holmes, C. M.; Ferrer, D.; Dyer, S. D.; Wang, X.; White-Hull, C. iSTREEM®: An approach for broad-scale in-stream exposure assessment of "down-the-drain" chemicals. *Integr. Environ. Assess. Manage.* **2016**, *12*, 782−792.
(52) Sumpter, J. P.; Johnson, A. C.; Williams, R. J.; Kortenkamp, A.; Scholze, M. Modeling effects of mixtures of endocrine disrupting chemicals at the river catchment scale. *Environ. Sci. Technol.* **2006**, *40*, 5478−5489.
(53) Jensen, K. M.; Korte, J. J.; Kahl, M. D.; Pasha, M. S.; Ankley, G. T. Aspects of basic reproductive biology and endocrinology in the fathead minnow (*Pimephales promelas*). *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2001**, *128*, 127−141.

DOI: 10.1021/acs.est.8b05655
Environ. Sci. Technol. 2019, 53, 3429−3440

(54) Ankley, G. T.; Villeneuve, D. L. The fathead minnow in aquatic toxicology: past, present and future. *Aquat. Toxicol.* **2006**, *78*, 91−102.
(55) Panter, G. H.; Thompson, R. S.; Sumpter, J. P. Adverse effects in male fathead minnows (*Pimephales promelas*) exposed to environmentally relevant concentrations of the natural oestrogens oesteadiol and oestrone. *Aquat. Toxicol.* **1998**, *42*, 243−253.
(56) Brian, J.; Harris, C. A.; Scholze, M.; Backhaus, T.; Booy, P.; Lamoree, M.; Pojana, G.; Jonkers, J.; Runnalls, T.; Bonfa, A.; Marcomini, A.; Sumpter, J. P. Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals. *Environ. Health Perspect.* **2005**, *113*, 721−728.
(57) Thorpe, K. L.; Cummings, R. I.; Hutchinson, T. H.; Scholze, M.; Brighty, G.; Sumpter, J. P.; Tyler, C. R. Relative potencies and combination effects of steroidal estrogens in fish. *Environ. Sci. Technol.* **2003**, *37*, 1142−1149.
(58) Caldwell, D. J.; Mastrocco, F.; Anderson, P. D.; Länge, R.; Sumpter, J. P. Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol. *Environ. Toxicol. Chem.* **2012**, *31*, 1396−1406.
(59) Ankley, G. T.; Bennett, R. S.; Erickson, R. J.; Hoff, D. J.; Hornung, M. W.; Johnson, R. D.; Mount, D. R.; Nichols, J. W.; Russom, C. L.; Schmieder, P. K.; Serrano, J. A.; Tietge, J. E.; Villeneuve, D. L. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ. Toxicol. Chem.* **2010**, *29*, 730−741.
(60) Barber, L. B.; Lee, K. E.; Swackhamer, D. L.; Schoenfuss, H. L. Reproductive responses of male fathead minnows exposed to wastewater treatment plant effluent, effluent treated with XAD8 resin, and an environmentally relevant mixture of alkylphenol compounds. *Aquat. Toxicol.* **2007**, *82*, 36−46.
(61) Vajda, A. M.; Barber, L. B.; Gray, J. L.; Lopez, E. M.; Bolden, A. M.; Schoenfuss, H. L.; Norris, D. O. Demasculinization of male fish by wastewater treatment plant effluent. *Aquat. Toxicol.* **2011**, *103*, 213−221.
(62) Barber, L. B.; Vajda, A. M.; Bertolatus, D. W.; Dietze, J. E.; Hladik, M. L.; Iwanowicz, L. R.; Jasmann, J. R.; Jastrow, A.; Keefe, S. H.; Kolpin, D. W.; Kraus, J. M.; Melberg, A. K.; Meyer, M. T.; Rapp, J. L.; Roth, D. A.; Sperry, A.; Tokranov, A. K. Assessment of endocrine disruption in the Shenandoah River Watershed—Chemical and biological data from mobile laboratory fish exposures and other experiments conducted during 2014, 2015, and 2016. U.S. Geol. Survey data release: U.S. Geol. Survey: Washington, DC, 2019. [https://doi.org/10.5066/F7QF8S22].
(63) R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2017.
(64) Barber, L. B.; Antweiler, R. C.; Flynn, J. L.; Keefe, S. H.; Kolpin, D. W.; Roth, D. A.; Schnoebelen, D. J.; Taylor, H. E.; Verplanck, P. L. Lagrangian mass-flow investigations of inorganic contaminants in wastewater-impacted streams. *Environ. Sci. Technol.* **2011**, *45*, 2575−2583.
(65) Washburn, S. J.; Blum, J. D.; Demers, J. D.; Kurz, A. Y.; Landis, R. C. Isotopic characterization of mercury downstream of historic industrial contamination in the South River. *Environ. Sci. Technol.* **2017**, *51*, 10965−10973.
(66) U.S. Environmental Protection Agency. *TRI basic data files: Calendar years 1987−2017*. Toxics Release Inventory (TRI) Program. Accessed August 21, 2018 [https://www.epa.gov/toxics-release-inventory-tri-program/tri-basic-data-files-calendar-years-1987-2017].
(67) U.S. Environmental Protection Agency. Water pollution search of the discharge monitoring report (DMR). Accessed August 21, 2018 [https://echo.epa.gov/trends/loading-tool/water-pollution-search accessed on 08/21/2018].
(68) Hasenmueller, E. A.; Criss, R. E. Multiple sources of boron in urban surface waters and groundwaters. *Sci. Total Environ.* **2013**, *447*, 235−247.
(69) Dyer, S. D.; Caprara, R. J. A method for evaluating consumer product ingredient contributions to surface and drinking water: Boron as a test case. *Environ. Toxicol. Chem.* **1997**, *16*, 2070−2081.
(70) Neal, C.; Williams, R. J.; Bowes, M. J.; Harrass, M. C.; Neal, M.; Rowland, P.; Wickham, H.; Thacker, S.; Harman, S.; Vincent, C.; Jarvie, H. P. Decreasing boron concentrations in UK rivers: Insights into reductions in detergent formulations since the 1990s and within-catchment storage issues. *Sci. Total Environ.* **2010**, *408*, 1374−1385.
(71) Wind, T.; Werner, U.; Jacob, M.; Haul, A. Environmental concentrations of boron, LAS, EDTA, NTA and triclosan simulated with GREAT-ER in the river Itter. *Chemosphere* **2004**, *54*, 1135−1144.
(72) Fox, K. K.; Daniel, M.; Morris, G.; Holt, M. S. The use of measured boron concentration data from the GREAT-ER validation study (1996−1998) to generate predicted regional boron concentrations. *Sci. Total Environ.* **2000**, *251-252*, 305−316.
(73) Schoderboeck, L.; Mühlegger, S.; Losert, A.; Gausterer, C.; Hornek, R. Effects assessment: Boron compounds in the aquatic environment. *Chemosphere* **2011**, *82*, 483−487.
(74) Clara, M.; Strenn, B.; Kreuzinger, N. Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behavior of carbamazepine in wastewater treatment and during groundwater infiltration. *Water Res.* **2004**, *38*, 947−954.
(75) Cunningham, V. L.; Perino, C.; D'Aco, V. J.; Hartmann, A.; Bechter, R. Human health risk assessment of carbamazepine in surface waters of North America and Europe. *Regul. Toxicol. Pharmacol.* **2010**, *56*, 343−351.
(76) Johnson, A. C.; Williams, R. J. A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol in sewage treatment works. *Environ. Sci. Technol.* **2004**, *38*, 3649−3658.
(77) Johnson, A. C.; Williams, R. J.; Matthiessen, P. The potential steroid hormone contribution of farm animals to freshwaters, the United Kingdom as a case study. *Sci. Total Environ.* **2006**, *362*, 166−178.
(78) Liu, Z.; Kanjo, Y.; Mizutani, S. Urinary excretion rates of natural estrogens and androgens from humans, and their occurrence and fate in the environment: A review. *Sci. Total Environ.* **2009**, *407*, 4975−4985.
(79) Hannah, R.; D'Aco, V. J.; Anderson, P. D.; Buzby, M. E.; Caldwell, D. J.; Cunningham, V. L.; Ericson, J. F.; Johnson, A. C.; Parke, N. J.; Samuelian, J. H.; Sumpter, J. P. Exposure assessment of 17α-ethinylestradiol in surface waters of the United States and Europe. *Environ. Toxicol. Chem.* **2009**, *28*, 2725−2732.
(80) Caldwell, D. J.; Mastrocco, F.; Hutchinson, T. H.; Länge, R.; Heijerick, D.; Janssen, C.; Anderson, P. D.; Sumpter, J. P. Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17α-ethinyl estradiol. *Environ. Sci. Technol.* **2008**, *42*, 7046−7054.
(81) Ankley, G. T.; Feifarek, D.; Blackwell, B.; Cavallin, J. E.; Jensen, K. M.; Kahl, M. D.; Poole, S.; Randolph, E.; Saari, T.; Villeneuve, D. L. Re-evaluating the significance of estrone as an environmental estrogen. *Environ. Sci. Technol.* **2017**, *51*, 4705−4713.
(82) Shappell, N. W.; Feifarek, D. J.; Rearick, D. C.; Bartell, S. E.; Schoenfuss, H. L. Do environmentally factors affect male fathead minnow (*Pimephales promelas*) response to estrone? Part 2. Temperature and food availability. *Sci. Total Environ.* **2018**, *610−611*, 32−43.
(83) The Endocrine Disruption Exchange. TEDX list of potential endocrine disruptors. Accessed August 2018. [https://endocrinedisruption.org/interactive-tools/tedx-list-of-potential-endocrine-disruptors/search-the-tedx-list].
(84) Jobling, S.; Williams, R.; Johnson, A.; Taylor, A.; Gross-Sorokin, M.; Nolan, M.; Tyler, C. R.; van Aerle, R.; Santos, E.; Brighty, G. Predicted exposures to steroid estrogens in UK rivers correlate with widespread sexual disruption in wild fish populations. *Environ. Health Perspect.* **2006**, *114*, 32−39.
(85) Williams, R. J.; Keller, V. D. J.; Johnson, A. C.; Young, R. A.; Holmes, M. G. R.; Wells, C.; Gross-Sorokin, M.; Benstead, R. A. A national risk assessment for intersex in fish arising from steroid estrogens. *Environ. Toxicol. Chem.* **2009**, *28*, 220−230.
(86) Anderson, P. D.; Johnson, A. C.; Pfeiffer, D.; Caldwell, D. J.; Hannah, R.; Mastrocco, F.; Sumpter, J. P.; Williams, R. J. Endocrine disruption due to estrogens derived from humans predicted to be low in the majority of U.S. surface waters. *Environ. Toxicol. Chem.* **2012**, *31*, 1407−1415.

DOI: 10.1021/acs.est.8b05655
Environ. Sci. Technol. 2019, 53, 3429−3440

(87) Johnson, A. C.; Yoshitani, J.; Tanaka, H.; Suzuki, Y. Predicting national exposure to a point source chemical: Japan and endocrine disruption as an example. *Environ. Sci. Technol.* **2011**, *45*, 1028−1033.
(88) Barber, L. B.; Murphy, S. F.; Verplanck, P. L.; Sandstrom, M. W.; Taylor, H. E.; Furlong, E. T. Chemical loading into surface water along a hydrological, biogeochemical, and land use gradient - A holistic watershed approach. *Environ. Sci. Technol.* **2006**, *40*, 475−486.
(89) Barber, L. B.; Keefe, S. H.; Brown, G. K.; Furlong, E. T.; Gray, J. L.; Kolpin, D. W.; Meyer, M. T.; Sandstrom, M. W.; Zaugg, S. D. Persistence and potential effects of complex organic contaminant mixtures in wastewater-impacted streams. *Environ. Sci. Technol.* **2013**, *47*, 2177−2188.
(90) Jones, O. A. H.; Voulvoulis, N.; Lester, J. N. Potential ecological and human health risks associated with the presence of pharmaceutically active compounds in the aquatic environment. *Crit. Rev. Toxicol.* **2004**, *34*, 335−350.
(91) Cunningham, V. L.; Binks, S. P.; Olson, M. J. Human health risk assessment from the presence of human pharmaceuticals in the aquatic environment. *Regul. Toxicol. Pharmacol.* **2009**, *53*, 39−45.
(92) Krstolic, J. L. Data collection and simulation of ecological habitat and recreational habitat in the Shenandoah River, Virginia. U.S. Geol. Survey Sci. Invest. Report 2015−5005: U.S. Geol. Survey: Washington, DC, 2015.
(93) Sanseverino, J.; Gupta, R. K.; Layton, A. C.; Patterson, S. S.; Ripp, S. A.; Saidak, L.; Simpson, M. L.; Schultz, T. W.; Sayler, G. S. Use of Saccharomyces cerevisiae BLYES expressing bacterial bioluminescence for rapid, sensitive detection of estrogenic compounds. *Appl. Environ. Microb.* **2005**, *71*, 4455−4460.
(94) Young, J.; Iwanowicz, L.; Sperry, A.; Blazer, V. A landscape-based reconnaissance survey of estrogenic activity in streams of the upper Potomac, upper James, and Shenandoah Rivers. *Environ. Monit. Assess.* **2014**, *186*, 5531−5545.

DOI: 10.1021/acs.est.8b05655
Environ. Sci. Technol. 2019, 53, 3429−3440