Use of high-throughput screening results to prioritize chemicals for potential adverse biological effects within a West Virginia watershed

Levi D. Rose [a],[*], Denise M. Akob [b],[**], Shea R. Tuberty [c], Steven R. Corsi [d], Laura A. DeCicco [d], Jeffrey D. Colby [a], Derek J. Martin [a]

[a] Appalachian State University, Department of Geography and Planning, NC 28607, USA
[b] U.S. Geological Survey, Reston, VA 20192, USA
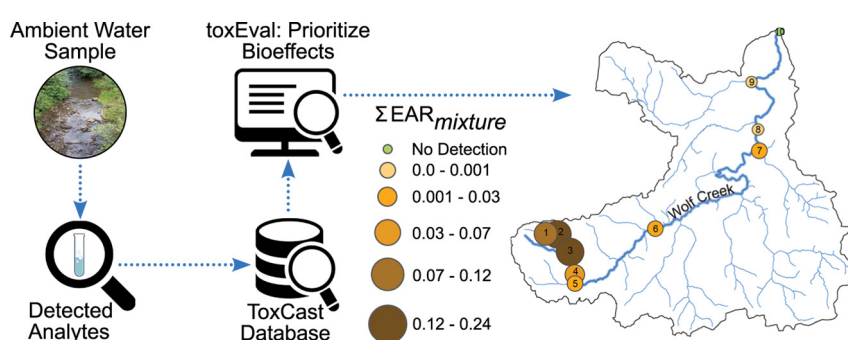[c] Appalachian State University, Department of Biology, NC 28607, USA
[d] U.S. Geological Survey, Middleton, WI 53562, USA

HIGHLIGHTS

- Exposure activity ratio approach was used to screen contaminants in source water.
- Organic compounds occurred as complex mixtures of 10 or more at multiple sites.
- toxEval was used to rapidly analyze and screen environmental monitoring data.
- Exposure-activity ratios were observed for chemicals without water quality benchmarks.
- Water quality impacts were observed downstream from a wastewater disposal facility.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Organic chemicals from industrial, agricultural, and residential activities can enter surface waters through regulated and unregulated discharges, combined sewer overflows, stormwater runoff, accidental spills, and leaking septic-conveyance systems on a daily basis. The impact of point and nonpoint contaminant sources can result in adverse biological effects for organisms living in or near surface waters. Assessing the adverse or toxic effects that may result when exposure occurs is complicated by the fact that many commonly used chemicals lack toxicity information or water quality standards. To address these challenges, an exposure-activity ratio (EAR) screening approach was used to prioritize environmental chemistry data in a West Virginia watershed (Wolf Creek). Wolf Creek is a drinking water source and recreation resource with documented water quality impacts from point and nonpoint sources. The EAR screening approach uses high-throughput screening (HTS) data from ToxCast as a method of integrating environmental chemical occurrence and biological effects data. Using water quality schedule 4433, which targets 69 organic waste compounds typically found in domestic and industrial wastewater, chemicals were screened for potential adverse biological affects at multiple sites in the Wolf Creek watershed. Cumulative EAR mixture values were greatest at Sites 2 and 3, where bisphenol A (BPA) and pentachlorophenol exhibited maximum EAR values of 0.05 and 0.002, respectively. Site 2 is downstream of an unconventional oil and gas (UOG) wastewater disposal facility with documented water quality impacts. Low-level organic contaminants were found at all sample sites in Wolf Creek, except Site 10, where Wolf Creek enters

* Correspondence to: L.D. Rose, 108 West 15th Avenue, Spokane, WA 99203, USA.
** Correspondence to: D.M. Akob, U.S. Geological Survey, National Research Program, 12201 Sunrise Valley Dr., MS 430, Reston, VA 20192, USA.
E-mail addresses: levidrose@gmail.com (L.D. Rose), dakob@usgs.gov (D.M. Akob).

the New River. The application of an EAR screening approach allowed our study to extend beyond traditional environmental monitoring methods to identify multiple sites and chemicals that warrant further investigation.

1. Introduction

In the United States, 40,655 of the 86,228 (47%) chemicals in the Toxic Substances Control Act inventory are currently in commerce (GAO, 2019; US EPA, 2019). Many of the chemicals in commerce lack toxicity information for evaluating their safety and have been the driver for considering new risk assessment approaches (Judson et al., 2009; Villeneuve et al., 2019). The lack of toxicity information is exacerbated by organic waste compounds (OWCs) that can enter surface waters through regulated and unregulated discharges, combined sewer overflows, stormwater runoff, accidental spills, and leaking septic-conveyance systems on a daily basis (Baldwin et al., 2016; Foreman et al., 2015; Orem et al., 2017; Rogers, 2016). To overcome the extensive gap in up-to-date toxicity information, modern technologies such as high-throughput toxicity testing are shifting the reliance on whole-animal toxicity testing towards greater use of in vitro bioassays (Schroeder et al., 2016; Villeneuve et al., 2019).

Development of high-throughput toxicity testing has led to the expansion of biological effects data for an extensive range of chemicals. For example, the U.S. Environmental Protection Agency's (EPA) ToxCast research program has expanded coverage on >9000 chemicals and information on approximately 1000 assay end points (US EPA, 2016a). Using automated chemical screening approaches, such as high-throughput screening (HTS) assays, living cells or isolated proteins are exposed to chemicals in a standardized manner. For each assay, chemicals are tested using a consistent dose-response design and this information is used to determine point of departure estimates, such as the chemical-specific half-maximal activity concentration (AC50) or activity concentration at cutoff (ACC) for each chemical—assay combination (Blackwell et al., 2017). Many of the chemicals that have been studied include industrial and environmentally relevant chemicals for which traditional human health or ecological effects data are lacking (Blackwell et al., 2017). While several studies have generally viewed the feasibility of HTS of environmental chemicals to be successful (Judson et al., 2015; Kleinstreuer et al., 2014; Leung et al., 2016), the techniques to process and identify insights from large, complex toxicity databases still remains a significant challenge facing the toxicology community (Benigni, 2013; Rovida et al., 2015; Shah and Greene, 2014; Zhu et al., 2014).

To rapidly process and identify insights from large chemical toxicity databases, new tools and approaches are being developed. Exposure-activity ratio (EAR) approaches (Becker et al., 2015; Blackwell et al., 2017) using the ToxCast database have demonstrated how HTS data can be applied as a standardized method of integrating chemical occurrence and biological effects data for the prioritization of environmental monitoring data. The process of integrating environmental monitoring datasets with ToxCast has been streamlined with the development of toxEval. toxEval, a bioeffects monitoring software tool, allows users to rapidly analyze and screen environmental monitoring data for potential adverse biological effects (DeCicco et al., 2018). The present work applies a ToxCast-based EAR approach in combination with screening water quality benchmarks and potential endocrine disrupting chemicals (EDCs) to a study focused on two primary objectives: (1) screen, characterize, and prioritize OWCs and other contaminants across streams within the Wolf Creek watershed, and (2) identify possible areas of concern (AOCs) based upon our evaluation. The Wolf Creek watershed in West Virginia is a drinking water source and a unique area because the first documented water quality impacts from UOG wastewater disposal activities occurred in the headwaters of Wolf Creek

(Akob et al., 2016). It provides an ideal location to evaluate OWCs using an EAR approach and toxEval as a rapid bioeffects screening tool. This study was part of an ongoing effort to monitor water quality impacts from activities at a UOG wastewater disposal facility (Akob et al., 2016; Kassotis et al., 2016; Orem et al., 2017) in the headwaters of Wolf Creek.

2. Materials and methods

2.1. Site description

Wolf Creek is a second-order stream that drains approximately 4430 ha into the lower reaches of the New River (Fig. 1). The land cover is predominantly forested (75%), followed by grass/pasture/agriculture (17%), barren/developed/roads (7%), and water/wetlands (1%) (Strager, 2011; Tables S1 & S2). Wolf Creek flows approximately 18 km from Oak Hill through Fayetteville and into the New River at Fayette Station rapid, a popular recreational area (Lukacs et al., 2011). Approximately 6 km downstream of Fayette Station rapid, the New River Water Treatment System provides primary drinking water supply to approximately 24,466 people (West Virginia American Water, 2016). In June 2016, West Virginia American Water updated their Source Water Protection Plan for the New River Water Treatment System, as per new requirements for public water systems following revisions to the state code in 2014 (West Virginia American Water, 2016). A requirement of Source Water Protection Plan submittals is to identify all potential significant contaminant sources located within the zone of critical concern (ZCC) and the zone of peripheral concern (ZPC). The ZCC generally extends upstream of a public water intake for the length that water in that stream can travel over a five-hour period, and 1.6 km downstream. The ZPC generally extends upstream of a public water intake for the length that water in that stream can travel over a ten-hour period, and 1.6 km downstream. Both zones are buffered 152 m from the center of the stream. Portions of the ZCC extend into the Wolf Creek watershed, and the ZPC covers all streams within the Wolf Creek watershed (West Virginia American Water, 2016). Geographic Information System (GIS) shapefiles of potential contaminant sources were obtained from the West Virginia Bureau for Public Health (Table S3) and were analyzed within the drainage boundary of the Wolf Creek watershed. Between the headwaters and the mouth of Wolf Creek, a diverse range of potential contaminant sources have been identified including legacy mining, UOG wastewater disposal, wastewater treatment plant sewage outlets, aboveground storage tanks with chemicals, and numerous National Pollutant Discharge Elimination System (NPDES) outlets (Fig. 1; Table S1). Failing onsite septic systems and leaking wastewater infrastructure have also been documented throughout the Wolf Creek watershed (Hansen et al., 2014; Lukacs et al., 2011). Detailed GIS methods are described in the Supplemental Information (SI) Methods.

Mining activities within the Wolf Creek watershed include areas that have been surface mined, underground mined, and used for coal refuse disposal. Water quality has been severely degraded by acid mine drainage (AMD) from the Summerlee abandoned mine land site (Fig. 1), and the Town of Fayetteville deemed Wolf Creek unsuitable as their primary public water source when the state identified water quality impairments in 2002 (Hansen et al., 2014). Wolf Creek also supported trout, but water quality impairments resulted in the West Virginia Department of Natural Resources to remove Wolf Creek from its trout stocking list (Hansen et al., 2014). The mainstem of Wolf Creek and some of its
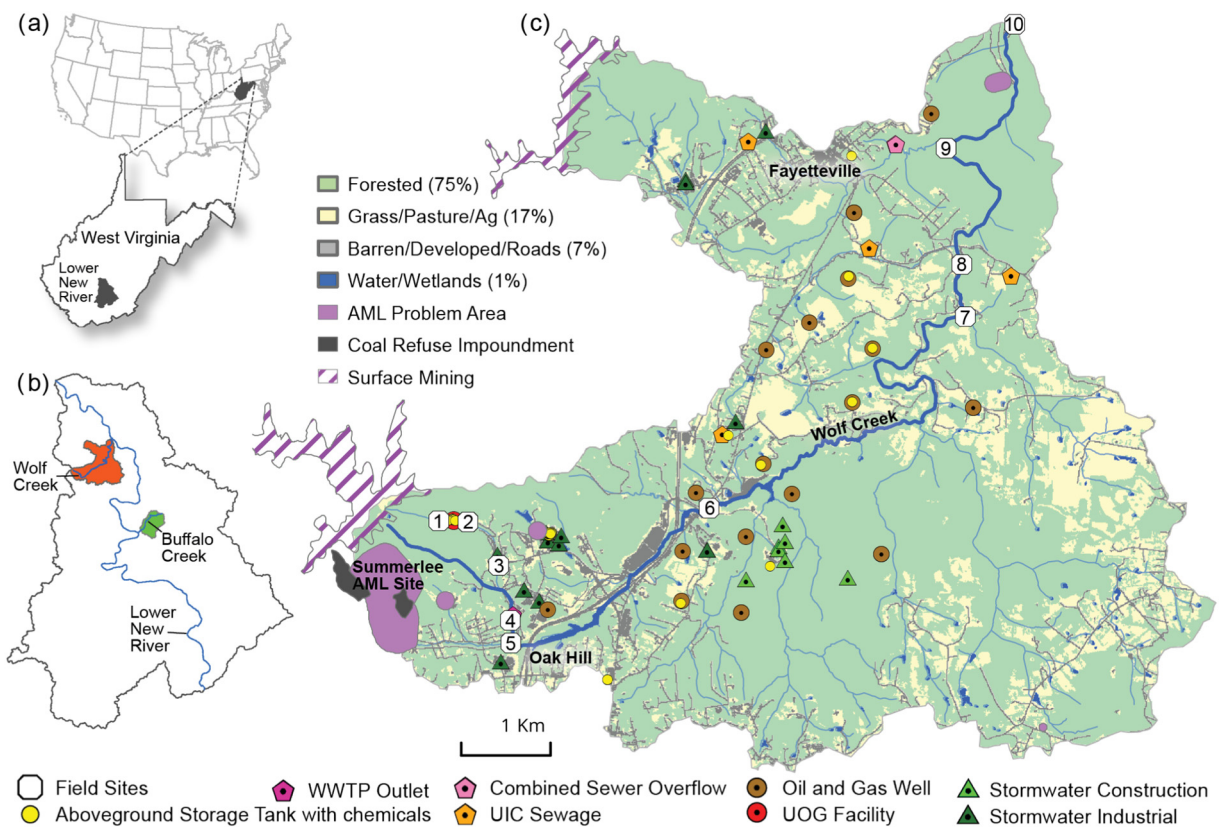
Fig. 1. Location of the Lower New River drainage in West Virginia (1a). Within the Lower New River location map (1b), the Wolf Creek watershed is highlighted red and the reference drainage, Buffalo Creek, is green. Location of sampling sites, land use/land cover, and potential contaminant sources (1c). Abandoned mine land (AML), underground injection control (UIC), unconventional oil and gas (UOG).
Sources: West Virginia Bureau for Public Health, 2016; West Virginia Department of Environmental Protection, 2016; and West Virginia University, 2016.

tributaries are impaired by high levels of iron, aluminum, fecal coliform bacteria, and low pH (Hansen et al., 2014). Additionally, organic enrichment and sedimentation have resulted in biological impairments (Hansen et al., 2014). More recently, impacts from UOG wastewater disposal activities were documented (Akob et al., 2016; Kassotis et al., 2016; Orem et al., 2017).

2.2. Sample collection and analysis

To ensure the quality of water samples and prevent contamination, numerous quality assurance/quality control (QA/QC) procedures were followed during sampling. Before sampling field equipment was rigorously cleaned, and between sampling sites field equipment was cleaned/sprayed with clean, deionized water. Disposable gloves were worn during the entire process, and were changed as necessary and between sites. QA/QC water samples were collected at Site 2, including a duplicate and blank sample (Table S4). The field equipment blank was prepared with deionized water and was preserved as required for each analyte. The field duplicate was collected using the same methods as required for each analyte. Grab samples were collected and processed in a manner consistent with minimal contamination. This included cleaning of equipment before fieldwork and between sites, and changing disposable gloves as necessary and between sites.

From September 18–20, 2016, approximately 1.18 in. of rain was observed in the region, as reported by the Beckley, Raleigh County Memorial Airport (NOAA, 2016). The Raleigh County Memorial Airport is located approximately 24 km south of Wolf Creek and 17 km south of Buffalo Creek. On September 19, 2016, the day samples were collected in Wolf Creek, a light rain was observed in the morning for approximately an hour. Several headwater streams in the Wolf Creek watershed were observed with no flow including Crooked Run, House

Branch, Levisee Creek, and Short Creek. Streamflow conditions were visually observed and measured as being low flow in both Wolf Creek and Buffalo Creek watersheds (Table S4). These observations are further supported by below average baseflow conditions, as monitored by the U.S. Geological Survey (USGS) gage on the New River at Thurmond (USGS Site 03185400). The Thurmond gage site is approximately 22 km upstream from the mouth of Wolf Creek and approximately 7 km downstream from the mouth of Buffalo Creek. The average baseflow on the New River at Thurmond in September over a 38-year period from 1981 to 2016 was 3544 ft^3/s (USGS, 2016). On September 19 and 20, 2016, streamflows were reported on the New River at Thurmond as 1760 ft^3/s and 1800 ft^3/s respectively (USGS, 2016).

One surface water grab sample was collected from ten sites along Wolf Creek on September 19, 2016, and one surface water grab sample was collected from an unimpacted reference drainage on September 20, 2016 (Fig. 1). The reference site is within the Lower New River drainage and was recommended by the West Virginia Department of Environmental Protection (WVDEP) Watershed Assessment Branch. Buffalo Creek (Site 11) was chosen as the reference drainage because it was characterized as having minimal human impact and was within close proximity to Wolf Creek. Field measurements of dissolved oxygen, oxidation-reduction potential, pH, specific conductance, stream flow, and water temperature were recorded in the field using a lab calibrated YSI instrument (YSI Pro Plus multiparameter meter, YSI, Inc., Yellow Springs, OH). At sites with adequate water depth, stream flow rates (velocity ft/s) were recorded in the field using an OTT MF Pro Water Flow Meter (OTT Hydromet Inc., Kempten, Germany). Wetted width was recorded as the wetted stream width during the time of sampling. Surface water grab samples were collected from the approximate center of the stream for analysis of trace OWCs, anions, cations, and trace metals. Glass bottles were used for the collection of OWCs and high-density

polyethylene (HDPE) bottles were used for the collection of all other samples. Organic samples were chilled at 4 °C, shipped overnight to the USGS National Water Quality Laboratory (NWQL) in Denver Colorado, and analyzed using Schedule 4433, which targets 69 OWCs typically found in domestic and industrial wastewater (Table S5). Trace metal samples were preserved at pH = 2 with 1% v/v ultra-pure nitric acid (HNO_3). Anion, cation, and trace metal samples were chilled at 4 °C and delivered to the Appalachian State University Ecotoxicology Lab in Boone, North Carolina for analysis. Detailed analytical methods are further described in SI Methods. All water quality data are presented in SI Tables S4 and S6 are also available through a USGS data release (Rose et al., 2018).

2.3. Screening potential chemical contaminants

2.3.1. Exposure activity ratios

Several screening tools were used to evaluate potential adverse biological effects within the Wolf Creek watershed including EARs, water quality benchmarks and toxicity quotients, and potential EDCs. EARs were used as the primary method of screening OWCs in surface water samples for potential toxicity. Prior to field sampling, the 69 OWCs in Schedule 4433 were cross-referenced in ToxCast (US EPA, 2018) using chemical abstract service (CAS) numbers, which resulted in a match of 62 chemicals being identified (Table S5). The CAS numbers of the 62 matched chemicals were used in toxEval to process environmental monitoring datasets and prioritize EARs. An EAR is the quotient of the environmental concentration divided by the activity concentration at cutoff (ACC) (Eq. (1)).

$$\text{Exposure Activity Ratio} = \frac{Environmental\ Concentration\ (\mu M)}{ACC\ Concentration\ (\mu M)} \quad (1)$$

The ACC is a point of departure estimate used in EAR calculations, and estimates the chemical concentration at which a defined cutoff value is achieved within an assay (Blackwell et al., 2017; Filer et al., 2017). The activity cutoff is an assay-specific metric determined as a multiplier of the baseline median absolute deviation of measured activity in the assay. The derivation of this metric is more thoroughly described in other studies (Filer et al., 2017; Judson et al., 2010). Because this metric is less prone to violating assumptions underlying relative potency estimation compared to using the half-maximal activity concentration (AC50), its use has been preferred in recent applications of ToxCast data (Blackwell et al., 2017; Fay et al., 2018). For this reason, the ACC (parameter name "modl_acc" in the ToxCast database) was used as the final end point for comparison against water quality data.

toxEval was used to calculate EARs (Eq. (1)) for each detected environmental chemical (Table S7). The CAS number of each detected chemical was used to reference ACC values from dose-response concentration models published in ToxCast. toxEval allows the user to establish an EAR threshold to help screen and prioritize chemicals of concern for further investigation. As an initial screen of detected OWCs, we selected an EAR = 0.001 as a conservative (protective) effects-screening threshold. Threshold exceedances, called EAR "hits", are not necessarily indicative of a hazard, but are an indication of the potential for adverse biological effects. To illustrate a sense of scale, it has been shown that an EAR ≥1 typically indicates that a chemical is present at a high enough concentration to trigger the observed biological activity (Schroeder et al., 2016), whereas an EAR ≤1 suggests proportionally lower probability of biological activity (Bradley et al., 2018). However, this over simplification does not account for other processes such as absorption, distribution, metabolism, and elimination that control bioavailability within the organism. Previous studies (Blackwell et al., 2017) established an EAR screening threshold by focusing on EARs that represented the upper 10% of EAR values, while others (Bradley et al., 2019) have used a conservative effects-screening threshold defined by an EAR = 0.001. In either case, caution should be used in establishing a

predefined EAR screening threshold without first reviewing the range of EAR responses in a dataset.

Many of the same QA considerations used by Blackwell et al. (2017) were used as guidelines in this study including: excluding the platforms Apredica and Bioseek because they largely target nonspecific endpoints, removing Attagene assays reporting in the "loss" direction and Novascreen assays reporting in the "gain" direction from the final EAR results, as these platforms are not optimized or designed to report for the given assay direction, and censoring chemical−assay pairs from the final data set using ToxCast data quality flags that indicated false positives (data with flag identifications 6, 7, 10, 11, 15, and 17 were removed; Table S8). Chemicals with abnormal dose-response curves were excluded (Table S9), which primarily consisted of unflagged false positives that were not recognized by ToxCast data quality flags. For more information on interpreting ToxCast dose-response curves and data quality, Ryan (2017) produced a technical white paper in consultation with American Chemistry Council's Computational Profiling Work Group.

Detected OWCs and EAR hits were further characterized by chemical class. Many of the OWCs have multiple uses, but for the purposes of this study, OWCs were grouped into 15 primary chemical classes: antimicrobial disinfectants, antioxidants, detergent metabolites, dyes and pigments, fire retardants, flavors and fragrances, fuels, herbicides, insecticides, miscellaneous, nonprescription drugs (human), PAHs, plasticizers, solvents, and sterols (Table S5). These classes and methods have been used in previous studies (Baldwin et al., 2013, 2016), originally based on tables developed by Sullivan et al. (2005).

2.3.2. Water quality benchmarks and inorganic chemical signatures

To support OWC screening and prioritization, particularly at sites where EARs were below the screening threshold, chemical occurrence data were compared to water quality benchmarks and inorganic chemical signatures. In previous studies, Baldwin and others (Baldwin et al., 2013, 2016) created a table of water quality benchmarks for acute and chronic exposure to aquatic life that were compiled from a variety of sources, including the U.S. EPA (Berry et al., 2003; US EPA, 1996, 2006, 2014, 2015a, 2016b), the National Oceanic and Atmospheric Administration (Buchman, 2008), Oak Ridge National Laboratory (Suter and Tsao, 1996), and the Canadian Council of Ministers of the Environment (CCME, 2015). This table was expanded here as SI Table S10 to also include water quality criteria for human health (US EPA, 2015b, 2016c; West Virginia Department of Environmental Protection, 2016). From the 69 OWCs in Schedule 4433, 28 chemicals were identified as having an aquatic toxicity benchmark, and 10 chemicals were identified as having water quality criteria for human health (Table S10). Toxicity quotients (TQ) were computed by dividing the greatest measured concentration of a chemical at a particular site by the lowest known water quality benchmark for that chemical (Table S11). A TQ ≥ 1 indicates a toxicity criterion exceedance and the potential for adverse biological effects. It should be noted that potential adverse biological effects can exist in TQs ≤ 1 caused by mixture effects with other chemicals and low dose effects (Liess et al., 2013; Vandenberg et al., 2012). In addition to OWCs, 13 inorganic chemicals were reviewed for their potential to cause adverse biological effects to aquatic life and human health (Table S12).

Specific conductance, pH, and inorganic chemical signatures were used as indicators of UOG wastewater and acid mine drainage (AMD), as well as documenting water quality criteria exceedances. Brantley et al. (2014) and Akob et al. (2016) have demonstrated that the combination of Ba^{2+}, Br^-, Cl^-, and Sr can function as a local tracer of UOG wastewater impacts, and this approach was used to characterize surface waters downstream of an UOG wastewater disposal facility at Site 2. In waters with documented AMD impacts (Hansen et al., 2014; Larson et al., 2014a, 2014b), the combination of Al, Fe, Mn, and SO_4^{2-} were used to characterize surface waters downstream of an AMD source at Site 5.

2.3.3. Endocrine disrupting chemicals

The Endocrine Disruption Exchange (TEDX) is a nonprofit research institute that identifies chemicals that have shown evidence of endocrine disruption in scientific research. Endocrine disrupting chemicals (EDCs) occur both naturally and synthetically, and refer to exogenous chemicals that can interfere with any aspect of hormone action (Zoeller et al., 2012). Chemicals are evaluated by searching the publicly available scientific literature and identifying peer-reviewed research that has shown effects on endocrine signaling (TEDX, 2018a). The scientific literature is maintained in a searchable database (TEDX, 2018b) of potential endocrine disruptors, defined as chemicals with at least one study demonstrating endocrine disrupting properties. Chemicals in the TEDX database can be searched by chemical name or CAS number and filtered by their source or use. Information is obtained from several sources including the Hazardous Substances Data Bank, Haz-Map, the Household Products Database, PubChem, US EPA Agency for Toxic Substances and Disease Registry (ATSDR), Chemical and Product Categories database, the Compendium of Pesticide Common Names, PAN pesticide database, manufacturer information, primary peer-reviewed literature, and patent information. To date, TEDX has identified 1484 potential EDCs (TEDX, 2018a).

There are several ways to assess the endocrine disrupting activities of environmental contaminants, as demonstrated by a recent study in the Wolf Creek Watershed by Kassotis et al. (2016). The approach described here demonstrates a preliminary screening technique to identify potential EDCs for further investigation. Using CAS numbers, 39 chemicals from Schedule 4433 were identified as potential EDCs in the TEDX List of Potential Endocrine Disruptors database (TEDX, 2018a). This information was used on a site-by-site basis to cross reference detected OWCs and support prioritization of chemicals and AOCs.

3. Results

3.1. Chemical occurrence summary

Understanding the detection frequency, magnitude, and spatial distribution of OWCs are important steps towards prioritizing chemicals of concern. Approximately 33 of 69 (48%) distinct OWCs were detected at least once in waters sampled from the Wolf Creek watershed. The most frequently detected chemicals in the watershed, occurring in at least 4 of 10 (40%) samples from at least one site, were bisphenol A (BPA; antioxidants), camphor (flavors/fragrances), indole (flavors/fragrances), N,N-diethyl-meta-toluamide (DEET; insecticides), methyl salicylate (miscellaneous), isophorone (solvent), and cholesterol (sterols) (Table 1). From the most frequently detected chemicals, BPA, indole, and methyl salicylate are documented EDCs (Benísek et al., 2008; Kassotis et al., 2015; Zhang et al., 2012). One or more OWCs were detected in 9 of 10 (90%) samples collected in Wolf Creek. There were no OWCs detected at Site 10 near the mouth of Wolf Creek, or at Site 11 near the mouth of Buffalo Creek (reference drainage). Within the Wolf Creek watershed OWCs were generally observed at low concentrations. Total OWC sample concentrations had a mean of 1.28 μg/L and a median of 0.729. However, mixtures of ten or more chemicals were detected at Site 6 (19 OWCs), Site 4 (18 OWCs), and Site 3 (12 OWCs). Even at low concentrations, it has been shown that the synergistic effects of multiple OWCs can result in adverse biological effects (Silva et al., 2002; Vandenberg et al., 2012; Walter et al., 2002). The highest total concentration of OWCs was observed at Site 4 (6.62 μg/L), collected immediately downstream of a wastewater treatment outlet, and Site 6 (1.81 μg/L), collected immediately downstream from a large shopping area. Wastewater discharges can be a common source for anthropogenic OWCs, including disinfection by-products, manufacturing additives, personal care and domestic-use products, and plant or animal derived

Table 1
Occurrence of detected organic waste compounds (OWCs) in the Wolf Creek watershed per site, potential endocrine disrupting chemical (EDC; yes denotes detection and listing in The Endocrine Disruption Exchange database), exposure-activity ratio (EAR) hit count above screening threshold (0.001), aquatic life (Aq. Life) water quality exceedance count, and ambient water quality criteria (AWQC) exceedance count. Maximum (Max.) and median (MEd.) concentrations are reported in μg/L. DL= detection limit. *2-Column fitting.

Chemical Class	Organic Waste Compound	Detection (n=10)	Max.	Med.	EDC	EAR Hit	Aq. Life	AWQC
Antimicrobial Disinfectants	p-Cresol	10%	0.100	< DL	yes			
	Phenol	10%	0.086	< DL	yes			
Antioxidants	5-Methyl-1H-benzotriazole	10%	0.087	< DL				
	Bisphenol A	40%	0.280	< DL	yes	73		
Detergent Metabolites	4-Cumylphenol	20%	0.022	< DL		1		
Dyes/Pigments	Anthraquinone	10%	0.301	< DL				
Fire Retardants	Tri(2-Butoxyethyl) phosphate	30%	4.340	< DL	yes	1		
	Tris(Dichloroisopropyl) phosphate	10%	0.060	< DL	yes			
Flavors/ Fragrances	3-Methyl-1H-indole	30%	0.013	< DL				
	Benzophenone	20%	0.122	< DL	yes	1		
	Camphor	90%	0.178	0.037				
	Hexahydrohexamethyl cyclopentabenzopyran	20%	0.126	< DL	yes			
	Indole	40%	0.022	< DL	yes			
	3,4-Dichlorophenyl isocyanate	10%	0.423	< DL				
Herbicides	Pentachlorophenol	10%	0.291	< DL	yes	3		1
	Prometon	10%	0.010	< DL				
Insecticides	Carbazole	10%	0.042	< DL				
	N,N-Diethyl-meta-toluamide (DEET)	60%	0.144	0.009				
Miscellaneous	Methyl Salicylate	80%	0.665	0.017	yes	1		
	Caffeine	20%	0.439	< DL	yes	2		
Nonprescription Drugs	Cotinine	20%	0.059	< DL				
	Menthol	30%	0.168	< DL				
PAH	Anthracene	20%	0.016	< DL	yes		2	
	Fluoranthene	30%	0.035	< DL	yes			
	Phenanthrene	20%	0.028	< DL	yes			
	Pyrene	30%	0.021	< DL	yes			
Plasticizers	Bis(2-Ethylhexyl) phthalate	10%	0.420	< DL	yes		1	1
	Tri(2-Chloroethyl) Phosphate	20%	0.029	< DL				
	Triethyl Citrate	10%	0.049	< DL				
	Triphenyl phosphate	10%	0.049	< DL	yes			
Solvents	Isophorone	70%	0.052	0.016				
Sterols	3-Beta-Coprostanol	20%	0.372	< DL				
	Cholesterol	80%	0.517	0.145				

biochemicals (Kingsbury et al., 2008). Runoff from parking lot surfaces can carry coal-tar pavement sealant to receiving waters and be a primary source of PAHs, resulting in adverse effects to benthic organisms (Baldwin et al., 2017).

3.2. Exposure activity ratio screening

3.2.1. EAR site prioritization

For OWCs analyzed in this study, 62 of 69 (90%) chemicals from Schedule 4433 were identified in ToxCast and 33 of the 62 (53%) chemicals were detected in Wolf Creek. The EAR approach can be used to estimate the integrated effects of organic contaminant mixtures by summing individual contaminant EARS, providing a site-specific cumulative EAR (Blackwell et al., 2017; Schroeder et al., 2016). As an initial screen of the chemical occurrence data, a summation of all EARs per site were calculated (Table S13). Sites 2 and 3 were identified as having the highest cumulative EAR mixture value, followed by Site 1 (Fig. 2; Table 2). The cumulative EAR mixture values at Sites 1–3 were 0.12, 0.24, and 0.24 respectively (Table 2). In addition, Sites 1–3 also exhibited the greatest number of EAR hits per site, 18, 25, and 24 respectively (Table 2). Site 1 is upstream of an UOG wastewater disposal facility, and downstream from an area with previous surface mining (Fig. 1). At Site 1, BPA resulted in 18 EAR hits, with 0.03 being the highest EAR. This suggests that there is a possible contamination source upstream of the UOG wastewater disposal facility. Possible uses of BPA include manufacturing polycarbonate resins, antioxidants, and flame retardants (Focazio et al., 2008; Table S5), but the source is unknown. Site 2 is downstream of an UOG wastewater disposal facility with documented water quality impacts (Akob et al., 2016; Kassotis et al., 2016; Orem et al., 2017), where BPA and pentachlorophenol exhibited EAR values of 0.05 and 0.002, respectively. BPA has been identified in hydraulic fracturing fluids according to data received from oil and gas exploration and production companies (US EPA, 2012), and is known to be an estrogen receptor agonist (Kassotis et al., 2015). Pentachlorophenol is a widespread environmental pollutant commonly used as a pesticide

and disinfectant, and known to effect reproductive and metabolic hormone function (Rawlings et al., 1998). The UOG wastewater disposal facility could be a possible source of BPA and pentachlorophenol at Site 2. Site 3 is located 0.6 km downstream from Site 2 and water samples were collected directly below an NPDES outlet from an auto recycling facility. At Site 3, BPA resulted in 21 EAR hits, with 0.06 being the highest EAR, and 4-cumylphenol resulted in one EAR hit at 0.001. 4-cumylphenol is a nonionic detergent metabolite that is present in cleaners and sealers (Morace, 2012). The auto recycling facility could be a possible source of 4-cumylphenol and BPA at Site 3. As a result of the cumulative EAR values at Sites 1–3, the potential for cumulative effects of mixed organic exposures, and undetermined sources, further investigation at Sites 1–3 is warranted.

3.2.2. EAR chemical prioritization

EAR calculations can also be used to prioritize individual chemicals for monitoring or impact. In this approach, the maximum concentrations for each chemical were used to calculate EARs (Table S13). From the chemical occurrence data, 7 of 33 (21%) detected chemicals were identified in one or more assays. In the current study, an EAR >0.001 was used as a conservative threshold to screen potential chemicals of concern. BPA had the highest EAR value of 0.06 (at maximum concentration 0.28 µg/L) and the greatest number of EARs >0.001 (73). The constitutive androstane receptor (CAR) antagonist assay resulted in the greatest EAR for BPA. The CAR is a nuclear receptor that regulates xenobiotic metabolism, including detoxification enzymes and transporters in the liver and intestine (Basketter and Kimber, 2010). In total, six additional chemicals have one or more EARs >0.001 including 4-cumylphenol, benzophenone, caffeine, methyl salicylate, pentachlorophenol, and tris(2-butoxyethyl) phosphate. Tris(2-butoxyethyl) phosphate, a fire retardant, shows the greatest activity in the "ATG_PXRE_CIS_up" assay. The pregnane X receptor (PXR) is a xenobiotic and steroid sensing nuclear receptor and regulates xenobiotic metabolism through transcription of cyctochrome P450. Comparison of elevated EAR values with EDC literature was used as a complementary
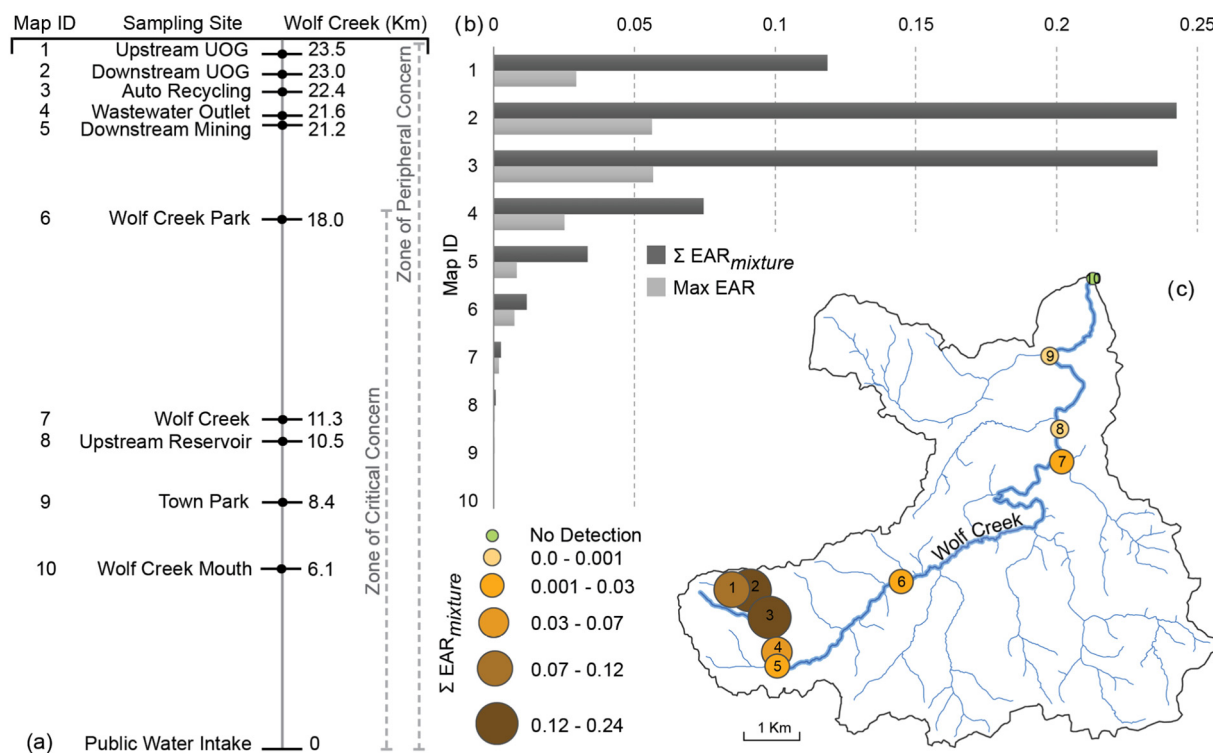

Fig. 2. Sampling locations with respect to the Zones of Critical Concern (ZCC) and Peripheral Concern (ZPC) (2a). Cumulative EAR mixture values and maximum EAR values by site (2b). Cumulative EAR values in the Wolf Creek watershed represented by graduated symbols (2c). The numbers on map (2c) correspond to the Map ID and sampling site labeled in (2a).

Table 2
Detection of organic waste compounds (OWCs) and total OWC concentration by site, exposure-activity ratio (EAR) hit count above screening threshold (0.001) and cumulative EAR mixture values by site, detection of endocrine disrupting compounds (EDCs) and total EDC concentration by site, and organic and inorganic water quality criteria exceedances by site. To clarify how results are reported, the Zone of Critical Concern (ZCC) is defined as sites (6-10) and the Zone of Peripheral Concern (ZPC) is defined as sites (1-5). *2-Column fitting.

Watershed	Map ID	Source Water Protection Zone	OWCs Detected	$\sum_{OWC}$ (µg/L)	EAR ≥ 0.001	$\sum_{EAR}$ Mixture	EDCs Detected	$\sum_{EDCs}$ (µg/L)	Organic Aquatic Chronic	Organic Drinking Water	Organic Ambient Water	Inorganic Aquatic Acute	Inorganic Aquatic Chronic	Inorganic Drinking Water
Wolf Cr.	1	ZPC	5	0.36	18	0.12	2	0.16	0	0	0	0	1	0
Wolf Cr.	2	ZPC	8	0.86	25	0.24	3	0.59	0	0	1	0	4	4
Wolf Cr.	3	ZPC	12	0.47	24	0.24	8	0.41	1	0	0	0	1	0
Wolf Cr.	4	ZPC	18	6.62	6	0.07	8	5.13	1	0	0	0	1	0
Wolf Cr.	5	ZPC	9	0.63	8	0.03	5	0.29	0	0	0	1	3	2
Wolf Cr.	6	ZCC	19	1.81	1	0.01	9	0.47	0	0	0	0	0	0
Wolf Cr.	7	ZCC	6	1.02	1	0.003	4	0.71	0	0	0	0	0	0
Wolf Cr.	8	ZCC	7	0.83	0	0.0007	2	0.43	1	0	1	0	0	0
Wolf Cr.	9	ZCC	7	0.22	0	0.0003	1	0.02	0	0	0	0	0	0
Wolf Cr.	10	ZCC	0	0	0	0	0	0	0	0	0	0	1	0
Wolf Cr.	1-5	ZPC	25	8.94	81	0.700	14	6.58	2	0	1	1	10	6
Wolf Cr.	6-10	ZCC	23	3.88	2	0.014	10	1.63	1	0	1	0	1	0
Buffalo Cr.		ZPC	0	0	0	0	0	0	0	0	0	0	1	0

approach to further prioritize individual chemicals. Endocrine disruption research on flame retardants demonstrated that Tris(2-butoxyethyl) phosphate displayed PXR agonistic activity, which may have the potential for endocrine disrupting effects (Kojima et al., 2013).

3.3. Water quality screening

3.3.1. Water quality benchmarks

To enhance screening and prioritization, particularly at sites where EARs were below the screening threshold, chemical occurrence data were compared to water quality benchmarks. Pentachlorophenol, anthracene, and bis(2-ethylhexyl) phthalate concentrations exceeded water quality criteria for aquatic or human health (Table S11). At Site 2, pentachlorophenol measured 0.291 µg/L and exceeded the ambient water quality criteria of 0.03 µg/L established by the EPA. Possible uses of pentachlorophenol include pesticides or fungicides (Rawlings et al., 1998), but the source is unknown. At Sites 3 and 4, anthracene (PAH) measured 0.0138 µg/L and 0.0157 µg/L respectively, and exceeded an aquatic water quality benchmark of 0.012 µg/L established by the Canadian Council of Ministers of the Environment (CCME, 2015; Table S10). Possible uses of anthracene include wood preservative; component of tar, diesel, and crude oil; or combustion product (Lorah et al., 2008). Upstream from Sites 3 and 4, seven NPDES outlets and one UOG wastewater disposal (underground injection control) facility were identified (Table S1) and could be possible sources of anthracene. At Site 8, bis(2-ethylhexyl) phthalate, a plasticizer, measured 0.42 µg/L and exceeded the ambient water quality criteria of 0.32 µg/L established by the EPA, as well as an aquatic water quality benchmark of 0.3 µg/L established by the National Oceanic and Atmospheric Administration (Buchman, 2008; Table S10). Possible uses of bis(2-ethylhexyl) phthalate include plasticizers for polymers and resins, and a major component of vinyl (Lorah et al., 2008), but sources of this compound are less clear. Using this approach, bis(2-ethylhexyl) phthalate and anthracene were identified using water quality benchmarks even though they were below the EAR threshold that was used in this study (0.001).

Inorganic water quality criteria exceedances were primarily concentrated in the headwaters at Sites 1–5 (Table 2), which has a history of extractive land use including mining and oil and gas (Fig. 1). Aquatic toxicity and drinking water exceedances were most notable at Sites 2 and 5, with eight exceedances at Site 2 and six exceedances at Site 5 (Table 2). Site 2 was sampled below an UOG wastewater disposal facility, and constituents associated with UOG wastewater (Ba^{2+}, Cl^-) exceeded water quality criteria for drinking water. Site 5 was sampled downstream of an AMD site, and constituents associated with AMD (Fe, Mn) exceeded water quality criteria for drinking water. At Site 10, specific conductance was elevated (562 µS/cm, Table S4) and the

chronic aquatic water quality criteria for lead (0.0025 mg/L) was exceeded. In addition, the chronic aquatic water quality criteria for lead was exceeded at Sites 1–5, and 11, which may be explained by widespread mining practices in the area (Larson et al., 2014a). The minerals galena, clausthalite, and pyrite are commonly found in coal and contain lead (Finkelman, 1988).

3.3.2. Inorganic chemical signatures

Samples collected from Site 2, located downstream from an UOG wastewater disposal facility, had clear differences in chemistry with respect to upstream samples collected at Site 1. Specific conductance measured at Site 2 was 10× higher (1018 µS/cm) than Site 1 (97 µS/cm), and 8× higher than the reference stream at Site 11 (123 µS/cm). Water samples at Site 2 also had elevated concentrations of several constituents (Ba^{2+}, Br^-, Cl^-, and Sr) that are known indicators of UOG wastewater impacts. When compared to Site 1, concentrations of Ba^{2+} increased by 15×, Br^- by 17×, Cl^- by 485×, and Sr by 22×. These findings are consistent with previous studies that sampled in the month of September (Akob et al., 2016) and indicate that downstream waters are still being impacted from nearby UOG wastewater disposal operations.

AMD from the Summerlee Abandoned Mine Land site has been characterized in several studies (Larson et al., 2014b, 2014a), demonstrating high concentrations of Al (20.3 mg/L), Fe (278 mg/L), Mn (15.5 mg/L), and SO_4^{2-} (547 mg/L). Water samples collected at Site 5, 1.3 km downstream from the Summerlee site, showed elevated concentrations of several constituents (Al, Fe, Mn, SO_4^{2-}) that are known indicators of AMD. Waters at Site 5 had the highest specific conductance (1566 µS/cm), Al (10.27 mg/L), Fe (27.5 mg/L), Mn (7.06 mg/L), SO_4^{2-} (712 mg/L), and lowest pH (3.14) of any of the water samples (Table S4). These observations provide evidence that AMD is still impacting the headwaters of Wolf Creek.

3.4. Potential endocrine disrupting chemicals

To further support screening and prioritization, particularly at sites where EARs were below the screening threshold or lacked water quality criteria, chemical occurrence data were compared to TEDX List of Potential Endocrine Disruptors database (TEDX, 2018b). Potential EDCs were detected at 9 of 10 sites in the Wolf Creek watershed (Table 2), and 17 EDCs were identified from at least one site (Table 1). Mixtures of two or more EDCs were observed at 8 of 10 sites, and a maximum of nine EDCs were observed at Site 6, followed by eight EDCs at Sites 3 and 4 (Table S14). Synergistic effects from compound mixtures have been observed in several EDC studies (Vajda et al., 2008; Vandenberg et al., 2012) and can have adverse biological effects even at low concentrations. Site 6 was sampled downstream of a large shopping center, and

surface runoff from parking lots is commonly known to contain PAHs and many other contaminants (Baun et al., 2006). Three PAHs were detected at Site 6 including fluoranthene, phenanthrene, and pyrene. The most frequently detected EDCs were methyl salicylate (miscellaneous), BPA (antioxidant), indole (flavors/fragrances), tri(2-butoxyethyl) phosphate (fire retardant), pyrene (PAH), and fluoranthene (PAH) (Table S14). In total, nine chemicals were identified as potential EDCs that were either below the EAR screening threshold or lacked water quality benchmarks (Table 1). By using the List of Potential Endocrine Disruptors database, chemicals were further prioritized, and SI Table 14 identifies EDCs that warrant further investigation.

3.5. Areas of concern

3.5.1. Source water protection: Zone of critical concern

Wolf Creek is a tributary of the New River, a drinking water source for communities in Fayette County and an important recreation resource. Several screening tools were used to evaluate potential adverse biological effects within the Zone of Critical Concern (ZCC) and Zone of Peripheral Concern (ZPC) including EAR hits, water quality benchmarks, and potential EDCs (Table 2). A portion of the ZCC for the New River Water Treatment System extends into the Wolf Creek watershed, and the ZPC covers all streams in the watershed (the ZPC overlaps the ZCC). To clarify how results are summarized, Sites 1–5 are located within the ZPC, and Sites 6–10 are located within the ZCC (Fig. 3). Within the ZCC, 23 of 69 (33%) OWCs were detected that resulted in two EAR hits, one human health ambient water quality criteria exceedance, one aquatic chronic water quality criteria exceedance, and ten EDCs (Table 2). The potential for adverse biological effects is greatest at Site 6, downstream from a large shopping area. Samples from Sites 8–10 had EAR values below the screening threshold and the data show a steady decline in OWC sample concentration after Site 6 (Table 2). Downstream of Site 8, Wolf Creek enters National Park Service lands, which may provide a barrier of protection from anthropogenic sources. At the mouth of Wolf Creek (Site 10), a popular recreation area, no OWCs were detected but lead exceeded the action level to

protect drinking water, and also exceeded aquatic chronic water quality criteria. Due to low flow conditions during the time of sampling, House Branch, a tributary upstream from the mouth, was not flowing. House Branch contains a combined sewer overflow that discharges stormwater runoff and untreated sewage during heavy rain events (Hansen et al., 2014). Combined sewer overflows have been shown to release contaminants such as pharmaceutical and personal care products, antimicrobial disinfectants, PAHs, organochlorine compounds, nutrients, and nonprescription drugs into receiving waters, adversely affecting water quality (Ellis, 2006; Phillips et al., 2012). In addition, stormwater runoff from urban development can carry organic contaminants during rain events, and is one of the major causes of decreased water quality (Burant et al., 2018). To better define the potential for adverse biological effects in the Wolf Creek watershed, it would be valuable to collect samples during baseflow conditions, stormwater runoff events, and combined sewer overflow events.

3.5.2. Source water protection: Zone of peripheral concern

All streams in the Wolf Creek watershed are within the ZPC (the ZPC overlaps the ZCC), but to eliminate redundancy in the results, we focus here on Sites 1–5. Within the ZPC, 25 of 69 (36%) OWCs were detected that resulted in 81 EAR hits, two aquatic chronic water quality criteria exceedances, one human health ambient water quality criteria exceedance, and 14 EDCs (Table 2). In addition, 17 inorganic water quality criteria exceedances were observed including one aquatic acute criteria, 10 aquatic chronic criteria, and six drinking water quality criteria (Table 2). Data from the present study indicate that the potential for adverse biological effects is greatest at Site 2, followed by Sites 3, 1, 5, and 4 (Fig. 2; Table 2). An examination of potential contaminant sources in the headwaters of Wolf Creek (Sites 1–5) show the presence of a wastewater treatment plant effluent discharge, seven industrial stormwater outlets, abandoned mine lands problem areas (59 ha), coal refuse impoundment (5 ha), legacy surface mining (13 ha), and an UOG wastewater disposal facility (Fig. 1; Table S1). This study supports the previous work done by others (Akob et al., 2016; Kassotis et al., 2016; Orem et al., 2017) and shows evidence of chemicals consistent with
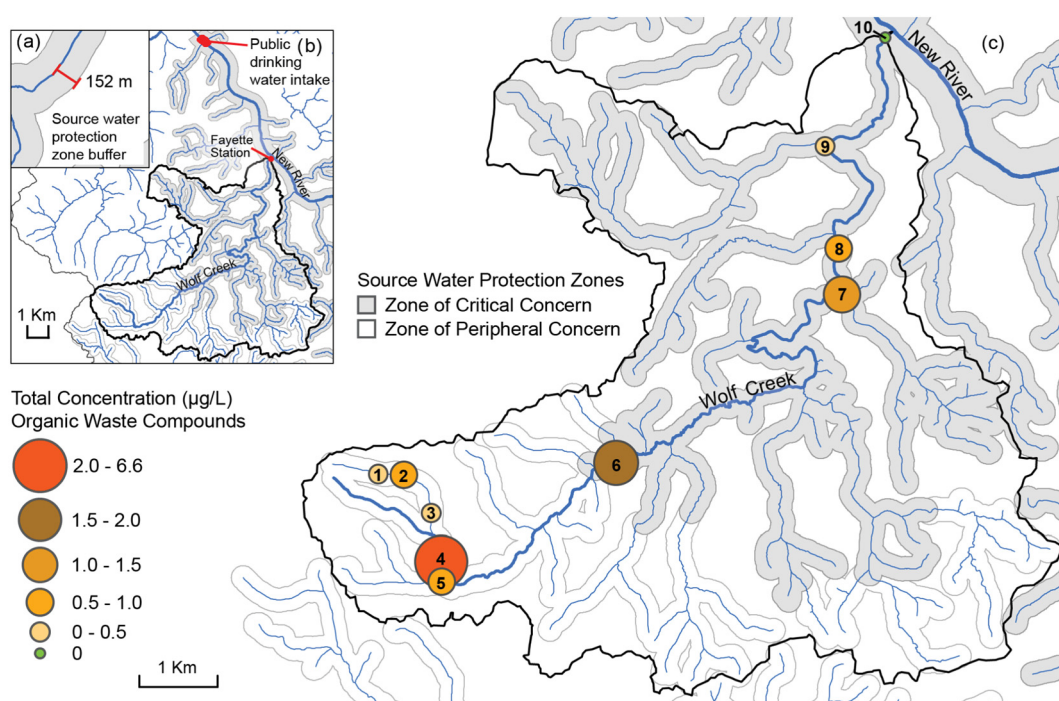

Fig. 3. Source water protection zones buffered 152 m from center of stream (3a). Overview of the source water protection zones for the New River Water Treatment System (3b). Total concentration of detected organic waste compounds at each sampling site (3c). The numbers on map (3c) correspond to the sampling site.
Source: West Virginia Bureau for Public Health, 2016.

UOG wastewaters in surface waters collected immediately downstream from a UOG wastewater disposal facility. Downstream of the UOG wastewater disposal facility, low-level organic contamination in headwater Sites 2–4 showed elevated EAR cumulative mixture values, demonstrating the potential for adverse biological affects for organisms living in or near the stream. The combination of legacy mining and current UOG wastewater disposal practices in headwater drainages could result in cumulative impacts. Further work evaluating cumulative impacts at headwater sites could improve our understanding of the risks present to human health and aquatic life.

4. Discussion

4.1. Adverse outcome pathways

toxEval can also be used to help prioritize molecular targets and biological pathways that demonstrate biological activity in the chemical occurrence data. However, this approach was not used in the current study, as the primary focus was to screen sampling sites and prioritize chemicals for further investigation. It is important to reiterate that an EAR hit does not indicate a hazard, but is an indication that the potential for adverse biological effects is present. Ultimately, biological targets or pathways associated with particular EAR values need to be credibly linked to adverse outcomes that serve as verification that a hazard is present. The adverse outcome pathways (AOP) framework provides a means to link a perturbed biological target or pathway to end points of regulatory concern (Blackwell et al., 2017). Identification of chemical initiators is the first step in the AOP conceptual framework (Organization for Economic Cooperation and Development, 2013). Once targets have been identified, the AOP-Knowledge Base (AOP-KB) can be queried for relevant AOPs (Society for Advancement of Adverse Outcome Pathways, 2018) and evaluated for the potential to adversely impact the development, growth, reproduction or survival of the organism being exposed (Schroeder et al., 2016). However, a recognized limitation of the EAR approach using ToxCast data is the capability to link molecular endpoints to AOPs at the organism or population scale, most of which are largely unknown (Bradley et al., 2019; Conolly et al., 2017; Schroeder et al., 2016; Villeneuve et al., 2014). In vitro to in vivo extrapolation models are still needed to better understand how pathway perturbations at the cell level translate to impacts for an intact organism (Villeneuve et al., 2019). Further, models translating in vitro concentration-response values to equivalent in vivo environmental exposure concentrations have been proposed (Fischer et al., 2017; Wetmore et al., 2015) but are currently limited by available assay-specific parameters and in vitro toxicokinetic models. While currently limited, the AOP-KB is progressing, and the current framework underscores the challenge associated with linking biological activity to hazards.

4.2. Utility of the exposure-activity ratio-based approach

EARs have been used in this study and by others (Blackwell et al., 2017; DeCicco et al., 2018) to identify potential adverse biological effects in water resources, but in order to prioritize impacted sites or drinking water treatment needs, it is essential to identify all of the chemicals responsible for biological activity in the environmental sample. It is important to understand that the approach used in this study can only be used to assess the chemicals that were actually targeted and quantified at a given site and does not account for any unknown chemicals that may be present. Tang and Escher (2014) refer to these as "iceberg mixtures," an analogy comparing the "tip of the iceberg" as representing known (or detected) chemicals to the submerged portion of the iceberg representing unknown chemicals. By comparing iceberg mixtures with the biological effects of water samples, several studies have demonstrated that only a small fraction of the effect in most bioassays was explained by targeted, detected chemicals (König et al., 2017;

Neale et al., 2017; Tang and Escher, 2014). Therefore, when analyzing environmental samples, an important consideration is using the full breadth of HTS effects-based in vitro assays to provide bioactivity information from the full sample matrix that can account for any unknown contaminants that may be present.

5. Conclusion

With >9000 chemicals in the ToxCast database, the "bioeffects surveillance" approach provides an estimate of the biological activity of an environmental sample across a broad range of pathways (Schroeder et al., 2016), and can be an important tool for the initial identification of potential hazards. In addition, EAR-based predictions about the biological targets being affected can be used to follow up with validation studies to confirm biological effects from environmental contaminants. As HTS databases continue to grow and EAR approaches become more common, use of environmental monitoring tools, such as toxEval, will be integral to rapidly process and identify insights from large chemical toxicity databases.

In this study, we present an approach that takes advantage of HTS data and a new bioeffects screening tool, toxEval, to identify potential adverse biological effects in water. This approach can be used to screen potentially harmful chemicals in water resources, inform the prioritization of future monitoring and research, and ultimately improve the efficiency and effectiveness of watershed management activities. The application of EARs allowed our study to extend beyond traditional environmental monitoring methods to identify multiple sites that have the potential for adverse biological effects in the Wolf Creek watershed. Our screening results highlight the need for future monitoring and research, which have been prioritized using EARs, water quality benchmarks, and potential EDCs. Absent from the current and previous research is the investigation of contaminant transport and fate in ground water. Studying the transport and fate of injected UOG wastewater in ground water, monitoring chemicals disposed of by the UOG wastewater disposal facility, and further work identifying unknown contaminants or hazards that might be present could improve our spatial understanding of the contaminants throughout the watershed and the potential risk associated with different land use activities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.04.180.

References

Akob, D.M., Mumford, A.C., Orem, W., Engle, M.A., Klinges, J.G., Kent, D.B., Cozzarelli, I.M., 2016. Wastewater disposal from unconventional oil and gas development degrades stream quality at a West Virginia injection facility. Environ. Sci. Technol. 50, 5517–5525. https://doi.org/10.1021/acs.est.6b00428.

Baldwin, A.K., Corsi, S.R., Richards, K.D., Geis, S.W., Magruder, C., 2013. Organic Waste Compounds in Streams: Occurrence and Aquatic Toxicity in Different Stream Compartments, Flow Regimes, and Land Uses in Southeast Wisconsin (2006–9).

Baldwin, A.K., Corsi, S.R., De Cicco, L.A., Lenaker, P.L., Lutz, M.A., Sullivan, D.J., Richards, K.D., 2016. Organic contaminants in Great Lakes tributaries: prevalence and potential aquatic toxicity. Sci. Total Environ. 554–555, 42–52. https://doi.org/10.1016/j.scitotenv.2016.02.137.

Baldwin, A.K., Corsi, S.R., Lutz, M.A., Ingersoll, C.G., Dorman, R., Magruder, C., Magruder, M., 2017. Primary sources and toxicity of PAHs in Milwaukee-area streambed sediment. Environ. Toxicol. Chem. https://doi.org/10.1002/etc.3694.

Basketter, D.A., Kimber, I., 2010. Comprehensive Toxicology, Comprehensive Toxicology. https://doi.org/10.1016/B978-0-08-046884-6.00623-0.

Baun, A., Eriksson, E., Ledin, A., Mikkelsen, P.S., 2006. A methodology for ranking and hazard identification of xenobiotic organic compounds in urban stormwater. Sci. Total Environ. 370, 29–38. https://doi.org/10.1016/j.scitotenv.2006.05.017.

Becker, R.A., Friedman, K.P., Simon, T.W., Marty, M.S., Patlewicz, G., Rowlands, J.C., 2015. An exposure: activity profiling method for interpreting high-throughput screening data for estrogenic activity-proof of concept. Regul. Toxicol. Pharmacol. 71, 39–408. https://doi.org/10.1016/j.yrtph.2015.01.008.

Benigni, R., 2013. Evaluation of the toxicity forecasting capability of EPA's ToxCast phase I data: can ToxCast in vitro assays predict carcinogenicity? J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 31, 201–212. https://doi.org/10.1080/10590501.2013.824188.

Benísek, M., Bláha, L., Hilscherová, K., 2008. Interference of PAHs and their N-heterocyclic analogs with signaling of retinoids in vitro. Toxicol. In Vitro https://doi.org/10.1016/j.tiv.2008.09.009.

Berry, W.J., Burgess, R.M., Hansen, D.J., 2003. Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms. Environ. Prot. Agency, Dieldrin.

Blackwell, B.R., Ankley, G.T., Corsi, S.R., Decicco, L.A., Houck, K.A., Judson, R.S., Li, S., Martin, M.T., Murphy, E., Schroeder, A.L., Smith, E.R., Swintek, J., Villeneuve, D.L., 2017. An "eAR" on environmental surveillance and monitoring: a case study on the use of exposure-activity ratios (EARs) to prioritize sites, chemicals, and bioactivities of concern in Great Lakes waters. Environ. Sci. Technol. 51, 8713–8724. https://doi.org/10.1021/acs.est.7b01613.

Bradley, P.M., Kolpin, D.W., Romanok, K.M., Smalling, K.L., Focazio, M.J., Brown, J.B., Cardon, M.C., Carpenter, K.D., Corsi, S.R., Decicco, L.A., Dietze, J.E., Evans, N., Furlong, E.T., Givens, C.E., Gray, J.L., Griffin, D.W., Higgins, C.P., Hladik, M.L., Iwanowicz, L.R., Journey, C.A., Kuivila, K.M., Masoner, J.R., McDonough, C.A., Meyer, M.T., Orlando, J.L., Strynar, M.J., Weis, C.P., Wilson, V.S., 2018. Reconnaissance of mixed organic and inorganic chemicals in private and public supply tapwaters at selected residential and workplace sites in the United States. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.8b04622.

Bradley, P.M., Journey, C.A., Berninger, J.P., Button, D.T., Clark, J.M., Corsi, S.R., DeCicco, L.A., Hopkins, K.G., Huffman, B.J., Nakagaki, N., Norman, J.E., Nowell, L.H., Qi, S.L., VanMetre, P.C., Waite, I.R., 2019. Mixed-chemical exposure and predicted effects potential in wadeable southeastern USA streams. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2018.11.186.

Brantley, S.L., Yoxtheimer, D., Arjmand, S., Grieve, P., Vidic, R., Pollak, J., Llewellyn, G.T., Abad, J., Simon, C., 2014. Water resource impacts during unconventional shale gas development: the Pennsylvania experience. Int. J. Coal Geol. 126, 140–156. https://doi.org/10.1016/j.coal.2013.12.017.

Buchman, M.F., 2008. NOAA Screening Quick Reference Tables, NOAA OR&R Report 08-1. Office of Response and Restoration Division, National Oceanic and Atmospheric Administration, Seattle WA (34 pages).

Burant, A., Selbig, W., Furlong, E.T., Higgins, C.P., 2018. Trace organic contaminants in urban runoff: associations with urban land-use. Environ. Pollut. https://doi.org/10.1016/j.envpol.2018.06.066.

CCME, 2015. Canadian Environmental Quality Guidelines, Water Quality Guidelines for the Protection of Aquatic Life [WWW Document]. URL. http://ceqg-rcqe.ccme.ca/en/index.html, Accessed date: 10 March 2019.

Conolly, R.B., Ankley, G.T., Cheng, W., Mayo, M.L., Miller, D.H., Perkins, E.J., Villeneuve, D.L., Watanabe, K.H., 2017. Quantitative adverse outcome pathways and their application to predictive toxicology. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.6b06230.

DeCicco, L., Corsi, S., Villeneuve, D., Blackwell, B., Ankley, G., 2018. toxEval: Evaluation of Measured Concentration Data Using the ToxCast High-throughput Screening Database or a User-defined Set of Concentration Benchmarks. https://doi.org/10.5066/P906UQ5I.

Ellis, J.B., 2006. Pharmaceutical and personal care products (PPCPs) in urban receiving waters. Environ. Pollut. 144, 184–189. https://doi.org/10.1016/j.envpol.2005.12.018.

Fay, K.A., Villeneuve, D.L., Swintek, J., Edwards, S.W., Nelms, M.D., Blackwell, B.R., Ankley, G.T., 2018. Differentiating pathway-specific from nonspecific effects in high-throughput toxicity data: a foundation for prioritizing adverse outcome pathway development. Toxicol. Sci. https://doi.org/10.1093/toxsci/kfy049.

Filer, D.L., Kothiya, P., Woodrow Setzer, R., Judson, R.S., Martin, M.T., 2017. tcpl: the ToxCast pipeline for high-throughput screening data. Bioinformatics 33. https://doi.org/10.1093/bioinformatics/btw680.

Finkelman, R.B., 1988. The inorganic geochemistry of coal: a scanning electron-microscopy view. Scanning Microsc. 97–105.

Fischer, F.C., Henneberger, L., König, M., Bittermann, K., Linden, L., Goss, K.U., Escher, B.I., 2017. Modeling exposure in the Tox21 in vitro bioassays. Chem. Res. Toxicol. https://doi.org/10.1021/acs.chemrestox.7b00023.

Focazio, M.J., Kolpin, D.W., Barnes, K.K., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Barber, L.B., Thurman, M.E., 2008. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States - II untreated drinking water sources. Sci. Total Environ. 402, 201–216. https://doi.org/10.1016/j.scitotenv.2008.02.021.

Foreman, W.T., Rose, D.L., Chambers, D.B., Crain, A.S., Murtagh, L.K., Thakellapalli, H., Wang, K.K., 2015. Determination of (4-methylcyclohexyl)methanol isomers by heated purge-and-trap GC/MS in water samples from the 2014 Elk River, West Virginia, chemical spill. Chemosphere 131, 217–224. https://doi.org/10.1016/j.chemosphere.2014.11.006.

GAO, U.S., 2019. Chemical Assessments: Status of EPA's Efforts to Produce Assessments and Implement the Toxic Substances Control Act. Rep. to Congr. Requestors.

Hansen, E., Betcher, M., Hereford, A., Boettner, F., Christ, M., Warren, M., 2014. Revised Watershed-based Plan for the Wolf Creek Watershed of the New River.

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

Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen, H.M., Reif, D.M., Rotroff, D.M., Shah, I., Richard, A.M., Dix, D.J., 2010. In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. Environ. Health Perspect. 118, 485–492. https://doi.org/10.1289/ehp.0901392.

Judson, R.S., Magpantay, F.M., Chickarmane, V., Haskell, C., Tania, N., Taylor, J., Xia, M., Huang, R., Rotroff, D.M., Filer, D.L., Houck, K.A., Martin, M.T., Sipes, N., Richard, A.M., Mansouri, K., Woodrow Setzer, R., Knudsen, T.B., Crofton, K.M., Thomas, R.S., 2015. Integrated model of chemical perturbations of a biological pathway using 18 in vitro high-throughput screening assays for the estrogen receptor. Toxicol. Sci. 148, 137–154. https://doi.org/10.1093/toxsci/kfv168.

Kassotis, C.D., Klemp, K.C., Vu, D.C., Lin, C.H., Meng, C.X., Besch-Williford, C.L., Pinatti, L., Zoeller, R.T., Drobnis, E.Z., Balise, V.D., Isiguzo, C.J., Williams, M.A., Tillitt, D.E., Nagel, S.C., 2015. Endocrine-disrupting activity of hydraulic fracturing chemicals and adverse health outcomes after prenatal exposure in male mice. Endocrinology 156, 4458–4473. https://doi.org/10.1210/en.2015-1375.

Kassotis, C.D., Iwanowicz, L.R., Akob, D.M., Cozzarelli, I.M., Mumford, A.C., Orem, W.H., Nagel, S.C., 2016. Endocrine disrupting activities of surface water associated with a West Virginia oil and gas industry wastewater disposal site. Sci. Total Environ. 557–558, 901–910. https://doi.org/10.1016/j.scitotenv.2016.03.113.

Kingsbury, J.A., Delzer, G.C., Hopple, J.A., 2008. Anthropogenic organic compounds in source water of nine community water systems that withdraw from streams, 2002–05. US Geol. Surv. Sci. Investig. Rep. 68.

Kleinstreuer, N.C., Yang, J., Berg, E.L., Knudsen, T.B., Richard, A.M., Martin, M.T., Reif, D.M., Judson, R.S., Polokoff, M., Dix, D.J., Kavlock, R.J., Houck, K.A., 2014. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat. Biotechnol. 32, 583–591. https://doi.org/10.1038/nbt.2914.

Kojima, H., Takeuchi, S., Itoh, T., Iida, M., Kobayashi, S., Yoshida, T., 2013. In vitro endocrine disruption potential of organophosphate flame retardants via human nuclear receptors. Toxicology 314, 76–83. https://doi.org/10.1016/j.tox.2013.09.004.

König, M., Escher, B.I., Neale, P.A., Krauss, M., Hilscherová, K., Novák, J., Teodorović, I., Schulze, T., Seidensticker, S., Kamal Hashmi, M.A., Ahlheim, J., Brack, W., 2017. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. Environ. Pollut. https://doi.org/10.1016/j.envpol.2016.11.011.

Larson, L.N., Sánchez-España, J., Burgos, W., 2014a. Rates of low-pH biological Fe(II) oxidation in the Appalachian Bituminous Coal Basin and the Iberian Pyrite Belt. Appl. Geochem. 47, 85–98. https://doi.org/10.1016/j.apgeochem.2014.05.012.

Larson, L.N., Sánchez-España, J., Kaley, B., Sheng, Y., Bibby, K., Burgos, W.D., 2014b. Thermodynamic controls on the kinetics of microbial low-pH Fe(II) oxidation. Environ. Sci. Technol. 48, 9246–9254. https://doi.org/10.1021/es501322d.

Leung, M.C.K., Phuong, J., Baker, N.C., Sipes, N.S., Klinefelter, G.R., Martin, M.T., McLaurin, K.W., Woodrow Setzer, R., Darney, S.P., Judson, R.S., Knudsen, T.B., 2016. Systems toxicology of male reproductive development: profiling 774 chemicals for molecular targets and adverse outcomes. Environ. Health Perspect. 124, 1050–1061. https://doi.org/10.1289/ehp.1510385.

Liess, M., Foit, K., Becker, A., Hassold, E., Dolciotti, I., Kattwinkel, M., Duquesne, S., 2013. Culmination of Low-dose Pesticide Effects.

Lorah, M., Soeder, D., Teunis, J., 2008. Summary of Organic Wastewater Compounds and Other Water-Quality Data in Charles County, Maryland, October 2007 Through August 2008.

Lukacs, H., St. John, E., Lewis, M., Rose, L., Schrayshuen, B., Stonum, S., Purvis, J., Shleser, T., Wait, C., Dupree, J., Gasper, M., Johnson, J., Boettner, F., Martin, R., Hereford, A., 2011. Lower New River State of the Watershed.

Morace, J.L., 2012. Reconnaissance of Contaminants in Selected Wastewater-Treatment-Plant Effluent and Stormwater Runoff Entering the Columbia River, Columbia River Basin, Washington and Oregon, 2008–10 (80p).

Neale, P.A., Munz, N.A., Aït-Aïssa, S., Altenburger, R., Brion, F., Busch, W., Escher, B.I., Hilscherová, K., Kienle, C., Novák, J., Seiler, T.B., Shao, Y., Stamm, C., Hollender, J., 2017. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2016.10.141.

NOAA, 2016. Climatological data for Beckley Area, West Virginia - September 2016 [WWW Document]. URL. https://w2.weather.gov/climate/xmacis.php?wfo=rlx, Accessed date: 10 March 2019.

Orem, W., Varonka, M., Crosby, L., Haase, K., Loftin, K., Hladik, M., Akob, D., Tatu, C., Mumford, A., Jaeschke, J., Bates, A., Schell, T., Cozzarelli, I., 2017. Organic Geochemistry and Toxicology of a Stream Impacted by Unconventional Oil and Gas Wastewater Disposal Operations. Appl. Geochemistry.

Organization for Economic Cooperation and Development, 2013. Adverse Outcome Pathways Knowledge Base. http://aopkb.org/background.html, Accessed date: 10 March 2019.

Phillips, P.J., Chalmers, A.T., Gray, J.L., Kolpin, D.W., Foreman, W.T., Wall, G.R., 2012. Combined sewer overflows: an environmental source of hormones and wastewater micropollutants. Environ. Sci. Technol. 46, 5336–5343. https://doi.org/10.1021/es3001294.

Rawlings, N.C., Cook, S.J., Waldbillig, D., 1998. Effects of the pesticides carbofuran, chlor-pyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, andpentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. J. Toxic. Environ. Health A https://doi.org/10.1080/009841098159006.

Rogers, K., 2016. Reduction of Fecal Coliform Bacteria Through the Elimination of Sewage Discharges in West Virginia Streams (PhD Thesis).

Rose, L., Akob, D., Tuberty, S., Corsi, S., DeCicco, L., Colby, J., Martin, D., 2018. Chemistry of Waters Collected in the Wolf Creek and Buffalo Creek Watersheds in West Virginia in 2016. https://doi.org/10.5066/P9WE8RDV.

Rovida, C., Asakura, S., Daneshian, M., Hofman-Huether, H., Leist, M., Meunier, L., Reif, D., Rossi, A., Schmutz, M., Valentin, J.P., Zurlo, J., Hartung, T., 2015. Toxicity testing in the 21st century beyond environmental chemicals. ALTEX 32, 171–181. https://doi.org/10.14573/altex.1506201.

Ryan, N., 2017. A User's Guide for Accessing and Interpreting ToxCast Data. https://lri.americanchemistry.com/Users-Guide-for-Accessing-and-Interpreting-ToxCast-Data.pdf, Accessed date: 10 March 2019.

Schroeder, A.L., Ankley, G.T., Houck, K.A., Villeneuve, D.L., 2016. Environmental surveillance and monitoring-the next frontiers for high-throughput toxicology. Environ. Toxicol. Chem. 35, 513–525. https://doi.org/10.1002/etc.3309.

Shah, F., Greene, N., 2014. Analysis of Pfizer compounds in EPA's ToxCast chemicals-assay space. Chem. Res. Toxicol. 27, 86–98. https://doi.org/10.1021/tx400343t.

Silva, E., Rajapakse, N., Kortenkamp, A., 2002. Something from "nothing" - eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. https://doi.org/10.1021/es0101227.

Society for Advancement of Adverse Outcome Pathways, 2018. Adverse Outcome Pathways. URL. https://aopwiki.org/aops, Accessed date: 10 March 2019.

Strager, M., 2011. Landuse/Landcover of West Virginia. Nat. Resour. Anal. Cent http://wvgis.wvu.edu/data/dataset.php?ID=451, Accessed date: 10 March 2019.

Sullivan, P.J., Agardy, F.J., Clark, J.J.J., 2005. The environmental science of drinking water. Environ. Sci. Drink. Water, 29–87 https://doi.org/10.1016/B978-075067876-6/50005-1.

Suter, G.W., Tsao, C.I., 1996. Toxicological Benchmarks for Screening Potential Contaminants of Concern for Effects on Aquatic Biota: 1996 Revision. (United States). https://doi.org/10.2172/259365.

Tang, J.Y.M., Escher, B.I., 2014. Realistic environmental mixtures of micropollutants in surface, drinking, and recycled water: herbicides dominate the mixture toxicity toward algae. Environ. Toxicol. Chem. https://doi.org/10.1002/etc.2580.

TEDX, 2018a. The Endocrine Disruption Exchange. https://endocrinedisruption.org/interactive-tools/tedx-list-of-potential-endocrine-disruptors/updates, Accessed date: 10 March 2019.

TEDX, 2018b. TEDX List of Potential Endocrine Disruptors. https://endocrinedisruption.org/interactive-tools/tedx-list-of-potential-endocrine-disruptors/search-the-tedx-list, Accessed date: 10 March 2019.

US EPA, 1996. Ecotox Thresholds. https://www.epa.gov/sites/production/files/2015-09/documents/v3no2.pdf, Accessed date: 10 March 2019.

US EPA, 2006. Freshwater Sediment Screening Benchmarks. https://www.epa.gov/risk/freshwater-sediment-screening-benchmarks, Accessed date: 10 March 2019.

US EPA, 2012. EPA's Study of Hydraulic Fracturing and its Potential Impact on Drinking Water Resources. https://www.epa.gov/hfstudy/appendix-chemicals-identified-hydraulic-fracturing-fluids-and-wastewater-excel-file, Accessed date: 10 March 2019.

US EPA, 2014. Aquatic Life Benchmarks for Pesticide Registration. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration#benchmarks, Accessed date: 10 March 2019.

US EPA, 2015a. Water Quality Benchmarks for Aquatic Life. https://archive.epa.gov/emergency/bpspill/web/html/water-benchmarks.html, Accessed date: 3 October 2019.

US EPA, 2015b. National Recommended Water Quality Criteria, Human Health Criteria Table. https://www.epa.gov/wqc/national-recommended-water-quality-criteria-human-health-criteria-table, Accessed date: 10 March 2019.

US EPA, 2016a. Toxicity Forecaster (ToxCast). https://www.epa.gov/sites/production/files/2016-11/documents/icss_factsheet_nov2016.pdf, Accessed date: 10 March 2019.

US EPA, 2016b. National Recommended Water Quality Criteria, Aquatic Life Criteria Table. https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table, Accessed date: 10 March 2019.

US EPA, 2016c. Table of Regulated Drinking Water Contaminants. https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants, Accessed date: 10 March 2019.

US EPA, 2018. ToxCast Dashboard. https://actor.epa.gov/dashboard/, Accessed date: 10 March 2019.

US EPA, 2019. TSCA Chemical Substance Inventory. https://www.epa.gov/tsca-inventory, Accessed date: 10 March 2019.

USGS, 2016. Water-year summary for site USGS 03185400. https://waterdata.usgs.gov/nwis/sw, Accessed date: 3 October 2019.

Vajda, M.A., Barber, L.B., Gray, J.L., Lopez, E.M., Woodling, J.D., Norris, D.O., 2008. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environ. Sci. Technol. 42, 3407–3414. https://doi.org/10.1021/es0720661.

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455. https://doi.org/10.1210/er.2011-1050.

Villeneuve, D.L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T.H., LaLone, C.A., Landesmann, B., Lettieri, T., Munn, S., Nepelska, M., Ottinger, M.A., Vergauwen, L., Whelan, M., 2014. Adverse outcome pathway (AOP) development I: strategies and principles. Toxicol. Sci. 142, 312–320. https://doi.org/10.1093/toxsci/kfu199.

Villeneuve, D.L., Coady, K., Escher, B.I., Mihaich, E., Murphy, C.A., Schlekat, T., Garcia-Reyero, N., 2019. High-throughput screening and environmental risk assessment: state of the science and emerging applications. Environ. Toxicol. Chem. 38, 12–26. https://doi.org/10.1002/etc.4315.

Walter, H., Consolaro, F., Gramatica, P., Scholze, M., Altenburger, R., 2002. Mixture toxicity of priority pollutants at no observed effect concentrations (NOECs). Ecotoxicology https://doi.org/10.1023/A:1020592802989.

West Virginia American Water, 2016. Source Water Protection Plan New River Water System (Fayette County, WV).

West Virginia Bureau for Public Health, 2016. Freedom of Information Act request: New River Water Treatment System (350 Capitol Street, Charleston, WV 25301, USA).

West Virginia Department of Environmental Protection, 2016. Requirements Governing Water Quality Standards. http://www.dep.wv.gov/WWE/Programs/wqs/Pages/default.aspx, Accessed date: 10 March 2019.

West Virginia University, 2016. West Virginia GIS Technical Center [WWW Document]. http://wvgis.wvu.edu/, Accessed date: 10 March 2019.

Wetmore, B.A., Wambaugh, J.F., Allen, B., Ferguson, S.S., Sochaski, M.A., Setzer, R.W., Houck, K.A., Strope, C.L., Cantwell, K., Judson, R.S., LeCluyse, E., Clewell, H.J., Thomas, R.S., Andersen, M.E., 2015. Incorporating high-throughput exposure predictions with dosimetry-adjusted in vitro bioactivity to inform chemical toxicity testing. Toxicol. Sci. https://doi.org/10.1093/toxsci/kfv171.

Zhang, Z., Jia, C., Hu, Y., Sun, L., Jiao, J., Zhao, L., Zhu, D., Li, J., Tian, Y., Bai, H., Li, R., Hu, J., 2012. The estrogenic potential of salicylate esters and their possible risks in foods and cosmetics. Toxicol. Lett. https://doi.org/10.1016/j.toxlet.2011.12.004.

Zhu, H., Zhang, J., Kim, M.T., Boison, A., Sedykh, A., Moran, K., 2014. Big Data in Chemical Toxicity Research: The Use of High-throughput Screening Assays to Identify Potential Toxicants.

Zoeller, T.R., Brown, T.R., Doan, L.L., Gore, A.C., Skakkebaek, N.E., Soto, A.M., Woodruff, T.J., Vom Saal, F.S., 2012. Endocrine-disrupting chemicals and public health protection: a statement of principles from the Endocrine Society. Endocrinology 153, 4097–4110. https://doi.org/10.1210/en.2012-1422.