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Endocrine Active Chemicals, Pharmaceuticals, and Other Chemicals of Concern in Surface Water, Wastewater-Treatment Plant Effluent, and Bed Sediment, and Biological Characteristics in Selected Streams, Minnesota—Design, Methods, and Data, 2009

Data Series 575

U.S. Department of the Interior
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Front cover. Industrial wastewater-treatment plant outflow in Worthington, Minnesota. Photograph by John Greene, U.S. Geological Survey, September 9 , 2009.

Back cover. Left: Duluth Minnesota wastewater-treatment plant, Duluth, Minnesota. Photograph by Laura Murphy, U.S. Geological Survey, October 1, 2009. Upper right: Metropolitan wastewater-treatment plant outflow, St. Paul , Minnesota. Photograph by Kathy Lee, U.S. Geological Survey, September 24, 2009. Lower right: East Grand Forks wastewater-treatment plant outflow, East Grand Forks, Minnesota. Photograph by John Greene, U.S. Geological Survey, October 21, 2009.

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KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

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Conversion Factors

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
micrometer (µm)	0.00003937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
square kilometer (km^2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
square meter (m^2)	10.76	square foot (ft^2)
square centimeter (cm^2)	0.1550	square inch (ft^2)
	Volume	
cubic meter (m^3)	6.290	barrel (petroleum, 1 barrel = 42 gal)
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
cubic meter (m^3)	264.2	gallon (gal)
cubic meter (m^3)	0.0002642	million gallons (Mgal)
liter (L)	61.02	cubic inch (in^3)
cubic meter (m^3)	35.31	cubic foot (ft^3)
cubic meter (m^3)	1.308	cubic yard (yd^3)
	Flow rate	
cubic meter per second (m^3/s)	70.07	acre-foot per day (acre-ft/d)
cubic meter per second (m^3/s)	35.31	cubic foot per second (ft^3/s)
cubic meter per second (m^3/s)	22.83	million gallons per day (Mgal/d)
meter per second (m/s)	3.281	foot per second (ft/s)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
	Pressure	
kilopascal (kPa)	0.1450	pound per square inch (lb/ft^2)

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

$$°F=(1.8\times°C)+32$$

Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

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

Altitude, as used in this report, refers to distance above the vertical datum.

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L), micrograms per liter (µg/L), or nanograms per liter (ng/L). Concentrations of chemical constituents in bed sediment are given in nanograms per gram (ng/g) or micrograms per kilogram (µg/kg).

Abbreviations and Acronyms

±	plus or minus
µL	microliter
µm	micrometer
mL/min	milliliter per minute
ng	nanogram
Ab	antibody
ASE	accelerated solvent extraction
^{13}C	carbon-13
CLLE	continuous liquid-liquid extraction
DEET	*N,N*-diethyl-*meta*-toluamide
D-loss	deuterium loss
E2	17-*beta*-estradiol
EAC	endocrine active chemical
EC_{10}	environmental concentration at which a chemical induces a response 10 percent above the baseline
EDTA	ethylenediaminetetraacetic acid
FBS	fetal bovine serum
GC/MS	gas chromatography/mass spectrometry
GC/MS/MS	gas chromatography/tandem mass spectrometry
GIS	geographic information system
HP	Hewlett Packard
HPLC	high-perfomance liquid chromatography
ICI	antiestrogen receptor
IDS	isotope dilution standard
LSW	Lake Superior water
MPCA	Minnesota Pollution Control Agency
MSTFA	*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide
MSTFA II	*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide activated with 2-(trimethylsilyl)ethanethiol and ammonium iodide
NLCD	National Land Cover Data
NP1EC	nonylphenolmonoethoxycarboxylate
NP4EC	nonlyphenoltetraethoxycarboxylate
NP1EO	4-nonylphenolmonoethoxylate
NP2EO	4-nonylphenoldiethoxylate

NP3EO	4-nonylphenoltetriethoxylate
NP4EO	4-nonylphenoltetraethoxylate
NTA	nitrilotriacetic acid
PBDE 47	2,2′,4,4′-tetrabromodiphenylether
r^2	coefficient of determination
RPD	relative percent difference
SCSU	St. Cloud State University
SIM	selected ion monitoring
SPE	solid-phase extraction
USGS	U.S. Geological Survey
USGS-IASED	U.S. Geological Survey Iowa Sediment Laboratory
USGS-NRPL	U.S. Geological Survey National Research Program Laboratory
USGS-NWQL	U.S. Geological Survey National Water Quality Laboratory
UST	University of St. Thomas
v/v	volume per volume
WWTP	wastewater-treatment plant

Endocrine Active Chemicals, Pharmaceuticals, and Other Chemicals of Concern in Surface Water, Wastewater-Treatment Plant Effluent, and Bed Sediment, and Biological Characteristics in Selected Streams, Minnesota—Design, Methods, and Data, 2009

By Kathy E. Lee[1], Susan K. Langer[1], Larry B. Barber[1], Jeff H. Writer[1], Mark L. Ferrey[2], Heiko L. Schoenfuss[3], Edward T. Furlong[1], William T. Foreman[1], James L. Gray[1], Rhiannon C. ReVello[1], Dalma Martinovic[4], Olivia P. Woodruff[1], Steffanie H. Keefe[1], Greg K. Brown[1], Howard E. Taylor[1], Imma Ferrer[5], and E. Michael Thurman[5]

Abstract

This report presents the study design, environmental data, and quality-assurance data for an integrated chemical and biological study of selected streams or lakes that receive wastewater-treatment plant effluent in Minnesota. This study was a cooperative effort of the U.S. Geological Survey, the Minnesota Pollution Control Agency, St. Cloud State University, the University of St. Thomas, and the University of Colorado. The objective of the study was to identify distribution patterns of endocrine active chemicals, pharmaceuticals, and other organic and inorganic chemicals of concern indicative of wastewater effluent, and to identify biological characteristics of estrogenicity and fish responses in the same streams.

The U.S. Geological Survey collected and analyzed water, bed-sediment, and quality-assurance samples, and measured or recorded streamflow once at each sampling location from September through November 2009. Sampling locations included surface water and wastewater-treatment plant effluent. Twenty-five wastewater-treatment plants were selected to include continuous flow and periodic release facilities with differing processing steps (activated sludge or trickling filters) and plant design flows ranging from 0.002 to 10.9 cubic meters per second (0.04 to 251 million gallons per day) throughout Minnesota in varying land-use settings. Water samples were collected from the treated effluent of the 25 wastewater-treatment plants and at one point upstream from and one point downstream from wastewater-treatment plant effluent discharges. Bed-sediment samples also were collected at each of the stream or lake locations. Water samples were analyzed for major ions, nutrients, trace elements, pharmaceuticals, phytoestrogens and pharmaceuticals, alkylphenols and other neutral organic chemicals, carboxylic acids, and steroidal hormones. A subset (25 samples) of the bed-sediment samples were analyzed for carbon, wastewater-indicator chemicals, and steroidal hormones; the remaining samples were archived.

Biological characteristics were determined by using an *in-vitro* bioassay to determine total estrogenicity in water samples and a caged fish study to determine characteristics of fish from experiments that exposed fish to wastewater effluent in 2009. St. Cloud State University deployed and processed caged fathead minnows at 13 stream sites during September 2009 for the caged fish study. Measured fish data included length, weight, body condition factor, and vitellogenin concentrations.

Introduction

Streams receiving wastewater-treatment plant (WWTP) effluent have been documented to contain chemicals used in private homes, industry, and agriculture. A subset of these chemicals, endocrine active chemicals (EACs) (Ahel and others, 1994a, b; Desbrow and others, 1998; Ternes, 1998; Kolpin and others, 2002) and pharmaceuticals (Lajeunesse and others, 2008; Schultz and Furlong, 2008) have been identified in WWTP effluents and in surface waters in Minnesota (Barber and others, 2000, 2007; Lee and others, 2004; Lee, Schoenfuss, and others, 2008; Lee, Yaeger, and others, 2008; Martinovic and others, 2008; Writer and others, 2010).

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EACs include natural and synthetic chemicals that mimic or block the function of natural hormone mediated systems in animals, including fish (Kime, 1998; National Research Council, 1999) and invertebrates (Gagnaire and others, 2009). Although no single list of EACs exists, laboratory studies have confirmed that certain classes of chemicals including natural and synthetic hormones, pesticides, trace metals, alkylphenols, alkylphenol ethoxylates, plastic components, phthalates, and phytoestrogens affect the endocrine systems of fish through biochemical, structural, and behavioral disruption (Jobling and Sumpter, 1993; Jobling and others, 1996; Ankley and others, 1998; Kime, 1998; Miles-Richardson and others, 1999; Bistodeau and others, 2006; Barber and others, 2007; Schoenfuss and others, 2008). The presence of pharmaceuticals in surface waters can alter normal body functions of aquatic species, including invertebrate reproduction (Nentwig, 2007) and fish behavior (Painter and others, 2010).

More than 500 WWTPs throughout Minnesota discharge treated wastewater to surface water (fig. 1). Approximately 75 percent of the WWTPs release effluent periodically (generally biannually during the spring and fall) with design flows less than 0.04 cubic meter per second (m^3/s; 1 million gallons per day (Mgal/d)). Approximately 60 WWTPs discharge continuously to receiving streams with average design flows greater than 0.04 m^3/s (Lee and others, 2010).

Results from several investigations (Barber and others, 2000, 2007; Lee, Schoenfuss, and others, 2008; Lee, Yaeger, and others, 2008; Martinovic and others, 2008; Lee and others, 2010) indicate that concentrations of EACs in Minnesota WWTP effluent and receiving streams greatly vary. For example, nonylphenol was detected in effluent from 9 of 11 previously studied WWTPs at concentrations ranging from less than the detection level to 18.2 micrograms per liter (µg/L) among all samples (Lee and others, 2010).

The variability in chemical occurrence and concentrations in WWTP effluent is dependent upon the influent type and processing techniques of each WWTP (Richardson and Bowron, 1985; Stumpf and others, 1996; Ternes, 1998). Huang and Sedlak (2001) and Drewes and others (2005) determined that tertiary wastewater treatment resulted in a 70-percent reduction of EACs, and advanced treatment with reverse osmosis resulted in a 96-percent reduction of EACs. Drewes and others (2005) determined that although sewage treatment reduced the overall estrogenicity, effluents still had sufficient estrogenicity to elicit a response from a human breast cancer cell assay.

Indicators of endocrine disruption including elevated concentrations of vitellogenin in male fish (an egg yolk protein present in female fish but generally absent in male fish) and intersex occurrence (oocytes present in testes tissue) have been observed downstream from wastewater discharges (Folmar and others, 1996, 2001; Lee and others, 2000, 2010; Goodbred and others, 1997; Lee and Blazer, 2005; Lee, Schoenfuss, and others, 2008; Lee, Yaeger, and others, 2008).

Although WWTP effluent has been identified as a contributor of EACs to the aquatic environment (Desbrow and others, 1998; Ternes and others, 1999; Johnson and Sumpter, 2001; Vajda and others, 2008), EACs also have been detected in streams and lakes with no obvious WWTP effluent discharges indicating that other sources of contamination are contributing EACs (Lee and others, 2004, 2010; Lee, Schoenfuss, and others, 2008; Writer and others, 2010). EACs and pharmaceuticals can enter aquatic systems through a variety of pathways in addition to WWTP effluent including industrial effluent discharge, runoff from agricultural and urban land surfaces, land application of human and animal waste and subsequent movement to groundwater or surface water, and septic system discharge.

Although research and monitoring efforts have identified EACs and pharmaceuticals in WWTP effluent and receiving streams in Minnesota, the number of WWTPs sampled among the various studies represent a small fraction (less than 5 percent) of the WWTPs in Minnesota. In addition, little is known about EACs in bed sediment, which may serve as a reservoir of these chemicals in aquatic environments.

In order to address these issues, the U.S. Geological Survey (USGS), Minnesota Pollution Control Agency (MPCA), St. Cloud State University (SCSU), University of St. Thomas (UST), and the University of Colorado with input from the Minnesota Department of Natural Resources, Minnesota Department of Health, University of Minnesota, and the Metropolitan Council, began an integrated chemical and biological study in July 2009 to look at the occurrence of a broad suite of chemicals including EACs and pharmaceuticals in WWTP effluent and at sites upstream and downstream from the WWTP effluent discharge. Samples of surface water, wastewater effluent, and bed sediment were collected throughout Minnesota during 2009 and analyzed for selected EACs, pharmaceuticals, and other chemicals of concern. Biological characteristics were determined by using an *in-vitro* bioassay to determine total estrogenicity in water samples and a caged fish study to determine characteristics of fish from experiments that exposed fish to wastewater effluent in 2009.

The chemicals analyzed in this study were selected because they are indicators of human- and animal-waste sources to the environment and can affect aquatic organisms, although many of the chemicals also have natural sources (Barnes and others, 2008). The presence of naturally occurring compounds alone may not indicate a human- or animal-waste source, and some of the naturally occurring chemicals are incorporated in commercial products. Details of the potential natural sources are beyond the scope of this report, but may come from microorganisms, plant or animal sources, and may include by-products of combustion or other natural processes (Barnes and others, 2008).

Purpose and Scope

This report presents the study design, methods, environmental data, and quality-assurance and quality-control data for the integrated chemical and biological study of concentrations of EACs, pharmaceuticals, and other organic and inorganic

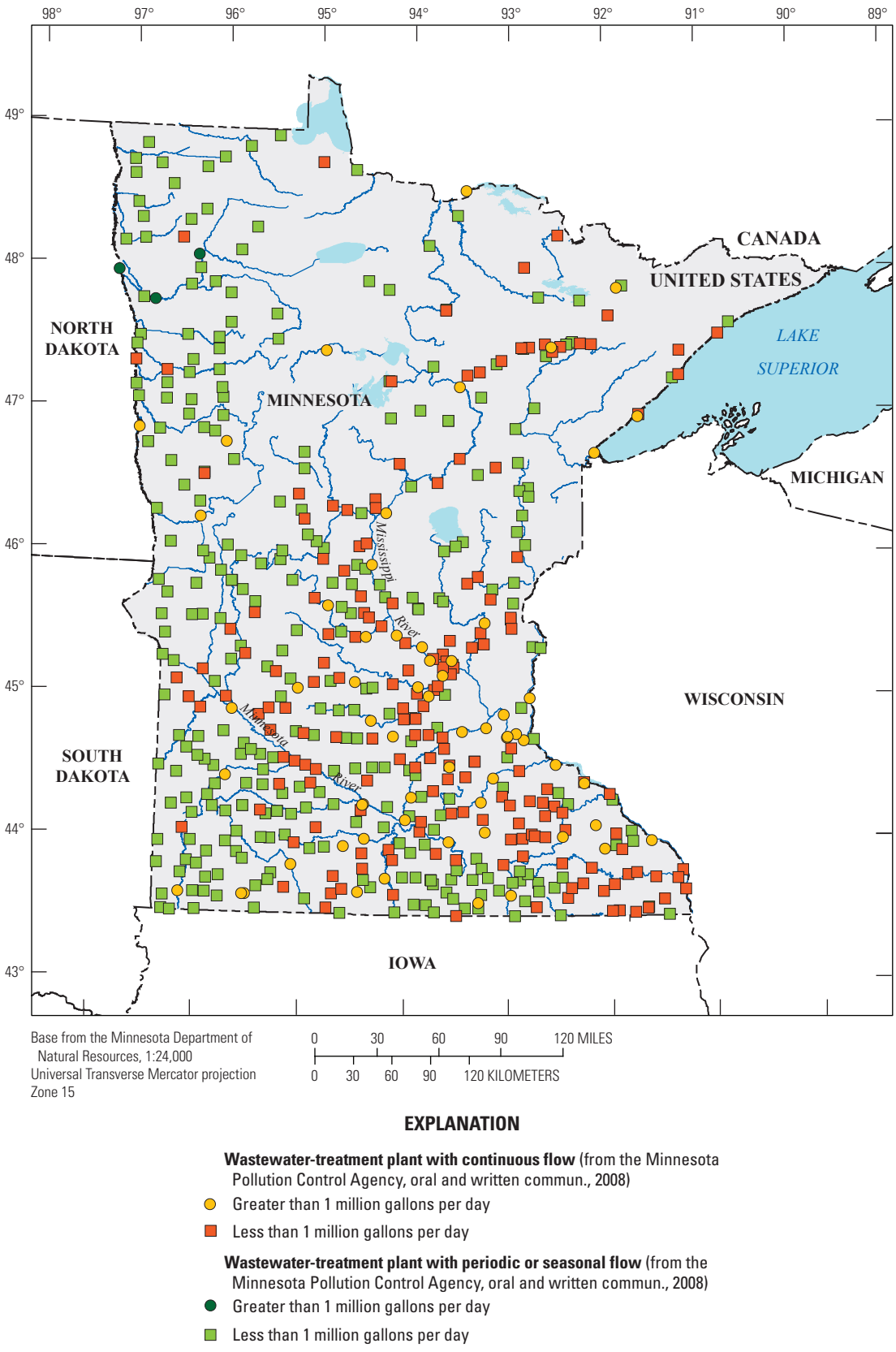

Figure 1. Locations of wastewater-treatment plants that discharge to surface waters in Minnesota.

chemicals in water samples collected from the effluents from 25 WWTPs and at 24 surface-water sites upstream and 24 sites downstream from WWTP effluent discharge in Minnesota during 2009. Environmental data and quality-assurance data also are presented for bed-sediment samples that were collected and analyzed at 25 sites. This report also provides data for biological characteristics: total estrogenicity estimates of each water sample using an *in-vitro* bioassay, and determined concentrations of plasma vitellogenin and other sex characteristics of caged fish from onsite exposure experiments.

Acknowledgments

This study was supported by numerous personnel from the 25 WWTPs sampled throughout Minnesota that graciously assisted with sampling and provided access for sampling. The authors also thank Paul Hoff, Leslie Goldsmith, and Gene Soderbeck from the MPCA; Dave Wright, Jack Enblom, Jan Wolf, and Allen Stevens from the Minnesota Department of Natural Resources; Kent Johnson and Mary Gale Scott from the Metropolitan Council Environmental Services; Hillary Carpenter from the Minnesota Department of Health; and Deborah Swackhamer and Paige Novak from the University of Minnesota for their advice on study design and site selection.

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Study Design

A longitudinal sampling design was utilized to sample the wastewater effluent and surface-water sites upstream and downstream from the effluent discharge in each receiving water (fig. 2). The upstream sample provides information about contaminants originating from sources other than the WWTP of interest, and the downstream sample provides information about contributions of contaminants from the wastewater effluent.

This study was designed to use chemical and biological measures to assess the presence and effects of EACs and pharmaceuticals in Minnesota WWTP effluents and receiving waters. Chemical analyses and *in-vitro* and *in-vivo* (estrogenicity) bioassays were used because each method has advantages and disadvantages addressing different questions. In this study, chemical data for EACs and other contaminants indicative of anthropogenic influence provided quantitative measures of the effects of WWTP effluents and land use on Minnesota surface waters. *In-vitro* cellular assays provide a method to measure the "total estrogenicity" of an aquatic environment (Martinovic and others, 2008), and *in-vivo* caged fish experiments provide a method to measure whole-organism responses.

Site Selection

Twenty-five WWTPs (and associated receiving streams) were selected (fig. 3, table 1). Site selection was based on WWTP and landscape characteristics. WWTPs of differing sizes, influent, and processing techniques were selected.

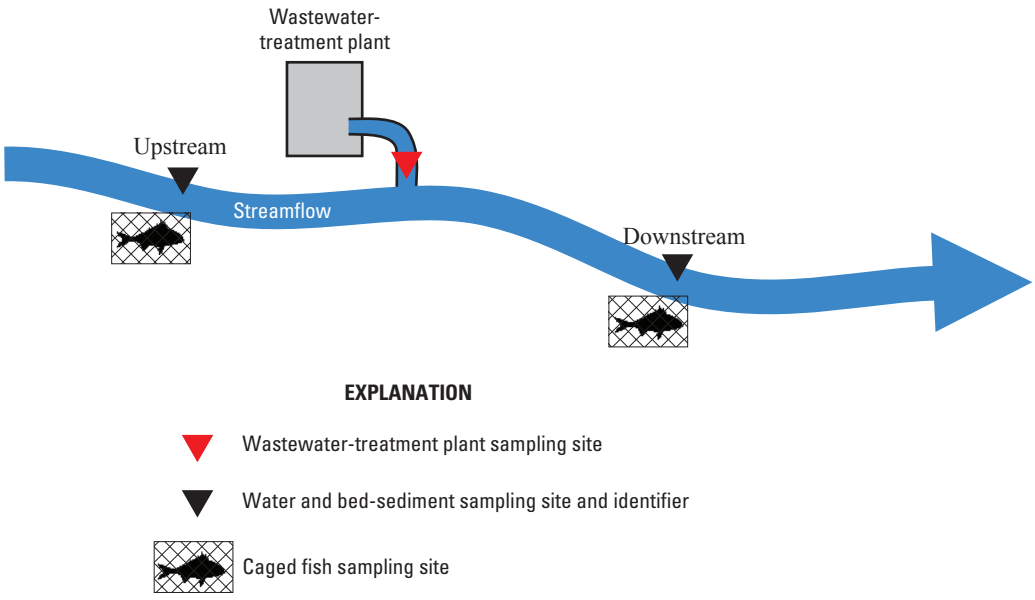

Figure 2. Relative locations of sampling sites upstream and downstream from discharge of wastewater-treatment plant effluent.

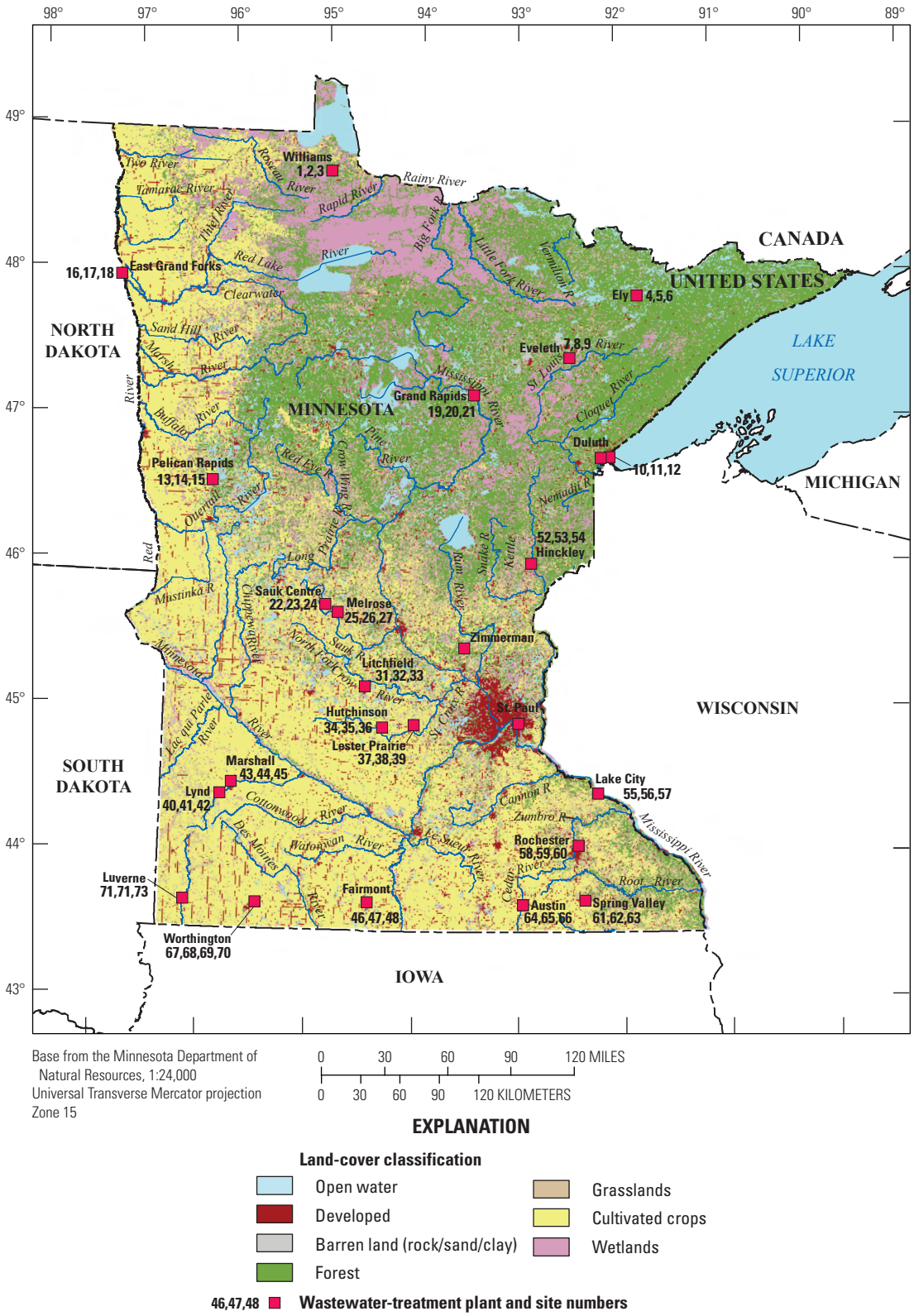

Figure 3. Locations during 2009. Each square typically represents three sites (one upstream, one downstream, and one wastewater-effluent sample).

Table 1. List of sampling sites, latitude, longitude, and sampling dates.

[USGS, U.S. Geological Survey; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; WWTP, wastewater-treatment plant; Minn., Minnesota; latitude and longitude are in degrees, minutes, seconds, and decimal seconds format; --, not published]

Site number (fig. 3)	USGS station identification number	USGS station name	Major river basin	Position in relation to wastewater-effluent discharge	Latitude	Longitude	Date water sampled
1	05137050	Williams Creek above WWTP at Williams, Minn.	Lake of the Woods	US	484626.53	945708.38	10/20/2009
2	484627094570801	Williams WWTP outflow at Williams, Minn.	Lake of the Woods	WWTP	--	--	10/20/2009
3	05137055	Williams Creek below WWTP at Williams, Minn.	Lake of the Woods	DS	484627.23	945708.80	10/20/2009
4	475504091545401	Shagawa Lake at mouth of Burntside River near Ely, Minn.	Rainy River	US	475505.23	915453.43	09/28/2009
5	475435091522601	Ely WWTP outflow at Ely, Minn.	Rainy River	WWTP	--	--	09/28/2009
6	475436091522501	Shagawa Lake near Ely WWTP outflow at Ely, Minn.	Rainy River	DS	475435.69	915226.35	09/28/2009
7	04018765	Elbow Creek above Eveleth WWTP at Eveleth, Minn.	St. Louis River	US	472737.50	923244.60	09/29/2009
8	472737092324501	Eveleth WWTP outflow at Eveleth, Minn.	St. Louis River	WWTP	--	--	09/29/2009
9	04018767	Elbow Creek below Eveleth WWTP at Eveleth, Minn.	St. Louis River	DS	472736.00	923246.40	09/29/2009
10	04024025	St. Louis River at Hwy. 23 above Fond Du Lac, Minn.	St. Louis River	US	463929.76	921702.14	10/01/2009
11	464538092072601	Western Lake Superior Sanitary District - WWTP at Duluth, Minn.	St. Louis River	WWTP	--	--	10/01/2009
12	464523092065501	Lake Superior in St. Louis Bay at Duluth, Minn.	St. Louis River	DS	464521.77	920715.42	10/01/2009
13	05040340	Pelican River above WWTP at Pelican Rapids, Minn.	Red River of the North	US	463411.89	960509.38	10/19/2009
14	463408096052401	Pelican Rapids WWTP outflow at Pelican Rapids, Minn.	Red River of the North	WWTP	--	--	10/19/2009
15	05040345	Pelican River below WWTP at Pelican Rapids, Minn.	Red River of the North	DS	463411.80	960534.00	10/19/2009
16	05082520	Red River of the North above WWTP at East Grand Forks, Minn.	Red River of the North	US	475831.78	970333.23	10/21/2009
17	475834097032002	Wastewater outflow at East Grand Forks, Minn.	Red River of the North	WWTP	--	--	10/21/2009
18	475854097032001	Red River of the North below WWTP at East Grand Forks, Minn.	Red River of the North	DS	475853.22	970321.79	10/21/2009
19	05211020	Mississippi River above WWTP at Grand Rapids, Minn.	Mississippi River	US	471357.60	933123.60	09/30/2009
20	471336093301801	Grand Rapids WWTP outflow at Grand Rapids, Minn.	Mississippi River	WWTP	--	--	09/30/2009
21	05211030	Mississippi River below WWTP at Grand Rapids, Minn.	Mississippi River	DS	471259.39	932917.10	09/30/2009
22	05270181	Sauk River above Sauk Centre WWTP at Sauk Centre, Minn.	Mississippi River	US	454308.18	945623.93	09/16/2009
23	454308094562601	Sauk Centre WWTP outflow at Sauk Centre, Minn.	Mississippi River	WWTP	--	--	09/16/2009
24	05270183	Sauk River below Sauk Centre WWTP at Sauk Centre, Minn.	Mississippi River	DS	454251.04	945617.75	09/16/2009
25	05270195	Sauk River above Melrose WWTP at Melrose, Minn.	Mississippi River	US	454037.14	944813.38	09/17/2009

Table 1. List of sampling sites, latitude, longitude, and sampling dates.—Continued

[USGS, U.S. Geological Survey; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; WWTP, wastewater-treatment plant; Minn., Minnesota; latitude and longitude are in degrees, minutes, seconds, and decimal seconds format; --, not published]

Site number (fig. 3)	USGS station identification number	USGS station name	Major river basin	Position in relation to wastewater-effluent discharge	Latitude	Longitude	Date water sampled
26	454040094480701	Melrose WWTP outflow at Melrose, Minn.	Mississippi River	WWTP	--	--	09/17/2009
27	05270197	Sauk River below Melrose WWTP at Melrose, Minn.	Mississippi River	DS	454045.20	944742.70	09/17/2009
28	05274850	Tibbets Brook above WWTP outflow at Zimmerman, Minn.	Mississippi River	US	452559.09	933454.50	09/03/2009
29	452559093345601	Zimmerman WWTP outflow at Zimmerman, Minn.	Mississippi River	WWTP	--	--	09/03/2009
30	05274855	Tibbets Brook below WWTP outflow at Zimmerman, Minn.	Mississippi River	DS	452558.54	933456.60	09/03/2009
31	05278080	Jewitts Creek at U.S. Hwy. 12 in Litchfield, Minn.	Mississippi River	US	450830.49	943122.53	10/08/2009
32	450833094311001	Litchfield WWTP outflow near Litchfield, Minn.	Mississippi River	WWTP	--	--	10/08/2009
33	05278083	Jewitts Creek near Litchfield, Minn.	Mississippi River	DS	450843.27	943059.71	10/08/2009
34	05278570	South Fork of the Crow River above WWTP at Hutchinson, Minn.	Mississippi River	US	445220.41	942124.86	09/14/2009
35	445220094212201	Hutchinson WWTP outflow at Hutchinson, Minn.	Mississippi River	WWTP	--	--	09/14/2009
36	05278580	South Fork of the Crow River below Hutchinson, Minn.	Mississippi River	DS	445202.91	942107.69	09/14/2009
37	05278650	South Fork Crow River above WWTP at Lester Prairie, Minn.	Mississippi River	US	445227.70	940303.70	09/15/2009
38	445243094020301	Lester Prairie WWTP outflow at Lester Prairie, Minn.	Mississippi River	WWTP	--	--	09/15/2009
39	05278655	South Fork Crow River below WWTP at Lester Prairie, Minn.	Mississippi River	DS	445251.93	940111.00	09/15/2009
40	05314985	Redwood River above Lynd WWTP near Lynd, Minn.	Mississippi River	US	442415.19	955231.69	11/23/2009
41	442415095523001	Lynd WWTP outflow near Lynd, Minn.	Mississippi River	WWTP	--	--	11/23/2009
42	05314988	Redwood River below Lynd WWTP near Lynd, Minn.	Mississippi River	DS	442419.29	955221.20	11/23/2009
43	05315045	Redwood River above WWTP below Marshall, Minn.	Mississippi River	US	442845.50	954633.79	10/07/2009
44	442846095463201	Marshall WWTP outflow at Marshall, Minn.	Mississippi River	WWTP	--	--	10/07/2009
45	05315050	Redwood River below WWTP near Marshall, Minn.	Mississippi River	DS	442912.10	954557.47	10/07/2009
46	05318170	Center Creek on Co. Rd. 143, at Fairmont, Minn.	Mississippi River	US	434021.78	942745.13	09/09/2009
47	434018094273301	Fairmont WWTP outflow at Fairmont, Minn.	Mississippi River	WWTP	--	--	09/09/2009
48	05318171	Center Creek below WWTP at Fairmont, Minn.	Mississippi River	DS	434022.72	942726.18	09/09/2009
49	05331005	Mississippi River at Industrial Mollasses, St. Paul, Minn.	Mississippi River	US	445511.04	930304.46	09/24/2009
50	445509093024301	Metro Plant (WWTP) outflow in St. Paul, Minn.	Mississippi River	WWTP	--	--	09/24/2009

Table 1. List of sampling sites, latitude, longitude, and sampling dates.—Continued

[USGS, U.S. Geological Survey; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; WWTP, wastewater-treatment plant; Minn., Minnesota; latitude and longitude are in degrees, minutes, seconds, and decimal seconds format; --, not published]

Site number (fig. 3)	USGS station identification number	USGS station name	Major river basin	Position in relation to wastewater-effluent discharge	Latitude	Longitude	Date water sampled
51	05331400	Mississippi River at South St. Paul, Minn.	Mississippi River	DS	445405.90	930217.60	09/24/2009
52	05337003	Grindstone River above WWTP near Hinckley, Minn.	Mississippi River	US	460106.86	925433.46	09/02/2009
53	460107092543101	Hinckley WWTP near Hinckley, Minn.	Mississippi River	WWTP	--	--	09/02/2009
54	05337005	Grindstone River below Hinckley, Minn.	Mississippi River	DS	460048.40	925323.57	09/02/2009
55	05355260	Mississippi River (Lake Pepin) above Lake City, Minn.	Mississippi River	US	442821.60	921624.80	09/23/2009
56	442626092152201	Lake City WWTP outflow at Lake City, Minn.	Mississippi River	WWTP	--	--	09/23/2009
57	05355331	Mississippi River (Lake Pepin) at Mile 771 near Lake City, Minn.	Mississippi River	DS	442624.90	921516.00	09/23/2009
58	05372995	South Fork Zumbro River at Rochester, Minn.	Mississippi River	US	440345.29	922754.75	09/22/2009
59	440350092275501	Rochester WWTP outflow at Rochester, Minn.	Mississippi River	WWTP	--	--	09/22/2009
60	05373005	South Fork Zumbro River below WWTP near Rochester, Minn.	Mississippi River	DS	440433.34	922759.58	09/22/2009
61	05383820	Spring Valley Creek above Spring Valley WWTP outflow at Spring Valley, Minn.	Mississippi River	US	434120.07	922250.52	09/21/2009
62	434122092225001	Spring Valley WWTP outflow at Spring Valley, Minn.	Mississippi River	WWTP	--	--	09/21/2009
63	05383822	Spring Valley Creek below Spring Valley WWTP outflow at Spring Valley, Minn.	Mississippi River	DS	434122.38	922246.33	09/21/2009
64	05455975	Cedar River above treatment plant at Austin, Minn.	Mississippi River	US	433924.80	925829.47	09/08/2009
65	433913092581601	Austin WWTP outflow at Austin, Minn.	Mississippi River	WWTP	--	--	09/08/2009
66	05455978	Cedar River below treatment plant at Austin, Minn.	Mississippi River	DS	433858.56	925825.74	09/08/2009
67	05474883	Okabena Creek above WWTP outflow at Worthington, Minn.	Mississippi River	US	433838.60	953443.60	09/09/2009
68	433838095344201	Worthington WWTP outflow at Worthington, Minn.	Mississippi River	WWTP-Domestic	--	--	09/09/2009
69	433847095330001	Industrial WWTP outflow near Worthington, Minn.	Mississippi River	WWTP-Industrial	--	--	09/09/2009
70	05474884	Okabena Creek below WWTP outflow at Worthington, Minn.	Mississippi River	DS	433848.54	953257.78	09/09/2009
71	06483005	Rock River above WWTP near Luverne, Minn.	Missouri River	US	433855.77	961154.54	10/06/2009
72	433856096115801	Luverne WWTP outflow near Luverne, Minn.	Missouri River	WWTP	--	--	10/06/2009
73	06483010	Rock River below WWTP near Luverne, Minn.	Missouri River	DS	433851.83	961157.03	10/06/2009

Streams or lakes with differing basin land use and varying percentages of streamflow composed of effluent were selected. Additionally, the existence of previous data and potential to work cooperatively with ongoing sample collection efforts were considered.

Site Characterization

Landscape characteristics such as basin drainage area, percentage of forested land, population, and the number of animal feedlots were generated for each sampling site using a geographic information system (GIS). The drainage basin and drainage area characteristics for each sampling site were generated from the point delineation and basin characteristics tools found on Minnesota's StreamStats Web site (U.S. Geological Survey, 2009). The sampling site drainage basin outlined the total upstream area that potentially contributes surface water to the sampling location. A GIS overlay of each drainage basin on land cover, feedlot, and population data was used to generate characteristics for each site.

Percentages of land-cover type were calculated for each drainage basin using 2001 National Land Cover Data (NLCD) (U.S. Geological Survey, 2006). The NLCD is a standardized nationwide dataset composed of 36 land-cover classes including cultivated crops, open water, and forested upland. In table 2, the 36 NLCD land-cover classes were consolidated into seven general categories: open water, developed, barren, forest, grassland, cropland, and wetland. The area of each consolidated land-cover category was summed and divided by the total drainage area to obtain percentages of each category within each drainage basin. The population per square kilometer was estimated by overlaying each drainage basin on the 2000 block-group population data (U.S. Census Bureau, 2002). The GIS was used to identify all block groups within each study basin and calculate the percentage of the total block-group area within each drainage area. Using GIS, the percentage value of the total block-group area was multiplied by the population of the block group to estimate the population of each block group within the drainage area. The population of all block groups represented within each drainage basin was then divided by the drainage area to calculate people per square kilometer within each basin (table 2).

A GIS point dataset of animal feedlots that contained tabular information of animal type and number of animals at each location was obtained from the Minnesota Pollution Control Agency (Minnesota Pollution Control Agency, 2009) and from the Iowa Department of Natural Resources (Iowa Department of Natural Resources, 2007). A GIS overlay of each drainage basin on feedlot locations was used to identify all feedlots within each drainage basin. A summary listing of the number of feedlots, animal types, and the total number of animals within each drainage basin is provided in table 3. Six sites (station numbers 05355331, 05355260, 05331400, 05331005, 05040345, and 05082520) on the Mississippi and Red Rivers had drainage areas that included parts of North Dakota, South Dakota, and Wisconsin. Information on animal feedlots was not available for these States, therefore, the overall number of animals for these drainage basins is likely greater than those provided in table 3.

Distances between the WWTP site and the upstream and downstream sampling sites were measured with a GIS by digitizing stream centerlines from 2008 aerial imagery (National Agriculture Imagery Program, 2008) at scales of 1:3,000–1:5,000. By study design, the characteristics of the WWTPs varied among the facilities sampled. A variety of WWTPs were selected to include those of differing treatment types (continuous flow or periodic releases), differing treatment processing steps (activated sludge or trickling filters), and plant design flows ranging from 0.002 to 10.9 m^3/s (0.04 to 251 Mgal/d) (table 4). The WWTPs selected also varied in the population served and the distribution of incoming influent between industrial and domestic sources.

Methods

This section of the report describes the data collection methods for hydrologic measurements, water-sample collection, and bed-sediment sample collection. Deployment methods for the caged fish are described. Methods for sample processing and analyses also are described.

Data Collection

During this study, hydrologic, chemical, and biological measurements or sample collections were made. Field measurements and sample collections were completed from September 2 to November 23, 2009. The types of samples collected and collection procedures are described in this section.

Hydrologic Measurements

Streamflow was measured at each stream site following USGS protocols (Rantz and others, 1982a, b; Morlock and others, 2002). Daily effluent discharge values from WWTPs were obtained from each facility at the time of sampling. Basic water-quality properties (dissolved oxygen, pH, specific conductance, temperature, and turbidity) were measured at each site using a submersible YSI (Yellow Springs Instruments) data sonde (Yellow Springs, Ohio). The data sonde was calibrated according to U.S. Geological Survey (variously dated) and manufacturer's specifications before and after sampling.

The fraction of wastewater effluent (f_{eff}) in the receiving stream was estimated as follows:

$$f_{eff} = \frac{Q_{eff}}{Q_{eff} + Q_{us}} \quad (1)$$

Table 2. Population and land-cover percentages for drainage basins for sampling sites.

[USGS, U.S.Geological Survey; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; WWTP, wastewater-treatment plant; Minn., Minnesota]

Site number (fig. 3)	USGS station identification number	USGS station name	Position in relation to wastewater-effluent discharge	Drainage area (square kilometer)	Number of people per square kilometer	Land-cover percentages						
						Open water	Developed	Barren	Forest	Grass-land	Crop-land	Wet-land
1	05137050	Williams Creek above WWTP at Williams, Minn.	US	32.8	1	0	2	0	63	6	1	28
3	05137055	Williams Creek below WWTP at Williams, Minn.	DS	32.8	1	0	2	0	63	6	1	28
4	475504091545401	Shagawa Lake at mouth of Burntside River near Ely, Minn.	US	184.9	2	21	1	0	71	5	0	2
5	475436091522501	Shagawa Lake near Ely WWTP outflow at Ely, Minn.	DS	240.8	4	20	1	0	73	4	0	2
7	04018765	Elbow Creek above Eveleth WWTP at Eveleth, MN	US	4.45	238	1	32	9	46	11	0	1
9	04018767	Elbow Creek below Eveleth WWTP at Eveleth, Minn.	DS	4.48	237	1	32	9	46	11	0	1
10	04024025	St. Louis River at Hwy. 23 above Fond Du Lac, Minn.	US	9,253	11	4	3	1	62	10	0	21
11	464523092065501	Lake Superior in St. Louis Bay at Duluth, Minn.	DS	9,583	14	4	3	1	62	10	0	20
13	05040340	Pelican River above WWTP at Pelican Rapids, Minn.	US	892.8	20	21	6	0	29	23	15	5
15	05040345	Pelican River below WWTP at Pelican Rapids, Minn.	DS	894.8	20	21	6	0	29	23	15	5
16	05082520	Red River of the North above WWTP at East Grand Forks, Minn.	US	77,337	6	6	4	0	8	15	56	10
18	475854097032001	Red River of the North below WWTP at East Grand Forks, Minn.	DS	77,343	6	6	4	0	8	15	56	10
19	05211020	Mississippi River above WWTP at Grand Rapids, Minn.	US	8,475	8	16	3	0	57	8	1	16
21	05211030	Mississippi River below WWTP at Grand Rapids, Minn.	DS	8,486	8	16	3	0	57	8	1	16
22	05270181	Sauk River above Sauk Centre WWTP at Sauk Centre, Minn.	US	970.5	10	6	5	0	7	25	51	5
24	05270183	Sauk River below Sauk Centre WWTP at Sauk Centre, Minn.	DS	972.2	11	6	5	0	7	25	51	5

Table 2. Population and land-cover percentages for drainage basins for sampling sites.—Continued

[USGS, U.S.Geological Survey; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; WWTP, wastewater-treatment plant; Minn., Minnesota]

Site number (fig. 3)	USGS station identification number	USGS station name	Position in relation to wastewater-effluent discharge	Drainage area (square kilometer)	Number of people per square kilometer	Land-cover percentages						
						Open water	Developed	Barren	Forest	Grass-land	Crop-land	Wet-land
25	05270195	Sauk River above Melrose WWTP at Melrose, Minn.	US	1,124	12	5	5	0	7	26	51	5
27	05270197	Sauk River below Melrose WWTP at Melrose, Minn.	DS	1,134	12	5	5	0	7	26	51	5
28	05274850	Tibbets Brook above WWTP outflow at Zimmerman, Minn.	US	18.5	96	11	10	0	25	28	18	8
30	05274855	Tibbets Brook below WWTP outflow at Zimmerman, Minn.	DS	18.5	96	11	10	0	25	28	18	8
31	05278080	Jewitts Creek at U.S. Hwy. 12 in Litchfield, Minn.	US	67.5	57	9	13	0	7	10	55	5
33	05278083	Jewitts Creek near Litchfield, Minn.	DS	67.9	58	9	13	0	7	10	55	5
34	05278570	South Fork of the Crow River above WWTP at Hutchinson, Minn.	US	1,160	23	7	6	0	3	8	74	3
36	05278580	South Fork of the Crow River below Hutchinson, Minn.	DS	1,162	23	7	6	0	3	8	74	3
37	05278650	South Fork Crow River above WWTP at Lester Prairie, Minn.	US	1,460	23	6	6	0	4	9	74	3
39	05278655	South Fork Crow River below WWTP at Lester Prairie, Minn.	DS	1,463	23	6	6	0	4	9	73	3
40	05314985	Redwood River above Lynd WWTP near Lynd, Minn.	US	660.5	5	4	6	0	1	19	67	3
42	05314988	Redwood River below Lynd WWTP near Lynd, Minn.	DS	660.5	5	4	6	0	1	19	67	3
43	05315045	Redwood River above WWTP below Marshall, Minn.	US	692	14	3	7	0	1	19	66	3
45	05315050	Redwood River below WWTP near Marshall, Minn.	DS	696.4	14	3	7	0	1	19	67	3
46	05318170	Center Creek on Co. Rd. 143, at Fairmont, Minn.	US	238.2	44	6	12	0	1	4	75	2
48	05318171	Center Creek below WWTP at Fairmont, Minn.	DS	238.5	44	6	12	0	1	4	75	2
49	05331005	Mississippi River at Industrial Molasses St. Paul, Minn.	US	95,703	35	6	6	0	20	14	45	9

Table 2. Population and land-cover percentages for drainage basins for sampling sites.—Continued

[USGS, U.S.Geological Survey; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; WWTP, wastewater-treatment plant; Minn., Minnesota]

Site number (fig. 3)	USGS station identification number	USGS station name	Position in relation to wastewater-effluent discharge	Drainage area (square kilometer)	Number of people per square kilometer	Land-cover percentages						
						Open water	Developed	Barren	Forest	Grass-land	Crop-land	Wet-land
51	05331400	Mississippi River at South St. Paul, Minn.	DS	95,712	36	6	6	0	20	14	45	9
52	05337003	Grindstone River above WWTP near Hinckley, Minn.	US	206.7	9		4	0	40	32	3	19
54	05337005	Grindstone River below Hinckley, Minn.	DS	209.4	9	2	4	0	40	32	4	19
55	05355260	Mississippi River (Lake Pepin) above Lake City, Minn.	US	122,558	34	5	6	0	25	15	39	9
57	05355331	Mississippi River (Lake Pepin) at Mile 771 near Lake City, Minn.	DS	122,578	34	5	6	0	25	15	39	9
58	05372995	South Fork Zumbro River at Rochester, Minn.	US	778.9	106	0	15	0	8	23	52	1
60	05373005	South Fork Zumbro River below WWTP near Rochester, Minn.	DS	807.9	111	0	15	0	8	24	51	1
61	05383820	Spring Valley Creek above Spring Valley WWTP outflow at Spring Valley, Minn.	US	40.5	40	0	11	0	2	6	81	0
63	05383822	Spring Valley Creek below Spring Valley WWTP outflow at Spring Valley, Minn.	DS	40.6	41	0	11	0	2	6	81	0
64	05455975	Cedar River above treatment plant at Austin, Minn.	US	631	29	0	9	0	1	6	82	2
66	05455978	Cedar River below treatment plant at Austin, Minn.	DS	631.6	30	0	9	0	1	6	82	2
67	05474883	Okabena Creek above WWTP outflow at Worthington, Minn.	US	8.1	672	0	66	0	0	3	30	1
70	05474884	Okabena Creek below WWTP outflow at Worthington, Minn.	DS	15.06	386	0	46	0	0	3	49	1
71	06483005	Rock River above WWTP near Luverne, Minn.	US	1,085	6	0	6	0	1	17	76	1
73	06483010	Rock River below WWTP near Luverne, Minn.	DS	1,085	6	0	6	0	1	17	76	1

Table 3. Number of feedlots and animal type summaries for drainage basins for each sampling site.

[USGS, U.S. Survey; WWTP, wastewater-treatment plant; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; Minn., Minnesota]

Site number (fig. 3)	USGS station identification number	USGS station name	Position in relation to WWTP discharge	Total number of upstream feedlots[1]	Number of animals[1]								
					Poultry	Bovine	Deer and elk	Goats and sheep	Horses	Llama	Pigs	Other animals	Total
1	05137050	Williams Creek above WWTP at Williams, Minn.	US	0	0	0	0	0	0	0	0	0	0
3	05137055	Williams Creek below WWTP at Williams, Minn.	DS	0	0	0	0	0	0	0	0	0	0
4	475504091545401	Shagawa Lake at mouth of Burntside River near Ely, Minn.	US	0	0	0	0	0	0	0	0	0	0
5	475436091522501	Shagawa Lake near Ely WWTP outflow at Ely, Minn.	DS	0	0	0	0	0	0	0	0	0	0
7	04018765	Elbow Creek above Eveleth WWTP at Eveleth, Minn.	US	0	0	0	0	0	0	0	0	0	0
9	04018767	Elbow Creek below Eveleth WWTP at Eveleth, Minn.	DS	0	0	0	0	0	0	0	0	0	0
10	04024025	St. Louis River at Hwy. 23 above Fond Du Lac, Minn.	US	38	75,012	3,847	0	68	53	0	2,852	0	81,832
11	464523092065501	Lake Superior in St. Louis Bay at Duluth, Minn.	DS	39	75,012	4,017	0	68	53	0	2,872	3	82,025
13	05040340	Pelican River above WWTP at Pelican Rapids, Minn.	US	68	539,418	7,093	0	301	52	3	1,400	15,005	563,272
15	05040345	Pelican River below WWTP at Pelican Rapids, Minn.	DS	68	539,418	7,093	0	301	52	3	1,400	15,005	563,272
16	05082520	Red River of the North above WWTP at East Grand Forks, Minn.[1]	US	1,134	4,062,803	164,990	16	16,941	1,135	3	123,868	93,012	4,462,768
18	475854097032001	Red River of the North below WWTP at East Grand Forks, Minn.[1]	DS	1,134	4,062,803	164,990	16	16,941	1,135	3	123,868	93,012	4,462,768
19	05211020	Mississippi River above WWTP at Grand Rapids, Minn.	US	95	410	12,673	80	2,480	331	2	103	2	16,081
21	05211030	Mississippi River below WWTP at Grand Rapids, Minn.	DS	95	410	12,673	80	2,480	331	2	103	2	16,081
22	05270181	Sauk River above Sauk Centre WWTP at Sauk Centre, Minn.	US	317	315,127	43,875	214	1,240	414	2	35,548	24,910	421,330
24	05270183	Sauk River below Sauk Centre WWTP at Sauk Centre, Minn.	DS	318	404,627	43,875	214	1,240	414	2	35,548	24,910	510,830
25	05270195	Sauk River above Melrose WWTP at Melrose, Minn.	US	411	669,162	58,331	214	1,868	454	2	37,316	24,910	792,257

Table 3. Number of feedlots and animal type summaries for drainage basins for each sampling site.—Continued

[USGS, U.S. Survey; WWTP, wastewater-treatment plant; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; Minn., Minnesota]

Site number (fig. 3)	USGS station identification number	USGS station name	Position in relation to WWTP discharge	Total number of upstream feedlots[1]	Number of animals[1]								
					Poultry	Bovine	Deer and elk	Goats and sheep	Horses	Llama	Pigs	Other animals	Total
27	05270197	Sauk River below Melrose WWTP at Melrose, Minn.	DS	419	669,162	58,844	214	1,868	454	2	37,316	24,910	792,770
28	05274850	Tibbets Brook above WWTP outflow at Zimmerman, Minn.	US	0	0	0	0	0	0	0	0	0	0
30	05274855	Tibbets Brook below WWTP outflow at Zimmerman, Minn.	DS	0	0	0	0	0	0	0	0	0	0
31	05278080	Jewitts Creek at U.S. Hwy. 12 in Litchfield, Minn.	US	13	1,314,275	568	0	110	4	0	364	0	1,315,321
33	05278083	Jewitts Creek near Litchfield, Minn.	DS	13	1,314,275	568	0	110	4	0	364	0	1,315,321
34	05278570	South Fork of the Crow River above WWTP at Hutchinson, Minn.	US	217	1,214,025	24,673	0	2,268	236	7	31,488	37	1,272,734
36	05278580	South Fork of the Crow River below Hutchinson, Minn.	DS	218	1,214,025	24,688	0	2,268	236	7	31,488	37	1,272,749
37	05278650	South Fork Crow River above WWTP at Lester Prairie, Minn.	US	308	1,217,502	36,562	0	4,325	362	32	36,032	77	1,294,892
39	05278655	South Fork Crow River below WWTP at Lester Prairie, Minn.	DS	309	1,217,652	36,566	0	4,345	366	32	36,032	77	1,295,070
40	05314985	Redwood River above Lynd WWTP near Lynd, Minn.	US	171	4,630	22,657	0	12,100	166	3	43,325	0	2,881
42	05314988	Redwood River below Lynd WWTP near Lynd, Minn.	DS	171	4,630	22,657	0	12,100	166	3	43,325	0	82,881
43	05315045	Redwood River above WWTP below Marshall, Minn.	US	174	4,630	23,727	0	12,100	169	3	44,714	0	85,343
45	05315050	Redwood River below WWTP near Marshall, Minn.	DS	174	4,630	23,727	0	12,100	169	3	44,714	0	85,343
46	05318170	Center Creek on County Road. 143, at Fairmont, Minn.	US	59	1,021	3,978	0	140	29	0	88,887	0	94,055
48	05318171	Center Creek below WWTP at Fairmont, Minn.	DS	59	1,021	3,978	0	140	29	0	88,887	0	94,055
49	05331005	Mississippi River at Industrial Molasses St. Paul, Minn.[1]	US	13,989	38,523,687	1,596,314	3,988	149,887	24,904	495	5,243,498	46,159	45,688,932
51	05331400	Mississippi River at South St. Paul, Minn.[1]	DS	13,989	38,523,687	1,596,314	3,988	149,887	24,904	495	5,243,498	46,159	45,688,932

Table 3. Number of feedlots and animal type summaries for drainage basins for each sampling site.—Continued

[USGS, U.S. Survey; WWTP, wastewater-treatment plant; US, upstream from wastewater-effluent discharge; DS, downstream from wastewater-effluent discharge; Minn., Minnesota]

Site number (fig. 3)	USGS station identification number	USGS station name	Position in relation to WWTP discharge	Total number of upstream feedlots[1]	Number of animals[1]								
					Poultry	Bovine	Deer and elk	Goats and sheep	Horses	Llama	Pigs	Other animals	Total
52	05337003	Grindstone River above WWTP near Hinckley, Minn.	US	9	0	1,078	0	0	10	0	0	0	1,088
54	05337005	Grindstone River below Hinckley, Minn.	DS	11	0	1,396	0	0	10	0	0	0	1,406
55	05355260	Mississippi River (Lake Pepin) above Lake City, Minn.[1]	US	16,035	41,163,689	1,794,522	4,974	163,778	29,066	585	5,571,698	87,257	49,115,569
57	05355331	Mississippi River (Lake Pepin) at Mile 771 near Lake City, Minn.[1]	DS	16,035	41,163,689	1,794,522	4,974	163,778	29,066	585	5,571,698	87,257	49,115,569
58	05372995	South Fork Zumbro River at Rochester, Minn.	US	220	99,599	21,747	0	1,154	394	0	63,428	0	86,322
60	05373005	South Fork Zumbro River below WWTP near Rochester, Minn.	DS	223	99,599	22,181	0	1,154	394	0	63,773	0	87,101
61	05383820	Spring Valley Creek above Spring Valley WWTP outflow at Spring Valley, Minn.	US	9	0	728	0	49	0	0	872	0	1,649
63	05383822	Spring Valley Creek below Spring Valley WWTP outflow at Spring Valley, Minn.	DS	9	0	728	0	49	0	0	872	0	1,649
64	05455975	Cedar River above treatment plant at Austin, Minn.	US	213	56,965	9,168	50	1,254	306	0	125,007	35	192,785
66	05455978	Cedar River below treatment plant at Austin, Minn.	DS	213	56,965	9,168	50	1,254	306	0	125,007	35	192,785
67	05474883	Okabena Creek above WWTP outflow at Worthington, Minn.	US	2	0	0	0	0	0	0	3,600	0	3,600
70	05474884	Okabena Creek below WWTP outflow at Worthington, Minn.	DS	3	0	150	0	0	0	0	3,600	0	3,750
71	06483005	Rock River above WWTP near Luverne, Minn.	US	447	71,066	65,950	0	6,247	228	1	178,376	3	321,871
73	06483010	Rock River below WWTP near Luverne, Minn.	DS	447	71,066	65,950	0	6,247	228	1	178,376	3	321,871

[1]Stations 05355331, 05355260, 05331400,05331005, 05040345, and 05082520 have contributing drainage areas in North Dakota, South Dakota, and Wisconsin. Specific location information for animal feedlots was not available for these States. Therefore, the overall numbers of animals for these stations are likely greater than those provided.

Table 4. Characteristics of wastewater-treatment plants sampled.

[NPDES, National Pollutant Discharge Elimination System; USGS, U.S. Geological Survey; Mgal/d, million gallons per day; m^3/s, cubic meters per second; Chl, chlorination; DEChl, dechlorination; UV, ultraviolet; WWTP, wastewater-treatment plant; Minn., Minnesota]

NPDES identification number	Site number (fig. 3)	USGS station name	Design flow (Mgal/d)	Design flow (m^3/s)	Type	Treatment processes	Disinfection[1]	Phosphorus limits exist?	Population served	Percent domestic	Percent industrial
MN0021679	2	Williams WWTP outflow at Williams, Minn.	0.08	0.0035	Continuous	Activated sludge, extended aeration	UV	Yes	865	100	0
MN0020508	5	Ely WWTP outflow at Ely, Minn.	1.5	.0657	Continuous	Activated sludge, extended aeration; sand filters	Chl/DEChl	Yes	3,900	100	0
MN0023337	8	Eveleth WWTP outflow at Eveleth, Minn.	1	.0438	Continuous	Activated sludge, extended aeration; sand filters	Chl/DEChl	Yes	3,900	99	1
MN0049786	11	Western Lake Superior Sanitary District—WWTP at Duluth, Minn	48.4	2.119	Continuous	Activated sludge, pure oxygen; sand filters	Chl/DEChl	Yes	111,203	55	45
MN0022225	13	Pelican Rapids WWTP outflow at Pelican Rapids, Minn.	.91	.0398	Continuous	Trickling filter; rotating biological contactor	Chl/DEChl	Yes	2,476	67	33
MN0021814	17	Wastewater outflow at East Grand Forks, Minn.	1.4	.0613	Controlled	Stabilization ponds	Chl/DEChl	No	8,000	80	20
MN0022080	20	Grand Rapids WWTP outflow at Grand Rapids, Minn.	15.2	.6657	Continuous	Activated sludge, contact stabilization, conventional, step feed	Chl/DEChl	No	12,000	10	90
MN0024821	23	Sauk Centre WWTP outflow at Sauk Centre, Minn.	.88	.0385	Continuous	Activated sludge, contact stabilization, conventional, step feed	Chl/DEChl	Yes	4,111	100	0
MN0020290	26	Melrose WWTP outflow at Melrose, Minn.	3	.1314	Continuous	Trickling filter; activated sludge, contact stabilization, conventional, step feed	Chl/DEChl	Yes	3,400	20	80
MN0042331	29	Zimmerman WWTP outflow at Zimmerman, Minn.	.45	.0197	Continuous	Activated sludge, conventional, sequencing batch reactors	UV	Yes	5,000	100	0
MN0023973	32	Litchfield WWTP outflow near Litchfield, Minn.	2.4	.1051	Continuous	Trickling filter; activated sludge, contact stabilization, convention, step feed	Chl/DEChl	Yes	7,500	50	50
MN0055832.	35	Hutchinson WWTP outflow at Hutchinson, Minn.	5.4	.2365	Continuous	Membrane bioreactor[2]	NA	No	13,900	68	32
MN0023957	38	Lester Prairie WWTP outflow at Lester Prairie, Minn.	.36	.0157	Continuous	Activated sludge, extended aeration, oxidation ditch	UV	Yes	1,774	90	10
MNG580030	41	Lynd WWTP outflow near Lynd, Minn.	.045	.0019	Controlled	Stabilization ponds	Chl/DEChl	No	410	90	10

Table 4. Characteristics of wastewater-treatment plants sampled.—Continued

[NPDES, National Pollutant Discharge Elimination System; USGS, U.S. Geological Survey; Mgal/d, million gallons per day; m^3/s, cubic meters per second; Chl, chlorination; DEChl, dechlorination; UV, ultraviolet; WWTP, wastewater-treatment plant; Minn., Minnesota]

NPDES identification number	Site number (fig. 3)	USGS station name	Design flow (Mgal/d)	Design flow (m^3/s)	Type	Treatment processes	Disinfection[1]	Phosphorus limits exist?	Population served	Percent domestic	Percent industrial
MN0022179	44	Marshall WWTP outflow at Marshall, Minn.	4.5	0.1971	Continuous	Trickling filter; activated sludge, contact stabilization, conventional, step fee; sand filter	UV	Yes	13,000	40	60
MN0030112	47	Fairmont WWTP outflow at Fairmont, Minn.	3.9	.1708	Continuous	Activated sludge, contact stabilization, conventional, step feed	UV	Yes	10,889	82	18
MN0029815	50	Metro Plant (WWTP) outflow in St. Paul, Minn.	251	10.99	Continuous	Activated sludge, contact stabilization, conventional, step feed	Chl/DEChl	Yes	1,800,000	na	na
MN0023701	53	Hinckley WWTP near Hinckley, Minn.	.5	.0219	Continuous	Activated sludge, extended aeration	UV	Yes	1,438	100	0
MN0020664	56	Lake City WWTP outflow at Lake City, Minn.	1.52	.066576	Continuous	Activated sludge, contact stabilization, conventional, step feed	UV	Yes	5,300	75	25
MN0024619	59	Rochester WWTP outflow at Rochester, Minn.	19.1	.83658	Continuous	Activated sludge, pure oxygen	Chl/DEChl	Yes	100,000	50	50
MN0051934	62	Spring Valley WWTP outflow at Spring Valley, Minn.	.94	.041172	Continuous	Activated sludge, extended aeration, oxidation ditch	Chl/DEChl	No	2,561	95	5
MN0022683	65	Austin WWTP outflow at Austin, Minn.	8.5	.3723	Continuous	Trickling filter	Chl/DEChl	No	23,000	67	33
MN0031186	68	Worthington WWTP outflow at Worthington, Minn.	4	.1752	Continuous	Trickling filter	Chl	Yes	11,283	97	3
MN0031178	69	Industrial WWTP outflow near Worthington, Minn.	2.29	.0876	Continuous	Anaerobic ponds; activated sludge extended aeration; sand filters	Chl	Yes	0	0	100
MN0020141	72	Luverne WWTP outflow near Luverne, Minn.	1.5	.0657	Continuous	Activated sludge; trickling filter	Chl/DEChl	No	4,617	90	10

[1] Minnesota WWTPs are required to disinfect seasonally according to State of Minnesota Rule 7053.0215 subpart 1 (Minnesota Office of the Revisor of Statutes, 2010). Disinfection was occurring during sampling.

[2] Effluent that had passed through the activated sludge membrane bio-reactor was sampled.

where

Q_{eff} (in cubic meters per second) is the total discharge of wastewater effluent on the day of sampling (provided by the wastewater operator) and

Q_{us} (in cubic meters per second) is the measured streamflow at the upstream site on the day of the sampling using USGS protocols (Rantz and others, 1982a, b).

Tracer studies using rhodamine WT dye (20-percent active ingredient, Ben Meadows®, Janesville, Wisc.) were performed at selected WWTPs. Using methods developed by Kilpatrick and Wilson (1989), a pre-determined volume of rhodamine WT dye (V_s; in liters) was added to the effluent channel of the WWTP, based on an estimate of streamflow (Q_m; in cubic feet per second), distance to the downstream sampling site (L; in miles), estimated stream velocity (u_{est}; in feet per second), and a desired peak concentration of less than 100 µg/L of dye at the downstream monitoring site (C_p):

$$V = 3.4x10^{-4}\left(\frac{Q_m L}{u_{est}}\right)C_p \quad (2)$$

After the rhodamine WT dye was added to the effluent channel, relative fluorescence was measured at the downstream site using either a YSI fluorometer (Yellow Springs, Ohio) or a Self-Contained Underwater Fluorescence Apparatus (SCUFA, Turner Designs, Sunnyvale, Calif.). Instruments were placed at 50 percent of the channel width. Median hydraulic transit times (t_{med}) were determined as the time when one-half of the measured mass of rhodamine WT dye (assuming constant streamflow) passed the observation point (Runkel, 2002).

Additionally, hydrologic properties such as downstream width, downstream depth and stream slope were measured at each site at which a tracer study was performed. Stream width and average stream depth at the downstream site were measured coincident with the streamflow measurement at each location. The wetted stream width was measured along the stream cross-section where the streamflow measurement was completed. Stream depths were measured at each location where stream velocities were recorded. Stream slope estimates were determined for each site by locating points where contour lines cross stream lines on USGS 1:24,000 topographic maps. The distance between contour crossings was measured using high-resolution national hydrography data (U.S. Geological Survey, 2008). The distance between contour crossings was divided by the change in altitude to obtain stream slope.

These hydraulic properties were used to calculate the theoretical mixing distance (L_m; in meters) at which a side discharge would be completely mixed. The distance L_m is dependent upon the longitudinal dispersion coefficient (E_{lat}; in meters squared per second; eq. 4), average velocity of the river (u_{est}; in meters per second), and stream width (B; in meters) (Fisher and others, 1979). The shear velocity (U*; in meters per second; eq. 5) defines these parameters, and can be computed from the gravitational constant (g; 9.8 meters per second per second), the depth of the river (H; in meters), and the stream slope (S; in meters per meter).

$$L_m = \frac{0.1u(2B)^2}{E_{lat}} \quad (3)$$

$$E_{lat} = (0.6)HU^* \quad (4)$$

$$U^* = \sqrt{gHS} \quad (5)$$

Water-Sample Collection

Integrated width-and depth-sampling techniques were used to collect water from streams (U.S. Geological Survey, variously dated). A modified approach was used to collect lake water samples including a depth integrated sample from 10 locations radiating from the effluent discharge location. Wastewater was collected directly from each WWTP effluent discharge channel. Following collection, water samples were composited into a Teflon® churn and chilled before processing. Chilled water samples were processed within 1 to 2 hours of collection before shipping to their respective laboratories.

USGS clean-sampling techniques were used to collect samples (U.S. Geological Survey, variously dated). In order to avoid contamination of samples, personnel avoided use of personal-care items such as insect repellent, sunscreen, cologne, aftershave, and perfume; and they did not consume caffeinated or tobacco products during (or immediately before) collection or processing of samples; and they wore powderless, disposable gloves during sample collection. All samples were collected with inert materials such as Teflon®, glass, or stainless steel. All collection and processing equipment was cleaned between sampling sites with a succession of native water, soapy (liquinox) tap water, tap water, deionized water, methanol, and organic-free water rinses.

Bed-Sediment Sample Collection

Bed-sediment samples were collected upstream and downstream from each WWTP according to established protocols (U.S. Geological Survey, variously dated). Bed sediment was collected at 5–10 locations at each site using techniques that obtained the most recent bed-sediment deposition (top 10 centimeters (cm)). Samples were collected with a stainless steel Eckman grab sampler or other stainless steel coring equipment. The bed-sediment sample was discarded and resampled if it contained a large amount of vegetation or appeared to be disturbed. Bed-sediment samples were transferred to a glass

bowl and homogenized with a stainless steel spoon for 5 minutes. Approximately 100–200 grams (g) of unsieved wet material was placed in wide-mouth, glass containers, and frozen. All but 25 samples were archived frozen at -4 degrees Celsius (°C) for potential future analyses; the subset of 25 samples underwent chemical analyses.

Caged Fish Deployment

Fathead minnows (*Pimphales promelas*) were obtained from a laboratory fish supplier (Environmental Testing and Consulting, Superior, Wisc.). The minnows were caged and deployed at 13 sites in three river systems during the fall of 2009. Cages were placed in the wastewater-effluent discharge channels from five WWTPs (Sauk Center, Melrose, Hutchinson, Lester Prairie, and Litchfield) in three river systems (Sauk River, South Fork of the Crow River, and Jewitts Creek). For each site, fish were caged upstream from the effluent discharge location, in the effluent discharge, and in the mixing zone downstream from the effluent discharge location. At each site, two cages (wire-mesh, 10 cm by 10 cm by 24 cm) were deployed. One cage contained 15 male fathead minnows, and the second cage contained 15 female fathead minnows. Fish were caged for 14 days before retrieval. In addition, 15 male and 15 female fathead minnows were processed prior to deployment to serve as a pre-deployment baseline (control) group.

Sample Processing and Analyses

This section describes the methods used to process and analyze water and bed-sediment samples. Methods for biological analyses also are described. Six laboratories performed analyses for samples in this study: (1) the USGS National Water Quality Laboratory (USGS-NWQL) in Denver Colo., (2) the USGS National Research Program Laboratory (USGS-NRPL) in Boulder, Colo., (3) the USGS Iowa Sediment Laboratory (USGS-IASED) in Iowa City, Iowa, (4) the University of Colorado Center for Environmental Mass Spectrometry in Boulder, Colo., (5) the UST Laboratory, and (6) the SCSU Aquatic Toxicology Laboratory.

Surface-Water and Wastewater-Effluent Chemical Analyses

The surface-water and wastewater-effluent samples (water samples) were split into numerous fractions for analyses. The USGS-NWQL analyzed water samples for major ions, nutrients, and pharmaceuticals. The USGS-IASED analyzed water samples for suspended sediments. The USGS-NRPL analyzed water samples for trace elements, major ions, alkylphenols and other neutral organic compounds, carboxylic acid compounds, and steroidal hormones. Water samples were sent to the UST Laboratory for estimation of total estrogenicity. Phytoestrogens, pharmaceuticals, and an antimicrobial compound were analyzed at the University of Colorado Center for Environmental Mass Spectrometry in Boulder, Colo.

Samples were analyzed for 2 major ions and 8 nutrients at the USGS-NWQL (table 5) using standard analytical techniques described in Patton and Truitt (1992), U.S. Environmental Protection Agency (1993), and Fishman and others (1994). Samples analyzed for dissolved major-ion and most nutrient concentrations were filtered using 0.45-micrometer (µm) pore-size encapsulated filters. Nutrient samples were preserved with 1 milliliter (mL) of 4.5 normal sulfuric acid and maintained at 4°C until analyzed at the USGS-NWQL. Samples analyzed to determine total nutrient concentrations were not filtered. Dissolved phosphorus was analyzed using U.S. Environmental Protection Agency method 365.1, low-level persulfate digestion (U.S. Environmental Protection Agency, 1993). Suspended-sediment samples were analyzed for particle size and concentrations at the USGS-IASED using methods described by Guy (1969).

Samples were analyzed for 54 trace elements and major ions (table 5). Samples were filtered (47-millimenter (mm) diameter, 0.4-µm polycarbonate filter, GE Waters and Process Technologies, Trevose, Penn.) and preserved by acidification with ultra-high purity nitric acid. Trace elements were determined at the USGS-NRPL by inductively coupled plasma/mass spectrometry using a Perkin Elmer model Elan 6000 mass spectrometer (Garbarino and Taylor,1994; Taylor, 2001). Multiple internal standards (indium, iridium, and rhodium) were used to normalize the inductively coupled plasma/mass spectrometry system for drift. Major ions were determined at the USGS-NRPL using inductively coupled plasma atomic-emission spectrometry using a Perkin Elmer model DV3300 emission spectrometer (Garbarino and Taylor, 1979). Concentrations were determined in triplicate for each sample and the results are an average of the three analyses. Interelement interference corrections were applied during post analysis data processing to optimize accuracy and precision.

Water samples were analyzed for 16 pharmaceuticals at the USGS-NWQL (table 5) using methods described by Furlong and others (2008). For this method, a water sample is filtered in the field using a 0.7-µm nominal pore-size glass-fiber filter, and then upon arrival at the laboratory the sample is amended with a method performance surrogate solution and passed through a solid-phase extraction (SPE) cartridge. Pharmaceuticals retained on the SPE bed are eluted from the cartridge sequentially with methanol followed by acidified methanol. The extract is reduced in volume using nitrogen evaporation, fortified with an internal standard solution, and analyzed using high-performance liquid chromatography/mass spectrometry to determine individual pharmaceutical concentrations ranging from 0.005 to 1.0 µg/L, based on the lowest and the highest calibration standards routinely used.

Water samples were analyzed for 10 phytoestrogens (Ferrer and others, 2009), 1 antimicrobial, and 8 additional pharmaceuticals at the University of Colorado Center for

Table 5. Properties and chemicals analyzed in water or bed-sediment samples.

[CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); mg/L, milligrams per liter; µg/L, micrograms per liter; nm, nanometers; ng/L, nanograms per liter; ng/g, nanograms per gram; g/kg, grams per kilogram; >, greater than; UV, ultraviolet; --, not applicable]

Property/chemical name	Laboratory reporting level	CASRN[1]
Basic water-quality properties measured in the field		
Air pressure (millimeters of mercury)	--	--
Air temperature (°C)	--	--
Discharge/streamflow (cubic meters per second; cubic feet per second)	--	--
Dissolved oxygen (mg/L)	--	--
pH	--	--
Specific conductance (µS/cm)	--	--
Water temperature (°C)	--	--
Turbidity (nephelometric turbidity units)	--	--
Major ions and nutrients analyzed in water at the USGS National Water Quality Laboratory and suspended sediment analyzed at the USGS Iowa Sediment Laboratory (mg/L)		
Filtered (dissolved) chloride	0.12	16887–00–6
Filtered (dissolved) sulfate	.18	14808–79–8
Filtered (dissolved) ammonia plus organic nitrogen as nitrogen	.1	17778–88–0
Unfiltered (total) ammonia plus organic nitrogen as nitrogen	.1	17778–88–0
Filtered (dissolved) ammonia as nitrogen	.02	7664–41–7
Filtered (dissolved) nitrate plus nitrite as nitrogen	.04	--
Filtered (dissolved) nitrite as nitrogen	.002	14797–65–0
Filtered (dissolved) orthophosphate as phosphorus	.008	14265–44–2
Filtered (dissolved) phosphorus	.006	7723–14–0
Unfiltered (total)phosphorus	.008	7723–14–0
Suspended sediment	--	--
Trace elements and major ions analyzed in water at the USGS National Research Program Laboratory (µg/L, unless noted)		
Aluminum	0.07	7429–90–5
Antimony	.004	7440–36–0
Arsenic	.02	7440–38–2
Boron	3.0	7440–42–8
Barium	.01	7440–39–3
Beryllium	.007	7440–41–7
Bismuth	.002	7440–69–9
Cadmium	.0007	7440–43–9
Calcium (mg/L)	.01	7440–70–2
Cerium	.0002	7440–45–1
Cesium	.002	7440–46–2
Chromium	.1	7440–47–3
Cobalt	.2	7440–48–4
Copper	.005	7440–50–8
Dysprosium	.0003	7429–91–6
Erbium	.0001	7440–52–0
Europium	.0001	7440–53–1

Table 5. Properties and chemicals analyzed in water or bed-sediment samples. —Continued

[CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); mg/L, milligrams per liter; µg/L, micrograms per liter; nm, nanometers; ng/L, nanograms per liter; ng/g, nanograms per gram; g/kg, grams per kilogram; >, greater than; UV, ultraviolet; --, not applicable]

Property/chemical name	Laboratory reporting level	CASRN[1]
Trace elements and major ions analyzed in water at the USGS National Research Program Laboratory (µg/L, unless noted)—Continued		
Gadolinium	0.0002	7440–54–2
Holmium	.0001	7440–60–0
Iron	1.3	7439–89–6
Lanthanum	.0001	7439–91–0
Lead	.001	7439–92–1
Lithium	.01	7439–93–2
Lutetium	.00007	7439–94–3
Magnesium (mg/L)	.01	7439–95–4
Manganese	.01	7439–96–5
Molybdenum	.03	7439–98–7
Neodymium	.0005	7440–00–8
Nickel	.4	7440–02–0
Phosphorus	12	7723–14–0
Potassium (mg/L)	.009	7440–09–7
Praseodymium	.0001	7440–10–0
Rhenium	.0001	7440–15–5
Rubidium	.002	7440–17–7
Samarium	.0006	7440–19–9
Selenium	.3	7782–49–2
Silica (mg/L)	.007	60676–86–0
Sodium (mg/L)	.1	7440–23–5
Strontium	.04	7440–24–6
Sulfur (mg/L)	.04	7704–34–9
Tellurium	.005	13494–80–9
Terbium	.00006	7440–27–9
Thallium	.007	7440–28–0
Thorium	.0005	--
Thulium	.00004	7440–30–4
Tin	.005	7440–31–5
Titanium	.2	7440–32–6
Tungsten	.003	7440–33–7
Uranium	.002	--
Vanadium	.1	7440–62–2
Ytterbium	.0002	7440–64–4
Yttrium	.0001	7440–65–5
Zinc	.4	7440–66–6
Zirconium	.003	7440–67–7

Table 5. Properties and chemicals analyzed in water or bed-sediment samples. —Continued

[CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); mg/L, milligrams per liter; µg/L, micrograms per liter; nm, nanometers; ng/L, nanograms per liter; ng/g, nanograms per gram; g/kg, grams per kilogram; >, greater than; UV, ultraviolet; --, not applicable]

Property/chemical name	Laboratory reporting level	CASRN[1]
Pharmaceuticals analyzed in water at the USGS National Water Quality Laboratory (µg/L)		
Acetaminophen	0.08	103–90–2
Albuterol	.06	18559–94–9
Caffeine	.2	58–08–2
Carbamazepine	.04	298–46–4
Codeine	.04	76–57–3
Cotinine	.026	486–56–6
Dehydronifedipine	.08	67035–22–7
Diltiazem	.08	42399–41–7
1,7-Dimethylxanthine	.12	611–59–6
Diphenhydramine	.04	58–73–1
Fluoxetine	.016	54910–89–3
Sulfamethoxazole	.16	723–46–6
Thiabendazole	.06	148–79–8
Trimethoprim	.02	738–70–5
Warfarin	.1	81–81–2
Carbamazepine-d_{10} surrogate standard (percent)	--	--
Ethyl nicotinate-d_4 surrogate standard (percent)	--	--
Phytoestrogens, pharmaceuticals, and an antimicrobial analyzed at the University of Colorado Center for Environmental Mass Spectrometry (ng/L)		
Biochanin A	5	491–80–5
Bupropion	10	34911–55–2
Carbamazepine	5	298–46–4
Coumestrol	1	479–13–0
Daidzein	1	486–66–8
Daidzin (sugar)	1	552–66–9
Equol	20	531–95–3
Fluoxetine	10	54910–89–3
Fluvoxamine	10	61718–82–9
Formononetin	1	485–72–3
Genistein	10	446–72–0
Genistin (sugar)	5	529–59–9
Glycitein	1	40957–83–3
Hydroxy-bupropion	1	--
Prunetin	5	552–59–0
Sulfamethoxazole	5	723–46–6
Triclocarban	20	101–20–2
Trimethoprim	5	738–70–5
Venlafaxine	5	93413–69–5

Table 5. Properties and chemicals analyzed in water or bed-sediment samples. —Continued

[CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); mg/L, milligrams per liter; µg/L, micrograms per liter; nm, nanometers; ng/L, nanograms per liter; ng/g, nanograms per gram; g/kg, grams per kilogram; >, greater than; UV, ultraviolet; --, not applicable]

Property/chemical name	Laboratory reporting level	CASRN[1]
Alkylphenols and other neutral organic chemicals analyzed in water at the USGS National Research Program Laboratory (ng/L)		
Acetyl hexamethyl tetrahydronaphthalene (AHTN)	5	21145–77–7
Bisphenol A	20	80–05–7
4-*tert*-Butylphenol	5	98–54–4
Caffeine	20	58–08–2
2,6-Di-*tert*-butyl-1,4-benzoquinone	300	719–22–2
1,3-Dichlorobenzene	5	541–73–1
1,4-Dichlorobenzene	5	106–46–7
N,N-diethyl-*meta*-toluamide (DEET)	10	134–62–3
Hexahydrohexamethylcyclopentabenzopyran (HHCB)	5	1222–05–5
5-Methyl-1H-benzotriazole	20	136–85–6
4-Nonylphenol (NP)	50	25154–52–3
4-Nonylphenolmonoethoxylate (NP1EO)	50	27986–36–3
4-Nonylphenoldiethoxylate (NP2EO)	50	9016–45–9
4-Nonylphenoltriethoxylate (NP3EO)	50	--
4-Nonylphenoltetraethoxylate (NP4EO)	50	--
4-*tert*-Octylphenol	5	140–66–9
4-*tert*-Octylphenolmonoethoxylate (OP1EO)	5	9036–19–5
4-*tert*-Octylphenoldiethoxylate (OP2EO)	5	--
4-*tert*-Octylphenoltriethoxylate (OP3EO)	5	--
4-*tert*-Octylphenoltetraethoxylate (OP4EO)	5	--
4-*tert*-Octylphenolpentaethoxylate (OP5EO)	5	--
4-*tert*-Pentylphenol	5	--
Triclosan	5	3380–34–5
4-*n*-Nonylphenol surrogate standard (percent)	--	104–40–5
4-*n*-Nonylphenolmonoethoxylate surrogate standard (percent)	--	--
4-*n*-Nonylphenoldiethoxylate surrogate standard (percent)	--	--
Bisphenol A-d_6 surrogate standard(percent)	--	--
Cholesterol-d_7 surrogate standard (percent)	--	--
Carboxylic acids analyzed in water at the USGS National Research Program Laboratory (µg/L)		
Ethylenediaminetetraacetic acid (EDTA)	1	60–00–4
Nitrilotriacetic acid (NTA)	1	139–13–9
4-Nonylphenolmonoethoxycarboxylate (NP1EC)	1	3115–49–9
4-Nonylphenoldiethoxycarboxylate (NP2EC)	1	106807–78–7
4-Nonylphenoltriethoxycarboxylate (NP3EC)	1	108149–59–3
4-Nonylphenoltetraethoxycarboxylate (NP4EC)	1	--
4-*n*-Nonylphenolmonoethoxycarboxylate surrogate standard (percent)	--	--
Steroidal hormones and other chemicals analyzed in water at the USGS National Research Program Laboratory (ng/L)		
4-Androstene-3,17-dione[2]	0.8	63–05–8
cis-Androsterone[2]	.8	53–41–8

Table 5. Properties and chemicals analyzed in water or bed-sediment samples. —Continued

[CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); mg/L, milligrams per liter; µg/L, micrograms per liter; nm, nanometers; ng/L, nanograms per liter; ng/g, nanograms per gram; g/kg, grams per kilogram; >, greater than; UV, ultraviolet; --, not applicable]

Property/chemical name	Laboratory reporting level	CASRN[1]
Steroidal hormones and other chemicals analyzed in water at the USGS National Research Program Laboratory (ng/L)—Continued		
Bisphenol A	20	80–05–7
Cholesterol	800	57–88–5
3-*beta*-Coprostanol	50	360–68–9
Diethylstilbestrol	.1	56–53–1
Equilenin	.1	517–09–9
Equilin[2]	2	474–86–2
17-*alpha*-Estradiol	.1	57–91–0
17-*beta*-Estradiol (E2)	.2	50–28–2
Estriol	0.1	50–27–1
Estrone[2]	2	53–16–7
17-*alpha*-Ethynylestradiol (EE2)	.1	57–63–6
Mestranol	.1	72–33–3
Norethindrone[2]	2	68–22–4
Progesterone[2]	2	57–83–0
Testosterone[2]	2	58–22–0
dihydro-Testosterone[2]	2	521–18–6
epi-Testosterone[2]	2	481–30–1
11-*keto*-Testosterone[2]	2	564–35–2
4-Androstene-3-17-dione-d_7 isotope dilution standard (percent)	--	--
Bisphenol A-d_{16} isotope dilution standard (percent)	--	96210–87–6
Cholesterol-d_7 isotope dilution standard (percent)	--	83199–47–7
trans-Diethylstilbestrol-d_8 isotope dilution standard (percent)	--	91318–10–4
17-*beta*-Estradiol-d_4 isotope dilution standard (percent)	--	--
Estriol-d_3 isotope dilution standard (percent)	--	--
Estrone-d_4 isotope dilution standard (percent)	--	--
17-*alpha*-Ethynylestradiol-2,4,16,16-d_4 isotope dilution standard (percent)	--	--
Mestranol-2,4,16,16-d_4 isotope dilution standard (percent)	--	--
Norethindrone-d_6 isotope dilution standard (percent)	--	--
Progesterone-d_9 isotope dilution standard (percent)	--	--
Testosterone-d_5 isotope dilution standard (percent)	--	--
Dihydrotestosterone-d_4 isotope dilution standard (percent)	--	--
Carbon and wastewater-indicator chemicals analyzed in bed-sediment samples at the USGS National Water Quality Laboratory (ng/g)		
Total carbon (g/kg)	--	--
Inorganic carbon (g/kg)	--	--
Organic carbon (g/kg)	--	--
Acetophenone[3]	150	98–86–2
Acetyl hexamethyl tetrahydronaphthalene (AHTN)	50	21145–77–7
Anthracene	50	120–12–7

Table 5. Properties and chemicals analyzed in water or bed-sediment samples. —Continued

[CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); mg/L, milligrams per liter; µg/L, micrograms per liter; nm, nanometers; ng/L, nanograms per liter; ng/g, nanograms per gram; g/kg, grams per kilogram; >, greater than; UV, ultraviolet; --, not applicable]

Property/chemical name	Laboratory reporting level	CASRN[1]
Carbon and wastewater-indicator chemicals analyzed in bed-sediment samples at the USGS National Water Quality Laboratory (ng/g)—Continued		
9,10-Anthraquinone	50	84–65–1
Atrazine	100	1912–24–9
Benzo[a]pyrene	50	50–32–8
Benzophenone	50	119–61–9
Bisphenol A[3]	50	80–05–7
Bromacil	500	314–40–9
3-*tert*-Butyl-4-hydroxyanisole (BHA)	150	25013–16–5
Camphor	50	76–22–2
Carbazole	50	86–74–8
Chlorpyrifos	50	2921–88–2
Cholesterol	250	57–88–5
3-*beta*-Coprostanol	500	360–68–9
p-Cresol (4-Methylphenol)[3]	250	106–44–5
4-Cumylphenol	50	599–64–4
Diazinon	50	333–41–5
1,4-Dichlorobenzene	50	106–46–7
Diethylhexyl phthalate[5]	250	117–81–7
Diethylphthalate[5]	100	84–66–2
2,6-Dimethylnaphthalene	50	581–42–0
Fluoranthene[3]	50	206–44–0
Hexahydrohexamethylcyclopentabenzopyran (HHCB) (galaxolide)[3]	50	1222–05–5
Indole[3]	100	120–72–9
Isoborneol	50	124–76–5
Isophorone[3]	50	78–59–1
Isopropylbenzene (cumene)[3]	100	98–82–8
Isoquinoline[3]	100	119–65–3
d-Limonene[3]	50	5989–27–5
Menthol	50	89–78–1
3-Methyl-1H-indole (skatol)	50	83–34–1
1-Methylnaphthalene	50	90–12–0
2-Methylnaphthalene	50	91–57–6
Metolachlor[3]	50	51218–45–2
Naphthalene	50	91–20–3
N,N-diethyl-*meta*-toluamide (DEET)[3]	100	134–62–3
4-Nonylphenol (NP) (all isomers)[4]	750	84852–15–3
4-Nonylphenoldiethoxylate (NP2EO)[4]	1000	--
4-Nonylphenolmonoethoxylate (NP1EO)[4]	500	--

Table 5. Properties and chemicals analyzed in water or bed-sediment samples. —Continued

[CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); mg/L, milligrams per liter; µg/L, micrograms per liter; nm, nanometers; ng/L, nanograms per liter; ng/g, nanograms per gram; g/kg, grams per kilogram; >, greater than; UV, ultraviolet; --, not applicable]

Property/chemical name	Laboratory reporting level	CASRN[1]
Carbon and wastewater-indicator chemicals analyzed in bed-sediment samples at the USGS National Water Quality Laboratory (ng/g)—Continued		
4-*n*-Octylphenol	50	1806–26–4
4-*tert*-Octylphenol (TOP)	50	140–66–9
4-*tert*-Octylphenoldiethoxylate (OP1EO)[4]	50	--
4-*tert*-Octylphenolmonoethoxylate (OP2EO)[4]	250	--
Phenanthrene	50	85–01–8
Phenol[3]	50	108–95–2
Prometon	50	1610–18–0
Pyrene	50	129–00–0
beta-Sitosterol	500	83–46–5
beta-Stigmastanol	500	19466–47–8
2,2',4,4'-Tetrabromodiphenylether (PBDE 47)	50	5436–43–1
Tributyl phosphate	50	126–73–8
Triclosan	50	3380–34–5
Triphenyl phosphate[3]	50	115–86–6
Tris(2-butoxyethyl) phosphate	150	78–51–3
Tris(2-chloroethyl) phosphate[3]	100	115–96–8
Tris(dichloroisopropyl) phosphate[3]	100	13674–87–8
Bisphenol A-d_3 surrogate standard (percent)	--	--
Decafluorobiphenyl surrogate standard (percent)	--	--
Fluoranthene-d_{10} surrogate standard (percent)	--	--
Steroidal hormones and other chemicals analyzed in bed sediment at the USGS National Water Quality Laboratory (ng/g)		
4-Androstene-3,17-dione[2]	0.1	63–05–8
cis-Androsterone[2]	.1	53–41–8
Bisphenol A	12	80–05–7
Cholesterol	250	57–88–5
3-*beta*-Coprostanol	250	360–68–9
trans-Diethylstilbestrol	.1	56–53–1
Dihydrotestosterone[2]	.1	521–18–6
Equilenin	.26	517–09–9
Equilin[2]	.5	474–86–2
17-*alpha*-Estradiol	.1	57–91–0
17-*beta*-Estradiol (E2)	.1	50–28–2
Estriol	.26	50–27–1
Estrone[2]	.1	53–16–7
17-*alpha*-Ethynylestradiol (EE2)	.1	57–63–6
Mestranol	.1	72–33–3
Norethindrone[2]	.1	68–22–4

Table 5. Properties and chemicals analyzed in water or bed-sediment samples. —Continued

[CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); mg/L, milligrams per liter; µg/L, micrograms per liter; nm, nanometers; ng/L, nanograms per liter; ng/g, nanograms per gram; g/kg, grams per kilogram; >, greater than; UV, ultraviolet; --, not applicable]

Property/chemical name	Laboratory reporting level	CASRN[1]
Steroidal hormones and other chemicals analyzed in bed sediment at the USGS National Water Quality Laboratory (ng/g)—Continued		
Progesterone[2]	0.5	57–83–0
Testosterone[2]	.1	58–22–0
epi-Testosterone[2]	.5	366495–94–5
11-*keto*-Testosterone[2]	.26	564–35–2
4-Androstene-3,17-dione-2,2,4,6,6,16,16-d_7 isotope dilution standard (percent)	--	--
Bisphenol-A-d_{16} isotope dilution standard (percent)	--	96210–87–6
Cholesterol-d_7 isotope dilution standard (percent)	--	83199–47–7
trans-Diethyl-1,1,1',1'-d_4-stilbesterol-3,3',5,5'-d_4 isotope dilution standard (percent)	--	91318–10–4
16-Epiestriol-2,4-d_2 (percent)	--	--
Dihydrotestosterone-1,2,4,5a-d_4 isotope dilution standard (percent)	--	--
Estriol-2,4,17-d_3 isotope dilution standard (percent)	--	--
Estrone-2,4,16,16-d_4 isotope dilution standard (percent)	--	--
Estrone-13,14,15,16,17,18-$^{13}C_6$ isotope dilution standard (percent)	--	--
17-*beta*-Estradiol-d_4 isotope dilution standard (percent)	--	--
17-*beta*-Estradiol-13,14,15,16,17,18-$^{13}C_6$ isotope dilution standard (percent)	--	--
17-*alpha*-Ethynylestradiol-2,4,16,16-d_4 isotope dilution standard (percent)	--	350820–06–3
Medroxy-progesterone-d_3 isotope dilution standard (percent)	--	--
Mestranol-2,4,16,16-d_4 isotope dilution standard (percent)	--	--
Nandrolone-16,16,17-d_3 surrogate (percent)	--	--
Norethindrone-2,2,4,6,6,10-d_6 isotope dilution standard (percent)	--	--
Progesterone-2,2,4,6,6,17a,21,21,21-d_9 isotope dilution standard (percent)	--	--
Testosterone-2,2,4,6,6-d_5 isotope dilution standard (percent)	--	--

[1]This report contains Chemical Abstract Service (CAS) Registry Numbers® (CASRNs), which is a Registered Trademark of the American Chemical Society. CAS recommends the verification of the CASRNs through CAS Client Services℠.

[2]Chemical that may have been affected by isotope dilution standard deuterium loss.

[3]Concentration is estimated because recovery is less than 60 percent or precision is greater than 25 percent relative standard deviation. This can be caused by instrumental or extraction difficulties.

[4]Concentration is estimated because the reference standard is from a technical mixture.

[5]Concentration is estimated because of potential blank contamination unless concentration is greater than 10 times the 95th percentile of all blank concentrations.

Environmental Mass Spectrometry (Boulder, Colo.) (table 5) using high-performance liquid chromatography/tandem mass spectrometry. Each water sample was spiked with 100 microliters (µL) of a solution containing a mix of the internal standards. Analytical standards were purchased from Sigma-Aldrich (St. Louis, Mo.), Cole Parmer (Vernon Hills, Ill.), and Cerilliant (Round Rock, Texas). Labeled internal standards (carbamazepine-d_{10}, daidzein-d_4, fluoxetine-d_6, genistein-d_4, sulfamethoxazole-$^{13}C_6$, triclocarban-$^{13}C_6$, and trimethoprim-$^{13}C_3$) were obtained from Cambridge Isotope Laboratories (Andover, Mass.). Individual pharmaceutical stock solutions (approximately 1,000 micrograms per millilter) were prepared in pure acetonitrile or methanol depending on the solubility of each individual compound, and stored at -18ºC, and working standard solutions were prepared by dilution with acetonitrile and water. High-performance liquid-chromatography (HPLC) grade acetonitrile, methanol, and water were obtained from Burdick and Jackson (Muskegon, Mich.). Acetic acid was obtained from Sigma-Aldrich (St. Louis, Mo.).

Water samples for analyses of phytoestrogens, additional pharmaceuticals, and an antimicrobial were extracted in the laboratory using SPE and eluted with methanol for

pre-concentration. The SPE was performed using an automated extraction column system (GX-271 ASPEC, Gilson, Middleton, Wisc.) fitted with a 25-mL syringe pump for dispensing the water samples through the SPE cartridges. Water samples were extracted with Oasis HLB cartridges (200 milligrams, 6 mL) obtained from Waters (Milford, Mass.). The cartridges were conditioned with 4 mL of methanol followed by 6 mL of HPLC-grade water at a flow rate of 1 mL per minute. The solvent was evaporated to 0.5 mL by using a stream of nitrogen at a temperature of 45ºC in a water bath with a Turbovap concentration workstation (Caliper Life Sciences, Mountain View, Calif.). The samples were transferred to vials and analyzed by liquid chromatography/tandem mass spectrometry. Separation of the analytes was carried out with a reversed-phase carbon-18 analytical column (Agilent Series 1290, Agilent Technologies, Santa Clara, Calif.) and acetonitrile and water with 0.1-percent acetic acid mobile phases. Column temperature was maintained at 25ºC. The injected sample volume was 20 microliters (µL). The HPLC was connected to a triple quadrupole mass spectrometer (Model 6460; Agilent Technologies, Santa Clara, Calif.) equipped with electrospray interface with Jet Stream technology operating in positive and negative ion mode. Samples were analyzed in positive and negative ion modes.

The USGS-NRPL analyzed 23 alkylphenols (4-nonylphenol, 4-nonylphenolmonoethoxylate (NP1EO) to 4-nonylphenoltetraethoxylate (NP4EO), 4-*tert*-butylphenol, 4-*tert*-octylphenol, and 4-*tert*-octylphenolmonoethoxylate to 4-*tert*-octylphenolpentaethoxylate), and other neutral organic contaminants, and five surrogate standards in unfiltered water samples (table 5). The following surrogate standards were added to the water samples before extraction by this method: cholesterol-d_7, bisphenol A-d_6, 4-*n*-nonylphenol, 4-*n*-nonylphenolmonoethoxylate, and 4-*n*-nonylphenoldiethoxylate. Acidification with hydrochloric acid was used to preserve samples for continuous liquid-liquid extraction (CLLE) analyses (Barber and others, 2000), and the samples were stored at 4˚C. Chemicals were isolated using CLLE with methylene chloride at a pH of 2 following ionic strength adjustments with sodium chloride. The extract was then concentrated by evaporation under a stream of nitrogen gas for analysis by gas chromatography/mass spectrometry (GC/MS).

The USGS-NRPL analyzed unfiltered water samples (preserved in the field using 1-percent formalin volume per volume) for six carboxylic acids, including ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), and 4-nonylphenolmonoethoxycarboxylate (NP1EC) to 4-nonylphenolmonotetraethoxycarboxylate (NP4EC), and one surrogate standard (table 5). Internal standard (ethylenediaminetetraacetic acid-d_{12}) and surrogate (4-*n*-nonylphenoldiethoxycarboxylate) were added to the samples prior to analysis. The acidic chemicals were isolated by evaporation and derivatized with acetyl chloride:propanol to form the propyl esters (Schaffner and Giger, 1984; Barber and others, 2000). The derivatized extracts were analyzed by GC/MS.

The CLLE and evaporation extracts were analyzed by electron impact GC/MS in the full-scan and selected ion monitoring (SIM) modes. The following were the general chromatographic conditions: Hewlett Packard (HP) 6890 GC gas chromatograph; column - HP Ultra II (5-percent phenylmethyl silicone), 25 meters (m) by 0.2 mm, 33-µm film thickness; carrier gas, ultra-high purity helium with a linear-flow velocity of 27 centimeters per second; injection port temperature, 300°C; initial oven temperature, 50°C; split vent open, 0.75 minute; ramp rate, 6°C per minute to 300°C; and hold time, 15 minutes at 300°C. The following were the mass spectrometer conditions: HP 5973 Mass Selective Detector; tuned with perflurotributylamine; ionization energy, 70 electron volts; source temperature, 250°C; and interface temperature, 280°C. Concentrations were calculated on the basis of SIM data using three diagnostic ions for each compound, which were identified on the basis of matching of retention times (plus or minus (±) 0.05 minutes) and ion ratios (± 20 percent) determined from analysis of authentic standards. External calibration curves and internal standard procedures were used for calculating concentrations.

The USGS-NRPL analyzed for 17 steroidal hormones and 3 other chemicals using 13 isotope dilution standards (IDSs) in unfiltered water samples (table 5). The following IDSs were added to samples prior to analysis: 4-androstene-3-17-dione-d_7, bisphenol A-d_{16}, cholesterol-d_7, *trans*-diethylstilbestrol-d_8, 17-*beta*-estradiol-d_4, estriol-d_3, estrone-d_4, 17-*alpha*-ethynylestradiol-d_4, mestranol-2,4,16,16-d_4, norethindrone-d_6, progesterone-d_9, testosterone-d_5, and dihydrotestosterone-d_4. Standards were obtained from Dr. Ehrenstorfer, GmbH (Augsburg, Germany), Cambridge Isotope Laboratories (Cambridge, Mass.), Sigma-Aldrich (Milwaukee, Wisc.), Supelco (Bellefonte, Pa.), and CDN Isotopes (Pointe-Claire, Canada). Samples were stored at 4°C, to reduce biodegradation before extraction by SPE within 14 days of spiking with the 13 IDSs. Steroidal hormones were isolated from the sample water using octadecylsilica SPE followed by elution with methanol (Barber and others, 2000; Barber, Furlong, and others, 2003; Furlong and others, 2010). The methanol extracts were cleaned up using Florisil, and the steroids were derivatized with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) activated with 2-(trimethylsilyl)ethanethiol and ammonium iodide (MSTFA II, Sigma Aldrich, Milwaukee, Wisc.) before analysis with gas chromatography/tandem mass spectrometry (GC/MS/MS) (Barber, Furlong, and others, 2003; Furlong and others, 2010).

The SPE extracts were analyzed by GC/MS/MS using a Quattro-micro-GC® instrument (Waters Corp., Milford, Mass.) with an Agilent 6890 GC gas chromatograph. Chromatography was on a 30-m by 0.25-mm internal diameter Rxi XLB gas chromatography column with a 0.25-µm film thickness (Restek Corp., Bellefonte, Pa.) and a helium flow rate of 1 milliliter per minute (mL/min) with the injection port maintained at 275°C. The gas chromatograph was programmed on a variable temperature gradient from 100°C to 310°C. For each target compound, the most abundant diagnostic ion in the

full-scan spectrum was selected as a precursor and appropriate conditions were selected to maximize the signal for three precursor-product transitions. All 20 chemicals were quantified relative to a specific IDS using an isotope-dilution quantification procedure that automatically corrects for procedural losses in the reported chemical concentration based on the absolute method recovery of the IDS.

The basic premise of isotope dilution quantification is that deuterium labeled surrogate standards behave in a similar manner as the unlabeled chemical during sample preparation and instrumental analysis. As a result, quantitation by isotope dilution compensates for losses that occur during sample storage (sorption and biotransformation), sample transfer, and sample processing (isolation, extraction, clean-up, and derivatization). Multiple assumptions go into this quantitation method, the discussion of which is beyond the scope of this report. One of the basic assumptions is that the isotopically labeled analog is stable during the analytical procedure, and this assumption was not met for certain standards during this study (as described further in the "Bed-Sediment Chemical Analyses" section).

Bed-Sediment Chemical Analyses

Bed-sediment samples were analyzed for 3 carbon types (total, inorganic, and organic) (Wershaw and others, 1987), a suite of 57 organic wastewater-indicator chemicals, and 3 surrogate standards at USGS-NWQL (table 5) using method schedule 5433 according to Burkhardt and others (2006). Briefly, the method used accelerated solvent extraction (ASE), subsequent analyte isolation and extract cleanup by SPE, and analysis by GC/MS operated in electron-impact mode with full-scan ion monitoring. The method identified individual chemicals using chromatographic retention times and mass spectral matches, and quantified the analytes using multi-point standard calibration curves. Chemicals analyzed included alkylphenol ethoxylate nonionic surfactants and several degradates, food additives, fragrances, antioxidants, flame retardants, plasticizers, industrial solvents, disinfectants, fecal and plant sterols, polycyclic aromatic hydrocarbons, and high-use domestic pesticides, and three surrogate standards (bisphenol A-d_3, decafluorobiphenyl, and fluoranthene-d_{10}). The concentrations of 20 chemicals are reported as estimated with the "E" remark code for one of three reasons: (1) unacceptably low-biased recovery (less than 60 percent) or highly variable method performance (greater than 25-percent relative standard deviation), (2) reference standards prepared from technical mixtures, or (3) potential blank contamination.

Custom analytical method 6434, which is under development at the USGS-NWQL, was used to analyze for 17 steroidal hormones and 3 other compounds in bed-sediment samples (table 5). Following receipt at the NWQL, samples were stored in a freezer at 5°C or less until the day preceding extraction when allowed to thaw at room temperature. Each sample was homogenized prior to sub-sampling for extraction or for separate dry-weight determination. Dry weight was obtained by weighing a sample aliquot, contained in a tarred aluminum pan, before and after heating at 130ºC for at least 16 hours. Amounts used for extraction of samples in this study ranged from 1.7 to 11.7 g of material (dry weight), with lesser amounts used for matrices anticipated to have high organic matter or method chemical concentrations. A subsample aliquot was placed in a tarred ASE cell (Dionex Corp., Sunnyvale, Calif.) and reweighed to determine the aliquot's wet weight prior to extraction. Reagent sand (precleaned by heating at 450°C for a minimum of 4 hours) was added to the cell, as needed, based on cell and sample size. The aliquot was fortified with 10–10,000 nanograms (ng; compound dependent) of the isotopically labeled compounds shown in table 6 that were used as IDSs. The sample aliquot was extracted by pressurized solvent extraction using the ASE instrument with a mixture of water:isopropanol (50:50 volume per volume [v/v]) at 120ºC and water:isopropanol (20:80 v/v) at 200ºC using three static cycles (40 minutes total) at each temperature at a pressure of 10.3 megaPascals (1,500 pounds per square inch). The resultant ASE extract portions were diluted using 100 mL of a pH 7 potassium phosphate buffer solution and sequentially passed through an OASIS® HLB (Waters Corp., Milford, Mass.) SPE column to isolate the method compounds on the column using the procedure given in Burkhardt and others (2006). The SPE column was dried with nitrogen gas at a flow of 2 liters per minute of nitrogen for 15 minutes. Method compounds were eluted from the OASIS® column and passed through a cleanup column containing 2 g of Florisil overlain with 2.5 g of sodium sulfate by using 25 mL of a dichloromethane-methanol (95:5 v/v) mixture. The resultant extract was concentrated to 1–2 mL by using nitrogen gas evaporation, and transferred to a silanized 5-mL reaction vial by using a 1.5-mL rinse with the dichloromethane-methanol (95:5 v/v) mixture. The extract was evaporated to dryness using nitrogen gas. The method chemicals were derivatized to trimethylsilyl or trimethylsilyl-enol ether analogs to increase compound volatility or to minimize compound interactions with active sites in the gas chromatography system. Derivation was accomplished by addition of 500 µL of MSTFA II, and heating of the MSTFA II solution to 65°C for 1 hour. The MSTFA solution also contains cholestane-d_6 and chrysene-d_{12} as injection internal standards. The extract was transferred to a gas-chromatography vial and the method compounds were determined by GC/MS/MS using a Quattro-micro-GC® instrument (Waters Corp., Milford, Mass.). Compounds were separated by using a 30-m by 0.25-mm internal diameter Rxi XLB gas chromatography column with 0.25-µm film thickness (Restek Corp., Bellefonte, Pa.) and a multiple ramp temperature program. Chemicals were detected by tandem mass spectrometry by monitoring the product ions of three specific precursor-to-product ion transitions. Positive identification required the presence of at least two unique transition product ions, with ion ratios not deviating from those in a standard by more than specified tolerances (Antignac and others, 2003).

Similar to the water samples analyzed for hormones, all 20 method chemicals were quantified relative to a specific

Table 6. Chemical and the corresponding isotope dilution standard (IDS) used for its quantification in bed sediment by U.S. Geological Survey National Water Quality Laboratory for custom analytical method 6434.

[The six chemicals with direct IDS analogs that were susceptible to deuterium loss (D-loss) are shown in ***bold italics***. The four chemicals quantified with non-direct IDS analogs susceptible to D-loss are shown in **bold**. Six IDSs that contain ketone functional groups (keto-IDSs) that can undergo D-loss are shown in **bold**]

Chemical	Isotope dilution standard used for 11 samples prepared in 2009	Isotope dilution standard used for 14 samples prepared in 2010[1]
4-androstene-3,17-dione	**4-androstene-3,17-dione-d_7**	estrone-$^{13}C_6$
***cis*-androsterone**	**dihydrotestosterone-d_4**	estrone-$^{13}C_6$
bisphenol A	bisphenol A-d_{16}	no change
3-*beta*-coprostanol	cholesterol-d_7	no change
cholesterol	cholesterol-d_7	no change
trans-diethylstilbestrol	*trans*-diethylstilbestrol-d_8	no change
dihydrotestosterone	**dihydrotestosterone-d_4**	estrone-$^{13}C_6$
epitestosterone	**testosterone-d_5**	estrone-$^{13}C_6$
equilenin	17-*beta*-estradiol-d_4	17-*beta*-estradiol-$^{13}C_6$
equilin	**estrone-d_4**	estrone-$^{13}C_6$
17-*alpha*-estradiol	17-*beta*-estradiol-d_4	17-*beta*-estradiol-$^{13}C_6$
17-*beta*-estradiol	17-*beta*-estradiol-d_4	17-*beta*-estradiol-$^{13}C_6$
estriol	estriol-d_3	16-epiestriol-d_2
estrone	**estrone-d_4**	estrone-$^{13}C_6$
17-*alpha*-ethynylestradiol	17-*alpha*-ethynylestradiol-d_4	no change
11-ketotestosterone	**testosterone-d_5**	estrone-$^{13}C_6$
mestranol	mestranol-d_4	no change
norethindrone	**norethindrone-d_6**	estrone-$^{13}C_6$
progesterone	**progesterone-d_9**	medroxyprogesterone-d_3
testosterone	**testosterone-d_5**	estrone-$^{13}C_6$

[1]Nandrolone-16,16,17-d_3 also was added to samples determined by custom analytical method 6434, but was used as a surrogate only and not as an isotope dilution standard in analytical method 6434.

IDS compound by using an isotope-dilution quantification procedure that automatically corrects for procedural losses in the reported chemical concentration based on the absolute method recovery of the IDS. During this study, two sets of IDS compounds were used for the bed-sediment analyses. For 11 samples prepared in 2009, 13 deuterium-labeled IDS compounds were used for the bed-sediment analyses that were exact isotopic analogs of method chemicals (table 6). The remaining seven method chemicals in these samples were quantified relative to one of the IDS compounds that has similar chemical functionality but is not a direct isotopic analog of the chemical (table 6).

Six of the original 13 IDS compounds—4-androstene-3,17-dione-d_7, dihydrotestosterone-d_4, estrone-d_4, norethindrone-d_7, testosterone-d_5, and progesterone-d_9—were found to be susceptible to deuterium-hydrogen exchange (deuterium loss, D-loss) under non-routine sample preparation conditions (water bath temperatures above ambient temperature of 25°C) or prolonged IDS standard storage periods (months) in methanol (Foreman and others, 2010). D-loss results in an underestimate of the IDS total mass in the sample extract and, thus, an underestimate of IDS absolute recovery, which produces a positive bias in the determined chemical concentration. Consequently, these six deuterium-labeled IDSs were removed from the method, and not used for the 14 samples prepared in 2010. Reported concentrations of chemicals normally determined using these six IDSs were censored to the laboratory reporting level or a raised reporting level, if needed, or were quantified relative to 17-*alpha*-ethynylestradiol-d_4 to eliminate risk of positive bias in chemical concentration for any of the 11 samples prepared in 2009 where D-loss was evident from follow-up GC/MS analysis or suspected based on comparison of recoveries of these six IDSs to three other IDSs in the sample that are not susceptible to D-loss.

The 14 samples prepared in 2010 were fortified with 10 deuterium- or carbon-13 (^{13}C)-labeled compounds (table 6), five of which were unchanged from those used for the 11 samples prepared in 2009. Nine of these compounds were used as

IDSs. Nandrolone-d_3 was used as a surrogate only for samples analyzed by custom analytical method 6434. Replacement IDSs contained either carbon-13 or were non-direct analogs of the chemicals that have deuterium labels in positions not adjacent to a ketone group and, thus, are not susceptible to D-loss. In addition, 17-*beta*-estradiol-$^{13}C_6$ replaced 17-*beta*-estradiol-d_4, and 16-epiestriol-d_2 replaced estriol-d_3 to further minimize risk of IDS interference with the chemical's parent ion at concentrations near the GC/MS/MS instrumental detection level. Six of the IDS compounds are exact isotopic analogs of method analytes. The remaining 14 method chemicals also were quantified by isotope dilution by using one of the IDSs that have similar related chemical functionality but are not direct isotopic analogs of the chemical (table 6).

Quality assurance was monitored in part by evaluation of IDS recoveries in each sample matrix, which represent absolute recoveries for the method. Quality-assurance sample types included at least one laboratory reagent blank sample and one laboratory reagent spike sample for each sample preparation set and were processed at the same time as the associated environmental samples. The laboratory reagent blank and laboratory reagent spike samples were prepared using baked (450ºC) reagent sand. The laboratory reagent blank was used to monitor for interferences and the possible introduction of method chemicals during sample preparation or analysis. The laboratory reagent spike was used to assess recovery performance for method chemicals. The IDS compounds are reported (in percent recovery) along with the chemicals' concentrations in the environmental samples or the analytes' recoveries in laboratory reagent-spike sample. However, these IDS measurements reflect absolute recoveries achieved during sample preparation, and are only corrected for injection variability by quantitation compared to chrysene-d_{12} or cholestane-d_6. Reported chemical concentrations (or recoveries in quality-control spike samples) are automatically recovery-corrected by using this isotope dilution quantification procedure.

Bioassay for Total Estrogenicity

Environmental water samples of surface water and wastewater effluent were collected for analyses of total estrogenicity, stored at -40ºC, and thawed on the same day that cell-based analyses of estrogenic activity were conducted. To prepare sample extracts for the cell-based estrogenicity analyses, 150 mL of water sample was filtered through a 2-µm glass-fiber filter and then concentrated at flow rate of approximately 5 mL/min, using a 6-mL high-capacity carbon-18 SPE column (Baker Bond, Phillipsburg, N.J.) that had been activated with 100-percent methanol and deionized water. The extract was eluted with 2 mL of 100-percent methanol. Positive controls were prepared by adding 12 or 50 nanograms (ng) of 17-*beta*-estradiol (E2) to 1 liter (L) of distilled water or matrix (Lake Superior water) frozen at -40ºC, and processed following the procedures for environmental sample preparation. The distilled water sample and Lake Superior water sample also were prepared in the same manner as environmental samples, and acted as negative controls.

Total estrogenic activity of the environmental samples was measured using an estrogen receptor transcriptional assay. The T47D estrogen-dependent human breast-cancer line stably transfected with an estrogen-responsive luciferase reporter, named T47D-KBluc, was developed by Wilson and others (2004). This assay quantifies hormonal activity of environmental samples based on their ability to bind to the steroid receptor and to induce or attenuate subsequent responses. Although this is a mammalian-based assay, recent work (Wilson and others, 2007) that compared properties of mammalian and fish estrogen receptor assays, indicated that the results obtained are comparable and that mammalian and fish receptor assays respond to the same chemicals.

T47D-KBluc cells were maintained in RPMI 1640 Media (GIBCO) supplemented with 1-percent Anti-Anti (antibiotic-antimycotic, GIBCO) and 10-percent fetal bovine serum (FBS; HyClone, Logan, Utah). Before conducting bioassays, the cells were cultured in RPMI 1640 media supplemented with 10-percent charcoal dextran filtered FBS (HyClone) and in the absence of antibiotics and antimycotics for 7 days, reaching approximately 90-percent confluence. The cells were removed from culture flasks by a 15-minute incubation in TrypLE™ Express (GIBCO) and transferred to assay media (RPMI supplemented with 5-percent charcoal dextran filtered FBS). The cells were then counted using a hemocytometer, diluted to 100,000 cells per milliliter in assay media, and seeded into 96 clear-bottomed well plates at 100 µL per well. Cells were allowed to attach for 1 hour at room temperature before being transferred to a 37°C, 5-percent carbon dioxide incubator for approximately 24 hours.

A standard curve using 17-*beta*-estradiol (E2), negative control (distilled water), and positive control (distilled water with E2 spike) were run with each plate and each assay. The E2 standard stock solutions were prepared in 100-percent methanol. The E2 standard curve with in-well concentrations ranging from 10^{-6} to 5 nanomolar was applied to the cells. The methanol eluates of the environmental samples were diluted in the assay media, and these diluted samples were used to dose the cells. All controls, standards, and samples were prepared so that the methanol concentrations in each plate well remained constant (0.25-percent solvent). All samples were analyzed in duplicate. All cell plating, dosing, and media preparation were conducted under a laminar flow hood to mitigate bacterial and fungal contamination.

After exposure to standard, sample, or control solutions, cells were incubated for 24 hours at 37°C and 5-percent carbon dioxide. Following the incubation period, the exposure medium was removed, and the cells were subjected to a cytotoxicity assay (Invitrogen Live/Dead® Viability/Cytotoxicity Kit, Eugene, Oreg.) using the protocol developed by the manufacturer. After the Live/Dead® reagent was removed, the cells were washed with 25 µL per well of Dulbecco's phosphate buffered saline (GIBCO, Grand Island, N.Y.) and lysed through a 45-minute room temperature incubation period in

25 µL per well of Luciferase Cell Culture Lysis Reagent (Promega, Madison, Wisc.). Luminescence, in relative light units, was determined with a luminometer (Biotek, Synergy 2, Winooski, Vt.; Gen5 Software) after the addition of 25 µL per well of reaction buffer (25 millimolar glycyglycine), 15 millimolar magnesium chloride, 5 millimolar adenosine triphosphate, 0.1 milligram per milliliter bovine serum albumin, pH 7.8) followed by 25 µL per well of luciferin (Promega, Madison, Wisc.).

Data from the *in-vitro* T47D-KBluc assays were used to estimate the total estrogenic equivalents in the effluent samples, relative to E2. The concentrations of the E2 standards used in the cell assays were adjusted for dilutions in the assay media and transformed using the base-10 logarithm. The estrogenic activity of the test samples was interpolated by the least-squares means procedure from a nonlinear sigmoidal dose response curve fit to the relative luminescence units of the E2 standards (using Prism 5.02., Graph Pad Software Inc., La Jolla, Calif.). The interpolated sample values were adjusted back to concentrations in nanograms per liter and adjusted for sample dilutions in assay media.

Caged Fish Processing

At the end of the 14-day deployment, fish were retrieved and returned to the laboratory in aerated coolers. Fish were sacrificed and sampled for blood and reproductive tissues within 15 hours of retrieval from the field sites. Animal use and care in all experiments was approved by the SCSU Animal Use and Care Committee. All fish (pre-deployment and caged fish) were assessed for morphological endpoints (total and standard length, weight, body condition factor) and plasma vitellogenin concentrations. Fish were deeply anesthetized in 0.1-percent MS-222 (Argent Laboratories, Redmond, Wash.) before all procedures. Fish were lightly blotted dry and then weighed to determine whole body weight (0.01-g precision, Acculab Vicon, Edgewood, N.Y.). Total and standard length was measured for each fish to the closest millimeter. Body weight in grams and total length in millimeters were used to calculate the body condition factor (*BCF*), which is a measure of the overall nutritional state of the animal:

$$BCF = [body\ weight/total\ length^3] \times 100{,}000 \qquad (6)$$

Plasma vitellogenin levels were measured by using a competitive antibody-capture enzyme-linked immunosorbent assay. The polyclonal anti-fathead minnow vitellogenin antiserum (1° antibody (Ab)) was provided by Gerald LeBlanc, North Carolina State University. The antiserum used in this study was produced in female New Zealand white rabbits by injecting plasma from estradiol-exposed fathead minnows. Specificity to vitellogenin was obtained by incubating antiserum with plasma from unexposed male fathead minnows, followed by centrifugation to remove antibodies that recognized other plasma proteins (Parks and others, 1999). Standard vitellogenin was purified by anion-exchange chromatography from estradiol-exposed fathead minnows. The standard curve was prepared as a seven-step two-fold serial dilution with a range of 0.075 to 4.8 microliters per milliliter. Microtiter wells were coated with the purified fathead minnow vitellogenin (200 µL of a 600-nanograms-per-milliliter solution) in a coating buffer of 0.35 molar sodium bicarbonate, 0.15 molar sodium carbonate, pH 9.6). Plasma samples/standards were pre-incubated in microcentrifuge tubes at a ratio of 1:1 sample dilution to 1° Ab (1:20,000 final dilution) at 25°C for 2 hours. Just before the completion of the pre-incubation, microtiter plates were washed three times with wash buffer in an automated plate washer, then 200 µL of each pre-incubation mix (1° Ab plus sample or standard) was loaded into the microtiter wells of the assay plate and incubated 1 hour at room temperature. Plates were washed and incubated for 1 hour at room temperature with 200 µL of horseradish peroxidase labeled anti-rabbit IgG 2° Ab (Sigma, St. Louis, Mo.). Plates were again washed and incubated with 200 µL of 3,3′,5,5′-tetramethylbenzidine substrate (Sigma, St. Louis, Mo.) for 15 minutes in the dark. Absorbance was read at 620 nanometers on a Multiskan EX (Thermo Electron). Standard curves were constructed and sample values were calculated using the accompanying Multiskan Ascent software. The standard curves produced were robust, with coefficient of determination (r^2) values routinely greater than 0.99. The lowest standard was periodically removed from the curve to maintain linearity. The samples were diluted 1:75, 1:825 and 1:7,700 in 0.075 molar phosphate-buffered saline assay buffer, giving an assay quantitation range of 8 to 5,000 micrograms per milliliter.

Data for Endocrine Active Chemicals, Pharmaceuticals, Other Chemicals of Concern, and Biological Characteristics

This section of the report presents the environmental and quality-assurance data collected for the chemical and biological study of EACs, pharmaceuticals, other organic and inorganic chemicals of concern, and biological characteristics. Data are presented for water samples (surface water and wastewater effluent), bed-sediment samples, an *in-vitro* bioassay for estrogenicity, and fish plasma vitellogenin concentrations.

Quality-assurance samples are collected for all USGS methods as part of ongoing research and evaluation of potential interferences and contamination sources, and were used to set or adjust laboratory reporting levels. Laboratory and field quality-assurance samples were collected as part of the study to assess potential sources of contamination and variability. Laboratory quality-assurance samples included distilled water blanks, distilled water spikes, and surrogate compounds

added to samples to monitor sample-specific performance during sample preparation and analysis. For the USGS-NRPL and USGS-NWQL hormone analytical methods, IDS compounds similarly were added to monitor performance and also to quantify the method chemicals. Field quality-assurance samples included blank, duplicate, and matrix-spike samples. The type and number of quality-assurance samples varied by laboratory and analytical method. Quality-assurance samples were used to review the environmental data and to censor the data if needed as described in the following sections of the report.

Water Data

Basic water-quality properties (dissolved oxygen, pH, specific conductance, temperature, and turbidity) are presented in appendix 1 for the surface-water and wastewater-effluent samples. Hydrologic properties measured or determined from the tracer studies are presented in table 7. Analytical results and quality-assurance data for water samples are presented in the following sections for major ions and nutrients, trace elements, pharmaceuticals, phytoestrogens and pharmaceuticals, alkylphenols and other neutral organic chemicals, carboxylic acids, and steroid hormones.

Major Ions and Nutrients

During this study, 73 environmental samples of surface water and wastewater effluent, 6 field-blank samples, and 2 field-duplicate sample pairs were analyzed for 2 major ions and 8 nutrients at the USGS-NWQL. Analytical results of major ions and nutrients are presented in appendix 2 for the environmental and quality-assurance samples.

Sulfate and chloride were not detected in any of the six field-blank samples analyzed for major ions. Among the six field-blank samples that were analyzed for nutrients, dissolved ammonia plus organic nitrogen was the only nutrient detected. Dissolved (filtered) ammonia plus organic nitrogen was detected in 50 percent of the blank samples at low estimated concentrations ranging from 0.05 to 0.07 milligram per liter (mg/L; table 8). Concentrations of filtered ammonia plus organic nitrogen in field-blank samples were much less than concentrations measured in environmental samples (0.18 to 13.0 mg/L).

Differences in duplicate sample pairs were assessed by calculating the average relative percent difference (*RPD*). The *RPD* was calculated as follows:

$$RPD = \left(\frac{ENV - FDUP}{\left(\frac{ENV + FDUP}{2} \right)} \right) x\,100 \qquad (7)$$

where

ENV is the concentration in an environmental sample, and

FDUP is the concentration in the corresponding field duplicate sample.

The average *RPD* values for the two duplicate sample pairs analyzed for chloride and sulfate were 0.3 and 0.1 percent, respectively (table 8). The average *RPD* for all the nutrients analyzed in the two duplicate sample pairs was low at 3.1 ± 3.9 percent indicating good agreement between the two duplicate pairs that were analyzed.

Trace Elements and Major Ions

During this study, 73 environmental samples of surface water and wastewater effluent, 6 field-blank samples, and one field-duplicate pair were analyzed for dissolved concentrations of 54 trace elements and major ions at the USGS-NRPL (appendix 3). Laboratory measurements were made in triplicate for each trace element and concentrations in appendix 3 are averages of these three measurements. In addition, known standard reference materials were analyzed randomly with the environmental samples at a frequency of no less than 20 percent. These standard reference materials (including USGS Standard Reference Water Samples), were selected to simulate typical water sample matrices (Taylor, 2001). To establish a measure of accuracy, the difference between the most probable values and measured values during sample analysis with chemicals occurring at nominal natural water concentration levels typically was 4 percent or better (Barber, Keefe, and others, 2003). Approximately 25 percent of samples analyzed for trace elements and major ions consisted of laboratory-blank samples. Concentrations of trace elements in laboratory-blank samples generally were less than the method detection levels (Ron Antweiler, U.S. Geological Survey, oral commun., January 2010).

Among the six field-blank samples, 30 of the 54 trace elements or major ions analyzed were detected at least once at low concentrations. That frequency of detections indicates a potential source of field or laboratory contamination (table 9). For these 30 trace elements and all chemicals in this report, a conservative approach was used to flag environmental data using the field-blank concentrations in order to assure that reported concentrations were minimally affected by contamination. The mean and standard deviation of the blank sample concentrations for each of the 30 trace elements were calculated using a maximum likelihood estimation technique (Helsel, 2005). This technique is useful for statistical estimation for datasets with censored and non-censored data and for datasets with multiple censoring levels. The technique provides estimates of the sample mean and standard deviation that account for concentration data less than the detection limit. Statistics were calculated using the survReg routine in TIBCO© Spotfire S+ statistical analysis software (Palo Alto, Calif). As an example to describe the technique, cadmium was quantified in three blank samples at concentrations of 0.0044,

Table 7. Hydrologic properties determined from tracer studies for selected sites.

[m/m, meter per meter; m/s, meters per second; m^2/s, meters squared per second; +, plus; WWTP, wastewater-treatment plant]

Wastewater-treatment plant location	Receiving water	Fraction of effluent[1] (f_{eff})	Date water sampled	Tracer study date	Volume RWT[2] (V_s) (liters)	Down-stream width (B) (meters)	Down-stream depth (H) (meters)	Slope[3] (S) (m/m)	Median hydraulic transit time[4] (t_{med}) (minutes)	Average velocity[5] (u_{est}) (m/s)	Longitudinal dispersion[6] (E_{lat}) (m^2/s)	Theoretical mixing distance[7] (L_m) (meters)
Williams	Williams Creek	0.06	10/20/2009	10/20/2009	0.01	2.5	0.21	0.002	10	0.02	0.01	6
Eveleth	Elbow Creek	.93	09/29/2009	09/29/2009	.03	1.59	.11	.008	7	.08	.01	10
Pelican Rapids	Pelican River	.01	10/19/2009	10/19/2009	.5	9.82	.64	.00066	26	.26	.02	400
Sauk Center	Sauk River	.03	09/16/2009	09/16/2009	2	21.04	.49	.0036	118	.14	.04	700
Melrose	Sauk River	.10	09/17/2009	09/17/2009	2+	18.29	.45	.0007	99	.16	.02	1,000
Zimmerman	Tibbets Brook	.53	09/03/2009	09/02/2009	.2	1.16	.37	.002	6	.08	.02	2
Litchfield	Jewitts Creek	.18	10/08/2009	10/08/2009	.13	4.21	.51	.0021	29	.22	.03	50
Hutchinson	South Fork Crow River	.16	09/14/2009	09/14/2009	.93	23.02	.24	.0005	73	.15	.01	6,000
Marshall	Redwood River	.19	10/07/2009	10/07/2009	1.25	11.89	.44	.0005	115	.3	.01	1,000
Fairmont	Center Creek	.84	09/09/2009	09/08/2009	.4	14.63	.37	.0005	217	.03	.01	300
Hinckley	Grindstone River	.04	09/02/2009	09/01/2009	.4	7.93	.42	.002	296	.12	.02	100
Rochester	Zumbro River	.27	09/22/2009	09/22/2009	4	15.24	.43	.008	170	.19	.05	400
Spring Valley	Spring Valley Creek	.08	09/21/2009	09/21/2009	.1	6.71	.22	.024	51	.08	.03	50
Austin	Cedar River	.12	09/08/2009	09/07/2009	1	18.6	.37	.0057	48	.18	.03	800
Luverne	Rock River	.02	10/06/2009	10/06/2009	.24	13.41	.76	.0087	11	.19	.12	100

[1]Fraction of wastewater was determined as discharge from the WWTP divided by the sum of the discharge from the WWTP plus the streamflow at the upstream site.

[2]Rhodamine WT dye with 20-percent active ingredient.

[3]Stream slope was determined for each site with a geographic information system by locating the points where contour lines cross blue stream lines on U.S. Geological Survey 1:24,000 topographic maps. The distance between contour crossings was measured using high-resolution National Hydrography data (U.S. Geological Survey, 2008). The distance between contour crossings was divided by the change in altitude to obtain stream slope.

[4]Time for 50 percent of the rhodamine WT dye to pass the observation point. Two instruments were used at Rochester, Austin, Marshall, Litchfield, Luverne, Pelican Rapids, and Williams; the values for these sites were determined as the average of two instruments. One instrument was used at the other locations.

[5]Determined by dividing distance from the WWTP to downstream site by the median hydraulic transit time.

[6]Longitudinal dispersion coefficient.

[7]Theoretical lateral mixing point for a side discharge.

Table 8. Quality-assurance summary for major ions and nutrients in water samples analyzed at the U.S. Geological Survey National Water Quality Laboratory.

[Filtered analyses indicate dissolved concentrations, and unfiltered analyses indicate total concentrations. *n*, number; mg/L, milligrams per liter; nd, not detected; E, estimated concentration less than the laboratory reporting level]

Chemical	Field blank samples (*n* = 6)		Environmental samples (*n* = 73)	Field duplicate samples (*n* = 2 pairs)
	Range in detected concentrations (mg/L)	Frequency of detection (percent)	Range in detected concentrations (mg/L)	Average relative percent difference (percent)
Filtered chloride	nd	0	4.35–523	0.3
Filtered sulfate	nd	0	2.11–1,050	.1
Filtered ammonia plus organic nitrogen as nitrogen	E0.05–E0.07	50	0.18–13.0	2.0
Unfiltered ammonia plus organic nitrogen as nitrogen	nd	0	0.25–14.0	.8
Filtered ammonia as nitrogen	nd	0	0.021–13.2	9.0
Filtered nitrate plus nitrite as nitrogen	nd	0	E0.03–170	1.8
Filtered nitrite as nitrogen	nd	0	E0.001–1.09	2.1
Filtered orthophosphate as phosphorus	nd	0	E0.004–8.7	5.7
Filtered phosphorus	nd	0	0.007–7.9	2.0
Unfiltered phosphorus	nd	0	0.012–8.41	1.2

0.0028, and 0.002 µg/L, and left-censored concentrations were <0.001, <0.001, and <0.0007 in the remaining three blank samples. The calculated mean and standard deviation of the blank sample concentrations using the maximum likelihood estimation technique were 0.001 and 0.0023, respectively. The mean plus two standard deviations for cadmium concentrations was 0.0057. All environmental sample concentrations of cadmium equal to or less than 0.0057 were flagged (marked with a "C" remark code preceding the value to indicate potential contamination) in the dataset (appendix 3). This procedure resulted in flagging four cadmium concentrations in environmental samples with "C." The same process was used for the other trace elements that were detected in blank samples. The average RPD for all trace elements in the one replicate pair was 34 ± 40 percent.

Pharmaceuticals

During this study, 73 environmental samples of surface water and wastewater effluent, 20 laboratory-blank samples, 20 laboratory-spike samples, 6 field-blank samples, 2 pairs of field-duplicate samples, and 2 field-spike samples were analyzed for 16 pharmaceuticals and 2 surrogate chemicals at the USGS-NWQL (appendix 4). Acetaminophen, caffeine, codeine, and diphenhydramine were detected in laboratory-blank samples (table 10). Concentrations of acetaminophen, caffeine, codeine, and diphenhydramine in laboratory-blank samples were used to flag environmental data that may have been affected by contamination. The mean and two standard deviations of the blank-sample concentrations for each chemical were calculated using maximum likelihood estimation techniques described in the "Trace Elements and Major Ions" section. Detected concentrations that were less than the calculated mean plus two standard deviations of the blank concentrations were flagged (a "C" qualifier code precedes the value) in the dataset (appendix 4); only detected environmental concentrations of acetaminophen and caffeine had to be flagged in the dataset as all detected concentrations of codeine and diphenhydramine were greater than the calculated mean plus two standard deviations of the blank samples.

Percent recoveries for pharmaceuticals in the laboratory-spike samples ranged from 8 to 146 percent, with an average recovery of 63 ± 15 percent among all pharmaceuticals. The percent recoveries for individual samples vary from the average making it important to look at individual results that are unusually high or low in subsequent analyses. Diltiazem and sulfamethoxazole in the laboratory-spike samples had the lowest average recoveries (less than 40 percent).

Two surrogate pharmaceuticals, carbamazepine-d_{10} and ethyl nicotinate-d_4, were added to all the samples. The surrogate recoveries were greater than 80 percent for both surrogates in all field-blank samples (appendix 4). The percent recoveries for carbamazepine-d_{10} ranged from 1.7 to 82.1 percent among all environmental samples, and on average were 38 ± 15 percent. The average recovery for ethylnicotinate-d_4 in the environmental samples was 60 ± 11 percent. No pharmaceuticals were detected in the six field-blank samples. The average RPD for all pharmaceuticals in the two replicate pairs was low at 9.9 ± 9.2 percent, indicating acceptable reproducibility.

Table 9. Quality-assurance summary for trace elements and major ions in water samples analyzed at the U.S. Geological Survey National Research Program Laboratory.

[All concentrations are dissolved concentrations and represent the average of three analyses. *n*, number; µg/L, micrograms per liter; mg/L, milligrams per liter; nd, not detected; --, not applicable]

Chemical	Field blank samples (*n* = 6)		Environmental samples (*n* = 73)	Field duplicate samples (*n* = 1 pair)
	Range in detected concentrations	Frequency of detection (percent)	Range in detected concentrations	Relative percent difference (percent)
Aluminum (µg/L)	0.13–0.93	100	1.2–298	87
Antimony (µg/L)	0.005	33	0.024–2.3	3
Arsenic (µg/L)	0.06	17	0.3–5.1	4
Barium (µg/L)	0.02–0.22	100	0.65–129	3
Beryllium (µg/L)	nd	--	0.02–0.03	--
Bismuth (µg/L)	0.002	17	0.003–0.19	--
Boron (µg/L)	nd	--	7–562	1
Cadmium (µg/L)	0.002–0.0044	50	0.006–0.34	52
Calcium (mg/L)	0.02–0.10	50	9.3–143	1
Cerium (µg/L)	0.0007–0.0013	67	0.002–0.36	93
Cesium (µg/L)	nd	--	0.003–8.6	6
Chromium (µg/L)	0.1–0.2	33	0.3–2.1	--
Cobalt (µg/L)	nd	--	0.1–2	--
Copper (µg/L)	0.08–0.51	100	0.46–30	--
Dysprosium (µg/L)	nd	--	0.0003–0.041	0
Erbium (µg/L)	nd	--	0.0004–0.045	--
Europium (µg/L)	0.0001	17	0.0002–0.007	--
Gadolinium (µg/L)	nd	--	0.0004–0.82	24
Holmium (µg/L)	nd	--	0.0001–0.011	100
Iron (µg/L)	4–9	33	13–1,090	--
Lanthanum (µg/L)	0.0004–0.0005	67	0.0011–0.17	--
Lead (µg/L)	0.005–0.026	67	0.027–0.62	37
Lithium (µg/L)	nd	--	0.9–78	8
Lutetium (µg/L)	nd	--	0.0001–0.013	--
Magnesium (mg/L)	nd	--	2.3–115	3
Manganese (µg/L)	0.02–0.27	100	0.57–602	120
Molybdenum (µg/L)	0.02	17	0.14–27	10
Neodymium (µg/L)	0.0005–0.0006	33	0.0017–0.20	55
Nickel (µg/L)	nd	--	0.4–11	--
Phosphorus (µg/L)	7	17	9–11,100	1
Potassium (mg/L)	nd	--	0.69–63	1
Praseodymium (µg/L)	0.0001–0.0002	33	0.0003–0.048	--
Rhenium (µg/L)	nd	--	0.0011–0.20	5
Rubidium (µg/L)	0.002–0.004	67	0.72–77	1
Samarium (µg/L)	nd	--	0.0007–0.043	--
Selenium (µg/L)	nd	--	0.1–7.0	--
Silica (mg/L)	0.021	17	3.1–38	7
Sodium (mg/L)	nd	--	3.3–539	4

Table 9. Quality-assurance summary for trace elements and major ions in water samples analyzed at the U.S. Geological Survey National Research Program Laboratory.—Continued

[All concentrations are dissolved concentrations and represent the average of three analyses. *n*, number; µg/L, micrograms per liter; mg/L, milligrams per liter; nd, not detected; --, not applicable]

Chemical	Field blank samples (*n* = 6)		Environmental samples (*n* = 73)	Field duplicate samples (*n* = 1 pair)
	Range in detected concentrations	Frequency of detection (percent)	Range in detected concentrations	Relative percent difference (percent)
Strontium (µg/L)	0.18	17	31–877	111
Sulfur (mg/L)	0.05	17	1.4–372	109
Tellurium (µg/L)	nd	--	0.006–0.08	67
Terbium (µg/L)	nd	--	0.0001–0.007	40
Thallium (µg/L)	0.002	17	0.004–0.03	--
Thorium (µg/L)	nd	--	0.0013–0.018	40
Thulium (µg/L)	nd	--	0.0001–0.009	--
Tin (µg/L)	0.005–0.007	50	0.008–0.44	--
Titanium (µg/L)	nd	--	0.4–0.9	--
Tungsten (µg/L)	0.0008	17	0.0015–1.6	--
Uranium (µg/L)	nd	--	0.009–19.1	8
Vanadium (µg/L)	0.23	17	0.3–5.1	--
Ytterbium (µg/L)	nd	--	0.0004–0.067	96
Yttrium (µg/L)	0.0002–0.0004	83	0.0023–0.27	10
Zinc (µg/L)	0.4–1.2	50	1.2–167	--
Zirconium (µg/L)	0.004–0.01	50	0.039–1.3	44

Phytoestrogens, Pharmaceuticals, and an Antimicrobial

During this study 73 environmental samples of surface water and wastewater effluent, 7 field-blank samples, and 2 field-duplicate pairs were analyzed for 10 phytoestrogens, 8 pharmaceuticals, and 1 antimicrobial (appendix 5) at the University of Colorado Center for Environmental Mass Spectrometry. No chemicals were detected in field-blank samples. Eight of the nine pharmaceuticals analyzed were detected in field-duplicate samples. The average RPDs were low for all pharmaceuticals ranging from 0.9 to 15 percent (table 11).

The accuracy and precision of the method were assessed at two different concentration levels in spiked-deionized water with five replicate samples each. Accuracy, expressed as the mean from the five measurements, ranged from 88 to 105 percent for all chemicals. Intra-day precision was calculated as the percent relative standard deviation from the five measurements and ranged between 2 and 5 percent. Inter-day precision was measured by analyzing spiked water extracts on five consecutive days and ranged from 4 to 11 percent relative standard deviation.

Standard solutions of chemicals were added to tap water at seven different concentrations of 5, 10, 20, 40, 80, 200, and 400 nanograms per liter (ng/L) to obtain the standard calibration curves, all of which went through the SPE system and were treated in an identical manner as the environmental samples. These internal standards were used to account for recovery losses during SPE and any suppression from the matrix of the samples. An aliquot of 100 µL of the mixture containing the surrogate-labeled standards was added to each calibration sample and to each environmental sample. For the calibration curves, only the area of the quantifying transition was taken into account. All the calibration curves were linear between the concentrations studied with correlation coefficients higher than 0.99 for all the chemicals analyzed. The method detection limit was defined as the lowest concentration of the chemical that yielded minimum ion signal-to-noise ratios of 3:1 for both the quantitation and the confirmatory ions.

Table 10. Quality-assurance summary for pharmaceuticals in water samples analyzed at the U.S. Geological Survey National Water Quality Laboratory.

[*n*, number; µg/L, microgram per liter; nd, not detected; E, estimated concentration less than the laboratory reporting level; ±, plus or minus]

Chemical	Laboratory-blank samples (*n* = 20)		Laboratory-spike samples (*n* = 20)	Field-blank samples (*n* = 6)	Environmental samples (*n* = 73)	Field-duplicate samples (*n* = 2 pairs)	Field-spike samples (*n* = 2)
	Range in detected concentrations (µg/L)	Frequency of detection (percent)	Average recovery and standard deviation (percent)	Range in detected concentrations (µg/L)	Range in detected concentrations (µg/L)	Average relative percent difference (percent)	Average recovery and standard deviation (percent)
Acetaminophen (µg/L)	E0.0012–E0.0322	15	57 ± 14	nd	nd	nd	9 ± 5
Albuterol (µg/L)	nd	nd	57 ± 11	nd	nd	nd	16 ± 8
Caffeine (µg/L)	E0.0108–E0.0364	15	91 ± 15	nd	E0.051–E11.5	4	49 ± 8
Carbamazepine (µg/L)	nd	nd	77 ± 11	nd	E0.002–0.393	2	35 ± 14
Codeine (µg/L)	E0.0013	5	65 ± 11	nd	E0.006–0.079	4	47 ± 5
Cotinine (µg/L)	nd	nd	77 ± 15	nd	E0.009–0.22	nd	34 ± 2
Dehydronifedipine (µg/L)	nd	nd	69 ± 14	nd	E0.004	0	60 ± 3
Diltiazem (µg/L)	nd	nd	36 ± 12	nd	E0.011–E0.046	21	14 ± 4
1,7-Dimethylxanthine (µg/L)	nd	nd	93 ± 24	nd	E0.073–E4.32	nd	30
Diphenhydramine (µg/L)	E0.002	5	66 ± 11	nd	E0.009-0.127	24	15 ± 1
Fluoxetine (µg/L)	nd	nd	na	nd	E0.002–E0.066	nd	1 ± 0.3
Ranitidine (µg/L)	nd	nd	na	nd	E0.015–E0.13	nd	7 ± 2
Sulfamethoxazole (µg/L)	nd	nd	32 ± 21	nd	E0.004–E0.289	16	14 ± 10
Thiabendazole (µg/L)	nd	nd	53 ± 11	nd	nd	nd	9
Trimethoprim (µg/L)	nd	nd	82 ± 14	nd	E0.006–E0.35	8	37 ± 5
Warfarin (µg/L)	nd	nd	46 ± 13	nd	nd	nd	27 ± 1

Table 11. Quality-assurance summary for phytoestrogens, pharmaceuticals, and an antimicrobial chemical in water samples analyzed at the University of Colorado Center for Environmental Mass Spectrometry.

[*n*, number; µg/L, microgram per liter; nd, not detected; ±, plus or minus]

Chemical	Field-blank samples (*n* = 7)		Environmental samples (*n* = 73)	Field-duplicate samples (*n* = 2 pairs)
	Range in detected concentrations	Frequency of detection (percent)	Range in detected concentrations (ng/L)	Average relative percent difference and standard deviation (percent)
Biochanin A[1]	nd	0	nd	nd
Bupropion[2]	nd	0	10.4–4,298	15 ± 4
Carbamazepine[2]	nd	0	5.7–1,475	0.9 ± 0.3
Coumestrol[1]	nd	0	1.4–6	nd
Daidzein[1]	nd	0	4.1	nd
Daidzin (sugar)[1]	nd	0	nd	nd
Equol[1]	nd	0	nd	nd
Fluoxetine[2]	nd	0	10–76	2.11
Fluvoxamine[2]	nd	0	16–21	nd
Formononetin[1]	nd	0	1.0–4.6	nd
Genistein[1]	nd	0	nd	nd
Genistin (sugar)[1]	nd	0	nd	nd
Glycitein[1]	nd	0	11.0–14.6	nd
Hydroxy-bupropion[2]	nd	0	1–1,975	9 ± 6
Prunetin[1]	nd	0	nd	nd
Sulfamethoxazole[2]	nd	0	5–4,166	4 ± 2
Triclocarban[3]	nd	0	20–416	14 ± 4
Trimethoprim[3]	nd	0	6–1,463	7 ± 6
Venlafaxine[2]	nd	0	5–5,451	4 ± 5

[1]Phytoestrogen.

[2]Pharmaceutical.

[3]Antimicrobial.

Alkylphenols and Other Neutral Organic Chemicals

During the course of this study, 73 environmental samples of surface water and wastewater effluent, 24 field-duplicate samples, 20 field-spike samples, 7 field-blank samples, and 3 laboratory-blank samples were analyzed at the USGS-NRPL for 23 alkylphenols and other neutral organic chemicals (appendix 6). Laboratory reporting levels for the chemicals ranged from less than 5 to 300 ng/L (table 5), and were defined as the concentration equivalent to three times the mean value detected in method laboratory blanks or three times the baseline signal, whichever was greater.

In general, few chemicals were detected in field-blank samples confirming that the collection and processing techniques are appropriate for chemicals that were analyzed in this study (table 12). The chemical 4-nonylphenol was detected in one field-blank sample, 4-nonylphenoltriethoxylate was detected in two field-blank samples, 4-*tert*-octylphenol was detected in one field-blank sample, and 4-*tert*-octylphenol-diethoxylate was detected in one field-blank sample. Three chemicals were detected in the field-blank sample from one site (station number 434122092225001) indicating that there was a particular contamination problem for that sample that was not a pervasive contamination problem among all samples. The chemical *N,N*-diethyl-*meta*-toluamide (DEET) was detected in one of three laboratory-blank samples at a concentration of 14 ng/L. Concentrations of the chemicals detected in blank samples were used to flag environmental data that may have been affected by contamination. The mean and two standard deviations of the blank-sample concentrations for each chemical were calculated using maximum likelihood estimation techniques described in the "Trace Elements and Major Ions" section. Detected environmental concentrations that

Table 12. Quality-assurance summary for alkylphenols and other neutral organic chemicals in water samples analyzed at the U.S. Geological Survey National Research Program Laboratory.

[*n*, number; ng/L, nanograms per liter; nd, not detected; --, not applicable; ±, plus or minus]

Chemical	Laboratory-blank samples (*n* = 3)		Field-blank samples (*n* = 7)		Environmental samples (*n* = 73)	Field-duplicate samples (*n* = 24 pairs)	Field-spike samples (*n* = 20)
	Range in detected concentrations (ng/L)	Frequency of detection (percent)	Range in detected concentrations (ng/L)	Frequency of detection (percent)	Range in detected concentrations (ng/L)	Relative percent difference (percent)	Average recovery and standard deviation (percent)
Acetyl hexamethyl tetrahydronaphthalene (AHTN)	nd	--	nd	--	1–110	20 ± 17	--
Bisphenol A	nd	--	nd	--	23–22,000	33 ± 31	73 ± 27
4-*tert*-Butylphenol	nd	--	nd	--	6–110	23 ± 28	58 ± 20
Caffeine	nd	--	nd	--	21–9,900	20 ± 21	61 ± 24
2,6-Di-*tert*-butyl-1,4-benzoquinone	nd	--	nd	--	400–2,200	34 ± 24	162 ± 75
1,3-Dichlorobenzene	nd	--	nd	--	26–30	nd	45 ± 14
1,4-Dichlorobenzene	nd	--	nd	--	8–280	24 ± 13	56 ± 18
N,N-diethyl-*meta*-toluamide (DEET)	14	33	nd	--	16–780	24 ± 28	73 ± 19
Hexahydrohexamethylcyclopentabenzopyran (HHCB)	nd	--	nd	--	6–640	18 ± 14	--
5-Methyl-1H-Benzotriazole	nd	--	nd	--	24–1,000	74 ± 40	37 ± 25
4-Nonylphenol (NP)	nd	--	110	14	100–10,000	21 ± 23	60 ± 19
4-Nonylphenolmonoethoxylate (NP1EO)	nd	--	nd	--	52–2,200	44 ± 39	61 ± 15
4-Nonylphenoldiethoxylate (NP2EO)	nd	--	nd	--	96–3,400	28 ± 29	65 ± 18
4-Nonylphenoltriethoxylate (NP3EO)	nd	--	990–5,300	29	4300–4,900	nd	56 ± 15
4-Nonylphenoltetraethoxylate (NP4EO)	nd	--	nd	--	46–300	68 ± 3	41 ± 14
4-*tert*-Octylphenol (TOP)	nd	--	18	14	14–430	29 ± 11	63 ± 21
4-*tert*-Octylphenolmonoethoxylate (OP1EO)	nd	--	nd	--	6–31	44 ± 37	66 ± 17
4-*tert*-Octylphenoldiethoxylate (OP2EO)	nd	--	23	14	24–850	99	77 ± 21
4-*tert*-Octylphenoltriethoxylate (OP3EO)	nd	--	nd	--	29	nd	60 ± 20
4-*tert*-Octylphenoltetraethoxylate (OP4EO)	nd	--	nd	--	50–78	nd	48 ± 17
4-*tert*-Octylphenolpentaethoxylate (OP5EO)	nd	--	nd	--	220–4,100	174	34 ± 19
4-*tert*-Pentylphenol	nd	--	nd	--	9–51	nd	57 ± 19
Triclosan	nd	--	nd	--	11–410	24 ± 25	60 ± 17

were less than the calculated mean plus two standard deviations of the blank concentrations were flagged (a "C" qualifier code precedes the value) in the dataset (appendix 6).

The average recovery of the five surrogate standards for this method was 56 ± 17 percent. Surrogate recoveries vary among samples because of sample matrix effects, losses due to analytical processing, and degradation during sample storage. The average RPD for alkylphenols and other neutral organic chemicals that were detected in field-duplicate samples was 29 ± 30 percent.

Average recovery for the alkylphenols and neutral organic chemicals from the field-spike samples was 63 ± 25 percent. The recoveries were less than 50 percent for 5-methyl-1H-benzotriazole, 4-nonylphenoltetraethoxylate, 4-*tert*-octylphenoltetraethoxylate, and 4-*tert*-octylphenolpentaethoxylate. The recoveries for all chemicals in the field spike from one WWTP sample (station number 433856096115801) were consistently low, and the surrogate recoveries for this sample were low indicating a complex sample matrix or some other interference with quantitation.

Carboxylic Acids

During the course of this study 73 environmental samples of surface water and wastewater effluent, 13 field-duplicate samples, 7 field-blank samples, 3 laboratory-blank samples, 8 field-spike samples, and 1 laboratory-spike sample were analyzed at the USGS-NRPL for carboxylic acids by the evaporation method (appendix 7). Samples were preserved in the field with 1-percent formalin to limit biodegradation. The laboratory reporting level for ethylenediaminetetraacetic acid, nitrilotriacetic acid, and the NP1EC to NP4EC was 1 µg/L (table 5), and was defined as the concentration equal to three times the mean value detected in method blanks or three times the baseline signal, whichever was greater. No chemicals were detected in laboratory-blank samples, and ethylenediaminetetraacetic acid was the only carboxylic acid detected in the field-blank samples (table 13). Concentrations of ethylenediaminetetraacetic acid detected in blank samples were used to flag environmental data for this chemical that may have been affected by contamination. The mean and two standard deviations of the blank-sample concentrations for this chemical were calculated using maximum likelihood estimation techniques described in the "Trace Elements and Major Ions" section. Detected concentrations that were less than the calculated mean plus two standard deviations of the blank concentrations were flagged (a "C" qualifier code precedes the value) in the dataset (appendix 7).

Average percent recovery of the surrogate standard (4-*n*-nonylphenolmonoethoxycarboxylate) was 103 ± 31 percent. The average RPD for carboxylic acids detected in field-duplicate samples was 13 ± 16 percent indicating acceptable reproducibility. Average recovery for method compounds from the field- and laboratory-spike samples was 84 ± 13 percent.

Steroidal Hormones and Other Chemicals

During the course of this study, 73 environmental samples of surface water and wastewater effluent, 23 field-duplicate samples, 15 field-spike samples, 3 laboratory-blank samples, and 7 field-blank samples were analyzed at the USGS-NRPL for 17 steroidal hormones and 3 other chemicals in unfiltered water by the SPE method (appendix 8). Laboratory reporting levels for the 20 chemicals ranged from 0.1 to 2 ng/L (table 5), defined as the concentration equal to three times the mean value detected in method blanks or three times the baseline signal, whichever was greater.

There were potential analytical issues related to the loss of one or more deuterium atoms in IDSs. In order to correct a potential positive bias due to D-loss, a conservative approach was used. Reported concentrations of chemicals determined using these six IDSs potentially susceptible to D-loss were censored to the laboratory reporting level, if needed, or were quantified relative to 17-*alpha*-ethynylestradiol-d_4 (surrogate standard recoveries less than 10 percent) for any of the 73 environmental samples where D-loss was evident or suspected.

Bisphenol A, cholesterol, coprostanol, 17-*beta*-estradiol, epitestosterone, and estriol were detected in either laboratory- or field-blank samples (table 14). Concentrations of these chemicals detected in blank samples were used to flag environmental data that may have been affected by contamination. The mean and two standard deviations of the blank-sample concentrations for these chemicals were calculated using maximum likelihood estimation techniques described in the "Trace Elements and Major Ions" section. Detected concentrations that were less than the calculated mean plus two standard deviations of the blank concentrations were flagged (a "C" qualifier code precedes the value) in the dataset (appendix 8).

Average recovery of the 13 IDSs and surrogate standards was 28 ± 19 percent. The average RPD for target compounds that were detected in 23 duplicate samples was 16 ± 7 percent. The average RPD for the surrogate standards between duplicate pairs was 17 ± 24 percent. Average recovery for the 20 compounds from the field-spike samples was 51 ± 41 percent.

Bed-Sediment Data

Analytical results and quality-assurance data for bed-sediment samples are presented in the following sections. The bed-sediment samples were analyzed for three carbon types, organic wastewater-indicator chemicals, and steroidal hormones.

Carbon and Wastewater-Indicator Chemicals

During this study, 25 environmental samples of bed sediment, 4 laboratory-blank samples, and 4 laboratory-spike samples were analyzed for 3 carbon types, 57 wastewater-indicator chemicals, and 3 surrogate standards at the USGS-NWQL

Table 13. Quality-assurance summary for carboxylic acids in water samples analyzed at the U.S. Geological Survey National Research Program Laboratory.

[*n*, number; µg/L, micrograms per liter; nd, not detected; --, not applicable; ±, plus or minus]

Chemical	Laboratory-blank samples (*n* = 3)		Field-blank samples (*n* = 7)		Environmental samples (*n* = 73)	Field-duplicate samples (*n* = 13 pairs)	Field-spike samples (*n* = 8)
	Range in detected concentrations (µg/L)	Frequency of detection (percent)	Range in detected concentrations (µg/L)	Frequency of detection (percent)	Range in detected concentrations (µg/L)	Relative percent difference (percent)	Average recovery and standard deviation (percent)
Ethylenediaminetetraacetic acid (EDTA)	nd	--	5–24	100	27–580	7.0 ± 4.1	82.5 ± 14
Nitrilotriacetic acid (NTA)	nd	--	nd	--	2–10	7.7 ± 5.5	56.4 ± 34.3
4-Nonylphenolmonoethoxycarboxylate (NP1EC)	nd	--	nd	--	2–110	18.9 ± 12.1	93.2 ± 10.6
4-Nonylphenoldiethoxycarboxylate (NP2EC)	nd	--	nd	--	2–130	18.6 ± 29.3	83.2 ± 17.1
4-Nonylphenoltriethoxycarboxylate (NP3EC)	nd	--	nd	--	2–19	--	82.4 ± 17.2
4-Nonylphenoltetraethoxycarboxylate (NP4EC)	nd	--	nd	--	2–7	--	97.3 ± 17.4

Table 14. Quality-assurance summary for steroidal hormones and other chemicals in water samples analyzed at the U.S. Geological Survey National Research Program Laboratory.

[*n*, number; ng/L, nanograms per liter; nd, not detected; ±, plus or minus; --, not applicable]

Chemical	Laboratory-blank samples (*n* = 3)		Field-blank samples (*n* = 7)		Environmental samples (*n* = 73)	Field-duplicate samples (*n* = 23 pairs)	Field-spike samples (*n* = 15)
	Range in detected concentrations (ng/L)	Frequency of detection (percent)	Range in detected concentrations (ng/L)	Frequency of detection (percent)	Range in detected concentrations (ng/L)	Relative percent difference (percent)	Average recovery and standard deviation (percent)
4-androstene-3,17-dione[1]	nd	--	nd	--	nd	--	19 ± 32
cis-Androsterone[1]	nd	--	nd	--	3.0–4.6	15 ± 14	0 ± 1
Bisphenol A[3]	35	33	nd	--	46–6,200	5	38 ± 75
Cholesterol[2]	1,600–2,000	66	2,400	14	2,500 –92,000	21 ± 30	49 ± 56
3-*beta*-Coprostanol[2]	52–210	66	65–82	29	230–14,000	25 ± 33	70 ± 90
Diethylstilbestrol[1]	nd	--	nd	--	0.1–1.0	--	35 ± 56
Equilenin[1]	nd	--	nd	--	0.3–8.2	11 ± 3	37 ± 37
Equilin[1]	nd	--	nd	--	nd	--	39 ± 31
17-*alpha*-Estradiol[1]	nd	--	nd	--	0.4–2.5	27	94 ± 77
17-*beta*-Estradiol[1]	nd	--	0.375	14	0.4–10.2	19 ± 28	64 ± 44
Estriol[1]	nd	--	0.4	14	0.2–11	--	40 ± 31
Estrone[1]	nd	--	nd	--	2.1–38	7 ± 7	166 ± 113
17-*alpha*-Ethynylestradiol[1]	nd	--	0.296	14	0.1–1.5	16 ± 9	118 ± 43
Mestranol[1]	nd	--	nd	--	0.2	--	102 ± 57
Norethindrone[1]	nd	--	nd	--	nd	--	75 ± 49
Progesterone[1]	nd	--	nd	--	nd	--	8 ± 24
Testosterone[1]	nd	--	nd	--	0.6	--	15 ± 33
dihydro-Testosterone[1]	nd	--	nd	--	nd	--	18 ± 35
epi-Testosterone[1]	nd	--	3.15	14	nd	--	18 ± 36
11-*keto*-testosterone[1]	nd	--	nd	--	nd	--	17 ± 25

[1]Steroidal hormones.

[2]Sterols.

[3]Plastic component.

(appendix 9). The average percent recovery for surrogate standards was 62 ± 36 percent. Eighteen of the 57 wastewater-indicator chemicals were detected in laboratory-blank samples (appendix 9) at low concentrations. The environmental sample sizes vary, and thus the reporting levels change (are scaled) on the basis of sample weight extracted relative to reporting levels that assume a default 10-g sample size. The blank samples are composed of a 10-g sample. Because blank-sample and environmental-sample size differ from each other, a comparison of these samples was made on total mass of a chemical rather than on concentrations, which can be misleading. For example, a concentration of 0.14 ng/g for a 1-g environmental sample size is the same as a concentration of 0.014 ng/g for a 10-g laboratory-blank sample. The mass of all chemicals in laboratory-blank samples and environmental samples was calculated by multiplying the concentrations of the sample by the weight of the sample. The mean and two standard deviations of the blank-sample masses for these chemicals were calculated using maximum likelihood estimation techniques described in the "Trace Elements and Major Ions" section. There were no cases where the environmental sample concentration was less than the mean plus two standard deviations of the concentrations in the blank samples. The average percent recovery for the 57 organic wastewater-indicator chemicals among the four laboratory-spike samples was 77 ± 25 percent. The chemicals 2,2',4,4'-tetrabromodiphenylether (PBDE 47), bisphenol A, isophorone, and tris(dichloroisopropyl) phosphate all had recoveries less than 40 percent in the laboratory-spike samples (appendix 9).

Steroidal Hormones and other chemicals

During this study, 25 environmental samples of bed sediment, 4 laboratory-blank samples, and 4 laboratory-spike samples were analyzed for 17 steroidal hormones and three othe chemicals at the USGS NWQL (appendix 10). For the 11 samples extracted in 2009, 13 IDSs were used (table 6). For the 14 samples extracted in 2010, 5 of the original 13 IDSs were used with 4 alternative chemicals substituted as described in the "Bed-Sediment Chemical Analyses" section, resulting in the use of 9 compounds as IDSs, plus nandrolone-d_3 as a surrogate only (table 6). The average percent recovery for all 18 IDSs in environmental samples was 47 ± 35 percent. The standards 17-*beta*-estradiol-d_4, cholesterol-d_7, dihydrotestoterone-d_4, estriol-d_3, nandrolone-d_3, norethindrone-d_6, progesterone-d_9, and testosterone-d_5 all had low average recoveries (less than 40 percent).

Similar to the analyses of steroidal hormones in water, there was a potential analytical performance issue that was related to the loss of one or more deuterium atoms for the six IDSs shown in table 6 for the subset of the environmental samples extracted in 2009 (Foreman and others, 2010). Because chemical concentrations are recovery-corrected using the IDSs, low-biased recoveries of the IDSs due to D-loss could have resulted in a positive bias in concentrations for the following 10 chemicals: 4-androstene-3,17-dione, *cis*-androsterone, dihydrotestosterone, equilin, estrone, norethindrone, progesterone, testosterone, epi-testosterone, 11-*keto*-testosterone. In order to correct this potential positive bias, a conservative approach was used. For affected samples, reported concentrations were quantified relative to 17-*alpha*-ethynylestradiol-d_4 to eliminate positive concentration bias, or were censored (particularly those with detected concentrations less than the laboratory reporting level) to reduce the risk of false positives for the 6 of the 10 chemicals noted above that had direct IDS analogs (table 6). As a consequence, it is possible that selected chemicals such as estrone and possibly 4-androstene-3,17-dione were censored when they were actually present in the sample at a concentration less than the "less than" value listed in appendix 10. A reported "less than" value does not always constitute a non-presence.

Bisphenol A, cholesterol, and 3-*beta*-coprostanol were routinely detected in the laboratory-blank samples at less than 10 nanograms per gram (ng/g) for a 10-g sample and, thus, have higher minimum reporting levels than other chemicals (William Foreman, U.S. Geological Survey, oral commun., January 2011). The remaining 17 analytes were detected in one or more laboratory-blank samples at low concentrations ranging from 0.004 to 0.046 ng/g. The mass of all laboratory-blank samples and environmental samples was calculated by multiplying the concentrations of the sample by the weight of the sample. The mean and two standard deviations of the blank-sample masses for these chemicals were calculated using maximum likelihood estimation techniques described in the "Trace Elements and Major Ions" section. The masses of the environmental samples were compared to the blank sample masses and bisphenol A was the only chemical (in one sample) that had a detected concentration less than the mean plus two standard deviations of the blank concentrations. This concentration was flagged with a "C" qualifier code that precedes the value (appendix 10). Percent recoveries for laboratory-spike samples in baked Ottawa reagent sand averaged 95 ± 27 percent.

Biological Characteristics

Analytical results and quality-assurance data for biological characteristics are presented in the following sections. Two biological measures are described: (1) *in-vitro* cellular assays of "total estrogenicity," and (2) *in-vivo* caged fish experiments to measure whole-organism responses.

Total Estrogenicity

During the study, 56 environmental samples of surface water and wastewater effluent, four distilled water blanks, four distilled water samples spiked with 17-*beta*-estradiol, three distilled water samples spiked with a mixture of 17-*beta*-estradiol and antiestrogen receptor (ICI) (0.1 micromolar), two matrix samples with Lake Superior water (LSW), two 17-*beta*-estradiol matrix (LSW) spike samples, and two

17-*beta*-estradiol matrix (LSW) spike samples treated with ICI (0.1 micromolar) were analyzed for total estrogencity in units of 17-*beta*-estradiol equivalents at the UST Laboratory (appendix 11). The distilled water samples, LSW matrix samples, distilled water co-treated with 17-*beta*-estradiol and ICI, and LSW matrix samples co-treated with 17-*beta*-estradiol and ICI did not exhibit any estrogenic activity in the cell assay. The average and standard deviation of the measured-to-expected ratio in the distilled water samples spiked with 17-*beta*-estradiol was 0.896 ± 0.06. In matrix (LSW) spikes, it was 0.72 ± 0.11. The quantification limit of the method was calculated by calculating the environmental concentration at which a chemical induces a response 10 percent above the baseline (EC_{10}). The EC_{10} was calculated from a nonlinear sigmoidal dose response curve fit to the relative luminescence units of the 17-*beta*-estradiol standards (using Prism 5.02., Graph Pad Software Inc., La Jolla, Calif.). The quantification limit was 0.211 ng/L, which was similar to the results of Wilson and others (2004), who developed and tested the T47D-KBluc assay for estrogenic activity. The detection limit of the assay was the equivalent of 0.03 ng of the 17-*beta*-estradiol standard.

Caged Fish Data

Fish length, weight, body condition factor, and plasma vitellogenin concentrations were determined in fish (fathead minnows) from the caged fish study by the SCSU Aquatic Toxicology Laboratory (appendix 12). A total of 236 fish plasma samples were analyzed for vitellogenin (appendix 12). Biological samples for analysis of plasma vitellogenin concentrations were coded to ensure that the analyst was unaware of the sample location. Plasma samples were analyzed by enzyme-linked immunosorbent assay. All plasma samples were analyzed at three dilutions in duplicate and referenced against a multi-point standard curve (acceptable standard curve r^2 greater than 0.95). Each assay included an aliquot analyzed in duplicate from a composite blood sample to determine interassay variation.

Summary

This report presents the study design, environmental data, and quality-assurance data for an integrated chemical and biological study of selected streams or lakes that receive wastewater in Minnesota. Datasets for this study are located in appendixes 1 through 12. Data for appendixes 1–12 are available in Microsoft Excel format on the report's Web page at *http://pubs.usgs.gov/ds/575/downloads/*.

This study was a cooperative effort of the U.S. Geological Survey, the Minnesota Pollution Control Agency, St. Cloud State University, University of St. Thomas, and the University of Colorado. The objective of the study was to identify distribution patterns of chemicals of concern including endocrine-active chemicals, pharmaceuticals, and other organic chemicals of concern input to streams and lakes, and to identify biological characteristics (estrogenicity and fish responses) in the same streams.

The U.S. Geological Survey collected and analyzed water samples (surface water and wastewater effluent), bed-sediment samples, and quality-assurance samples and measured or recorded streamflow once from September through November 2009. Twenty-five wastewater-treatment plants were selected to include those of differing treatment types (continuous flow or periodic releases), differing processing steps (activated sludge or trickling filters), and plant design flows ranging from 0.001 to 10.9 cubic meters second (0.04 to 251 million gallons per day) throughout Minnesota in varying land-use settings.

Water samples were collected from the treated effluent of 25 wastewater-treatment plants and at one point upstream from and one point downstream from wastewater-effluent discharges. Bed-sediment samples also were collected at each of the stream or lake locations. Water samples were analyzed for major ions, nutrients, trace elements, pharmaceuticals, phytoestrogens and pharmaceuticals, alkylphenols and other neutral organic chemicals, carboxylic acids, and steroidal hormones. A subset (25 samples) of the bed-sediment samples were analyzed for carbon, wastewater-indicator chemicals, and steroidal hormones; the remaining samples were archived.

Biological characteristics were determined by using an *in-vitro* bioassay to determine total estrogenicity in water samples and a caged fish study to determine characteristics of fish from experiments that exposed fish to wastewater effluent in 2009. St. Cloud State University deployed and processed caged fathead minnows at 13 stream sites during September 2009 for the caged fish study. Measured fish data included length, weight, body condition factor, and vitellogenin concentrations.

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Appendixes 1–12

These data files are included with the U.S. Geological Survey (USGS) Data Report 575 and are available for download at *http://pubs.usgs.gov/ds/575/downloads*. See report text for details about the study. The data tables are available for download in Microsoft© Excel (.xls) format. The first row of each data table contains information about the data. There are 12 appendixes included in this report.

Appendix 1. Basic field properties and hydrologic characteristics of sampling sites

The Excel spreadsheet appendix_1.xls contains data on basic field properties and hydrologic characteristics for 73 sites sampled during September–November 2009. Instantaneous streamflow, effluent discharge, and basic water-quality properties of dissolved oxygen, pH, specific conductance, and water temperature are contained in the spreadsheet. This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_1.xls*.

Appendix 2. Concentrations of major ions and nutrients in water samples and quality-assurance samples analyzed at the U.S. Geological Survey National Water Quality Laboratory, and suspended sediment analyzed at the U.S. Geological Survey Iowa Sediment Laboratory

The Excel spreadsheet appendix_2.xls contains concentration data for 2 major ions and 6 nutrients for which water samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of water samples collected from 73 sites during September–November 2009 ("Environmental" worksheet) and the analytical results of the associated quality-assurance samples ("Quality Assurance" worksheet). This Excel spreadsheet can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_2.xls*.

Appendix 3. Dissolved concentrations of trace elements and major ions in water samples and quality-assurance samples analyzed at U.S. Geological Survey National Research Program Laboratory.

The Excel spreadsheet appendix_3.xls contains dissolved concentration data for 54 trace elements and major ions for which water samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of water samples collected from 73 sites during September–November 2009 ("Environmental" worksheet) and the analytical results of the associated quality-assurance samples ("Quality Assurance" worksheet). This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_3.xls*.

Appendix 4. Concentrations of pharmaceuticals in water samples and quality-assurance samples analyzed at the U.S. Geological Survey National Water Quality Laboratory

The Excel spreadsheet appendix_4.xls contains concentration data for 16 pharmaceuticals and 2 surrogate standards for which water samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of water samples collected from 73 sites during September–November 2009 ("Environmental" worksheet) and associated quality-assurance samples ("Quality Assurance" worksheet). This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_4.xls*.

Appendix 5. Concentrations of phytoestrogens, pharmaceuticals, and an antimicrobial in water samples and quality-assurance samples analyzed by the University of Colorado Center for Environmental Mass Spectrometry

The Excel spreadsheet *appendix_5.xls* contains concentration data for 10 phytoestrogens, 8 pharmaceuticals, and 1 antimicrobial for which water samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of water samples collected from 73 sites during September–November 2009 ("Environmental" worksheet) and associated quality-assurance samples ("Quality Assurance" worksheet). This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_5.xls*.

Appendix 6. Concentrations of alkylphenols and other neutral organic chemicals in water and quality-assurance samples analyzed at the U.S. Geological Survey National Research Program Laboratory

The Excel spreadsheet appendix_6.xls contains concentration data for 23 alkylphenols, other neutral organic chemicals, and 5 surrogate standards for which water samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of water samples collected from 73 sites during September–November 2009 ("Environmental" worksheet) and associated quality-assurance samples ("Quality Assurance" worksheet). This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_6.xls*.

Appendix 7. Concentrations of carboxylic acids in water and quality-assurance samples analyzed at the U.S. Geological Survey National Research Program Laboratory

The Excel spreadsheet appendix_7.xls contains concentration data for six chemicals and one surrogate standard for which water samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of water samples collected from 73 sites during September–November 2009 ("Environmental" worksheet) and associated quality-assurance samples ("Quality Assurance" worksheet). This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_7.xls*.

Appendix 8. Concentrations of steroidal hormones in water and quality-assurance samples analyzed at the U.S. Geological Survey National Research Program Laboratory

The Excel spreadsheet appendix_8.xls contains concentration data for 20 chemicals and 13 surrogate standards for which water samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of water samples collected from 73 sites during September–November 2009 ("Environmental" worksheet) and associated quality-assurance samples ("Quality Assurance" worksheet). This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_8.xls*.

Appendix 9. Concentrations of carbon and organic wastewater-indicator chemicals analyzed in bed-sediment samples and quality-assurance samples at the U.S. Geological Survey National Water Quality Laboratory

The Excel spreadsheet appendix_9.xls contains concentration data for 3 carbon types, 57 organic wastewater-indicator chemicals, and 3 surrogate standards for which bed-sediment samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of bed-sediment samples collected from 25 sites during September–October 2009 ("Environmental" worksheet) and associated quality-assurance samples ("Quality Assurance" worksheet). This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_9.xls*.

Appendix 10. Concentrations of steroidal hormones and other chemicals analyzed in bed-sediment samples at the U.S. Geological Survey National Water Quality Laboratory

The Excel spreadsheet appendix_10.xls contains concentration data for 17 steroid hormones, 3 other chemicals,and 18 isotope dilution standards for which bed-sediment samples in this study were analyzed. This spreadsheet contains separate worksheets for the analytical results of bed-sediment samples collected from 25 sites during September–October 2009 ("Environmental" worksheet) and associated quality-assurance samples ("Quality Assurance" worksheet). This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_10.xls*.

Appendix 11. Total estrogenicity in water samples analyzed at University of St. Thomas Laboratory

The Excel spreadsheet appendix_11.xls contains total estrogenicity data for selected water samples in this study. The "Environmental" worksheet contains the analytical results of water samples collected from 73 sites during September–November 2009. This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_11.xls*.

Appendix 12. Length, weight, body condition factor, and vitellogenin concentrations in caged fathead minnows analyzed at the St. Cloud State University Aquatic Toxicology Laboratory

The Excel spreadsheet appendix_12.xls contains analyses of length, weight, body condition factor, and vitellogenin concentrations in samples of caged fathead minnows collected from 13 sites during September 2009. This Excel file can be accessed at *http://pubs.usgs.gov/ds/575/downloads/appendix_12.xls*.

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