Prepared in cooperation with St. Cloud State University, Minnesota Department of Health, Minnesota Pollution Control Agency, Minnesota Department of Natural Resources, Metropolitan Council Environmental Services, and the University of Minnesota

Endocrine Active Chemicals and Endocrine Disruption in Minnesota Streams and Lakes—Implications for Aquatic Resources, 1994–2008

Scientific Investigations Report 2010–5107

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

Specific conductance measurement at the outflow from the Hutchison, Minn. wastewater treatment plant. Photograph by John Greene (USGS) September 14, 2009.

Water sample collection from the Mississippi River. Photograph by Kathy Lee (USGS) July 3, 2006.

Collection of water samples from the Redwood River near Marshall, Minn. Photograph by Christine Yaeger (USGS) February 27, 2007.

Calibration of water quality monitoring equipment. Photograph by Irina Comardicea (USGS) July 5, 2006.

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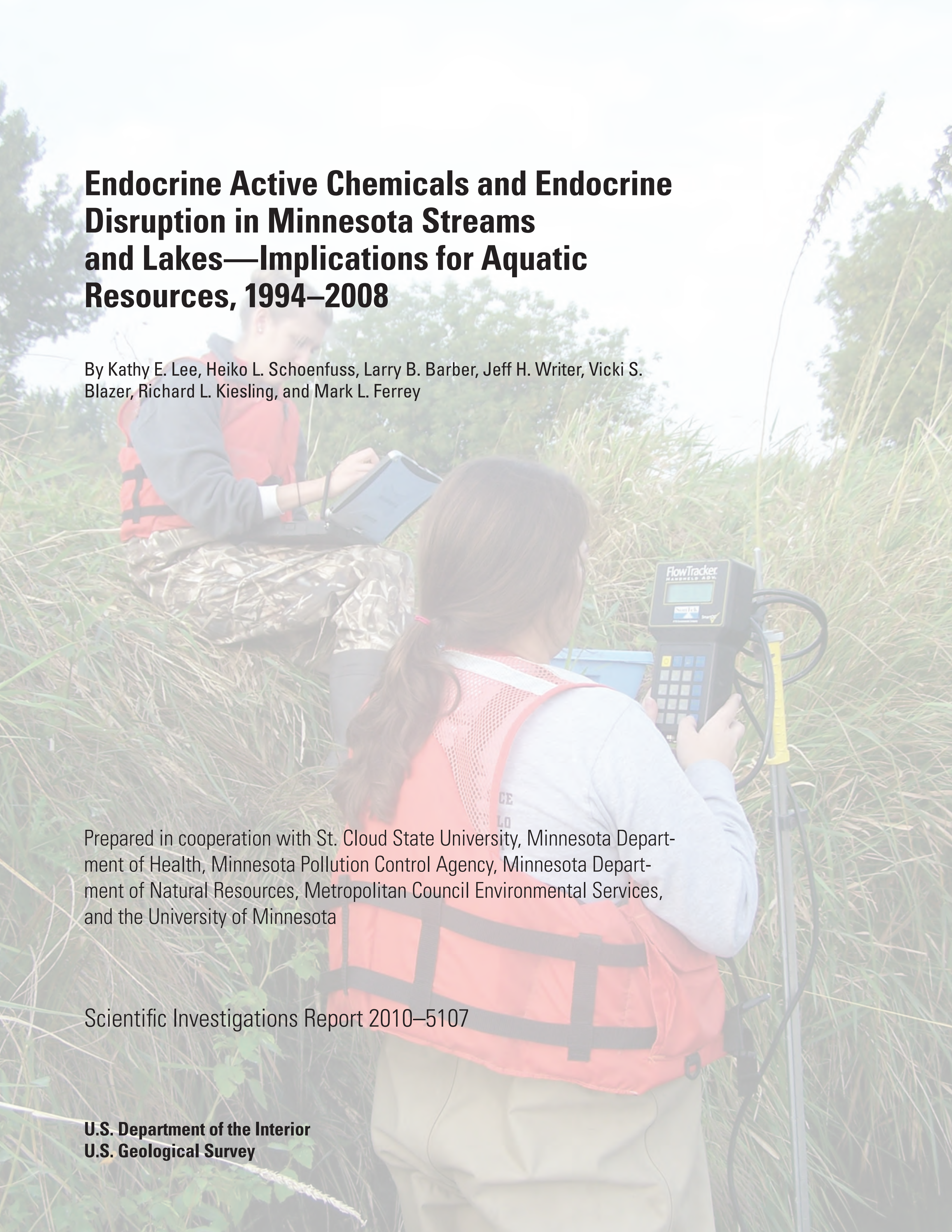
By Kathy E. Lee, Heiko L. Schoenfuss, Larry B. Barber, Jeff H. Writer, Vicki S. Blazer, Richard L. Kiesling, and Mark L. Ferrey



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Conversion Factors, Abbreviations, and Datums

Multiply	By	To obtain
	Length	
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
inch (in)	2.54	centimeter (cm)
	Area	
square mile (mi^2)	259.0	hectare (ha)
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
gallon (gal)	3.785	liter (L)
million gallons (Mgal)	3,785	cubic meter (m^3)
cubic foot (ft^3)	0.02832	cubic meter (m^3)
	Flow rate	
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)
gallon per day (gal/d)	0.003785	cubic meter per day (m^3/d)
million gallons per day (Mgal/d)	0.04381	cubic meter per second (m^3/s)

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

$$°F=(1.8\times°C)+32$$

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

$$°C=(°F-32)/1.8$$

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.

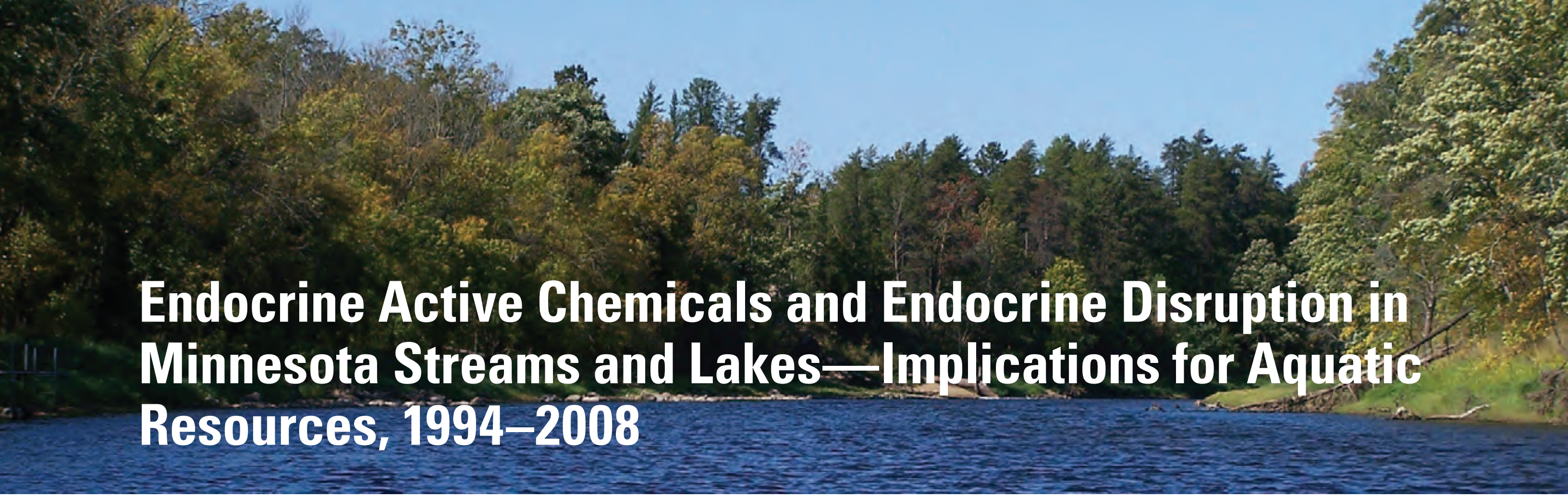
Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L), micrograms per liter (µg/L), nanograms per liter (ng/L), nanograms per milliliter (ng/mL).

Concentrations of chemical constituents in bed sediment are given in nanograms per gram (ng/g).

Abbreviations and Acronyms

AHTN	acetyl-hexamethyl-tetrahydronaphthalene
BPA	bisphenol A
EAC	endocrine active chemical
GnRH	gonadotropin-releasing hormone
GtH	gonadotrophins
HHCB	hexahydrohexamethyl-cyclopenta-benzopyran
MPCA	Minnesota Pollution Control Agency
NP	4-nonylphenol
NP1EC	4-nonylphenolmonoethoxycarboxylate
NP2EC	4-nonylphenoldiethoxycarboxylate
NP1EO	4-nonylphenolmonoethoxylate
NP2EO	4-nonylphenoldiethoxylate
OP1EO	4-*tert*-octylphenolmonoethoxylate
OP2EO	4-*tert*-octylphenoldiethoxylate
SCSU	St. Cloud State University
TOP	4-*tert*-octylphenol
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WWTP	wastewater-treatment plant



Endocrine Active Chemicals and Endocrine Disruption in Minnesota Streams and Lakes—Implications for Aquatic Resources, 1994–2008

By Kathy E. Lee[1], Heiko L. Schoenfuss[2], Larry B. Barber[1], Jeff H. Writer[1], Vicki S. Blazer[1], Richard L. Kiesling[1], and Mark L. Ferrey[3]

Abstract

The U.S. Geological Survey, in cooperation with St. Cloud State University, Minnesota Department of Health, Minnesota Pollution Control Agency, Minnesota Department of Natural Resources, Metropolitan Council Environmental Services, and the University of Minnesota, has conducted field monitoring studies and laboratory research to determine the presence of endocrine active chemicals and the incidence of endocrine disruption in Minnesota streams and lakes during 1994–2008. Endocrine active chemicals are chemicals that interfere with the natural regulation of endocrine systems, and may mimic or block the function of natural hormones in fish or other organisms. This interference commonly is referred to as endocrine disruption. Indicators of endocrine disruption in fish include vitellogenin (female egg yolk protein normally expressed in female fish) in male fish, oocytes present in male fish testes, reduced reproductive success, and changes in reproductive behavior.

The results from a series of studies during 1994–2008 demonstrate that endocrine active chemicals are present in Minnesota surface waters, indicating that aquatic organism exposure is likely. Endocrine active chemicals have been identified in wastewater-treatment plant effluent and surface waters downstream from discharge of wastewater-treatment plant effluent throughout Minnesota at low concentrations.

Biological indicators of endocrine disruption have been detected in wild fish throughout Minnesota at sites directly downstream from wastewater-treatment plant effluent, indicating that endocrine active chemicals in effluent contribute to endocrine disruption in fish. This finding was confirmed in a controlled study exposing fathead minnows to wastewater-treatment plant effluent at an onsite fish exposure laboratory. During this controlled study, changes in biological responses coincided with changes in wastewater-treatment plant effluent composition demonstrating that effluent effects on fish endocrine systems are temporally variable. Although chemicals contributing to endocrine disruption in fish are complex, several laboratory studies have further confirmed that certain classes of chemicals, such as hormones and alkylphenols, which are components of wastewater-treatment plant effluent, affect the endocrine systems of fish through biochemical, structural, and behavioral disruption.

Although these studies indicate that wastewater-treatment plant effluent is a conduit for endocrine active chemicals to surface waters, endocrine active chemicals also were present in surface waters with no obvious wastewater-treatment plant effluent sources. Endocrine active chemicals were detected and indicators of endocrine disruption in fish were measured at numerous sites upstream from discharge of wastewater-treatment plant effluent. These observations indicate that other unidentified sources of endocrine active chemicals exist, such as runoff from land surfaces, atmospheric deposition, inputs from onsite septic systems, or other groundwater sources. Alternatively, some endocrine active chemicals may not yet have been identified or measured. The presence of biological indicators of endocrine disruption in male fish indicates that the fish are exposed to endocrine active chemicals. However indicators of endocrine disruption in male fish does not indicate an effect on fish reproduction or changes in fish populations.

Introduction

Concern that selected chemicals in aquatic environments may act as endocrine active chemicals (EACs) is widespread (Colburn and Clement, 1992; Ankley and others, 1998; Kime, 1998). EACs interfere with the natural regulation of fish endocrine systems by either mimicking or blocking the function of natural hormones (Kime, 1998; National Research Council, 1999). This interference is commonly referred to as endocrine disruption.

Numerous lists of EACs have been created for various purposes (Illinois Environmental Protection Agency, 1997;

[1] U.S. Geological Survey

[2] St. Cloud State University

[3] Minnesota Pollution Control Agency

Table 1. Descriptions of commonly used indicators of endocrine disruption in fish.

Indicator	Description
Vitellogenin	Vitellogenin is a protein that is the precursor to egg yolk proteins. In male fish, vitellogenin production is stimulated in the liver when they are exposed to various natural and synthetic estrogens.
Sex steroid hormones	Chemical messengers that stimulate vitellogenin production, secondary sex characteristics, development of gametes, and spawning.
Gonado-somatic index	Percentage of body weight composed of gonad tissue, indicating reproductive status and chemical exposure.
Gonad histopathology	Microscopic examination for the presence of abnormalities such as oocytes in male testes.
Secondary sexual characteristics	Development of secondary sexual characteristics such as tubercles, dorsal pads, and coloration in male fish.
Behavioral indicators	Courtship behavior including the ability of male fish to construct and defend nesting sites.
Reproductive success	Number of surviving offspring that carry genes derived from parents into the next generation.

Colborn and others, 1993); however, no definitive regulatory-based list of EACs is available. The U.S. Environmental Protection Agency (USEPA) is in the process of evaluating chemicals for their potential to disrupt estrogen, androgen, and thyroid systems through the Endocrine Disruption Screening Program (U.S. Environmental Protection Agency, 2009). Although no single list exists, laboratory studies have confirmed that certain classes of chemicals including natural and synthetic hormones, pesticides, 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). EACs have been identified in wastewater-treatment plant (WWTP) effluents and surface waters worldwide (Ahel, Giger, and Koch, 1994; Ahel, Giger, and Schaffner, 1994; Desbrow and others, 1998; Kolpin and others, 2002), and more specifically in Minnesota (Barber and others, 2000, 2007; Lee and others, 2004; Lee, Yaeger, and others, 2008; Lee, Schoenfuss, and others, 2008; Martinovic and others, 2008). EACs can enter the environment through many pathways including WWTP effluent, industrial effluent discharge, runoff from agricultural and urban land surfaces, application of human and animal waste, and septic system discharge and subsequent movement to groundwater or surface water. In addition, EACs are not completely removed by wastewater-treatment systems (Richardson and Bowron, 1985; Stumpf and others, 1996; Ternes, 1998), resulting in potentially continuous sources of EACs to groundwater, surface water, and drinking water.

Aquatic organisms including fish are exposed directly to EACs on a potentially continual basis through dermal and gill surface contact and food consumption. Signaling within the endocrine system can be modified or disrupted by EACs at many levels. Fish reproduction includes a complex chain of hormonal events (fig. 1). In female fish, external signals stimulate the hypothalamus gland to produce gonadotropin-releasing hormone (GnRH) that stimulates the pituitary gland to produce gonadotropins (GtH), which then stimulate the synthesis of sex steroids hormones (testosterone and 17β-estradiol) in the ovaries (Kime, 1998). A primary role of 17β-estradiol in females is to stimulate the liver to produce vitellogenin, which is a protein that is the precursor to egg yolk proteins. In male fish, vitellogenin production is stimulated in the liver when they are exposed to various natural and synthetic estrogens (Jobling and Sumpter, 1993). Concentrations of measurable amounts of plasma vitellogenin in male fish usually are low (nanograms per milliliter) or undetectable, thus making the presence of vitellogenin in male fish an indicator of the presence of estrogen or estrogenic chemicals in the environment (Purdom and others, 1994; Sumpter and Jobling, 1995; Folmar and others, 1996).

EACs may disrupt normal function of the endocrine system in a variety of ways including direct cellular damage to organs, damage to neurons or the nervous system that control the organs, modification of hormone or enzyme synthesis, or interference with the feedback regulation of hormones (Kime, 1998). In normal endocrine system operation, hormones bind

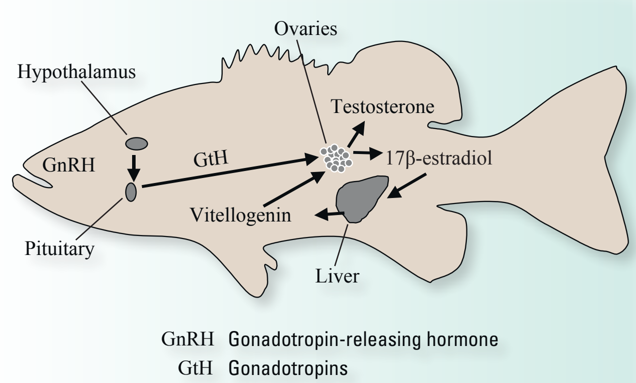


Figure 1. Endocrine system control of vitellogenin induction in female fish.

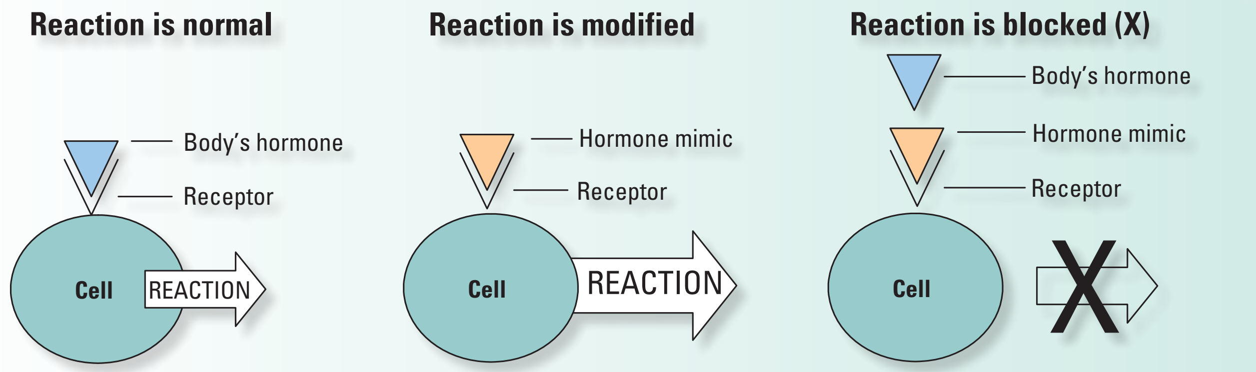


Figure 2. The mechanism of action for hormones and endocrine active chemicals (modified from Streets and others, 2008).

to receptors to elicit reactions within a cell (fig. 2). An EAC that acts as a hormone mimic can modify the reaction so that it is stronger or weaker than normal, or occurs at an inappropriate time. An EAC also may bind with the receptor and block the reaction.

A variety of receptor binding assays and whole-organism methods can determine whether or not a chemical is interacting with the endocrine system. For example, estrogen receptor binding assays are designed to identify chemicals that bind to an estrogen receptor and, therefore, might result in changes to normal function (National Institute of Environmental Health Sciences, 2003; Bolger and others, 1993). Some commonly used receptor binding methods include the yeast estrogen screen reporter gene assay (Routledge and Sumpter, 1996) and the human estrogen receptor positive MCF-7 breast cancer cell line (E-screen) proliferation test (Soto and others, 1991; Koerner and others, 1999). Numerous whole-organism indicators of endocrine disruption in fish also are available ranging from those that indicate exposure to EACs but not necessarily a negative effect on reproduction such as vitellogenin, to those that indicate a physical, behavioral, or reproductive alteration (table 1).

The U.S. Geological Survey (USGS), in cooperation with St. Cloud State University, Minnesota Department of Health, Minnesota Pollution Control Agency, Minnesota Department of Natural Resources, Metropolitan Council Environmental Services, and the University of Minnesota, have completed a series of studies to determine the presence of EACs and endocrine disruption in Minnesota streams and lakes during 1994–2008. Combinations of field monitoring and controlled laboratory studies have been completed by this interdisciplinary group. The long-term goals of the cooperative studies are to determine the occurrence and distribution of EACs and endocrine disruption in Minnesota surface waters, factors contributing to EAC occurrence and fate in surface waters, factors related to endocrine disruption occurrence, source pathways of EACs to organisms, and population-level effects on fish and other organisms. The purpose of this report is to summarize the findings of the cooperative studies completed during 1994–2008.

Approach and Methods

A series of individual cooperative studies were completed during 1994–2008 by the USGS and one or more research partners (appendix 1). A combination of water, bed sediment, and fish was sampled at 135 stream or lake sites in Minnesota, and treated effluent was sampled from 12 WWTPs that discharge to streams in Minnesota (figs. 3*A* and 3*B*; appendix 2).

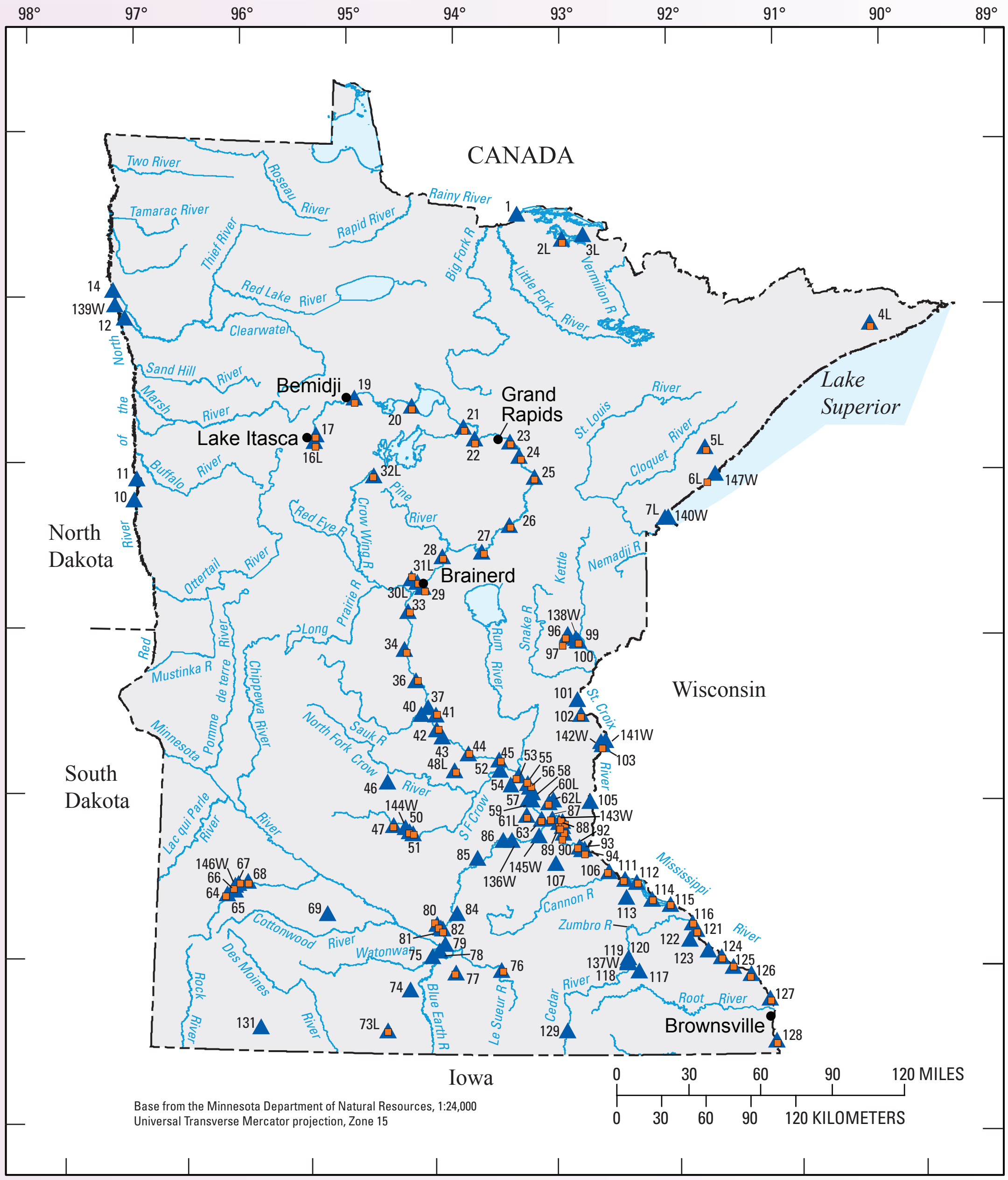
General Approach

Initial studies were broadly focused on the occurrence of biological responses or the occurrence of EACs and other contaminants in surface water, groundwater, wastewater, or drinking water in Minnesota. The focus developed into integrated studies of chemistry, hydrology, and biological responses to better understand the fate and effects of EACs. The increased attention on more narrowly focused studies to investigate specific chemical classes and specific environmental settings has provided additional information on sources and occurrence of EACs and on the biological responses to EACs.

Datasets from each study were combined into three datasets based on sampling media (water, bed sediment, and fish) for the analyses in this report (appendixes 3, 4, and 5, respectively). Data for appendixes 3–5 are available in Microsoft Excel format on the report's Web page at *http://pubs.usgs.gov/sir/2010/5107/*. Measurements made for each study varied with the study objectives. Analyses were done at multiple laboratories, which introduces variability. Site selection was not random, and many of the sites were chosen specifically because they were downstream from a WWTP discharge or represented a specific land-use category.

Chemical analyses were performed at two USGS laboratories and the detection limits and methods varied between laboratories. Chemical identification for all methods had to meet qualitative and quantitative criteria including positive identification based on elution within expected retention times and sample spectra and ion abundance had to match reference

A.



EXPLANATION

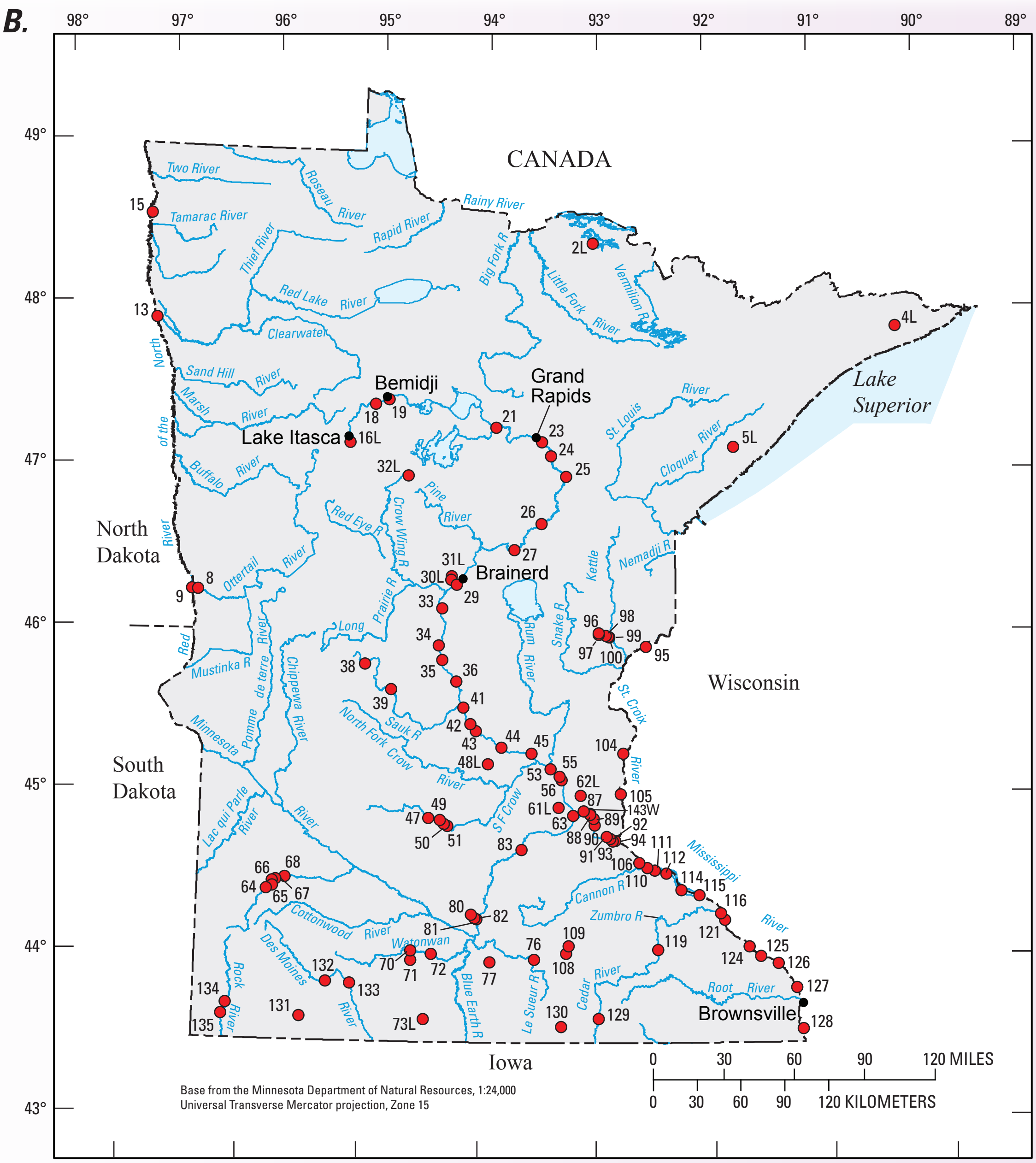
74 ▲ **Site where water was sampled from streams, lakes, or wastewater treatment plants**—Number is sampling site identifier shown in appendix 2

128 ■ **Site where bed sediments were sampled from streams or lakes**—Number is sampling site identifier shown in appendix 2

Figure 3. Locations where *A*, water, bed sediment, or *B*, fish were sampled during 1994–2008.

analytes (Barber and others, 2000, 2003, 2007; Lee and others, 2000, 2004; Lee, Yaeger, and others, 2008; Lee, Schoenfuss, and others, 2008). Concentrations were coded as estimated values when average recoveries were less than acceptable limits, when they were routinely found in blanks, when concentrations were above or below a calibration curve, or when standards were prepared from a technical mixture. There is more uncertainty in estimated concentrations (Zaugg and others, 2006); however, reporting the concentrations as estimated does not decrease confidence in the qualitative identification of a chemical.

Differences in detection limits and estimated values provide challenges to making comparisons among sites. Censored concentrations (those with a less than symbol and a concentration, for example “<0.05”), does not necessarily indicate that the chemical was not in the water sample but rather that the concentration was less than quantitation limits. For the purpose of this report, data were not censored at one



EXPLANATION

131 **Fish endocrine disruption characterization in stream and lakes—** Number is sampling site identifier shown in appendix 2

Figure 3. Locations where *A*, water, bed sediment, or *B*, fish were sampled during 1994–2008.—Continued

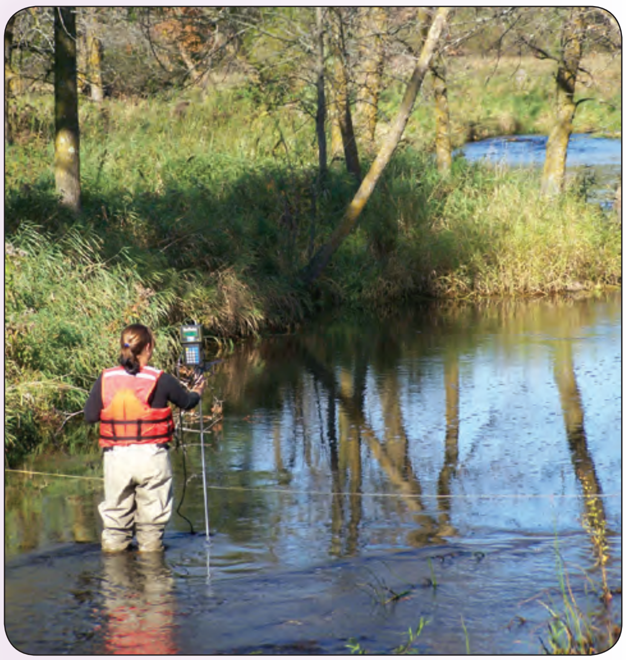
detection limit, but rather concentrations (estimated or not) were counted as detections. This approach is somewhat conservative as there may be more true environmental detections (that occurred at concentrations less than detection limits) than summarized in this report. Although these limitations provide challenges to interpretations, the combined datasets are satisfactory for analyses of general trends as presented in this report.

Sample Collection and Analytical Methods

All sampling was conducted using established protocols and described in detail in Goodbred and others (1997), Barber and others (2000, 2003, 2007), Lee and others (2000, 2004), Lee, Yaeger, and others (2008), and Lee, Schoenfuss, and others (2008). Streamflow was measured using USGS protocols (Rantz and others, 1982a, 1982b; Morlock and others, 2002).

Water samples were collected by wading, by drilling through ice, from boats, or from bridges depending on the flow conditions at the time of sampling. Water samples were collected using integrated width-and-depth sampling techniques (Edwards and Glysson, 1988; U.S. Geological Survey, variously dated) to obtain representative samples. Automatic samplers were used for sites where more frequent collection was required. Water samples were processed immediately and sent to USGS laboratories for analyses.

Bed-sediment samples were collected according to established USGS protocols (U.S. Geological Survey, variously dated). Bed-sediment samples were collected with stainless-steel sampling equipment from the top 20 centimeters of bed sediment. Samples were collected from 5 to 10 locations within a stream reach and composited to obtain a representative sample. Following collection, chilled samples were processed within 1 to 2 hours after collection before they were shipped to USGS laboratories.



Stream flow measurement at the Grindstone River below Hinckley, Minn. (USGS station number 05337005, photograph by John Greene (USGS) on September 27, 2007).

To avoid sample contamination, personnel who collected and processed water and bed-sediment samples 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 samples with a succession of native water, soapy tap water, tap water, deionized water, methanol, and organic-free water rinses (U.S. Geological Survey, variously dated).

Among all studies, water and bed-sediment samples were analyzed for a wide variety of chemicals including fragrances, pesticides, metal complexing agents, surfactant degradation products, plastic components, fire retardants, antioxidants, caffeine, antimicrobials, and steroids that are indicators of industrial, domestic, and agricultural wastewaters. These chemicals were selected for these studies on the basis of usage, toxicity, potential endocrine activity, and persistence in the environment (Barnes and others, 2002; Kolpin and others, 2002). Water samples were analyzed at the USGS National Water Quality Laboratory in Denver, Colo., and at the USGS National Research Laboratory in Boulder, Colo., as described in Barber and others (2000, 2003, 2007); Kolpin and others (2002); Zaugg and others (2002); Lee, Yaeger, and others (2008); and Lee, Schoenfuss, and others (2008). Bed-sediment samples were analyzed at the USGS National Water Quality Laboratory and at the USGS National Research Laboratory as described in Burkhardt and others (2006) and Barber and others (2000), respectively. The chemicals analyzed were not identical among studies because of differences in study scopes and purposes. Analytical detection limits varied between the laboratories and varied with time within one laboratory. The chemicals summarized in this report have been shown to be EACs in laboratory studies, and were analyzed in water and bed-sediment samples for most of the studies (table 2).

Quality-assurance samples were collected for water and bed-sediment analyses and discussed in detail in other reports (Goodbred and others,1997; Barber and others, 2000; Barber and others, 2003; Barber and others, 2007; Lee and others, 2000; Lee and others, 2004; Lee, Yaeger, and others, 2008; and Lee, Schoenfuss, and others, 2008). Quality-assurance samples included field blanks (water analyses) and field replicates (water and bed-sediment analyses). Field blanks were prepared at the sampling sites before the collection of the corresponding environmental sample. Blank samples were prepared by processing high-performance liquid-chromatography-grade organic-free water (Baker Analyzed, J.T. Baker Co.) through the same equipment used to collect and process field samples. Field replicate samples were used to determine variability of detections and concentrations that result from sample processing techniques (sample splitting, filtration, and transport). Replicate samples consist of a split of the environmental sample, so the environmental and replicate samples should be nearly equal in composition. Replicate samples measure the combined precision of sampling and laboratory analyses.

The quality-assurance data are included in appendixes 3 and 4 for water and bed-sediment samples, respectively. Among all the studies, 20 blank samples for water analyses were collected (appendix 3). In general, detections in blank samples were infrequent and almost all were at estimated concentrations. A few EACs were detected in the blank samples: 4-nonylphenol (4 detections at estimated concentrations), 4-nonylphenolmonoethoxylate (NP1EO; 2 detections), 4-*tert*-octylphenolmonoethoxylate (OP1EO; 1 detection at an estimated concentration), 4-*tert*-octylphenoldiethoxylate (OP2EO; 1 detection at an estimated concentration), acetyl-hexamethyl-tetrahydronaphthalene (AHTN; 1 detection at an estimated concentration), bisphenol A (1 detection at an

Table 2. List of endocrine active chemicals summarized.

Chemical name	Abbreviation	Possible chemical uses or sources	Sources
4-Nonylphenol	NP	Surfactant metabolite	Van den Belt and others, 2004; Brian and others, 2005; Preuss and others, 2006.
4-Nonylphenolmonoethoxylate (total)	NP1EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-Nonylphenoldiethoxylate (total)	NP2EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-Nonylphenoltriethoxylate (total)	NP3EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-Nonylphenoltetraethoxylate (total)	NP4EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-Nonylphenolmonoethoxycarboxylate	NP1EC	Surfactant metabolite	Routledge and Sumpter, 1996.
4-Nonylphenoldiethoxycarboxylate	NP2EC	Surfactant metabolite	Routledge and Sumpter, 1996.
4-Nonylphenoltriethoxycarboxylate	NP3EC	Surfactant metabolite	Routledge and Sumpter, 1996.
4-Nonylphenoltetraethoxycarboxylate	NP4EC	Surfactant metabolite	Routledge and Sumpter, 1996.
4-*normal*-Octylphenol	NOP	Plasticizer	Bonefeld-Jørgensen and others, 2007.
4-*tert*-Octylphenol	TOP	Surfactant metabolite	Bonefeld-Jørgensen and others, 2007; Soto and others, 1995; Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-*tert*-Octylphenolmonoethoxylate (total)	OP1EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-*tert*-Octylphenoldiethoxylate (total)	OP2EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-*tert*-Octylphenoltriethoxylate (total)	OP3EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-*tert*-Octylphenoltetraethoxylate (total)	OP4EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
4-*tert*-Octylphenolpentaethoxylate (total)	OP5EO	Surfactant metabolite	Jobling and Sumpter, 1993; Routledge and Sumpter, 1996.
Acetyl-hexamethyl-tetrahydronaphthalene	AHTN	Polycyclic musk fragrance	Schreurs and others, 2005; Yamauchi and others, 2008.
Bisphenol A	BPA	In polycarbonate resins	Bonefeld-Jørgensen and others, 2007; Brian and others, 2005; Schultz and others, 2000; Terasaki and others, 2005.
Hexahydrohexamethyl-cyclopenta-benzopyran	HHCB	Polycyclic musk fragrance	Schreurs and others, 2005; Yamauchi and others, 2008.

estimated concentration), and hexahydrohexamethyl-cyclopenta-benzopryan (HHCB; 1 detection at an estimated concentration). Many of the detections in blank samples occurred during one study in 2005 indicating sources of contamination in the laboratory or in the field during that study. Among all the studies, 15 replicate samples were collected for analyses of water and one replicate sample was collected for analyses of bed sediment. In general, the analytical results of the replicate samples agreed well (mean relative percent difference of 21 percent) with the results of the paired environmental samples.

Fish were collected from streams and rivers using electrofishing techniques (Moulton and others, 2002). Fish were processed immediately after collection (Goodbred and others, 1997; Lee and others, 2000; Lee, Yaeger, and others, 2008; Lee, Schoenfuss, and others, 2008). Fathead minnows used for laboratory and caged fish studies were obtained from Environmental Consulting and Testing Laboratory (Superior, Wisc.) and from the USEPA (Duluth, Minn.). Fish were exposed at the Aquatic Toxicology Laboratory at St. Cloud State University (SCSU; St. Cloud, Minn.). Fish plasma and tissues were analyzed at St. Cloud State University and the University of Florida according to established protocols (Lee and others, 2000; Bistodeau and others, 2006; Barber and others, 2007; Schoenfuss and others, 2008) and guidelines established by the USEPA (U.S. Environmental Protection Agency, 2006).

Stream flow measurement at the South Fork Crow River at Highway 22 near Biscay, Minn. (USGS station number 05337005, photograph taken by John Greene (USGS) February 28, 2007).

Endocrine Active Chemicals and Endocrine Disruption

The data summarized in this report provide information on the distribution and temporal variability of EACs in Minnesota WWTP effluent, streams, lakes, and bed sediment. Information on biological responses, fate, and transport of EACs; endocrine disruption responses in wild-caught and caged fish in Minnesota streams; and the results of controlled laboratory studies to define endocrine disruption responses also are provided.

Distribution of Endocrine Active Chemicals in Wastewater-Treatment Plant Effluent

EACs can enter the environment through many pathways including WWTP effluent. EACs are not completely removed by wastewater-treatment systems (Richardson and Bowron, 1985; Stumpf and others, 1996; Ternes, 1998), resulting in potentially continuous sources of EACs to surface water. More than 400 WWTPs discharge effluent to surface waters throughout Minnesota (Minnesota Pollution Control Agency, oral and written commun., 2008; fig. 4). Most of the WWTPs have periodic releases (generally twice per year during the spring and fall) of less than 1 million gallons per day (Mgal/d). Approximately 60 WWTPs with average design flows greater than 1 Mgal/d discharge continually to receiving streams (Minnesota Pollution Control Agency, oral and written commun., July 14, 2006).

Twelve WWTPs that discharge to Minnesota waters were sampled from 1997 to 2008 for several studies (Barber and others, 2000, 2007; Lee and others, 2004; Lee, Schoenfuss, and others, 2008; Ferrey and others, 2009; Abigail Tomasek, U.S. Geological Survey, written commmun., 2009). Not all EACs were measured at each site because of differences in study objectives, so sample sizes were not equal. The 12 WWTPs discharging to Minnesota streams that were sampled were located in the Minnesota cities of Shakopee, Rochester, Hinckley, East Grand Forks, Duluth, Taylors Falls, St. Paul, Hutchinson, Eagan, Marshall, and Two Harbors, and the Wisconsin city of St. Croix Falls (sites 136W–147W) from 1997 through 2008 (fig. 4, appendix 3).

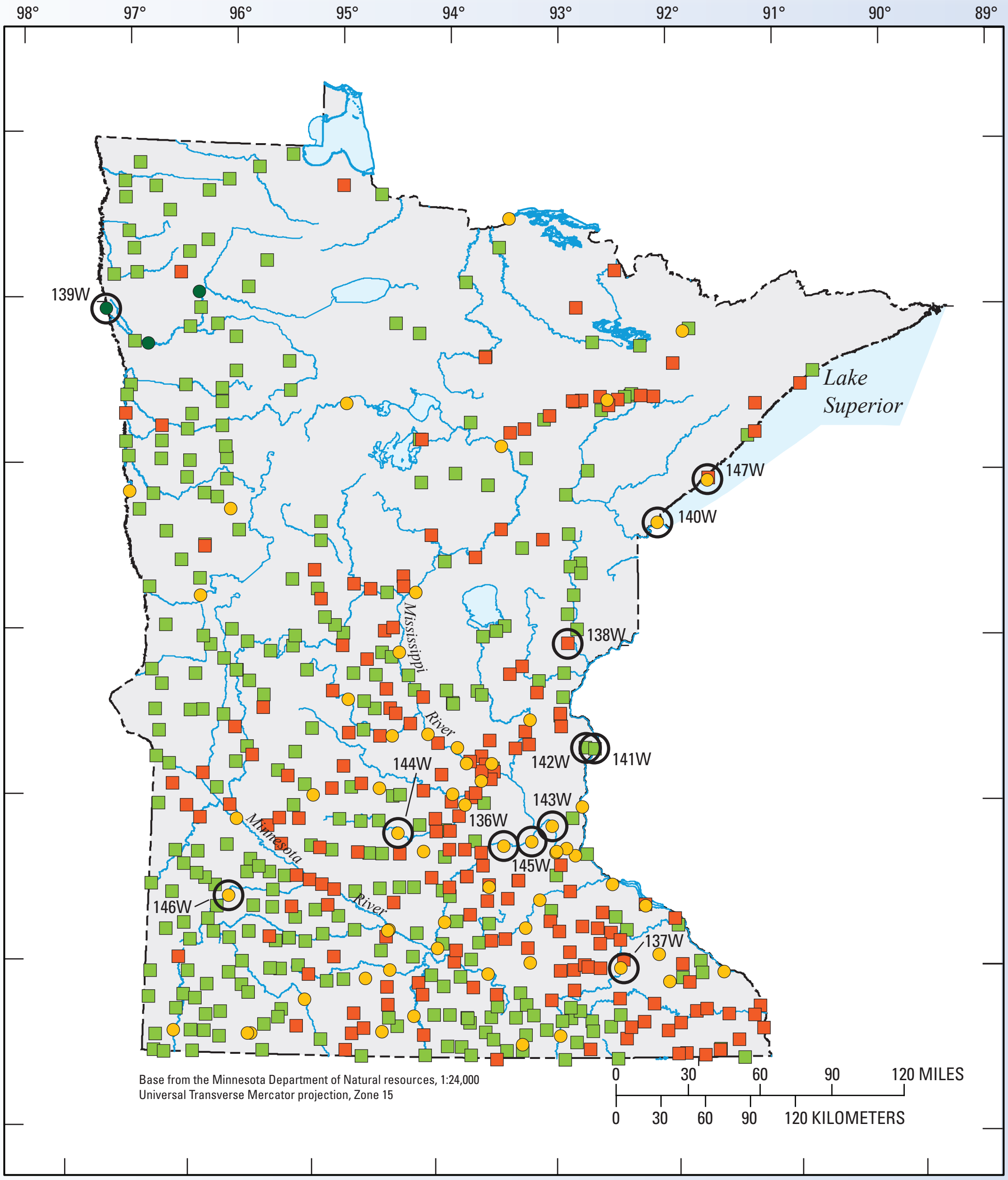
WWTP effluent is a complex mixture of multiple EACs and other organic and inorganic contaminants. On average, six EACs were detected per WWTP effluent sample, although this average may be low because the full suite of EACs was not analyzed for samples from each site. The chemicals 4-nonylphenoldiethoxycarboxylate (NP2EC), hexahydrohexamethyl-cyclopenta-benzopyran (HHCB), acetyl-hexamethyl-tetrahydronaphthalene (AHTN), 4-nonylphenolmonoethoxycarboxylate (NP1EC), and 4-nonylphenol (NP) were the five most frequently detected EACs in WWTP effluent samples (fig. 5). EAC concentrations varied among WWTPs (Barber and others, 2000, 2007; Lee and others, 2004). Most of the EACs that were measured occurred in more than 30 percent of the samples with detectable concentrations ranging from 0.003 to 183.7 micrograms per liter (µg/L). NP1EC and NP2EC had the greatest average concentrations of 41.8 and 54.3 µg/L, respectively. Concentrations of EACs for the WWTP effluents summarized in this study are similar to concentrations reported by Glassmeyer and others (2005) for effluent samples from 10 WWTPs across the United States.

Concentrations of EACs varied among WWTPs. For example, NP was detected in the treated effluent from 10 of the 12 WWTPs sampled at concentrations that varied by more than one order of magnitude, from 0.1 to 18.2 µg/L among all samples (fig. 6). In most of the WWTP effluent sampled, concentrations of EACs also varied with time. For example, detectable concentrations of NP at site 143W ranged from 0.19 to 1.9 µg/L during 2000–2002. Differences in the types and concentrations of EACs among WWTP effluent samples and among sampling periods at each WWTP are likely due to influent differences that each WWTP receives or processing technique differences.

The 12 WWTPs sampled for the various studies summarized in this report represent a small fraction of the WWTPs in Minnesota, and thus may not be representative of all WWTPs in Minnesota. The number of samples per WWTP generally was low with the exception of a few WWTPs (fig. 6).

Distribution of Endocrine Active Chemicals in Minnesota Stream Water

EACs were detected in water samples collected during 1997–2008 from streams throughout Minnesota. EACs were detected in streams (fig. 7) draining different land uses, with different drainage areas, and with different contributions of point sources such as WWTP effluent discharge (Barber and others, 2000; Lee and others, 2004; Lee, Schoenfuss, and



EXPLANATION

Wastewater-treatment plant with continuous flow (from the Minnesota Pollution Control Agency, oral and written commun., 2008)

- Greater than 1 million gallons per day
- Less than 1 million gallons per day

Wastewater-treatment plant with periodic or seasonal flow (from the Minnesota Pollution Control Agency, oral and written commun., 2008)

- Greater than 1 million gallons per day
- Less than 1 million gallons per day

137W Effluent sample collected—Number is sampling site identifier

Figure 4. Location of wastewater-treatment plants that discharge to surface waters in Minnesota (modified from Lee, Schoenfuss, and others, 2008).

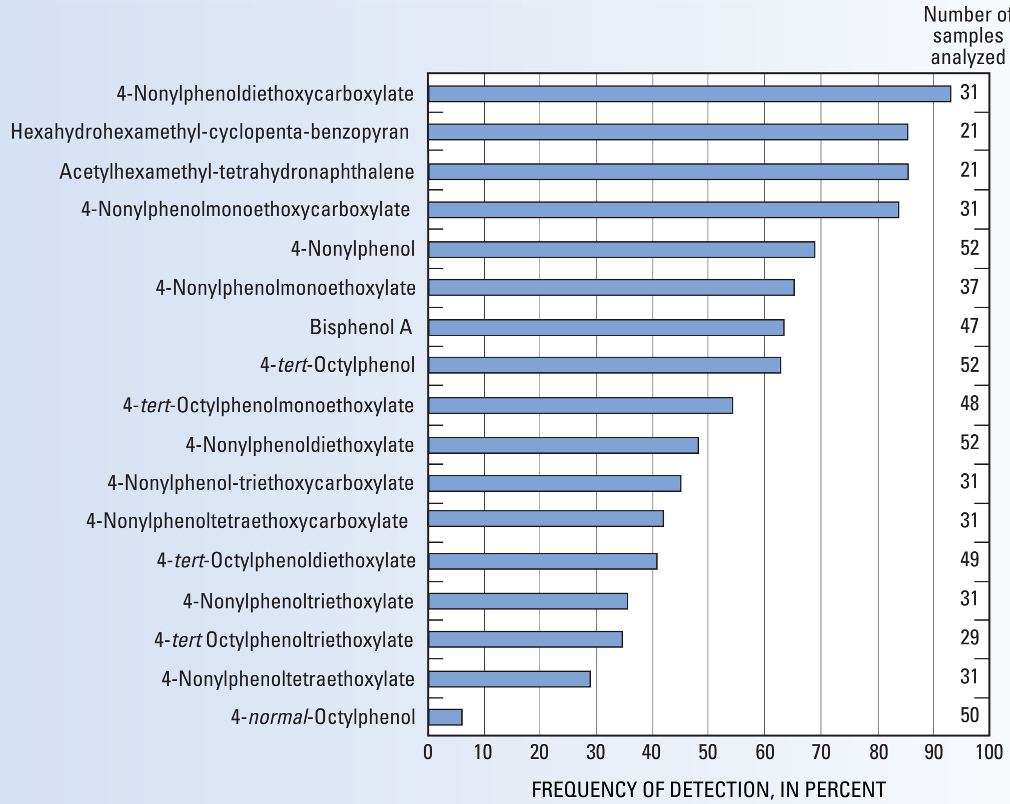


Figure 5. Detection frequencies of endocrine active chemicals among all effluent samples collected during 1997–2008 from 12 wastewater-treatment plants that discharge to surface waters in Minnesota.

others, 2008; Lee, Yaeger, and others, 2008; Ferrey and others, 2009; U.S. Geological Survey, 2009).

A wide variety of EACs were detected in Minnesota stream samples. The most frequently detected EACs (fig. 8) among all stream samples were an alkylphenol (NP), a plastic component (bisphenol A; BPA), and two synthetic musk fragrances (AHTN and HHCB). Streams contained unique mixtures of EACs (0–11 detected per sample). More than 80 percent of the detected EAC concentrations were less than 1 µg/L. Some concentrations of NP, 4-nonylphenoldiethoxylate (NP2EO), NP1EC, and NP2EC were greater than 1 µg/L (appendix 3).

The detection frequencies and median concentrations of BPA, NP, 4-nonylphenolmonoethoxylate (NP1EO), NP2EO, 4-*tert*-octylphenolmonoethoxylate (OP1EO), and 4-*tert*-octylphenoldiethoxylate (OP2EO) found in Minnesota streams summarized in this report were less than those reported by Kolpin and others (2002) in a study of 139 streams throughout the United States. This pattern may be due to differences in the types of sites sampled because Kolpin and others (2002) sampled more streams that were considered susceptible to point and nonpoint source contamination.

In general, more EACs were detected in water at sites directly downstream from WWTPs than at sites with no obvious source of effluent, confirming that WWTP effluent is a source of EACs to surface waters (Lee and others, 2004; Lee, Yaeger, and others, 2008; Lee, Schoenfuss, and others, 2008). EACs were detected in water samples collected from

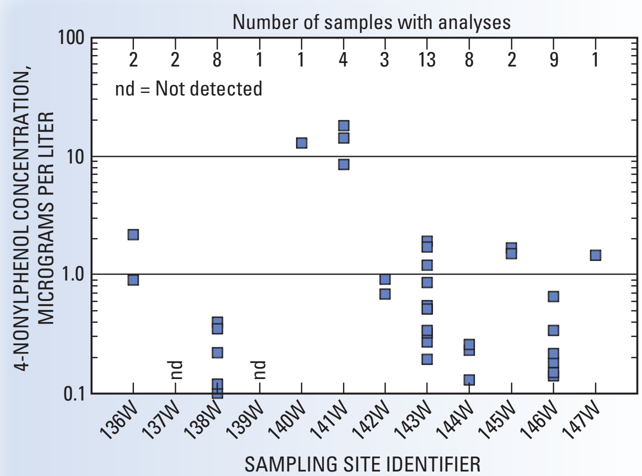
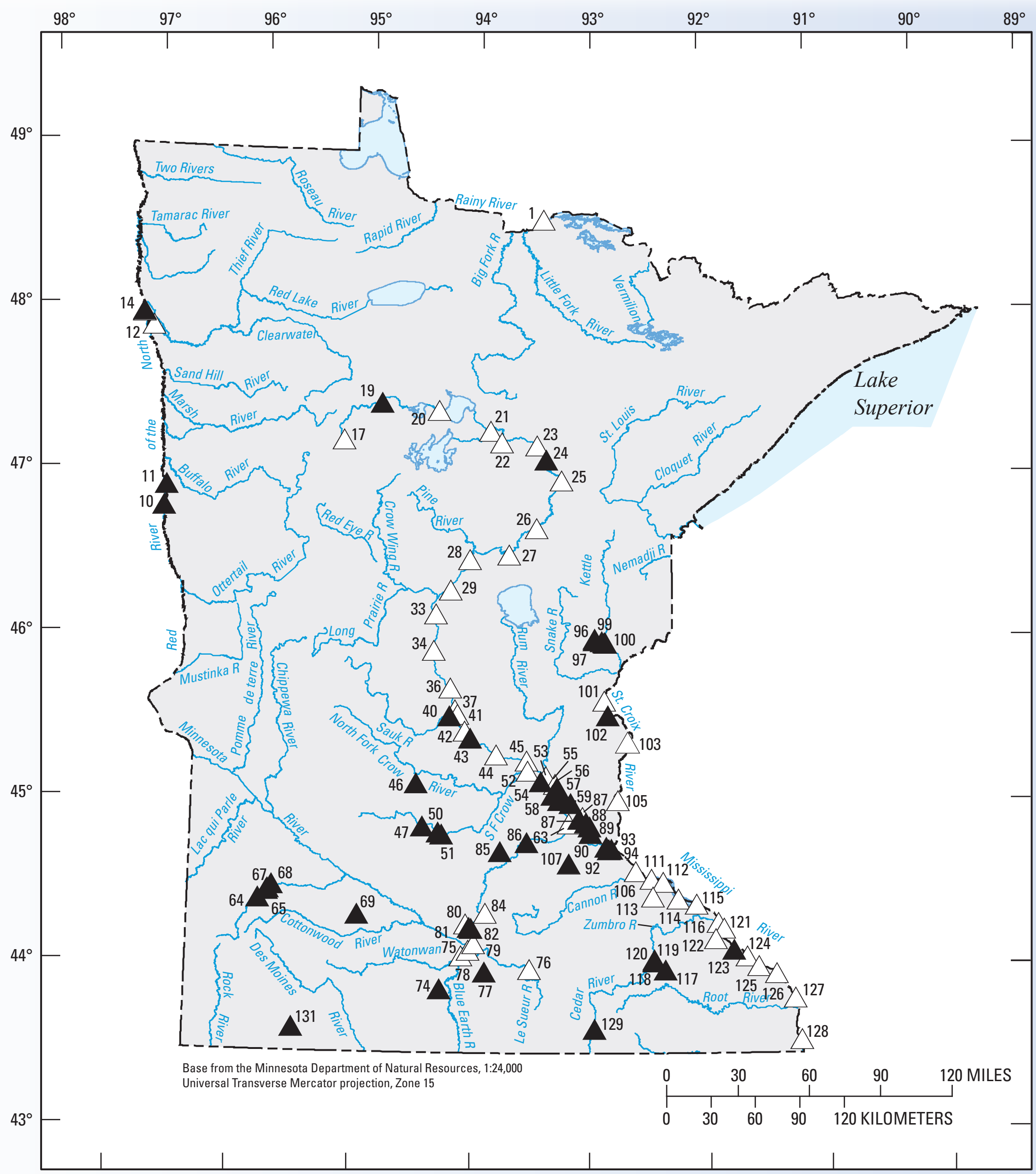


Figure 6. Concentrations of 4-nonylphenol in wastewater-treatment plant effluent samples collected during 1997–2008 from 12 facilities that discharge to Minnesota surface waters.



EXPLANATION

76 △ **Endocrine active chemicals analyzed in streams and not detected**—Number is sampling site identifier shown in appendixes 2 and 3

131 ▲ **Endocrine active chemicals analyzed in streams and detected**—Number is sampling site identifier shown in appendixes 2 and 3

Figure 7. Locations where endocrine active chemicals were analyzed in stream water samples, 1997–2008.

small streams more commonly than in larger rivers, potentially because EAC concentrations in larger rivers were diluted below detection limits (Lee and others, 2004). The presence of EACs in streams with no wastewater source indicates that there are EAC sources other than WWTP effluent. Alternative sources may include runoff from land surfaces, atmospheric deposition, or inflow from contaminated groundwater into streams, such as from on-site septic systems.

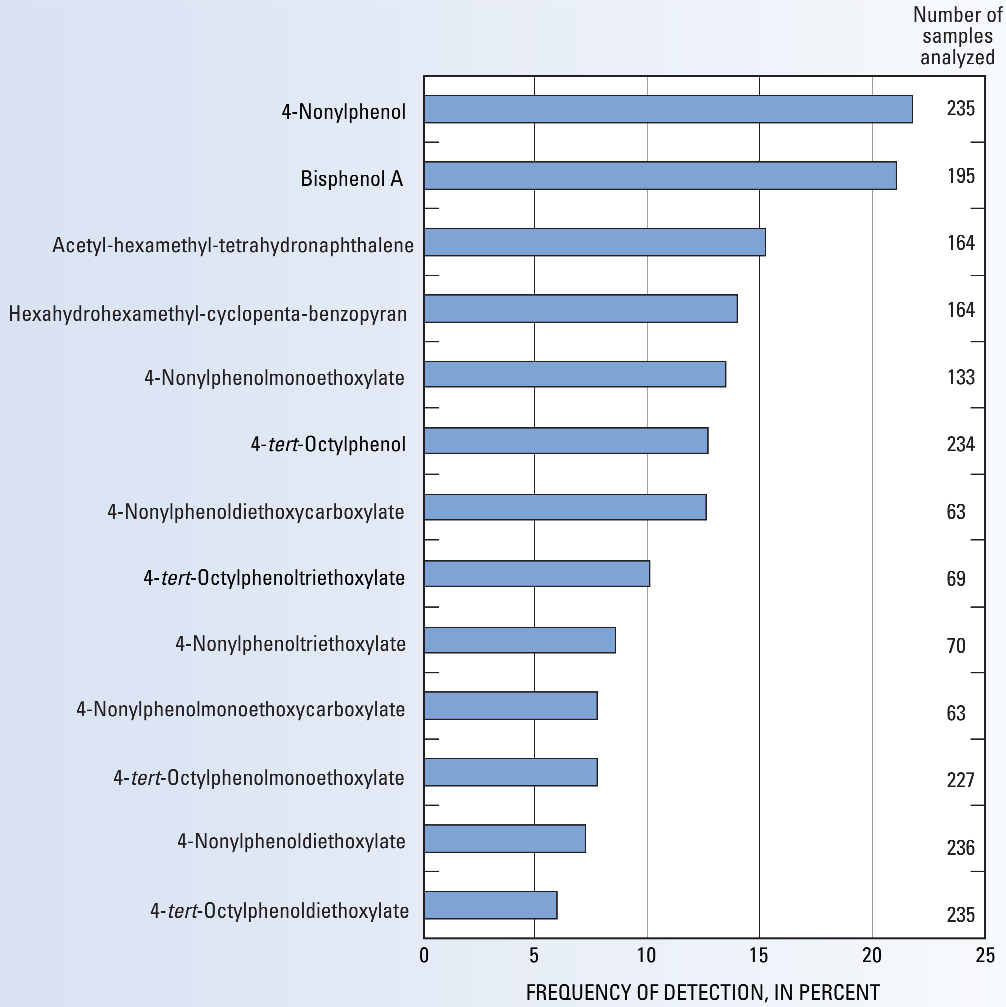


Figure 8. Frequency of detection of endocrine active chemicals among all samples collected from streams in Minnesota, 1997–2008.

Temporal Variability of Endocrine Active Chemicals in Streams

The types and numbers of EACs detected varied temporally at sites sampled more than once. EACs were measured in samples from 12 streams (sites 10, 11, 12, 14, 37, 40, 43, 52, 58, 78, 79, 93; fig. 3*A*) and 1 lake sample (site 60L; fig 3*A*) 2 to 4 times during 2000 and 2001 to determine the occurrence and temporal variability of a broad suite of chemicals including selected EACs associated with agricultural, industrial, and household use (Lee and others, 2004). Most of these sites have drainage areas greater than 1,000 square miles (mi^2) and drain agricultural land. Generally, few EACs were detected (0 to 3 per sample) at these study sites; however, 50 percent of the sites had a detection of at least one EAC during the sampling period at low concentrations (less than 1 µg/L). The type of chemicals detected varied among sampling periods. For example, BPA, OP1EO, and NP were detected during the fall sample; BPA, NP, and HHCB were detected in the summer sample; and no EACs were detected in the spring sample at the Mississippi River near Hastings, Minn. (site 93).

One stream location, the Mississippi River below I-694 at Fridley, Minn. (site 57), was sampled for 9 selected chemicals during 2004–06 (appendix 3). The number and types of EACs detected in stream samples collected at site 57 varied from 0 to 5 during the sampling period (fig. 9). The number of chemicals detected at site 57 did not correspond directly to streamflow or season. The chemicals NP, AHTN, and OP1EO were the most frequently detected EACs (detected in 29, 19, and 18 percent of the water samples, respectively) among all sampling periods. The mixture of EACs detected was not consistent among sampling periods potentially because of variations in upstream sources, in-stream degradation processes, and potential dilution to concentrations less than detection limits.

A study of three streams (South Fork Crow, Redwood, and Grindstone Rivers) that receive wastewater in Minnesota was conducted to identify temporal patterns of EACs (Lee, Schoenfuss, and others, 2008). Water samples were collected six times upstream from and at two successive points

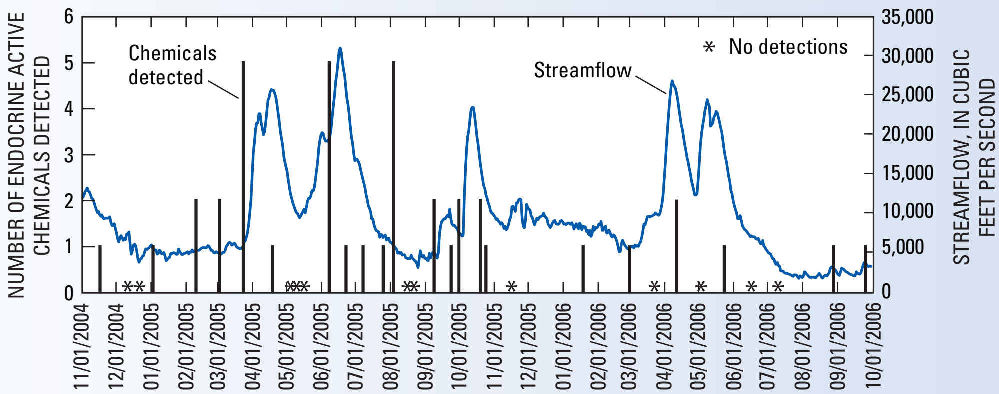


Figure 9. Endocrine active chemicals detected in samples collected from the Mississippi River below I-694 at Fridley, Minn., 2004–06.

downstream from discharge of WWTP effluent and from treated effluent from February through September 2007 (fig. 10). The number of EACs detected in these smaller streams (using the USGS National Research Laboratory data) ranged from 0 to 11 per site (Lee, Schoenfuss, and others, 2008) (sites 47, 50, 51, 64, 67, 68, 96, 97, 99 and 100; appendix 3). Similar to other streams, the types and concentrations of EACs detected varied temporally. For example, concentrations of NP at a given site varied by as much as four times during the sampling period (fig. 11). A noticeable seasonal pattern in the number of EACs detected at sites along the South Fork of the Crow River (sites 47, 50, and 51) and the Redwood River (sites 64, 67, and 68) was observed. More EACs were detected in samples collected during the winter or early spring under ice conditions than during the summer and fall at the upstream and first downstream site. This pattern indicates that aquatic organisms are exposed to variable concentrations and chemical mixtures throughout the year.

Fate and Transport of Endocrine Active Chemicals in Aquatic Environments

The fate and transport of EACs also was investigated at the South Fork Crow, Redwood, and Grindstone Rivers (Lee, Schoenfuss, and others, 2008). The expectation was that EACs would be found more frequently and at higher concentrations at sites downstream from WWTP discharge than at upstream sites and that concentrations and detections would decrease as a function of distance downstream from WWTP

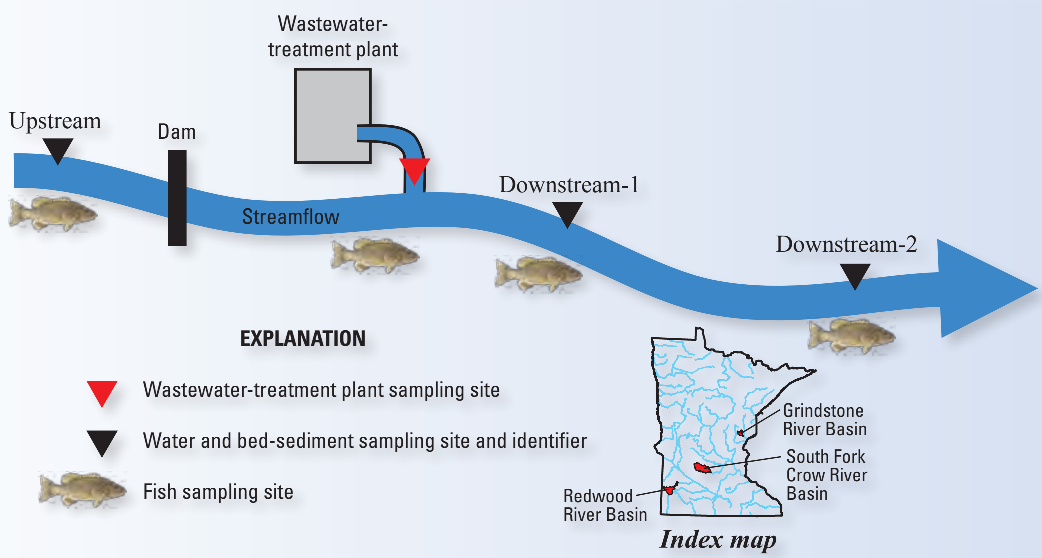


Figure 10. Relative locations of sampling sites upstream and downstream from discharge of wastewater-treatment plant effluent along three Minnesota streams (modified from Lee, Schoenfuss, and others, 2008).

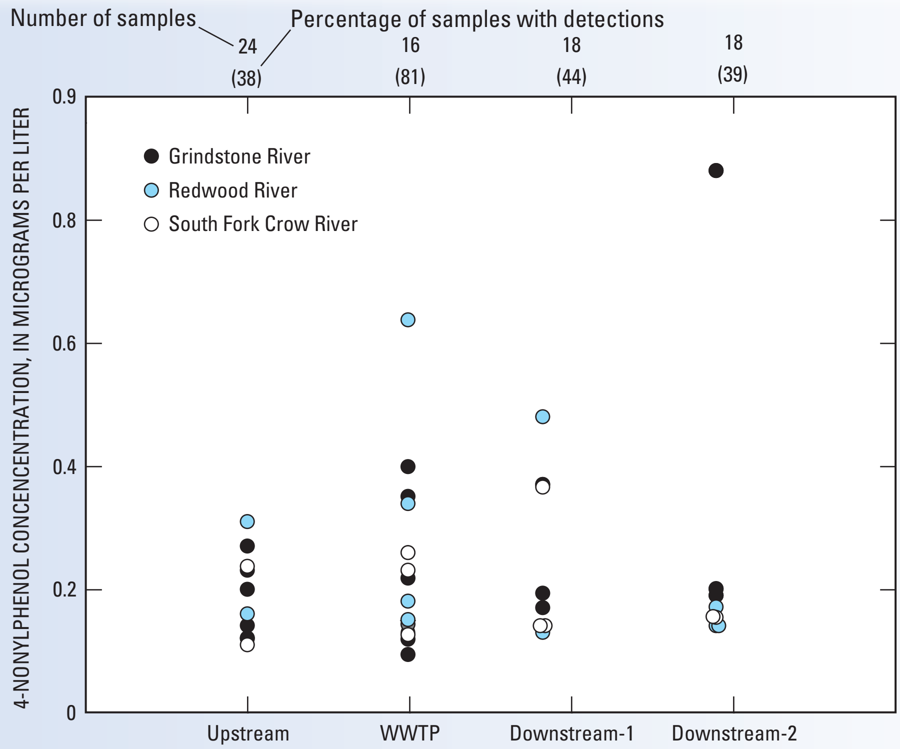


Figure 11. Concentrations of 4-nonylphenol in stream and wastewater-treatment plant (WWTP) effluent samples collected from three stream systems in 2007.

discharge locations. In general, that pattern was observed for most chemicals such as NP (fig. 11). Interestingly, some EACs persisted for more than 6 miles (10 kilometers) downstream. Unexpectedly, EACs also were detected at upstream sites on each river, indicating upstream sources potentially including effluent from other upstream WWTPs, onsite-septic system effluent, runoff from land surfaces, or groundwater influent. These results indicate that aquatic organism exposure occurs beyond the point of WWTP discharge.

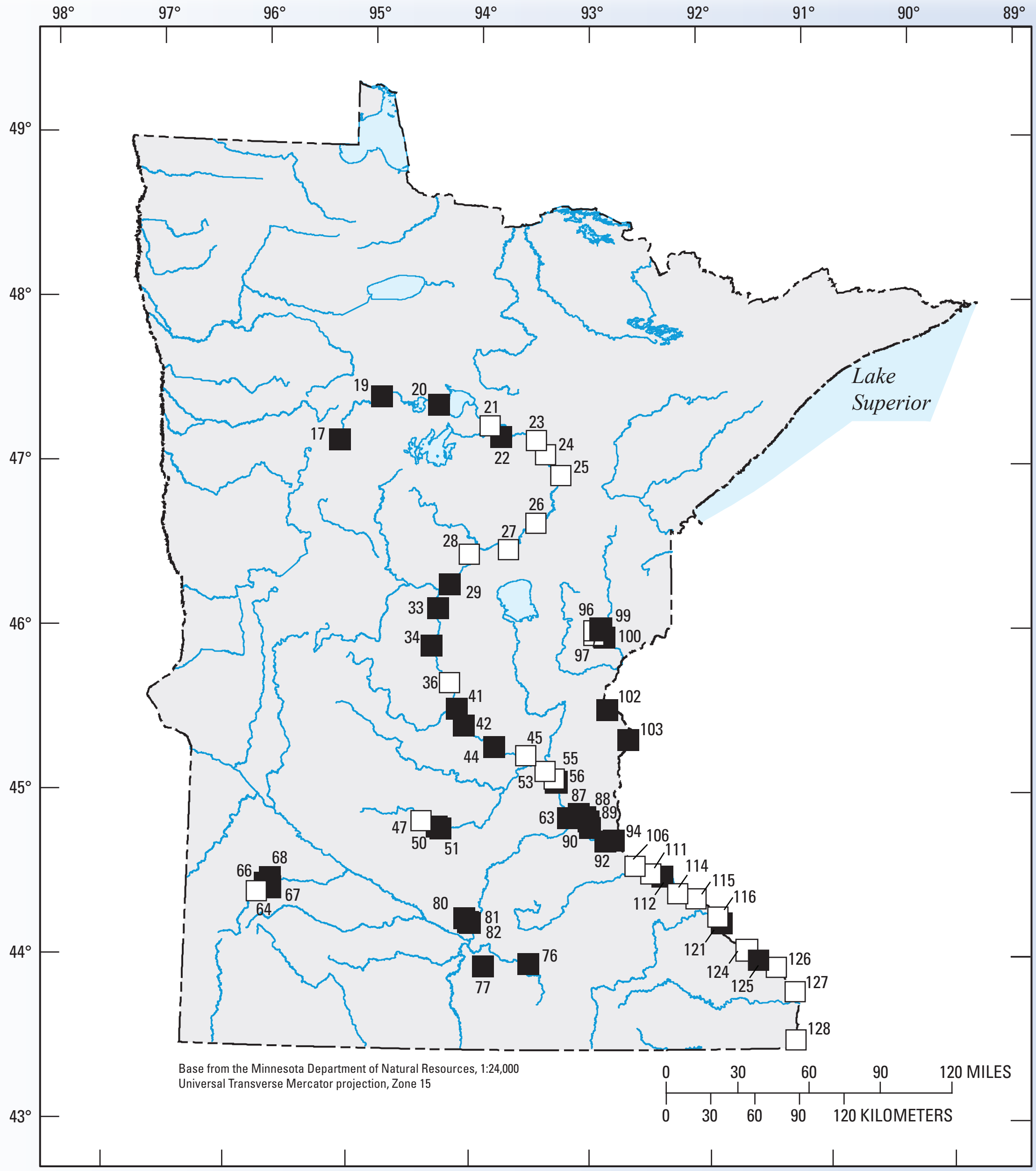
Distribution of Endocrine Active Chemicals in Minnesota Stream Bed Sediments

EACs were detected in greater than 50 percent of the bed-sediment samples collected from streams throughout Minnesota (appendix 4, fig. 12), indicating that bed sediment is a storage location of EACs in stream ecosystems. In general, a mixture of EACs was detected at each site. The most frequently detected chemicals in bed sediments were NP, NP1EO, and BPA (detected in 24, 24, and 17 percent, respectively, of the stream bed-sediment samples collected) (fig. 13). EAC concentrations in bed sediment ranged from 1.21 to 2,024 nanograms per gram (ng/g) (Lee, Yaeger, and others, 2008; Lee, Schoenfuss, and others, 2008).

Most of the bed-sediment samples were collected from the main stem of the Mississippi River (fig. 12; appendix 4). EACs were detected in bed sediments throughout the Mississippi River reach from the headwaters of the Mississippi River (site 17) near the outlet of Lake Itasca and upstream from any wastewater discharge to Brownsville Minn. (site 128), near the Minnesota and Iowa border. Although EACs were detected in bed sediments along the entire reach of the Mississippi River, more EACs were detected near Bemidji, Minn. (site 19) and along the river from Brainerd (site 29) through site 92 in the Twin Cities Metropolitan Area than in other locations. The presence of EACs in bed sediments along the entire river reach reflects the multiple point- and nonpoint-source discharges of EACs that exist along the river. EAC presence from Brainerd downstream (sites 29–92) generally coincides with human population density increases and a change from forested to agricultural and urban land use along the river.

Bed-sediment samples also were collected upstream and downstream from WWTP effluent discharge on three small streams in Minnesota: the South Fork of the Crow, Redwood, and Grindstone Rivers (Lee, Schoenfuss, and others, 2008). EACs were detected in bed sediment only at sites downstream from effluent discharge on these small streams.

Results indicate that EACs partition onto or accumulate in bed sediment. Bed sediments serve as a storage location of EACs that potentially provide a continual source of EACs to aquatic organisms that live in proximity to bed sediments or depend on food from bed sediments. EACs may be transported during high-flow events when bed sediment is suspended, resulting in redistribution of EACs in the aquatic environment.



EXPLANATION

128 ☐ **Endocrine active chemicals analyzed in bed sediment and not detected**—Number is sampling site identifier shown in appendixes 2 and 4

77 ■ **Endocrine active chemicals analyzed in bed sediment and detected**—Number is sampling site identifier shown in appendixes 2 and 4

Figure 12. Locations where endocrine active chemicals were analyzed in stream bed-sediment samples, 1997–2008.

Endocrine Disruption Responses in Wild-Caught Fish in Minnesota Streams

Male and female fish of 11 different species were collected from 86 stream sites to characterize endocrine disruption in Minnesota surface waters during 1994 to 2008 (Goodbred and others, 1997; Lee and others, 2000; Lee and Blazer, 2005; Lee, Yaeger, and others, 2008; Lee, Schoenfuss, and others, 2008; Ferrey and others, 2009). Several indicators of endocrine disruption were used among all studies (table 1). A compilation of data from all studies for vitellogenin and oocyte presence in male fish testes is shown in

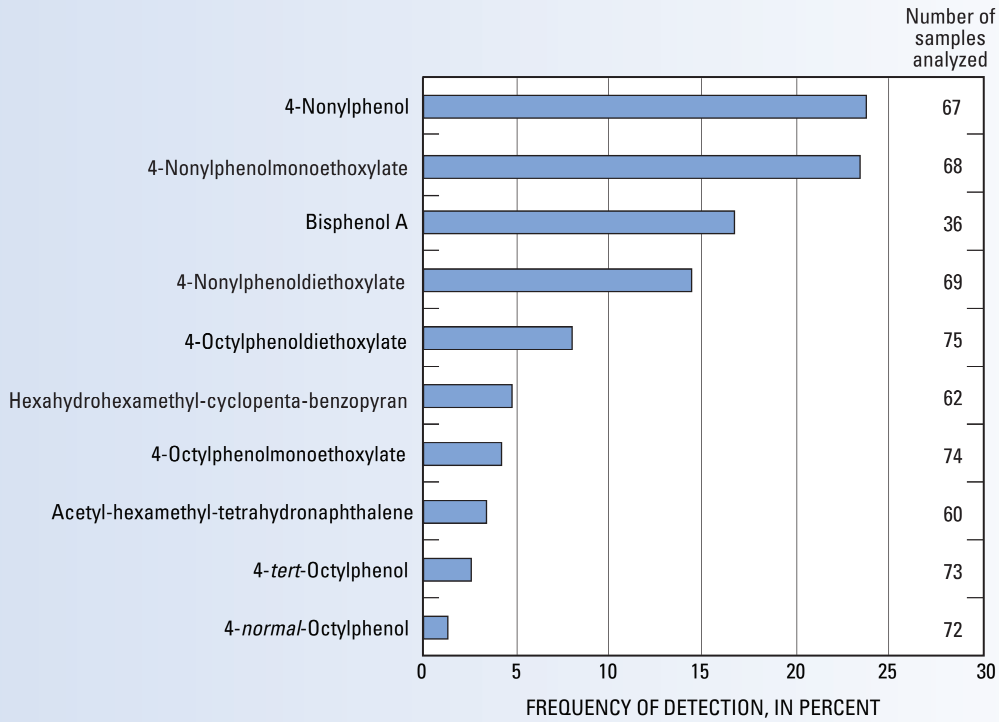


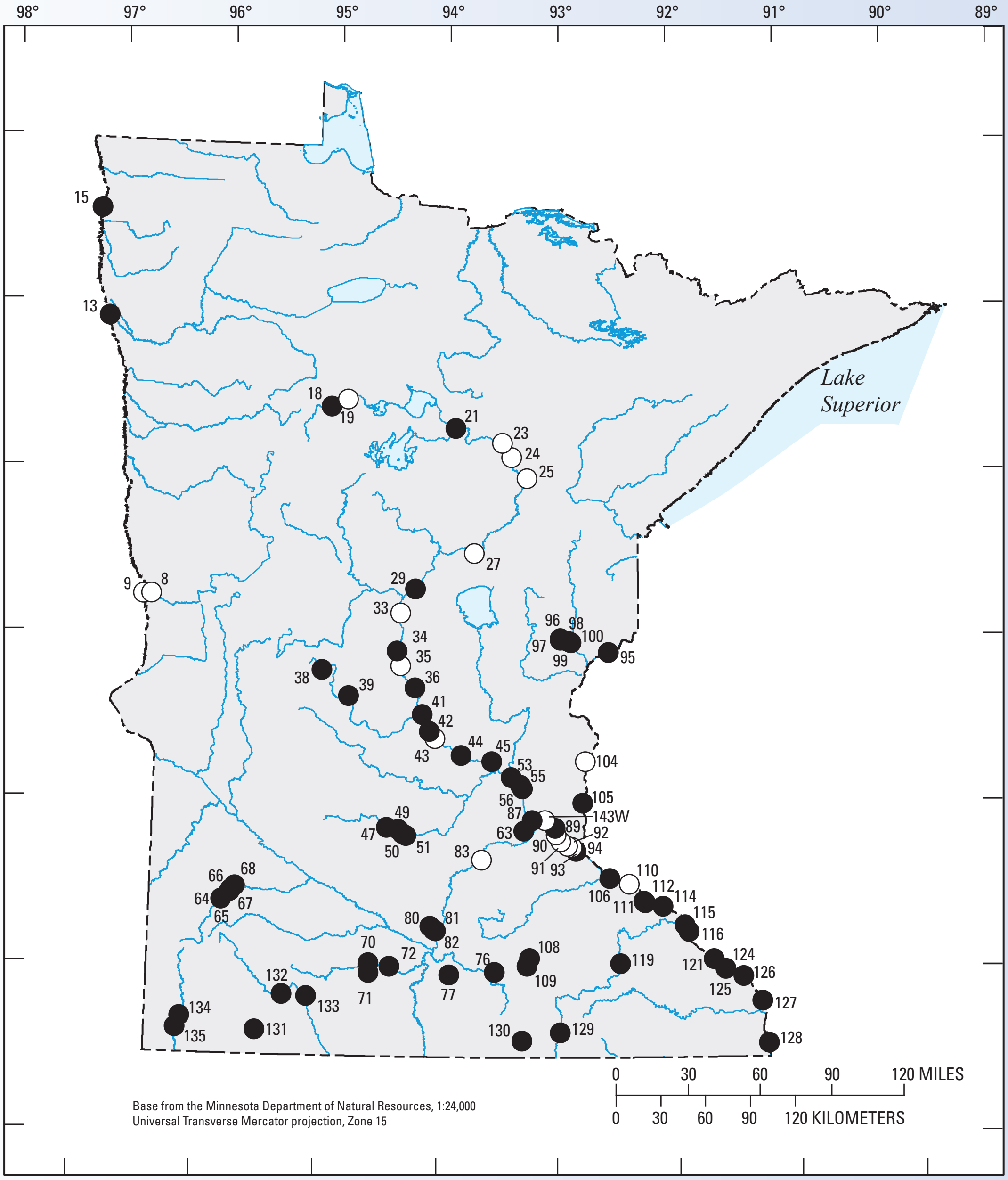
Figure 13. Frequency of detection of EACs among all bed-sediment samples collected from streams in Minnesota, 1997–2008.

figures 14 and 15, and the data are included in appendix 5. Sites with greater than 25 percent of the male fish of one species collected during one sample event with the presence of vitellogenin or oocytes were considered to have the presence of endocrine disruption.

Results indicate that EACs in streams are interacting with the endocrine systems of native fish in diverse environmental settings, from small streams draining agricultural land use to large rivers, such as the Mississippi River, draining mixed land uses. Vitellogenin was present in the plasma of at least one species of male fish during one sampling period at more than 40 percent of sampled sites and occurred in streams of various sizes and environmental settings (fig. 14). Oocytes were present in at least one species of male fish during one sampling period at 10 sites throughout Minnesota ranging from small streams to large rivers (fig. 15). The presence of oocytes in male testes tissue varied temporally. Oocytes were found in the testes of at least 25 percent of the smallmouth bass (*Micropterus dolomieu*) in all six sites (29, 34, 43, 55, 110, 114) measured along the Mississippi River during 1998, but were not detected in male smallmouth bass at corresponding sites (29, 34, 55, and 114) that were measured during a subsequent study along the Mississippi River in 2006. Differences in fish responses at sites over time could indicate differences in EAC presence and fish exposure or differences in fish sensitivity.

Several additional focused studies helped identify potential sources of EACs to streams. The USGS, in cooperation with the Minnesota Pollution Control Agency (MPCA) and the Minnesota Department of Natural Resources, conducted a study between August 3 and September 13, 1999, to investigate the presence of vitellogenin and other indicators of endocrine disruption in common carp (*Cyprinus carpio*) exposed to WWTP effluent and runoff from agricultural and forested land (Lee and others, 2000). The study was a paired site approach targeting sites upstream and downstream from discharges of WWTP effluent with a dam in between to prevent fish migration (fig. 16). Paired upstream/downstream sites were selected on seven different streams. Fish were collected at an additional eight sites located downstream from discharge of WWTP effluent with no paired upstream site due to the absence of fish.

Several biological indicators of endocrine disruption were detected in male fish upstream and downstream from discharge of WWTP effluent (Lee and others, 2000). The number of biological indicators of endocrine disruption present and the values for a particular indicator varied considerably among sites, because of differences in EAC presence, fish sensitivity, or fish exposure time. The site with the greatest number of indicators of endocrine disruption was in a WWTP effluent channel (site 143W) with 100 percent effluent; however, for the remaining 21 sites, the percentage of streamflow composed of WWTP effluent did not correlate well with the number of indicators of endocrine disruption present. This indicates that, although the percentage of streamflow composed of effluent is important, other factors, such as the composition of

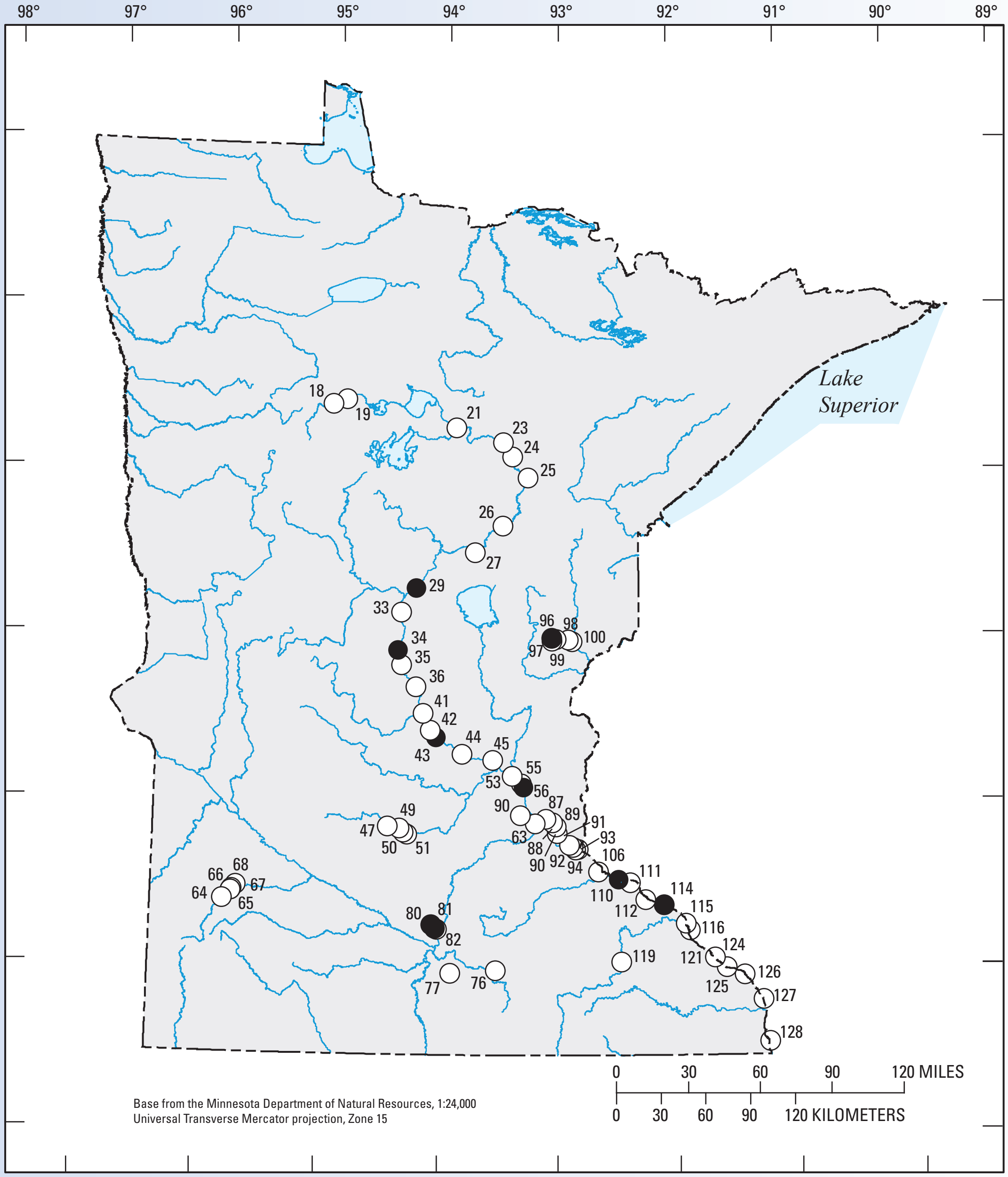


EXPLANATION

128 ● **Location where 25 percent or more of at least one species of male fish during one sampling period had plasma vitellogenin detections**—Number is sampling site identifier shown in appendixes 2 and 5

83 ○ **Location where less than 25 percent of at least one species of male fish during one sampling period had plasma vitellogenin detections**—Number is sampling site identifier shown in appendixes 2 and 5

Figure 14. Stream locations where vitellogenin was present in male fish during 1994–2008.



EXPLANATION

112 ● **Location where 25 percent or more of at least one species of male fish during one sampling period had oocytes in testes tissue**—Number is sampling site identifier shown in appendixes 2 and 5

128 ○ **Location where less than 25 percent of at least one species of fish during one sampling period had oocytes in testes tissue**—Number is sampling site identifier shown in appendixes 2 and 5

Figure 15. Stream locations where oocytes were present in male fish testes during 1994–2008.

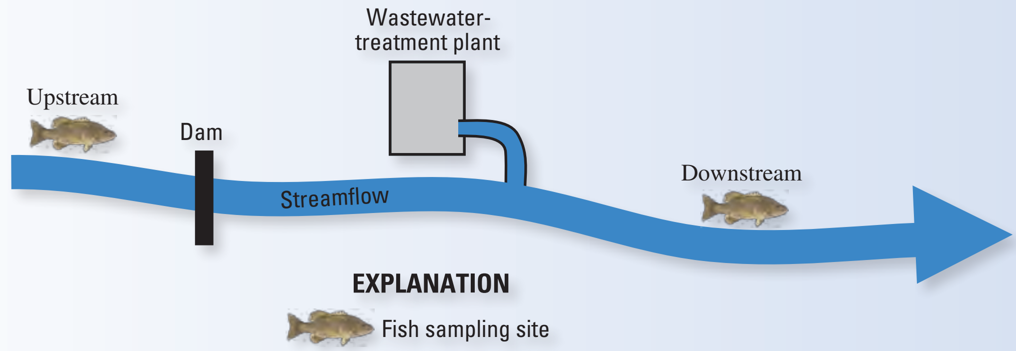


Figure 16. Relative sampling locations for carp study in 1999 (modified from Lee and others, 2000).

the effluent and organism exposure, may be more important factors controlling endocrine disruption. Neither of these other factors was measured in this study.

Because the dominant chemicals at upstream sites likely affect downstream sites, endocrine disruption measures were expected to be greater at downstream sites because of the combination of dominant upstream factors and WWTP effluent. Contrary to expectations, vitellogenin concentrations in male carp plasma were greater at some of the upstream sites draining primarily agricultural land than at the paired site on the same stream downstream from discharge of WWTP effluent (fig. 17). These results indicate that WWTP effluent is not the only source of EACs to streams, and that the unknown sources could cause a greater biological response than the effluent in some cases. Potential sources of EACs at upstream sites include unknown wastewater discharges, onsite-septic system effluent, runoff from the agricultural landscape, or influent from groundwater.

The USGS, in cooperation with SCSU and MPCA, conducted a field study sampling water, bed sediment, and fish at 43 sites along the Mississippi River (Lee, Yaeger, and others, 2008). Results from this study indicate that the frequency of occurrence of endocrine disruption in multiple fish species along the Mississippi River was greatest from site 36 downstream to site 128 (fig. 14). This pattern coincided with greater human population density and a change from forested to agricultural and urban land use. The presence of endocrine disruption in fish did not directly coincide with the presence of EACs in water or bed sediment at the same site. This may result from the migration of fish throughout the river, differences in fish sensitivity to EACs, or the EACs that elicited a response were not measured.

Additional longitudinal studies of three small streams (Redwood River, South Fork of the Crow River, and the Grindstone River; Lee, Schoenfuss, and others, 2008) demonstrated that endocrine disruption occurred in wild fish downstream from discharge of WWTP effluent, indicating that effluent is one source of EACs to the aquatic environment. However, endocrine disruption, as indicated by vitellogenin induction in male fish plasma, was not limited to fish downstream from discharge of WWTP effluent on these three streams, but also was measured in fish at sites with no obvious wastewater sources.

The presence of vitellogenin or oocytes in male fish indicates that the fish are exposed to EACs. However, their presence in male fish does not indicate an effect on fish reproduction or changes in fish populations. The diffuse occurrence of endocrine disruption in wild fish indicates sources of EACs other than WWTP effluent to surface waters in Minnesota. It is difficult to define the specific chemicals that elicited endocrine disruption responses in field studies because fish exposure is largely unknown.

Distribution of Endocrine Active Chemicals in Water and Bed Sediments in Minnesota Lakes and Biological Responses

Fifteen lakes in Minnesota were investigated during October 2000 through August 2008 by the USGS in cooperation with SCSU, the MPCA, and the Minnesota Department of Health, to assess the presence and concentrations of a diverse group of organic chemicals (pharmaceuticals, pesticides, and EACs) commonly associated with wastewater contamination in water and bed sediment (Lee and others, 2004; Ferrey and others, 2009; Jeff Writer, U.S. Geological Survey, written commun., 2009). Water samples were collected from 14 lakes from 2000–08. Bed sediment and fish were sampled from a subset of the lakes during 2008. No EACs were detected in the water samples collected from the two lakes—Vadnais Lake (site 60L) and Ek Lake (site 3L)—sampled during 2000 and 2001 (fig. 18, appendix 3). Vadnais Lake is a water-supply lake for the City of St. Paul, Minn., and Ek lake is a remote lake in Voyageurs National Park. EACs were detected in the water samples from St. Louis River Bay of Lake Superior (site 7L) during 2001 (fig. 18).

Twelve lakes (sites 2L, 4L, 5L, 6L, 16L, 30L, 31L, 32L, 48L, 61L, 62L, and 73L) representing various trophic levels,

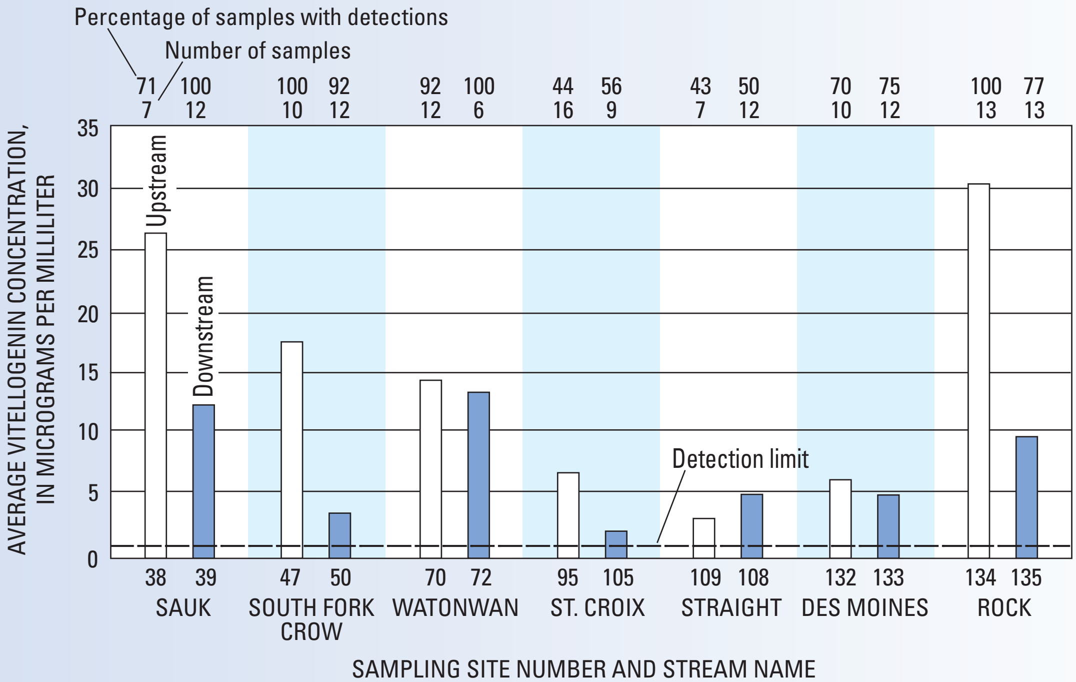


Figure 17. Graph showing average vitellogenin concentrations in male fish plasma at sites located upstream and downstream from discharge of wastewater-treatment plant effluent in 1999.

different land use and development, and different regions of Minnesota were sampled during 2008 (Ferrey and others, 2009; Jeff Writer, U.S. Geological Survey, written commun., 2009). Water samples were collected from 11 of the 12 lakes and bed sediments were collected from all 12 lakes. Wild-caught fish (fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis macrochirus*), common shiner (*Luxilus cornutus*), and yellow perch (*Perca flavescens*)) collected from these lakes and caged fathead minnows that were deployed at 11 of the 12 lakes for 3 weeks were examined for evidence of endocrine disruption. Measures of endocrine disruption in this study included induction of vitellogenin in male fish and the presence of oocytes in male fish testes.

A wide variety of EACs were detected in water samples among the 11 lakes sampled during 2008 (Ferrey and others, 2009; Jeff Writer, U.S. Geological Survey, written commun., 2009; appendix 3). The most frequently detected EACs in lake water samples were BPA (42 percent), NP1EO (25 percent), and OP2EO (25 percent). Ferrey and others (2009) also reported the presence of estrone and 17β-estradiol in 82 and 55 percent, respectively, of the lake water samples.

EACs were detected in the bed sediments of about 90 percent of the 12 lakes measured in 2008 (fig. 18) at concentrations ranging from 3.1 to 223.9 ng/g (appendix 4). For lake bed-sediment samples, the most frequently detected EACs were BPA (83 percent), 4-*tert*-octylphenol (TOP; 42 percent), and OP2EO (33 percent). Ferrey and others (2009) also reported the occurrence of two hormones—estrone (82 percent) and 17β-estradiol (55 percent)—in the bed-sediment samples collected.

EACs are present in lakes that lack obvious sources of contamination and in lakes with substantial residential development. Sources of EACs to lakes in this study are not known; however, the detection of these chemicals indicates that WWTP effluent is not the only source of EACs in surface waters because the lakes in this study are not affected by this source. Potential sources of EACs to lakes include onsite-septic system effluent, runoff from agricultural and urban land surfaces, and, for 17β-estradiol and estrone, excretion by vertebrates.

Lake in an urban setting. Minneapolis, Minn. in the background. Photograph courtesy of the Minneapolis Park and Recreation Board.

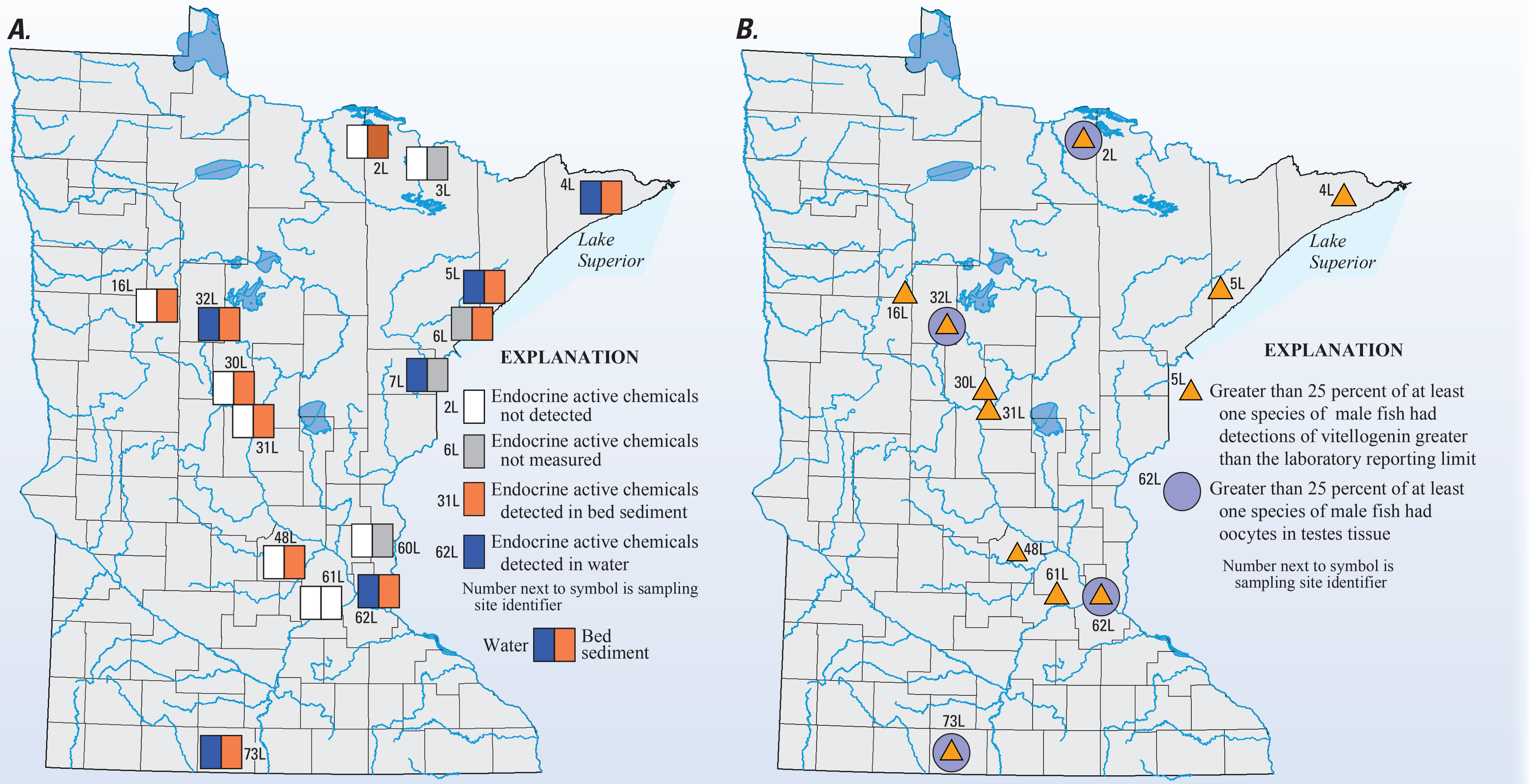


Figure 18. Occurrence of (*A*) endocrine active chemicals in water and bed-sediment samples collected during 2000–08, and (*B*) endocrine disruption of wild-caught or caged fish collected during 2008 from lake sites.

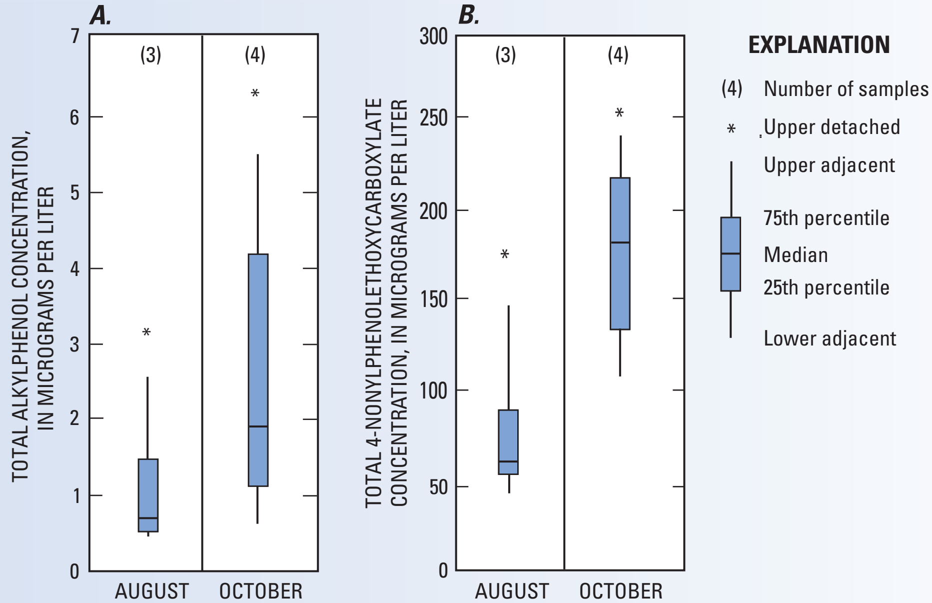


Figure 19. Concentrations of (*A*) total alkylphenols and (*B*) total 4-nonylphenolethoxycarboxylates in wastewater-treatment plant effluent collected in August and October 2002 (modified from Barber and others, 2007).

Biological responses in fish in the sampled lakes indicate exposure to EACs in the 11 lakes sampled (fig. 18*B*). Plasma vitellogenin concentrations in male fishes collected from the 11 Minnesota lakes varied considerably among sites. Vitellogenin induction was observed in at least 25 percent of the male fish of one species collected from the 11 lakes sampled, and ovatestes (oocytes in testes tissue) were observed in at least 25 percent of the male fish of one species collected at 4 of the 11 lakes. Similar to studies of Minnesota rivers (Folmar and others, 1996, 2001; Lee and others, 2004; Lee, Yaeger, and others, 2008; Lee, Schoenfuss, and others, 2008), the results from the lake studies indicate that low concentrations of EACs are present in Minnesota lakes regardless of region or land use, and wild-caught and caged fish show evidence of endocrine disruption in diverse aquatic environments in Minnesota.

Controlled Laboratory Studies to Define Endocrine Disruption Responses

To better understand EAC exposure and effects, additional controlled exposure studies were conducted collaboratively with SCSU. Onsite, continuous-flow experiments were conducted during August and October 2002, at a major metropolitan WWTP in Minnesota (site 143W) to determine if effluent exposure induced endocrine disruption in sexually mature male fathead minnows (Barber and others, 2007). Two individual sets of fish were exposed to WWTP effluent; one in August and one in October. Treated wastewater discharged from the WWTP was pumped continuously through aquariums for 28 days during both of those experiments. This design allowed the fish to be exposed to the normal day-to-day changes in the complex mixture of chemicals contained in wastewater. Parallel experiments were conducted at the Aquatic Toxicology Laboratory at SCSU exposing fish to groundwater for a control.

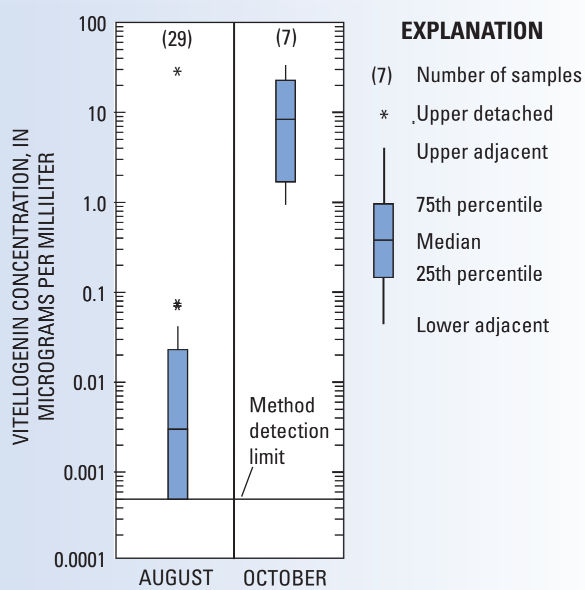


Figure 20. Vitellogenin concentrations in male fathead minnows exposed to wastewater-treatment plant effluent during August and October 2002 (modified from Barber and others, 2007).

Effluent composition varied temporally, and the continuous-flow experiments captured the range of chemical variability that occurred during normal WWTP operations. Wastewater contained several chemicals known to be endocrine active chemicals, such as 4-nonylphenolethoxycarboxylates (NP1EC, NP2EC, NP3EC), alkylphenols (NP, NP1EO, NP2EO, OP1EO), 17β-estradiol, and BPA (Barber and others, 2007). Concentrations of total alkylphenols, total 4-nonylphenolethoxycarboxylates (fig. 19), and likely other EACs such as 17β-estradiol, were greater in October than in August, reflecting a difference in effluent composition.

Exposure to WWTP effluent resulted in vitellogenin induction in male fathead minnows, with greater response in October than in August (fig. 20). In contrast to expectations, the gonado-somatic index (proportion of fish weight composed of testicular tissue) in males exposed to WWTP effluent was greater than in fish exposed to groundwater controls, possibly because of greater nutrient concentrations in wastewater that resulted in increased testicular growth (Barber and others, 2007).

This controlled exposure study highlights the potential effects of wastewater on wild fish. In some cases, beneficial effects (such as increased gonado-somatic index) were observed along with detrimental effects (such as male minnows producing vitellogenin). Although an endocrine disruption response was observed in the fish exposed to wastewater, determining the exact causative factors, or which chemicals within the mixture of chemicals in the effluent were responsible for the response, was difficult. This difficulty arose because of continual changes in the presence and concentrations of chemicals and nutrients in the wastewater and the corresponding changes in the multiple responses of the fish (Barber and others, 2007).

The USGS assisted SCSU in fathead minnow larvae exposure studies at SCSU's Aquatic Toxicology Laboratory (Bistodeau and others, 2006; Schoenfuss and others, 2008). In the first study, fathead minnow larvae were exposed for 64 days to a mixture of alkylphenols, which closely matched the alkylphenol concentrations and composition of the effluent at site 143W. Target exposure included total alkylphenol concentrations of 200, 100, and 50 percent of WWTP effluent concentrations at site 143W. The final exposure concentrations were 148, 73.9, and 38.1 µg/L respectively for the 200 percent, 100 percent, and 50 percent treatments. The stock solution was composed of eight chemicals (0.2 percent octylphenol, 2.8 percent NP, 5.1 percent NP1EO, 9.3 percent NP2EO, 0.9 percent OP1EO, 3.1 percent OP2EO, 33.8 percent NP1EC, 44.8 percent NP2EC).

Following exposure, larvae were raised to maturity in groundwater and allowed to compete with males that were not exposed to alkylphenols as larvae. Male fathead minnows normally display aggressive behavior against other males to defend a nest site for spawning. Most of the larvae died after 4-weeks of exposure to the 200-percent alkylphenol treatment (Bistodeau and others, 2006). There was a substantial decrease in the ability of many of the previously exposed males to defend and hold a nest site for both the 100-percent and 50-percent exposures (Bistodeau and others, 2006). These results indicate that the life stage when the exposure occurs is critical, as alkylphenol mixtures have an effect on the reproductive competence of male fathead minnows exposed as larvae.

In a follow-up experiment to further define the effects of NP, Schoenfuss and others (2008) examined the ability of NP to alter reproductive competence in male fathead minnows after a 28-day flow-through exposure in a range of environmentally relevant concentrations bracketing the USEPA toxicity-based NP chronic exposure criterion of 6.6 µg/L. Exposure to NP at concentrations equal to and greater than 6.6 µg/L resulted in an induction of plasma vitellogenin in male fish within 14 days. Schoenfuss and others (2008) reported that male fish exposed to lower concentrations of NP out-competed control males, and indicate that NP at the lower concentrations affected the males similar to pheromones released from female fathead minnows. At greater NP exposure concentrations, control males out-competed exposed males indicating that the effects of NP are dependent on concentration.

Results of these controlled studies confirm that WWTP effluent does result in endocrine disruption in male fathead minnows, that the life stage during exposure is critical, and that alkylphenols including NP are one group of chemicals that elicit an endocrine disruption response. Results of these controlled laboratory studies highlight the complexity of endocrine disrupting effects and the need for multiple analysis levels to assess the effects of these chemicals on aquatic resources.

Implications

The results of research and monitoring studies in Minnesota indicate that EACs in streams are interacting with the endocrine systems of native fish, and that multiple sources of EACs exist in addition to WWTP effluent. The presence of EACs in the water column and bed sediments indicate multiple pathways for aquatic organism exposure. The accumulation or presence of EACs in bed sediment results in a more

Fathead minnow (*Pimephales promelas*). Photograph by Konrad Schmidt.

permanent exposure for organisms that live in close contact with the sediments such as benthic insects and mussels. The exposure of aquatic organisms to EACs also is expected to be in constant flux based on the variability in EACs measured at sites sampled more than once. This variability in EAC occurrence and concentrations may be caused by differences in the inputs from point and nonpoint sources, and also in-stream physical, chemical, and biological processes. The presence of multiple EACs in surface waters and bed sediments indicates that organisms are exposed to mixtures of EACs. Although concentrations generally were low for most EACs in water, the combined effects of numerous organic contaminants on fish is largely unknown.

The widespread occurrence of endocrine disruption responses at sites where no EACs were detected in water or bed sediment indicates that some chemicals acting as EACs were not measured during the studies summarized in this report. Many other organic chemicals, such as plant sterols, fire retardants, antimicrobial chemicals, and pesticides were detected during these studies but have not been directly linked to endocrine disruption in fish. In field studies, the sources or specific chemicals that elicited the endocrine disruption responses are difficult to define because of fish movement and inherent sensitivity of different individuals or species.

Controlled laboratory studies confirmed that WWTP effluent elicits endocrine disruption responses, and the biological responses correspond to differences in WWTP composition, which varies because of differences in influent and treatment type or efficiency at an individual WWTP. Controlled laboratory studies also confirmed that selected EACs detected in WWTP effluent, such as hormones and alkylphenols, are contributors to endocrine disruption responses in fish and that the effects depend on fish life stage. Additionally, the effects of WWTP effluent on fish were beneficial and detrimental. For example, nutrients in effluent provide food that is incorporated as fish biomass, whereas chemicals that are not removed in treatment, such as EACs or other chemicals in wastewater, act as endocrine disruptors.

Human exposure to EACs through dermal contact, water consumption, or fish consumption is possible based on the ubiquitous distribution of EACs in aquatic environments. Lee and others (2004) detected EACs in the source waters for six drinking-water facilities that use surface water as source waters in Minnesota, but only detected AHTN and NP in one finished-water sample. Tornes and others (2007) reported AHTN, OP1EO, and HHCB in untreated groundwater used as source water for drinking water and no detections of EACs in finished-water samples. Focazio and others (2008) reported NP2EO, BPA, and HHCB in 2.7, 9.5, and 16.2 percent, respectively, of the 25 groundwater and 49 surface-water sources of drinking water sampled across the United States.

Few EACs were detected in source or finished drinking water in Minnesota and the effects of EACs on human health is largely unknown. There are indications that EACs might be contributing to increasing incidences of breast, prostate, and testicular cancers (Glass and Hoover, 1990; Davis and others, 1993; Adami and others, 1994) and to precocious puberty, hypospadias, and decreased sperm counts (Carlsen and others, 1992; Sharpe and Skakkabaek,1993); however, other investigators have concluded that there is no evidence for effects in humans (National Research Council, 1999; Safe, 2004).

The results from these studies of endocrine active chemicals and endocrine disruption in Minnesota streams and lakes provide information useful to understand sources, fate, and effects of EACs. An expansion of a combined multidisciplinary approach to sample existing resources combined with controlled studies at a larger scale is necessary to continue to better define the effects on aquatic resources. Numerous samples have been collected across Minnesota; however, these samples represent a relatively small percentage of possible sampling locations.

The studies summarized in this report were designed with differing objectives and differing analytical techniques, which provides challenges to interpretation. The establishment of fixed sites with long-term sampling and consistent analytical techniques is necessary to better understand temporal and spatial variability of EACs and biological responses.

The presence of biological indicators of endocrine disruption in male fish indicates that the fish are exposed to EACs. However, their presence in male fish does not indicate an effect on fish reproduction or changes in fish populations. Studies that better define the specific effects and modes of action of EACs on aquatic and terrestrial organisms are crucial. Many of the biological indicators currently (2010) used are indicators of exposure and are not predictive of reproductive success. Controlled studies with large fish in more natural conditions would allow for a more thorough investigation of population-level effects on fish and other organisms. Equally as important is the quantification of the effects of mixtures of EACs on aquatic organisms because this is the environmental exposure regime of most organisms.

Summary

This report summarizes a series of field monitoring studies and laboratory research conducted from 1994 through 2008 by the U.S. Geological Survey in cooperation with St. Cloud State University, Minnesota Department of Health, Minnesota Pollution Control Agency, Minnesota Department of Natural Resources, Metropolitan Council Environmental Services, and the University of Minnesota to determine the occurrence, fate, and effects of endocrine active chemicals (EACs) and the incidence of endocrine disruption in Minnesota streams and lakes. EACs are chemicals that interfere with the natural regulation of endocrine systems and may mimic or block the function of natural hormones in fish or other organisms. This interference commonly is referred to as endocrine disruption. Indicators of endocrine disruption in fish include vitellogenin (female egg yolk protein normally expressed in female fish) in male fish, depressed vitellogenin in female fish, oocytes present in

male fish testes, reduced reproductive success, and changes in reproductive behavior.

The long-term goals of these cooperative studies were to determine the occurrence and distribution of EACs and endocrine disruption in Minnesota surface waters, factors contributing to EAC occurrence and fate in surface waters, factors related to endocrine disruption occurrence, source pathways of EACs to organisms, and population-level effects on fish and other organisms. Select EACs were analyzed in water and bed-sediment samples, and endocrine disruption was measured through a series of biological indicators in fish.

Results of these studies indicate ubiquitous distribution of selected EACs in the aquatic environment that originate from numerous sources and pathways. The data indicate that wastewater-treatment plant effluent (WWTP) is a primary pathway of EACs to surface waters. The types and concentrations of EACs vary among WWTPs and vary temporally within one WWTP likely because of variations in the influent received and treatment operations.

In general, EAC occurrence and concentrations in streams are greater at sites directly downstream from discharge of WWTP effluent. Although WWTP is a primary conduit of EACs to streams, EACs also were detected in streams with no obvious sources of WWTP discharge, indicating other sources. Alternative sources may include runoff from land surfaces, atmospheric deposition, or inflow from groundwater into streams.

Another important finding of these studies is that EACs were detected in stream bed sediment at 50 percent of the sites sampled, indicating that bed sediment is a storage location of EACs in stream ecosystems. Aquatic organism exposure to EACs is expected based on the widespread presence of EACs in wastewater, water, and bed sediments in Minnesota streams that were sampled. The exposure of aquatic organisms to EACs also is expected to be in constant flux based on the variability in EACs measured at sites sampled more than once. This variability in EAC occurrence and concentrations likely is because of differences not only in the inputs from point and nonpoint sources, but also in-stream physical, chemical, and biological processes.

Indicators of endocrine disruption, such as the presence of vitellogenin in male fish, have been observed at more than 40 percent of the sites sampled in Minnesota. The presence of biological indicators of endocrine disruption in male fish indicates that the fish are exposed to EACs. However, their presence in male fish does not indicate an effect on fish reproduction or changes in fish populations. Endocrine disruption was observed in wild fish downstream from discharge of WWTP effluent, indicating that effluent is one source of EACs in the aquatic environment. This finding was confirmed in a controlled study exposing fathead minnows to WWTP effluent at an onsite fish exposure laboratory. During this controlled study, changes in biological responses coincided with changes in WWTP effluent composition and strength, demonstrating that effluent effects on fish endocrine systems are temporally variable. Although chemicals contributing to endocrine disruption in fish are complex, several laboratory studies have further confirmed that certain chemical classes, such as hormones and alkylphenols, which are components of WWTP effluent, affect the endocrine systems of fish through biochemical, structural, and behavioral disruption.

Endocrine disruption was observed in wild fish from diverse environmental settings ranging from small streams draining agricultural land use to large rivers such as the Mississippi River draining mixed land uses. The results of these field studies indicate that EACs in streams are interacting with the endocrine systems of native fish. This pattern also indicates multiple sources of EACs in addition to WWTP effluent. The presence of EACs in water and bed sediments indicates multiple pathways for aquatic organism exposure. The accumulation or presence of EACs in bed sediment results in a more permanent exposure for organisms such as benthic insects and mussels that live in close contact with the sediments. Although few EACs were detected in source or finished drinking water in Minnesota, the effect of EACs on human health is largely unknown.

Although these studies indicate that WWTP effluent is a conduit for EACs to surface waters, EACs also were present in surface waters with no obvious WWTP sources. EACs were detected and indicators of endocrine disruption in fish were measured at numerous streams upstream from discharge of WWTP effluent and in lakes with no WWTP discharge. These observations indicate that other unidentified sources of EACs exist, such as runoff from land surfaces, atmospheric deposition, inputs from onsite-septic systems, or other groundwater sources. Alternatively, some EACs may not have been identified or measured.

The complex results from these field and laboratory experiments indicate that multidisciplinary research is crucial to gain a better understanding of the effects EACs on exposed aquatic organisms. Although numerous samples have been collected across Minnesota, these samples represent a relatively small percentage of possible sampling locations. Although the results from these studies provide information useful to understand sources, fate, and effects of EACs, a continuation and expansion of a combined multidisciplinary approach to sample existing resources, combined with controlled studies at a larger scale, is necessary to continue to better define the effects on aquatic organisms.

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Appendix 1

Appendix 1. U.S. Geological Survey studies summarized in this report.

[NA, not applicable; WWTP, wastewater-treatment plant; USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; MCES, Metropolitan Council Environmental Services; MDH, Minnesota Department of Health; MPCA, Minnesota Pollution Control Agency; LCCMR, Legislative and Citizens Commission on Minnesota Resources; NPS, National Park Service; SCSU, St. Cloud State University; UM, University of Minnesota]

Study	Publication	Description	Cooperators	Study identification number
Reconnaissance of 17-estradiol, 11-ketotestosterone, vitellogenin, and gonad histopathology in common carp of U.S. streams—potential for contaminant-induced endocrine disruption	Goodbred and others, 1997; *http://water.usgs.gov/nawqa/pnsp/pubs/ofr96-627/*	Common carp sampled at 25 sites across the United States to assess whether endocrine disruption may be occurring in fish in the United States	NA	1
Potential endocrine disrupting organic chemicals in treated municipal wastewater and river water	Barber and others, 2000	Selected organic contaminants were measured in effluents and streams throughout the Nation including 2 WWTPs and 2 river locations in Minnesota	USEPA	2
Use of biological characteristics of common carp (*Cyprinus carpio*) to indicate exposure to hormonally active agents in selected Minnesota streams, 1999	Lee and others, 2000; *http://mn.water.usgs.gov/publications/pubs/00-4202.pdf*	The presence of endocrine disruption was measured in common carp exposed to WWTP effluent and runoff from agricultural and forested land. Fish were sampled at 22 sites in Minnesota	MPCA, LCCMR	3
Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000—a national reconnaissance	Kolpin and others, 2002; Barnes and others, 2002	Pharmaceuticals, hormones, and other organic contaminants associated with wastewater were measured in 139 streams across 30 States including Minnesota during 1999 and 2000	NA	4
Presence and distribution of organic wastewater compounds in wastewater, surface water, groundwater, and drinking water, Minnesota, 2000–02	Lee and others, 2004; *http://pubs.usgs.gov/sir/2004/5138/*	Household, industrial, and agricultural-use compounds, pharmaceuticals, antibiotics, and sterols and hormones were measured at 65 sites in Minnesota including WWTP influent and effluent; landfill and feedlot lagoon leachate; surface water; groundwater; and the intake of and finished drinking water from drinking-water facilities	MDH, MPCA	5
Reconnaissance of endocrine disruption at sites sampled as part of the National Water-Quality Assessment Program in the Upper Mississippi River study unit	Lee and Blazer, 2005; published in this report	Common carp and smallmouth bass collected from 14 sites in the Upper Mississippi River Basin to determine if endocrine disruption was occurring in Minnesota streams	NA	6
Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows	Bistodeau and others, 2006	In this controlled study, conducted at the SCSU Aquatic Toxicology Research Laboratory, fathead minnow larvae were exposed to a mixture of alkylphenols, which closely matched the composition of effluent from a major metropolitan WWTP	SCSU	7
Reproductive responses of male fathead minnows exposed to WWTP effluent, effluent treated with XAD8 resin, and an environmentally relevant mixture of alkylphenol compounds	Barber and others, 2007	On-site, continuous-flow experiments were conducted during August and October 2002 at a major metropolitan WWTP in Minnesota to determine if effluent exposure induced endocrine disruption in sexually mature male fathead minnows (*Pimephales promelas*)	USEPA, MCES, UM	8

Appendix 1. U.S. Geological Survey studies summarized in this report.—Continued

[NA, not applicable; WWTP, wastewater-treatment plant; USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; MCES, Metropolitan Council Environmental Services; MDH, Minnesota Department of Health; MPCA, Minnesota Pollution Control Agency; LCCMR, Legislative and Citizens Commission on Minnesota Resources; NPS, National Park Service; SCSU, St. Cloud State University; UM, University of Minnesota]

Study	Publication	Description	Cooperators	Study identification number
Occurrence of endocrine active compounds and biological responses in the Mississippi River—Study design and data, June–August 2006	Lee, Yaeger, and others, 2008; *http://pubs.usgs.gov/ds/368/pdf/DS368.pdf*	Integrated biological and chemical study of endocrine disruption in fish in the Mississippi River. Water, bed sediment, and fish were collected at 43 sites along the river from the headwaters to 14 miles downstream from Brownsville, Minnesota, during June through August 2006	MPCA, SCSU	9
Alkylphenols, other endocrine active chemicals, and fish responses in three streams in Minnesota—study design and data, February–September 2007	Lee, Schoenfuss, and others, 2008; *http://pubs.usgs.gov/ds/405/*	Integrated chemical and biological study of three streams (South Fork Crow River, Redwood River, and Grindstone River) in Minnesota that receive wastewater to identify distribution patterns of endocrine active chemicals and to identify fish responses. Samples were collected from February through September 2007	MPCA, SCSU	10
Impairment of the reproductive potential of male fathead minnows by environmentally relevant exposures to 4-nonylphenol	Schoenfuss and others, 2008	In this controlled study, conducted at the SCSU Aquatic Toxicology Research Laboratory, the ability of 4-nonylphenol to alter reproductive competence in male fathead minnows was examined	SCSU	11
Emerging contaminants in the St. Croix River	Abigail Tomasek, U.S. Geological Survey, written commun., 2009; U.S. Geological Survey, 2009; *http://waterdata.usgs.gov/nwis/*	Occurrence of organic contaminants in two WWTPs that discharge to the St. Croix River, and their occurrence at two locations within the St. Croix River that coincide with concentrated mussel populations	NPS	12
Landscape Indicators Project	U.S. Geological Survey, 2009; *http://waterdata.usgs.gov/nwis/*	Evaluation of the relations between hydrologic landscapes in the upper Midwest, and pesticides, nutrients, endocrine active chemicals, and aquatic invertebrates in small streams during base-flow conditions	USEPA	13
Endocrine active chemical occurrence in rivers and lakes of Minnesota	Jeff Writer, U.S. Geological survey written commun., 2009	Occurrence and fate of selected contaminants in 12 lakes and 4 rivers in Minnesota	MPCA, SCSU	14
USGS, National Water-Quality Assessment Program, Source Water Investigation	U.S. Geological Survey, 2009; *http://waterdata.usgs.gov/nwis/*	Occurrence and temporal variability in organic contaminants in water used as source water for drinking-water facilities	NA	15

Appendix 2

Appendix 2. List of sites, site locations, site types, and media sampled.

[USGS, U.S. Geological Survey; STR, stream; na, not available; STRDW, stream directly downstream from discharge of wastewater-treatment plant effluent; na, not available; WWEF, wastewater-treatment plant effluent; WWTP, wastewater-treatment plant; Y, yes, sample was collected; N, no, sample was not collected; L, lake; W, wastewater-treatment plant]

Site number (shown on figs. 3*A* and 3*B*)	Latitude (decimal degrees)	Longitude (decimal degrees)	USGS station identification number	Site name	Site type	Reference[1]	Media sampled		
							Water	Bed-sediment	Fish
1	48.593611	-93.440000	05129510	Rainy River below International Falls, Minn.	STR	5	Y	N	N
2L	48.444230	-93.0284	482639093014201	Lake Kabetogama in Peterson Bay, Minn.	LAKE	14	Y	Y	Y
3L	48.470000	-92.835556	482812092500801	Ek Lake near International Falls, Minn.	LAKE	5	Y	N	N
4L	47.908070	-90.2521	475429090150801	Northern Light Lake near Grand Marais, Minn.	LAKE	14	Y	Y	Y
5L	47.182460	-91.75473	471057091451701	Stewart Lake near Two Harbors, Minn.	LAKE	14	Y	Y	Y
6L	47.015833	-91.666389	470103091395101	Lake Superior at Agate Bay near Two Harbors, Minn.	LAKE	14	N	Y	N
7L	46.756389	-92.115278	464523092065501	Lake Superior in St. Louis Bay at Duluth, Minn.	LAKE	5	Y	N	N
8	46.261667	-96.545833	05046450	Ottertail River above Breckenridge, Minn.	STR	1	N	N	Y
9	46.264167	-96.598861	na	Red River of the North near Breckenridge, Minn.	STR	1	N	N	Y
10	46.803889	-96.796389	05053800	Red River of the North above Fargo, N. Dak.	STR	5	Y	N	N
11	46.930556	-96.784722	05054020	Red River of the North below Fargo, N. Dak.	STR	5	Y	N	N
12	47.896667	-96.961667	05082000	Red Lake River at State Hwy 220 above East Grand Forks, Minn.	STR	5	Y	N	N
13	47.926944	-97.028889	05082500	Red River of the North at Grand Forks, N. Dak.	STR	1	N	N	Y
14	47.981667	-97.055556	475854097032001	Red River of the North below WWTP at East Grand Forks, Minn.	STR	5	Y	N	N
15	48.572222	-97.147222	05092000	Red River of the North near Drayton, N. Dak.	STR	1	N	N	Y
16L	47.196460	-95.22157	471147095131801	Elk Lake near Douglas Lodge, Minn.	LAKE	14	Y	Y	Y
17	47.239167	-95.209167	05200010	Mississippi River at Lake Itasca, Minn.	STR	9	Y	Y	N
18	47.436389	-95.001944	05200400	Mississippi River below County Road 7 near Bemidji, Minn.	STR	9	N	N	Y
19	47.465833	-94.879444	05200430	Mississippi River at State Hwy 197 in Bemidji, Minn.	STR	9	Y	Y	Y
20	47.425278	-94.364444	05200945	Mississippi River near Schley, Minn.	STR	9	Y	Y	N
21	47.302222	-93.901667	05207600	Mississippi River at Willow Beach at Ball Club, Minn.	STR	9	Y	Y	Y
22	47.232778	-93.802222	05210050	Mississippi River below Days High Landing near Deer River, Minn.	STR	9	Y	Y	N

Appendix 2. List of sites, site locations, site types, and media sampled.—Continued

[USGS, U.S. Geological Survey; STR, stream; na, not available; STRDW, stream directly downstream from discharge of wastewater-treatment plant effluent; na, not available; WWEF, wastewater-treatment plant effluent; WWTP, wastewater-treatment plant; Y, yes, sample was collected; N, no, sample was not collected; L, lake; W, wastewater-treatment plant]

Site number (shown on figs. 3*A* and 3*B*)	Latitude (decimal degrees)	Longitude (decimal degrees)	USGS station identification number	Site name	Site type	Reference[1]	Media sampled		
							Water	Bed-sediment	Fish
23	47.216111	-93.487500	05211000	Mississippi River at Grand Rapids, Minn.	STR	6,9	Y	Y	Y
24	47.128611	-93.404444	05213600	Mississippi River near Philbin, Minn.	STRDW	9	Y	Y	Y
25	47.001389	-93.268611	05217650	Mississippi River below Jacobson, Minn.	STR	9	Y	Y	Y
26	46.708889	-93.484167	05220600	Mississippi River at Palisade, Minn.	STR	9	Y	Y	Y
27	46.547500	-93.725000	05227510	Mississippi River below County Highway 1 in Aitkin, Minn.	STR	9	Y	Y	Y
28	46.517778	-94.074444	05240000	Mississippi River below Mission Creek near Trommald, Minn.	STR	9	Y	Y	N
29	46.327778	-94.240556	05242320	Mississippi River below Brainerd, Minn.	STR	6,9	Y	Y	Y
30L	46.359910	-94.2874	462136094171501	White Sand Lake near Baxter, Minn.	LAKE	14	Y	Y	Y
31L	46.381030	-94.2861	462252094171001	Red Sand Lake near Baxter, Minn.	LAKE	14	Y	Y	Y
32L	46.999360	-94.68737	465958094411501	Shingobee Lake near Akeley, Minn.	LAKE	14	Y	Y	Y
33	46.180833	-94.366944	05261000	Mississippi River near Fort Ripley, Minn.	STR	9	Y	Y	Y
34	45.950833	-94.389722	05263400	Mississippi River above Pike Creek at Little Falls, Minn.	STRDW	6,9	Y	Y	Y
35	45.861111	-94.358333	05267000	Mississippi River near Royalton, Minn.	STR	6	N	N	Y
36	45.728889	-94.229722	05268300	Mississippi River below Rice, Minn.	STR	9	Y	Y	Y
37	45.598889	-94.186944	05270010	Mississippi River above Sauk River near Sauk Rapids, Minn.	STR	5	Y	N	N
38	45.830833	-95.037778	na	Sauk River upstream from Sauk Center, Minn.	STR	3	N	N	Y
39	45.676944	-94.803611	na	Sauk River downstream from Melrose, Minn.	STR	3	N	N	Y
40	45.559722	-94.233333	05270500	Sauk River near St. Cloud, Minn.	STR	5	Y	N	N
41	45.571111	-94.165833	05270600	Mississippi River at Sauk Rapids, Minn.	STR	9	Y	Y	Y
42	45.467500	-94.100278	05272500	Mississippi River above Plum Creek near Clearwater, Minn.	STRDW	9	Y	Y	Y
43	45.425833	-94.049444	05272800	Mississippi River above Clearwater River near Clearwater, Minn.	STR	5,6	Y	N	Y
44	45.325278	-93.823889	05273650	Mississippi River above Monticello, Minn.	STR	9	Y	Y	Y
45	45.289167	-93.559444	05275500	Mississippi River at Elk River, Minn.	STRDW	9	Y	Y	Y
46	45.145556	-94.516667	05278083	Jewitt's Creek near Litchfield, Minn.	STR	5	Y	N	N

Appendix 2. List of sites, site locations, site types, and media sampled.—Continued

[USGS, U.S. Geological Survey; STR, stream; na, not available; STRDW, stream directly downstream from discharge of wastewater-treatment plant effluent; na, not available; WWEF, wastewater-treatment plant effluent; WWTP, wastewater-treatment plant; Y, yes, sample was collected; N, no, sample was not collected; L, lake; W, wastewater-treatment plant]

Site number (shown on figs. 3*A* and 3*B*)	Latitude (decimal degrees)	Longitude (decimal degrees)	USGS station identification number	Site name	Site type	Reference[1]	Media sampled		
							Water	Bed-sediment	Fish
47	44.885000	-94.457500	05278560	South Fork Crow River above Otter Lake near Hutchinson, Minn.	STR	3,10	Y	Y	Y
48L	45.220040	-93.94226	451312093563201	Sullivan Lake near Buffalo, Minn.	LAKE	14	Y	Y	Y
49	44.872222	-94.356111	na	South Fork Crow River at the WWTP	STRDW	10	N	N	Y
50	44.848611	-94.321944	05278580	South Fork Crow River below Hutchinson, Minn.	STR	3,10	Y	Y	Y
51	44.836389	-94.287778	05278590	South Fork Crow River at Hwy. 22 near Biscay, Minn.	STR	10	Y	Y	Y
52	45.226944	-93.551944	05280400	Crow River below State Hwy 101 at Dayton, Minn.	STR	5	Y	N	N
53	45.192222	-93.393611	05283400	Mississippi River at Dayton, Minn.	STR	9	Y	Y	Y
54	45.163333	-93.436389	05287890	Elm Creek near Champlin, Minn.	STR	5	Y	N	N
55	45.146389	-93.315278	05288400	Mississippi River above Coon Rapids Dam in Coon Rapids, Minn.	STR	6,9	Y	Y	Y
56	45.126667	-93.296667	05288500	Mississippi River near Anoka, Minn	STR	2,5,9	Y	Y	Y
57	45.049444	-93.277222	05288650	Mississippi River below I-694 at Fridley, Minn.	STR	15	Y	N	N
58	45.090278	-93.276389	05288610	Rice Creek at County Road 1 in Fridley, Minn.	STR	5	Y	N	N
59	45.050000	-93.310000	05288705	Shingle Creek at Queen Ave. in Minneapolis, Minn.	STR	5	Y	N	N
60L	45.049444	-93.094444	450258093054001	Vadnais Lake at Pumping Station in Vadnais Heights, Minn.	LAKE	5	Y	N	N
61L	44.956160	-93.3211	445722093193101	Cedar Lake near Cedar Lake Parkway, Minneapolis, Minn.	LAKE	14	Y	Y	Y
62L	45.031330	-93.13004	450153093074801	Lake Owasso near Country Road C, Roseville, Minn.	LAKE	14	Y	Y	Y
63	44.906667	-93.195278	05289900	Mississippi River above Minnesota River at Fort Snelling, Minn.	STR	9	Y	Y	Y
64	44.430278	-95.845278	05315000	Redwood River near Marshall, Minn.	STR	10	Y	Y	Y
65	44.479111	-95.776111	na	Redwood River upstream 15 meters from WWTP effluent discharge	STR	14	Y	N	Y
66	44.479444	-95.775556	na	Redwood River at WWTP	STRDW	10,14	N	Y	Y

Appendix 2. List of sites, site locations, site types, and media sampled.—Continued

[USGS, U.S. Geological Survey; STR, stream; na, not available; STRDW, stream directly downstream from discharge of wastewater-treatment plant effluent; na, not available; WWEF, wastewater-treatment plant effluent; WWTP, wastewater-treatment plant; Y, yes, sample was collected; N, no, sample was not collected; L, lake; W, wastewater-treatment plant]

Site number (shown on figs. 3*A* and 3*B*)	Latitude (decimal degrees)	Longitude (decimal degrees)	USGS station identification number	Site name	Site type	Reference[1]	Media sampled		
							Water	Bed-sediment	Fish
67	44.486667	-95.765556	05315050	Redwood River below WWTP near Marshall, Minn.	STRDW	3, 10, 14	Y	Y	Y
68	44.512500	-95.735833	05315250	Redwood River at 280th Ave. near Marshall, Minn.	STRDW	10,14	Y	Y	Y
69	44.341944	-94.998333	05316983	County Ditch 24 near Clements, Minn.	STR	13	Y	N	N
70	44.064722	-94.589444	05318700	Watonwan River at La Salle, Minn.	STR	3	N	N	Y
71	44.005556	-94.589167	na	St. James Creek downstream from St. James, Minn.	STR	3	N	N	Y
72	44.044722	-94.412222	na	Watonwan River downstream from Madelia, Minn.	STR	3	N	N	Y
73L	43.639650	-94.47441	433823094282801	Budd Lake, Fairmont, Minn.	LAKE	14	Y	Y	Y
74	43.890000	-94.289167	05318283	Judicial Ditch 85 near Willow Creek, Minn.	STR	13	Y	N	N
75	44.095556	-94.109167	05320000	Blue Earth River near Rapidan, Minn.	STR	5	Y	N	N
76	44.014444	-93.527222	05320070	Le Sueur River near Wilton, Minn.	STR	14	Y	Y	Y
77	43.996667	-93.908333	05320270	Little Cobb River near Beauford, Minn.	STR	5,13,14	Y	Y	Y
78	44.128333	-94.048611	05321990	Blue Earth River at County Road 90 near Mankato, Minn.	STR	5	Y	N	N
79	44.168889	-94.003056	05325000	Minnesota River at Mankato, Minn.	STR	5	Y	N	N
80	44.291100	-94.075580	na	Sevenmile Creek upstream from St Hwy 99 near St Peter, Minn.--upstream	STR	14	Y	Y	Y
81	44.268070	-94.04393	na	Sevenmile Creek 1.6 miles upstream from US Hwy 169 near St Peter, Minn.--mid-stream	STR	14	Y	Y	Y
82	44.261111	-94.025556	na	Sevenmile Creek downstream from US Hwy 169 near St Peter, Minn.--downstream	STR	14	Y	Y	Y
83	44.693056	-93.641667	05330000	Minnesota River near Jordan, Minn.	STR	6	N	N	Y
84	44.357778	-93.908889	05325580	Cherry Creek near Ottawa, Minn.	STR	13	Y	N	N
85	44.691389	-93.737222	05329898	Silver Creek near East Union, Minn.	STR	13	Y	N	N
86	44.801944	-93.520556	05330713	Minnesota River (RM 25) near Shakopee, Minn.	STR	2	Y	N	N
87	44.933889	-93.103889	05330990	Mississippi River at I-35E Bridge in St. Paul, Minn.	STR	9	Y	Y	Y
88	44.912222	-93.045833	05331400	Mississippi River at South St. Paul, Minn.	STR	9	Y	Y	Y

Appendix 2. List of sites, site locations, site types, and media sampled.—Continued

[USGS, U.S. Geological Survey; STR, stream; na, not available; STRDW, stream directly downstream from discharge of wastewater-treatment plant effluent; na, not available; WWEF, wastewater-treatment plant effluent; WWTP, wastewater-treatment plant; Y, yes, sample was collected; N, no, sample was not collected; L, lake; W, wastewater-treatment plant]

Site number (shown on figs. 3*A* and 3*B*)	Latitude (decimal degrees)	Longitude (decimal degrees)	USGS station identification number	Site name	Site type	Reference[1]	Media sampled		
							Water	Bed-sediment	Fish
89	44.886389	-93.015556	05331540	Mississippi River at Highway 494, in Newport, Minn.	STR	9	Y	Y	Y
90	44.846944	-93.008611	05331555	Mississippi River at 11th Avenue, in St. Paul Park, Minn.	STR	9	Y	Y	Y
91	44.776111	-92.902222	05331568	Spring Lake West End, Rosemount, Minn.	STR	9	N	N	Y
92	44.762222	-92.873333	05331570	Mississippi River at Ninninger, Minn.	STR	9	Y	Y	Y
93	44.746667	-92.852222	05331580	Mississippi River below Lock and Dam 2 at Hastings, Minn.	STR	4,5,6	Y	N	Y
94	44.749722	-92.830278	0533158010	Mississippi River below Clay Street at Hastings, Minn.	STR	9	Y	Y	Y
95	45.951389	-92.555556	na	St. Croix River near Cloverdale, Minn.	STR	3	N	N	Y
96	46.034167	-92.971111	05336900	North Branch Grindstone River near Hinckley, Minn.	STR	10	Y	Y	Y
97	46.024444	-92.971111	05336990	South Branch Grindstone River near Hinckley, Minn.	STR	10	Y	Y	Y
98	46.018611	-92.908611	na	Grindstone at WWTP	STRDW	10	N	N	Y
99	46.013611	-92.889444	05337005	Grindstone River below Hinckley, Minn.	STR	10	Y	Y	Y
100	46.011111	-92.876944	05337010	Grindstone River near Hinckley, Minn.	STR	10	Y	Y	Y
101	45.656111	-92.890278	05339721	Rush Creek above mouth near Rush City, Minn.	STR	13	Y	N	N
102	45.567222	-92.858611	05340200	St. Croix River below Sunrise River near Sunrise, Minn.	STR	12	Y	Y	N
103	45.381944	-92.675278	05340540	St. Croix River above Rock Island near Franconia, Minn.	STRDW	12	Y	Y	N
104	45.290556	-92.759444	0534055940	St. Croix River at Cedar Bend below Osceola, Wisc.	STR	6	N	N	Y
105	45.037500	-92.778333	05341551	St. Croix River below Stillwater, Minn.	STR	3,5	Y	N	Y
106	44.613056	-92.621944	05344980	Mississippi River at Lock and Dam 3 near Red Wing, Minn.	STR	9	Y	Y	Y
107	44.663611	-93.075000	05344998	Vermillion River below Empire WWTP near Empire, Minn.	STR	5	Y	N	N
108	44.098611	-93.229722	na	Straight River downstream from Owatona, Minn.	STR	3	N	N	Y

Appendix 2. List of sites, site locations, site types, and media sampled.—Continued

[USGS, U.S. Geological Survey; STR, stream; na, not available; STRDW, stream directly downstream from discharge of wastewater-treatment plant effluent; na, not available; WWEF, wastewater-treatment plant effluent; WWTP, wastewater-treatment plant; Y, yes, sample was collected; N, no, sample was not collected; L, lake; W, wastewater-treatment plant]

Site number (shown on figs. 3*A* and 3*B*)	Latitude (decimal degrees)	Longitude (decimal degrees)	USGS station identification number	Site name	Site type	Reference[1]	Media sampled		
							Water	Bed-sediment	Fish
109	44.052778	-93.250833	na	Straight River upstream from Owatona, Minn.	STR	3	N	N	Y
110	44.582222	-92.550556	05355250	Mississippi River near Redwing, Minn.	STR	6	N	N	Y
111	44.566389	-92.486389	05355251	Mississippi River at mile 788 near Red Wing, Minn.	STRDW	9	Y	Y	Y
112	44.546389	-92.387222	05355260	Mississippi River (Lake Pepin) above Lake City, Minn.	STR	9	Y	Y	Y
113	44.457778	-92.477222	05355307	Wells Creek near Belvidere Mills, Minn.	STR	13	Y	N	N
114	44.444722	-92.259167	05355331	Mississippi River (Lake Pepin) at mile 771 near Lake City, Minn.	STR	6,9	Y	Y	Y
115	44.411389	-92.104722	05355341	Mississippi River (Lake Pepin) above Read's Landing, Minn.	STR	9	Y	Y	Y
116	44.299722	-91.918889	05372510	Mississippi River below Lock and Dam 4 at Alma, Wisc.	STR	9	Y	Y	Y
117	44.011944	-92.369444	05372915	Bear Creek tributary near Chester, Minn.	STR	5	Y	N	N
118	44.061667	-92.466111	05372995	South Fork Zumbro River at Rochester, Minn.	STR	5	Y	N	N
119	44.066667	-92.465278	05373000	South Fork Zumbro River near Rochester, Minn.	STRDW	3,5	Y	N	Y
120	44.073889	-92.467500	05373005	South Fork Zumbro River below WWTP near Rochester, Minn.	STR	5	Y	N	N
121	44.256944	-91.887222	05375700	Mississippi River at mile 746 near Buffalo, Wisc.	STR	9	Y	Y	Y
122	44.200556	-91.944444	05377508	Trout Creek near Weaver, Minn.	STR	13	Y	N	N
123	44.134167	-91.788611	05377550	Deering Valley Creek near Whitman, Minn.	STR	13	Y	N	N
124	44.092222	-91.676944	05378490	Mississippi River at Lock and Dam 5A near Winona, Minn.	STR	9	Y	Y	Y
125	44.031667	-91.580833	05378500	Mississippi River at Winona, Minn.	STR	9	Y	Y	Y
126	43.986389	-91.429167	05380505	Mississippi River below Lock and Dam 6 near Trempealeau, Wisc.	STR	9	Y	Y	Y
127	43.833889	-91.276111	05380590	Mississippi River at Lock and Dam 7 near La Crescent, Minn.	STR	9	Y	Y	Y
128	43.579444	-91.229167	05386400	Mississippi River at Brownsville, Minn.	STR	9	Y	Y	Y

Appendix 2. List of sites, site locations, site types, and media sampled.—Continued

[USGS, U.S. Geological Survey; STR, stream; na, not available; STRDW, stream directly downstream from discharge of wastewater-treatment plant effluent; na, not available; WWEF, wastewater-treatment plant effluent; WWTP, wastewater-treatment plant; Y, yes, sample was collected; N, no, sample was not collected; L, lake; W, wastewater-treatment plant]

Site number (shown on figs. 3*A* and 3*B*)	Latitude (decimal degrees)	Longitude (decimal degrees)	USGS station identification number	Site name	Site type	Reference[1]	Media sampled: Water	Media sampled: Bed-sediment	Media sampled: Fish
129	43.649444	-92.973333	05455978	Cedar River below treatment plant at Austin, Minn.	STR	3,5	Y	N	Y
130	43.600556	-93.291667	na	Shell Rock River near Albert Lea, Minn.	STR	3	N	N	N
131	43.647500	-95.532500	05474885	Okabena Creek near Worthington, Minn.	STR	3,5	Y	N	N
132	43.865000	-95.313056	na	Des Moines River upstream from Windom, Minn.	STR	3	N	N	N
133	43.857500	-95.107778	na	Des Moines River downstream from Windom, Minn.	STR	3	N	N	N
134	43.717778	-96.163611	06482945	Rock River upstream from Luverne, Minn.	STR	3	N	N	N
135	43.647778	-96.200000	433852096120001	Rock River downstream from Luverne, Minn.	STR	3	N	N	N
136W	44.803333	-93.449722	na	Blue Lake WWTP in Shakopee, Minn.	WWEF	5	Y	N	N
137W	44.063889	-92.465278	440350092275501	WWTP outflow at Rochester, Minn.	WWEF	2	Y	N	N
138W	46.018611	-92.908611	460107092543101	Hinckley WWTP near Hinckley, Minn.	WWEF	10	Y	N	N
139W	47.976111	-97.055556	475834097032001	WWTP outflow at East Grand Forks, Minn.	WWEF	10	Y	N	N
140W	46.760556	-92.123889	464538092072601	WWTP outflow at Duluth, Minn.	WWEF	5	Y	N	N
141W	45.406667	-92.647222	452424092385001	St. Croix Falls Treatment Plant at St. Croix Falls, Wisc.	WWEF	12	Y	N	N
142W	45.400833	-92.685000	452403092410601	Taylors Falls Treatment Plant at Taylors Falls, Minn.	WWEF	12	Y	N	N
143W	44.919167	-93.045278	445509093024301	WWTP outflow in St. Paul, Minn.	WWEF	12	Y	N	N
144W	44.872222	-94.356111	445220094212201	Hutchinson WWTP outflow at Hutchinson, Minn.	WWEF	10	Y	N	N
145W	44.831667	-93.218611	na	Seneca WWTP in Eagan, Minn.	WWEF	10	Y	N	N
146W	44.479444	-95.775556	442846095463201	Marshall WWTP outflow at Marshall, Minn.	WWEF	2	Y	N	N
147W	47.017400	-91.66425	470103091395102	Two Harbors, Minn., WWTP effluent	WWEF	14	Y	N	N

[1]Reference is study identification number in appendix 1.

Appendix 3

http://pubs.usgs.gov/sir/2010/5107/Appendix_3_EAC_WATER.xlsx

Appendix 4

http://pubs.usgs.gov/sir/2010/5107/Appendix_4_EAC_SEDIMENTS.xlsx

Appendix 5

http://pubs.usgs.gov/sir/2010/5107/Appendix_5_EAC_FISH.xlsx

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