APPLICATION OF TOXCAST TO ASSESS POTENTIAL ADVERSE BIOLOGICAL EFFECTS IN AN IMPACTED WATERSHED

A Thesis
by
LEVI ROSE

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DECEMBER 2016

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Abstract

APPLICATION OF TOXCAST TO ASSESS POTENTIAL ADVERSE BIOLOGICAL EFFECTS IN AN IMPACTED WATERSHED

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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). The U.S. Environmental Protection Agency's ToxCast program uses a wide array of high-throughput screening assays to evaluate the potential toxicity of environmental chemicals. To prioritize organic contaminants with the potential for adverse biological effects, researchers at the U.S. Geological Survey have developed a bioeffects surveillance tool, ToxEval, that links environmental analytic chemistry to published toxicology data from the ToxCast program. ToxEval and other screening methods were used to evaluate environmental chemicals for potential adverse biological effects in an impacted watershed. In September 2016, water samples collected across an impacted watershed detected 91 organic waste compounds, 19 water quality benchmark exceedances were observed, and 17 endocrine disrupting chemicals were identified. Using ToxEval, we identified contaminants that may be potentially harmful to human health and aquatic life despite lacking water quality benchmarks. If this study was done in the traditional manner, the potential of these contaminants to cause adverse effects may have gone unnoticed. Given the large number of chemicals in common use without water quality benchmarks or toxicity information, the application of ToxCast is an effective tool that can be used to assess the potential adverse effects of environmental contaminants on aquatic life or human health.

Acknowledgments

This project was supported by funding from the Plateau Action Network and the U.S. Geological Survey Toxic Substances Hydrology Program. Many thanks are extended to Denise Akob and Isabelle Cozzarelli for their initial insights into the development of this project. The authors would also like to thank Steve Corsi and Laura De Cicco for extending the opportunity to participate in early use of the ToxEval application. Many thanks are extended to my Advisor, Jeff Colby, and committee members Derek Martin, Shea Tuberty, and Denise Akob for their support.

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Article for Submission: *Application of ToxCast to Assess Potential Adverse Biological Effects in an Impacted Watershed*

Foreword

The manuscript prepared for this thesis will be submitted for review to the journal, *Science of The Total Environment*, an international journal for publication of original research on the total environment, which includes the atmosphere, hydrosphere, biosphere, lithosphere, and anthroposphere. The manuscript has been formatted according to the style guide for that journal.

Introduction

The U.S. Geological Survey's (USGS) Toxic Substances Hydrology Program "provides objective scientific information on environmental contamination to improve characterization and management of contaminated sites, to protect human and environmental health, and to reduce potential future contamination problems" (USGS, 2016a). As part of the program's mission, USGS scientists and university researchers have been studying the potential impacts of unconventional oil and gas waste materials on water resources and ecosystems (Akob et al., 2016; Kassotis et al., 2016; Orem et al. 2016). From September 2013 to September 2014, USGS scientists from the National Research Program in Reston, Virginia investigated the potential impacts on a stream adjacent to an underground injection wastewater disposal facility in Lochgelly, West Virginia. Stream water and sediment samples were collected upstream and downstream of the wastewater disposal facility, and were analyzed for chemical and microbiological changes and potential toxicological effects. Waters collected downstream from the site had elevated concentrations in specific conductance, total dissolved solids, sodium, chloride, barium, strontium, and lithium compared to upstream waters, demonstrating that activities at the wastewater disposal facility were impacting the adjacent stream (Akob et al. 2016). In addition, sediment analysis downstream of the disposal facility indicated enriched radium and elevated bioavailable Fe(III) concentrations relative to upstream sediments (Akob et al. 2016). The study identified the need for additional research in light of the poorly understood effects of wastewater releases on environmental health, which is predicted to increase based upon future projections of unconventional oil and gas production (Akob et al. 2016).

In the Spring of 2016, collaborative research between Appalachian State University and the USGS began and Denise Akob joined my thesis committee under Affiliate Graduate Faculty status. The collaboration provided an opportunity to continue advancing previous research, and to study the fate and effects of contaminants downstream of a wastewater disposal facility. The collaboration was further expanded when we were invited to join a small group of researchers at the USGS Wisconsin Water Science Center to evaluate a new application, ToxEval, that can be used to assess organic chemicals for potential adverse effects. The tool allows researchers to evaluate the potential toxicity of measured environmental chemicals in a web-based dashboard, and includes mapping features that can be used to assess the spatial distribution of environmental chemicals or identify areas of concern, as well as graphical outputs that summarize what biological pathways or processes are most affected. The primary function of the application is to provide users with biologically-relevant prioritization of contaminants that can be used to identify emerging contaminants and locations of concern. Our study involved using ToxEval and other screening methods to evaluate environmental chemicals for potential adverse biological effects across the Wolf Creek watershed in south central West Virginia.

1. Introduction

Watersheds and the waterways within them provide essential functions to humans and other organisms including water supply, aquatic habitat, transportation, recreation, and wastewater disposal (Barber et al., 2015). Chemical contaminants 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 (Baldwin et al., 2016; Foreman et al., 2015; Orem et al., 2016 unpublished results; Rogers, 2016). In addition, states with extractive industries, like West Virginia, also manage large volumes of wastewater from unconventional oil and gas (UOG) operations (Akob et al., 2016; Kassotis et al., 2016; Orem et al., 2016 unpublished results), as well as discharges from active and legacy mining (Larson et al., 2014b; Lindberg et al., 2011). Managing water resources with multiple uses is a challenging task, and understanding the spatial distribution, sources, and potential adverse biological effects of chemical contaminants is vital for watershed management (Baldwin et al., 2016).

Numerous studies (Baldwin et al., 2016; Barber et al., 2015; Focazio et al., 2008; Orem et al., 2016 unpublished results) on organic waste compounds (OWCs) have found natural and synthetic organic compounds such as pharmaceuticals, surfactants, flame retardants, plasticizers, steroids, herbicides, polycyclic aromatic hydrocarbons (PAHs), and other trace organics from UOG wastewater present in surface water. Trace organic compounds can have adverse effects on aquatic life and potentially human health at very low (sub parts per billion) concentrations (Liess et al., 2013; Schultz et al., 2011; Vandenberg et al., 2012). Many OWCs are persistent organic pollutants that do not readily degrade in the environment (Johnson et al., 2013). Instead, they can pose multiple risks through bioaccumulation in the food chain (Jenkins et al., 2014), or be difficult to remove at water treatment plants (Kingsbury et al., 2008; Stackelberg et al., 2004; Yoon and Amy, 2014), thus creating an exposure route for humans. Many of the OWCs sampled in this study are known or suspected endocrine-disrupting chemicals (EDCs) (TEDX, 2015).

In the United States and Canada, an estimated 30,000 chemicals are widely distributed throughout the environment (Judson et al., 2009; Karmaus et al., 2016; U.S. EPA, 2016a), but many chemicals in common use lack toxicity information or water-quality standards (Baldwin et al., 2016; Judson et al., 2009; Kleinstreuer et al., 2014). Until recently, the ability to provide timely and relevant screening of toxic chemicals was a costly and laborious task. 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). The U.S. Environmental Protection Agency's (U.S. EPA) ToxCast research program uses a wide array of high-throughput screening (HTS) assays to evaluate the potential toxicity of environmental chemicals. Since 2007, the ToxCast program has expanded coverage on 3,800 chemicals, using more than 700 different bioassays (Richard et al., 2016). While several studies have generally viewed the feasibility of high-throughput screening 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 prioritize organic contaminants with the potential for adverse biological effects, researchers at the U.S. Geological Survey (USGS) have developed a bioeffects surveillance tool, ToxEval, that links environmental analytic chemistry to published toxicology data from the ToxCast program (Corsi et al., 2017, unpublished results). The application uses concentration-response data to provide important information about the relationship between chemical concentration and bioactivity. Bioactivity is the effect of a given agent, such as an environmental contaminant, on a living organism or tissue. Examples are many and include processes that can be perturbed when a xenobiotic mimics the action of natural ligands (agonist) or block the action of those ligands (antagonist) (Judson et al., 2015). This in turn can effect signaling pathways and networks that are key components of complex biological systems, resulting in adverse outcomes on growth, health, reproduction, and survival (Kassotis et al., 2016; Zoeller et al., 2012) The primary function of the application is to provide users with biologically-relevant prioritization of contaminants that can be used to identify emerging contaminants and locations of concern.

Recently published studies (Akob et al., 2016; Kassotis et al., 2016; Orem et al., 2016 unpublished results) have highlighted water quality impacts to a tributary of Wolf Creek from UOG wastewaters at an underground injection control (UIC) well near Lochgelly, West Virginia. The studies concluded that more research was needed to identify contaminant sources and assess adverse biological effects downstream of a wastewater disposal facility. In addition, several potential contaminant sources have been identified within the watershed. The goals of this study were to evaluate environmental chemicals for potential adverse biological effects across the Wolf Creek watershed using ToxCast and other screening methods. The results from this research are a first level screening to narrow down potential chemicals, locations, and biological pathways of concern.

2. Materials and Methods

2.1 Site Description

Wolf Creek is a second order stream that drains approximately 4430 hectares (17 mi2) into the lower reaches of the New River (Fig. 1). The land cover is predominantly forested (75%), followed by grass/pasture/ag (17%), barren/developed/roads (7%), and water/wetlands (1%). Wolf Creek flows approximately seventeen kilometers from Oak Hill through Fayetteville and into the New River at Fayette Station rapid, a popular area to swim and boat (Lukacs et al., 2011). Approximately six kilometers (3.7 mi) 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 requirements of Senate Bill 373 (West Virginia American Water, 2016). In the plan, potential sources of significant contamination were identified, and this information was acquired from the West Virginia Bureau of Public Health for further analysis in a geographic information system (GIS) (West Virginia Bureau for Public Health, 2016). 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 SI-1). GIS methods are described in supplemental information (SI).

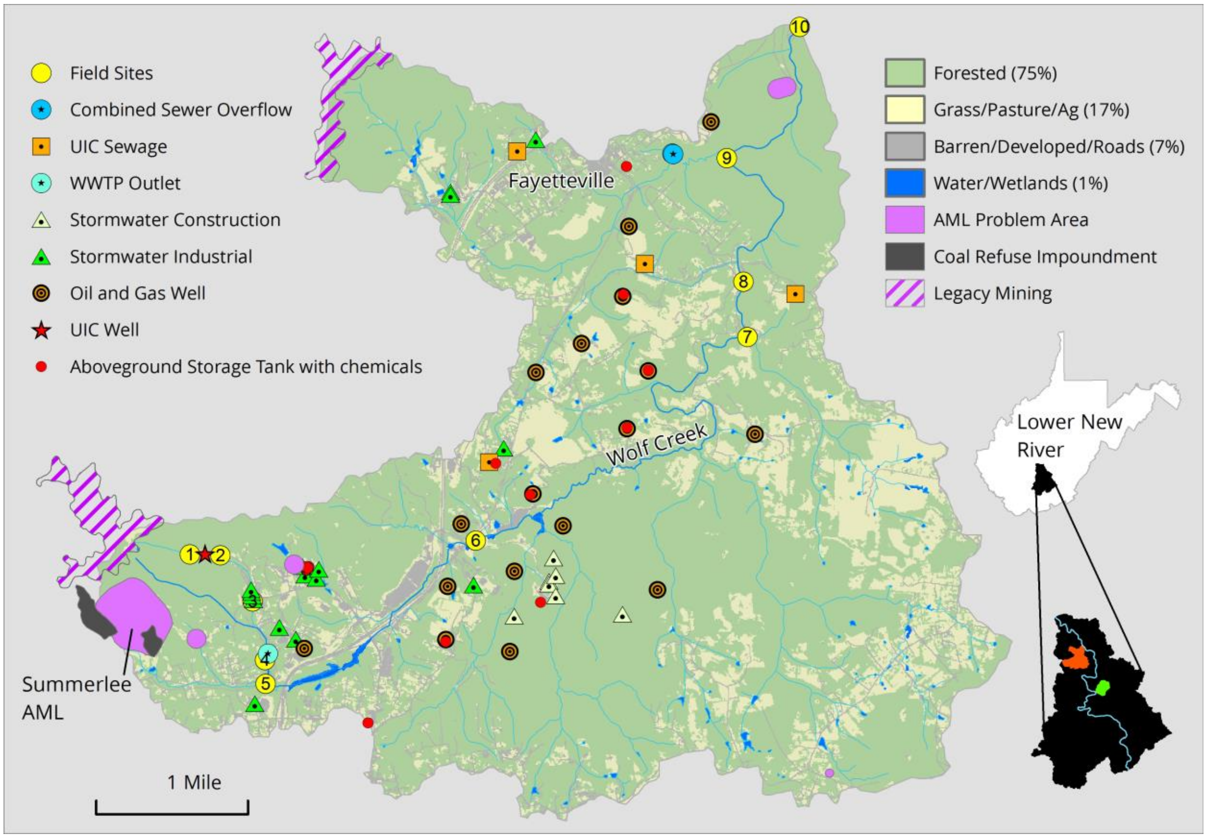

Fig. 1 Location of sampling sites, land use/land cover, and potential contaminant sources. Within the Lower New River location map, Wolf Creek watershed is red and the reference drainage, Buffalo Creek, is green. Sources: WV Bureau for Public Health, WV Department of Environmental Protection, WV Geological and Economic Survey, and WV GIS Technical Center.

Mining activities within Wolf Creek include areas that have been strip 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 (Hansen et al., 2014), and the Town of Fayetteville deemed Wolf Creek unsuitable as their primary public water source when the state identified water quality impairments in 2002. 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 tributaries are impaired by high levels of iron, aluminum, fecal coliform bacteria, and low pH. Additionally, organic enrichment and sedimentation have resulted in biological impairments (Hansen et al., 2014). More recently, evidence from unconventional oil and gas wastewater impacts have been documented by the USGS (Akob et al., 2016; Orem et al., 2016).

2.2 Site Sampling

Ten sites along Wolf Creek were sampled in September 2016, and one sample was collected from a non-impacted reference drainage (Fig. 1). Reference sites within the Lower New River drainage were provided by the West Virginia Department of Environmental Protection (WVDEP) Watershed Assessment Branch, and Buffalo Creek (Site 11) was chosen as the reference drainage in this study because of its close proximity to Wolf Creek. Surface water samples were collected from the approximate center of the stream for analysis of anions, cations, trace inorganic elements, and trace organic waste compounds. 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/sec) 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. Samples were collected and processed in a manner consistent with minimal contamination. Glass or Teflon equipment was used during sample collection and processing. Anions, cations, and trace inorganic elements samples were preserved to at least pH 2 with ultra-pure nitric acid (HNO3) and stored in HDPE containers. Organic samples were chilled at 4 °C and shipped overnight to the USGS National Water Quality Lab (NWQL) for analysis. Anions, cations, and trace inorganic elements were chilled at 4 °C and delivered to the Appalachian State University Ecotoxicology Lab for analysis.

Organic samples were analyzed in whole water samples at the USGS NWQL in Denver, Colorado using NWQL Schedule 4433, which targets 69 OWCs typically found in domestic and industrial wastewater (Table SI-2 and SI-3). Compounds were extracted using continuous liquid-liquid extraction and methylene chloride solvent, then determined by capillary-column gas chromatography/mass spectrometry (Zaugg et al., 2006). Anions, cations, and inorganic trace elements were analyzed at Appalachian State University in Boone, North Carolina. Anions, cations, and inorganic elements in water samples were prepared by microwave assisted acid digestion following U.S. EPA Method 3015A (U.S. EPA, 2007). Cation and inorganic elements (Al, As, B, Ba, Cd, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, S, Se, Sr, W, Zn) in water samples were analyzed using inductively coupled plasma-optical emission spectroscopy with a Varian 710-ES ICP-OES by EPA Protocol SW-846 Method 6010C (Manning and Grow, 2000). Anion element concentrations of (Br, Cl, F, NO3, SO4) in water samples were determined by ion chromatography with a DionexTM ICS-3000 by EPA Method 300.0 (Pfaff, 1993). Detailed quality assurance/quality control procedures are described in the SI Methods.

2.3 Data Analysis

A component of the Source Water Protection Plan for the New River Water Treatment System is to identify all potential significant contaminant sources located within the zone of critical concern (ZCC) and the zone of peripheral concern (ZPC) (West Virginia American Water, 2016). 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 ¼ mile 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 ¼ mile downstream. Both zones are buffered 500 feet from the center of stream. Portions of the ZCC extend into the Wolf Creek watershed, and the ZPC covers all waters within the Wolf Creek watershed (West Virginia American Water, 2016). GIS shapefiles of potential contaminant sources were obtained from the West Virginia Bureau for Public Health and were analyzed within the drainage boundary of the Wolf Creek watershed (Table SI-1). Detailed GIS methods are described in SI methods.

Schedule 4433 contains a total of 69 OWCs, and they were reviewed for their potential to cause adverse biological effects. Using chemical abstract service (CAS) numbers, 62 compounds were identified in the ToxCast (U.S. EPA, 2016b), 40 compounds were identified as potential endocrine disrupting chemicals (TEDX, 2015), 27 compounds had water quality benchmarks for aquatic toxicity, and 10 compounds had water quality benchmarks for human health (Table SI-4). In addition, 13 inorganic elements were reviewed for their potential to cause adverse effects to aquatic life and human health (Table SI-5). Total sample concentrations were calculated by summing all detected concentrations, using zeros for non-detected compounds. Organic waste compounds were grouped into 15 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 SI-2). These classes and methods have been used in previous studies (Baldwin et al., 2016, 2013), and were originally based on tables developed by Sullivan and others (Sullivan et al., 2005).

Failing onsite septic systems and leaking wastewater infrastructure has been documented throughout the Wolf Creek watershed (Hansen et al., 2014; Lukacs et al., 2011), as well as several NPDES sites that are permitted to discharge domestic wastewater (Table SI-1). To identify areas that are commonly associated with OWCs from failing onsite septic systems or leaking wastewater infrastructure, a subset of 20 wastewater indicator compounds from Schedule 4433 were analyzed (Baldwin et al., 2013). However, it is possible that these compounds could be from other sources. The subset of compounds includes all of the detergent metabolites and fire retardants, several of the flavors/fragrances, and the antimicrobial disinfectant triclosan. (Table SI-2). The total concentration of domestic wastewater indicator compounds in each water sample was calculated by summing all detected concentrations, and using zeros for non-detected compounds. Specific conductance, pH, and trace inorganic constituents were used as indicators of AMD and UOG wastewater, as well as documenting water quality benchmark exceedances. The combination of Ba, Br, Cl, and Sr can function as a local tracer of UOG wastewater impacts (Brantley et al., 2014), and was successfully used to characterize impacts downstream of an UOG wastewater disposal facility (Fig.1, Sites 1 and 2) (Akob et al., 2016). In waters with documented AMD impacts (Hansen et al., 2014; Larson et al., 2014a, 2014b), the combination of Al, Fe, Mn, and SO_4 were used to characterize waters downstream of an AMD source (Fig. 1, Site 5).

ToxEval was used to evaluate organic compounds in ToxCast for potential adverse biological effects. The application was developed using the R programming language, and uses several curated R packages that are available through the Geological Survey R Archive Network (GRAN) repository (USGS, 2016b). The application is currently available for download and installation in the public domain (DeCicco, 2016), but importing new data is currently only available to project participants. Detailed methods for ToxEval data preparation are described in SI Methods. The CAS number of the chemical compound is used to reference half-maximal activity concentration (AC50) values from dose-response concentration models published in ToxCast. The AC50 parameter in the Hill Equation model (Hill, 1910) is a common approach used to approximate chemical potency in toxicity testing (Shockley et al., 2016). In ToxCast, the AC50 is used to estimate the concentration at which a chemical produces the half-maximal response along a sigmoidal curve in an in vitro bioassay (Schroeder et al., 2016). An exposure activity ratio (EAR) is the quotient of the environmental concentration divided by the AC50 concentration.

$$\textit{Exposure Activity Ratio} = \frac{\textit{Environmental Concentration } (\mu M)}{\textit{AC50 Concentration } (\mu M)}$$

In ToxEval the EAR “hit” threshold can be defined by the user, and in this study the hit threshold was defined as an EAR > 0.1, indicating that the measured concentration is 10% of the AC50 or greater (Corsi et al., 2017, unpublished results) EARs were used to identify emerging chemicals of concern and adverse outcome pathways for further investigation.

In previous studies (Baldwin et al., 2016, 2013) a table of water quality benchmarks for acute and chronic exposure to aquatic life were compiled from a variety of sources, including the U.S. EPA (U.S. EPA, 2016c, 2014, 1996), 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 to also include water quality benchmarks for human health (U.S. EPA, 2016d; West Virginia Department of Environmental Protection, 2016) (Table SI-4).

3. Results and Discussion

3.1 Organic Waste Compounds

Understanding the detection frequency, magnitude, and spatial distribution of OWCs is an important step towards identifying contaminants of emerging concern. Lab analyses detected 33 unique OWCs from waters sampled in the Wolf Creek watershed. The most frequently detected compounds in the watershed, occurring in 40-90% of samples, were bisphenol A (antioxidants), camphor (flavors/fragrances), indole (flavors/fragrances), DEET (insecticides), methyl salicylate (miscellaneous), isophorone (solvent), and cholesterol (sterols) (Table 1). Of these compounds, bisphenol A, indole, and methyl salicylate are known EDCs. One or more OWCs were detected in 90% of the samples (n=10) collected in the Wolf Creek watershed, with the exception being zero detections at Site 10. Site 10 was collected near the mouth of Wolf Creek and due to low flow conditions, an upstream tributary and potential source of OWCs to Wolf Creek was not flowing. At Site 11, the reference drainage, zero OWCs were detected near the mouth of Buffalo Creek. Across the Wolf Creek watershed OWCs were generally observed at low concentrations, however, mixtures of ten or more compounds were detected at Site 6 (19 OWCs), Site 4 (18 OWCs), and Site 3 (12 OWCs) (Fig. 2). Even at low concentrations, it has been shown that the synergistic effects of multiple compounds can result in adverse biological effects (Vandenberg et al., 2012).

Domestic wastewater indicator compounds can enter surface waters through failing onsite septic systems, leaking wastewater infrastructure or NPDES discharges, and were observed at their highest total concentration at Site 4 (4.8 µg/L), followed by Site 6 (0.19 µg/L) and Site 5 (0.18 µg/L) (Table SI-6). Site 4 is located approximately 284 feet

Table 1. Occurrence of detected organic waste compounds in the Wolf Creek watershed, and concentrations that resulted in a ToxEval hit or water quality (WQ) exceedance. Endocrine disrupting chemical (EDC). *Domestic wastewater indicator compound.

			Concentration (µg/L)				
Chemical Class	Compound	Occurrence (n=10)	Max.	Med.	ToxEval Hit	WQ Exceedance	EDC
Antimicrobial Disinfectants	*p* -Cresol	10%	0.100	0			**X**
	Phenol	10%	0.086	0			**X**
Antioxidants	5-Methyl-1H-benzotriazole	10%	0.087	0			
	Bisphenol A	40%	0.280	0	**X**		**X**
Detergent Metabolites	4-Cumylphenol*	20%	0.022	0			
Dyes/Pigments	Anthraquinone	10%	0.301	0			
Fire Retardants	Tri(2-Butoxyethyl) phosphate*	30%	4.340	0			**X**
	Tris(Dichloroisopropyl) phosphate*	10%	0.060	0			**X**
Flavors/ Fragrances	3-Methyl-1H-indole	30%	0.013	0			
	Benzophenone*	20%	0.122	0			**X**
	Camphor*	90%	0.178	0.037			
	Hexahydrohexamethyl cyclopentabenzopyran*	20%	0.126	0			**X**
	Indole	40%	0.022	0			**X**
Herbicides	3,4-Dichlorophenyl isocyanate	10%	0.423	0			
	Pentachlorophenol	10%	0.291	0			**X**
	Prometon	10%	0.010	0			
Insecticides	Carbazole	10%	0.042	0			
	N,N-Diethyl-meta-toluamide (DEET)	60%	0.144	0.009	**X**		
Miscellaneous	Methyl Salicylate	80%	0.665	0.017			**X**
Nonprescription Drugs	Caffeine	20%	0.439	0			**X**
	Cotinine	20%	0.059	0			
	Menthol	30%	0.168	0			
PAH	Anthracene	20%	0.016	0		**X**	**X**
	Fluoranthene	30%	0.035	0			**X**
	Phenanthrene	20%	0.028	0			**X**
	Pyrene	30%	0.021	0			**X**
Plasticizers	Bis(2-Ethylhexyl) phthalate*	10%	0.420	0		**X**	**X**
	Tri(2-Chloroethyl) Phosphate	20%	0.029	0			
	Triethyl Citrate	10%	0.049	0			
	Triphenyl phosphate	10%	0.049	0	**X**		**X**
Solvents	Isophorone	70%	0.052	0.016			
Sterols	3-Beta-Coprostanol	20%	0.372	0			
	Cholesterol	80%	0.517	0.145			

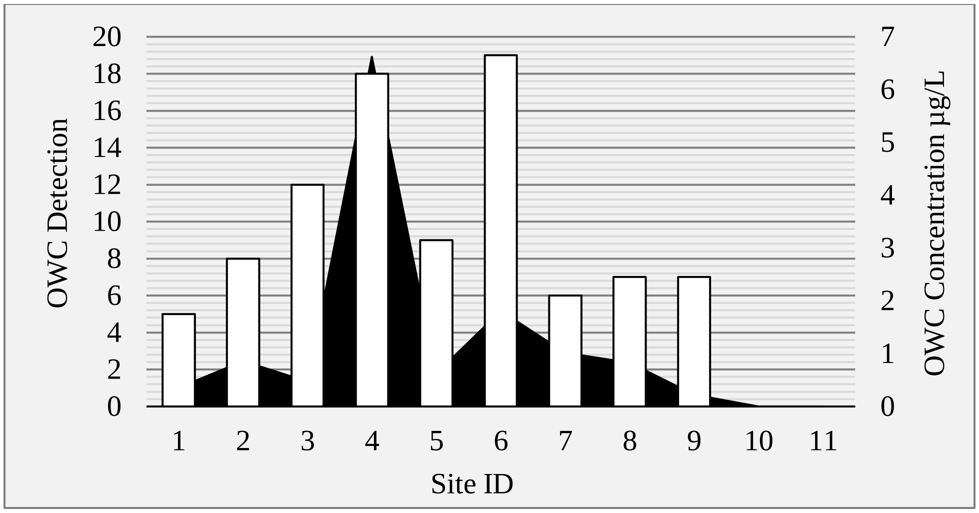

Fig. 2 Organic waste compound detection (bars) and total sample concentration (area) by site.

feet downstream from an NPDES wastewater treatment plant outlet, which could be the primary source of domestic wastewater indicator compounds.

At Site 3, approximately 2,200 feet upstream from Site 4, five compounds were detected at both sites, indicating another possible source. Tris(2-butoxyethyl) phosphate, a flame retardant, had the highest detected concentration of any wastewater indicator (4.34 µg/L), and made up 91% of the total sample concentration at Site 4. In a recent study using the same methodology for domestic wastewater indicator compounds (Baldwin et al., 2016), the study reported a mean total sample concentration in nonurban watersheds at 0.4 µg/L, and 1.22 µg/L in urban watersheds. For general comparison, all sites within Wolf Creek were below the nonurban watershed mean concentration, except Site 4, which was approximately 3.5x greater than the mean concentration reported in urban watersheds.

3.2 Inorganic water characterization

In September 2016, field sampling revealed elevated specific conductance levels at Sites 2, 5, 6, and 10 compared to background reference drainage Site 11 (Table SI-7). Waters downstream from an UOG wastewater disposal facility (Site 2) had elevated specific conductance (1018 µS/cm) compared to waters sampled upstream from the facility (Site 1), which had specific conductance (97 µS/cm) in line with reference Site 11 (123 µS/cm). A 10.5x increase in specific conductance is strong indication that downstream waters are still being impacted from nearby UOG wastewater disposal operations. Upstream and downstream water samples showed clear differences in chemistry with respect to the UOG wastewater disposal facility. Water samples collected in September 2016 show elevated concentrations of several constituents (Ba, Br, Cl, and Sr) that are known indicators of UOG wastewater impacts, consistent with previous studies that sampled in the month of September (Akob et al., 2016) (Table SI-8). Between Site 1 (upstream) and Site 2 (downstream) the concentrations of Ba increased by (15x), Br by (17x), Cl by (485x), and Sr by (22x).

AMD from the Summerlee Abandoned Mine Land site has been characterized in several studies (Larson et al., 2014a, 2014b), demonstrating high concentrations of Al (20.3 mg/L), Fe (278 mg/L), Mn (mg/L), and SO_4 (547 mg/L). Water samples collected in September 2016, 1.3 km (0.8 miles) downstream from the Summerlee site, show elevated concentrations of several constituents (Al, Fe, Mn, SO_4) that are known indicators of AMD (Table SI-9). 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 (712 mg/L), and lowest pH (3.14) of any of the water samples (Table SI-7). These observations provide evidence that AMD is still impacting headwaters, and the long distance that pollutants can be transported from the source.

3.3 Potential Adverse Biological Effects

The potential for organic contaminants to cause adverse biological effects was evaluated using EARs in ToxEval. OWCs with bioactivity above the threshold (EAR > 0.1) were observed in 60% of the water samples in the Wolf Creek watershed (Fig. 3). The highest EAR was observed at Site 4 (EAR 1.2) from an insecticide commonly referred to as DEET. In total, there were eight EAR hits observed above the threshold from three compounds DEET, bisphenol A, and triphenyl phosphate. DEET has an acute aquatic toxicity benchmark that was referenced from the U.S. EPA's Aquatic Life Benchmarks for Pesticide Registration (Table SI-4). However, both bisphenol A and triphenyl phosphate lack water quality benchmarks, but both have been identified as endocrine disruptors.

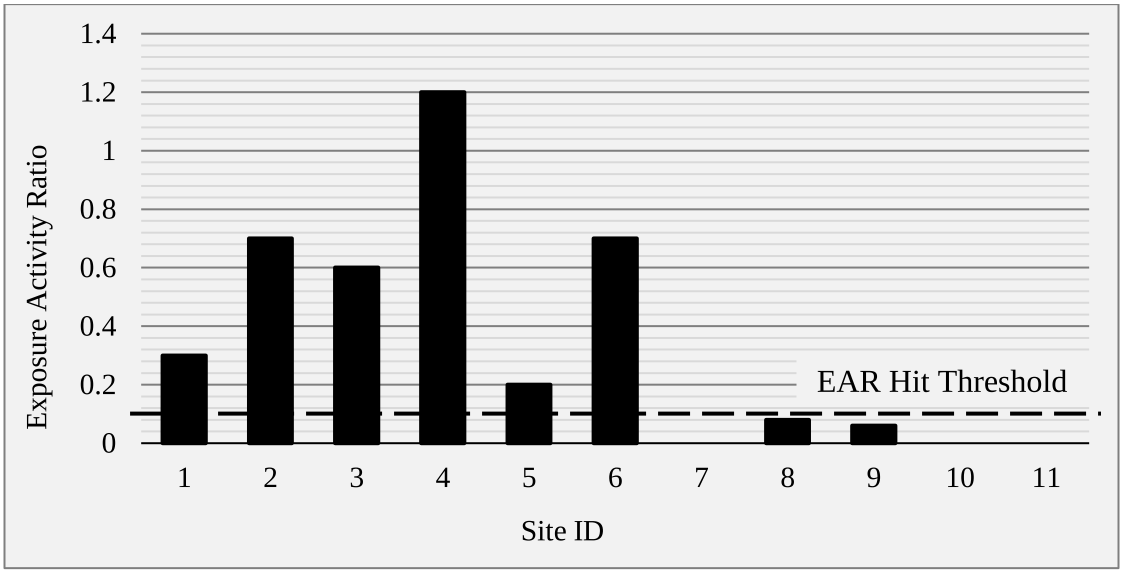

Fig. 3 Maximum exposure activity ratio calculated at each site. The EAR hit threshold (> 0.1).

Between Sites 1-4 a corridor of potential contaminant sources includes a UOG wastewater disposal facility, six industrial stormwater outlets, and a wastewater treatment plant sewage outlet just upstream of Site 4. A culmination of these factors may explain the high EAR hit at Site 4. The remaining sites, Sites 7, 8, 9, and 10, were below the EAR threshold, and the data show a precipitous drop in OWC sample concentration after Site 8 (Table SI-10). Downstream of Site 8, Wolf Creek enters National Park Service lands, which may provide a barrier of protection from anthropogenic sources.

At sites 2, 4, 5, and 6 DEET (insecticide) interacted with the endpoint target peroxisome proliferator-activated receptor gamma (PPARg), indicating receptor-ligand binding activity. PPARg is a nuclear hormone receptor involved in the regulation of energy homeostasis, primarily fatty acid metabolism (Tyagi et al., 2011). Relevant information about the assay endpoint can be reviewed in the Interactive Chemical Safety for Sustainability (iCSS) ToxCast Dashboard, including an assay summary describing the organism, intended target family, and biological process target (U.S. EPA, 2016b). Triphenyl phosphate (plasticizer) also interacted with PPARg at Site 6, indicating possible competitive receptor-ligand binding from at least two contaminants. At sites 1, 2, and 3 bisphenol A interacted with members of the cytochrome P450 family, CYP1A1 and CYP1A2, indicating inducible reporter genes. CYP1A1 and CYP1A2 are hemoproteins involved with xenobiotic detoxification and can be induced when exposed to environmental chemicals (Newman, 2015).

It is important to clarify that EAR hits do not indicate a hazard, but serve as a screening tool to identify potential contaminants of concern. Identification of chemical initiators is the first step in the Adverse Outcome Pathway (AOP) conceptual framework (Organization for Economic Cooperation and Development, 2013). Once targets have been identified (E.G. CYP1A1, PPARg), the AOP Knowledge Base (AOP-KB) can be queried for relevant AOPs (Society for Advancement of Adverse Outcome Pathways, 2016) and evaluated for the potential to adversely impact the development, growth, reproduction or survival of the organism being exposed (Schroeder et al., 2016). For example, in this study, PPARg was identified with elevated EARs at several sites and was queried in the AOP-KB. Two studies were identified (AOP 72 and 163), however both were under development.

Further work that corroborates in vitro results with in vivo toxicology testing is needed to substantiate potential adverse effects at Sites 1-6. While currently limited, the AOP-KB is growing, and underscores the challenge associated with linking biological activity to hazards.

Across the watershed OWCs were generally observed at low concentrations, and most of the compounds were below water quality benchmarks. None of the samples had OWCs that exceeded Federal or State protections for aquatic life or human health. However, anthracene (PAH) exceeded water quality benchmarks established by the Canadian Council of Ministers of the Environment anthracene at Sites 3 and 4 (Table SI-4). Bis(2-ethylhexyl) phthalate (plasticizer) exceeded water quality benchmarks established by the National Oceanic and Atmospheric Administration at Site 8 (Table SI-4). Possible uses or sources of anthracene include, wood preservative, component of tar, diesel, crude oil or combustion product (Lorah et al., 2008). Upstream from Sites 3 and 4, seven NPDES outlets and one underground injection control facility were identified (Table SI-1), and could be possible sources of anthracene. Possible uses or sources of Bis(2-ethylhexyl) phthalate include plasticizers for polymers and resins and a major component of vinyl (Lorah et al., 2008). Possible sources of this compound are less clear.

Inorganic water quality criteria exceedances were primarily concentrated in the headwaters (Sites 1-5) (Table SI-7), 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 (Fig. 4; Table SI-10). Site 2 was sampled below an UOG wastewater disposal facility, and constituents associated with UOG wastewater (Ba, Cl) exceeded drinking water standards (Fig. 5). Site 5 was sampled downstream of an AMD site, and constituents associated with

AMD (Fe, Mn) exceeded drinking water standards (Fig. 5). At Site 10 specific conductance was elevated (562 µS/cm) and the chronic aquatic toxicity criteria for lead (0.0025 mg/L) was exceeded. The chronic aquatic toxicity criteria for lead (0.0025 mg/L) was exceeded at Sites 1, 2, 3, 4, 5, 10, and 11, which may be explained by widespread mining practices in the area. The minerals galena, clausthalite, and pyrite are commonly found in coal and contain lead (Finkelman, 1988).

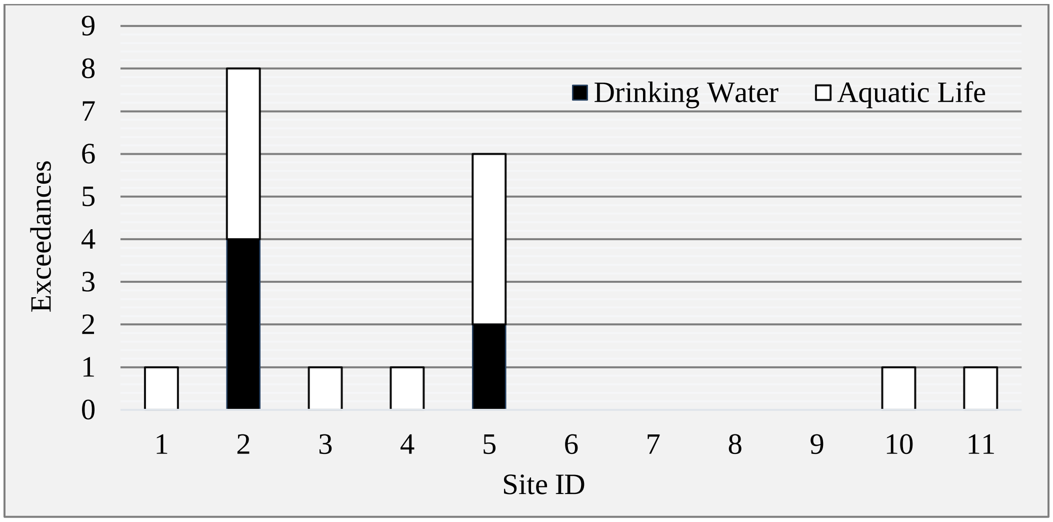

Fig. 4 Drinking water and aquatic life criteria exceedances by site.

Endocrine disrupting chemicals (EDCs) were detected in 90% of the water samples in the Wolf Creek watershed, and 17 unique EDCs were identified (Table 1). Mixtures of two or more EDCs were observed at 80% of the sites, and a maximum of 9 EDCs was observed at Site 6. 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, phenathrene, and pyrene. Synergistic effects from compound mixtures have been observed in several EDC studies (Vajda et al., 2008; Vandenberg et al., 2012), and can have adverse effects even at low concentrations. The most frequently detected EDCs were methyl salicylate

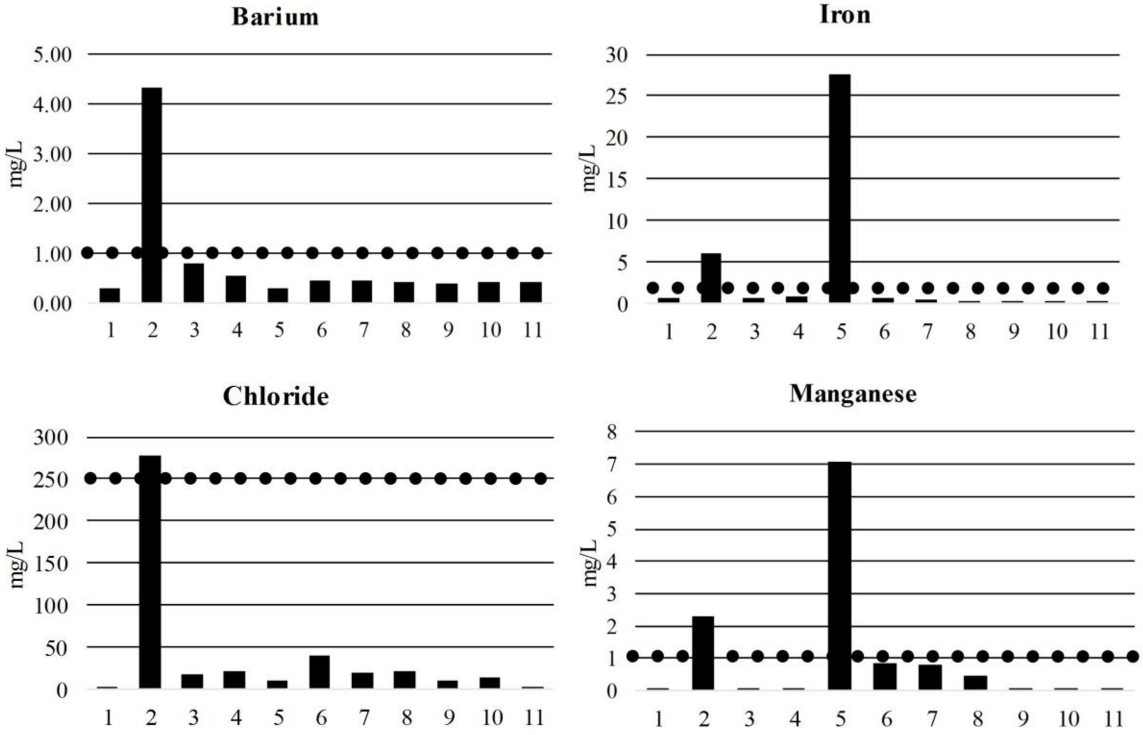

Fig. 5 Inorganic elements with drinking water exceedances by site. Round dashed line indicates water quality criteria.

(miscellaneous), bisphenol A (antioxidant), indole (flavors/fragrances), fluoranthene (PAH), pyrene (PAH), and tri(2-butoxyethyl) phosphate (fire retardant) (Table 1).

3.4 Areas of Concern

Wolf Creek is a tributary of the New River, a drinking water source for communities in Fayette County and an important recreational area. A portion of the Zone of Critical Concern (ZCC) for the New River Water Treatment System extends into the Wolf Creek watershed, and the Zone of Peripheral Concern (ZPC) covers all waters in the watershed (Fig. 6). Sites 1-5 are located within the ZPC, and Sites 6-10 are located within the ZCC. Several screening tools were used to evaluate potential adverse effects within the ZCC and

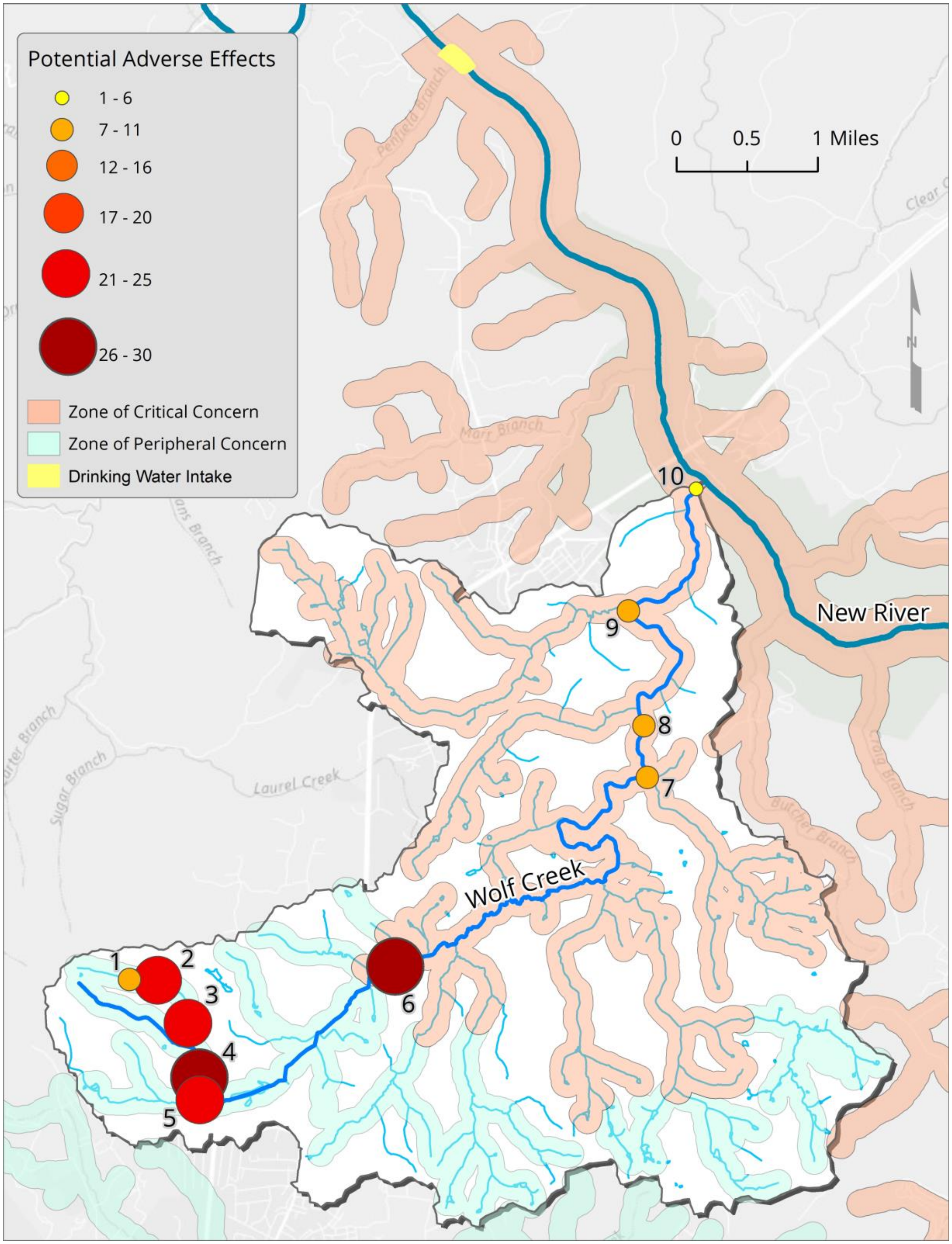

Fig. 6 Symbols represent the combined total of detected organic waste compounds, ToxEval hits, detected endocrine disrupting chemicals, and water quality benchmark exceedances at each site relative to the source water protection zones for the New River Water Treatment System. It is approximately 3.7 miles from the mouth of Wolf Creek to the drinking water intake. Sources: Esri, West Virginia Bureau for Public Health.

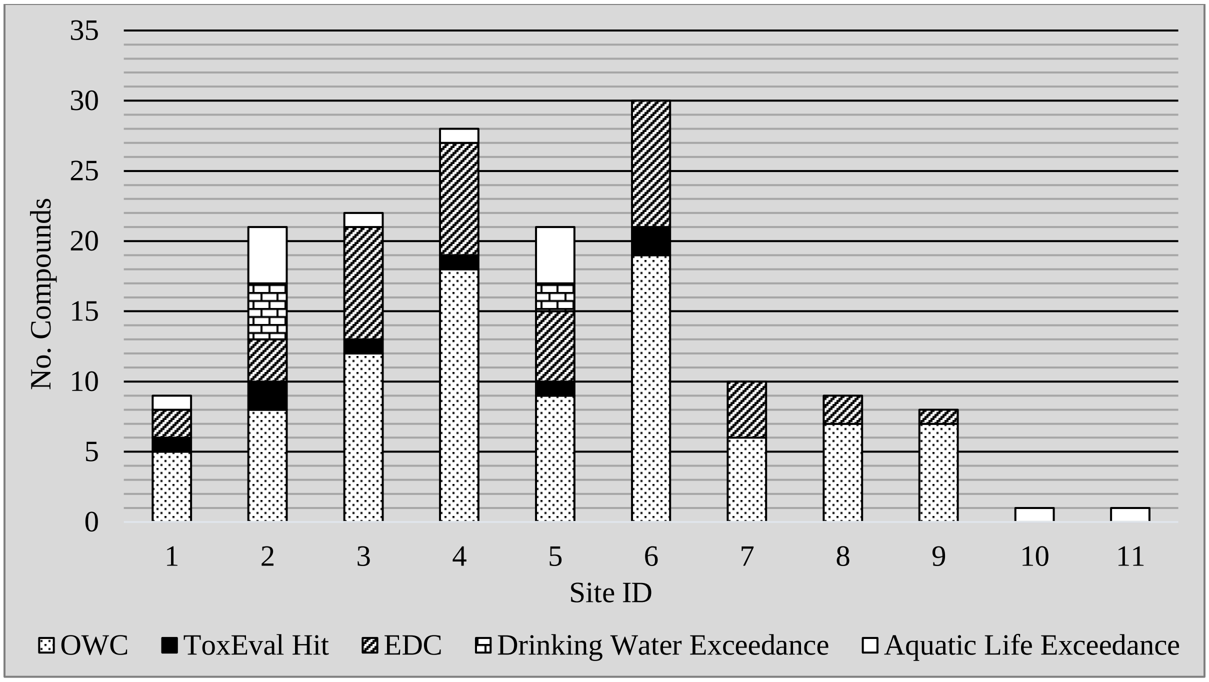

Fig. 7 Detection of organic waste compounds (OWC), ToxEval hits above the exposure activity ratio (>0.1), detected endocrine disrupting chemicals, and water quality exceedances by site.

ZPC including, ToxEval hits, water quality benchmarks, and potential endocrine disrupting compounds (Fig. 7).

Within the ZCC, 39 OWCs were detected and resulted in two ToxEval hits above the EAR threshold, one water quality benchmark exceedance was observed, and nine EDCs were identified (Table SI-10). The potential for adverse effects is greatest at Site 6, and decreases moving downstream to Site 10. At the mouth of Wolf Creek (Site 10), a popular recreation area, no OWCs were detected but two water quality benchmark exceedances were observed for lead. Due to low flow conditions during the time of sampling, a tributary upstream from the mouth, House Branch, was not flowing. House Branch contains a combined sewer overflow that discharges stormwater runoff and untreated sewage during heavy rain events. 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 that adversely affect water quality (Ellis, 2006; Phillips et al., 2012). Further work should involve sampling on House Branch during baseflow conditions and during combined sewer overflow events to evaluate the potential for adverse biological effects.

All waters in the Wolf Creek watershed are within the ZPC, but to reduce redundancy of the results, we focus here on Sites 1-5. Within the ZPC, 52 OWCs were detected that resulted in six ToxEval hits above the EAR threshold, seventeen water quality benchmark exceedances were observed, and 14 EDCs were identified (Table SI-10). The most impacted areas occurred at Sites 2, 3, 4, and 5 (Fig. 5). An examination of potential contaminate sources in the headwaters (Sites 1-5), show a wastewater treatment plant sewage outlet, seven industrial stormwater outlets, abandoned mine lands problem areas (119 acres), coal refuse impoundment (12 acres), legacy strip mining (26 acres), and an underground injection control well (Fig. 1; Table SI-1). The density of potential contaminate sources in headwater drainages could result in cumulative stressors that adversely affect aquatic organisms and human health. Further work evaluating cumulative impacts at headwater sites could improve our understanding of the risks that are present to aquatic organisms and human health.

4. Conclusion

Using ToxEval, we identified contaminants that may be potentially harmful to human health and aquatic life despite lacking water quality benchmarks. This study targeted 69 organic waste compounds that are typically found in domestic and industrial wastewater, but only 27 of the compounds have water quality benchmarks. Three compounds, bisphenol A, DEET, and triphenyl phosphate, were observed above the ToxEval EAR threshold, and two compounds, bisphenol A and triphenyl phosphate, lack water quality benchmarks. If this study was done in the traditional manner, the potential of these compounds to cause adverse effects may have gone unnoticed.

We report multiple sites that demonstrated the potential for adverse biological effects. The most impacted sites occurred in the headwaters at Sites 2, 3, 4, and 5, and downstream of a large shopping center at Site 6 (Fig. 5). OWCs were observed at low concentrations across the watershed, however mixtures of ten or more compounds were detected at Site 6 (19), Site 4 (18), and Site 3 (12). Water quality benchmark exceedances were greatest at Sites 2 and 5, and strengthen evidence that contaminants are present at concentrations that could cause adverse effects to aquatic life and human health. Within the zone of critical concern (Sites 6-10), we show multiple lines of evidence that demonstrate the potential for adverse biological effects including mixtures of OWCs, ToxEval hits, water quality benchmark exceedances, and mixtures of endocrine disrupting chemicals (Fig. 6).

One sampling event can’t account for all the variability that may be present, including seasonal differences and fluctuations in flow regimes. Further work should involve seasonal sampling across different flow regimes, building upon this study to develop a comprehensive monitoring plan that identifies contaminants of emerging concern. Additional work should include biological monitoring and in vivo toxicology testing to confirm these results and assess water resources for the New River Water Treatment System in a more comprehensive manner. Given the large number of chemicals in common use without water quality benchmarks or toxicity information, the application of ToxCast is an effective tool that can be used to assess the potential adverse effects of environmental contaminants on aquatic life or human health.

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. doi:http://dx.doi.org/10.1021/acs.est.6b00428

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. doi:10.1016/j.scitotenv.2016.02.137

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.

Barber, L.B., Loyo-Rosales, J.E., Rice, C.P., Minarik, T.A., Oskouie, A.K., 2015. Endocrine disrupting alkylphenolic chemicals and other contaminants in wastewater treatment plant effluents, urban streams, and fish in the Great Lakes and Upper Mississippi River Regions. Sci. Total Environ. 517, 195–206. doi:10.1016/j.scitotenv.2015.02.035

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. doi:10.1016/j.scitotenv.2006.05.017

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. Heal. Part C-Environmental Carcinog. Ecotoxicol. Rev. 31, 201–212. doi:10.1080/10590501.2013.824188

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. doi:10.1016/j.coal.2013.12.017

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

CCME, 2015. Canadian Environmental Quality Guidelines [WWW Document]. URL http://ceqg-rcqe.ccme.ca/en/index.html (accessed 3.11.16).

Corsi, S., De Cicco, L., Baldwin, A., Alvarez, D., Schroeder, A., Villeneuve, D., Blackwell, B., Ankley, G., Lenaker, P., 2017. Application of ToxCast to Evaluate Potential Biological Effects from Organic Contaminants in Great Lakes Tributaries. Unpubl. results.

DeCicco, L., 2016. GitHub ToxEval [WWW Document]. URL https://github.com/USGS-R/toxEval (accessed 3.11.16).

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

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. doi: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. doi:10.1016/j.chemosphere.2014.11.006

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.

Hill, A., 1910. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. Physiol 4–7.

Jenkins, J.A., Olivier, H.M., Draugelis-Dale, R.O., Eilts, B.E., Torres, L., Patiño, R., Nilsen, E., Goodbred, S.L., 2014. Assessing reproductive and endocrine parameters in male largescale suckers (Catostomus macrocheilus) along a contaminant gradient in the lower Columbia River, USA. Sci. Total Environ. 484, 365–378. doi:10.1016/j.scitotenv.2013.09.097

Johnson, L.L., Anulacion, B.F., Arkoosh, M.R., Burrows, D.G., da Silva, D.A.M., Dietrich, J.P., Myers, M.S., Spromberg, J., Ylitalo, G.M., 2013. Effects of Legacy Persistent Organic Pollutants (POPs) in Fish-Current and Future Challenges. Fish Physiol. 33, 53–140. doi:10.1016/B978-0-12-398254-4.00002-9

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. doi:10.1289/ehp.0800168

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. doi:10.1093/toxsci/kfv168

Karmaus, A.L., Filer, D.L., Martin, M.T., Houck, K.A., 2016. Evaluation of food-relevant chemicals in the ToxCast high-throughput screening program. Food Chem. Toxicol. 92, 188–196. doi:10.1016/j.fct.2016.04.012

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. doi: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. Sci. Investig. Report. U.S. Geol. Surv. 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, 583–91. doi:10.1038/nbt.2914

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. Geochemistry 47, 85–98. doi: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. doi: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. doi:10.1289/ehp.1510385

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

Lindberg, T.T., Bernhardt, E.S., Bier, R., Helton, A.M., Merola, R.B., Vengosh, A., Di Giulio, R.T., 2011. Cumulative impacts of mountaintop mining on an Appalachian watershed. Proc Natl Acad Sci U S A 108, 20929–20934. doi:10.1073/pnas.1112381108

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.

Manning, T., Grow, W., 2000. Inductively coupled plasma-atomic emission spectrometry. Chem. Educ. 2, 1–19. doi:citeulike-article-id:3214328

Newman, M., 2015. Fundamentals of Ecotoxicology: The Science of Pollution, Fourth. ed. CRC Press.

Orem, W., Varonka, M., Crosby, L., Haase, K., Loftin, K., Hladik, M., Akob, D., Tatu, C., Mumford, A., Jaeschke, J., Bates, A., Schell, T., Cozzeralli, I., 2016. 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 [WWW Document]. URL http://aopkb.org/background.html (accessed 6.11.16).

Pfaff, J.D., 1993. Method 300.0 Determination of Inorganic Anions By Ion Chromatography. Stand. Methods 28.

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. doi:10.1021/es3001294

Richard, A.M., Judson, R.S., Houck, K.A., Grulke, C.M., Volarath, P., Thillainadarajah, I., Yang, C., Rathman, J., Martin, M.T., Wambaugh, J.F., Knudsen, T.B., Kancherla, J., Mansouri, K., Patlewicz, G., Williams, A.J., Little, S.B., Crofton, K.M., Thomas, R.S., 2016. ToxCast Chemical Landscape: Paving the Road to 21st Century Toxicology. Chem. Res. Toxicol. 29, 1225–1251. doi:10.1021/acs.chemrestox.6b00135

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

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. doi:http://dx.doi.org/10.14573/altex.1506201

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. doi:10.1002/etc.3309

Schultz, M.M., Painter, M.M., Bartell, S.E., Logue, A., Furlong, E.T., Werner, S.L., Schoenfuss, H.L., 2011. Selective uptake and biological consequences of environmentally relevant antidepressant pharmaceutical exposures on male fathead minnows. Aquat. Toxicol. 104, 38–47. doi:10.1016/j.aquatox.2011.03.011

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

Shockley, K.R., Inglese, J., Reinhold, W.C., Zhu, H., Tice, R.R., Austin, C.P., Kavlock, R.J., Bucher, J.R., Beam, A., Motsinger-Reif, A., Hsieh, J.H., Sedykh, A., Huang, R., Xia, M., Tice, R.R., Shockley, K.R., Thomas, R.S., Hill, A. V., Shockley, K.R., Bergeron, C., Moore, G., Krein, M., Breneman, C.M., Bennett, K.P., Fujii, Y., Narita, T., Tice, R.R., Takeda, S., Yamada, R., Conolly, R.B., Lutz, W.K., Peddada, S.D., Haseman, J.K., Crump, K.S., Woutersen, R.A., Jonker, D., Stevenson, H., Biesebeek, J.D. te, Slob, W., Shannon, C.E., Fuhrman, S., Schug, J., Zhang, Y., Huang, R., Vivacqua, A., Macarron, R., Collins, F.S., Gray, G.M., Bucher, J.R., Kevorkov, D., Makarenkov, V., Malo, N., Hanley, J.A., Cerquozzi, S., Pelletier, J., Nadon, R., Ilouga, P.E., Hesterkamp, T., Shockley, K.R., Altman, D.G., Bland, J.M., 2016. Estimating Potency in High-Throughput Screening Experiments by Maximizing the Rate of Change in Weighted Shannon Entropy. Sci. Rep. 6, 27897. doi:10.1038/srep27897

Society for Advancement of Adverse Outcome Pathways, 2016. Adverse Outcome Pathways [WWW Document]. URL https://aopwiki.org/aops

Stackelberg, P.E., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Henderson, A.K., Reissman, D.B., 2004. Persistence of pharmaceutical compounds and other organic\nwastewater contaminants in a conventional drinking-watertreatment\nplant. Sci. Total Environ. 329, 99–113.

Sullivan, P.J., Agardy, F.J., Clark, J.J.J., 2005. The Environmental Science of Drinking Water. Environ. Sci. Drink. Water 29–87. doi: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. doi:10.2172/259365

TEDX, 2015. TEDX List of Potential Endocrine Disruptors [WWW Document]. URL http://endocrinedisruption.org/endocrine-disruption/tedx-list-of-potential-endocrine-disruptors/overview (accessed 3.11.16).

Tyagi, S., Gupta, P., Saini, A.S., Kaushal, C., Sharma, S., 2011. The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. J. Adv. Pharm. Technol. Res. 2, 236–40. doi:10.4103/2231-4040.90879

U.S. EPA, 2016a. Toxicity Forecaster (ToxCast) [WWW Document]. URL https://www.epa.gov/sites/production/files/2013-12/documents/toxcast-fact-sheet.pdf

U.S. EPA, 2016b. ToxCast Dashboard [WWW Document]. URL https://actor.epa.gov/dashboard/ (accessed 3.11.16).

U.S. EPA, 2016c. National Recommended Water Quality Criteria, Aquatic Life Criteria Table [WWW Document]. URL https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table (accessed 3.11.16).

U.S. EPA, 2016d. Table of Regulated Drinking Water Contaminants [WWW Document]. URL https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants (accessed 3.11.16).

U.S. EPA, 2014. Aquatic Life Benchmarks for Pesticide Registration [WWW Document].

URL https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration#benchmarks (accessed 3.11.16).

U.S. EPA, 2007. Method 3015a - Microwave Assisted Acid Digestion of Aqueous Samples and Extracts. doi:10.1017/CBO9781107415324.004

U.S. EPA, 1996. Ecotox Thresholds [WWW Document]. URL https://www.epa.gov/sites/production/files/2015-09/documents/v3no2.pdf (accessed 3.11.16).

USGS, 2016a. About the Toxic Substances Hydrology Program [WWW Document]. URL http://toxics.usgs.gov/about.html

USGS, 2016b. Geological Survey R Archive Network [WWW Document]. URL https://owi.usgs.gov/R/gran.html (accessed 3.11.16).

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. doi:10.1210/er.2011-1050

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 [WWW Document]. URL http://www.dep.wv.gov/WWE/Programs/wqs/Pages/default.aspx (accessed 3.11.16).

Yoon, M.K., Amy, G.L., 2014. Reclaimed water quality during simulated ozone-managed aquifer recharge hybrid [WWW Document]. Environ. Earth Sci. doi:10.1007/s12665-014-3412-5

Zaugg, S.D., Smith, S.G., Schroeder, M.P., 2006. Determination of Wastewater Compounds in Whole Water by Continuous Liquid-Liquid Extraction and Capillary-Column Gas Chromatography/Mass Spectrometry, Techniques and Methods.

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. doi:10.1210/en.2012-1422

Vita

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