Impact of Indirect Potable Reuse on Endocrine Disrupting Compounds in the Potomac River Basin

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ABSTRACT

The Potomac River Basin is significant for both public and ecological health as it flows directly into the ecologically-sensitive Chesapeake Bay. It is a drinking water source for about 5 million people living in Maryland, Virginia, and Washington D.C. The discovery of intersex fish, an indicator of poor ecological health, in the Chesapeake Bay occurred in the 2000s, and has led to a series of studies in the watershed to determine the sources and magnitude of endocrine disruption. Endocrine disrupting compounds (EDCs) are exogenous chemicals that interfere with the endocrine system and can cause detrimental health effects at low concentrations. This study aims to understand a best management practice referred to as planned indirect potable reuse (IPR) and its impacts on EDCs. The Occoquan Watershed is a planned IPR subwatershed of the Potomac River Basin. Water samples were collected at the water reclamation plant discharge (Upper Occoquan Service Authority), up- and downstream of that location along Bull Run, and at the water treatment plant intake (Frederick P. Griffith WTP) in the Occoquan Watershed to assess planned IPR. Samples were also collected at a water treatment plant (James J. Corbalis WTP) along the Potomac River for comparison as an unplanned IPR location. These two groups of samples were analyzed for EDCs (categorized into two groups: estrogen hormones and other synthetic organic compounds (SOCs)), nutrients, and other water quality parameters. The infrequency of estrogen hormones and SOC patterns indicate planned and unplanned IPR are both viable approaches to provide safe drinking water.

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GENERAL AUDIENCE ABSTRACT

Our river systems are important to maintain both for human and environmental health. The Potomac River Basin is the area of land drained by the Potomac River and its tributaries. The Potomac River Basin is significant for both public and ecological health as it flows directly into the ecologically-sensitive Chesapeake Bay. It is a drinking water source for about 5 million people living in Maryland, Virginia, and Washington D.C.

The discovery of intersex fish, or when a single fish has both male and female characteristics, occurred in the Chesapeake Bay in the 2000s. Fish health is often an indicator of poor environmental health, and in this case endocrine disruption. This discovery led to a series of studies in the watershed to determine the sources and magnitude of endocrine disruption. Endocrine disrupting compounds (EDCs) are external chemicals that interfere with the endocrine system once they enter the body of a human or another organism, and can cause detrimental health effects even at low concentrations.

This study aims to understand a best management practice, or a type of water pollution control, referred to as *planned* indirect potable reuse (IPR) and its impacts on EDCs. IPR occurs when wastewater from a community is discharged into to a river or a reservoir, and then downstream it is withdrawn from that same source for drinking water purposes. This can be either *planned* or *unplanned*. *Planned* IPR is becoming more common as population, especially in urban areas, increases.

The Occoquan Watershed is a *planned* IPR subwatershed of the Potomac River Basin. Water samples were collected at the water reclamation plant discharge (Upper Occoquan Service Authority), up- and downstream of that location along Bull Run, and at the water treatment plant intake (Frederick P. Griffith WTP) in the Occoquan Watershed to assess *planned* IPR. Samples were also collected at a water treatment plant (James J. Corbalis WTP) along the Potomac River for comparison as an *unplanned* IPR location.

These two groups of samples were analyzed for EDCs (categorized into two groups: estrogen hormones and other synthetic organic compounds (SOCs)), nutrients, and other water quality parameters. The infrequency of estrogen hormones and SOC patterns indicate planned and unplanned IPR are both viable approaches to provide safe drinking water.

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ABBREVIATIONS

ALB	Acute Level Benchmark
BMP	Best Management Practice
BR.D	Bull Run Downstream
BR.U	Bull Run Upstream
CI	Confidence Interval
CWA	Clean Water Act
DIC	Dissolved Inorganic Carbon
DOC	Dissolved Organic Carbon
EDC	Endocrine Disrupting Compound
EDSP	Endocrine Disruptor Screening Program
FFDCA	Federal Food Drug and Cosmetic Act
FQPA	Food Quality Protection Act
GC-MS/MS	Gas Chromatography- Mass Spectrometry/Mass Spectrometry
HBSL	Health-Based Screening Level
HDPE	High Density Polyethylene
IPR	Indirect Potable Reuse
LC-MS/MS	Liquid Chromatography- Mass Spectrometry/Mass Spectrometry
MCL	Maximum Contaminant Level
OWML	Occoquan Watershed Monitoring Laboratory
PCA	Principal Component Analysis
POTW	Publicly Owned Water Treatment Works
PPCPs	Pharmaceuticals and Personal Care Products
SOC	Synthetic Organic Compound
SPE	Solid Phase Extraction
SRP	Soluble Reactive Phosphorus
SWDA	Safe Water Drinking Act
TDN	Total Dissolved Nitrogen
TO	Testicular Oocytes
U.S. EPA	United States Environmental Protection Agency
UMD	University of Maryland
UOSA	Upper Occoquan Service Authority
USGS	United States Geological Survey
WRF	Water Reclamation Facility
WTP	Water Treatment Plant

INTRODUCTION

As population increases, management solutions for higher water quality and potable water quantity are evolving. A best management practice (BMP) that addresses this issue at a watershed level is planned indirect potable reuse (IPR). Indirect potable water reuse is defined as the succession of communities returning reclaimed wastewater to a water body, and then withdrawing surface water for potable use from the same source (Spellman 2017). Unplanned IPR, or *de facto* reuse, occurs throughout the United States and is not considered a geographically isolated event (Rice et al. 2015). When *de facto* reuse occurs, water reclamation facilities (WRFs; previously called wastewater treatment plants) tend not to be designed with downstream water use in mind (Da Silva et al. 2014). A planned IPR system typically involves an advanced water reclamation plant in place of a conventional WRF (Rice et al. 2015). The reclaimed water is returned to a natural water source, such as a river or a reservoir, to store, transport, and provide an additional method of human health protection, before use as a source for potable treatment (Kayhanian and Tchobanoglous 2016).

A considerable concern since the 1990s, endocrine disrupting compounds (EDCs) are commonly found in WRF discharge (Daughton and Ternes 1999; Hudson et al. 2005; Pal et al. 2014). EDCs are chemicals that can interfere with the endocrine system of humans and wildlife, even at low concentrations (Change et al. 2009; Young et al. 2014). Clear evidence of effects from EDCs have been identified in fish and other wildlife, but less is known about the effects on humans (Blazer et al. 2012; Iwanowicz et al. 2009; U.S. EPA 2019c). Fish, a common indicator of aquatic health, have been found with intersex, testicular oocytes, in the Potomac River Basin (Alvarez et al. 2009; Iwanowicz et al. 2009; Young et al. 2014). This is of particular concern as the Potomac River flows into the Chesapeake Bay, a vital and ecologically sensitive estuary. Intersex severity has been found to be associated with both WRF discharge and agriculture sources (Blazer et al. 2012).

EDCs monitored from water sampling sites in the Occoquan Watershed were compared to a site along the Potomac River, a *de facto* IPR system. A subwatershed of the Potomac River Basin, the Occoquan Watershed became a planned IPR system in the 1970s (Randall and Grizzard 1995). EDCs analyzed in this study were classified as estrogen hormones and synthetic organic compounds (SOCs), consisting of insecticides, herbicides, and industrial chemicals. This study investigates (1) sources of EDCs, along with nutrients and water quality parameters, in a planned IPR system: the Occoquan Watershed, and (2) the effects of a planned IPR versus a *de facto* reuse system.

LITERATURE REVIEW

History of the Potomac River Basin and Occoquan Watershed

The Potomac River Basin is located on the East Coast of the United States, spanning 14,670 mi^2 through four states (Virginia, Maryland, West Virginia, and Pennsylvania) and Washington D.C. (ICPRB 2019). As the second largest basin in the Chesapeake Bay watershed, it is important to protect its ecological health. The Chesapeake Bay is an ecologically sensitive estuary. The Potomac Basin is about 55% forest, 14% developed land, 26% agriculture, and 6% water and wetlands (ICPRB 2019). With a population of more than 6 million people living in the Potomac River Basin, more than 5 million (85%) live in the Washington Metropolitan area. This area uses an average of 486 million gallons of water per day for drinking water. Of the Basin's population, 86% receive drinking water from public water supplies and 13% receive drinking water from wells (ICPRB 2019). There are 117 water reclamation facilities (WRFs) located in the Maryland section of the Basin alone (Alvarez et al. 2009).

The Occoquan Watershed is a 570 mi^2 subwatershed of the Potomac Basin, located in Northern Virginia, southwest of Washington D.C (Randall and Grizzard 1995). The Occoquan Watershed includes four counties and three cities. It flows into the Potomac River, and thus into the Chesapeake Bay. The Occoquan Watershed land cover is primarily forests and shrubs (40%), urban (27.5%), and cultivated crops and hay (24%) (USGS 2020). The Occoquan Reservoir was established with the construction of the high dam in 1957 near the mouth of the Occoquan River. A reservoir is a body of water formed by human activity to provide a reliable and controllable water resource (Chapman 1996). The main services of the Occoquan Reservoir are drinking water supply, with additional recreational fishing, boating, and other recreational uses allowed. The Reservoir is riverine in nature, but behaves similarly to a lake as it approaches the dam. This is supported by the occurrence of annual thermal stratification, most prevalently observed at the Occoquan Watershed Monitoring Laboratory (OWML) monitoring stations in the downstream sections of the reservoir (Cubas et al. 2014).

Eutrophication in the Occoquan Reservoir was observed 10 years after the dam was constructed and was linked to the discharge of publicly owned treatment works (POTWs) (Randall and Grizzard 1995). This led to the implementation of the *Occoquan Policy* in 1971 (9VAC25-410). The *Policy* included the recommendation that the POTWs be removed, and instead implement one advanced water reclamation plant. This led to the establishment of the Upper Occoquan Sewage Authority, now called the Upper Occoquan Service Authority (UOSA). The *Policy* also included the recommendation that a strategy for indirect potable reuse be adopted. The Occoquan Reservoir is currently one of the sources of water owned by Fairfax Water. The Occoquan Watershed Monitoring Program was established by the *Policy*, as well, to monitor water quality by collecting data before and after the POTWs were replaced by UOSA and ensure that the plants were complying with all treatment regulations. The establishment of the Occoquan Watershed Monitoring Laboratory (OWML) facilitated regular sampling of the Reservoir and streams, as well as the establishment of stream monitoring stations (Randall and Grizzard 1995).

Potable Reuse

Of the freshwater on Earth, only about 0.027% is available for human consumption (Spellman 2017). From this available amount, an increase in potable water is needed as human population increases. Potable water is water that can be safely used for drinking, cooking, washing, and other household applications (Spellman 2017). An urban area can change the characteristics of the hydrologic cycle because of the services that are needed, such as water supply, drainage, and wastewater collection and management. (Karamouz et al. 2010). Urbanization is the increase in human population density in an area, which leads to an increase in consumption of natural resources and extensive modification of the natural landscape (Karamouz et al. 2010). Urbanization has a large impact on the quality and quantity of the water, soil, and air of the local climate. The negative impacts on reservoirs can be from acidification, salinity, eutrophication, pathogens, and toxicity (Karamouz et al. 2010). With the many ways in which a drinking water source can deteriorate comes the need for best management practices (BMPs) that address the entire hydrologic cycle of the area.

An example of a BMP being adopted across the U.S. is potable water reuse (Sedlak 2014; U.S. EPA 2017b). There are two types of potable reuse: direct and indirect. Direct potable reuse is the deliberate use of reclaimed waters without dilution by natural waters (Karamouz et al. 2010). Indirect potable reuse (IPR) occurs when a community or succession of communities withdraw surface water and then return reclaimed wastewater to the same source (Spellman 2017). Water reclamation is the treatment of municipal wastewater to make it acceptable for reuse (Kayhanian and Tchobanoglous 2016). The process of IPR to return water to a natural water system allows for natural purification to occur (Karamouz et al. 2010). The river is used to provide storage, transport, and possibly to act as an additional buffer to protect human health. However, there is concern that IPR could contaminate the environment (Kayhanian and Tchobanoglous 2016).

Indirect potable water reuse can be a planned or unplanned occurrence. *De facto* reuse is the unplanned reuse of wastewater that has been discharged upstream for drinking water (Kayhanian and Tchobanoglous 2016). When a community resides in a *de facto* reuse system, the WRF tends to not be constructed with downstream water demands in mind (Da Silva et al. 2014). *De facto* reuse occurs throughout the world, and is not considered a geographically isolated phenomenon (Rice et al. 2015). On the other hand, planned IPR systems are equipped with reclamation facilities designed with a higher level of treatment and scrutiny. An example of such scrutiny is pathogen control by a 12/10/10-log thresholds required for groundwater recharge and surface augmentation in California (Pecson et al. 2017). These regulatory approaches have been developed around the country for different models (ranging from direct, to engineered barriers, to natural barriers). Whether reuse is planned or unplanned, those communities residing in smaller watersheds become more dependent on WRF discharge during low-flow events (Rice et al. 2015). For instance, in the Occoquan Watershed WRF discharge can make up 80% of the water supply during an extreme drought (Sedlak 2014). With wastewater discharge supplementing the water supply, it is imperative to understand the impacts on human and ecological health.

Models have been created to understand the effects of WRFs on source water, but there are many uncertainties that remain (Barber et al. 2019). One fugacity-based model suggested that higher concentrations of endocrine disrupting compounds (EDCs) were present in fish than the water in the reservoir modeled (Cao et al. 2010). In 1962, the first permanent planned IPR occurred in the Los Angeles County Sanitation District, motivated by water scarcity (Sedlak 2014). The Occoquan

Reservoir became the next IPR system established, not because of water scarcity, but because of water quality. The state-of-the-art reclamation system that was designed has improved water quality dramatically, and is still being monitored by the OWML (Randall and Grizzard 1995). Models and implemented IPR systems help to gain better understanding of the diverse impacts of this BMP on public and environmental health.

History of Water Quality and EDC Monitoring

The Federal Water Pollution Control Act, initially enacted in 1948, was strengthened in 1972 to include surface water pollution legislature (Spellman 2017). This Act led to the establishment of the Clean Water Act, and later the U.S. EPA. The Clean Water Act (CWA) was passed in 1972 with the purpose to restore and maintain the health of the U.S. waters by ending the discharge of pollutants into navigable waters and to make national waters fishable and swimmable by 1985. It was found that this was not true for a third of the U.S. rivers and half of its lakes in 1997 (Spellman 2017).

In 1974, the Safe Drinking Water Act (SDWA) was passed to establish federal standards for drinking water quality and underground water sources, and a federal and state cooperation system to ensure compliance with the law (Spellman 2017). The U.S. EPA has since established safety standards for over 80 contaminants. The national drinking water regulations developed by the U.S. EPA are divided into the National Primary Drinking Water Regulations and the National Secondary Drinking Water Regulations. The former details enforceable maximum contaminant level (MCLs), which is the highest concentration of a contaminant in water that is delivered to a public water system. EDCs are among those listed amongst these MCLs. The National Secondary Drinking Water Regulations are unenforceable guidelines for drinking water. They have no known health effects but are based on aesthetic or cosmetic effects of the water. The U.S. EPA also provides technical and financial assistance to states and municipalities for management of their drinking water programs. The states are required to ensure drinking water meets SDWA standards. Public water systems treat and monitor drinking water according to the 1986 amendments.

EDCs first started becoming an issue due to incomplete removal of steroids in WRFs in the 1960s (Stumm-Zollinger and Fair 1965). This concern became more prevalent in the 1980s and escalated significantly in the 1990s, with the discharge of PPCPs, EDCs, and pesticides and herbicides from WRFs (Daughton and Ternes 1999; Halling-Sørensen et al. 1998; Stumm-Zollinger and Fair 1965). In 1996, the Food Quality Protection Act (FQPA) amended the SDWA and Federal Food Drug and Cosmetic Act (FFDCA). The FQPA included a requirement for the U.S. EPA to screen pesticides for effects that may mimic naturally produced estrogen (U.S. EPA 2019c). Currently, the EPA has an Endocrine Disruptor Screening Program (EDSP) in place to screen pesticides, chemicals, environmental contaminants, and their potential effects on the endocrine system.

The Potomac Basin was analyzed for synthetic organic compounds (SOCs) beginning in the 1970s (USGS 1997). SOCs have been monitored in the Occoquan Watershed since 1982 (Hall 1996). Concentrations were analyzed from surface water, sediments, and fish samples. In the Occoquan Watershed, about 50 SOCs are monitored as they have been identified as possible anthropogenic contaminants (Hall 1996). The Potomac Basin is not monitored solely by one facility, as the Occoquan is, and so a variety of SOCs are monitored throughout the region. In the 2000s, male fish with female eggs in their body were discovered in the Chesapeake Bay (Konkel 2016). This led to a series of studies in the watershed to determine the magnitude and sources of endocrine disruption. Although much has been learned, there is still a lot of progress that needs to be made.

What is an EDC?

The endocrine system is made up of glands that produce and secrete hormones in the body. These hormones send signals throughout the body to help it function properly. The endocrine glands are the adrenal glands, gonads, hypothalamus, pituitary gland, thyroid gland, and pancreas (U.S. EPA 2019c). Endocrine disrupting compounds (EDCs) are exogenous chemicals that interfere with the endocrine system and can cause detrimental health effects even at low concentrations (Change et al. 2009; Young et al. 2014). Examples of such health effects are development malformations, interference with reproduction, increased cancer risk, and disturbances in the immune and nervous system function (Gogoi et al. 2018; U.S. EPA 2019c). There are three methods by which EDCs can interfere with the endocrine system: mimic natural hormones in the body, block natural hormones by binding to a receptor, or interfere with natural hormone production (U.S. NIEHS 2019). Fish and other wildlife have shown clear evidence of endocrine disruption, but less is known about the effects of EDCs on humans (Blazer et al. 2012; Iwanowicz et al. 2009; U.S. EPA 2019c). EDCs are of high concern since their long-term impacts on humans are unknown (Gogoi et al. 2018).

EDCs are often classified as emerging contaminants, also known as contaminants of emerging concern (Noguera-Oviedo and Aga 2016). EDCs can belong to the following categories: hormones (natural and synthetic), pharmaceuticals and personal care products (PPCPs), industrial chemicals, and pesticides. Examples of EDCs that are PPCPs include synthetic hormones, cosmetics, fragrances, preservatives, antibiotics, medications, toiletries, and more (Tijani et al. 2013). Industrial compounds that have been found to be EDCs include bisphenol A and nonylphenols among others (Change et al. 2009). Many pesticides (particularly triazine based pesticides such as atrazine) have been shown to exhibit EDC behavior (Hayes et al. 2002). Finally, natural hormones such as estrogen, testosterone, and others, from human or animal sources, are also EDCs when released into the environment (Khanal et al. 2006). Concentrations in parts per billion and parts per trillion can cause potential estrogenic responses. This is concerning considering these compounds are detected at WRFs, surface water, and other aquatic environments at concentration units of ng/L. EDCs can be estrogenic, adrogenic, or thyroidal in nature (Change et al. 2009). The majority are estrogenic, fewer are androgenic, and even less having effects on other endocrine glands (Burkhardt-Holm 2010). EDCs are also categorized as emerging pollutants, which are synthetic or naturally occurring compounds that are not monitored but may cause adverse effects on humans or the environment (Geissen et al. 2015). Additionally, EDCs are categorized as emerging organic contaminants, which are considered indicator compounds because they cover a broad range of chemical properties, structures and environmental behaviors that all have the potential to impact human and ecological health (Pal et al. 2014). In this study EDCs analyzed were broken into two categories: estrogen hormones and synthetic organic compounds (SOCs). SOCs are manufactured chemicals that may include herbicides, pesticides, and industrial compounds (Spellman 2017).

Landscape sources of EDCs include point and non-point locations. Agriculture activities and, in an urban environment, WRF sources correlate with EDC estrogenic activity. (Young et al. 2014). Water reclamation facilities (WRFs) are common point sources of EDCs (Alvarez et al. 2009; Da Silva et al. 2014; Daughton and Ternes 1999; Hudson et al. 2005; Iwanowicz et al. 2009; Pal et al. 2014). Other urban sources include surface water run-off and leachate from septic tanks and landfill sites (Burkhardt-Holm 2010). Besides WRF discharge, a main source of EDCs is agricultural runoff (Falconer et al. 2006). EDCs have been found in stormwater canals, with increased concentrations during rainfall events, and can be used as indicators of sewer contamination (Boyd et al. 2004). EDCs are found throughout the environment, but the greatest sinks are rivers, lakes, and other water bodies (Esperanza et al. 2004).

Because WRFs are designed specifically to the water characteristics of the sewershed, EDC removal is never guaranteed. It is noted that EDCs are not completely removed in the conventional WRF processes (Kim et al. 2007). This may be due to the chemical makeup of the compounds (Change et al. 2009). Secondary and advanced treatments have proven to be necessary for EDC removal. Biological processes, specifically activated sludge, in a WRF can be effective processes for the removal of many EDCs (Barber et al. 2012; Iwanowicz et al. 2009). Efficiency of removal depends on the age of the activated sludge, hydraulic detention time, organic loading, redox potential, cultivated environmental microorganisms, season, and size and population density of the sewershed (Burkhardt-Holm 2010). Advanced treatment processes, specifically granular activated carbon, membrane filtration, reverse osmosis, and nanofiltration are efficient methods for removing EDCs (Barber et al. 2012; Burkhardt-Holm 2010; Chen et al. 2013; Khanal et al. 2006; Kim et al. 2007; Watts et al. 2019). On the other hand, advanced treatment processes such as chlorination, ozonation, and advanced oxidation processes may be efficient at removing EDCs, but they may have by-products that could be more estrogenic (Auriol et al. 2005). By-products are also a concern when considering water treatment processes for drinking water. Many compound concentrations are reduced or removed altogether by physical or chemical processes in the water treatment plants (Coupe and Blomquist 2004).

There is evidence of EDCs negatively impacting wildlife. Fish are commonly used as an indicator of the health of an aquatic ecosystem. The Potomac Basin is an essential spawning habitat for both migratory and resident fish (Alvarez et al. 2009). EDCs have been cited as being the cause for masculinized female fish (Denton et al. 1985), feminized male fish (Gimeno and Komen 1997), and issues with spawning (Kramer et al. 1998). In the Potomac River Basin, there are male bass present with intersex, or testicular oocytes (TO), which is used as an indicator for EDCs (Blazer et al. 2012). TO severity has been found to be associated with WRF discharge and agriculture (i.e., total number of animal feeding operations, number of poultry houses, and animal density) within a watershed (Blazer et al. 2012). In mammals, EDC effects are shown to be passed down to rats' offspring by evidence of permanently impaired spermatogenesis in males and persistent neurobehavioral effects (Kuriyama et al. 2005). For all species, the impacts of EDCs are still largely unknown (Hudson et al. 2005).

Details of Relevant EDCs

A list of targeted analytes in this study are included in Table S6. EDCs are structurally diverse, but many have one or more aromatic rings (Burkhardt-Holm 2010). EDCs in this report are broken into two main subcategories: SOCs and estrogen hormones. The SOCs are broken into types: herbicide, insecticide, and industrial compounds. See Table 1 for SOC respective types, compound class, and general descriptions.

Because only two estrogen hormones were detected at low concentrations and infrequently, they were not analyzed further (Figure S1). The estrogen hormones regularly detected included estrone and its sulfonide conjugate, estrone-3-sulfate. These are associated with PPCPs and are observed in WRF discharge (Hudson et al. 2005).

Table 1. Description of SOCs**.** Icluded are the synthetic organic compounds (SOCs) monitored during this study and their corresponding type, class, general description, and references. SOCs with dark gray box were not found above the detection limit in this study. SOC names shown in red are on the list of banned compounds.

SOCs	Type	Compound Class	General Description	References
4,4 DDD	Insecticide Metabolite	Organochlorine	Metabolite of DDT (a banned insecticide to control malaria, typhus, and other insect-transmitted diseases). Classification B2- Probable human carcinogen.	(U.S. EPA 1988)
4,4 DDE	Insecticide Metabolite	Organochlorine	Metabolite of DDT (a banned insecticide to control malaria, typhus, and other insect-transmitted diseases). Classification B2- Probable human carcinogen.	(U.S. EPA 2000b)
4-Nonylphenol	Industrial Compound	Alkylphenol	Used in pesticides, anti-oxidants in plastics and rubbers, biodegradation product of nonylphenol ethoxylates (NPE) used in detergents. Main source is from treated wastewater discharge and land application of biosolids.	(U.S. EPA 2009a)
4-*Tert*-Octylphenol	Industrial Compound	Alkylphenol	Used to manufacture alkylphenol ethoxylates (also anionic surfactants used in detergents, industrial cleaners, and emulsifiers). Can enter water body directly from manufacturing waste streams.	(CDC 2017a)
Acetamiprid	Insecticide	Neonicotinoid	Used to control sucking-type insects on agricultural and residential plants. Not expected to be persistent in the environment.	(U.S. EPA 2002a)
Acetochlor	Herbicide	Chloroacetanilide	selective pre- and early post-emergent control of broadleaf weeds/grasses for corn.	(U.S. EPA 2009b)
Alachlor	Herbicide	Chloroacetanilide	Used for weed control on corn, soybeans, sorghum, peanuts, beans.	(U.S. EPA 1998)
Atrazine	Herbicide	Triazine	Applied before and after agricultural planting to control broadleaf/grassy weeds.	(U.S. EPA 2020a)
Bisphenol A (BPA)	Industrial Compound	Bisphenol	Industrial chemical used in production of epoxy resins and polycarbonate plastics. Primary exposure for humans is diet.	(U.S. EPA 2017a) (U.S. EPA 2020b)

SOCs	Type	Compound Class	General Description	References
Bromacil	Herbicide	Organophosphorus	Broad spectrum herbicide used to control weeds in agricultural food crops. Bromacil and its lithium salts are used to control weeds/brush in non-agricultural areas (utility right-of-ways, railroads, electrical switching stations, and industrial yards).	(U.S. EPA 1996a)
Butylate	Herbicide	Thiocarbamate	Selective herbicide for corn to control grassy/broadleaf weeds and nutsedge. Commonly used in conjunction with atrazine and/or cyanazine.	(U.S. EPA 1993)
Chlorpyrifos	Insecticide	Organophosphorus	Used for corn primarily and other row crops. Other uses are golf courses, turf, green houses, mosquito adulticide, roach/ant bait stations, and in child resistant packaging.	(U.S. EPA 2019b)
Cis-Chlordane	Insecticide Metabolite	Organochlorine	It is a chlordane isomer that makes up about 15% of the chlordane mixture. Chlordane was an insecticide for agricultural crops, livestock, lawns and gardens that was banned in 1988. Classified at B2- Probable human carcinogen.	(U.S. EPA 1997)
Clothianidin	Insecticide	Neonicotinoid	Used for seed treatment use on corn and canola.	(U.S. EPA 2003a)
Coumaphos	Insecticide	Organophosphorus	Used to control arthropod pests on cattle, goats, horses, sheep, and swine. Persistent in the environment.	(U.S. EPA 1996b)
Cycloate	Herbicide	Thiocarbamate	A broad-spectrum, pre-emergent herbicide for controlling annual grasses, some perennial grasses, and broadleaf weeds on garden beets, spinach, sugarbeets (+90% of usage)	(U.S. EPA 2004a)
Diazinon	Insecticide	Organophosphorus	Used on fruit, vegetable, orchard and ornamental crops. Residential use was banned in 2004.	(U.S. EPA 2008a)
Dichlorvos	Insecticide	Organophosphorus	Insecticide for crops, stored products, animals, pest-strips in homes. Anthelmintic (worming agent) for dogs, swine, horses, and flea collars for dogs. In 1995 residential uses were banned.	(U.S. EPA 2000c)

SOCs	Type	Compound Class	General Description	References
Dieldrin	Insecticide	Organochlorine	A banned (in 1987) stereoisomer of endrin. Originally a broad-spectrum insecticide, it was inserted directly into the soil for crops, and to protect wooden structures against ants and termites.	(U.S. EPA 2003b)
Dimethoate	Insecticide	Organophosphorus	Uses on alfalfa, wheat, cotton, and corn crops are more than 60% of the use in the USA. Residential and non-agricultural uses were cancelled in 2000.	(CDC 2017b)
Dinotefuran	Insecticide	Neonicotinoid	Applied to agriculture leafy vegetables, residential, and industrial outdoor areas.	(U.S. EPA 2004b)
Endrin Aldehyde	Insecticide	Organochlorine	Used to control insects, rodents, birds. It was banned along with DDT in 1986. The largest use was for control of lepidopteron larvae attacking cotton crops in southeastern and Mississippi delta states.	(U.S. EPA 1980)
Ethoprop	Insecticide	Organophosphorus	Controls wireworms/nematodes in potatoes, sugar cane, sweet potatoes, tobacco, and less use on corn, beans, cucumbers, cabbage, fruit, ornamentals.	(U.S. EPA 2002b)
Fenchlorphos	Insecticide	Organophosphorus	Also known as Ronnel. Used as an insecticide on livestock.	(U.S. EPA 2000a) (Cornell 1985)
Fipronil	Insecticide	Phenylpyrazole	Agricultural uses: potatoes, turnips, rutabagas. Other uses: lawn care operators and pest control operators to treat golf courses and food handling establishments, residential: spot treatment on domestic animals. and ant mount treatment. Group C - Possible human carcinogen.	(U.S. EPA 2011)
Heptachlor	Insecticide	Organochlorine	Initially applied as broad spectrum insecticide for agriculture and home use. All uses were banned in 1988. Causes cancer and birth defects in laboratory mice and rats. Persists in soil for many years and bioaccumulates throughout the	(U.S. EPA 2000d)

SOCs	Type	Compound Class	General Description	References
			food chain. Present in chlordane mixture.	
Heptachlor Epoxide	Insecticide Metabolite	Organochlorine	Created when heptachlor is released into the environment and mixes with oxygen. Also causes cancer/birth defects in lab mice and rats.	(U.S. EPA 2019d)
Imidacloprid	Insecticide	Neonicotinoid	Under review (along with all neonicotinoids) to assess risk (especially to pollinators) in 2017 with planned completion in 2020. Agricultural and non- agricultural uses.	(U.S. EPA 2020c)
Methyl Parathion	Insecticide	Organophosphorus	In 1996, residential use was banned. In 1992, many crop uses were voluntarily cancelled. 12 methyl parathion products are currently registered.	(U.S. EPA 2009c)
Metolachlor	Herbicide	Chloroacetanilide	Broad spectrum herbicide used for general weed control on agricultural crops, lawns and turfs. Classified as Group C - possible human carcinogen.	(U.S. EPA 1995)
Parathion	Insecticide	Organophosphorus	Used on fruit, cotton, wheat, vegetables, and nut crops. Classified as Group C- possible human carcinogen.	(U.S. EPA 2000e)
Prometon	Herbicide	Triazine	Non-selective herbicide that targets annual and perennial grasses, and broadleaf weeds. It is applied as pre- and post-emergence spot treatments.	(U.S. EPA 2008b)
Simazine	Herbicide	Triazine	Pre-emergent application for broad-leaf/grassy weeds on variety of crops and turf grasses. Additionally used to control algae in ponds and fish hatcheries. Often used in combination with atrazine when used for corn. Typically enters source waters through runoff about 1-3 months following agricultural application.	(U.S. EPA 2009d)
Trans-Chlordane	Insecticide Metabolite	Organochlorine	Chlordane isomer that makes up about 15% of the chlordane mixture. Chlordane was an insecticide for agricultural crops, livestock, lawns and gardens that was banned in 1988. Classified at B2- Probable human carcinogen.	(U.S. EPA 1997)

SOCs	Type	Compound Class	General Description	References
Tribufos (Tributylphosphorothithiote)	Herbicide	Organophosphorus	Defoliant used for cotton crops. No residential uses.	(U.S. EPA 2000f)

METHODS AND MATERIALS

Site Description and Sample Collection

Sample sites from the larger Potomac Basin and the Occoquan Watershed were chosen to study the impacts of planned and unplanned IPR (Figure 1). The Occoquan Watershed land cover is primarily forests and shrubs (40%), urban (27.5%), and cultivated crops and hay (24%) (USGS 2020). The Potomac Basin land cover is mainly forests (55%), agriculture (26%) and developed land (14%) (ICPRB 2019). The monitoring locations along the Potomac River are designed to represent an “unplanned indirect potable reuse (IPR)” system, while the Occoquan Watershed sites are representative of a “planned IPR” system. The sampling sites in the Occoquan Watershed analyzed to assess the impact on planned IPR were Bull Run Upstream (upstream of UOSA), UOSA (product water from within UOSA), and Bull Run Downstream (downstream of UOSA discharge) (Figure 2). The Frederick P. Griffith Water Treatment Plant intake (Griffith WTP) is located near dam of the Occoquan Reservoir and is the location of the withdrawal from the Reservoir that completes the planned IPR. The Griffith site was not directly compared to UOSA due to the long hydraulic retention time in the reservoir. The Griffith site was instead compared to the James J. Corbalis Water Treatment Plant (Corbalis WTP) unplanned IPR site. The Corbalis WTP intake is located on the Potomac River, downstream of many communities whose wastewater discharge flows into the Potomac. Of people in the Potomac Basin, 86% receive their potable water from public water suppliers (ICPRB 2019).

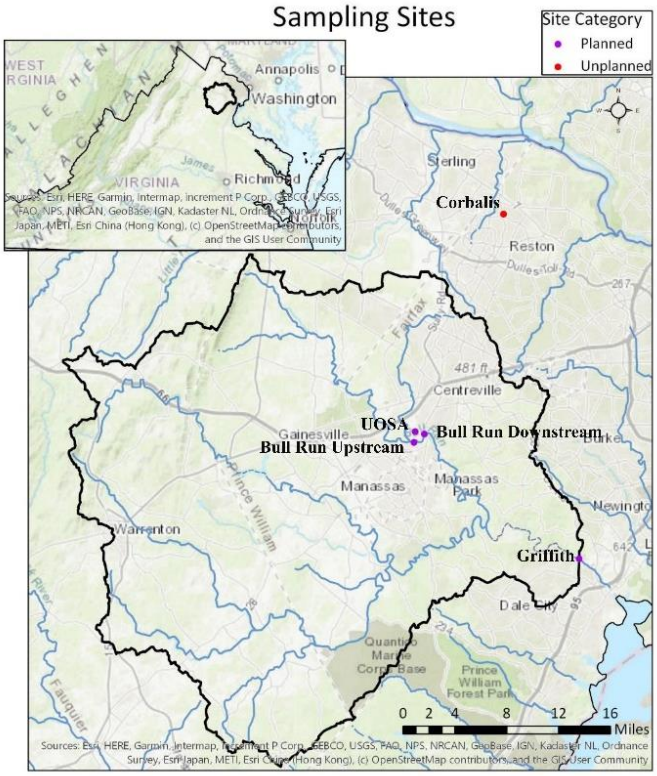
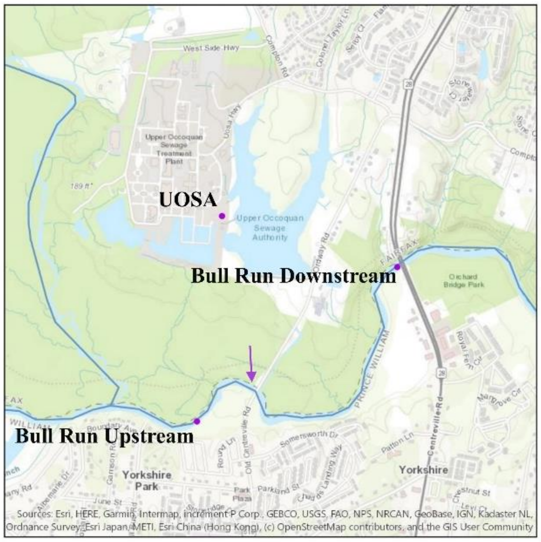


Figure 1. Map of sampling sites. Occoquan Watershed in Virginia outlined in black. All planned IPR sites (in purple) reside in the Occoquan Watershed. The unplanned site (red, Corbalis) is south of the Potomac River and represents treated Potomac River water.



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

Figure 2. Map of UOSA and Bull Run sampling sites. Detailed view of sampling sites above, below, and at UOSA. UOSA discharge enters Bull Run at the location indicated by the purple arrow, but was sampled within the facility. Bull Run Upstream is located before the discharge enter the river, and Bull Run Downstream is located after the discharge has entered the river.

Discrete water samples were collected monthly from October 2017 to September 2018 (12 months). At least one duplicate was taken per sampling effort. The EDC (both estrogen hormones and SOC) concentrations for the duplicate site were averaged and presented as one data point. Two trip blanks and one field blank were also tested for EDCs.

Temperature, dissolved oxygen, conductivity, and pH were measured at each site with a Hach HQ40D meter (Hach, Loveland, Colorado). Qualitative notes concerning flow were recorded at each location, as high, medium, or low flow. Three 0.5 L grab samples were obtained at each site for analyses at the State University of New York at Buffalo (EDCs) and University of Maryland (nutrients). Each bottle was prepared by rinsing with 10% hydrochloric acid solution. Glass bottles with Teflon-lined caps were used for EDC analysis. At the sampling location, the bottle was rinsed three times with stream water before collecting the water sample. At some sampling locations, a stainless steel bucket was required to collect the water sample. In those cases, the bottle was rinsed three times with water from the bucket subsequent to the bucket being rinsed three times with the stream water. Sample bottles were stored in a cooler with ice during transportation.

HDPE Nalgene bottles were used for nutrient analysis. Concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured on a Shimadzu Total Organic Carbon Analyzer (TOC-L CPH/CPN), using a high temperature catalytic oxidation method with a TNM-L unit for nitrogen (Álvarez-Salgado and Miller 1998). Concentrations of dissolved inorganic carbon (DIC) were also measured on the Shimadzu TOC-L CPH/CPN, using phosphoric acid to release CO_2 from DIC in stream water. Three injections (maximum of 5) were run for each sample to obtain a coefficient of variation less than 0.2. Soluble reactive phosphorus (SRP) was automatically measured on a QuikChem 8500 Series 2 FIA System and the ascorbic acid-molybdate blue method (Murphy and Riley 1962). Blanks and standards were run every 15 samples as a quality control measure to check the accuracy of the analyses.

Daily discharge flow was available from UOSA and an Occoquan Watershed Monitoring Lab (OWML) stream gaging station provided average daily flow for Bull Run Downstream. Bull Run Upstream flow was calculated by subtracting the UOSA flow from the Downstream flow (Figure S3). The flows could then be multiplied by SOC concentrations to determine the respective loads. Figure S4 presents the mean and 95% confidence intervals in theoretical and actual Bull Run Downstream SOC loads. SOCs without a 95% interval overlap are significantly different. Those with an overlap are not significantly different, suggesting that estimated SOC loads reflect actual loads well.

Filtration and Solid Phase Extraction (SPE)

The most common method to analyze EDCs is a mass-based method using mass spectrometry (MS). This is due to the fact that it has relatively low detection limit compared to other popular methods (Change et al. 2009). Various instruments can be used to increase MS results, such as GC-MS, LC-MS, HPLC-MS, or LC-UV (Change et al. 2009). The general procedure is to complete a pretreatment or extraction step, followed by the instrumental analysis. Extraction methods can be solid phase extraction (SPE), solid phase microextraction, and liquid phase microextraction (Change et al. 2009). In this project, the SPE method was used. A solid phase is employed as a sorbent in a cartridge for targeted analytes from the water sample to adsorb to the surface. Elution is the following step when analytes are removed from the cartridge in preparation for methods such as gas-chromatography or liquid-chromatography in tandem with mass spectrometry. GC-MS/MS and LC-MS/MS are advanced and sensitive instruments that target specific compounds, but are not commonly used for routine monitoring (Geissen et al. 2015).

To begin prepping samples for SPE, water samples being analyzed for EDCs were filtered and extracted before they were sent to the University at Buffalo. The samples were filtered within 24 hours of sampling. A 0.7-µm glass fiber filter, which had been placed in a muffle furnace for a 12 hours at 450°C, was used. Water samples were run through the filter using a glass filtration assembly. Once filtered, water samples were stored at 4°C until extraction.

The water samples were brought to room temperature for acidification and extraction. They were adjusted to pH 4 ±0.2 with sulfuric acid. Acidification was a necessary step to remove charged species of the targeted EDCs and favor neutral compounds and retention of these species in the cartridge. The samples were then spiked with 100 µL surrogate standard mix prepared by Diana Aga at the University at Buffalo containing 250 ng/mL surrogate mixture solution. The surrogate was added as a quality assurance measure to measure the extraction efficiency. After preparing the samples, the sample lines of the Dionex^{TM} Autotrace^{TM} Solid-Phase Extraction Instrument (Thermo Scientific^{TM}, USA). were cleaned with pure methanol, and then Milli-Q® water. Solvent lines and syringe were primed with methanol and then acidified (pH 4) water. The Oasis HLP SPE cartridges (500 mg, 6 cc) (Waters, Milford, MA, USA) were conditioned with 6 mL methanol followed by 10 mL acidified water. The sample was then loaded onto the cartridge at 10 mL/min. 10 mL of water/methanol (95/5 v/v) was used to rinse the sample bottle and was sorbed onto the cartridge. SPE cartridges were dried for 60 minutes The sample lines were cleaned with Milli-Q® water and then methanol after each run. Cartridges were stored at 18°C until shipped on ice. Samples were eluted at the University at Buffalo for GC-MS/MS or LC-MS/MS analysis.

SOC analysis methods were changed from gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS) to liquid-chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) in January 2018 (Month 4 of sampling). During this transition, 12 SOCs being analyzed by GC-MS/MS methods were replaced by SOCs that were more applicable to the region. Because of this change in the SOCs analyzed, data was categorized into three groups. Group 1 includes the 11 SOCs remained unchanged, that were analyzed by GC-MS/MS and then LC-MS/MS. Group 2 includes the 12 SOCs that were analyzed by LC-MS/MS in the later nine months of the study. Group 3 includes the 12 SOCs that were analyzed in the first four months of the study by GC-MS/MS (Table S6).

The GC separation was conducted with the Trace GC Ultra instrument in tandem with the TSQ Quantum XLS mass spectrometer (Thermo Scientific^{TM}, USA). LC separation was conducted with the Agilent 1200 HPLC system in tandem with the TSQ Quantum™ Ultra Triple Quadrupole Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA and Thermo Scientific^{TM}, USA).

Statistical Methods

EDCs were classified as estrogen hormones or synthetic organic compounds (SOCs). Because there were very few estrogen hormones found above the detection limit, they were not analyzed further (Figure S1). Non-detect data were converted to half of the detection limit (0.1 ng/L) (Domagalski and Munday 2003; Rippy et al. 2017). Because SOC data were positively skewed they were log transformed prior to analysis.

SOC concentrations were compared to public and aquatic health standards to understand them in the context of environmental and human safety. U.S. EPA maximum contaminant levels (MCLs) were referred to for public health standards, and U.S. EPA aquatic life benchmarks (ALBs) for fish, invertebrates, plants, and algae were referred to for aquatic ecosystem health standards ((U.S. EPA 2019a); Table S7).

PCA

Principal Component Analysis (PCA) was used to identify dominant patterns in SOCs. Only SOCs that were detected >2 times were included, as PCA is sensitive to outliers (Babamoradi et al. 2013). PCA identifies linear combinations of variables (in this case patterns of SOCs) called PC modes that are orthogonal to one-another and collectively represent the totality of data variance. Each successive mode captures the maximum possible amount of the data variance remaining such that earlier modes are more likely to represent physically meaningful processes than later modes, which often capture random noise. A resampling based stopping rule was used to determine which PC modes were meaningful (i.e., significant) at desired significance thresholds, and not capturing random noise. The thresholds were 50th percentile, (random), 90th percentile (marginally significant), 95th percentile (significant). PC modes above the 95th percentile threshold were analyzed. A nonparametric bootstrap method was used to determine 95% confidence bounds for each meaningful PC mode (Babamoradi et al. 2013). Because the number of months sampled and number of detections for group 3 was low (31 at IPR sites; 10 at WTP intake sites), it was not included in the PCA analysis.

Agglomerative Hierarchical Clustering and Heat Maps

Agglomerative hierarchical clustering was used to identify dominant patterns in SOC data and heat maps were used to visualize those patterns. Agglomerative hierarchical clustering uses distance measures to identify variables (in this case suites of SOC concentrations) that are similar across observations (in this case location and/or time) and/or observations that are similar with respect to variables. Data were standardized prior to clustering to avoid SOCs with higher magnitudes of concentrations driving the clustering.

The goal was to identify clusters of observations with similar concentration magnitudes, so a non-correlation based distance measure was used: Euclidean distance. A pairwise distance matrix was created to compute dissimilarity between every pair of observations. Agglomerative hierarchical clustering was conducted to partition groups based on their similarity and create a dendrogram. As with PCA, only groups 1 and 2 were analyzed due to the lack of observations in group 3. Agglomerative hierarchical clustering begins by assigning each observation a cluster of its own (the leaves of the dendrogram), and progressively linking similar observations until there is one big cluster (the root of the dendrogram). The following linkage methods were evaluated to determine which linkage measure most faithfully reproduced (i.e., had the strongest cophenetic correlation with) the original distance matrix for each dataset: complete, single, average, and Ward’s minimum variance. Scree plots and the gap statistic (Figure S10) were used to identify the appropriate number of clusters for each dendrogram (i.e., those that could be meaningfully interpreted).

RESULTS AND DISCUSSION

SECTION 1: PLANNED INDIRECT POTABLE REUSE

The Upper Occoquan Service Authority (UOSA) is an advanced WRF located in the Occoquan Watershed. SOC concentrations, SOC loads, nutrients, and water quality parameters sampled from UOSA discharge, as well as upstream and downstream of Bull Run (the river system receiving the discharge), were analyzed to gain a greater understanding of planned IPR.

Magnitude/Variability of SOC Concentrations

SOCs concentrations were plotted as box and whisker plots to be compared to toxicity benchmark values set by the U.S. EPA (Figure 3). All SOCs were below their respective benchmarks, indicating that **SOCs in WRF discharge were at acceptable levels**. All SOCs with a U.S. EPA maximum contaminant level (MCL), were detected below the MCL values. SOCs with MCLs include atrazine, *cis*-chlordane, heptachlor, heptachlor epoxide, simazine, *trans*-chlordane and Tribufos. Sampling sites had about the same number of detections of all SOCs monitored (UOSA (35%), Bull Run Downstream (BR.D; 33%), Bull Run Upstream (BR.U; 32%)). Although UOSA had slightly more detections, a 3% difference suggests it is not a major source of SOCs in the watershed.

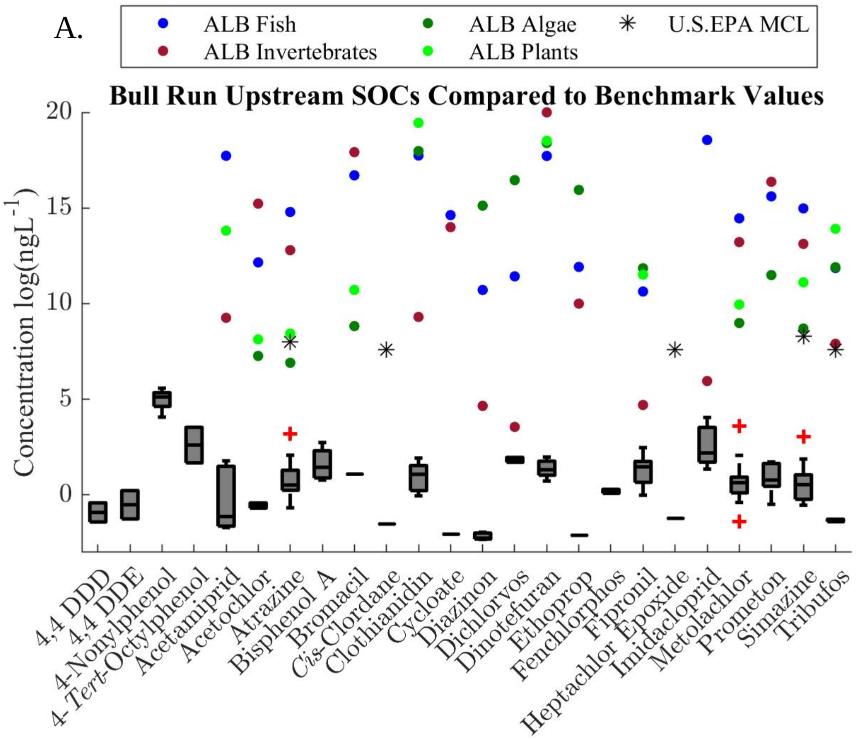
4-Nonylphenol, an alkyphenol industrial compound, was the highest SOC at all three locations, but still below a USGS noncancer health-based screening level (HBSLs) of 600,000 ng/L. These are non-enforceable benchmarks considering chronic noncancer effects. ALBs were not available for this compound since it is not a pesticide (USGS 2018). This compound may need to be monitored in the future to ensure levels do not significantly increase. 4-*tert*-octylphenol, also an alkylphenol, had the greatest variation of the SOCs plotted, with a range of 17.68 ng/L at UOSA, although it was only detected two out of nine times (Table S1).

Insecticides dichlorvos, fipronil, and imidacloprid box plots are plotted closest to their respective ALBs. Dichlorvos, an organophosphorus insecticide, was not often detected, but, when present, was detected at high concentrations. Detections in May and November at all sites indicate bi-annual usage. Dichlorvos is banned from being used residentially, and is used primarily for agricultural crops and livestock (U.S. EPA 2000). Other SOCs detected infrequently were: cycloate (detected once at BR.U in April at 0.13 ng/L), bromacil (detected once at BR.U as well, in January at 2.96 ng/L), Dimethoate (detected once at UOSA in May at 0.15 ng/L), and ethoprop (detected at two location in May: BR.U at 0.12 ng/L and UOSA at 0.23ng/L) (Table S1-3).

Only one banned SOC was detected at all three locations: heptachlor epoxide, which is a metabolite of organochlorine insecticide heptachlor. Heptachlor epoxide is created when heptachlor is released into the environment and reacts with oxygen (U.S. EPA 2019d). This compound was still below its MCL and ALBs, and so is considered of tolerable risk.

All SOCs with >1 detection from group 3 (only monitored in the initial 4-month period, October 2017 to January 2018) are insecticides or metabolites of an insecticide. They are 4,4 DDD, 4,4

DDE, *cis*-chlordane, fenchlorphos, heptachlor, heptachlor epoxide, and *trans*-chlordane. They were detected relatively consistently at low concentrations. The minimum was 0.13 ng/L of *trans*-chlordane and the maximum was 2.13 ng/L of fenchlorphos, both at UOSA (Table S1). The majority of detections for SOCs monitored in the initial 4-month period occurred at UOSA (48% of the 31 detections). They appear to be of tolerable risk with respect to MCLs and ALBs, but should be monitored. Fenchlorphos, an organophosphorus insecticide used on livestock, was frequently detected (twice in four samples at Bull Run Sites and thrice in four samples at UOSA). 4,4 DDD and 4,4 DDE are metabolites of DDT, which was banned in 1972 (Table 1). They were detected 50% (twice in four) of the sampling events at all locations.



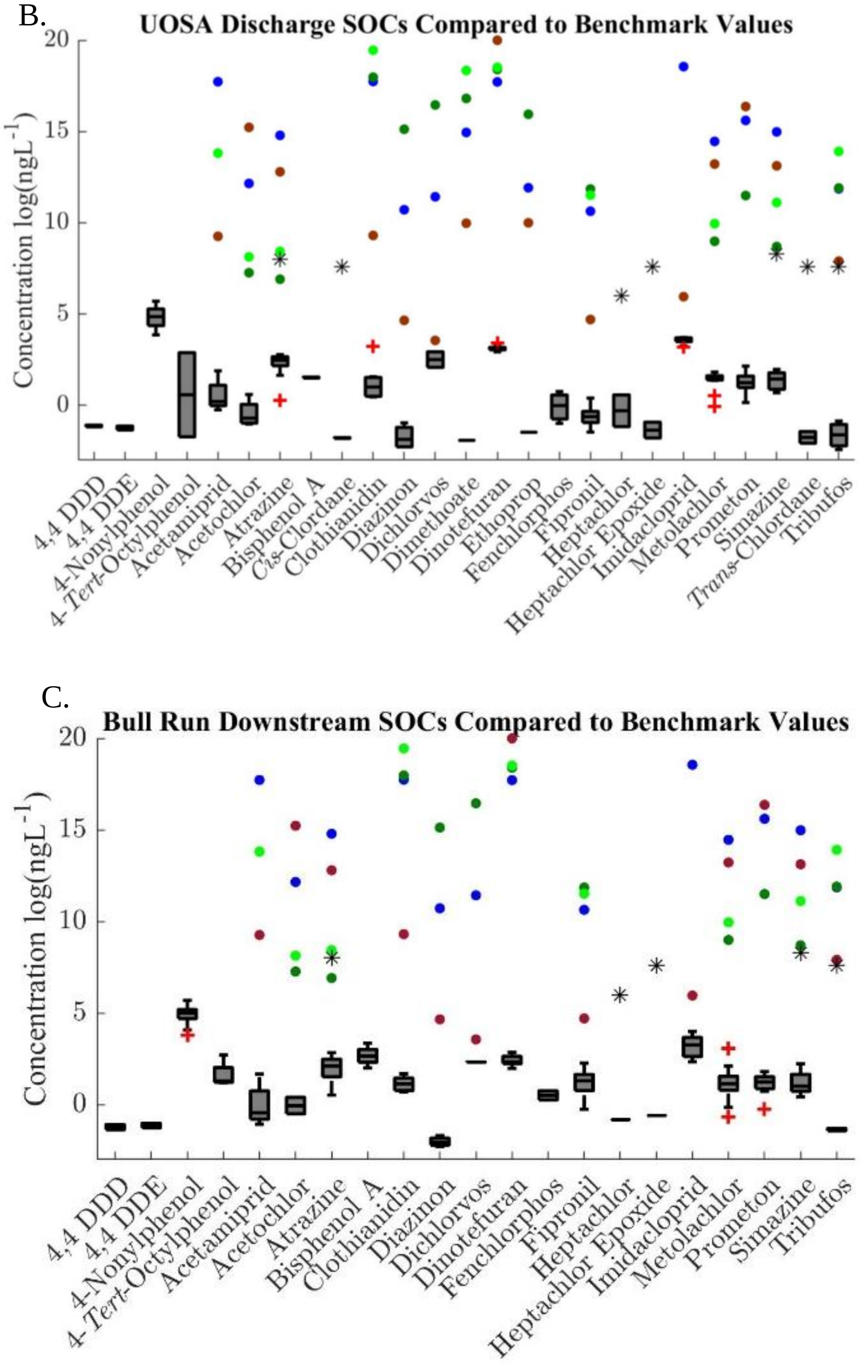


Figure 3. Box and whisker plots of SOCs from IPR sites compared to U.S. EPA benchmark values. The box and whisker plots represent the logged SOC concentrations (labeled along the x-axis). The center line of the boxplot represents the median, and the bottom and top represent the 25th and 75th percentile, respectively. The whiskers represent the remaining data, with outliers represented as red plus signs. U.S. EPA Aquatic Life Benchmarks (ALBs) are represented as dots, categorized as fish (blue), invertebrates (red), algae (dark green), and plants (lime green). The U.S. EPA Maximum Contaminant Levels (MCLs) are black asterisks. All benchmark values were standardized. SOCs detected at **A)** Bull Run Upstream, **B)** UOSA, and **C)** Bull Run Downstream sampling locations.

Patterns of SOCs Not Detected

SOCs that were not detected at UOSA or either of the Bull Run sites are: alachlor and chlorpyrifos (group 1); coumaphos (group 2); butylate, dieldrin, endrin aldehyde, methyl parathion, and parathion (group 3). Group 3 has the majority of SOCs that were not detected as they were the SOCs that were replaced in the fourth month of sampling in order to analyze SOCs that were more applicable to the region. Alachlor and butylate are agricultural herbicides. Alachlor has an MCL of 2 µg/L established by the U.S. EPA (U.S. EPA 1998). Chlorpyrifos, coumaphos, methyl parathion, and parathion are organophosphorus insecticides. Endrin aldehyde and dieldrin are chlorinated insecticides that were banned in the late 1980s. Endrin aldehyde has an MCL of 2 µg/L established by the EPA (U.S. EPA 1980).

SOCs not detected at UOSA were bromacil, cycloate (broad-spectrum agricultural herbicides); at BR.U were dimethoate, heptachlor, *trans*-chlordane; and at BR.D were bromacil, *cis*-chlordane, cycloate, dimethoate, ethoprop, *trans*-chlordane. Interestingly, SOCs were not detected Downstream that were detected either at Bull Run Upstream (2.96 ng/L bromacil in January; 0.13 ng/L cycloate in April) and not UOSA; or at neither BR.U and UOSA (0.22 and 0.16 ng/L *cis*-chlordane in January; 0.12 and 0.23 ng/L ethoprop in May, respectively). This could indicate dilution or another transportation mechanism occurred. Three out of the four SOCs had values close to the detection limit of 0.1 ng/L (cycloate, cis-chlordane, and ethoprop), which could be due to natural variation in the detection analysis.

Dominant SOC Patterns (Concentration)

SOC Group 1 (All SOCs Monitored for the Complete 12-month Period)

Question 1: What SOCs from group 1 tend to co-occur with one-another in water samples from the Occoquan IPR system?

This question was addressed using PCA, which allows for identification of dominant patterns of association among variables (in this case SOCs). PCA revealed one significant pattern in SOC concentrations that explained 40% of data variance (Figure 4). SOCs that co-occur in water samples have eigenvector values of the same sign and same approximate magnitude. Black vectors in Figure 4 illustrate the eigenvector values for each SOC in group 1. All SOCs, except dichlorvos, contributed positively to PC1, with contributions from the triazine herbicides atrazine, simazine, and prometon being significant at a 95% level (Table S8-A). This indicates that **all group 1 SOCs, except dichlorovos, tend to co-occur in water samples and that this co-association is strongest for the triazine herbicides**. Patterns for dichlorovos were unlike those of any other group 1 SOC.

Question 2: Where are group 1 SOCs coming from? Are they consistent across sampling locations (comparable at BR.U, UOSA and BR.D), coming from the sewershed (elevated at UOSA), or from alternate watershed sources (elevated at BR.D, but not UOSA or BR.U)?

The extent to which particular water samples contain more or less acetochlor, atrazine, dizinanon, metolachlor, prometon, and simazine is indicated by the position of their principal component scores relative to PC1 (colored symbols in Figure 4). Positive scores (i.e., loading with SOC eigenvectors) indicates water samples with more group 1 SOCs (excepting dichlorovos) and negative PC1 scores (i.e., loading opposite SOC eigenvectors) indicates fewer group 1 SOCs (again excepting dichlorovos). Less than half (33%) of samples collected Upstream of UOSA (square symbols in Figure 4) were in positive PC1 space, indicating that samples from BR.U are more likely to have lower concentations of group 1 SOCs than not. On the other hand, 75% of samples from UOSA (diamonds in Figure 4) and 67% from BR.D (circles in Fig. 4) were in positive PC1 space. **This suggests that the sewershed (UOSA discharge) may be contributing group 1 SOCs (particularly triazines, which were significant for PC1 – see above) to the Occoquan IPR system, albeit at very low concentrations (below MCLs and ABLs; Figure 3).** Triazine herbicides are used in both agricultural and urban settings, and may be entering the the sewershed via inflow and infiltration (I&I). I&I occurs as a sewer system ages and underground infrastructure degrades, allowing external pollutants from groundwater and stormwater to enter the system (Thapa et al. 2019).

Question 3: Are SOC concentrations stable throughout the year or do they vary by season?

Sample PC scores varied by season, with samples collected during fall and winter skewed towards negative PC1 and samples collected during spring and summer skewed towards positive PC1. This indicates that concentrations of group 1 SOCs are generally higher in spring and summer months. Triazine herbicides are often applied in early spring/summer, consistent with this observation (Graymore et al. 2001).

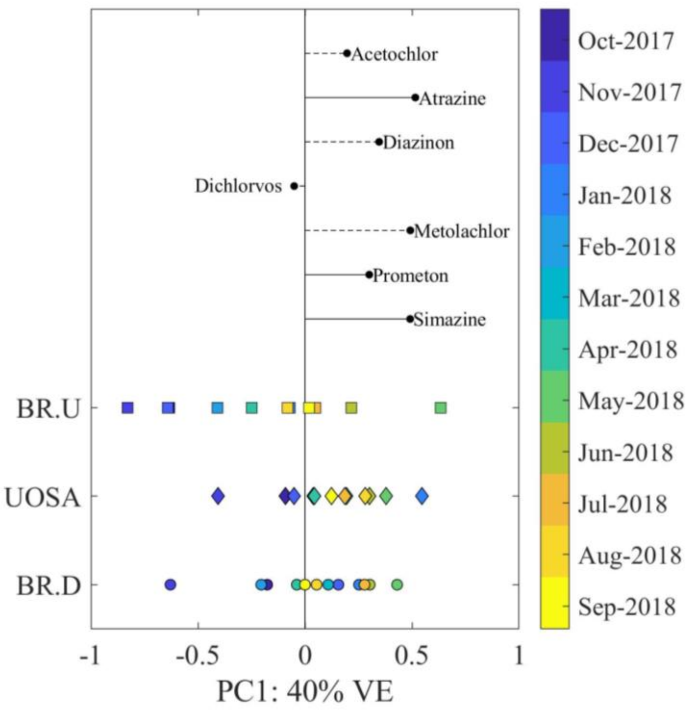


Figure 4. Principal component analysis of SOCs monitored for complete 12-month period. SOCs monitored from October 2017 to September 2018. The x-axis is the dominant mode PC1. PC scores are distinguished by shape to correlate with sampling location (see legend), and by color to correlate with the month sampled (see color bar). SOC loading vectors are represented by black lines if significant at a 95% confidence level, and by dashed black lines if not statistically significant.

SOC Group 2 (SOCs Monitored from January to September, 2018)

Question 1: What SOCs from group 2 tend to co-occur with one-another in water samples from the Occoquan IPR system?

Three significant PC modes captured 68% of the variance across SOCs analyzed during the last 9 months of sampling (PC1: 26%, PC2: 23%, PC3: 19%; Figure 5-A). The neonicotinoid insecticides clothianidin, dinotefuran, and imidacloprid loaded positively on PC1 and were the only SOCs that contributed significantly to this mode (95% confidence level; Table S8-B). Other SOCs were weakly positive (Tribufos, bisphenol A, and the neonicotinoid pesticide acetamiprid) or weakly negative (4-Nonylphenol, its putative replacement 4-*tert*-octylphenol, and fipronil). **This indicates that the positive SOCs tend to co-occur, with a co-association strongest for neonicotinoids clothianidin, dinotefuran, and imidacloprid**. **They have a different pattern from the negative loading group of SOCs 4-nonylphenol, 4-*tert*-octylphenol, and fipronil**.

Four SOCs contributed significantly to PC2 and can be broken up into two groups of strong co-association: **4-Nonylphenol and Tribufos exhibited positive loadings (and a strong co-association with each other)** and **4-*tert*-octylphenol and acetamiprid exhibited negative loadings (and a strong co-association with each other**; Figure 5-B).

Three SOCs contributed significantly (and positively) to PC3: **Bisphenol A, fipronil, and clothianidin, suggesting that these compounds are co-associated in a subset of samples**, despite their apparent orthogonality in PC1 vs. PC2 space (Figure 5-B).

Question 2: Where are group 2 SOCs coming from? Are they consistent across sampling locations (comparable at BR.U, UOSA and BR.D), coming from the sewershed (elevated at UOSA), or from alternate watershed sources (elevated at BR.D, but not UOSA or BR.U)?

Consistent with the group 1 PCA, all UOSA discharge samples and most (78%) of samples collected at BR.D exhibited positive PC1 loadings for group 2 (elevated concentrations of neonicotinoids), whereas samples collected at BR.U exhibited primarily negative PC1 loadings (lower concentrations of neonicotinoids; 78% of samples). **Neonicotinoid insecticides, in addition to triazine herbicides discussed in group 1, are increased in Bull Run by the presence of UOSA, an advanced WRF, suggesting a sewershed source.**

Most (78%) samples from UOSA discharge exhibited negative PC3 loadings (low concentrations of bisphenol A, fipronil, and clothianidin) whereas most (78%) of samples collected at BR.D exhibited positive PC3 loadings (higher concentrations of bisphenol A, fipronil, and clothianidin). **Elevated concentrations at BR.D, but not UOSA or BR.U suggests that these compounds have a watershed (not a sewershed) source. Because samples reflecting the watershed contribution upstream of UOSA don't exhibit strong positive skew towards PC3, this watershed source is likely new runoff inputs from the portion of the Bull Run watershed downstream of UOSA.** Sources may be runoff from Ordway Road bridge crossing Bull Run, or from residential or urban runoff near the stream. Since discharge samples were taken within the plant, pollutants could enter UOSA discharge from the polishing pond or as it flows to Bull Run. These SOCs (bisphenol Al, fipronil, and clothianidin) were not impacted by the presence of the WRF. This statement can be extended to these SOCs not being impacted by a planned reuse system in this study.

Question 3: Are SOC concentrations stable throughout the year or do they vary by season?

PC2 of group 2 SOCs displayed the most apparent tendency to be driven by underlying seasonal patterns. Samples containing 4-nonylphenol and Tribufos were primarily detected in winter and early spring, while samples containing 4-*tert*-octylphenol and acetamiprid were detected primarily in late spring through summer.

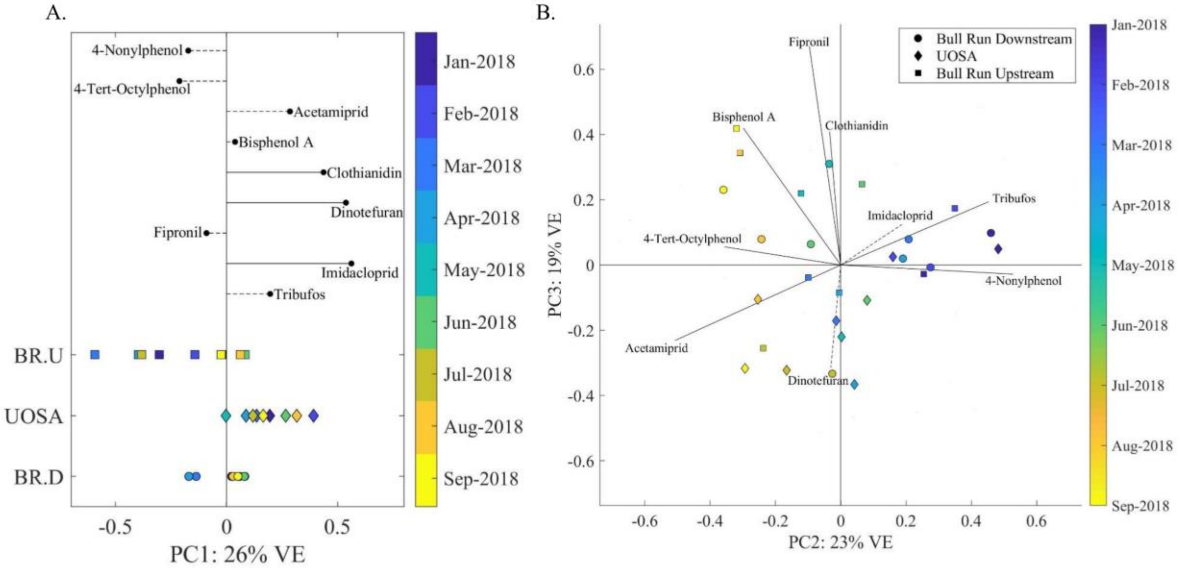


Figure 5. Principal component analysis of SOCs monitored for later, 9-month period. SOCs monitored from January 2018 to September 2018. A) Dominant mode PC1 (x-axis) plotted. **B)** Tertiary mode PC3 (y-axis) plotted against secondary mode PC2 (x-axis). PC scores are distinguished by shape to correlate with sampling location (see legend), and by color to correlate with the month sampled (see color bar). SOC loading vectors are represented by black lines if significant at a 95% confidence level, and by dashed black lines if not statistically significant.

Elevated concentrations of triazine herbicides and neonicotinoid insecticides can be attributed to UOSA discharge. However, the discharge levels and the levels in Bull Run are still magnitudes lower than their corresponding MCLs (if applicable) and ALBs. WRFs are a known source of EDCs, but in this planned IPR system, an advanced WRF seems to have the technologies to sufficiently remove the studied SOCs to concentrations of minimal impact. In cases of unplanned IPR systems, conventional WRFs, which lack advanced technologies that remove EDCs, may be established upstream of the drinking water plant (Kim et al. 2007), and may or may not successfully remove SOCs. A planned IPR, with an advanced WRF, appears to be an acceptable solution to reduce the risk of high EDC concentrations. Additionally, three SOCs studied were attributed to a watershed source, indicating another source that may have a greater impact on Bull Run than that from UOSA, depending on the compound.

Load Contribution

SOC loads at each site were calculated in order to gain a greater understanding of the impact of the upstream sources on Bull Run Downstream. It is important to look at UOSA discharge, not only as a source from a mass standpoint, but as a source as it contributes to flow. If UOSA contributes minimally to the flow, its impact on the watershed is diminished. While daily average flows were available from UOSA and BR.D sampling sites, they were calculated for BR.U (see methods). We expect our estimated loads to reflect actual loads at BR.U well, because estimated and actual loads at other sites (e.g., BR.D) were not significantly different at a 95% confidence level (Figure S4).

Figure 6 illustrates the relative contribution of various upstream sources of SOCs to downstream SOC loads at BR.D. Black circles indicate the average ratio of both known upstream loads (UOSA plus BR.U) to loads at BR.D. Values equal to 1 with 95% confidence (i.e., error bars spanning 1) indicate that SOC loads from these two upstream sources approximately equal SOC loads at BR.D. This was true for diazinon, dichlorovos, metolachlor, prometon, and simazine (group 1) and 4-nonylphenol, 4-*tert*-octylphenol, bisphenol A, and imidacloprid (group 2; Figure 6). Black circles with values that exceed 1 indicate that loads from both sources are in excess of BR.D, suggesting that decay or dilution of SOCs occurs between upstream sources and BR.D. This was true for acetochlor and atrazine (group 1; Figure 6). Finally, black circles with values less than 1 indicate that loads from both sources are lower than loads at BR.D, suggesting that an additional unquantified source of SOCs contributes to BR.D SOC loading. This was true for several group 2 SOCs (acetamiprid, clothianidin, dinotefuran, fipronil, and Tribufos).

Red and cyan symbols in Figure 6 illustrate which of the two known upstream sources (UOSA: red and BR.U: cyan) contribute more to BR.D. If UOSA contributes significantly more to SOC loading at BR.D than BR.U, red diamonds will be located to the right of the cyan squares in Figure 6 and the 95% confidence bounds about each symbol will not overlap. This was observed for atrazine (group 1), and acetamiprid and Tribufos (group 2). If BR.U contributes significantly more to SOC loading at BR.D than UOSA, then cyan squares will be to the right of the red diamonds in Figure 6 and the 95% confidence bounds about each will not overlap. This was observed for 4-nonylphenol (group 2). For all other SOCs, loads from BR.U and UOSA were not significantly different.

Taken together, the results presented in Figure 6 suggest that the following six scenarios are occurring in this watershed. They appear in order of their commonality in measured SOCs (most to least common):

1) There are only two, roughly equal upstream contributors to SOC loading at BR.D (UOSA and BR.U) and there is no evidence of decay or additional sources. This was true for half (8/16) SOCs, including diazinon, dichlorovos, metolachlor, prometon, simazine, 4-*tert*-octylphenol, bisphenol A, and imidacloprid;
2) There is an unknown source that contributes to SOC loading at BR.D, and loads from UOSA and BR.U are approximately equal (this was true for three group 2 SOCs, including clothianidin, dinotefuran, and fipronil).
3) There is an unknown source that contributes to SOC loading at BR.D, and loads from UOSA exceed BR.U (this was true for two group 2 SOCs, including acetamiprid and Tribufos)
4) There are only two, roughly equal contributors to SOC loading at BR.D (UOSA and BR.U), and some dilution or decay appears to occur prior to BR.D (this was only true for the group 1 SOC acetochlor)
5) There are only two upstream contributors to SOC loading at BR.D (UOSA and BR.U) and loads from BR.U exceed UOSA (this was only true for the group 2 SOC 4-nonylphenol)
6) There are only two upstream contributors to SOC loading at BR.D (UOSA and BR.U), loads from UOSA exceed BR.U, and some decay or dilution appears to occur prior to BR.D (this was only true for the group 1 SOC atrazine)

It is notable that of the sixteen SOC loads calculated, only three had contributions from UOSA exceeding BR.U, and for two of those an additional unknown source was present. This suggests that in terms of SOC loading, the advanced reclamation plant does not have a disproportionate impact on the natural river system.

Individual monthly mean SOC loads for each of the three ratios were analyzed for a seasonal pattern (Figure S5). However, it could only be concluded that pattern depended on flow, rather than season (Figure S3).

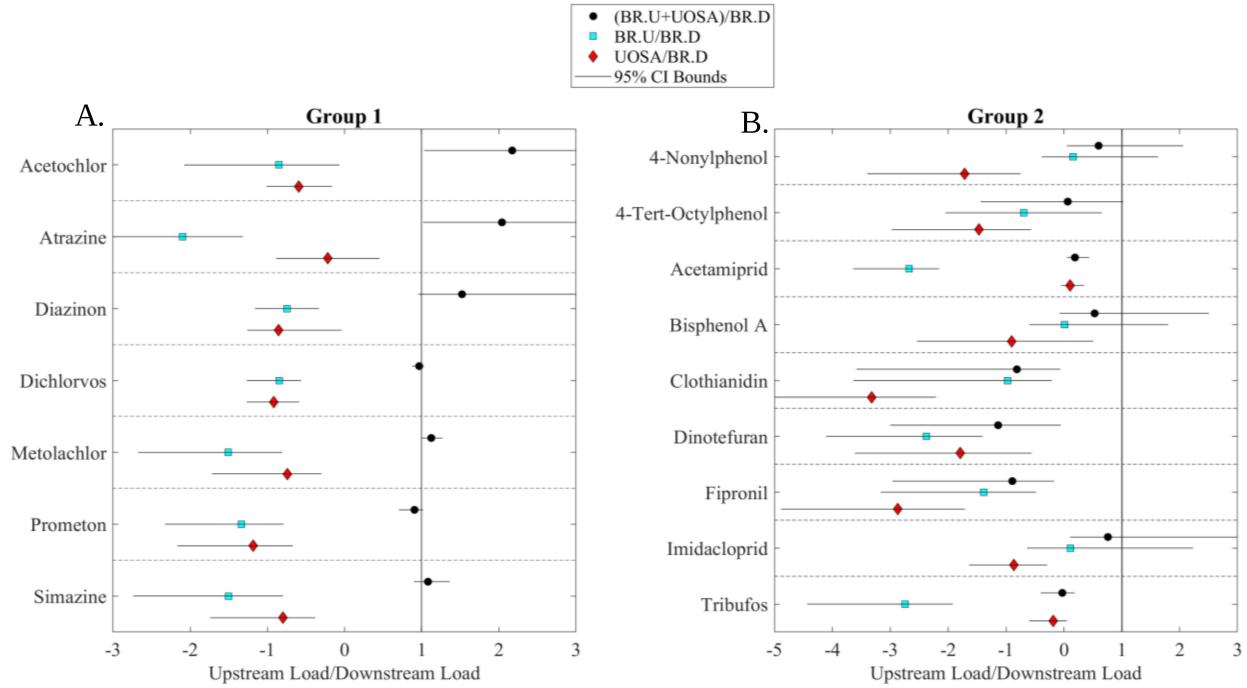


Figure 6. Comparison of Upstream and Downstream SOC loads. The mean load for each SOC was calculated for three scenarios: the ratio of Bull Run Upstream and UOSA to Bull Run Downstream ((BR.U+UOSA)/BR.D), the ratio of Bull Run Upstream to Bull Run Downstream (BR.U/BR.D), and the ratio of UOSA to Bull Run Downstream (UOSA/BR.D). 95% bootstrap confidence intervals are represented by black error bars. **A)** Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018).

As for SOC concentrations, PCA was also performed on SOC loads to determine 1) which SOCs had similar load patterns, 2) what the primary contributors to SOCs load were (a double check on the load analysis described above), and 3) if SOC loads were stable or varied by season.

Question 1: What SOCs have similar load patterns?

PCA revealed one significant pattern in SOC loads for group 1, explaining 60% of the variance (Figure 7-A). All SOCs contributed positively to PC1, with contributions from the triazine herbicides (atrazine, prometon, and simazine) and metolachlor being significant at a 95% confidence level (Table S9-A). **This suggests that all SOC loads co-varied (presumably in part**

due to co-varying flow), with the triazines and metolachlor exhibiting the strongest co-association. Triazines also had the strongest co-association in terms of mass (see prior PCA).

PCA also revealed one significant pattern that captured 49% of the variance for group 2 SOCs (Figure 7-B). All SOCs contributed positively to PC1 suggesting their loads co-varied (again, this is presumably in part due to co-varying flow). **This covariance was significant for 4-nonlylphenol and the neonicotinoid insecticides clothianidin, dinotefuran, and imidacloprid, suggesting they have the strongest co-association** (Table S9-B). The neonicotinoids had a strong tendency to co-occur in terms of mass (see prior PCA), but 4-nonylphenol did not, suggesting that its inclusion with the neonicotinoids may be due to similarities in flow rather than mass; indeed clothianidin, imidacloprid, and 4-nonylphenol were all slightly elevated at BR.U and therefore may have shared a common flow signature (see Figure 6B).

Question 2: What are the primary contributors to SOC loading?

PCA results regarding primary contributors to SOC loading are largely consistent with the results presented in Figure 6. **UOSA and BR.U were roughly evenly split about PC1 (slight negative skew, for BR.U), indicating that both sources contribute to overall loading of group 1 SOCs**. The somewhat negative skew of BR.U presumably reflects its lesser contribution to atrazine loading (see Figure. 6). PC scores for downstream samples were slightly positively skewed for PC1, which may be driven by an outlier SOC load (i.e., an extremely high load of metolachlor from a single time-point at BR.D that comes from BR.U, not UOSA) (see Figure 7).

For group 2 SOCs, UOSA exhibited primarily negative loadings (89% negative scores) whereas upstream and downstream Bull Run scores were mostly (67%) positive. Recall that the following four group 2 SOCs contributed significantly to PC1: 4-nonylphenol, clothianidin, dinotefuran, and imidacloprid. **Taken together, this suggests that 4-nonylphenol, clothianidin, dinotefuran, and imidacloprid were lower at UOSA than in Bull Run (i.e., not sourced from UOSA).** This finding consistent with the results presented in Figure 6, and the idea that advanced WRF does not contribute significantly to loading of these SOCs in this planned IPR system.

Question 3: Are SOC loads stable throughout the year or do they vary by season?

Sample scores varied by season for both group 1 and group 2, with samples collected in the winter and fall skewed towards negative PC1 and samples collected in spring and summer skewed towards positive PC1. Essentially, loads (especially of herbicides) were higher in spring and summer months, consistent with the prior PCA results for SOC mass (see Figure 4).

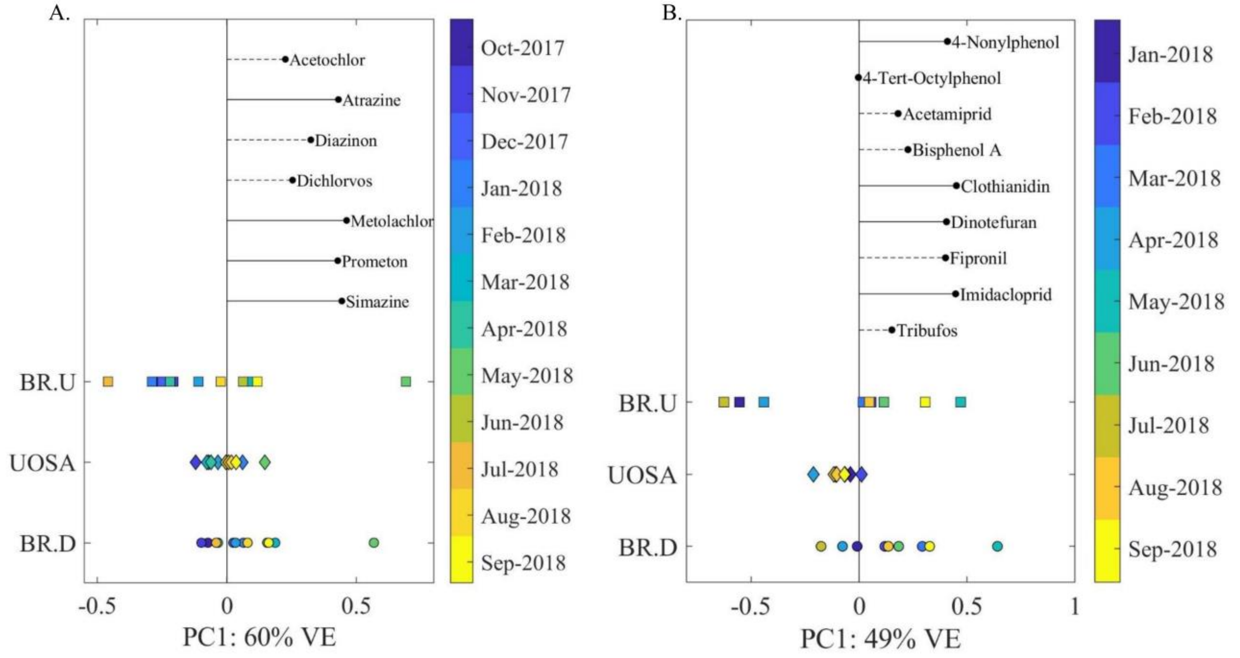


Figure 7. Principal component analysis of UOSA and Bull Run loads. The x-axis is dominant mode PC1 with the corresponding SOC loading vectors represented as black lines if significant at a 95% confidence level, and by dashed black lines if not statistically significant. Bull Run Upstream (BR.U), UOSA, and Bull Run Downstream (BR.D) PC scores are plotted as symbols distinguished by shape to correlate with sampling location, and by color to correlate with the month sampled (see color bar). **A)** Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018).

Nutrients at Planned Indirect Potable Reuse Sampling Sites

Nutrient concentration levels between UOSA and the Bull Run sites were compared. The dissolved inorganic carbon (DIC) concentrations were relatively constant, with a few outliers present (Figure 8-A). UOSA concentrations seemed to have little effect on Bull Run DIC. Generally, dissolved organic carbon (DOC) in the UOSA product water was less than that of Bull Run, with all DOC measurement less than 7 mg/L for all three sampling sites (Figure 8-B). Carbon levels in Bull Run (both DIC and DOC) were improved by UOSA discharge, because of the addition of water with low carbon concentrations. In Figure 7-D, it can be noted that UOSA SRP was consistently higher than the Bull Run stream sites, but does not seem to have a negative impact on SRP downstream. UOSA TDN levels are greater than that of Bull Run Upstream (Figure 7-C). In Figure S74, it is evident that this mainly occurs in the summer months. Since the first summer of UOSA's operation, it has discharging nitrified discharge to the waters of the Occoquan. The waters of Bull Run, where the UOSA discharge was, were generally cooler (hence denser) that the summertime surface waters of the reservoir, and thus this nitrified discharge entered the hypolimnion of the stratified reservoir. It was discovered that the nitrate, in the absence of dissolved oxygen, acted as an alternate terminal electron acceptor, and became denitrified in the process. Meanwhile, it prevented the system from going anaerobic, and poised the oxidation-reduction potential high enough to maintain oxidizing conditions and thus preventing the release of phosphorus, iron and manganese that occurs under reduced conditions. Due to this beneficial effect of nitrate during summer, UOSA continues to discharge nitrified waters during the summer months, and thus helps maintain the water quality in the reservoir (Cubas et al. 2014).

The planned potable reuse system is effective in terms of nutrients, due to increase in water quality concerning DOC, DIC, and very little to no change in SRP. In the Occoquan Watershed, the discharge of nitrate in the summer effectively controls the release of unwanted compounds in the Occoquan reservoir when it is stratified. A planned potable reuse system allows for nutrients levels to be appropriately impacted by the WRF, to help the Reservoir upstream of the drinking water plant to be of higher quality.

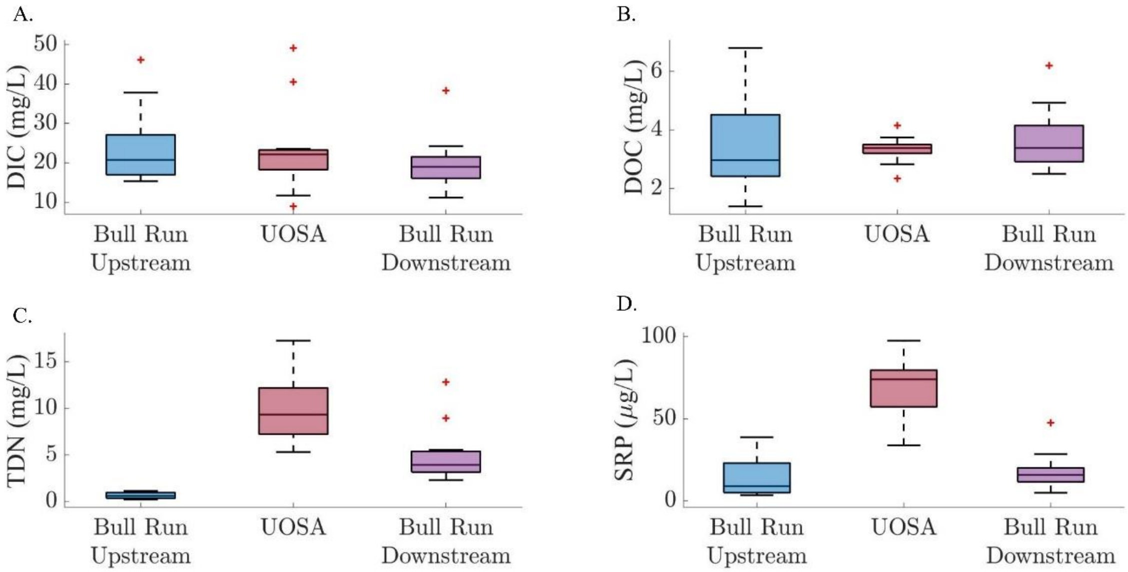


Figure 8. Box and whisker plots representing nutrient concentrations at planned IPR sites. The center line of the boxplot represents the median nutrient concentration, and the bottom and top represent the 25th and 75th percentile, respectively. The whiskers represent the remaining data, with outliers represented as red plus signs. **A)** Dissolved Inorganic Carbon (DIC) in mg/L. **B)** Dissolved Organic Carbon (DOC) in mg/L. **C)** Total Dissolved Nitrogen (TDN) in mg/L. **D)** Soluble Reactive Phosphorus (SRP) in µg/L.

In Situ Water Quality Parameters at Planned Indirect Potable Reuse Sites

The water quality parameters measured *in situ* at UOSA discharge and Bull Run were compared. The UOSA median temperature was higher than the Bull Run stream sites, although more consistent, as is to be expected when comparing a regulated discharge to a stream (Figure 9-A). UOSA discharge may slightly increase the temperature of Bull Run, as the median and the 25th and 75th percentiles represented by the box plot of Bull Run Downstream are higher than that of Bull Run Upstream. The median temperature of Bull Run increased from 14.35°C Upstream to 17.02°C Downstream. This increase in temperature is minimal, and may also be due to other factors. pH from UOSA remained around neutral, which was slightly lower than the Bull Run stream sites (Figure 9-B). Dissolved oxygen was higher and more variable in Bull Run than in UOSA discharge (Figure 9-C). The conductivity of UOSA stayed within a minimal, but elevated, range (600 to 1000 µS/cm) when compared to Bull Run conductivity (Figure 9-D). Bull Run Downstream conductivity is elevated compared to Bull Run Upstream, so it may have a sewershed source (UOSA discharge). *In situ* water quality parameter analysis showed UOSA was a source of conductivity, but decreased dissolved oxygen, in Bull Run. Overall, UOSA does not negatively impact Bull Run *in situ* water quality characteristics.

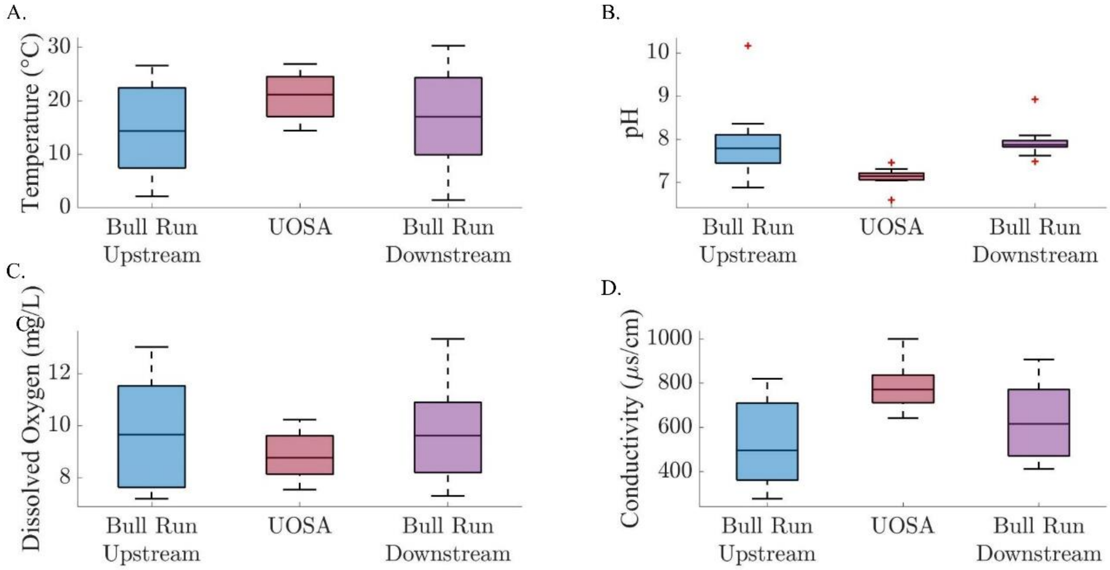


Figure 9. Box and whisker plots representing water quality parameters measured *in situ* at planned IPR sites. The center line of the boxplot represents the median parameter measurement, and the bottom and top represent the 25th and 75th percentile, respectively. The whiskers represent the remaining data, with outliers represented as red plus signs. **A)** Temperature (°C) **B)** pH **C)** Dissolved Oxygen (DO) in mg/L **D)** Conductivity in µS/cm.

SECTION 2: INDIRECT POTABLE REUSE IMPACT ON WTPS

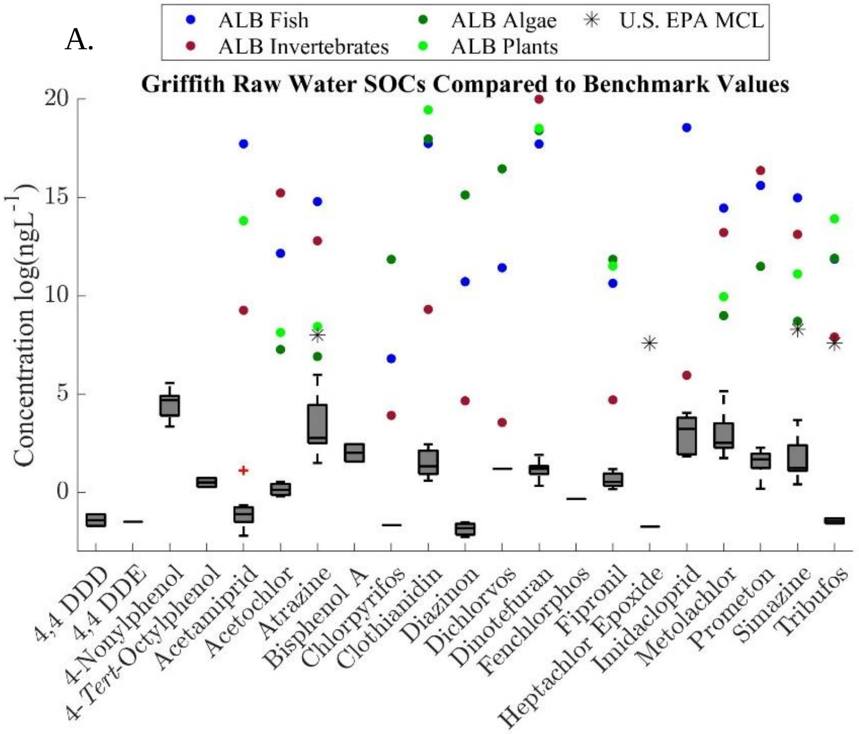
The Frederick P. Griffith Water Treatment Plant (Griffith WTP), located on the Occoquan Reservoir, was chosen as the planned indirect potable reuse sample site. The SOC concentrations were compared to the unplanned indirect potable reuse intake site at the James J. Corbalis Water Treatment Plant (Corbalis WTP). Both sites are located in the Potomac River Basin, with the Griffith WTP located in a subwatershed of the Potomac Basin, at the outlet of the Occoquan Reservoir.

Magnitude/Variability of SOC Concentrations

Both WTP intakes had high concentrations of industrial compound 4-nonylphenol, and herbicides atrazine, metolachlor, and simazine occur during the spring and summer months. The Griffith raw water experienced higher concentrations of atrazine than that of Corbalis. No raw water intake SOC levels were above their corresponding U.S. EPA ALB levels nor U.S. EPA MCLs values, indicating that WRFs and other watershed sources of SOCs were not significant enough to deteriorate water quality at the drinking plant intakes (Figure 10). SOCs with MCLs include atrazine, heptachlor epoxide, simazine, and Tribufos. The WTPs had about the same number of detections of all SOCs monitored (50% at each). This suggests that both planned and unplanned IPR scenarios are equally effective, although magnitude of SOC concentrations are an important factor that is considered as well.

SOCs detected infrequently were: chlorpyrifos (detected once at Griffith in April at 0.19 ng/L), dichlorvos (detected at Corbalis at 3.96 ng/L and Griffith at 3.32 ng/L in May), and Tribufos (detected in August and September at Corbalis and Griffith at 0.2 – 0.3 ng/L). SOCs not detected at either WTP intake site were: alachlor, bromacil, and cycloate (herbicides; group 1); coumaphos, dimethoate, and ethoprop (organophosphorus insecticides; group 2); butylate, dieldrin, endrin aldehyde, heptachlor, methyl paration, parathion, *trans*-chlordane, and *cis*-chlordane (eight of twelve SOCs monitored during this time period; group 3).

All SOCs with >1 detection that were only monitored in the initial 4-month period, October 2017 to January 2018 (group 3), are insecticides or metabolites of an insecticide. They are 4,4 DDD, 4,4 DDE, fenchlorphos, and heptachlor epoxide. The minimum was 0.17 ng/L of heptachlor epoxide (at Griffith) and the maximum was 0.40 ng/L of 4,4 DDE (at Corbalis).



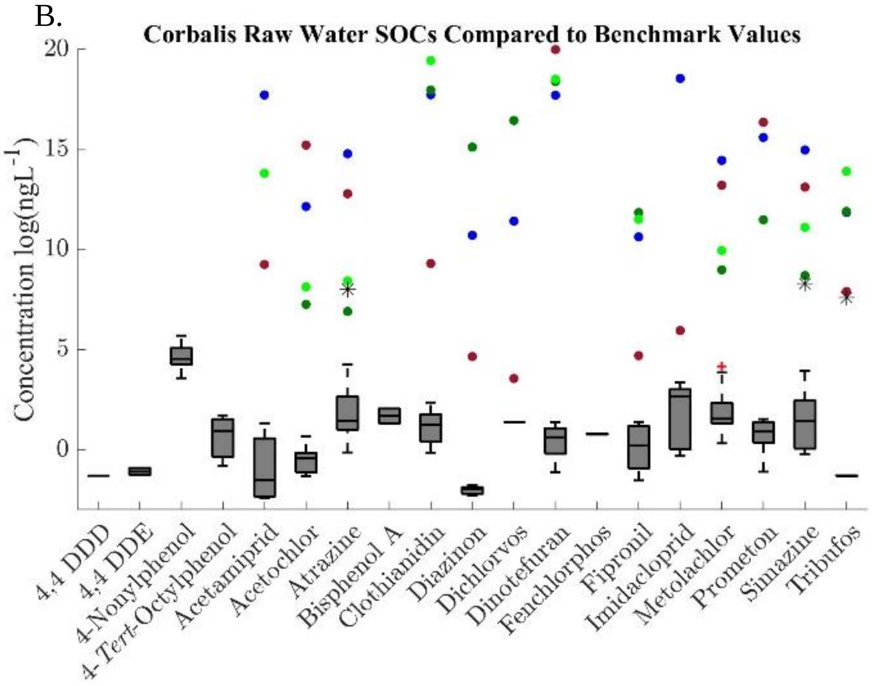


Figure 10. Box and whisker plots of WTP SOCs compared to U.S. EPA benchmark values. The box and whisker plots represent the logged SOC concentrations (labeled along the x-axis). The center line of the boxplot represents the median, and the bottom and top represent the 25th and 75th percentile, respectively. The whiskers represent the remaining data, with outliers represented as red plus signs. U.S. EPA Aquatic Life Benchmarks (ALBs) are represented as dots, categorized as fish (blue), invertebrates (red), algae (dark green), and plants (lime green). The U.S. EPA Maximum Contaminant Levels (MCLs) are black asterisks. All benchmark values were standardized. **A)** SOCs detected at Griffith WTP intake and **B)** SOCs detected at Corbalis WTP intake sampling locations.

<ins>Dominant SOC Patterns (Concentration)</ins>

Question 1: What SOCs tend to co-occur with one-another in water samples from an IPR system (whether planned or unplanned), within the Potomac River Basin?

One significant PC mode captured the variance across SOCs analyzed for group 1. All SOCs in group 1 positively contribute to PC1, which captures 57% of the data variance (Figure 11-A). Triazine herbicides (atrazine, simazine, and prometon) contribute significantly at a 95% level (Table S10-A). **This indicates that all SOCs of group 1 tend to co-occur, with the strongest co-association occurring among the triazines.**

Group 2 SOC concentrations also only had one significant PC mode that captured the variance (34%; Figure 11-B). **Alkylphenol industrial compounds, 4-nonylphenol and 4-*tert*-otylphenol, exhibited negative loadings onto PC1, indicating these SOCs tended to co-occur in samples.** All other SOCs exhibited positive loadings with neonicotinoid insecticides clothianidin, dinotefuran, and imidacloprid, as well as phenylpyrazole insecticide fipronil, contributed significant loadings onto PC1 (Table S10-B; 95% confidence level). **These SOCs tended to co-**

occur, and the significant SOCS (clothianidin, dinotefuran, imidacloprid, and fipronil) had the strongest co-association.

Question 2: Where are the SOCs coming from? Are they consistent across sampling locations (comparable at Griffith and Corblias intakes), coming from the a planned IPR system (elevated at Griffith intake), or from an unplanned IPR system (elevated at Corbalis intake)?

For group 1, **most (58%) of Griffith's scores skewed positively, reflecting elevated concentrations of all group 1 SOCs, especially of the triazine herbicides.** Most sample (67%) scores from Corbalis skewed negatively, reflecting **Corbalis was more likely to have lower SOC concentrations**. Griffith (the planned IPR site) was more likely to have higher levels of the triazine herbicides, which were attributed to UOSA discharge in Section 1 (see Figure 4). Because they are herbicides used for agricultural and residential use, the prevalence in UOSA may be due to more widespread use in the Occoquan Watershed, or higher levels of I&I as noted earlier. It is important to note the concentration levels are magnitudes lower than their U.S. EPA benchmark values.

For group 2 SOCs, **the WTPs scores both skewed negatively (55% for both) indicating SOC patterns were similar between planned and unplanned IPR systems.** These samples had lower concentrations of the insecticides (clothianidin, dinotefuran, imidacloprid, and fipronil) and higher concentrations of 4-nonylphenol and 4-*tert*-octylphenol. Since the WTPs appear to show similar patterns for group 2 SOCs, it is possible that the SOCs in this case relate to the background of the water supply from the Potomac.

Question 3: Are SOC concentrations stable throughout the year or do they vary by season?

Samples collected in spring and summer months of group 1 tended to be at higher magnitudes of PC1 at both WTPs. Winter and fall samples skewed negatively towards PC1, more clearly at Corbalis than at Griffith. This indicates SOC concentrations were higher during spring and summer, which occurred for group 1 SOC concentrations and loads at UOSA and Bull Run sites, as well (Figure 4; 7).

For group 2 SOCs, most samples from the WTPs exhibited negative loadings and were primarily detected from winter and early spring samples (55% of samples). This corresponds with the time period insecticides are applied: in the spring and summer months.

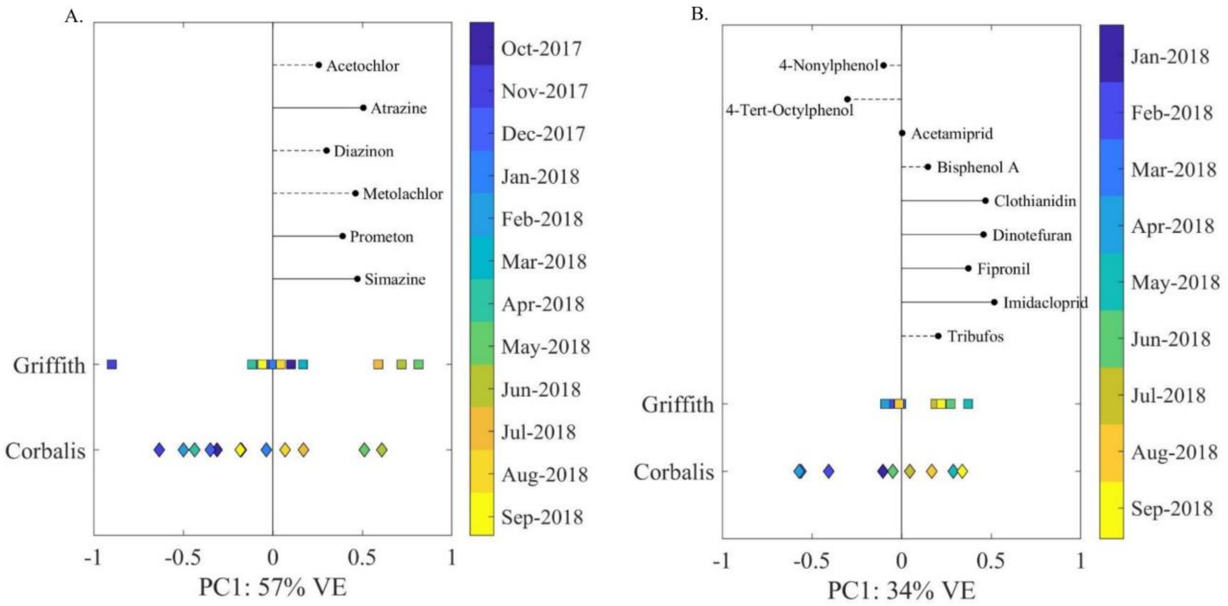


Figure 11. Principal component analysis of SOCs at water treatment plant intakes. The x-axis is dominant mode PC1 with the corresponding SOC loading vectors represented as black lines if significant at a 95% confidence level, and by dashed black lines if not statistically significant. Griffith WTP and Corbalis WTP PC scores are plotted as symbols distinguished by shape to correlate with sampling location, and by color to correlate with the month sampled (see color bar). **A)** Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018).

The average concentration of each SOC was calculated for the WTP intake sites (Figure 12). Corbalis WTP intake had higher average concentrations of industrial compounds, 4-nonylphenol and 4-*tert*-octylphenol; insecticides, acetamiprid, dichlorvos, and fenchlorphos; and triazine herbicide simazine. Griffith WTP intake had higher concentrations of the other triazine herbicides, atrazine and prometon; neonicotinoids, clothianidin, dinotefuran, and imidacloprid; industrial compound bisphenol A; and herbicide metolachlor. Most SOC differences tend to be attributed to Griffith. This supports prior PCA that higher concentrations of the triazines were more likely to occur at Griffith, but may not always be the case for simazine. In order to gain a greater understanding, the difference between the sum of the total amount of each respective SOC was found between the two water treatment plants (Figure S9). This figure supports the conclusions from Figure 12. While it was not possible to determine a watershed or sewershed source upstream of the WTP intakes for the SOCs, it is apparent that the water supply upstream of Griffith tended to have higher concentrations of SOCs. Future studies should determine the upstream sources of WTPs in planned vs. unplanned IPR systems.

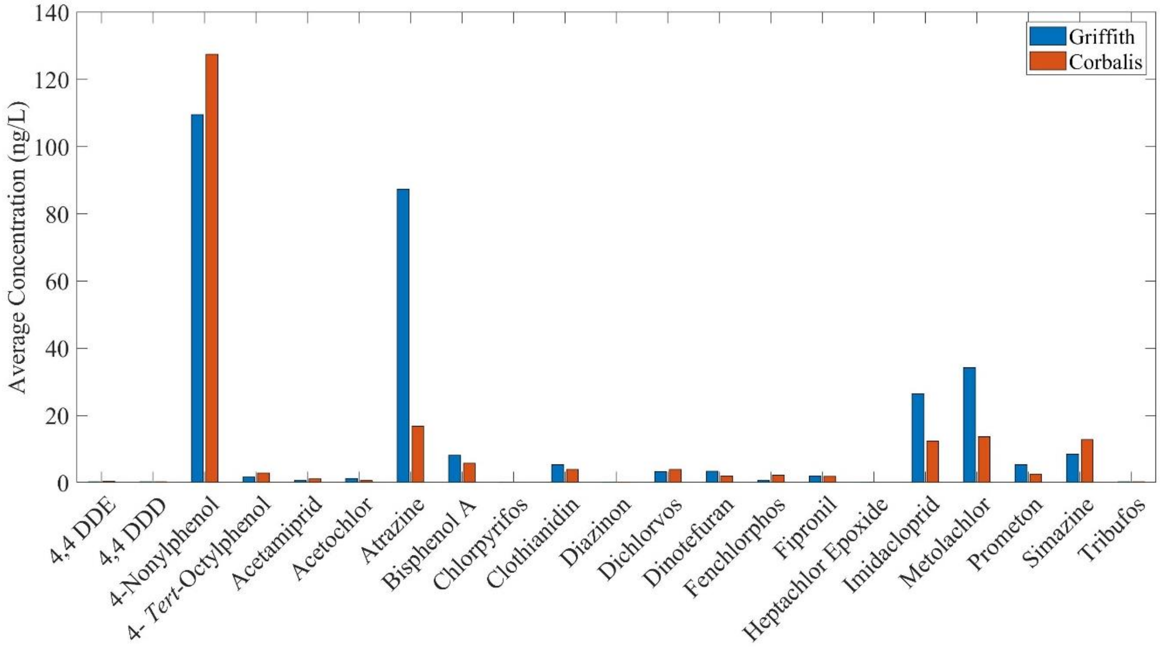


Figure 12. Average SOC concentrations at water treatment plant raw water intakes. Calculated average concentrations of SOCs detected in samples from Griffith and Corbalis WTP raw water intakes.

The heat maps are another visual to compare the difference in SOC concentrations. The SOCs that were sampled for all 12 months showed very clear patterns (Figure 13-A). The SOCs concentrations showed clear seasonality, with highest concentrations in early summer, and lowest concentrations in winter. The lowest concentrations appear to have occurred at Corbalis, except for Griffith in November. Diazinon and acetochlor concentrations are high during spring and summer months, but also sporadically during the winter. SOCs monitored for the later 9-months show clear seasonality as well, with high concentrations in the spring and summer months (Figure 13-B). This is especially true for neonicotinoid insecticides and fipronil (also an insecticide). The lowest concentrations of these insecticides occurred at Corbalis WTP intake in the winter. Other SOCs were sporadically detected at very high concentrations. Overall, lower SOC concentrations can be attributed to Corbalis SOC (the unplanned IPR site) and higher concentrations can mostly be attributed to Griffith (the planned IPR site).

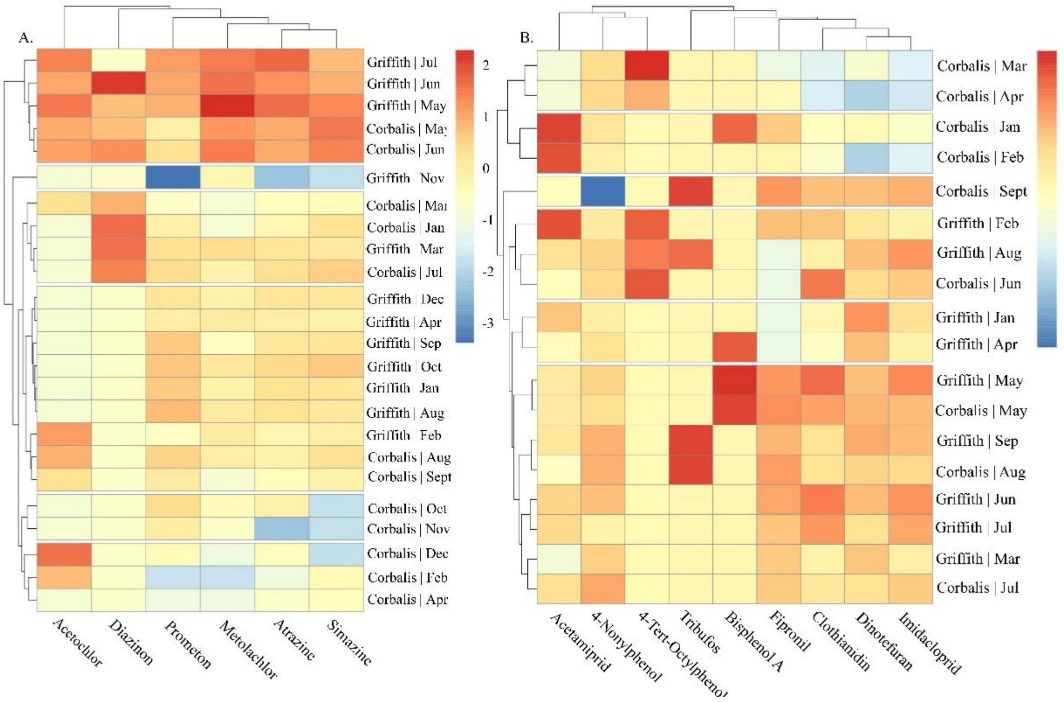


Figure 13. Heat map of SOC concentrations monitored for at WTP intakes. Logged SOC concentration magnitudes correlate with colors (see color bars). The x-axis is SOC type and the y-axis indicates sample location and month. **A)** Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018).

Griffith WTP raw water tended to have higher average concentrations, excluding 4-nonylphenol and simazine as the largest differences associated with Corbalis WTP intake. Several reasons for the higher concentrations at the Griffith WTP intake site may be: 1) This may be attributed to the type of IPR system, 2) differences in developed land as many SOCs are used in this setting (the Potomac is about 14% developed and the Occoquan Watershed is about 28% developed), or 3) differences in type of agriculture and proximity to the water source, which is about 25% of the land cover in both watersheds. The Griffith WTP intake (the planned IPR site) demonstrated the lack of difference in efficiency of planned IPR system compared to the Corbalis WTP intake (the unplanned IPR site) in the Potomac River Basin. While most concentrations were higher in the planned IPR site, they were not by differences of magnitudes. There is also greater natural dilution for the unplanned IPR site. All concentrations detected at both sites were magnitudes lower than their corresponding MCLs (if applicable) and ALBs. Both planned and unplanned IPR systems are sufficient in keeping SOC concentrations at minimal levels, so that there is a positive impact associated with either system.

There were no significant patterns between Corbalis and Griffith WTP intakes regarding *in situ* water quality parameters or nutrients data (Figure S11 and Figure S12).

CONCLUSIONS

- The planned potable reuse system water quality is as high quality as the “natural” Potomac River system. Comparison between the impacts of planned versus unplanned IPR in the Potomac River Basin indicates that both approaches are viable with respect to providing generally safe drinking water quality. However, it must be kept in mind that there is greater dilution of any WRF discharge into the Potomac River than there is in the Occoquan system, and this may account for the similar impacts.
- Impacts of the sewershed, including endocrine disrupting impacts, on the water quality are lessened by an advanced, more highly sophisticated WRF such as UOSA.
  - There were similar number of SOC detections at all three planned IPR sites (UOSA, and Bull Run Upstream and Downstream).
  - SOC concentrations at all three sites were significantly below intolerable levels (indicated by U.S. EPA maximum contaminant levels and acute level benchmarks).
  - UOSA discharge may be responsible for the triazine herbicide and neonicotinoid insecticides in terms of <u>mass</u>.
  - However, only 3/16 SOC <u>loads</u> calculated can be attributed to UOSA discharge (acetamiprid, atrazine, and Tribufos), indicating the low flow from UOSA minimizes it impacts on the natural system than analyses about mass indicate.
  - Nutrients and in situ water quality parameters were not impacted in Bull Run by UOSA discharge, except for conductivity.
- The watershed land use may have as much of an impact on the water quality of an indirect potable reuse (IPR) system as a WRF.
  - Both the unplanned (Corbalis WTP) and planned (Griffith WTP) intakes are from different sources and yet saw similar impacts from EDCs (both SOCs and estrogen hormones). Although located in different sub-watersheds, both WTPs are within the Potomac River Basin.
  - SOC mass and loads analyses of the Occoquan IPR sites (UOSA discharge and upstream and downstream) pointed to the occurrence of other watershed sources, whether upstream or downstream of UOSA.
- IPR, whether planned or unplanned, positively impacts EDCs.
  - Concentrations were significantly below intolerable levels (indicated by U.S. EPA maximum contaminant levels and acute level benchmarks).
  - The WTPs had about the same number of detections of SOCs monitored.
  - There was very little difference between the WTP intakes regarding nutrients and *in situ* water quality parameter data.

<u>Recommendations</u>

- A more intensive sampling schedule, spread out over a longer period of time (say, two years) will provide better and more solid data to buttress the conclusions reached in this study. More studies need to be done to tease apart these differences.

- Finding a way to factor in the size of the watershed in terms of water quantity into which WRF discharge will provide a means of more directly comparing technologies and planned versus unplanned IPR.
- Another watershed should be chosen to represent unplanned IPR, in order to more directly compare watersheds. This would also allow for a WRF and WTP to be chosen that can more directly contrast the Occoquan Watershed in terms of land use and plant technologies.

REFERENCES

Álvarez-Salgado XA, Miller AEJ. 1998. Simultaneous determination of dissolved organic carbon and total dissolved nitrogen in seawater by high temperature catalytic oxidation: conditions for precise shipboard measurements. Marine Chemistry 62(3):325-333.

Alvarez DA, Cranor WL, Perkins SD, Schroeder VL, Iwanowicz LR, Clark RC, Guy CP, Pinkney AE, Blazer VS, Mullican JE. 2009. Reproductive health of bass in the Potomac, USA, drainage: Part 2. Seasonal occurrence of persistent and emerging organic contaminants. Environmental Toxicology and Chemistry 28(5):1084-1095.

Auriol M, Filali-Meknassi Y, Tyagi RD, Adams CD, Surampalli RY. 2005. Endocrine disrupting compounds removal from wastewater, a new challenge. Process Biochemistry 41:525-539.

Babamoradi H, van den Berg F, Rinnan Å. 2013. Bootstrap based confidence limits in principal component analysis — A case study. Chemometrics and Intelligent Laboratory Systems 120:97-105.

Barber LB, Rapp JL, Kandel C, Keefe SH, Rice J, Westerhoff P, Bertolatus DW, Vajda AM. 2019. Integrated Assessment of Wastewater Reuse, Exposure Risk, and Fish Endocrine Disruption in the Shenandoah River Watershed. Environmental Science and Technology 53(7):3429-3440.

Barber LB, Vajda AM, Douville C, Norris DO, Writer JH. 2012. Fish endocrine disruption responses to a major wastewater treatment facility upgrade. Environmental Science and Technology 46(4):2121-2131.

Blazer VS, Iwanowicz LR, Henderson H, Mazik PM, Jenkins JA, Alvarez DA, Young JA. 2012. Reproductive endocrine disruption in smallmouth bass (Micropterus dolomieu) in the Potomac River basin: spatial and temporal comparisons of biological effects. Environmental Monitoring and Assessment 184(7):4309-4334.

Boyd GR, Palmeri JM, Zhang S, Grimm DA. 2004. Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana USA. The Science of the Total Environment 333(1):137-148.

Burkhardt-Holm P. 2010. Endocrine disruptors and water quality: A state-of-the-art review. International Journal of Water Resources Development 26(3):477-493.

Cao Q, Yu Q, Connell DW. 2010. Fate simulation and risk assessment of endocrine disrupting chemicals in a reservoir receiving recycled wastewater. Science of The Total Environment 408(24):6243-6250.

CDC. 4-tert-Octylphenol Biomonitoring Summary [Internet]. [visited 2020 March 23]. Available from: https://www.cdc.gov/biomonitoring/Octylphenol_BiomonitoringSummary.html

CDC. Dimethoate biomonitoring summary [Internet]. [visited 2020 March 23]. Available from: https://www.cdc.gov/biomonitoring/DimethoateOmethoate_BiomonitoringSummary.html

Change H-S, Choo K-H, Lee B, Choi S-J. 2009. The methods of identification, analysis, and removal of endocrine disrupting compounds (EDCs) in water. Journal of Hazardous Materials 172(1):1-12.

Chapman DV. 1996. Water Quality Assessments : A Guide to the Use of Biota, Sediments and Water in Environmental Monitoring. Baton Rouge, United States: CRC Press.

Chen W, Xu J, Lu S, Jiao W, Wu L, Chang AC. 2013. Fates and transport of PPCPs in soil receiving reclaimed water irrigation. Chemosphere 93(10):2621-2630.

Cornell. Ronnel [Internet]. [visited 2020 March 16]. Available from: http://pmep.cce.cornell.edu/profiles/insect-mite/propetamphos-zetacyperm/ronnel/insect-prof-ronnel.html

Coupe RH, Blomquist JD. 2004. Water-soluble pesticides in finished water of community water supplies. Journal - AWWA 96(10):56-68.

Cubas FJ, Novak JT, Godrej AN, Grizzard TJ. 2014. Effects of nitrate input from a water reclamation facility on the occoquan reservoir water quality. Water Environment Research 86(2):123-133.

Da Silva A, Navarrete E, Bell K. Potable reuse: Defining source water quality requirements. 87th Annual Water Environment Federation Technical Exhibition and Conference, WEFTEC 2014, September 27, 2014 - October 1, 2014; 2014; New Orleans, LA, United States: Water Environment Federation. pp. 5505-5520.

Daughton CG, Ternes TA. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environmental Health Perspectives Supplements 107:907.

Denton TE, Howell WM, Allison JJ, McCollum J, Marks B. 1985. Masculinization of female mosquitofish by exposure to plant sterols andMycobacterium smegmatis. Bulletin of Environmental Contamination and Toxicology 35(1):627-632.

Domagalski JL, Munday C. 2003. Evaluation of Diazinon and Chlorpyrifos Concentrations and Loads, and Other Pesticide Concentrations, at Selected Sites in the San Joaquin Valley, California, April to August, 2001. United States.

Escher BI, Allinson M, Altenburger R, Bain PA, Balaguer P, Busch W, Crago J, Denslow ND, Dopp E, Hilscherova K et al. 2014. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. Environ Sci Technol 48(3):1940-56.

Esperanza M, Suidan MT, Nishimura F, Wang Z-M, Sorial GA, Zaffiro A, McCauley P, Brenner R, Sayles G. 2004. Determination of Sex Hormones and Nonylphenol Ethoxylates in the Aqueous Matrixes of Two Pilot-Scale Municipal Wastewater Treatment Plants. Environmental Science & Technology 38(11):3028-3035.

Falconer IR, Chapman HF, Moore MR, Ranmuthugala G. 2006. Endocrine-disrupting compounds: A review of their challenge to sustainable and safe water supply and water

Geissen V, Mol H, Klumpp E, Umlauf G, Nadal M, van der Ploeg M, van de Zee SEATM, Ritsema CJ. 2015. Emerging pollutants in the environment: A challenge for water resource management. International Soil and Water Conservation Research 3(1):57-65.

Gimeno S, Komen H. 1997. Disruption of sexual differentiation in genetic male common carp (*Cyprinus carpio*) exposed to an Alkylphenol during Different Life Stages. Environmental Science & Technology 31(10):2884.

Gogoi A, Mazumder P, Tyagi VK, Tushara Chaminda GG, An AK, Kumar M. 2018. Occurrence and fate of emerging contaminants in water environment: A review. Groundwater for Sustainable Development 6:169-180.

Graymore M, Stagnitti F, Allinson G. 2001. Impacts of atrazine in aquatic ecosystems. Environment International 26(7):483-495.

Hall EL. 1996. Pesticide distribution in water, sediment, and fish of the Occoquan Watershed [Masters thesis]. Virginia Tech.

Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A. 2002. Feminization of male frogs in the wild. Nature 419(6910):895-896.

Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE. 1998. Occurrence, fate and effects of pharmaceutical substances in the environment- A review. Chemosphere 36(2):357-393.

Hudson B, Beller H, Bartel CM, Kane S, Campbell C, Grayson A. 2005. Environmental Transport and Fate of Endocrine Disruptors from Non-Potable Reuse of Municipal Wasterwater. United States.

ICPRB. Potomac Basin Facts [Internet]. [visited 2019 17 Oct]. Available from: https://www.potomacriver.org/potomac-basin-facts/

Iwanowicz LR, Blazer VS, Guy CP, Pinkney AE, Mullcan JE, Alvarezw DA. 2009. Reproductive health of bass in the potomac, USA, drainage: Part 1. exploring the effects of proximity to wastewater treatment plant discharge. Environmental Toxicology and Chemistry 28(5):1072-1083.

Karamouz M, Moridi A, Nazif S. 2010. Urban water engineering and management. CRC Press.

Kayhanian M, Tchobanoglous G. 2016. Water reuse in Iran with an emphasis on potable reuse. Sharif University of Technology 23(4):1594-1617.

Khanal SK, Xie B, Thompson ML, Sung S, Ong S-K, van Leeuwen J. 2006. Fate, Transport, and Biodegradation of Natural Estrogens in the Environment and Engineered Systems. Environmental Science & Technology 40(21):6537-6546.

Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA. 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Research 41(5):1013-1021.

Konkel L. Why are these male fish growing eggs? [Internet]. National Geographic; [visited 2020 March]. Available from: https://www.nationalgeographic.com/news/2016/02/160203-feminized-fish-endocrine-disruption-hormones-wildlife-refuges/

Kramer VJ, Miles-Richardson S, Pierens SL, Giesy JP. 1998. Reproductive impairment and induction of alkaline-labile phosphate, a biomarker of estrogen exposure, in fathead minnows (Pimephales promelas) exposed to waterborne 17β-estradiol. Aquatic Toxicology 40(4):335-360.

Kuriyama SN, Talsness CE, Grote K, Chahoud I. 2005. Developmental Exposure to Low-Dose PBDE-99: Effects on Male Fertility and Neurobehavior in Rat Offspring. Environmental Health Perspectives 113(2):149-154.

Murphy J, Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36.

Noguera-Oviedo K, Aga DS. 2016. Lessons learned from more than two decades of research on emerging contaminants in the environment. Journal of Hazardous Materials 316:242-251.

Pal A, He Y, Jekel M, Reinhard M, Gin KY-H. 2014. Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. Environment International 71:46-62.

Pecson BM, Triolo SC, Olivieri S, Chen EC, Pisarenko AN, Yang C-C, Olivieri A, Haas CN, Trussell RS, Trussell RR. 2017. Reliability of pathogen control in direct potable reuse: Performance evaluation and QMRA of a full-scale 1 MGD advanced treatment train. Water Research 122:258-268.

Randall CW, Grizzard TJ. 1995. Management of the Occoquan river basin: A 20-year case history. Water Science and Technology 32(5):235-243.

Rice J, Via SH, Westerhoff P. 2015. Extent and impacts of unplanned wastewater reuse in US rivers. American Water Works Association 107(11):571-581.

Rippy MA, Deletic A, Black J, Aryal R, Lampard J-L, Tang JY-M, McCarthy D, Kolotelo P, Sidhu J, Gernjak W. 2017. Pesticide occurrence and spatio-temporal variability in urban run-off across Australia. Water Research 115:245-255.

Sedlak D. 2014. Water 4.0: the past, present, and future of the world's most vital resource. New Haven & London: Yale University Press.

Spellman FR. 2017. The drinking water handbook, third edition. CRC Press.

Stumm-Zollinger E, Fair GM. 1965. Biodegradation of Steroid Hormones. Journal Water Pollution Control Federation, 37(11):1506-1510.

Thapa JB, Jung JK, Yovichin RD. 2019. A Qualitative Approach to Determine the Areas of Highest Inflow and Infiltration in Underground Infrastructure for Urban Area. Advances in Civil Engineering 2019:2620459 (11 pp.).

Tijani JO, Fatoba OO, Petrik LF. 2013. A Review of Pharmaceuticals and Endocrine-Disrupting Compounds: Sources, Effects, Removal, and Detections. Water, Air & Soil Pollution 224(11):1-29.

U.S. EPA. Ambient Water Quality Criteria for Endrin [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2019-03/documents/ambient-wqc-endrin-1980.pdf

U.S. EPA. p,p'-Dichlorodiphenyl dichloroethane (DDD); CASRN 72-54-8 [Internet]. [visited 2020 March]. Available from: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0347_summary.pdf

U.S. EPA. Butylate R.E.D. Facts [Internet]. [visited 2020 March]. Available from: https://archive.epa.gov/pesticides/reregistration/web/pdf/butylate-2.pdf

U.S. EPA. Metolachlor R.E.D. Facts [Internet]. [visited 2020 March]. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-108801_1-Apr-95.pdf

U.S. EPA. Bromacil R.E.D Facts [Internet]. [cited 2020 March]. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-012301_1-Aug-96.pdf

U.S. EPA. Coumaphos R.E.D. Facts [Internet]. [visited 2020 March]. Available from: https://archive.epa.gov/pesticides/reregistration/web/pdf/0018fact.pdf

U.S. EPA. Toxicological Review of Chlrodane (Technical) [Internet]. [visited 2020 March]. Available from: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0142tr.pdf

U.S. EPA. Alachlor R.E.D. Facts [Internet]. [visited 2020 March]. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-090501_1-Dec-98.pdf

U.S. EPA. 2,4,5-Trichlorophenol [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2016-09/documents/2-4-5-trichlorophenol.pdf

U.S. EPA. DDE [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2016-09/documents/dde.pdf

U.S. EPA. Dichlorvos [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2016-09/documents/dichlorvos.pdf

U.S. EPA. Heptachlor [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2016-09/documents/heptachlor.pdf

U.S. EPA. Parathion [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2016-09/documents/parathion.pdf

U.S. EPA. Tribufos Facts [Internet]. [visited 2020 March]. Available from: https://archive.epa.gov/pesticides/reregistration/web/pdf/2145iredfact.pdf

U.S. EPA. Acetamiprid Pesticide Fact Sheet [Internet]. [visited 2020 March]. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-099050_15-Mar-02.pdf

U.S. EPA. Ethoprop Facts [Internet]. [visited 2020 March]. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-041101_1-Jun-02.pdf

U.S. EPA. Clothianidin Pesticide Fact Sheet [Internet]. [visited 2020 March]. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-044309_30-May-03.pdf

U.S. EPA. Health effects support document for Aldrin/Dieldrin [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2014-09/documents/support_cc1_aldrin-dieldrin_healtheffects.pdf

U.S. EPA. Cycloate R.E.D. Facts [Internet]. [visited 2020 March]. Available from: https://archive.epa.gov/pesticides/reregistration/web/pdf/cycloate_red_fs.pdf

U.S. EPA. Dinotefuran pesticide fact sheet [Internet]. [visited 2020 March]. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-044312_01-Sep-04.pdf

U.S. EPA. Problem formulation for the environmental fate and ecological risk, endangered species and drinking water assessments in support of the registration review of Diazinon [Internet]. [visited 2020 March]. Available from: https://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-057801_26-Mar-08_a.pdf

U.S. EPA. Reregistration elgibility decision for prometon [Internet]. [visited 2020 March]. Available from: https://archive.epa.gov/pesticides/reregistration/web/pdf/prometon-red.pdf

U.S. EPA. 4-Nonylphenol [Internet]. [visited 2020 March]. Available from: https://iaspub.epa.gov/tdb/pages/contaminant/contaminantOverview.do?contaminantId=10220

U.S. EPA. Acetochlor [Internet]. [visited 2020 March]. Available from: https://ofmpub.epa.gov/tdb/pages/contaminant/contaminantOverview.do;jsessionid=GjjhQsTLcj2GGhwY6LrVTCQjDHGDSRp9Q32tLgZTWLv1QG2TJ4By!989777045?contaminantId=10041

U.S. EPA. Acute exposure guidline levels (AEGLs): Methyl Parathion [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2014-08/documents/methyl_parathion_interim_sep_09_v1.pdf;pl

U.S. EPA. Simazine and Simazine Degradates [Internet]. [visited 2020 March]. Available from: https://iaspub.epa.gov/tdb/pages/contaminant/contaminantOverview.do?contaminantId=10260

U.S. EPA. Fipronil Summary Document Registration Review [Internet]. [visited 2020 March]. Available from: https://www.regulations.gov/document?D=EPA-HQ-OPP-2011-0448-0003

U.S. EPA. Biomonitoring Bisphenol A (BPA) [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/sites/production/files/2017-08/documents/ace3_bpa_updates_8-4-17.pdf

U.S. EPA. Aquatic Life Benchmarks and Ecological Risk Assessments for Registered Pesticides [Internet]. [visited 2020 February]. Available from: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk#benchmarks

U.S. EPA. Chlorpyrifos [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/ingredients-used-pesticide-products/chlorpyrifos

U.S. EPA. Endocrine Disruption [Internet]. [visited 2019 October]. Available from: https://www.epa.gov/endocrine-disruption

U.S. EPA. Heptachlor Epoxide [Internet]. [visited 2020 March]. Available from: https://archive.epa.gov/epawaste/hazard/wastemin/web/pdf/hepchlep.pdf

U.S. EPA. Atrazine [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/ingredients-used-pesticide-products/atrazine

U.S. EPA. Norman List: Bisphenol Compounds [Internet]. [visited 2020 March]. Available from: https://comptox.epa.gov/dashboard/chemical_lists/bisphenols

U.S. EPA. Schedule Review for Neonicotinoid Pesticides [Internet]. [visited 2020 March]. Available from: https://www.epa.gov/pollinator-protection/schedule-review-neonicotinoid-pesticides

U.S. EPA CS. 2017b. 2017 Potable Reuse Compendium.

U.S. NIEHS. Endocrine Disruptors [Internet]. [visited 2019 October]. Available from: https://www.niehs.nih.gov/health/topics/agents/endocrine/index.cfm

USGS. 1997. Water-quality assessment of the Potomac River Basin: Analysis of available pesticide data, 1972-90.

USGS. Health-Based Screening Levels for Evaluating Water-Quality Data [Internet]. [visited 2020. Available from: https://cida.usgs.gov/hbsl/apex/f?p=104:1:::NO:::

USGS. Streamstats [Internet]. [visited 2019 October]. Available from: https://streamstats.usgs.gov/ss/

Watts M, Wickramasinghe SR, Qian X, Sardari K, Jones S. A bridge to potable reuse - How the biological treatment train at a WRRF affects the selection of additional advanced treatment and the removal of EDCs. 91st Annual Water Environment Federation Technical Exhibition and Conference, WEFTEC 2018, September 29, 2018 - October 3, 2018; 2019; New Orleans, LA, United states: Water Environment Federation. pp. 1984-1996.

Young J, Iwanowicz L, Sperry A, Blazer V. 2014. A landscape-based reconnaissance survey of estrogenic activity in streams of the upper Potomac, upper James, and Shenandoah Rivers, USA. Environmental Monitoring Assessment 186:5531-5545.

SUPPLEMENTARY INFORMATION

Table S1. UOSA SOC concentrations Summary.

UOSA									
Synthetic Organic Compounds	**Times Above Detection Limit**	**Times Analyzed**	**Percent Detection**	**Mean (ng/L)**	**Median (ng/L)**	**Maximum (ng/L)**	**Minimum (ng/L)**	**Range (ng/L)**	**Standard Deviation (ng/L)**
4,4 DDD	2	4	50%	0.32	0.32	0.34	0.31	0.03	0.01
4,4 DDE	2	4	50%	0.29	0.29	0.32	0.26	0.06	0.03
4-Nonylphenol	9	9	100%	140.74	128.71	300.50	47.53	252.97	79.39
4-Tert-Octylphenol	2	9	22%	9.02	9.02	17.86	0.18	17.68	8.84
Acetamiprid	9	9	100%	2.26	1.23	6.62	0.78	5.84	1.88
Acetochlor	4	12	33%	0.79	0.51	1.81	0.36	1.45	0.59
Alachlor	0	12	0%						
Atrazine	12	12	100%	10.97	11.89	16.10	1.29	14.81	4.22
Bisphenol A	2	9	22%	4.57	4.57	4.69	4.45	0.24	0.12
Bromacil	0	12	0%						
Butylate	0	4	0%						
Chlorpyrifos	0	12	0%						
Cis -Chlordane	2	4	50%	0.17	0.17	0.17	0.16	0.01	0.00
Clothianidin	9	9	100%	5.48	2.72	25.27	1.58	23.68	7.11
Coumaphos	0	9	0%						
Cycloate	0	12	0%						
Diazinon	4	12	33%	0.21	0.17	0.38	0.10	0.28	0.12
Dichlorvos	2	12	17%	13.36	13.36	18.73	7.99	10.74	5.37
Dieldrin	0	4	0%						
Dimethoate	1	9	11%	0.15	0.15	0.15	0.15	0.00	0.00
Dinotefuran	9	9	100%	23.23	22.44	30.65	18.73	11.92	3.52
Endrin Aldehyde	0	4	0%						
Ethoprop	1	9	11%	0.23	0.23	0.23	0.23	0.00	0.00
Fenchlorphos	3	4	75%	1.16	0.98	2.13	0.37	1.76	0.73
Fipronil	5	9	56%	0.66	0.54	1.49	0.23	1.26	0.43
Heptachlor	2	4	50%	1.04	1.04	1.76	0.31	1.45	0.73
Heptachlor Epoxide	2	4	50%	0.28	0.28	0.40	0.17	0.23	0.11
Imidacloprid	9	9	100%	35.33	36.36	41.17	23.99	17.19	5.90
Methyl Parathion	0	4	0%						
Metolachlor	12	12	100%	4.23	4.61	6.17	0.93	5.24	1.44
Parathion	0	4	0%						
Prometon	11	12	92%	3.91	3.46	8.58	1.17	7.41	1.89
Simazine	10	12	83%	4.26	4.23	7.18	1.99	5.18	1.81
Trans -Chlordane	2	4	50%	0.18	0.18	0.24	0.13	0.11	0.06
Tributylphosphorothithiote	3	9	33%	0.24	0.20	0.42	0.09	0.34	0.14

Table S2. Bull Run Upstream SOC concentrations Summary.

Bull Run Upstream									
Synthetic Organic Compounds	**Times Above Detection Limit**	**Times Analyzed**	**Percent Detection**	**Mean (ng/L)**	**Median (ng/L)**	**Maximum (ng/L)**	**Minimum (ng/L)**	**Range (ng/L)**	**Standard Deviation (ng/L)**
4,4 DDD	2	4	50%	0.45	0.45	0.66	0.24	0.42	0.21
4,4 DDE	2	4	50%	0.77	0.77	1.26	0.28	0.97	0.49
4-Nonylphenol	9	9	100%	161.47	167.87	264.66	58.37	206.29	62.30
4-Tert-Octylphenol	2	9	22%	19.81	19.81	34.27	5.36	28.91	14.46
Acetamiprid	5	9	56%	2.14	0.32	5.93	0.18	5.75	2.41
Acetochlor	2	12	17%	0.58	0.58	0.66	0.50	0.15	0.08
Alachlor	0	12	0%						
Atrazine	10	12	83%	4.61	1.72	24.60	0.51	24.09	6.97
Bisphenol A	4	9	44%	6.74	4.64	15.53	2.17	13.36	5.35
Bromacil	1	12	8%	2.96	2.96	2.96	2.96	0.00	0.00
Butylate	0	4	0%						
Chlorpyrifos	0	12	0%						
Cis -Chlordane	1	4	25%	0.22	0.22	0.22	0.22	0.00	0.00
Clothianidin	9	9	100%	3.32	2.93	6.86	0.96	5.91	2.05
Coumaphos	0	9	0%						
Cycloate	1	12	8%	0.13	0.13	0.13	0.13	0.00	0.00
Diazinon	5	12	42%	0.12	0.10	0.14	0.10	0.04	0.02
Dichlorvos	2	12	17%	6.31	6.31	7.10	5.51	1.59	0.80
Dieldrin	0	4	0%						
Dimethoate	0	9	0%						
Dinotefuran	9	9	100%	4.23	3.73	7.23	2.06	5.16	1.79
Endrin Aldehyde	0	4	0%						
Ethoprop	1	9	11%	0.12	0.12	0.12	0.12	0.00	0.00
Fenchlorphos	2	4	50%	1.21	1.21	1.34	1.09	0.25	0.12
Fipronil	8	9	89%	4.71	4.45	11.87	0.99	10.88	3.24
Heptachlor	0	4	0%						
Heptachlor Epoxide	1	4	25%	0.29	0.29	0.29	0.29	0.00	0.00
Imidacloprid	9	9	100%	21.71	9.00	57.39	3.85	53.54	19.08
Methyl Parathion	0	4	0%						
Metolachlor	11	12	92%	5.33	1.89	37.29	0.25	37.05	10.29
Parathion	0	4	0%						
Prometon	11	12	92%	2.96	2.17	5.61	0.61	5.00	1.81
Simazine	9	12	75%	4.03	1.71	20.68	0.58	20.10	6.13
Trans -Chlordane	0	4	0%						
Tributylphosphorothithiote	2	9	22%	0.26	0.26	0.28	0.25	0.03	0.02

Table S3. Bull Run Downstream SOC concentrations Summary.

Bull Run Downstream									
Synthetic Organic Compounds	**Times Above Detection Limit**	**Times Analyzed**	**Percent Detection**	**Mean (ng/L)**	**Median (ng/L)**	**Maximum (ng/L)**	**Minimum (ng/L)**	**Range (ng/L)**	**Standard Deviation (ng/L)**
4,4 DDD	2	4	50%	0.29	0.29	0.33	0.25	0.08	0.04
4,4 DDE	2	4	50%	0.32	0.32	0.36	0.28	0.08	0.04
4-Nonylphenol	9	9	100%	153.80	148.49	295.77	43.72	252.05	75.29
4-Tert-Octylphenol	4	9	44%	6.35	3.61	14.91	3.28	11.63	4.94
Acetamiprid	8	9	89%	1.64	0.64	5.28	0.34	4.94	1.85
Acetochlor	2	12	17%	1.04	1.04	1.47	0.60	0.87	0.43
Alachlor	0	12	0%						
Atrazine	11	12	92%	8.18	8.20	16.90	1.68	15.21	4.39
Bisphenol A	4	9	44%	15.97	14.01	28.48	7.37	21.11	7.72
Bromacil	0	12	0%						
Butylate	0	4	0%						
Chlorpyrifos	0	12	0%						
Cis -Chlordane	0	4	0%						
Clothianidin	9	9	100%	3.37	3.10	5.34	1.98	3.36	1.18
Coumaphos	0	9	0%						
Cycloate	0	12	0%						
Diazinon	4	12	33%	0.13	0.13	0.18	0.10	0.08	0.03
Dichlorvos	2	12	17%	10.16	10.16	10.24	10.08	0.16	0.08
Dieldrin	0	4	0%						
Dimethoate	0	9	0%						
Dinotefuran	9	9	100%	11.49	10.28	17.34	7.19	10.15	3.30
Endrin Aldehyde	0	4	0%						
Ethoprop	0	9	0%						
Fenchlorphos	2	4	50%	1.69	1.69	2.10	1.28	0.82	0.41
Fipronil	8	9	89%	4.08	3.69	9.63	0.78	8.86	2.57
Heptachlor	1	4	25%	0.44	0.44	0.44	0.44	0.00	0.00
Heptachlor Epoxide	1	4	25%	0.55	0.55	0.55	0.55	0.00	0.00
Imidacloprid	9	9	100%	27.12	25.61	54.03	10.33	43.71	13.92
Methyl Parathion	0	4	0%						
Metolachlor	12	12	100%	4.79	3.13	21.39	0.50	20.89	5.37
Parathion	0	4	0%						
Prometon	12	12	100%	3.47	3.42	6.01	0.77	5.24	1.53
Simazine	10	12	83%	3.78	2.75	9.22	1.52	7.71	2.28
Trans -Chlordane	0	4	0%						
Tributylphosphorothithiote	2	9	22%	0.25	0.25	0.27	0.24	0.03	0.01

Table S4. Corbalis SOC concentrations Summary.

Corbalis									
Synthetic Organic Compounds	**Times Above Detection Limit**	**Times Analyzed**	**Percent Detection**	**Mean (ng/L)**	**Median (ng/L)**	**Maximum (ng/L)**	**Minimum (ng/L)**	**Range (ng/L)**	**Standard Deviation (ng/L)**
4,4 DDD	1	4	25%	0.27	0.27	0.27	0.27	0.00	0.00
4,4 DDE	2	4	50%	0.34	0.34	0.40	0.28	0.12	0.06
4-Nonylphenol	8	9	89%	127.47	94.09	297.87	35.55	262.32	89.05
4-Tert-Octylphenol	3	9	33%	2.85	2.56	5.53	0.45	5.08	2.08
Acetamiprid	7	9	78%	1.10	0.22	3.75	0.09	3.66	1.49
Acetochlor	7	12	58%	0.76	0.65	1.92	0.26	1.66	0.52
Alachlor	0	12	0%						
Atrazine	11	12	92%	16.85	4.23	70.15	0.87	69.28	25.30
Bisphenol A	2	9	22%	5.74	5.74	7.77	3.71	4.06	2.03
Bromacil	0	12	0%						
Butylate	0	4	0%						
Chlorpyrifos	0	12	0%						
Cis -Chlordane	0	4	0%						
Clothianidin	9	9	100%	3.97	3.46	10.53	0.86	9.67	3.04
Coumaphos	0	9	0%						
Cycloate	0	12	0%						
Diazinon	5	12	42%	0.13	0.14	0.17	0.10	0.06	0.02
Dichlorvos	1	12	8%	3.96	3.96	3.96	3.96	0.00	0.00
Dieldrin	0	4	0%						
Dimethoate	0	9	0%						
Dinotefuran	7	9	78%	1.97	1.87	3.96	0.33	3.63	1.20
Endrin Aldehyde	0	4	0%						
Ethoprop	0	9	0%						
Fenchlorphos	2	4	50%	2.18	2.18	2.23	2.14	0.09	0.04
Fipronil	7	9	78%	1.88	1.21	4.02	0.21	3.80	1.42
Heptachlor	0	4	0%						
Heptachlor Epoxide	0	4	0%						
Imidacloprid	9	9	100%	12.38	14.47	28.85	0.73	28.12	10.48
Methyl Parathion	0	4	0%						
Metolachlor	12	12	100%	13.68	4.69	63.80	1.38	62.42	19.15
Parathion	0	4	0%						
Prometon	12	12	100%	2.54	2.46	4.59	0.33	4.26	1.33
Simazine	9	12	75%	12.85	4.15	51.76	0.81	50.95	18.82
Trans -Chlordane	0	4	0%						
Tributylphosphorothithiote	2	9	22%	0.27	0.27	0.28	0.26	0.01	0.01

Table S5. Griffith SOC concentrations Summary.

Griffith									
Synthetic Organic Compounds	**Times Above Detection Limit**	**Times Analyzed**	**Percent Detection**	**Mean (ng/L)**	**Median (ng/L)**	**Maximum (ng/L)**	**Minimum (ng/L)**	**Range (ng/L)**	**Standard Deviation (ng/L)**
4,4 DDD	2	4	50%	0.25	0.25	0.33	0.18	0.15	0.07
4,4 DDE	1	4	25%	0.22	0.22	0.22	0.22	0.00	0.00
4-Nonylphenol	9	9	100%	109.55	109.09	259.04	28.32	230.72	70.05
4-Tert-Octylphenol	2	9	22%	1.70	1.70	2.08	1.31	0.76	0.38
Acetamiprid	8	9	89%	0.65	0.33	3.06	0.11	2.95	0.92
Acetochlor	4	12	33%	1.21	1.15	1.71	0.82	0.90	0.36
Alachlor	0	12	0%						
Atrazine	11	12	92%	87.40	15.95	394.13	4.44	389.69	134.24
Bisphenol A	2	9	22%	8.20	8.20	11.63	4.77	6.86	3.43
Bromacil	0	12	0%						
Butylate	0	4	0%						
Chlorpyrifos	1	12	8%	0.19	0.19	0.19	0.19	0.00	0.00
Cis -Chlordane	0	4	0%						
Clothianidin	9	9	100%	5.32	3.76	11.56	1.81	9.76	3.38
Coumaphos	0	9	0%						
Cycloate	0	12	0%						
Diazinon	3	12	25%	0.16	0.16	0.22	0.10	0.11	0.05
Dichlorvos	1	12	8%	3.32	3.32	3.32	3.32	0.00	0.00
Dieldrin	0	4	0%						
Dimethoate	0	9	0%						
Dinotefuran	9	9	100%	3.39	3.31	6.72	1.40	5.32	1.46
Endrin Aldehyde	0	4	0%						
Ethoprop	0	9	0%						
Fenchlorphos	1	4	25%	0.72	0.72	0.72	0.72	0.00	0.00
Fipronil	6	9	67%	1.97	1.70	3.25	1.17	2.08	0.73
Heptachlor	0	4	0%						
Heptachlor Epoxide	1	4	25%	0.17	0.17	0.17	0.17	0.00	0.00
Imidacloprid	9	9	100%	26.46	25.27	56.87	6.22	50.65	18.39
Methyl Parathion	0	4	0%						
Metolachlor	12	12	100%	34.25	12.39	171.07	5.70	165.37	46.65
Parathion	0	4	0%						
Prometon	11	12	92%	5.33	5.36	9.66	1.20	8.46	2.42
Simazine	11	12	92%	8.53	3.42	39.45	1.50	37.95	10.62
Trans -Chlordane	0	4	0%						
Tributylphosphorothithiote	2	9	22%	0.24	0.24	0.27	0.20	0.06	0.03

Table S6. Targeted analytes and their corresponding limits of reporting. Gas chromatography–mass spectrometry/mass spectrometry (GC-MS/MS) and Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) methods limits of reporting (LOR) of estrogen hormones and synthetic organic compounds (SOCs).

Targeted analytes	Abbreviation	Compound class	Method of Analysis	LOR (ng/L)
Estrone	E1	Estrogen	LC-MS/MS	0.1
17α-estradiol	E2α	Estrogen	LC-MS/MS	0.1
17β-estradiol	E2β	Estrogen	LC-MS/MS	0.1
17β-Estriol	E3	Estrogen	LC-MS/MS	0.1
17α-Ethinylestradiol	EE2	Estrogen	LC-MS/MS	0.1
estrone-3-sulfate	E1-3S	Estrogen	LC-MS/MS	0.1
estrone-3-glucuronide	E1-3G	Estrogen	LC-MS/MS	0.1
17β-estradiol -17-sulfate	E2-17S	Estrogen	LC-MS/MS	0.1
17α-estradiol -3-sulfate	E2α-3S	Estrogen	LC-MS/MS	0.1
17β-estradiol -3-sulfate	E2β-3S	Estrogen	LC-MS/MS	0.1
17β-estradiol-3-glucuronide	E2-3G	Estrogen	LC-MS/MS	0.1
17α-ethinylestradiol-3-glucuronide	EE2-3G	Estrogen	LC-MS/MS	0.1
17α-Ethinylestradiol-d4	EE2-d4 (ISTD)	Estrogen (standard)	LC-MS/MS	0.1
17β-estradiol-d3	E2-d3 (ISTD)	Estrogen (standard)	LC-MS/MS	0.1
Estrone-d4	E1-d4 (ISTD)	Estrogen (standard)	LC-MS/MS	0.1
17β-estradiol-3-sulfate-d4	E2-3S-d4 (ISTD)	Estrogen (standard)	LC-MS/MS	0.1
17β-estadiol-3-glucuronide-d3	E2-3G-d3 (ISTD)	Estrogen (standard)	LC-MS/MS	0.1
Estrone-3-sulfate-d4	E1-3S-d4 (ISTD)	Estrogen (standard)	LC-MS/MS	0.1
4,4 DDD		SOC	GC-MS/MS	0.1
4,4 DDE		SOC	GC-MS/MS	0.1
4-Nonylphenol		SOC	LC-MS/MS	0.1
4-*Tert* -Octylphenol		SOC	LC-MS/MS	0.1
Acetamiprid		SOC	LC-MS/MS	0.1
Acetochlor		SOC	GC-MS/MS and LC-MS/MS	0.1
Alachlor		SOC	GC-MS/MS and LC-MS/MS	0.1
Atrazine		SOC	GC-MS/MS and LC-MS/MS	0.1
Bisphenol A		SOC	LC-MS/MS	0.1
Bromacil		SOC	GC-MS/MS and LC-MS/MS	0.1
Butylate		SOC	GC-MS/MS	0.1
Chlorpyrifos		SOC	GC-MS/MS and LC-MS/MS	0.1
Cis -Chlordane		SOC	GC-MS/MS	0.1
Clothianidin		SOC	LC-MS/MS	0.1
Coumaphos		SOC	LC-MS/MS	0.1
Cycloate		SOC	GC-MS/MS and LC-MS/MS	0.1
Diazinon		SOC	GC-MS/MS and LC-MS/MS	0.1
Dichlorvos		SOC	GC-MS/MS and LC-MS/MS	0.1
Dieldrin		SOC	GC-MS/MS	0.1
Dimethoate		SOC	LC-MS/MS	0.1
Dinotefuran		SOC	LC-MS/MS	0.1
Endrin Aldehyde		SOC	GC-MS/MS	0.1
Ethoprophos	Ethoprop	SOC	LC-MS/MS	0.1
Fenchlorphos		SOC	GC-MS/MS	0.1
Fipronil		SOC	LC-MS/MS	0.1
Heptachlor		SOC	GC-MS/MS	0.1
Heptachlor Epoxide		SOC	GC-MS/MS	0.1
Imidacloprid		SOC	LC-MS/MS	0.1
Methyl Parathion		SOC	GC-MS/MS	0.1
Metolachlor		SOC	GC-MS/MS and LC-MS/MS	0.1
Parathion		SOC	GC-MS/MS	0.1
Prometon		SOC	GC-MS/MS and LC-MS/MS	0.1
Simazine		SOC	GC-MS/MS and LC-MS/MS	0.1
Trans -Chlordane		SOC	GC-MS/MS	0.1
Tributylphosphorotrithioate	Tribufos	SOC	LC-MS/MS	0.1

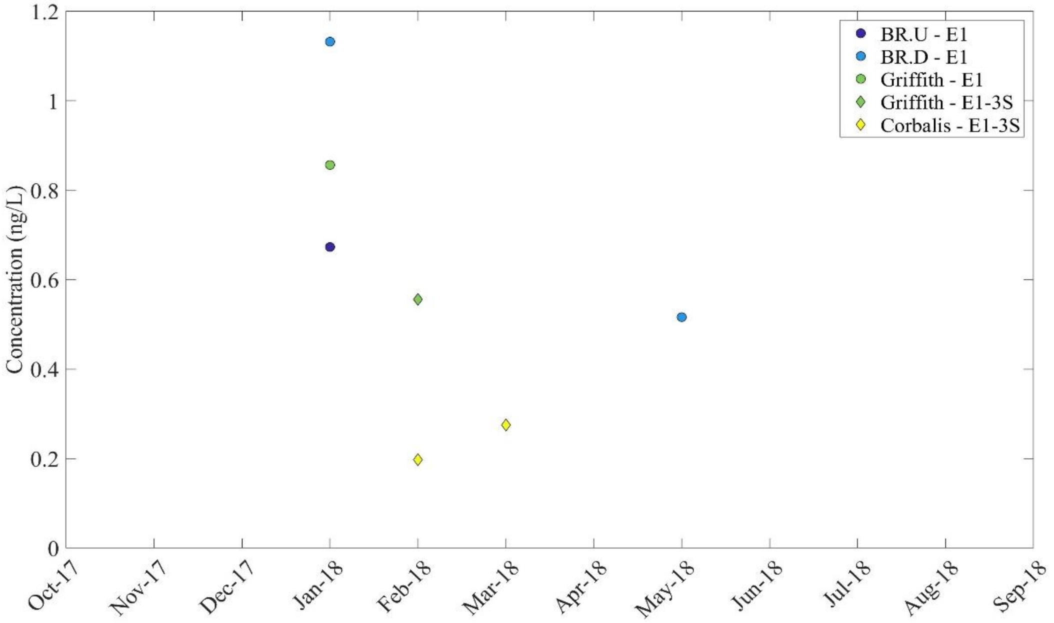


Figure S1. Estrogen hormone concentrations detected at Bull Run Downstream, Bull Run Upstream, Griffth and Corbalis sampling sites. Estrogen hormones concentrations (y-axis) are plotted according to the sampling month detected (x-axis). The symbol color corresponds with location and shape corresponds with type: E1(estrone) and E1-3S (estrone-3-sulfate) (see legend). No estrogen analytes were detected at UOSA.

Table S7. Values for U.S. EPA and USGS Benchmarks. Available U.S. EPA acute aquatic life benchmarks (ALBs) and maximum contaminant levels (MCLs) were presented for available SOCs (U.S. EPA 2019a).

		U.S. EPA Acute Aquatic Life Benchmarkers (ug/L) (freshwater)			
SOCs	U.S. EPA MCL (ug/L)	Fish	Invertebrates	Nonvascular Plants	Vascular Plants
4,4 DDD					
4,4 DDE					
4-Nonylphenol					
4-Tert-Octylphenol					
Acetamiprid		50000	10.5	1000	1000
Acetochlor		190	4100	1.43	3.4
Alachlor	2	900	1250	1.64	2.3
Atrazine	3	2650	360	1	4.6
Bisphenol A (BPA)					
Bromacil		18000	60500	6.8	45
Butylate		105	5500		4600
Chlorpyrifos		0.9	0.05	140	
Cis -Chlordane	2				
Clothianidin		50750	11	64000	280000
Coumaphos		170	0.037		166
Cycloate		2250	1200		
Diazinon		45	0.105	3700	
Dichlorvos		91.5	0.035	14000	
Dieldrin					
Dimethoate		3100	21.5	20000	92600
Dinotefuran		49550	484150	97600	110000
Endrin Aldehyde	2				
Ethoprop		150	22	8400	
Fenchlorphos					
Fipronil		41.5	0.11	140	100
Heptachlor	0.4				
Heptachlor Epoxide	2				
Imidacloprid		114500	0.385		
Methyl Parathion		925	0.485	15000	18000
Metolachlor		1900	550	8	21
Parathion					
Prometon		6000	12850	98	
Simazine	4	3200	500	6	67
Trans -Chlordane	2				
Tribufos	2	141.5	2.7	148	1100

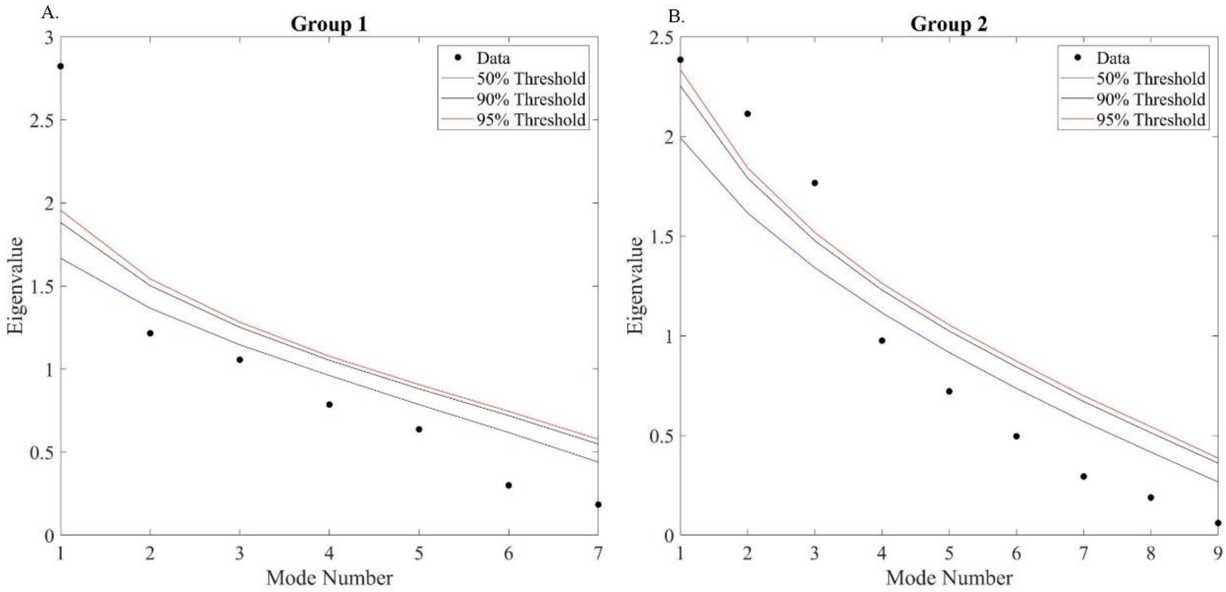


Figure S2. Stopping rule for SOC concentrations at UOSA discharge and Bull Run sites. A) Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018). Principal component modes (x-axis) are plotted by their eigenvalue. Eigenvalue data (black dots) are plotted along with resampling-based stopping rule resulting thresholds (blue, black, and red lines). In Group 1, one PC mode is above the 95% threshold, so it is significant at a $p<0.05$ level (red line). In Group 2, three PC modes are significant at a $p<0.05$ level. The remaining PC modes below the 50% threshold (blue line) and are considered random.

Table S8. Bootstrapped 95% confidence interval bounds for for SOC concentrations at UOSA discharge and Bull Run sites. Confidence interval bounds calculated by using non-parametric bootstrapping method determined by Babamoradi et al. 2013. **A)** Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018).

A.

	SOCs Sampled 12 months	
	Lower 95% CB	**Upper 95% CB**
Acetochlor	-0.349	0.819
Atrazine	0.418	0.693
Diazinon	-0.337	0.907
Dichlorvos	-0.730	0.453
Metolachlor	-0.053	0.761
Prometon	0.063	0.954
Simazine	0.396	0.812

B.

	SOCs Sampled 9 months					
	PC1		**PC2**		**P3**	
	Lower 95% CB	**Upper 95% CB**	**Lower 95% CB**	**Upper 95% CB**	**Lower 95% CB**	**Upper 95% CB**
4-Nonylphenol	-0.592	0.263	0.397	0.789	-0.510	0.375
4-tert-octylphenol	-0.706	0.321	-0.754	-0.029	-0.479	0.548
Acetamiprid	-0.137	0.651	-0.777	-0.269	-0.570	0.223
Bisphenol A	-0.739	0.663	-0.743	0.160	0.107	0.876
Clothianidin	0.191	0.846	-0.492	0.356	0.250	0.748
Dinotefuran	0.461	0.723	-0.419	0.436	-0.615	0.108
Fipronil	-0.573	0.332	-0.514	0.381	0.603	0.856
Imidacloprid	0.452	0.806	-0.299	0.517	-0.275	0.524
Tribufos	-0.356	0.655	0.195	0.830	-0.317	0.586

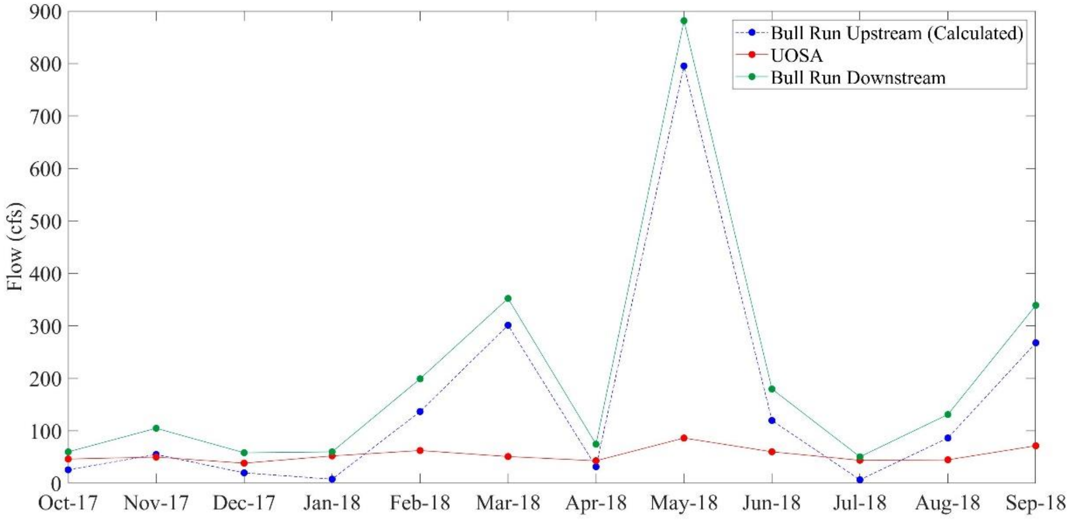


Figure S3. Flow of UOSA discharge and Bull Run. Daily flow for the dates sampled plotted for UOSA discharge (red solid line), Bull Run Downstream (green solid line), and Bull Run Upstream calculated flow (dashed blue line).

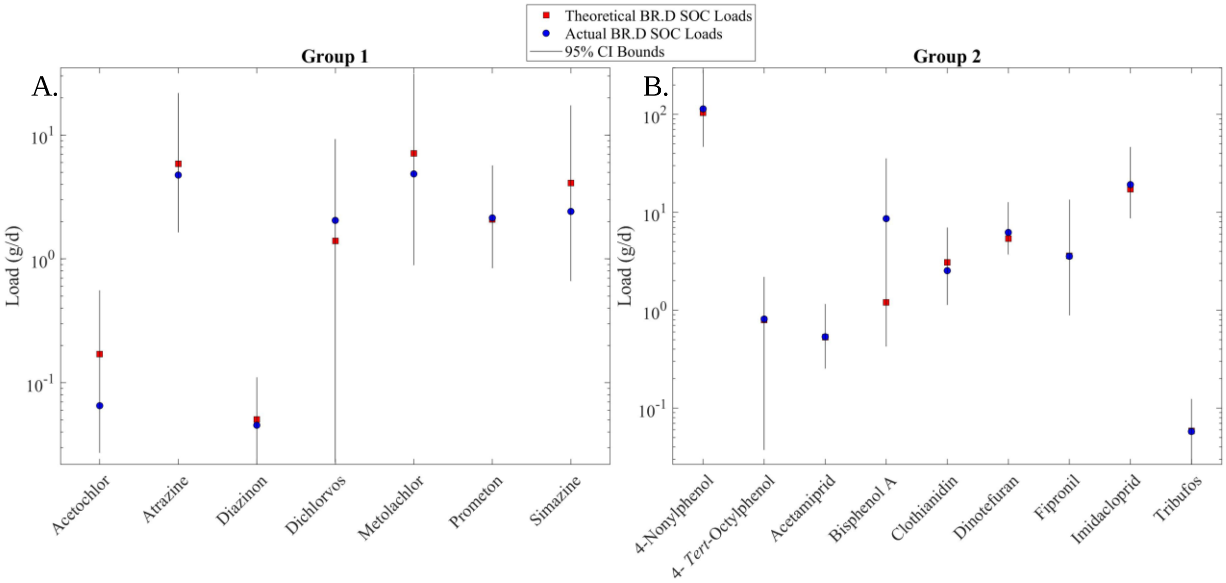


Figure S4. Significance of theoretical and actual SOC loads at Bull Run Downstream. Mean and bootsrapped 95% confidence intervals for SOC loads for the actual and theoretical load values at Bull Run Downstream. **A)** Shows Group 1 SOC loads and **B)** Shows Group 2 SOC loads.

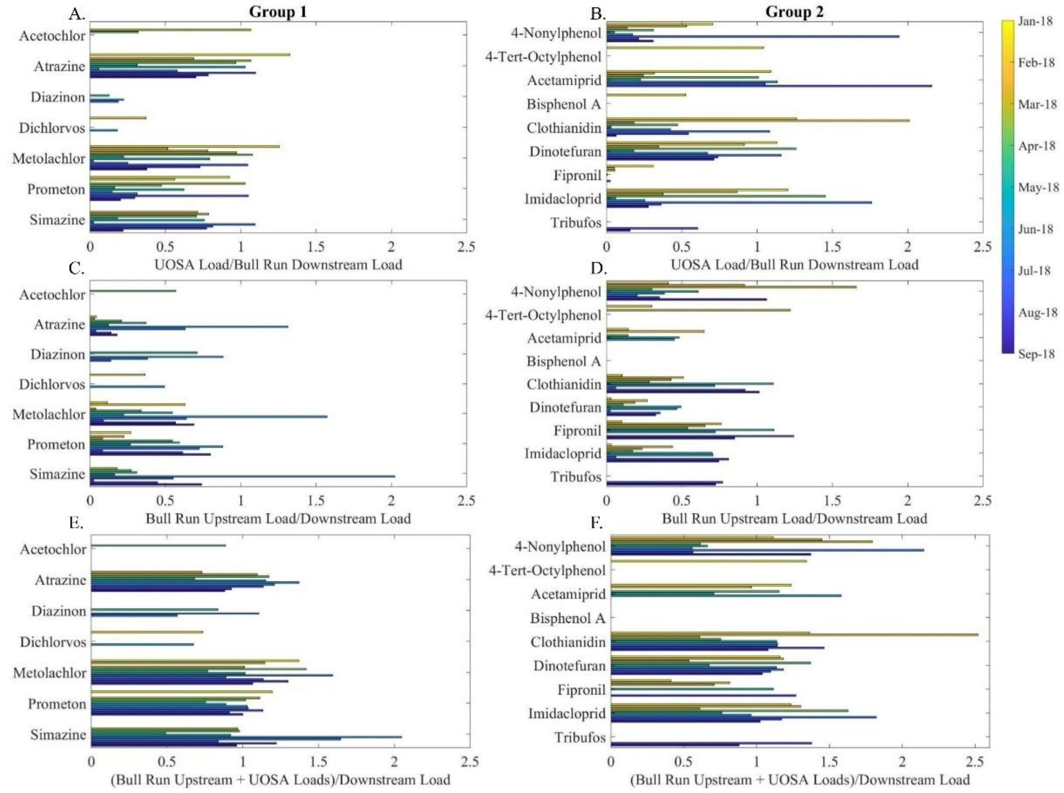
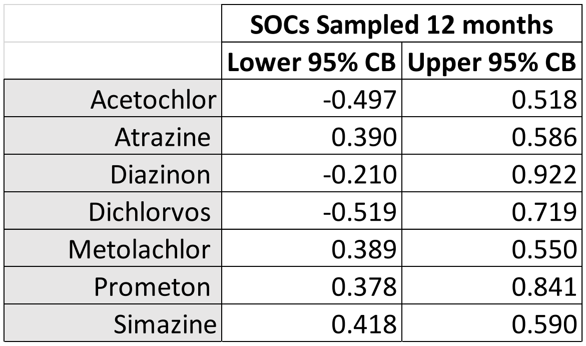


Figure S5. Average SOC loads for 3 scenarios. The mean load was calculated for each SOC in Group 1 and Group 2 for three scenarios: **A-B)** the ratio of Bull Run Upstream and UOSA to Bull Run Downstream ((BR.U+UOSA)/BR.D), **C-D)** the ratio of Bull Run Upstream to Bull Run Downstream (BR.U/BR.D), and **E-F)** the ratio of UOSA to Bull Run Downstream (UOSA/BR.D).

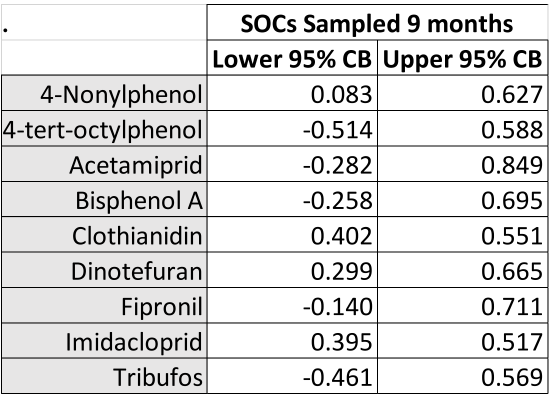
Table S9. Bootstrapped 95% confidence interval bounds for SOC loads at UOSA discharge and Bull Run sites. Confidence interval bounds calculated by using non-parametric bootstrapping method determined by Babamoradi et al. 2013. **A)** Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018).

A.



	SOCs Sampled 12 months	
	Lower 95% CB	Upper 95% CB
Acetochlor	-0.497	0.518
Atrazine	0.390	0.586
Diazinon	-0.210	0.922
Dichlorvos	-0.519	0.719
Metolachlor	0.389	0.550
Prometon	0.378	0.841
Simazine	0.418	0.590

B.



	SOCs Sampled 9 months	
	Lower 95% CB	Upper 95% CB
4-Nonylphenol	0.083	0.627
4-tert-octylphenol	-0.514	0.588
Acetamiprid	-0.282	0.849
Bisphenol A	-0.258	0.695
Clothianidin	0.402	0.551
Dinotefuran	0.299	0.665
Fipronil	-0.140	0.711
Imidacloprid	0.395	0.517
Tribufos	-0.461	0.569

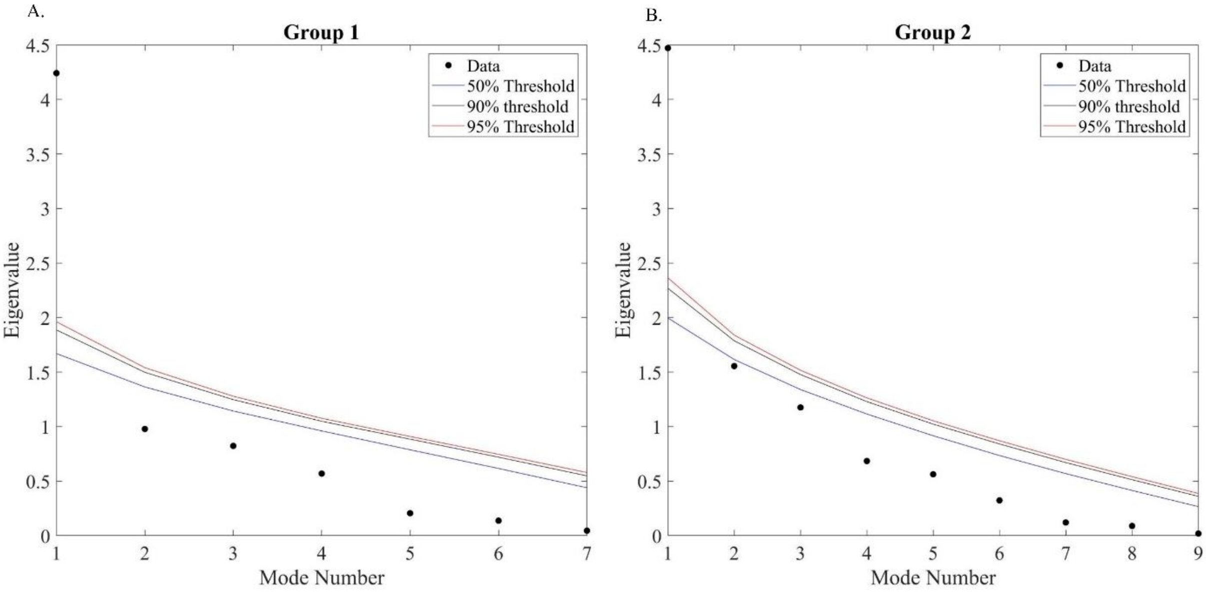


Figure S6. Stopping rule for SOC loads at UOSA discharge and Bull Run sites. A) Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018). Principal component modes (x-axis) are plotted by their eigenvalue. Eigenvalue data (black dots) are plotted along with resampling-based stopping rule resulting thresholds (blue, black, and red lines). In both Group 1 and Group 2, one PC mode is above the 95% threshold, so it is significant at a p<0.05 level (red line). The remaining PC modes below the 50% threshold (blue line) and are considered random.

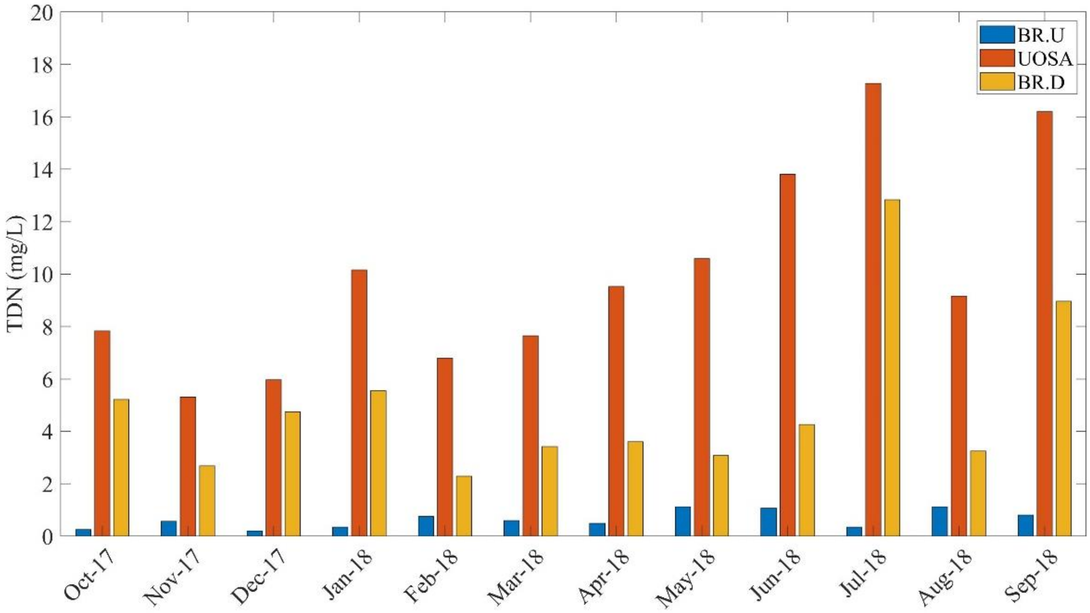


Figure S7. TDN at UOSA discharge and Bull Run sites. Total dissolved nitrogen (TDN) in mg/L from samples at Bull Run Upstream (BR.U), UOSA discharge, and Bull Run Downstream (BR.D) plotted along the sampling timeline (blue, red, and orange bars).

Table S10. Bootstrapped 95% confidence interval bounds for SOC concentrations at WTP intakes. Confidence interval bounds calculated by using non-parametric bootstrapping method determined by Babamoradi et al. 2013. **A)** Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018).

A.

	SOCs Sampled 12 months	
	Lower 95% CB	**Upper 95% CB**
Acetochlor	-0.781	0.428
Atrazine	0.333	0.640
Diazinon	-0.541	0.592
Metolachlor	-0.383	0.602
Prometon	0.191	0.754
Simazine	0.054	0.658

B.

	SOCs Sampled 9 months	
	Lower 95% CB	**Upper 95% CB**
4-Nonylphenol	-0.983	0.079
4-tert-octylphenol	-0.704	0.002
Acetamiprid	-0.540	0.444
Bisphenol A	-0.218	0.661
Clothianidin	0.319	0.770
Dinotefuran	0.217	0.664
Fipronil	0.112	0.698
Imidacloprid	0.438	0.628
Tribufos	-0.102	0.626

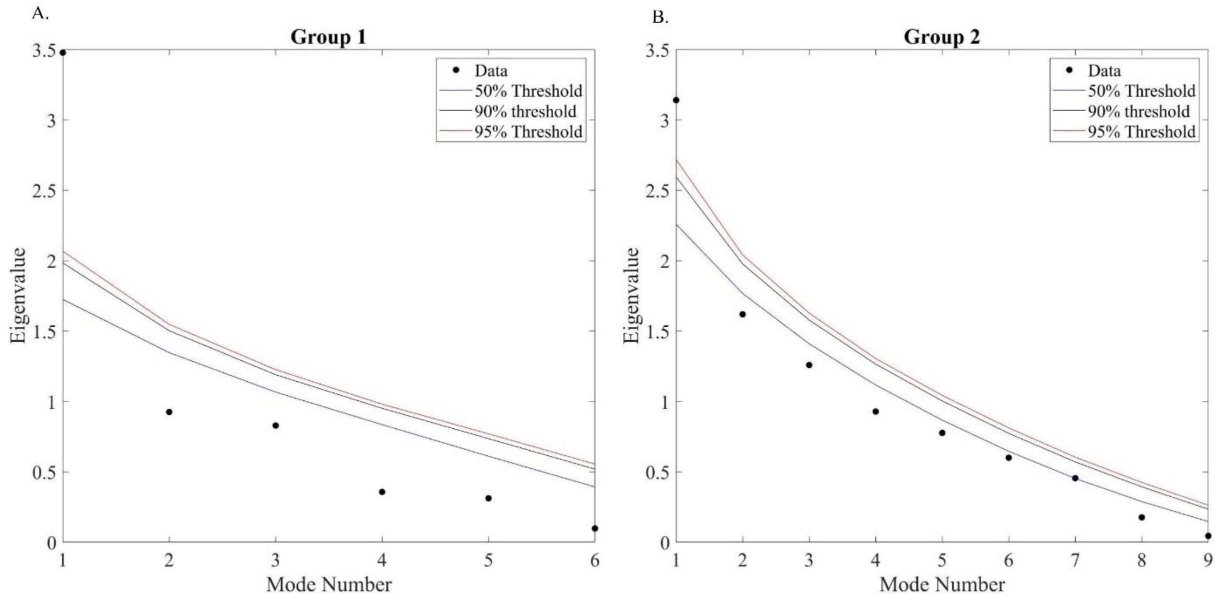


Figure S8. Stopping rule for SOC concentrations at WTP raw water intakes. A) Group 1 SOCs (monitored for complete 12-month period) **B)** Group 2 SOCs (monitored from January to September, 2018). Principal component modes (x-axis) are plotted by their eigenvalue. Eigenvalue data (black dots) are plotted along with resampling-based stopping rule resulting thresholds (blue, black, and red lines). In both Group 1 and Group 2, one PC mode is above the 95% threshold, so it is significant at a $p<0.05$ level (red line). The remaining PC modes below the 50% threshold (blue line) and are considered random.

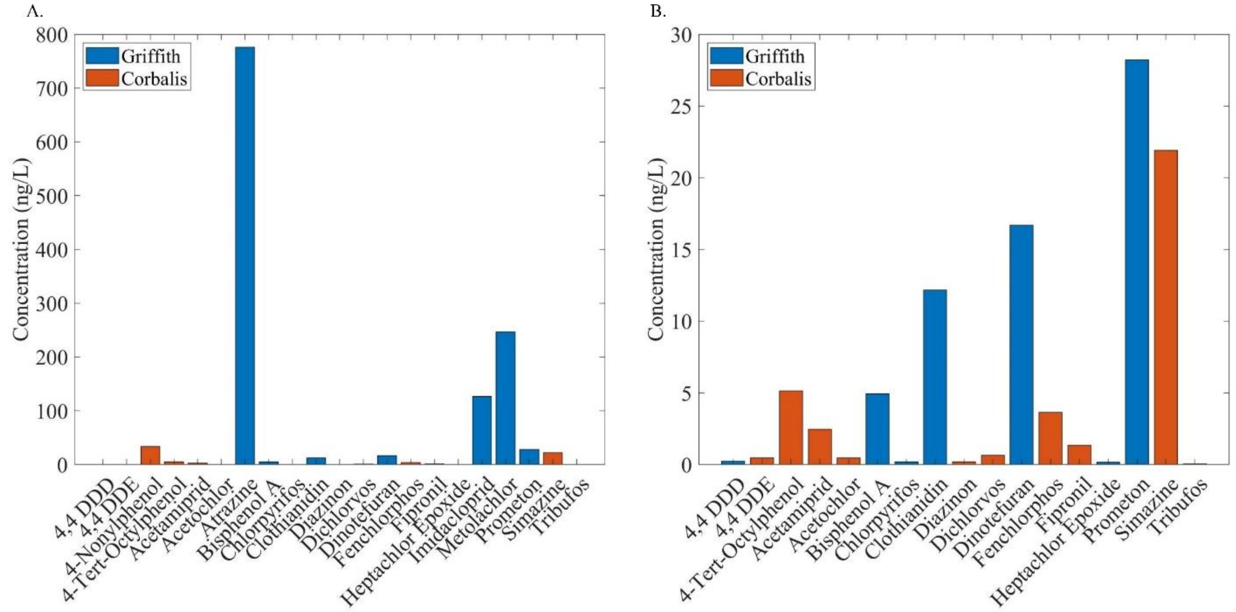


Figure S9. Difference in concentration per SOC at WTP Intakes. **A)** The difference between the sum of each SOC at each water treatment plant was plotted. If the difference was attributed to Griffith, the bar is blue. If it was attributed to Corbalis, the bar is orange. **B)** 4-Nonylphenol, atrazine, imidacloprid, and metolachlor were removed from to see SOCs present at lower concentrations.

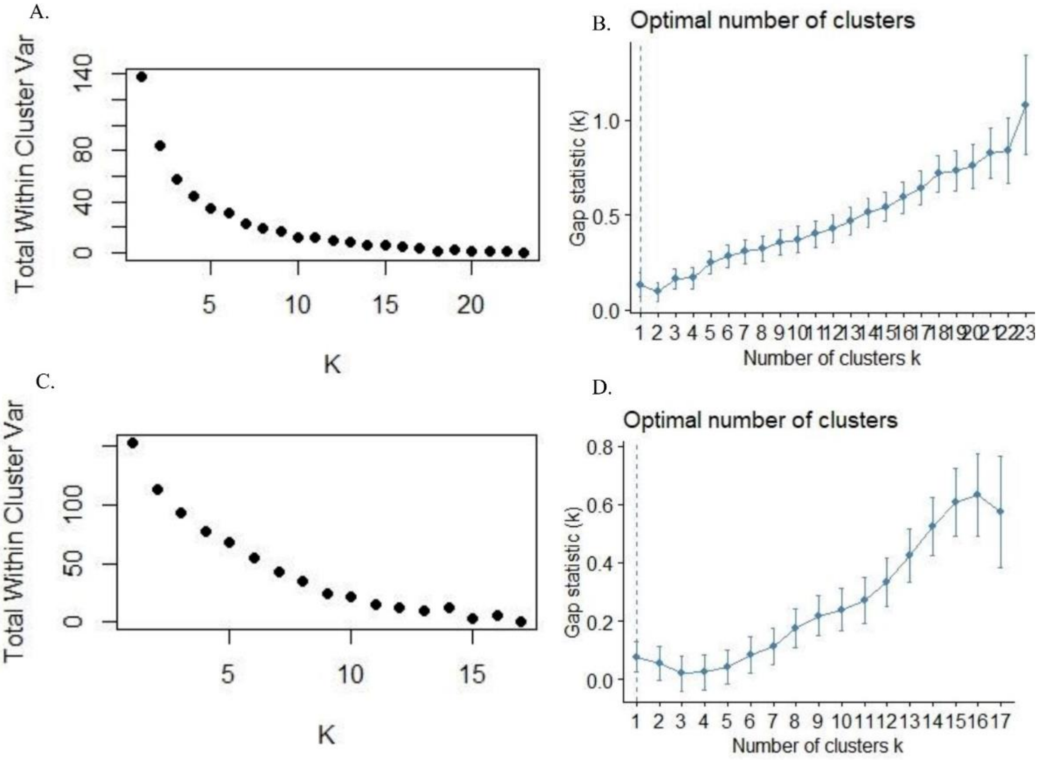


Figure S10. Visual methods to determine number of clusters for heat maps. Group 1, SOCs monitored for the full 12 months, heat map had 6 clusters determined by the elbows in **A)** Group 1 scree plot and **B)** Group 1 gap statistics plot. Group 2, SOCs monitored for the 9 months, heat map also had 6 clusters determined by the elbows in **C)** Group 2 scree plot and **D)** Group 2 gap statistics plot.

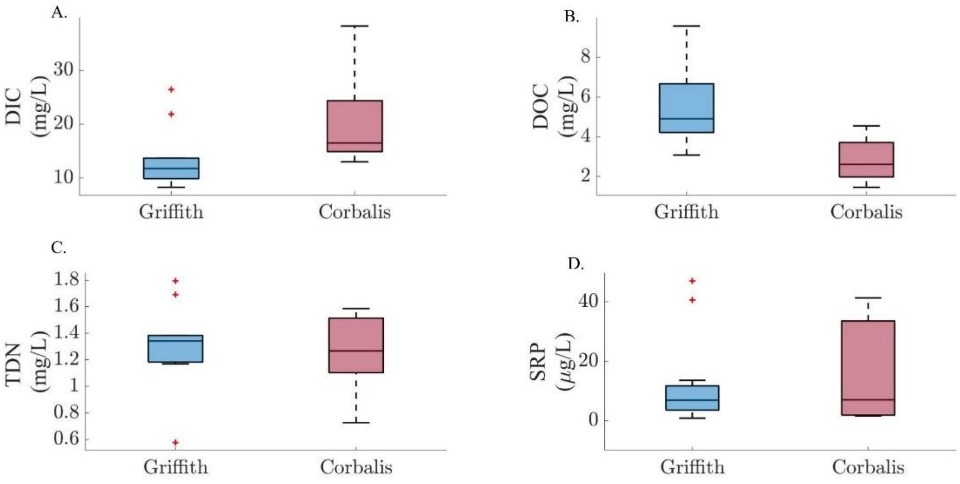


Figure S11. Box and whisker plots representing nutrient concentrations at WTP intakes. The center line of the boxplot represents the median nutrient concentration, and the bottom and top represent the 25th and 75th percentile, respectively. The whiskers represent the remaining data, with outliers represented as red plus signs. **A)** Dissolved Inorganic Carbon (DIC) in mg/L. **B)** Dissolved Organic Carbon (DOC) in mg/L. **C)** Total Dissolved Nitrogen (TDN) in mg/L. **D)** Soluble Reactive Phosphorus (SRP) in µg/L.

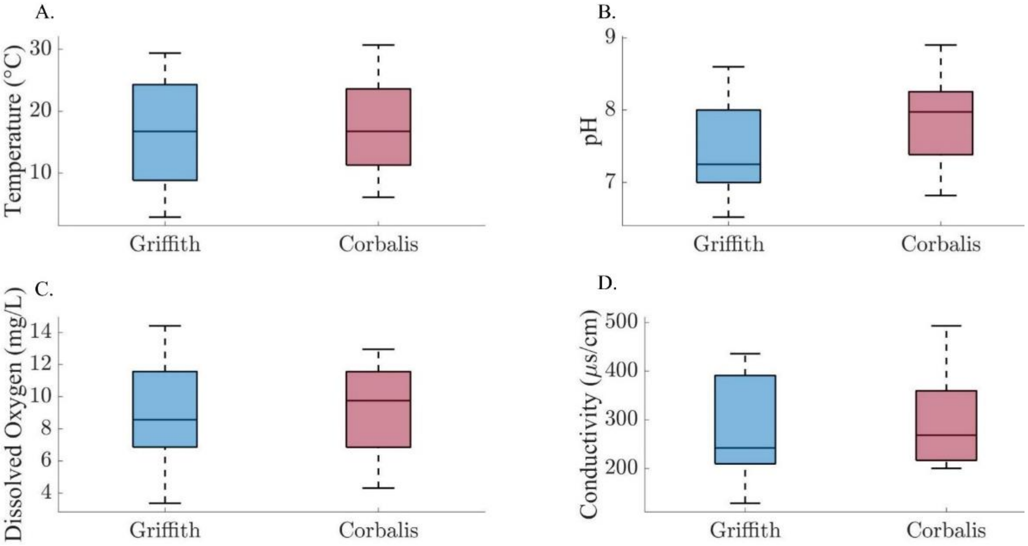


Figure S12. Box and whisker plots representing water quality parameters measured *in situ* at WTP intakes. The center line of the boxplot represents the median parameter measurement, and the bottom and top represent the 25th and 75th percentile, respectively. The whiskers represent the remaining data, with outliers represented as red plus signs. **A)** Temperature (°C) **B)** pH **C)** Dissolved Oxygen (DO) in mg/L **D)** Conductivity in µg/cm.

Month	April	August	September
Location	**Trip Blank**	**Trip Blank**	**Bull Run D Field Blank**
E1	ND	ND	ND
E1-3S	ND	ND	ND
4-Nonylphenol	255.30	ND	ND
4-tert-octylphenol	ND	ND	ND
Acetamiprid	ND	ND	ND
Acetochlor	ND	ND	ND
Alachlor	ND	ND	ND
Atrazine	ND	ND	ND
Bisphenol A	1.75	ND	ND
Bromacil	ND	ND	ND
Chlorpyrifos	ND	ND	ND
Clothianidin	0.08	ND	ND
Coumaphos	ND	ND	ND
Cycloate	ND	ND	ND
Diazinon	ND	ND	ND
Dichlorvos	ND	ND	ND
Dimethoate	ND	ND	ND
Dinotefuran	0.92	ND	ND
Ethoprop	ND	ND	ND
Fipronil	ND	ND	ND
Imidacloprid	ND	ND	ND
Metolachlor	1.64	ND	1.35
Prometon	0.20	0.09	0.09
Simazine	0.06	ND	0.13
Tributylphosphorothithiote	ND	ND	ND

Figure S13. Trip blanks and field blank EDC results. Their results showed no significant detections.