In cooperation with the West Virginia Department of Environmental Protection, Division of Water and Waste Management and the West Virginia Division of Natural Resources

A Reconnaissance for Emerging Contaminants in the South Branch Potomac River, Cacapon River, and Williams River Basins, West Virginia, April-October 2004

By Douglas B. Chambers and Thomas J. Leiker

Open-File Report 2006-1393

U.S. Department of the Interior
U.S. Geological Survey

U.S. Department of the Interior
Dirk Kempthorne, Secretary

U.S. Geological Survey
Mark D. Myers, Director

U.S. Geological Survey, Reston, Virginia 2006

For product and ordering information:
World Wide Web: http://www.usgs.gov/pubprod
Telephone: 1-888-ASK-USGS

For more information on the USGS—the Federal source for science about the Earth, its natural and living resources, natural hazards, and the environment:
World Wide Web: http://www.usgs.gov
Telephone: 1-888-ASK-USGS

Suggested citation:
Chambers, D.B., and Leiker, T. J., 2006, A Reconnaissance for Emerging Contaminants in the South Branch Potomac River, Cacapon River, and Williams River Basins, West Virginia, April-October 2004: U.S. Geological Survey Open-File Report 2006-1393, 23 p. http://pubs.usgs.gov/of/2006/1393

Contents

Figures

Tables

Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Area	
acre	0.4047	hectare (ha)
square mile (mi^2)	259.0	hectare (ha)
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
gallon (gal)	3.785	liter (L)
cubic foot (ft^3)	0.02832	cubic meter (m^3)
cubic yard (yd^3)	0.7646	cubic meter (m^3)
	Mass	
ounce, avoirdupois (oz)	28.35	gram (g)
pound, avoirdupois (lb)	0.4536	kilogram (kg)
	Pressure	
inch mercury (in Hg)	25.4	Torr

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

°F=(1.8×°C)+32

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

°C=(°F-32)/1.8

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

A Reconnaissance for Emerging Contaminants in the South Branch Potomac River, Cacapon River, and Williams River Basins, West Virginia, April-October 2004

By Douglas B. Chambers, and Thomas J. Leiker

Abstract

In 2003 a team of scientists from West Virginia Division of Natural Resources and the U. S. Geological Survey found a high incidence of an intersex condition, oocytes in the testes, among smallmouth bass (*Micropterus dolomieu*) in the South Branch Potomac River and the Cacapon River of West Virginia, indicating the possible presence of endocrine-disrupting compounds (EDCs). Possible sources of EDCs include municipal and domestic wastewater, and agricultural and industrial activities. Several sampling strategies were used to identify emerging contaminants, including potential EDCs, and their possible sources in these river basins and at an out-of-basin reference site. Passive water-sampling devices, which accumulate in-stream organic chemical compounds, were deployed for 40-41 days at 8 sampling sites. Sampler extracts were analyzed for a broad range of polar and non-polar organic compounds including pesticides, flame retardants, pharmaceuticals, and personal-care products. Analysis of passive-sampler extracts found 4 compounds; hexachloro-benzene; pentachloroanisole; 2,2',4,4',5-penta-bromo-diphenyl ether (BDE 47); and 2,2',4,4',6-penta-bromo-diphenyl ether (BDE 99) to be present at every sampled site, including the reference site, and several sites had detectable quantities of other compounds. No detectable quantity of any antibiotics was found in any passive-sampler extract. Effluent samples were analyzed for 39 antibiotics as tracers of human and agricultural waste. Additionally, poultry-processing plant effluent was sampled for roxarsone, an organoarsenic compound used as a poultry-feed additive, and other arsenic species as tracers of poultry waste. Antibiotics were detected in municipal wastewater, aquaculture, and poultry-processing effluent, with the highest number of antibiotics and the greatest concentrations found in municipal effluent. Arsenate was the only arsenic species detected in the poultry-processing plant effluent, at a concentration of 1.0 µg/L. Water samples were collected from 7 stream sites and analyzed for arsenic species, including roxarsone. Arsenate was detected in samples from 6 of the 7 stream samples, in concentrations ranging from 0.3 to 0.5 µg/L. Additionally, the analysis of smallmouth bass blood plasma for potential EDCs indicated the presence of several compounds including some found in the passive sampler extracts, specifically BDE 47 and BDE 99. Data from this reconnaissance will help to focus efforts for further studies of the occurrence of emerging contaminants, EDCs, and intersex in smallmouth bass in these Potomac River tributaries.

Introduction

In 2003 West Virginia Division of Natural Resources (WVDNR) officials and United States Geological Survey (USGS) Leetown Science Center scientists examining fish kills and widespread incidences of fish lesions in the South Branch Potomac River (fig. 1), discovered many reproductive anomalies among smallmouth bass (*Micropterus dolomieu*) (Vicki Blazer, USGS, written communication, 2004). These anomalies were both widespread and pervasive within communities and included oocytes, cells that are precursors to eggs, in the testes of male fish. Abnormal development of reproductive tissues may result from disruptions of the endocrine system brought about by exposure to anthropogenic sources of hormones and synthetic compounds that mimic hormones including ethynyl estradiol, nonyl phenol, polybrominated diphenyl ethers. Earlier studies by USGS researchers had found feminization of male common carp (*Cyprinus carpio*) in the Shenandoah River near Millville, WV, another tributary of the Potomac River (Goodbred and others, 1997).

Several classes of synthetic organic compounds to abnormal development or disturbances of the endocrine system in fish and other aquatic vertebrates (Jobling and others, 2002; Sheahan and others, 2002; Orlando and others, 2004; Nash and others, 2004; Scheurs and others, 2004; Melo and Ransdell, 2001; Kavanagh and others, 2004; Toft and others, 2003; Bevan and others, 2003). These compounds, known as endocrine-disrupting compounds (EDCs), mimic natural hormones and interfere with an organism's internal signaling and regulatory systems. Excess quantities of natural hormones, derived from people or livestock, can also enter the environment and potentially effect aquatic organisms (Nichols and others, 1997; Dyer and others, 2001; Carballa and others, 2004; Hutchison and others, 2005; Raman and others, 2004; Hanselman and others, 2003). EDCs and natural hormones can enter the environment through agricultural, industrial, municipal, and domestic wastes (Kolpin and others, 2002, Kolodziej and others, 2004).

Histopathologic examinations of smallmouth bass testes collected from the South Branch Potomac, Potomac, and Cacapon Rivers in 2004 (fig. 1) found an intersex condition, oocytes in the testes, affecting about 80% of the male fish sampled (Vicki Blazer, USGS, written communication, 2004). While there is no widely accepted normal rate of intersex occurrence, it is clear that observed rates exceed expected values and may be due to the presence of hormones or other EDCs in the environment. In 2004 the USGS, in cooperation with the West Virginia Department of Environmental Protection, Division of Water and Waster Management and the West Virginia Division of Natural Resources, initiated a study to characterize the chemical environment of the South Branch Potomac River and the Cacapon River Basins, with a reference site in the Williams River Basin, all in West Virginia (fig. 1). This study collected and analyzed water and smallmouth bass blood-plasma samples, and used innovative passive sampling devices (Alvarez and others, 2004) to identify emerging contaminants, including EDCs, which may be present in these watersheds.

Purpose and Scope

The purpose of this report is to present results of the analyses of passive-sampler extracts, stream-water samples, wastewater effluent samples, and smallmouth bass blood-plasma samples from sites in the South Branch Potomac River, the Cacapon River, and Williams River Basins of West Virginia (fig. 1) in 2004. These samples were

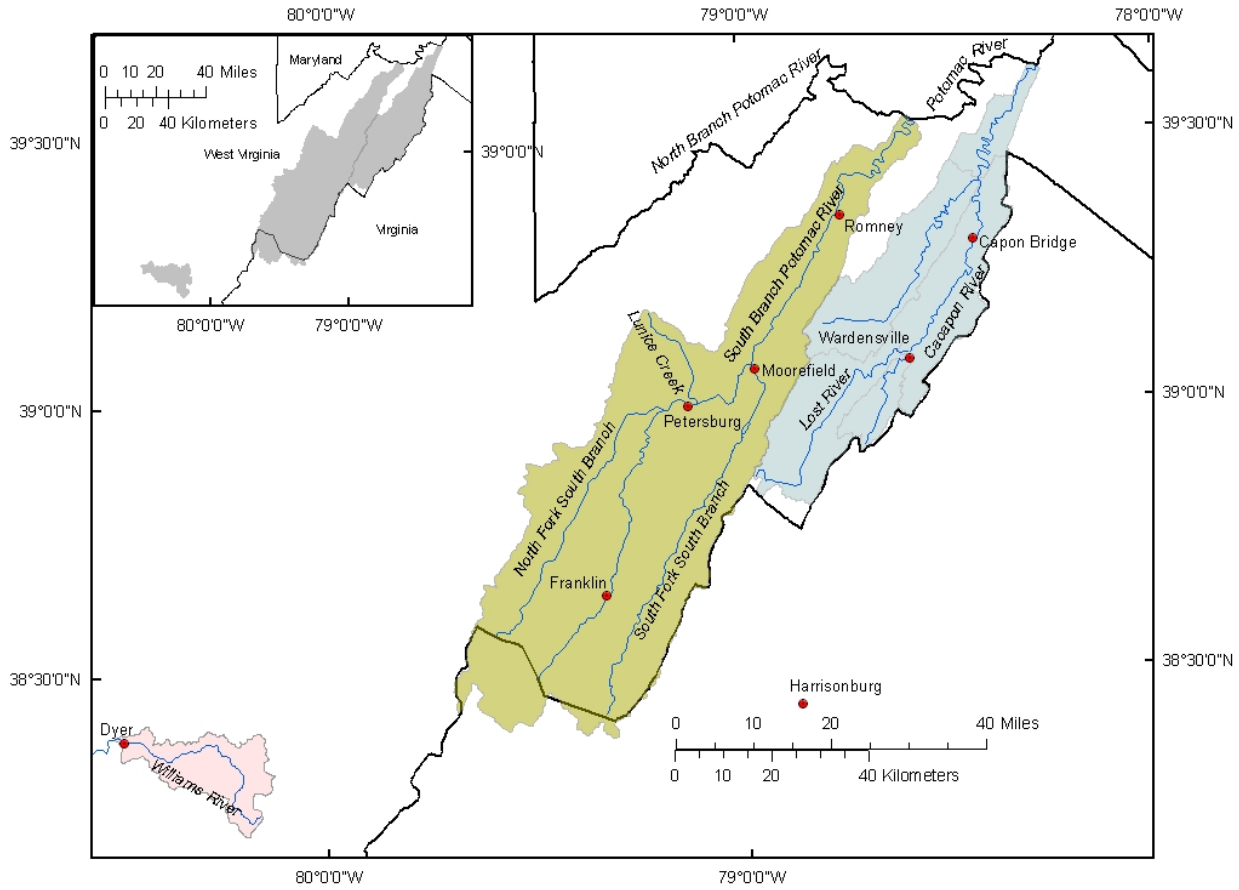

Figure 1. Emerging contaminant reconnaissance study areas in the South Branch Potomac River, Cacapon River, and Williams River Basins.

collected to detect emerging contaminants, including possible EDCs that may result in increased intersex in native male smallmouth bass.

Passive samplers, both semipermeable membrane devices (SPMDs) and polar organic compound integrative samplers (POCIS) (Alvarez and others, 2004; Petty and others, 2004), were deployed at six sites in the Potomac River Basin, one site in the Cacapon River Basin, and one reference site in the Williams River Basin. Extracts derived from the passive samplers were analyzed for several classes of emerging contaminants including antibiotics and personal-care products. Stream-water samples were analyzed for both organic and inorganic species of arsenic. All samples collected from wastewater effluents were analyzed for antibiotics, and one also was analyzed for arsenic species. Blood-plasma samples collected from smallmouth bass were analyzed for several classes of emerging contaminants including personal-care products.

Description of Study Area

The study area includes the South Branch Potomac River and Cacapon River Basins located in the Eastern Panhandle of West Virginia, and the Williams River Basin in central West Virginia (fig. 1). The initial discovery of intersex condition in smallmouth bass in the Potomac River Basin was in the

South Branch Potomac and the Cacapon River Basins (Vicki Blazer, USGS, written communication, 2004). The Williams River site was selected as an out-of-basin reference condition.

The South Branch Potomac River drains 1,506 mi^2 of West Virginia and Virginia. Population density is low, with an estimated mean population density of 20 people per mi^2, and an estimated total population in 2000 of approximately 30,100 (U.S. Census Bureau, 2002). The largest communities in the basin are Moorefield, population 2,375; Petersburg, population 2,423; Romney, population 1,940; and Franklin, population 797, all in West Virginia (U.S. Census Bureau, 2002). The area is rural and agricultural activities, primarily poultry, cattle and sheep rearing, are significant sources of income. Poultry-rearing and poultry-processing operations are major employers. Other important agricultural activities include low-density cattle and sheep rearing. Tourism also plays a significant role in the economy. Many people travel to the area for outdoor recreation activities such as backpacking, rock climbing, and trout and smallmouth bass angling.

The Cacapon River Basin (fig 1.) is adjacent to the South Branch Potomac River and has similar land uses and population densities; it drains 681 mi^2 of West Virginia's Eastern Panhandle. Population density is low, with an estimated mean population density of 23 people per mi^2, and an estimated total population in 2000 of approximately 15,500. The largest community in this basin is Great Cacapon, population 1,379 (U.S. Census Bureau, 2002). Poultry rearing is an important source of income. The highest density of poultry rearing houses in the study area is in the Lost River basin, an upper Cacapon River tributary (fig. 1).

Samples were also collected from the Williams River at Dyer, WV. The Williams River is in the Kanawha River Basin of the Ohio River Basin, and drains 129 mi^2 of a remote portion of West Virginia. This site served as a comparative reference site for the Kanawha-New River NAWQA Study (Paybins and others, 2000) and was selected as an out-of-basin reference site for this study. There are no incorporated communities located in the Williams River drainage. The mean population density is less than 8 people per mi^2 and the estimated population is less than 1,000 (U.S. Census Bureau, 2002). The Williams River site was selected as a reference due to its size, the presence of native smallmouth bass, and lack of potential sources of contaminants. No site in the South Branch Potomac River Basin offered this combination of characteristics.

Potential Sources of Contaminants

Many potential sources of contaminants discharge to the South Branch of the Potomac and Cacapon Rivers. Chief among these are runoff from agricultural activities, municipal and domestic wastewater effluent (both treated and untreated), industrial wastewater, and gypsy moth control programs using dimilin (diflubenzuron).

Agricultural Runoff

Livestock rearing is the principal agricultural activity in the South Branch Potomac and Cacapon River Basins. Chicken and turkey rearing are the principal livestock activities followed by low-intensity beef cattle rearing, with approximately 82,000 cattle raised in the area, and a lesser amount of sheep rearing, with approximately 11,000 sheep in the area. Crop production is limited; corn is the primary grain, with some wheat production; there are approximately 57,000 acres and 3,700 acres of land in row crop production, respectively, in the South Branch Potomac and Cacapon River Basins.

The Eastern Panhandle of West Virginia is a center of poultry production. According to the National Agricultural Statistics Service's 2002 West Virginia Census of Agriculture (2004), more than 90,000,000 birds were sold from the region in

2002 and approximately 450 chicken and turkey rearing facilities were found throughout the South Branch Potomac and Cacapon River Basins in 2002. When the birds reach market size they are transported to a processing plant for slaughter and packaging. Currently a poultry-processing plant operates in Moorefield, WV, in the South Branch Potomac River Basin that slaughters and processes most of the chickens raised in the area. Most of the turkeys reared in the area are slaughtered and processed at a facility in Harrisonburg, VA. After the birds have left the rearing house, the house litter (a mixture of pine shavings, excrement, and other residue) is removed from the house and composted. The composted litter is later spread on pastures and crop fields as both a fertilizer and method of waste disposal, with some composted litter trucked out of basin for land application elsewhere.

Two primary concerns associated with runoff from poultry-litter applications are 17β-estradiol and arsenic. The estradiol is a naturally occurring reproductive hormone that is shed in poultry feces and persists through composting (Nichols and others, 1997; Hanselman and others, 2003). Arsenic is introduced to the poultry as roxarsone, an organoarsenic compound originally used as anti-parasitic agent and now used to improve the color and texture of chicken and turkey meat (U. S. Food and Drug Administration, 2000).

Roxarsone does not accumulate in the poultry meat but is shed in the feces, the birds are taken off of roxarsone-amended feed five days before slaughter to reduce body burdens. Roxarsone and other arsenic species resulting from biotransformation of roxarsone are present in the composted poultry-house litter that is subsequently applied to pastures and crop fields. Studies of arsenic in a small agricultural basin indicate that much of this arsenic strongly sorbs to soils, but a fraction of the arsenic is washed into streams by storm events following the springtime application of poultry-house litter (Brown and others, 2005; Schreiber and others 2004).

Antibiotics are another suite of compounds associated with poultry production, used for both disease treatment and growth promotion. Macrolides (virginiamycin, lincomycin) and tetracyclines are the most extensively used antibiotic classes in poultry rearing. The primary release of poultry-rearing antibiotics to the environment is through the spreading of poultry-house litter on croplands and pastures (Chapman and Johnson, 2002; Campagnolo and others, 2002).

Point-Source Pollution

Wastewater effluent and other environmental releases from municipal, industrial and residential sources represent potentially significant inputs of contaminants in the South Branch Potomac and Cacapon River Basins. There are no point discharges permitted under the National Pollutant Discharge Elimination System (NPDES) or other regulated waste releases in the Williams River Basin (WVDEP, 2001, GIS coverage).

In the South Branch Basin there are 73 NPDES discharges permitted by West Virginia Department of Environmental Protection (WVDEP, 2001, GIS coverage). These permits are for industrial storm-water runoff, general sewage, aquaculture facilities, landfill leachate, and various other effluent types. The largest facilities are the wastewater-treatment plants for the towns of Moorefield, Romney, Franklin, and Petersburg, and the Cherry Grove U.S. Naval Station.

The West Virginia Department of Environmental Protection has granted NPDES permits to 24 discharges in the Cacapon River drainage for general sewage, industrial storm water runoff, sanitary landfill leachate, and various other discharges. The largest permitted discharges are wastewater-treatment plants for the towns of Wardensville and Capon Bridge, and East

Hardy High School (WVDEP, 2001, GIS coverage).

In the South Branch Potomac, Cacapon, and Williams River Basins, a large but unquantified portion of residential sewage is either treated by septic tank/leach field systems or discharged directly to surface waters without treatment. The "straight-pipe" untreated discharges are a problem throughout West Virginia (WVDEP, 2006).

The South Branch Potomac River Basin is the only study basin with a significant amount of industry. This includes a polymer-compounding plant in Petersburg and poultry-feed mill, poultry-processing plant and cabinetry factory in Moorefield. Compounds emitted by these facilities, according to the 2002 USEPA toxic release inventory, include organic compounds such as xylene, toluene, methanol, and ethylbenzene, and inorganic compounds such as nitrates, lead, copper, and manganese compounds (U.S. Environmental Protection Agency Toxic Release Inventory, 2004).

Methods

A broad program of sampling, designed to account for several potential sources of emerging contaminants, including endocrine disrupting compounds, was initiated in late April 2004. Water quality was assessed through the use of passive samplers, the collection of water samples from streams and waste-water discharges, and the collection of smallmouth bass blood plasma (table 1).

Passive Samplers

Passive-sampling technology typically uses an encased media that can accumulate and sequester compounds of interest over a period of time (Alvarez and others, 2004). Passive-sampling approaches offer several advantages; chief among these are the ability to integrate exposure over time and a range of hydrologic conditions, and the ability to accumulate a detectable mass of a compound that may be present in a water sample at concentrations below the method detection level (Alvarez and others, 2005). Several passive-sampling methods have been tested and used in aquatic environments; HPLC-grade hexane contained in dialysis tubing for accumulating hydrophobic organic compounds, lipids encased in plastic tubing for hydrophobic organic compounds, and waxes that exude from tubing to sorb metals and other inorganic constituents. This study used semipermeable membrane devices (SPMDs) to accumulate hydrophobic organic compounds (Huckins and others, 2002) and polar organic compound integrative samplers (POCIS) (Alvarez and others, 2004) to accumulate hydrophilic organic compounds.

SPMD

SPMDs consist of a small volume of neutral synthetic lipid encased in polyethylene tubing (Huckins and others, 2002). The SPMDs are designed to maximize the surface area to volume ratio, thereby maximizing the uptake efficiency of the device. The SPMDs used in this study were made by Environmental Sampling Technologies, Inc. of St. Joseph, MO[1] and were 91.4 cm by 2.5 cm lay-flat polyethylene tubing made without additives and filled with ultra-high purity synthetic triolein, a high molecular weight neutral lipid. Dissolved phase hydrophobic organic compounds sorb to the surface of the SPMD. A fraction of the sorbed material will move through channels in the polyethylene membrane to the internal triolein phase, the remainder of the sorbed material will remain sorbed to the exterior of the membrane or be held in the transient channels through the membrane.

[1] The use of tradenames is only for identification purposes and does not imply endorsement by the U. S. Geological Survey.

Table 1. Media sampled at emerging-contaminant reconnaissance sites in the South Branch Potomac River, Cacapon River, and Williams River Basins, April-October 2004.

[x, media sampled at each site; SPMD, semipermeable membrane device; POCIS, polar organic compound integrative sampler]

Site name	Map index number (fig. 1)	USGS Station identification number	Sample media collected and analyzed			
			Passive Samplers (SPMD and POCIS)	Water		Smallmouth bass blood plasma
				Antibiotics	Arsenic species	
South Branch Potomac River near Upper Tract, WV	1	384829079163401	x		x	x
North Fork South Branch Potomac River at Cabins, WV	2	01606000	x		x	x
South Branch Potomac River at Petersburg, WV	3	01606500				x
Spring Run Hatchery effluent near Masonville, WV	4	385509079050601		x		
Mill Creek near Petersburg, WV	5	01607200			x	
Lunice Creek at Petersburg, WV	6	385938079064301			x	
Petersburg wastewater treatment plant discharge at Petersburg, WV	7	385930079064501		x		
South Branch Potomac River at Petersburg Gap, WV	8	390011079044501	x			x
South Fork South Branch Potomac River near Moorefield, WV	9	01608000			x	
Moorefield wastewater treatment plant discharge at Moorefield, WV	10	390424078582301		x		
SH discharge at Moorefield, WV	11	390329078583001		x	x	
South Branch Potomac River near Moorefield, WV	12	01608070	x			x
South Branch Potomac River at railroad bridge at Sycamore, WV	13	390909078545201	x		x	
South Branch Potomac River near Springfield, WV	14	01608500	x			
Lost River at McCauley near Baker, WV	15	01610200	x		x	
Williams River at Dyer, WV	16	03186500	x			x

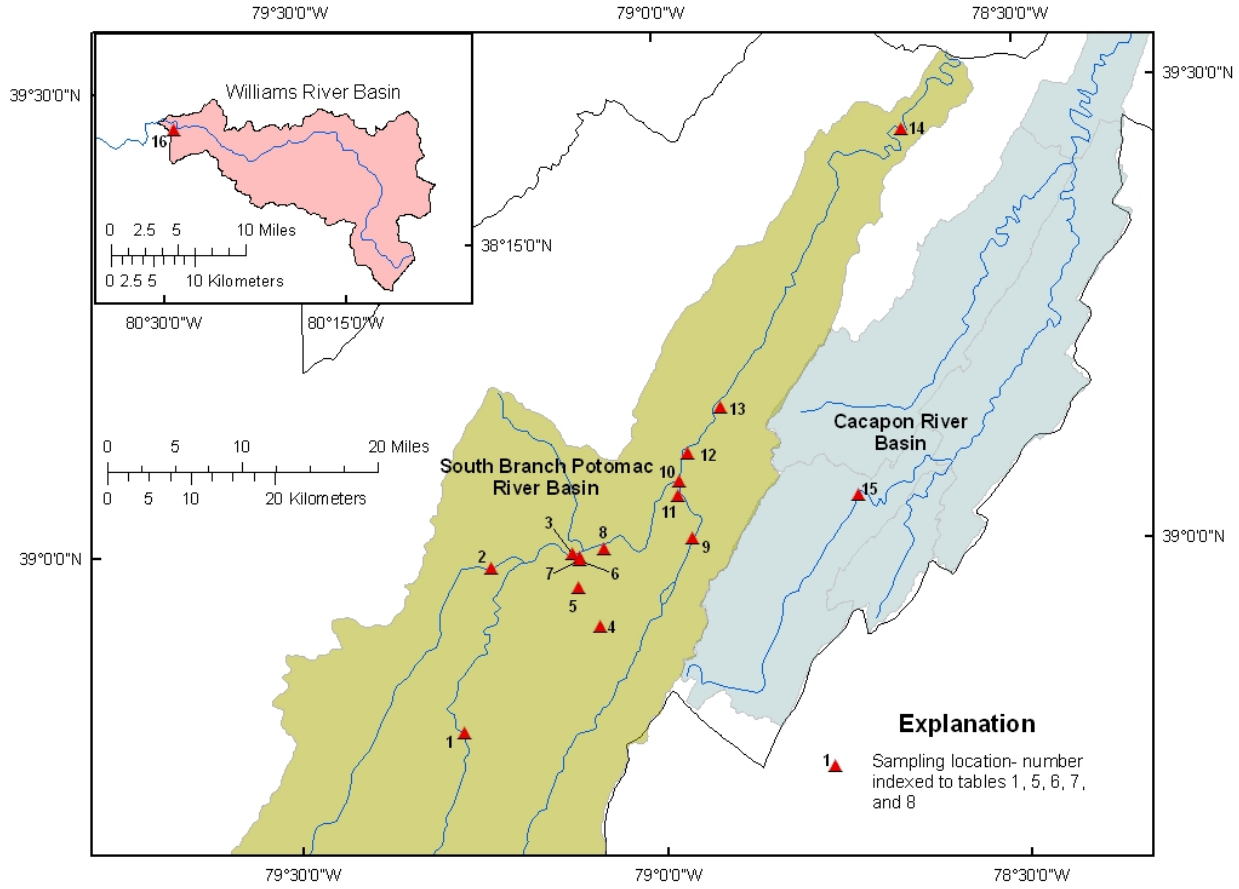

Figure 2. Locations of sites in the South Branch Potomac River, Cacapon River, and Williams River Basins where stream water, wastewater effluent, or smallmouth bass blood plasma were collected or passive samplers deployed, April-October 2004. Williams River Basin is shown in inset.

POCIS

POCIS sequester polar organic compounds in a solid phase media encased in a porous membrane (Alvarez and others, 2004). The POCIS consist of a mixed solid sequestration phase sealed in a hydrophilic microporous membrane. Water penetrates the encasing membrane and infiltrates the sequestration polymers, which sorb and immobilize water-soluble compounds throughout the exposure time. The POCIS devices used in this study were also manufactured by Environmental Sampling Technologies, Inc.

Passive-Sampler Deployment

Passive samplers were deployed at a total of eight sites, six stream sites in the South Branch Potomac River Basin, one site in the Cacapon River Basin, and one site in the Williams River Basin (table 1 and fig. 2). At each site three SPMDs and three POCIS were deployed in perforated stainless steel protective canisters. Care was taken to place the canisters where they would likely remain submerged for the duration of the deployment and in flowing water, avoiding backwater areas that may not be representative of main channel flow and water quality. Samplers were deployed at the South Branch Potomac

River near Springfield, near Moorefield, at Petersburg Gap, below Upper Tract, and in the North Fork South Branch Potomac River near Cabins during April 29-30, 2004 and retrieved June 7-8, 2004. Streamflow during deployment was typical for the season, moderately high flows punctuated with higher storm flows. Samplers were deployed at the Williams River at Dyer reference site on May 20, 2004 and retrieved July 1, 2004, a period when springtime flows were receding to typical summertime flows. The South Branch Potomac River at Sycamore and Lost River at Baker passive samplers were deployed on May 24-25, 2004 and retrieved on July 7-8, 2004, again during a period when springtime flows are receding to summertime lows. At the end of the 39-44 days of in-stream deployment, the passive samplers were removed from the deployment canisters, rinsed with native water, placed in sealed containers, chilled, and transported to the USGS West Virginia Water Science Center laboratory.

Because SPMDs can efficiently accumulate airborne contaminants, a trip-blank SPMD was used at each site. An individually packed trip-blank SPMD was removed from its sealed container and exposed to ambient air for the time interval during initial deployment when the sample SPMDs were exposed to the air, from removal from their sealed container to immersion in stream water. The trip-blank SPMDs were returned their sealed containers and kept until retrieval of the passive samplers, at which time the trip-blank SPMDs were again exposed to ambient air, from the time the sample SPMDs were removed from the water until they were returned to their sealed containers. Two polybrominated diphenyl ethers, 2,2',4,4'-tetra-bromo-diphenyl ether (BDE 47) and 2,2',4,4',5-penta-bromo-diphenyl ether (BDE 99), were found in trip blanks SPMD from three sites; South Branch Potomac River near Springfield (3.9 ng/g SPMD BDE 47 and 4.87 ng/g SPMD BDE 99), at Sycamore (6.24 ng/g SPMD BDE 47 and 6.26 ng/g SPMD BDE 99), and near Upper Tract (4.9 ng/g SPMD BDE 47 and 5.1 ng/g SPMD BDE 99).

Stream-Water and Effluent Sampling

Stream-water and effluent samples were collected at selected sites in the South Branch of the Potomac River and Cacapon River Basins; samples from four sites were analyzed for antibiotics and eight sites were sampled for arsenic species (table 1 and fig. 2). Stream-water samples were collected by West Virginia Department of Environmental Protection (WVDEP) personnel from five equidistant points across a stream using a weighted-bottle sampler. WVDEP personnel received effluent discharge as composited samples from the plant operator. Samples collected for analysis of roxarsone and other arsenic species were filtered at 0.45 μm, and 100 μL of EDTA solution was added to preserve ambient valence states of all arsenic species (Garbarino and others, 2000). Unfiltered samples were collected in 1-L amber glass bottles, chilled to 4° C, and shipped to the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas for analysis of antibiotics.

Blood-Plasma Collection

Smallmouth bass were collected by electrofishing using USGS Biomonitoring of Environmental Status and Trends (BEST) protocols (Schmitt and others, 1999) at 5 sites in the South Branch Potomac and one site at the Williams River at Dyer (table 1 and fig. 2). Captured smallmouth bass were weighed, measured, and sacrificed. Blood was collected from the caudal artery using a 5-mL syringe. Withdrawn blood was transferred to 5-mL heparinized vacutainers and centrifuged for 10 min. at 1,000 x g (where the constant g is the acceleration due to gravity). Supernatant plasma was transferred to microcentrifuge tubes and stored at -70°C until analysis. A subset of samples, blood plasma from 30 individuals from six sites,

was selected for analysis based on fish gender and degree of intersex condition.

Analytical Methods

Passive-Sampler Extracts

Extracts for SPMD and POCIS devices are prepared using different techniques. SPMD were extracted through dialysis of the entire SPMD into pesticide-grade hexane. Hydrophobic compounds preferentially partition into the dialysis solvent, the dialysis solvent now containing the hydrophobic compounds is the SPMD extract. POCIS extracts were prepared by eluting hydrophilic compounds from the POCIS sequestration medium with HPLC-grade methanol. Following dialysis or elution, the SPMD and POCIS extracts were concentrated, in their original containers under a gentle stream of filtered nitrogen at ambient temperature, to approximately 0.250 mL. After the extracts were concentrated, they were transferred to 1.8 ml gas-chromatographic (GC) amber vials with Teflon lined screw caps, each containing a 400 microliter (μL) insert. The volume of the extract was adjusted with hexane to 400 μL. Internal standards and injection internal standards were added to the extracts just prior to gas chromatographic and mass spectral (GC/MS) analysis.

The extracts were analyzed by capillary GC/MS under electron ionization and electron-capture negative ionization. Table 2 lists the compounds that were analyzed for in the passive-sampler extracts. The conditions for the gas chromatographic and mass spectral analysis are described below.

Capillary gas chromatographic analysis: Chromatographic separations for all mass spectral analysis were made on an RTX-5MS 30 M X 0.25 mm ID, 0.25 μm df fused silica capillary column. The injection port temperature was held at 285°C. The oven temperature program was as follows; the oven was held at 50°C for 5 min, the oven temperature was raised to 125°C at 10°C/min then raised to 200°C at 1.5°C/min, then raised up to 325°C at 8°C/min, and held at this temperature for 30 min. The transfer line temperature was held at 285°C. A 1-μL splitless injection of the concentrated extract was injected into the GC/MS for analysis. Ultrapure helium was the carrier gas. The carrier gas was filtered through moisture traps and traps for hydrocarbons and oxygen prior to entering the gas chromatograph.

Mass spectral analysis:. For all mass spectrometric analysis the instrument was repetitively scanned from 50 to 600 Daltons at a rate of 2.69 scans per second. The electron-multiplier was set at 2,000 volts. Application and specific conditions for each ionization mode are described below.

First ionization: Electron-capture negative ionization (ECNI) mass spectrometry mode was used to measure pesticides, PCBs, polybrominated diphenyl ethers, and halogenated organic compounds in general in the extracts. The source temperature was held at 160°C. The modifying gas was methane. The source pressure was 2×10^{-4} Torr.

Second ionization: Electron ionization mass spectrometry is the conventional method for analyzing extracts via mass spectrometry. The source temperature was held at 200°C. The source pressure was 4×10^{-5} Torr.

Table 2. Chemical compounds analyzed for in passive-sampler (SPMD and POCIS) extracts and associated estimated detection levels.

[ng/sampler, nanograms per sampler; ND, no detection; compounds detected in extracts from at least one site are italicized; SPMD, semipermeable membrane device; POCIS, polar organic compound integrative sampler]

Chemical compound	Estimated detection level, in ng/sampler	Chemical compound	Estimated detection level, in ng/sampler
Trifluralin	4	o,p'-DDT	ND
Benfluralin	4	*cis-Nonachlor*	128
alpha-hexachlorohexane	16	Endrin Aldehyde	64
Hexachlorobenzene	4	Endosulfan Sulfate	96
Pentachloroanisole	4	p,p'-DDT	32
beta-HCH	ND	Endrin Ketone	32
gamma-HCH	8	Mirex	4
delta-HCH	32	PCB70	4
desulfinyl fipronil	16	PCB 101	4
Aldrin	8	PCB 110	64
Chlorpyrifos	4	PCB 118	16
DCPA	4	PCB 138	4
Octachlorostyrene	16	PCB 146	4
Heptachlor-epoxide	16	PCB 149	4
Oxychlordane	16	PCB 151	4
fipronil sulfide	8	PCB 170	4
fipronil	64	PCB 174	4
trans-Chlordane	8	PCB 177	4
o,p'-DDE	8	PCB 180	4
Endosulfan I	8	PCB 183	4
cis-Chlordane	96	PCB 187	4
trans-Nonachlor	64	PCB 194	4
p,p'-DDE	128	PCB 206	4
Dieldrin	128	Toxaphene	2,500
o,p'-DDD	16	*BDE 47*	4
Endrin	ND	*BDE 99*	4
desulfinylfipronil amide	ND	*BDE 100*	4
fipronil sulfone	8	BDE 153	4
Endosulfan II	64	BDE 154	4
p,p'-DDD	8		

Compound identification: Identifications of the compounds detected in the extracts were classified as target or non-target (unknown). Target compounds were identified by comparing the mass spectra retention times for the sample extracts with the same properties from authentic standards, which are used to generate the standard curve for quantization. Unknown compounds were identified by comparing the mass spectrum of the compound detected with a library of mass spectra, and if necessary, tentative identification was based on classical mass spectral interpretation.

Quantitation: Quantization of the target compound was conducted using a 6-point calibration curve derived using linear regression, and authentic standards. Because authentic standards for the unknown compounds were not available, the quantization of an unknown compound is an estimate that is based on the response of the internal injection standard that is used for the quantitation of the target compounds. The quantization of the unknown compounds is a

semi-quantitative/estimated value. Although the reported concentration of the unknown compounds is an estimated value, the data may still be used to establish associations with toxicity tests.

Blood Plasma

Blood-plasma samples were extracted by solvent exchange, concentrated, and then analyzed for the compounds listed in table 3 following procedures described in the preceding section, "Passive-Sampler Extracts."

Antibiotics

Concentrations of antibiotics in water samples and POCIS extracts were determined by the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas. A total of 39 antibiotics in five antibiotic classes were analyzed for in both water samples and POCIS extracts (table 4). Samples were analyzed using online solid-phase extraction and Agilent 1100 Series liquid chromatography/mass spectrometers equipped with diode array detectors (Brown and others, 1999; Lindsey and others, 2001).

Roxarsone and Other Arsenic Species

Water concentrations of roxarsone, arsenate, arsenite, monomethyl arsonate, and dimethyl arsinate were determined by the USGS National Water Quality Laboratory (NWQL) in Lakewood Colorado, according to Garbarino and others (2002). Samples were filtered and preserved with EDTA, and then analyzed using inductively coupled plasma-mass spectrophotometry.

Results

The results of passive-sampler extract analysis, smallmouth bass blood-plasma analysis, stream-water analysis, and effluent analysis are presented in tables 5, 6, 7, and 8. Additionally, tables 2, 3, and 4 list all compounds except inorganic arsenic and organoarsenic compounds that were analyzed for in the various sample media, though not all compounds were detected. Compounds that were not detected in any environmental sample were omitted from the results tables.

Passive-Sampler Results

Both SPMDs and POCIS were deployed at six sites in the South Branch Potomac River Basin and one site each in the Cacapon River and Williams River Basins (table 1 and fig. 2). Table 2 lists 59 compounds analyzed for in the passive-sampler extracts; results of the analyses are shown in table 5. Four compounds were found at all sites; hexachloro-benzene (HCB), a fungicide banned from use in the United States; pentachloroanisole (PCA), a degradation product of other industrial phenols; 2,2',4,4'-tetra-bromo-diphenyl ether (BDE 47), a flame retardant; and 2,2',4,4',5-penta-bromo-diphenyl ether (BDE 99), another flame retardant. Only these four compounds were detected in passive samplers deployed at Williams River at Dyer, the out-of-basin reference site and the site with the fewest detected compounds. Other compounds detected in passive-sampler extracts are trifluralin, a dinitroanaline herbicide; gamma hexa-chloro-hexane (γHCH), a pesticide; chlorpyrifos, an organo-phosphate pesticide; trans- and cis-chlordane, organo-chlorine pesticides; trans- and cis-nonachlor, also organo-chlorine pesticides; and the flame retardant 2,2',4,4',6-penta-bromo-diphenyl ether (BDE 100). Of the compounds detected the polybrominated diphenyl ethers (Meerts and others, 2001, Lilienthal and others 2006), trifluralin (Fox, 2004), HCB (Ralph and others, 2003), γHCH (Kojima and others, 2004), chlorpyrifos (Kojima and others, 2004) cis-nonaclor (Fox, 2004), and cis- and trans-chlordane (Kojima and others, 2004) are either known or

Table 3. Chemical compounds analyzed for in smallmouth bass blood-plasma samples and associated detection levels.

[µg/kg, micrograms per kilogram; **, indicates compounds identified in reagent blanks, compounds identified in at least one plasma sample are italicized.]

Chemical compound	Detec-tion level, in µg/kg	Chemical compound	Detec-tion level, in µg/kg	Chemical compound	Detec-tion level, in µg/kg
bromoform	25	AHTN (tonalide)	50	o,p'-DDD	32
cumene	25	HHCB	50	p,p'-DDD	64
phenol	25	OPEO-1	100	o,p'-DDT	64
1,4-dichlorobenzene	25	carbaryl	250	p,p'-DDT	64
d-limonene	25	metalaxyl	250	dieldrin	16
acetophenone	25	anthraquinone	250	endosulfan I	8
para-cresol	25	bromacil	1,000	endosulfan II	8
isophorone	25	metolachlor	250	endrin	16
camphor	25	NPEO-1-total	1,500	endrin aldehyde	16
isoborneol	25	fluoranthene	25	endrin ketone	128
menthol	25	chlorpyrifos	100	endosulfan sulfate	4
napthalene	25	pyrene	25	fipronil	128
methyl salicylate	25	triclosan	250	alpha-HCH	32
dichlorvos	25	OPEO-2	250	beta-HCH	64
isoquinoline	25	bisphenol A	250	gamma-HCH	8
indole	25	NPEO-2-total	2,000	delta-HCH	32
2-methylnapthalene	25	tri (dichloroisopropyl) phosphate	250	heptachlor-epoxide	16
2-methyl benzothiophene	100	triphenyl phosphate	250	hexachlorobenzene	2
3,4-dichlorophenyl isocyanate	100	ethanol, 2-butoxy-, phosphate	250	trans-nonachlor	8
1-methylnapthalene	25	*diethylhexyl phthalate***	50	cis-nonachlor	4
skatol	25	beta-coprostanol	200	mirex	16
2,6-dimethylnapthalene	25	*cholesterol*	200	octachlorostyrene	2
butylated hydroxyanisole	25	beta-sitosterol	200	oxychlordane	16
5-methyl-1H-benzotriazole	800	stigmastanol	400	pentachloroanisole	2
diethyltoluamide (DEET)	25	aldrin	16	PCB70	64
*diethyl phthalate***	25	benfluralin	2	PCB 101	32
4-tert-octylphenol	50	*BDE 47*	2	PCB 110	32
benzophenone	25	BDE 66	16	PCB 118	4
ethyl citrate	100	BDE 71	8	PCB 138	16
tributylphosphate	500	BDE 85	4	PCB 146	2
cotinine	200	*BDE 99*	2	PCB 149	16
para-nonylphenol (total)	200	BDE 100	4	PCB 151	8
pentachlorophenol	2,000	BDE 138	4	PCB 170	2
prometon	100	BDE 153	2	PCB 174	2
atrazine	50	BDE 154	4	PCB 177	2
phenanthrene	25	BDE 183	4	PCB 180	2
4-octylphenol	50	chlorpyrifos	8	PCB 183	2
tri (2-chloroethyl) phosphate	100	trans-chlordane	2	PCB 187	2
anthracene	25	cis-chlordane	16	PCB 194	2
diazinon	100	DCPA	2	PCB 206	2
carbazole	25	o,p'-DDE	32	trifluralin	2
caffeine	50	p,p'-DDE	64	toxaphene	2,500

Table 4. Antibiotics analyzed for in passive sampler extracts and in wastewater effluent in the South Branch Potomac River, Cacapon River, and Williams River Basins.

[*, degradation products; †, detected in effluent discharge; µg/L, micrograms per liter]

Analyte	Laboratory reporting level	Analyte	Laboratory reporting level
Beta lactams		**Sulfonamides**	
Amoxicillin	0.01µg/L	Sulfachloropyridazine	0.005µg/L
Ampicillin	0.01µg/L	Sulfadiazine	0.005µg/L
Cefotaxime	0.01µg/L	Sulfadimethoxine	0.005µg/L
Claxacillin	0.01µg/L	Sulfamerazine	0.005µg/L
Ocacillin	0.01µg/L	Sulfamethazine	0.005µg/L
Penicillin G	0.01µg/L	Sulfamethoxazole†	0.005µg/L
Penicillin V	0.01µg/L	Sulfathiazole	0.005µg/L
Macrolides and degradation products*		**Tetracyclines and degradation products***	
Erythromycin†	0.005µg/L	Chlorotetracycline	0.01µg/L
Anhydro-erythromycin*†	0.005µg/L	*Anhydro-chlorotetracycline	0.01µg/L
Lincomycin†	0.005µg/L	*Demeclocycline	0.01µg/L
Ormetoprim	0.005µg/L	Doxycycline	0.01µg/L
Roxithromycin	0.005µg/L	Minocycline	0.01µg/L
Trimethoprim†	0.005µg/L	Oxytetracycline	0.01µg/L
Tylosin†	0.005µg/L	Tetracycline	0.01µg/L
Virginiamycin	0.005µg/L	*Anhydro-tetracycline	0.01µg/L
Quinolines		**Other groups**	
Ciprofloxacin†	0.005µg/L	Carbadox	0.005µg/L
Clinafloxacin	0.005µg/L		
Flumequine	0.005µg/L		
Lomefloxacin	0.005µg/L		
Norfloxacin	0.005µg/L		
Ofloxacin†	0.005µg/L		
Oxolinic acid	0.005µg/L		
Sarafloxacin	0.005µg/L		

suspected endocrine disruptors. The greatest number of compounds detected came from passive samplers deployed in the South Branch Potomac River at Petersburg Gap, with 12 compounds detected. POCIS extracts were also analyzed for the antibiotics listed in table 4. However, no detectable concentration of any antibiotic was identified in any POCIS extract. This finding was contrary to expectations, as samplers were deployed during spring flows when run-off events are common and following land application of poultry-house litter.

Blood-Plasma Results

A total of 30 smallmouth bass blood-plasma samples were collected from six sites (table 1 and fig. 2) and analyzed for the analytes shown in table 3. Ten of the analytes were present in detectable concentrations. Plasma samples were selected from both male and female mature fish without intersex characteristics and from male fish with intersex characteristics to represent the range of intersex conditions found at that site. Results of the analyses are shown in table 6. One compound, diethyl-hexyl phthalate, was found in every plasma sample analyzed. Diethyl-hexyl phthalate and diethyl phthalate were also detected in the reagent blanks. Reagent blank concentrations of diethyl-hexyl phthalate were well below concentrations found in plasma samples. However, reagent blank concentrations of diethyl phthalate (62 mg/kg) approached those found in several plasma samples, therefore concentrations of diethyl phthalate in plasma maybe an artifact of reagent contamination. In addition to diethyl-hexyl phthalate and diethyl phthalate, the following compounds were detected in at least one plasma sample: phenol, an industrial compound with many applications; d-limonene, used as a solvent and also as a fragrance; acetophenone, used as a fragrance, a flavoring, and an industrial solvent; napthalene and 2-methyl-napthalene, used in polyvinyl chloride production and in both moth balls and toilet deodorizer blocks; 4-tert-octyl-phenol, used as an industrial surfactant, plasticizer and in the synthesis of many end products; and the polybrominated diphenyl ether flame retardants 2,2',4,4'-tetra-bromo-diphenyl ether (BDE 47) and 2,2',4,4',5-penta-bromo-diphenyl ether (BDE 99). Analysis of plasma samples from the Williams River reference site detected fewer compounds and those compounds were at lower concentrations than in the South Branch Potomac River, with the exception of phenol, which was found only in a plasma sample from the Williams River. The polybrominated diphenyl ethers, known endocrine disruptors, were found at all sites except the reference site. Diethyl-hexyl phthalate, a known endocrine disruptor, was found in every plasma sample. Patterns in diethyl phthalate, another known endocrine disruptor, are harder to discern due to possible reagent blank contamination.

Water-Sample Results

Water samples collected at the sites listed in table 1 and shown in fig. 2 were analyzed for concentrations of roxarsone and other arsenic species and antibiotics. The results of the analysis of seven samples of stream water and one effluent sample are shown in table 7. Arsenate, found in samples from six streams in the South Branch Potomac and Cacapon River Basins, in concentrations ranging from 0.3 µg/L to 1 µg/L, was the only arsenic species detected. Arsenic concentrations in stream water samples from the South Branch Potomac River and Cacapon River Basins previously collected and analyzed by the USGS are typically low, with concentrations in dissolved-fraction samples ranging from 2 µg/L to below detection in most samples. It was not possible to determine the source of the arsenate in the samples, whether from natural sources, coal combustion, or photodegradation of roxarsone.

Table 5. Chemical compounds in passive samplers (SPMD and POCIS) deployed at sites in the South Branch Potomac River, Cacapon River, and Williams River Basins, April to June 2004.

[ng/sampler, nanograms per sampler; <, less than; SPMD, semipermeable membrane device; POCIS, polar organic compound integrative sampler; RR, railroad]

Map index number (fig. 1)	Site name	Trifluralin, in ng/sampler	Hexa-chloro-benzene, in ng/sampler	Penta-chloro-anisole, in ng/sampler	gamma-Hexa-chloro-hexane, in ng/sampler	Chlor-pyrifos, in ng/sampler	trans-Chlordane, in ng/sampler	cis-Chlordane, in ng/sampler	trans-Nonachlor, in ng/sampler	cis-Nonachlor, in ng/sampler	2,2',4,4'-tetra-bromo-diphenyl ether (BDE 47), in ng/sampler	2,2',4,4',5-penta-bromo-diphenyl ether (BDE 99), in ng/sampler	2,2',4,4',6-penta-bromo-diphenyl ether (BDE 100), in ng/sampler
1	So. Br. Potomac R near Upper Tract, WV	< 4	3.4	13.6	33.4	< 4	11.7	12.3	12.2	< 4	16.8	14.4	6.74
2	N. F. South Br. Potomac R. at Cabins, WV	< 4	7.7	14.9	34.5	20.1	8.14	9.4	9.4	< 4	14.9	11	< 4
8	So. Br. Potomac. R.. at Petersburg Gap, WV	75.4	5.34	12.3	21.3	15.1	9.94	11.4	10.6	4.8	13	9.6	5.5
9	South Branch Potomac River near Moorefield, WV	18.7	4.28	11.8	40.7	< 4	9.95	10.2	9.87	< 4	19.2	16.1	< 4
13	So Br Potomac R at RR bridge at Sycamore, WV	28.8	4.24	15	< 8	15.9	12.8	14.2	12.2	6.45	21.4	14	< 4
14	So. Br. Potomac R. near Springfield, WV	10.1	7.45	15.4	43.6	< 4	12.5	15.1	12.2	< 4	27.4	19.4	8.21
15	Lost River at McCauley near Baker, WV	< 4	2.88	25.3	< 8	70.8	6.51	<8	< 8	< 4	10.9	3.86	< 4
16	Williams River at Dyer, WV	< 4	4.13	10.3	< 8	< 4	< 8	< 8	< 8	< 4	10.5	7.21	< 4

Table 6. Chemical compounds in smallmouth bass blood-plasma samples from the South Branch Potomac River and the Williams River Basins, May to October 2004.

[µg/kg, micrograms per kilogram; ng/mL, nanograms per milliter; <, less than]

Map Index Number (fig. 1)	Site name	Fish sample ID	phenol, in µg/kg	d-limonene, in µg/kg	aceto-phenone, in µg/kg	nap-thalene, in µg/kg	2-methyl-nap-thalene, in µg/kg	diethyl phthalate, in µg/kg	4-tert-octyl-phenol, in µg/kg	diethyl-hexyl phthalate, in µg/kg	2,2',4,4'-tetra-bromo-diphenyl ether (BDE 47), in ng/mL	2,2',4,4',5-penta-bromo-diphenyl ether (BDE 99), in ng/mL
1	South Branch Potomac River near Upper Tract, WV	SMB 1	<25	127	<25	41.5	47.5	<25	73	644	<2	<2
		SMB 2	<25	152	<25	41.9	47.1	<25	94.3	261	3.1	<2
		SMB 5	<25	<25	<25	41.8	44.5	<25	<50	335	8.4	2.79
		SMB 7	<25	120	<25	25.6	42.7	<25	<50	638	<2	<2
		SMB10	<25	<25	<25	<25	34	<25	<50	452	2.79	<2
2	North Fork South Branch Potomac River at Cabins, WV	SMB 1	<25	177	95.4	<25	<25	108	<50	670	<2	4.11
		SMB 2	<25	74.2	62.8	<25	<25	66.8	<50	458	3.04	3.38
		SMB 4	<25	85.5	<25	<25	<25	75.9	<50	339	13.13	2.64
		SMB 6	<25	161	106	<25	<25	86.6	<50	884	<2	<2
		SMB 7	<25	92.5	73.8	<25	<25	<25	<50	285	<2	<2
		SMB 12	<25	163	<25	29.1	41.5	<25	97.8	715	<2	<2
		SMB 14	<25	101	<25	26.9	38	<25	76	536	4.2	<2
		SMB 16	<25	113	80.3	27.4	35.4	<25	<50	519	<2	<2
3	South Branch Potomac River at Petersburg, WV	SMB 1	<25	146	<25	29	30.6	<25	71.2	376	7.72	<2
		SMB 4	<25	<25	<25	<25	<25	<25	70.1	338	<2	<2
		SMB 5	<25	<25	<25	28.3	36.8	<25	69.1	460	3.49	<2
		SMB 6	<25	145	<25	33	50.9	<25	85.7	6,320	<2	<2
8	South Branch Potomac River at Petersburg Gap, WV	SMB 5	<25	75.8	<25	<25	<25	78.8	<50	670	<2	<2
		SMB 7	<25	225	123	<25	<25	150	<50	304	<2	2.9
		SMB 9	<25	141	<25	<25	<25	88.7	<50	366	2.5	<2
		SMB 10	<25	115	78.8	<25	<25	103	<50	672	6.8	4.8
		SMB 15	<25	93	63.4	<25	<25	74.2	<50	2,070	2.9	<2
12	South Branch Potomac River near Moorefield, WV	SMB 1	<25	134	86.5	<25	<25	<25	71.7	341	18.6	3.94
		SMB 4	<25	135	<25	<25	<25	114	<50	216	26.3	5.23
		SMB 9	<25	91.8	69.4	<25	<25	<25	67.4	281	12.02	7.34
		SMB 15	<25	188	120	<25	<25	<25	131	1,080	7.43	5.4
		SMB 17	<25	90.9	<25	<25	<25	128	82.1	378	<2	<2
16	Williams River at Dyer, WV	SMB 2	38.9	32.8	55.8	<25	<25	94	<50	1,520	<2	<2
		SMB 6	<25	69.8	103	<25	<25	110	<50	767	<2	<2
		SMB 7	<25	<25	<25	<25	<25	150	<50	1,180	<2	<2

Samples from 4 selected effluent discharges were analyzed for the list of 39 human and veterinary antibiotics and antibiotic degradation products in table 7, eight of which were detected in at least one sample (table 8). Only one antibiotic was detected in the sample from the slaughter house at Moorefield's poultry processing plant discharge, ofloxacin, a human antibiotic not approved for veterinary use (U.S. Food and Drug administration, Center for Veterinary Medicine, FDA Approved Animal Drug Products (Green Book) web page, http://www.fda.gov/cvm/greenbook.html, accessed October 12, 2006) and its presence in the discharge was likely due to a human source. Fish hatcheries have been found to be sources of antibiotics (Dietze and others, 2005); in this study both ofloxacin and anhydro-erythromycin were detected in the Spring Run hatchery discharge. The Petersburg sewage-treatment plant (STP) effluent sample contained detectable concentrations of seven antibiotics and one antibiotic degradate and the Moorefield STP had detections of six antibiotics and one antibiotic degradate. Concentrations of all antibiotics detected in the municipal STP effluents were above the median values in Glassmeyer and others (2005), except for ciprofloxacin, which was below the reporting level in the Moorefield STP effluent sample.

Summary

Passive water sampling devices used at eight stream sites detected the presence of emerging contaminants, including pesticide, flame-retardant, and personal-care product residues in water. No antibiotics or antibiotic-degradation products were found in passive sampler extracts from any site. Several of the compounds detected in passive sampler extracts are known or suspected as endocrine disrupting compounds (EDCs). These same compounds were also identified in blood plasma collected from smallmouth bass, including fish with intersex characteristics. Of the various arsenic species analyzed for, only arsenate was found in detectable concentrations. Antibiotics were found in the various wastewater effluents sampled, with municipal effluent having seven to eight compounds, whereas the fish-hatchery and the poultry-processing plant effluents were found to have detections of two and one antibiotics, respectively. This study found that some EDCs are nearly ubiquitous in the environment, also being present at sites where fish are not exhibiting intersex conditions. Other investigations have shown that some EDCs, including some found in both passive samplers and smallmouth bass blood plasma, act additively or synergistically, where mixtures of several compounds have a greater effect than a single compound (Rajapakse and others, 2002; Payne and others, 2000). Studies are currently (2006) under way to further define the occurrence of EDCs in the South Branch of the Potomac basin and to determine the potential for environmental mixtures of these compounds to interfere with normal endocrine function.

Table 7. Inorganic arsenic and organoarsenic compounds in stream-water and effluent samples collected at sites in the South Branch Potomac River and Cacapon River Basins, September 2004.

[wf, water filtered using 0.45 µm capsule filter; µg/L, micrograms per liter; <, less than; SH, slaughter house]

Map index number (fig. 1)	Site name	Sample date	Sample time	Arsenate, wf, in µg/L as As	Arsenite, wf, in µg/L as As	Dimethyl-arsinate, in µg/L as As	Mono-methyl-arsonate, in µg/L as As	Rox-arsone, in µg/L
1	South Branch Potomac River near Upper Tract, WV	9/9/2004	1400	0.5	<0.3	<0.2	<0.2	<0.2
2	North Fork South Branch Potomac River at Cabins, WV	9/9/2004	1515	.4	<.3	<.2	<.2	<.2
5	Mill Creek near Petersburg, WV	9/9/2004	1600	.4	<.3	<.2	<.2	<.2
6	Lunice Creek at Petersburg, WV	9/9/2004	1630	<.3	<.3	<.2	<.2	<.2
9	South Fork South Branch Potomac River near Moorefield, WV	9/9/2004	1720	.3	<.3	<.2	<.2	<.2
11	SH Discharge At Moorefield, WV	9/10/2004	1020	1	<.3	<.2	<.2	<.2
13	South Branch Potomac River at railroad bridge at Sycamore, WV	9/10/2004	800	.4	<.3	<.2	<.2	<.2
15	Lost River at McCauley near Baker, WV	9/9/2004	1820	.4	<.3	<.2	<.2	<.2

Table 8. Antibiotics and antibiotic degradation products in composite samples collected from wastewater effluent in the South Branch Potomac River Basin, June 7-8, 2004.

[µg/L, micrograms per liter; <, less than; SH, slaughter house; STP, sewage treatment plant; *, antibiotic degradation product]

Map index number (fig. 1)	Site name	Macrolides and degradation products					Quinolines		Sulfonamides
		Erythromycin in water, in µg/L	Anhydro-erythromycin* in water, in µg/L	Lincomycin in water, in µg/L	Trimethoprim in water, in µg/L	Tylosin in water, in µg/L	Ciprofloxacin in water, in µg/L	Ofloxacin in water, in µg/L	Sulfameth-oxazole in water, in µg/L
4	Spring Run Hatchery	<0.005	0.029	<0.005	<0.005	<0.005	<0.005	0.013	<0.005
7	Petersburg STP	.442	1.47	.011	.031	.006	.053	.242	.121
10	Moorefield STP	.149	1.011	.005	.117	.007	<.005	.067	.212
11	SH at Moorefield, WV	<.005	<.005	<.005	<.005	<.005	<.005	.009	<.005

References Cited

Alvarez, D.A., Petty, J.D., Huckins, J.N, Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., and Manahan, S.E., 2004, Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments: Environmental Toxicology and Chemistry, v. 23, p. 1640-1648.

Alvarez, D.A., Stackelberg, P.E., Petty, J.D., Huckins, J.N., Furlong, E.T., Zaugg, S.D., and Meyer, M.T., 2005, Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream: Chemosphere, v. 61, p. 610-622.

Bevan, C. L., Porter, D. M., Prasad, Anita, Howard, M. J., and Henderson, L. P., 2003, Environmental estrogens alter early development in *Xenopus laevis*: Environmental Health Perspectives, v. 111, no. 4, p. 488-496.

Brown, B. L., Slaughter, A. D., and Schreiber, M. E., 2005, Controls on roxarsone transport in agricultural watersheds: Applied Geochemistry, v. 20, no. 1, p. 123-133

Brown, G. K., Zaugg, S. D., and Barber, L. B., 1999, Wastewater Analysis by Gas Chromatography/Mass Spectrometry, in Morganwalp, D.W. and H.T. Buxton, eds. U.S. Geological Survey Toxic Substances Hydrology Program—Proceedings of the technical meeting. Charleston, S.C., March 8-12, 1999. Volume 2 of 3—Contamination of hydrologic systems and related ecosystems: U.S. Geological Survey-Water Resources Investigations Report 99-4018B. W. Trenton, NJ, 471 pp., *http://toxics.usgs.gov/pubs/wri99-4018/Volume2/index.html*, p. 431-436

Campagnolo, E.R., Johnson, K. R., Karpati, Adam, Rubin, C.S., Kolpin, D. W., Meyer, M. T., Esteban, J.E., Currier, R.W, Smith Kathleen, Thu, K.M., and McGeehin, Michael, 2002, Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations: The Science of the Total Environment, v. 299, no. 1-3, p. 89-95

Carballa, Marta, Omil, Francisco, Lema, J.M., Llompart, Maria, Garcia-Jares, Carmen, Rodriguez, Issac, Gomez, Mariano, Ternes, Thomas, 2004, Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant: Water Research, v. 38, p. 2918-2926

Chapman, H.D. and Johnson, Z.B., 2002, Use of antibiotics and roxarsone in broiler chickens in the USA: Analysis for the years 1995 to 2000: Poultry Science, v. 81, p. 356-364

Dietze, J. E., Scribner, E. A., Meyer, M. T., and Kolpin, D. W., 2005, Occurrence of antibiotics in water from 13 fish hatcheries, 2001-2003: International Journal of Environmental Analytical Chemistry, v. 85, no. 15, p. 1141-1152.

Dyer, A. R., Raman, D. R., Mullen, M. D., Burns, R. T., Moody, L. B., Layton, A. C., and Saylrer, G. S., 2001, Determination of 17β-estradiol concentrations in runoff from plots receiving dairy manure: American Society of Agricultural Engineers Meeting Paper no. 01-2107, Sacramento, Ca.

Fox, Jennifer, 2004, Chemical communication threatened by endocrine-disrupting chemicals: Environmental Health Perspectives, v. 112, no. 6, pp 648-653

Garbarino, J.R., Bednar, A.J., and Burkhardt, M.R., 2002, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory---Arsenic speciation in natural-water samples using laboratory and field methods: U.S. Geological Survey Water-Resources Investigations Report 02-4144, 40 p.

Glassmeyer, S. T., Furlong, E. T., Kolpin, D. W., Cahill, J. D., Zaugg, S. D., Werner, S. L., Meyer, M. T., and Kryak, D. D., 2005, Transport of chemical and microbial compounds from known wastewater discharges- Potential use as indicators of

human fecal contamination: Environmental Science and Technology, v. 39, p. 5157-5169.

Goodbred, S. L., Gilliom, R. J., Gross, T. S., Denslow, N. P., Bryant, W. L., and Schoeb, T. R., 1997, Reconnaissance of 17β-estradiol, 11-ketotestosterone, vitellogenin, and gonad histopathology in common carp of United States streams: Potential for contaminant-induced endocrine disruption: U. S. Geological Survey Open-File Report 96-627, Sacramento, CA, 47 p.

Hanselman, T. A., Graetz, D. A., and Wilkie, A. C., 2003, Manure-borne estrogens as potential environmental contaminants: A review- Environmental Science and Technology, v. 37, no. 24, p. 5471-5478

Hutchison, M.L., Walters, L. D., Avery, S. M., Muro, F. and Moore, A., 2005, Analyses of livestock production, waste storage, and pathogen levels and prevalence in farm manures: Applied and Environmental Microbiology, v. 71, no. 3, pp. 1221-1236

Huckins, J., Petty, J., Prest, H., Clark, R., Alvarez, D., Orazio, C., Lebo, J., Cranor, W., Johnson, B., 2002, A guide for the use of semipermeable membrane devices (SPMDs) as samplers of waterborne hydrophobic organic contaminants.: API Publication 4690. American Petroleum Institute, Washington, DC

Jobling, S, Beresford, N, Nolan, M., Rodgers-Gray, R., Tyler, C.R. and Sumpter, J.P., 2002, Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents: Biology of Reproduction. V 66, no. 2, p. 272-281.

Kavanagh, R. J., Balch, G. C., Kiparissis, Niimi, A. J., Sherry, Jim, Tinson, Cheryl, and Metcalfe, C. D., 2004, Endocrine disruption and altered gonadal development in white perch (*Morone americana*) from the lower Great Lakes Region: Environmental Health Perspectives, v. 112, no. 8, p. 898-902.

Kojima, Hiroyuki, Katsura, Eiji, Takeuchi, Shinji, Niiyama, Kazuhito, and Kobayashi, Kunihiko, 2004, Screening for estrogen and androgen receptor activities in 200 pesticides in *In Vitro* reporter gene assays using Chinese hamster ovary cells: Environmental Health Perspectives, v. 112, no. 5, p. 524-531

Kolodziej, E. P., Harter, Thomas, and Sedlak, D. L., 2004, Dairy wastewater, aquaculture and spawning fish as sources of steroid hormones in the aquatic environment: Environmental Science and Technology, v. 38, no. 23, p. 6377-6384

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., and Buxton, H. T., 2002, Pharmaceuticals, hormones, and other organic wastewater contaminants in U. S. streams, 1999-2000- A national reconnasissance: Environmental Science and Technology, v. 36, no. 6, p. 1202-1211

Lilienthal, Helmuth, Hack, Alfons, Roth-Härer, Astrid, Grande, S. W., and Talsness, C. E., 2006, Effects of developmental exposure to 2,2',4,4',5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behaviour in rats: Environmental Health Perspectives, v. 114, no. 2, p.194-201

Meerts, I. A. T. M., Letcher, R. J., Hoving, Saske, Marsh, Göran, Bergman, Åke, Lemmen, J. G., van der Burg, and Brouwer, Abraham, 2001, *In vitro* estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds: Environmental Health Perspectives, v. 109, no. 4, p.399-407

Melo, A. C., and Ransdell, J. S., 2001, Sexual dimorphism of brain aromatase activity in Medaka- induction of a female phenotype by estradiol: Environmental Health Perspectives, v. 109, no. 3, p. 257-264.

Nash, J. P., Kime, D. E., Van der Ven, L. T. M., Wester, P. W., Brion, François, Maack, Gerd, Stahlschmidt-Allner, Petra, and

Tyler, C. R., 2004, Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish: Environmental Health Perspectives, v. 112, no. 17, p. 1725-1733.

Nichols, D. J., Daniel, T. C., Moore, P.A. Jr., Edwards, D. R., and Pote, D. H., 1997, Runoff of Estrogen hormone 17β-estradiol from poultry litter applied to pasture: Journal of Environmental Quality, v. 26, no. 4, pp 1002-1006.

Orlando, E. F., Kolok, A. S., Binzcik, G. A., Gate, J. L., Horton, M. K., Lambright, C. S., Gray, L. E. Jr., Soto, A. M., and Guilette, L. J. Jr., 2004, Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow: Environmental Health Perspectives, v. 112, no. 3, p. 353-358.

Paybins, K.S., Messinger, Terence, Eychaner, J.H., Chambers, D.B., and Kozar, M.D., 2000, Water Quality in the Kanawha–New River Basin West Virginia, Virginia, and North Carolina, 1996–98: U.S. Geological Survey Circular 1204, 32 p., on-line at http://pubs.water.usgs.gov/circ1204/

Payne, Joachim, Rajapakse, Nissanka, Wilkins, Megan, and Kortenkamp, Andreas, 2000, Prediction and assessment of the effects of mixtures of four xenoestrogens: Environmental Health Perspectives, v. 108, no. 10, p. 983-987.

Petty, J.D., Huckins, J.N., Alvarez, D. A., Brumbaugh, W. G., Cranor, W.L., Gale, R. W., Rastall, A. C., Jones-Lepp, T.L., Leiker, T.J., Rostad, C.E., and Furlong, E.T., 2004, A holistic passive integrative sampling approach for assessing the presence and potential impacts of waterborne environmental contaminants: Chemosphere, v. 54, no. 6, p. 695-705

Rajapakse Nissanka., Silva, Elisabete, and Kortenkamp Andreas, 2002, Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action: Environmental Health Perspectives V. 110, no. 9, 917-921.

Ralph, J. L., Orgebin-Crist, Marie-Claire, Lareyre, Jean-Jacques, and Nelson, C. C., 2003, Disruption of androgen regulation in the prostate by the environmental contaminant hexachlorobenzene: Environmental Health Perspectives, v. 111, no. 4, p. 461-466

Raman, D. R., Williams, E. L., Layton, A. C., Burns, R. T., Easter, J. P., Daugherty, A. S., Mullen, M. D., and Salyer, G. S., 2004, Estrogen content of dairy and swine wastes: Environmental Science and Technology, v. 38, no. 13, p. 3567-3573

Scheurs, R. H. M. M., Legler, Juliette, Artola-Garciano, Elsa, Sinnige, T. L., Lanser, P. H., Seinen, Willem, and Van ser Burg, Bart, 2004, *In vitro* and *in vivo* antiestrogenic effects of polycyclic musks in zebrafish: Environmental Science and Technology, v. 38, no. 4, pp 997-1002.

Schmitt, C.J., T.M. Bartish, V.S. Blazer, T.S. Gross, D.E. Tillitt, W.L. Bryant, and L.R. DeWeese, 1999, Biomonitoring of Environmental Status and Trends (BEST) Program: Contaminants and their effects in fish from the Mississippi, Columbia, and Rio Grande basins. Pp. 437-446 in Morganwalp, D.W. and H.T. Buxton, eds. U.S. Geological Survey Toxic Substances Hydrology Program—Proceedings of the technical meeting. Charleston, S.C., March 8-12, 1999. Volume 2 of 3—Contamination of hydrologic systems and related ecosystems: U.S. Geological Survey-Water Resources Investigations Report 99-4018B, W. Trenton, NJ, 471 pp. *http://toxics.usgs.gov/pubs/wri99-4018/Volume2/sectionD/2507_Schmitt/index.html*

Schreiber, M.E., Chambers, D.B., and White, J. S., 2004, Cycling of organoarsenic compounds in agricultural watersheds [*abs*]: Eos, Transactions, American Geophysical Union, v. 85, no. 47, Fall Meeting Supplement, Abstract H21C-1031.

Sheahan, D.A., Brighty, G.C., Daniel M, Jobling, S., Harries, JE., Hurst, M.R., Kennedy J., Kirby, S.J., Morris, S., Routledge, E. J., Sumpter, J.P., Waldock, M.J., 2002, Reduction in the estrogenic activity of a treated sewage effluent discharge to an English river as a result of a decrease in the concentration of industrially derived surfactants: Environmental Toxicology and Chemistry, v. 21, no. 3, p. 515-519.

Toft, Gunnar, Edwards, T. M., Baatrup, Erik, and Guillette, L. J. Jr., 2003, Disturbed sexual characteristics in male mosquitofish (*Gambusia holbrooki*) from a lake contaminated with endocrine disruptors: Environmental Health Perspectives, v. 111, no. 5, p. 695-701.

U.S. Census Bureau, 2002, Summary File 1, census of population and housing: *http://www.census.gov/Press-Release/www/2001/sumfile1.html*

U.S. Department of Agriculture, National Agricultural Statistics Service, 2004, West Virginia state and county data: 2002 census of agriculture, v. 1, Geographic Area Series part 48, 513 p. (*http://www.nass.usda.gov/census/census02/volume1/wv/WVVolume104.pdf*)

U.S. Environmental Protection Agency, 2004, 2002 Toxic Release inventory data release: *http://www.epa.gov/tri/tridata/tri02/index.htm*

U. S. Food and Drug Administration, 2000. Freedom of Information Summary NADA 140-445: U.S. Food and Drug Administration, *http://www/fda/gov/cvm/infores/foi/140445062888.html*

West Virginia Department of Environmental Protection, 2006, 2006 integrated water quality monitoring and assessment report: West Virginia Department of Environmental Protection, Charleston, WV, 53 p.