

Evaluation of Emerging Contaminants of Concern at the South District Wastewater Treatment Plant Based on Seasonal Sampling Events, Miami-Dade County, Florida, 2004

By A.C. Lietz and Michael T. Meyer

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Conversion Factors, Datum, Abbreviations, and Acronyms

Multiply	By	To obtain
	Length	
inch (in.)	25.4	millimeter (mm)
mile (mi)	1.609	kilometer (km)
	Area	
acre	0.004047	square kilometer (km ²)
	Flow rate	
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:

$$^{\circ}\text{F}=(1.8\times^{\circ}\text{C})+32$$

Datum:

Horizontal coordinate information is referenced to the North American Datum of 1927 (NAD 27).

Other Units of Abbreviation:

g	gram	mL/min	milliliter per minute
kPa	kilopascal	µg	microgram
L	liter	µm	micromho
L/min	liter per minute	µL	microliter
mm	millimeter	µg/L	microgram per liter
mg	milligram	ng/L	nanogram per liter
mg/L	milligram per liter	ng/mL	nanogram per milliliter
mg/mL	milligrams per milliliter	ng/µL	nanogram per microliter
mL	milliliter	nm	nanometer

Acronyms:

BLM	beta-lactams and macrolides
CERP	Comprehensive Everglades Restoration Plan
CLO ₂	chlorine dioxide
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionization
GC/MS	gas chromatography/mass spectrometry
HLB	hydrophobically and lipophilically balanced
HPLC	high-performance liquid chromatography
HRD	high-rate disinfection
LC/MS	liquid chromatography/mass spectrometry
M + H	molecular ion plus hydrogen ion
MDL	method detection limit
MeOH	methyl alcohol
MRL	minimum reporting level
MS	mass spectrometry
NWQL	National Water Quality Laboratory
RPD	relative percent difference
SDWWTP	South District Wastewater Treatment Plant
SPE	solid-phase extraction
SQ	sulphonamides and quinolones
TET	tetracyclines
USGS	U.S. Geological Survey

Evaluation of Emerging Contaminants of Concern at the South District Wastewater Treatment Plant Based on Seasonal Sampling Events, Miami-Dade County, Florida, 2004

By A.C. Lietz and Michael T. Meyer

Abstract

The Comprehensive Everglades Restoration Plan has identified highly treated wastewater as a possible water source for the restoration of natural water flows and hydroperiods in selected coastal areas, including the Biscayne Bay coastal wetlands. One potential source of reclaimed wastewater for the Biscayne Bay coastal wetlands is the effluent from the South District Wastewater Treatment Plant in southern Miami-Dade County. The U.S. Geological Survey, in cooperation with the Comprehensive Everglades Restoration Plan Wastewater Reuse Technology Pilot Project Delivery Team, initiated a study to assess the presence of emerging contaminants of concern in the South District Wastewater Treatment Plant influent and effluent using current wastewater-treatment methods.

As part of the study, 24-hour composite and discrete samples were collected at six locations (influent at plants 1 and 2, effluent pump, reuse train, chlorine dioxide unit, and ultraviolet pilot unit) at the plant during: (1) a dry-season, low-flow event on March 2-3, 2004, with an average inflow rate of 83.7 million gallons per day; (2) a wet-season, average-flow event on July 20-21, 2004, with an average inflow rate of 89.7 million gallons per day; and (3) high-rate disinfection tests on October 5 and 20, 2004, with average flow rates of 84.1 and 119.6 million gallons per day, respectively. During these four sampling events, 26, 27, 29, and 35 constituents were detected, respectively. The following transformations in concentration were determined in the waste stream: -100 to 180 percent at the effluent pump and -100 to 85 percent at the reuse train on March 2-3, 2004, and -100 to 1,609 percent at the effluent pump and -100 to 832 percent at the reuse train on July 20-21, 2004; -100 to -37 percent at the effluent pump, -100 to -62 percent at the reuse train, -100 to -56 percent at the chlorine dioxide unit, and -100 to -40 percent at the ultraviolet pilot unit on October 5, 2004; and -100 to -4 percent at the effluent pump, -100 to 17 percent at the reuse train, -100 to -40 percent at the chlorine dioxide unit, and -100 to -14 percent at the ultraviolet pilot unit on October 20, 2004.

Samples were tested for detection of household and industrial (organic) wastewater compounds, pharmaceutical compounds, antibiotic compounds, and hormones in influent. Two "known" endocrine disrupting compounds—17 beta-estradiol (E2) and diethoxynonylphenol—and four "suspected" endocrine-disrupting compounds—1,4-dichlorobenzene, benzophenone, tris(2-chloroethyl) phosphate, and tris(dichloroisopropyl) phosphate—were detected during these sampling events. Phenanthrene and indole showed the greatest concentration ranges and highest concentrations for the organic wastewater compounds. Acetaminophen showed the greatest concentration range and highest concentration, and warfarin showed the smallest concentration range for the pharmaceutical compounds. Sulfamethoxazole (a sulfonamide) showed the greatest concentration range and highest concentration, and sulfathiazole (also a sulfonamide) showed the smallest concentration range for the antibiotic compounds. Two hormones, 17 beta-estradiol (E2) and estrone (E1), were detected in influent.

Samples were also tested for detection of organic wastewater compounds, pharmaceutical compounds, antibiotic compounds, and hormones in effluent. Indole showed the greatest concentration range and highest concentration, and triphenyl phosphate showed the smallest concentration range for the organic wastewater compounds. Dehydronifedipine showed the greatest concentration range and highest concentration, and warfarin had the smallest concentration range for the pharmaceutical compounds. Anhydro-erythromycin (a macrolide degradation product) showed the greatest concentration range, and sulfadiazine (a sulfonamide) and tetracycline showed the lowest concentration ranges for the antibiotic compounds. One hormone, 17 beta-estradiol (E2), was detected in effluent.

A statistical approach using the Wilcoxon signed-ranks test was undertaken to determine if there were statistically significant differences at the 95-percent confidence level between concentrations from composite and discrete samples. Of the 83 constituents detected, only 3.5 percent showed any statistically significant differences at the 95-percent confidence level.

Quality-assurance samples were collected for all organic wastewater compounds and included field blanks, equipment blanks, a duplicate sample, and matrix spikes. Analytical results of field and (or) equipment blanks were less than the minimum reporting levels (MRLs) for aceptophenone, N,N-diethyl-*meta*-toluamide (DEET), and naphthalene. Phenol had a concentration greater than the MRL. One duplicate sample was collected for 63 organic wastewater compounds. The relative percent differences were not determined or estimated for 62 and 11 percent of the compounds, respectively, because of censored data and matrix interference; however, relative percent differences were estimated for 25 percent of the compounds because of data below the MRLs. Of the relative percent differences that were determined, values ranged from 0.0 to 97.

Quality-assurance samples were also collected for pharmaceutical compounds, antibiotic compounds, and hormones. Analytical results of field and (or) equipment blanks for pharmaceutical compounds had concentrations greater than the MRL for 1,7-dimethylxanthene, diphenhydramine, and fluoxetine. Four pharmaceutical compounds had concentrations less than the MRL including acetaminophen, diltiazem, thiabendazole, and trimethoprim. Lincomycin, ofloxacin, and tetracycline were the only antibiotic compounds with concentrations greater than the MRLs for equipment blanks. The hormone 17 beta-estradiol (E2) had concentrations greater than the MRL for field and equipment blanks.

Average matrix spike recoveries were determined for 55 organic wastewater compounds. Average spike recoveries for 22 compounds (40 percent) were outside of the expected spike recovery range, and 16 compounds (29 percent) were considered "estimated" because one or more values were below the MRLs because of matrix interference or multiple detection levels. Recoveries could not be determined for 22 compounds because of a predominance of censored data values and lack of recovery for two samples.

The enzyme-linked immunosorbent assay (ELISA) analyses for hormones were confirmed by liquid chromatography/mass spectrometry (LC/MS) results. For 17-beta estradiol (E2), the average concentration of spiked samples and the spiked minus unspiked samples was about twice as high for the ELISA tests as for the LC/MS results. For the estrone (E1) and ethenyl estradiol (EE2) analyses, the average concentrations for the spiked samples and spiked minus unspiked samples were similar.

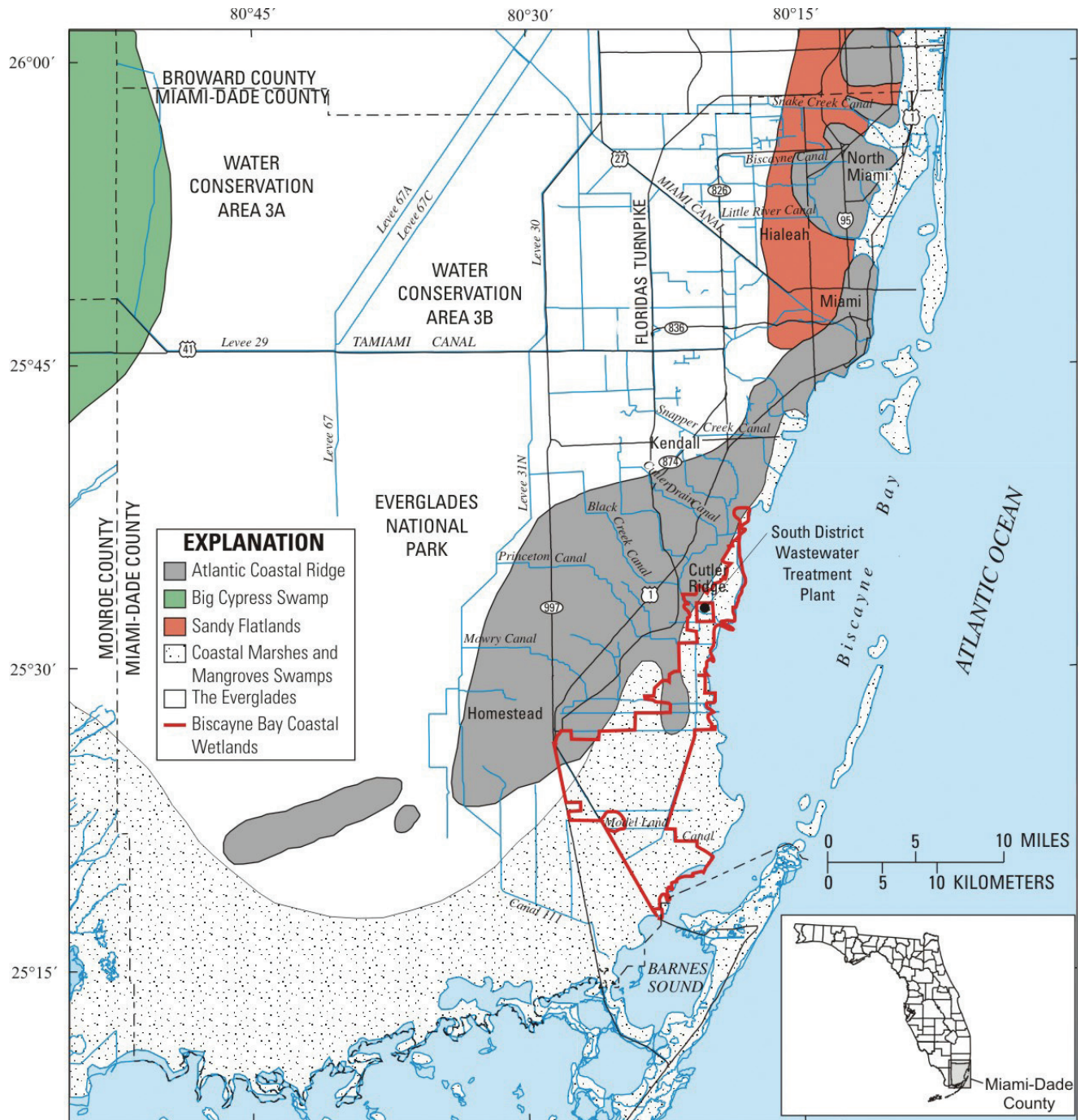
Surrogate performance was analyzed for selected compounds. Results indicated that decafluorobiphenyl, caffeine C-13, bisphenol A-d3, and fluoranthene d-10 were all within expected surrogate recovery ranges, indicating acceptable laboratory extraction and processing procedures.

Introduction

One objective of the Comprehensive Everglades Restoration Plan (CERP) is to restore natural water flows and historical hydroperiods to the southern Florida ecosystem. To accomplish this task, the Central and Southern Florida Restudy (U.S. Army Corps of Engineers, 1999) evaluated the use of reclaimed water to augment existing water supplies and identified highly treated wastewater as a potential source. The Wastewater Reuse Technology Pilot Project was established to: (1) identify advanced wastewater-treatment technologies capable of supplying reclaimed water (that will not adversely affect natural systems) in viable quantities to meet regional water demands as identified in CERP, and (2) investigate the effects of applying reclaimed water to ecosystems within Miami-Dade County.

In accordance with these objectives, CERP proposes to develop one of two advanced wastewater-treatment pilot facilities at the current South District Wastewater Treatment Plant (SDWWTP) in southeastern Miami-Dade County. Superior highly treated reuse wastewater from this facility will be used to replace and augment freshwater flows to the sensitive Biscayne Bay ecosystem, and restore more natural hydroperiods to the Biscayne Bay coastal wetlands area, which covers about 13,600 acres east of L-31E Canal in southeastern Miami-Dade County (fig. 1).

A critical concern that must be addressed in the application of reused water to ecosystems is the degree to which certain constituents in reclaimed water may adversely affect the aquatic biota of an ecosystem. The ecological and human health impacts of emerging contaminants of concern have received increased attention within the past decade, specifically, the endocrine disrupting chemicals and their effects on the aquatic biota. To address these issues, the U.S. Geological Survey (USGS), in cooperation with the CERP Wastewater Reuse Technology Pilot Project Delivery Team, initiated a study to evaluate: (1) the presence of emerging contaminants in influent and effluent at the SDWWTP, and (2) the degree of constituent transformation at selected locations in the waste stream using current (2004) treatment processes.



Base from U.S. Geological Survey digital data
 Universal Transverse Mercator projection, Zone 17, Datum NAD 27

Figure 1. Miami-Dade County showing location of the South District Wastewater Treatment Plant, physiographic provinces, and the Biscayne Bay coastal wetlands.

Purpose and Scope

The purposes of this report are to assess and document: (1) the occurrence and concentration of selected emerging contaminants in influent and effluent at the SDWWTP, based on seasonal sampling events, and (2) transformations in constituent concentration at selected locations in the waste stream during current treatment processes. The emerging contaminants analyzed at the effluent pump, reuse train, chlorine dioxide (CLO₂) unit, and ultraviolet irradiation pilot unit include: household and industrial (organic) wastewater compounds, pharmaceutical compounds (prescription and nonprescription drugs), antibiotic compounds, and hormones. The SDWWTP employs activated sludge as the primary treatment process.

Background information about the SDWWTP is provided, including the treatment processes used. Sampling and processing methods and the laboratory analytical methods are described, and analytical results are presented from dry- and wet-season sampling events and high-rate disinfection (HRD) tests utilizing the CLO₂ unit and ultraviolet pilot units. Results of composite and discrete samples are compared statistically, and results of field and laboratory quality-assurance procedures are presented.

Previous Studies

A number of studies conducted in the United States and other countries have examined the occurrence and concentration of selected emerging contaminants and their attenuation during the wastewater-treatment process and in the ambient environment. Boyd and Furlong (2002) investigated the occurrence of human-health pharmaceuticals in Lake Mead, Nevada and Arizona, and Las Vegas Wash, Nevada, from October 2000 to August 2001. The environmental fate of triclosan in the River Aire Basin, United Kingdom, was documented by Sabaliunas and others (2003). The fate of antibiotics in pharmaceutical wastewater by ozonation and the addition of hydrogen peroxide was investigated and documented in Turkey by Balcioglu and Ötker (2003). In the United States, the removal of antibiotics from surface and distilled water in conventional water-treatment processes was investigated by Adams and others (2002). The degradation of 17 beta-estradiol (E2) in water by titanium dioxide photocatalysis was investigated in Japan by Ohko and others (2002). The presence of pharmaceutical compounds in sewage-treatment plant effluents and their solar degradation in the aquatic environment was investigated in Italy, France, Greece, and Sweden by Andreozzi and others (2003). An environmental exposure assessment of fluoroquinolone antibacterial agents from sewage and soil environments in Switzerland was documented by Golet and others (2003). The adsorption of estrone on microfiltration hollow fiber membranes and the removal of estrone from aqueous solutions using nanofiltration and reverse osmosis were investigated by Chang and others (2003).

Description of the South District Wastewater Treatment Plant

The SDWWTP is located in southeastern Miami-Dade County, Florida (fig. 1), and has a permitted capacity of 112.5 Mgal/d. The plant currently (2004) uses influent screening, 4 aerated grit chambers, 6 oxygenation trains, 2 cryogenic oxygen plants, 6 final clarifiers, 1 chlorination system, 1 effluent pump station, and 17 deep-injection wells (fig. 2). The reuse train (not shown in fig. 2) is not one of the primary treatment processes; it is supplied water from one of the injection well headers and consists of four Dynasand filters and four chlorine contact chambers and provides water for irrigation and plant washdown uses (Steve Kronheim, South District Wastewater Treatment Plant, oral commun., 2006). The CLO₂ and ultraviolet irradiation pilot units (not shown in fig. 2) were mobile units temporarily deployed during the HRD tests and were located next to the reuse train.

Residuals stabilization is accomplished using six primary and six secondary digesters followed by two sludge dewatering centrifuges and composting facilities. Flow into the plant occurs through one 54-in.- and two 72-in.-diameter influent force mains. The plant has been constructed in three phases, two of which have been completed. Phase 1 involved the construction of plant 1, which was completed and operational by 1983. Phase 2 involved the construction of about 50 percent of plant 2; phase 3, currently in progress, involves the completion of plant 2. The completed project will consist of two treatment plants with a total capacity of 150 Mgal/d.

The SDWWTP treats influent using a purely biological method known as the "pure oxygen activated sludge" process. Influent enters the plant and travels through the treatment process, from the bar screens to the effluent pump building, by means of gravity-driven flow (fig. 2). The bar screens have 0.5-in.-diameter openings that trap small nonsoluble material and debris that are collected for eventual transport to a nearby landfill. Influent then flows to the grit chambers where inorganic material settles and is collected for eventual transport to a disposal facility. Influent subsequently enters the oxygen trains where 95-percent pure oxygen, generated by the cryogenic oxygen generators, is diffused into the waste stream to facilitate bacterial multiplication and biodegradation of organic solids. Influent then flows to the clarifier tanks where the bacterial mass is allowed to settle, and eventually, is pumped back into the waste stream to facilitate further biodegradation.

Following the above process, the waste is pumped into the effluent pump building and is discharged into 17 deep waste injection wells. Excess bacterial growth promoted by the activated sludge process is separated and pumped into concentrator tanks, developing into sludge (fig. 2) that eventually is transported to digesters for degradation into acids and eventually into methane gas, carbon dioxide gas, and water. The methane gas is used as fuel to operate a generator that supplies

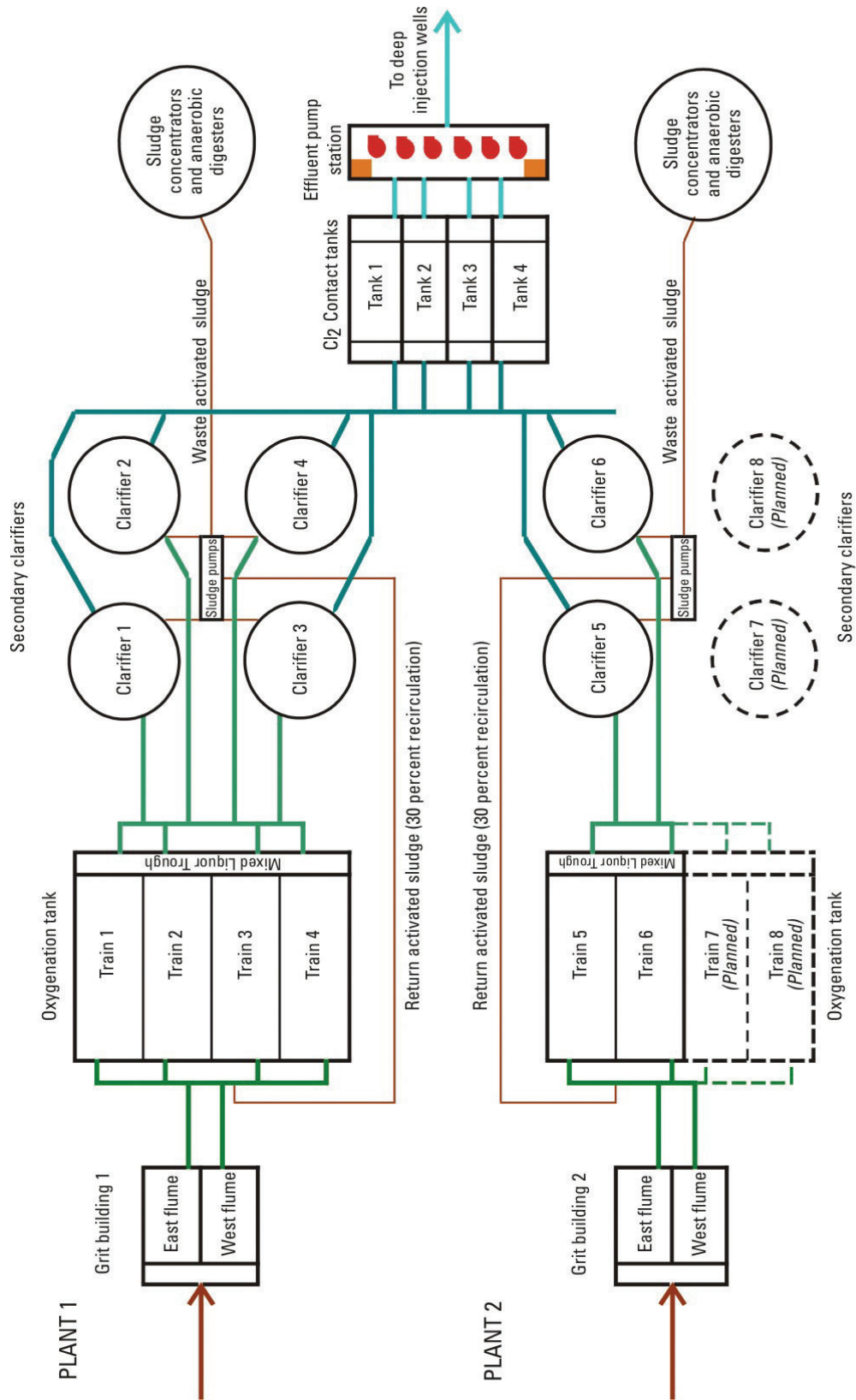


Figure 2. Treatment process at the South District Wastewater Treatment Plant.

electricity for the plant. The digested sludge is pumped into a centrifuge and thickened and removed to drying beds; the dried sludge is then composted and used as a soil conditioner.

Acknowledgments

The authors gratefully thank the South District Wastewater Treatment Plant personnel for their assistance with sample collection. Special gratitude is extended to Steve Kronheim, Assistant Plant Superintendent.

Methods of Investigation

For analysis of the organic wastewater compounds identified in this study, special sample collection and processing procedures were used to minimize the effects of contamination, bias, and uncertainty in the analytical results. Two types of samples were collected: 24-hour composites and discrete samples collected during the daily high-flow period (about 0730 time). These samples were compared statistically to determine whether a single discrete sample may adequately represent constituent concentrations in the waste stream. Samples were collected during dry-season, low-inflow conditions; wet-season, average-inflow conditions; and two HRD tests performed in the wet season. Both sample types were collected at the inflow to plants 1 and 2, effluent pump station, and reuse train during the dry-season, low-inflow event and the wet-season HRD tests. During the wet-season, average-inflow sampling event of July 20-21, 2004, only composite samples were collected. Additionally, samples were collected at the CLO₂ unit and ultraviolet irradiation pilot unit during the wet-season HRD tests.

Composite and discrete samples were collected using the same techniques during all sampling events, except for the sampling intervals used. Composite samples were collected as grab samples every 4 hours during the low-inflow and average-inflow sampling events using a 1-L stainless-steel, weighted-bottle sampler lowered into the waste stream. During the HRD tests, composite samples were collected in the same manner but at 2-hour intervals. All grab samples were composited in a Teflon churn splitter in the laboratory prior to filtration and processing. Contact with plastic was avoided during all sampling or processing procedures because many of the organic compounds adsorb to plastic surfaces, except during the HRD tests where this was impossible because certain components are composed of plastic. Discrete samples were collected in the same manner as composite samples during all sampling events.

Samples were analyzed for organic wastewater compounds, prescription and nonprescription pharmaceutical compounds, veterinary and human antibiotic compounds, and hormones. Analyses for samples of organic wastewater compounds and pharmaceutical compounds were performed at the USGS National Water Quality Laboratory (NWQL) in Denver,

Colorado. Analyses for samples of antibiotic compounds and hormones were performed at the Kansas Water Science Center, Organic Geochemistry Laboratory in Lawrence, Kansas.

Sampling and Processing Methods

To prevent contamination or sorption of analytes, water samples used to determine organic compounds should only have direct contact with inert materials such as glass, metal, fluorocarbon polymers (for example, Teflon), or ceramics during sampling and processing (Florida Department of Environmental Protection, 2004). In accordance with these requirements, water samples from the waste stream at the previously mentioned locations were collected using powderless nitrile laboratory gloves and a stainless-steel, weighted bottle sampler containing a 1-L amber borosilicate glass bottle with fluorocarbon polymer, fluorinated-ethylene-propylene lined caps. Because the chlorination process has been shown to facilitate degradation of certain organic constituents, samples collected at the reuse train and CLO₂ pilot unit were dechlorinated with ascorbic acid (Winslow and others, 2001).

Collected samples were chilled immediately to 4 °C and transported at the end of the 24-hour sampling period to the water-quality laboratory at the USGS Florida Integrated Science Center in Miami (now in Fort Lauderdale). Samples for each respective site and type were then composited in a 14-L fluoropolymer (Teflon) churn splitter for processing. Samples were processed according to protocol (U.S. Geological Survey, 2002). Processing equipment was decontaminated before and after samples were processed for each respective site according to protocol (U.S. Geological Survey, 2004).

Samples were processed in the following order based on their physical properties: organic wastewater compounds, pharmaceutical compounds, antibiotic compounds, and hormones. Sample bottles for collection of organic compounds were precleaned, baked and capped at the laboratory, and no further cleaning was required prior to use. Standard procedures for phase separation of trace organic compounds involved the use of an aluminum 142-mm-diameter plate filter assembly with 0.7- μ m nominal pore-size, glass-fiber filters; fluorocarbon polymer, fluorinated-ethylene-propylene tubing; and a ceramic-piston, valveless metering pump. Prior to decontaminating the equipment, a laboratory area was cleaned and lined with aluminum foil or fluorocarbon polymer sheeting. Disposable powderless nitrile gloves, a laboratory coat, and safety glasses were worn during the cleaning procedure.

The initial step in the cleaning procedure involved cleaning the equipment with a 0.2-percent nonphosphate, nonantibacterial grade laboratory detergent. This was followed by rinsing with: (1) deionized water, (2) pesticide-grade methanol, and (3) pesticide-grade organic-free water; all rinse agents were dispensed from fluorocarbon polymer rinse bottles. The equipment was then wrapped in aluminum foil and sealed in plastic bags prior to use.

Cleaning between sampling sites involved rinsing with: (1) 1-L 0.2-percent nonphosphate nonantibacterial detergent, (2) 1-L deionized water, (3) pesticide-grade methanol, and (4) pesticide-grade organic-free water. Prior to processing each sample, the filter assembly and glass-fiber filter were preconditioned by wetting with 10 to 20 mL of pesticide-grade organic-free water and preconditioned by processing 100 to 125 mL of the desired sample water.

Sampling for emerging contaminants required the use of special precautions to minimize the risk of compromising sample integrity. In accordance with this requirement, contact and consumption of specific compounds during sampling and processing for this study were avoided according to protocol (U.S. Geological Survey, 2002). These compounds include the following wastewater products: soaps and detergents, insect repellents, fragrances, sunscreen, caffeine, and tobacco products. Caution also was exercised in avoiding contact with the following pharmaceutical compounds: prescription drugs, medications and hormonal substances, nonprescription medications, and selected human and veterinary antibiotics. Powderless nitrile gloves were changed during activity changes and sample bottle changes. Direct contact between samples and clothing also was avoided during sampling and processing activities.

Laboratory Analytical Methods

The subsequent sections describe the analytical methods used to analyze household and industrial (organic) wastewater compounds, prescription and nonprescription pharmaceutical compounds, veterinary and human antibiotic compounds, and hormones. The analytical procedure used for the determination of organic wastewater compounds is an approved NWQL "production line" method. The procedures used for pharmaceutical compounds, antibiotic compounds, and hormones are provisional research methods not yet approved by the NWQL.

Organic Wastewater Compounds

As previously mentioned, the NWQL-approved method is designed for compounds in the dissolved phase; therefore, samples were filtered in the laboratory before shipment to the NWQL for analysis. When a sample is received by the laboratory, 60 g of sodium chloride is added prior to refrigeration at 4 °C to aid sample preservation (Zaugg and others, 2001). To ensure that samples are processed in a timely manner, the maximum holding time allowed for samples processed by this method is 14 days from the date of sample collection. This method is suitable for compounds that: (1) partition from the water phase onto a polystyrene-divinylbenzene organic phase, and (2) are sufficiently volatile and thermally stable for gas chromatography. This method is applicable to many compounds that typically are associated with industrial and household wastewater (Zaugg and others, 2001).

The NWQL analytical method used consists of several steps. Filtered 1-L water samples were extracted with disposable, polypropylene, solid-phase extraction (SPE) cartridges that contain a polystyrene-divinylbenzene phase. The SPE cartridges were dried by applying 2 L/min of pressurized nitrogen for about 45 minutes. Once the SPE cartridges were dry, the sample bottles were rinsed thoroughly using a 15-mL, 4:1 mixture of dichloromethane and diethyl ether. Sorbed compounds from the corresponding SPE cartridges were then eluted by this mixture rinsate. The extract was evaporated using a gentle stream of nitrogen to a final volume of 0.4 mL and transferred to an autosampler vial containing a 400-mL glass insert. Finally, the concentrated extracts were determined by capillary-column gas chromatography/mass spectrometry (GC/MS) (Zaugg and others, 2001). Recoveries in reagent water samples (fortified at 4 mg/L) averaged 74 percent, with 7-percent relative standard deviation for all method compounds. Initial method detection limits (MDLs) for single component compounds (excluding hormones and sterols) averaged 0.15 µg/L.

Pharmaceutical Compounds

The provisional analytical method used for the determination of pharmaceutical compounds utilizes combined SPE isolation and high-performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS). This method is sensitive for many compounds at concentrations less than 0.10 µg/L; MDLs average 0.022 µg/L (Cahill and others, 2004). Pharmaceutical compounds were isolated from 1-L filtered water samples using Waters Corp. Oasis hydrophobically and lipophilically balanced (HLB) 0.5-g, 6-mL SPE cartridges with a positive displacement pump (Fluid Metering Inc., Syosset, New York). A performance surrogate containing 1.0 µg of ¹³C-1-ethoxy-phenacetin in 100 mg/L of methanol was added to each filtered sample prior to extraction. Conditioning of the SPE cartridges was accomplished by: (1) rinsing with 6 mL of methanol, (2) vacuum drying at 1.6 kPa on a vacuum manifold for 10 to 15 seconds, and (3) rinsing with 6 mL of HPLC-grade water. Samples were processed through the SPE cartridge at 15 mL per minute.

After isolation, 1 mL of 5-percent methanol in reagent-grade water was passed through the cartridge using a vacuum manifold and discarded. Sequential elution of the cartridge with two 3-mL aliquots of methanol was followed by elution with two 2-mL aliquots of methanol acidified to a pH of 3.7 with sequanal-grade trifluoroacetic acid (Pierce Biotechnology Inc., Rockford, Illinois). The combined aliquots were evaporated until nearly dry with a Turbo Vap (Zymark, Hopkinton, Massachusetts) evaporative concentrator using nitrogen at a pressure of 34.5 kPa in a 40 °C bath. Samples were then reconstituted with 800 to 900 mL of formate buffer and 100 µL of caffeine-3-methyl-¹³C internal standard at a concentration of 0.010 mg/mL. The reconstituted samples were filtered through polytetrafluoroethylene/Teflon 0.2-mm syringe filters

(Acrodisc 13CR, Pall Corp., East Hills, New York). Environmental samples were then extracted in groups of 10 along with two laboratory quality-control samples: an HPLC-grade water sample containing only the performance surrogate, and an HPLC-grade water sample containing the performance surrogate and fortified at 1.0 mg/L.

The HPLC, coupled to mass spectrometry (HPLC/MS) using a Hewlett Packard (now Agilent Technologies, Inc., Palo Alto, California) Series 1100 HPLC/MSD, is used for analysis. A 10-mM ammonium formate/formic acid buffer (pH of 3.7) aqueous phase and acetonitrile were used to produce a multistep binary elution gradient. A flow rate of 0.200 mL/min was maintained, and all flow was directed to the MS. Separations were made using a Metasil Basic 3-mm, 150 by 20-mm, C-18 analytical column coupled to either a Metasil Basic Safeguard (MetaChem Technologies, Palo Alto, California), 3-mm, 2.0-mm guard column, or a New Guard RP-18, 7-mm, 15 by 3.2-mm guard column (Perkin-Elmer, Wellesley, Massachusetts).

The HPLC was interfaced with the MS using electrospray ionization (ESI) in the positive ionization mode. The ESI conditions were as follows: source temperature is 150 °C, nebulizer gas pressure is 100 kPa, drying gas flow rate is 9 L/min, and drying gas temperature is 350 °C. The potential difference between the source and capillary was held constant at 3,500 volts. Programmed capillary exit voltage changes were used to produce sufficient fragmentation for each compound so that characteristic fragments were produced. A series of flow-injection analyses were used for each compound to optimize the fragmentor voltage. For each compound, the optimal conditions for the protonated molecular ion and at least one confirming fragment ion were used for collecting data in the selected-ion-monitoring mode, thereby increasing the sensitivity of detection. A multipoint internal calibration standard, from 0.010 to 2.0 mg/L, was used for each sample set analyzed. Calibration was monitored through the use of continuing calibration verification samples, and if the calibration was within 20 percent, analysis of environmental and laboratory quality-control samples was continued.

Antibiotic Compounds

Water samples were analyzed for compounds in the macrolide, sulfonamide, quinoline, tetracycline, and beta-lactam classes of antibiotics. The beta-lactams and macrolides (BLM), sulfonamides and quinolones (SQ), and tetracyclines (TET) were analyzed separately using on-line SPE methods and liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) in positive-ion mode. Samples were extracted for the BLM and SQ methods using the HLB Prospekt cartridges (Waters Technologies, Milford, Massachusetts) and for the TET method using a proprietary Glyphosate Prospekt cartridge (Spark-Holland Inc., Plainsboro, New Jersey). Simatone was used as an internal standard for all three methods, and the surrogate standards were oleandomycin for the BLM method, nalidixic acid and ¹³C₆-sulfamethazine for

the SQ method, and meclocycline for the TET method. A 1.23-ng/μL solution of the internal standard, simatone, was diluted 1:20 for the BLM and SQ methods and 1:40 for the TET method. The diluted standard was transferred in an amber 2-mL chromatography vial and placed on the liquid chromatography autosampler tray.

Samples were prepared for analysis by pipetting a 10-mL aliquot of each sample into 11-mL glass crimp-top vials. About 75 mL of 6.67-ng/mL surrogate solutions were made in distilled water for the BLM and SQ methods and in a 0.7-percent EDTA-diNa solution for the TET method; 750 μL of the respective surrogate were added to each sample, standard, and blank. Standard curves were prepared for each method by diluting the respective 1-ng/μL standard mix to 1 ng/mL with distilled water. Standard solutions of 10 mL were then made at concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 μg/L.

A duplicate sample, 0.5-μg/L matrix spiked sample, and blank were analyzed after every 10th sample; a 1.0-μg/L standard solution was analyzed after every 20th sample. All standard solutions, blanks, and matrix spikes were treated the same as water samples. Sample modifier solutions were added to each sample just prior to extraction using the on-line SPE autosampler. The solutions were prepared for the SQ and TET methods by adding 250 μL of formic acid to 9.75 mL of deionized water, and for the BLM method by adding 10 mL of a 10-percent sodium chloride/0.5-percent EDTA-diNa solution to an 11-mL crimp-top vial.

All of the prepared samples were loaded into the on-line SPE Triathalon autosampler. During analysis, the on-line SPE lines were rinsed with solvents and solutions configured with the Triathalon autosampler and high-pressure dispenser. The cartridges were rinsed just prior to extraction. A water sample of 10 mL was pulled up through the sample vial into a Teflon sample loop and dispensed through the Prospekt SPE cartridge.

The antibiotics analyzed by each method were eluted and separated using a liquid-chromatograph gradient with the A and B mobile phases listed in the table below. The initial flow rates of the A and B mobile phases were decreased and contained a higher proportion of the B mobile phase to elute the Prospekt SPE cartridge. During the elution, the liquid-chromatography autosampler injected 20 μL of the internal standard. The isocratic mobile phase was used to increase the aqueous composition in the mobile phase stream to focus the compounds eluted from the SPE cartridge onto the head of the liquid-chromatography column. After the mobile phase flow was bypassed from the SPE cartridge, the flow rate was increased to 0.36 mL/min and the isocratic pump flow was turned off. A 3.0- by 150-mm Luna C18(2) SPE cartridge (Phenomenex) with 3-μm packing was used to separate the antibiotics for each of the three methods. The liquid-chromatography column was rinsed for 5 minutes with 100-percent mobile phase B solution at the end of the gradient and then equilibrated at initial conditions for 5 minutes before the next sample analysis. Mobile phases and the associated solvents for the TET, SQ, and BLM are as follows:

Mobile phase	Tetracyclines (solvent)	Sulfonamides and quinolines (solvent)	Macrolides and beta-lactams (solvent)
A	0.3-percent formic acid	0.3-percent formic acid	10 mM ammonium acetate
B	1.0-percent formic acid	Acetonitrile	Acetonitrile
C	Methanol	Not applicable	Not applicable
Isocratic	0.3-percent formic acid	0.3-percent formic acid	10 mM ammonium acetate

Individual antibiotic compounds were analyzed using selected ion monitoring and identified using retention times and the ratios of the quantifying ion to one to two confirming ions. The antibiotic compounds were then quantified using the base-peak-ion area ratio between the analyte and internal standard. The reporting levels ranged from 0.01 to 0.005 $\mu\text{g/L}$ for all of the antibiotic compounds analyzed.

Hormones

As previously mentioned, the analysis used for hormones is a provisional research method conducted by the Kansas Water Science Center, Organic Geochemistry Research Laboratory. Semiquantitative analyses of the hormones 17 beta-estradiol (E2), ethenyl estradiol (EE2), and estrone (E1) were made using an ELISA method and an SPE-LC/MS method.

Enzyme-Linked Immunosorbent Assays

The analysis of 17 beta-estradiol (E2) was performed using commercially available magnetic-particle ELISA kits (Abraxis, Warminster, Pennsylvania). The analytical protocol supplied by the manufacturer was followed, and the reported detection level is 1.5 ng/L. The analysis was initiated by adding 250 μL of either standard, control, or sample to a test tube containing 500 μL of estradiol antibody-coupled paramagnetic particles and vortexing for 1 to 2 seconds. After the samples were incubated for 30 minutes, 250 μL of estradiol enzyme conjugate was added to each sample and vortexed for 1 to 2 seconds. The samples were incubated for 90 minutes at room temperature and placed in a magnetic rack for 2 minutes to separate the magnetic particles from the water. Following separation, the test tubes were decanted and gently blotted to remove excess water. A 1-mL aliquot of washing solution was added to each sample for 2 minutes, followed by another round of decanting, blotting, and rinsing. The magnetic rack was then separated from the test tubes, and a color solution of 500 μL was added to each sample and vortexed for 1 to 2 seconds. The samples were incubated for 20 minutes at room temperature, followed by adding 500 μL of stopping solution and 1 mL of washing solution. The test tubes were then placed in a 450-nm wavelength spectrometer within 15 minutes after the stop solution was added to the samples.

The standard curve was obtained using a linear regression of the %B/Bo (mean standard absorbance for the 0, 2.5, 7.5, and 25 ng/L standards divided by the mean standard absorbance of the diluent/zero standard) on a logit Y variable against the log of the concentration as the X variable. The

correlation coefficient had to exceed 0.99 to achieve an acceptable relation.

Ethenyl estradiol (EE2) was analyzed using commercially available 96-well microtitre plate ELISA kits (Abraxis, Warminster, Pennsylvania). The analytical protocol supplied by the manufacturer was followed, and the

reported detection level is 0.05 $\mu\text{g/L}$. The analysis was initiated by reconstituting the antigen-enzyme conjugate powder in 7 mL of buffer solution, followed by diluting 50 mL of wash solution into 250 mL of distilled water. An ethenyl estradiol (EE2) standard or sample and an antigen-enzyme conjugate of 100 μL were then pipetted into wells of an uncoated 96-well microtitre plate and mixed. Each mixture of 100 μL was then pipetted into an antibody-coated 96-well microtitre plate, covered, and incubated for 1 hour at room temperature. Following incubation, the contents of the microtitre plate well were decanted and the wells rinsed three times with the diluted wash solution: once using 250 μL and twice using 200 μL . The substrate (color) solution of 100 μL was then pipetted into the microtitre plate wells and incubated for 30 minutes at room temperature. A stop solution of 100 μL was added to the microtitre plate wells containing the chromagen solution. The absorbance of each well was then analyzed using a 450-nm, 96-well microtitre plate reader.

The standard curve was obtained using a linear regression of the %B/Bo (mean standard absorbance for the 0, 0.05, 0.15, 0.5, and 3.0 $\mu\text{g/L}$ standards divided by the mean standard absorbance of the diluent/zero standard) on a logit Y variable against the log of the concentration as the X variable. The correlation coefficient had to exceed 0.99 to achieve an acceptable relation.

Estrone (E1) was analyzed with the same techniques used for ethenyl estradiol (EE2), except that a diluted chromagen-substrate solution was used instead of a substrate solution. The solution was prepared by adding 15 mL of substrate (color) to 150 μL of chromagen. The standard curve was obtained using a linear regression of the %B/Bo (mean standard absorbance for the 0, 0.05, 0.2, 0.5, 1.0, and 5.0 $\mu\text{g/L}$ standards divided by the mean standard absorbance of the diluent/zero standard) on a logit Y variable against the log of the concentration as the X variable. The correlation coefficient had to exceed 0.99 to achieve an acceptable relation.

Solid-Phase Extraction—Liquid Chromatography/Mass Spectrometry

Two subsets of samples, spiked and not spiked with estrone (E1), ethenyl estradiol (EE2), and 17 beta-estradiol (E2), were analyzed using SPE and LC/MS. Standard curves were prepared by spiking a 123-mL distilled-water sample using sample concentrations of 0.005, 0.01, 0.02, 0.05, 0.10, 0.2, 0.5, and 1.0 $\mu\text{g/L}$. Additionally, a set of 10 samples was spiked with a 0.2- $\mu\text{g/L}$ sample concentration.

Standard solutions, samples, and spiked samples were extracted using the following procedure. A C18 SPE column (6 mL, 500 mg, Waters Corp., Milford, Massachusetts) was conditioned with 2 mL of water, 2 mL of methyl alcohol (MeOH), 2 mL of dichloromethane, 2 mL of MeOH, and 2 mL of water. A sample of 100 mL was pumped through the SPE cartridge using a Zymark AutoTrace (Hopkington, Massachusetts). The column was dried for 1 minute and rinsed with 5 mL of hexane. The SPE cartridge was eluted twice with 4 mL of dichloromethane, and the eluate was evaporated to dryness in a Zymark turbopap at 50 °C and 103 kPa of molecular nitrogen. The samples were reconstituted with 3 mL of MeOH and vortexed for 5 seconds. An Amino-propyl cartridge (6 mL, 500 mg, Waters Corp., Milford, Massachusetts) was conditioned with 2 mL of MeOH, and the 3-mL MeOH sample eluate was pipetted onto the amino-propyl cartridge and eluted into a new 10-mL glass conical test tube. The SPE cartridge was eluted with 5 mL of MeOH. The sample eluate was evaporated to a volume of 100 µL and pipetted into a 2-mL amber vial with a 200-µL glass insert and stored at -4 °C until analysis.

The LC/MS analysis of the hormones was conducted at the Environmental Research Laboratory at the University of Missouri-Rolla. The MS was conducted using an Agilent 1100 Series LC/MS System (Model G1946D, Palo Alto, California) with a solvent degassing unit, gradient pump, autosampler, column temperature control, and MS. The analytes were separated on a Luna C18 (2) 150-mm-long, 4.6-mm-diameter column with 5-µm (average particle size) packing and a 4- by 3-mm-inside-diameter C18 precolumn security guard (Phenomenex Corp., Torrance, California). The initial composition of the mobile phase was 96-percent solution A (water) and 4-percent solution B (acetonitrile). The solvent ramp was linear as follows: 60 percent solvent B at 1 minute, 76 percent solvent B at 5 minutes, and 100 percent solvent B at 11 minutes where it was maintained for an additional 3 minutes to clean the column. The flow rate of the mobile phase was 0.7 mL per minute, and the injection volume was 30 µL.

The MS detection was performed by using ESI in negative-ion mode and selected ion monitoring mode for quantification. Nitrogen was used as the nebulizing, drying, and collision gas, and the MS nebulizer temperature was set at 350 °C. Compound identification was confirmed by: (1) the retention time of the compound of interest relative to the internal standard, and (2) the ratio of the area of the molecular ion plus hydrogen ion ($M + H$) relative to the area of at least one confirming ion. Quantitation was performed for each ion by the response ratio of the area of the $M + H$ relative to the area of the $M + H$ for the internal standard. Data processing was performed using Target 4.1 software on a Microsoft Windows platform (ThermoQuest Thru-Put Software, Orlando, Florida).

Evaluation of Emerging Contaminants of Concern in Influent and Effluent

As previously mentioned, the principal goals of this study were to assess: (1) the presence and concentrations of emerging contaminants of concern in SDWWTP influent and effluent, and (2) the percent transformations in concentration of contaminants at four locations in the waste stream—the effluent pump station, reuse train, CLO₂ unit, and the ultraviolet irradiation pilot unit. Table 1 lists the organic wastewater compounds analyzed for minimum reporting levels (MRLs), and possible sources or uses of compounds; table 2 lists the sampled pharmaceutical compounds and provisional MRLs; table 3 lists the antibiotic classes, sampled compounds, and MRLs; and table 4 lists the hormones and MRLs. As noted previously, samples were collected during four periods: a dry-season, low-inflow event on March 2-3, 2004; a wet-season, average-inflow event on July 20-21, 2004; and two HRD/CLO₂ and ultraviolet pilot tests on October 5 and 20, 2004. Composite and discrete samples were collected during the March 2-3, October 5, and October 20 sampling events; only composite samples were collected during the July 20-21 event. During the March and July sampling events, 24-hour composite samples were collected. During the October 5 and 20 sampling events, 3- and 6-hour composite samples, respectively, were collected corresponding to the length of the HRD tests. Table 5 lists sample types, sampling locations and events, and HRD tests for 2004. Table 6 lists the process flow rates and detention times corresponding to each sampling event at the SDWWTP.

Determination of the percent transformation in concentration at locations in the waste stream was accomplished by using the following formula:

$$\text{Percent transformation in concentration} = \frac{C - (A + B)}{A + B} \times 100, \quad (1)$$

where

C is the concentration of compound or constituent at a selected location in the waste stream,

A is the concentration of compound or constituent in inflow 1 from plant 1,

and

B is the concentration of compound or constituent in inflow 2 from plant 2.

Table 1. Organic wastewater compounds, method detection limits, minimum reporting levels, and possible sources or uses of compounds.

[Values shown in micrograms per liter]

Compound	Method detection limit	Minimum reporting level	Possible sources or uses of compound
1,4-Dichlorobenzene	0.28	0.5	Moth repellent, fumigate, deodorant
1-Methylnaphthalene	.13	.5	Gasoline, diesel fuel, crude oil
2,6-Dimethylnaphthalene	.10	.5	Present in diesel fuel, kerosene
2-Methylnaphthalene	.13	.5	Gasoline, diesel fuel, crude oil
3 beta-coprostanol	.52	2.0	Carnivore fecal indicator
3-Methyl-1H-indole (skatol)	.30	1.0	Fragrance, stench in feces, coal tar
3- <i>tert</i> -Butyl-4-hydroxyanisole (BHA)	3.06	5.0	Antioxidant, general preservative
4-Cumylphenol	.37	1.0	Nonionic detergent metabolite
4-Nonylphenol (total, NP)	.64	5.0	Nonionic detergent metabolite
4- <i>n</i> -Octylphenol	.33	1.0	Nonionic detergent metabolite
4- <i>tert</i> -Octylphenol	.37	1.0	Nonionic detergent metabolite
5-Methyl-1H-benzotriazole	.92	2.0	Antioxidant in antifreeze and deicers
Acetophenone	.09	.5	Fragrance in detergent, tobacco
Acetyl-hexamethyl-tetrahydro-naphthalene (AHTN)	.08	.5	Musk fragrance
Anthracene	.11	.5	Wood preservative, component of tar, diesel, crude oil
Anthraquinone	.11	.5	Used in manufacture of dye/textiles, seed treatment, bird repellent
Benzo[<i>a</i>]pyrene	.08	.5	Regulated polycyclic aromatic hydrocarbon, used in cancer research
Benzophenone	.12	.5	Fixative for perfumes and soaps
<i>beta</i> -Sitosterol	.60	2.0	Plant sterol
<i>beta</i> -Stigmastanol	.72	2.0	Plant sterol
Bisphenol A	.38	1.0	Used in manufacture of polycarbonate resins, antioxidant
Bromacil	.10	.5	General use pesticide
Caffeine	.09	.5	Beverages, diuretic
Camphor	.09	.5	Flavor, odorant, ointments
Carbaryl	.44	.5	Insecticide, crop and garden uses
Carbazole	.11	.5	Insecticide, manufacture of dyes, explosives, lubricants
Chlorpyrifos	.08	.5	Insecticide, domestic pest and termite control
Cholesterol	.71	2.0	Fecal indicator, plant sterol
Cotinine	.33	1.0	Primary nicotine metabolite
Diazinon	.07	.5	Insecticide, nonagricultural uses, ants, flies
Dichlorvos	.10	1.0	Insecticide, pet collars, flies
Diethoxynonylphenol (total NPEO2)	2.52	5.0	Nonionic detergent metabolite
Diethoxyoctylphenol (OPEO2)	.04	1.0	Nonionic detergent metabolite
<i>d</i> -Limonene	.05	.5	Fungicide, antimicrobial, antiviral, fragrance in aerosols
Fluoranthene	.08	.5	Component of coal tar and asphalt
Hexahydrohexamethyl cyclopentabenzopyran (HHCB)	.12	.5	Musk fragrance
Indole	.14	.5	Pesticide inert ingredient, fragrance in coffee
Isoborneol	.11	.5	Fragrance in perfumes, disinfectants

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Table 1. Organic wastewater compounds, method detection limits, minimum reporting levels, and possible sources or uses of compounds.—Continued

[Values shown in micrograms per liter]

Compound	Method detection limit	Minimum reporting level	Possible sources or uses of compound
Isophorone	.19	.5	Solvent for lacquer, plastic, oil, silicon, resin
Isopropylbenzene (cumene)	.04	.5	Manufacture of phenol/acetone, fuels, and paint thinner
Isoquinoline	.11	.5	Flavors and fragrances
Menthol	.08	.5	Cigarettes, cough drops, liniment, mouthwash
Metalaxyl	.08	.5	Herbicide, fungicide, mildew, blight, pathogens, golf/turf
Methyl salicylate	.08	.5	Liniment, food, beverage, ultraviolet-absorbing lotion
Metolachlor	.08	.5	Herbicide, indicator of agricultural drainage
Monoethoxyoctylphenol (OPEO1)	.29	1.0	Nonionic detergent metabolite
N,N-diethyl- <i>meta</i> -toluamide (DEET)	.14	.5	Mosquito repellent
Naphthalene	.15	.5	Fumigant, moth repellent
<i>para</i> -Cresol	.27	1.0	Wood preservative
Pentachlorophenol	.39	2.0	Herbicide, fungicide, wood preservative, termite control
Phenanthrene	.08	.5	Explosives, tar, diesel fuel, crude oil
Phenol	.11	.5	Disinfectant
Prometon	.12	.5	Herbicide, applied prior to blacktop
Pyrene	.08	.5	Component of coal tar and asphalt
Tetrachloroethylene	.03	.5	Solvent, degreaser, veterinary anthelmintic
Tribromomethane	.18	.5	Wastewater ozonation by-product, military explosives
Tributyl phosphate	.10	.5	Plasticizer, resin, wax, finish, roofing paper
Triclosan	.48	1.0	Disinfectant, antimicrobial
Triethyl citrate (ethyl citrate)	.09	.5	Cosmetics, pharmaceuticals
Triphenyl phosphate	.06	.5	Plasticizer, resin, wax, finish, roofing paper
Tris(2-butoxyethyl) phosphate	.20	.5	Flame retardant
Tris(2-chloroethyl) phosphate	.08	.5	Plasticizer, flame retardant
Tris(dichloroisopropyl) phosphate	.08	.5	Flame retardant

Table 2. Prescription and nonprescription pharmaceutical compounds and provisional minimum reporting levels.

[Values shown in micrograms per liter; ND, reporting level not yet established]

Compound	Provisional minimum reporting level
1,7-dimethylxanthine	0.140
Acetaminophen	.036
Azithromycin	.004
Caffeine	.016
Carbamazapine	.011
Cimetidine	.012
Codeine	.015
Cotinine	.014
Dehydronifedipine	.015
Diltiazem	.016
Diphenhydramine	.015
Erythromycin	.009
Fluoxetine	.014
Furosemide	ND
Gemfibrozil	.013
Ibuprofen	.042
Metformin	ND
Miconazole	.018
Ranitidine	.013
Salbutamol	.023
Sulfamethoxazole	.064
Thiabendazole	.011
Trimethoprim	.013
Warfarin	.012

Table 3. Antibiotic classes and compounds and minimum reporting levels.

[Values shown in micrograms per liter. Degradation product is in red]

Antibiotic and degradation products	Minimum reporting level	Antibiotic and degradation products	Minimum reporting level
Macrolides		Tetracyclines	
Azithromycin	0.005	Alpha apo-oxytetracycline	0.010
Anhydro-erythromycin	.005	Anhydro-chlorotetracycline	.010
Erythromycin	.005	Anhydro-tetracycline	.010
Lincomycin	.005	Beta-apo-oxytetracycline	.010
Ormetoprim	.005	Chlorotetracycline	.010
Roxithromycin	.005	Demeclocycline	.010
Trimethoprim	.005	Doxycycline	.010
Tylosin	.005	Epi-anhydro-chlorotetracycline	.010
Virginiamycin	.005	Epi-anhydro-tetracycline	.010
Quinolines		Epi-chlorotetracycline	.010
Ciprofloxacin	.005	Epi-oxytetracycline	.010
Clinafloxacin	.005	Epi-tetracycline	.010
Flumequine	.005	Epi-iso-chlorotetracycline	.010
Lomefloxacin	.005	Iso-chlorotetracycline	.010
Norfloxacin	.005	Minocycline	.010
Ofloxacin	.005	Oxytetracycline	.010
Oxolinic acid	.005	Tetracycline	.010
Sarafloxacin	.005	<i>beta lactams</i>	
Sulfonamides		Amoxicillin	.010
Sulfachlorpyridazine	0.005	Ampicillin	.010
Sulfadiazine	.005	Cefotaxime	.010
Sulfadimethoxine	.005	Cloxacillin	.010
Sulfamerazine	.005	Oxacillin	.010
Sulfamethazine	.005	Penicillin G	.010
Sulfamethoxazole	.005	Penicillin V	.010
Sulfathiazole	.005		

Table 4. Hormones and minimum reporting levels.

[Values shown in micrograms per liter, except for 17 beta-estradiol, which is in nanograms per liter]

Compound	Minimum reporting level
17 beta-estradiol (E2)	2.0
Ethenyl estradiol (EE2)	.05
Estrone (E1)	.05

Table 5. Sample types, sampling locations, average inflow, and high-rate disinfection (HRD) tests for four sampling events in 2004.

[Mgal/d, million gallons per day]

Sampling location	Dry-season low-inflow sampling event March 2-3, 2004 (83.7 Mgal/d)		Wet-season average-inflow sampling event July 20-21, 2004 (89.7 Mgal/d)		Wet-season HRD test October 5, 2004 (84.1 Mgal/d)		Wet-season HRD test October 20, 2004 (119.6 Mgal/d)	
	Composite sample (24 hours)	Discrete sample	Composite sample (24 hours)	Discrete sample	Composite sample (3 hours)	Discrete sample	Composite Sample (6 hours)	Discrete sample
Chlorine dioxide unit					x	x	x	x
Effluent pump station	x	x	x		x	x	x	x
Inflow plant 1	x	x	x		x	x	x	x
Inflow plant 2	x	x	x		x	x	x	x
Reuse station	x	x	x		x	x	x	x
Ultraviolet pilot unit					x	x	x	x

Table 6. Process flow rates and detention times at the South District Wastewater Treatment Plant during four sampling events in 2004.

[Schematic diagram showing different wastewater treatment stages is shown in figure 2. Sludge detention time is 20-30 days. Abbreviations: HRD, high-rate disinfection; Mgal/d, million gallons per day; N/A, not applicable]

Wastewater-treatment stage	Dry-season low-inflow sampling event March 2-3, 2004		Wet-season average-inflow sampling event July 20-21, 2004		Wet-season HRD test October 5, 2004		Wet-season HRD test October 20, 2004	
	Process flow rate (Mgal/d)	Detention time (hours)	Process flow rate (Mgal/d)	Detention time (hours)	Process flow rate (Mgal/d)	Detention time (hours)	Process flow rate (Mgal/d)	Detention time (hours)
Influent (plant 1)	54	N/A	58	N/A	55	N/A	78	N/A
Influent (plant 2)	29	N/A	31	N/A	29	N/A	42	N/A
Grit building 1, east and west flumes	27.2	.13	29.2	.13	27.3	.13	38.9	.09
Grit building 2, east and west flumes	14.6	.25	15.7	.23	14.7	.25	20.9	.18
Oxygenation tank 1 (train 1-4)	17.7	1.87	18.9	1.75	17.8	1.86	25.3	1.31
Oxygenation tank 2 (train 5-6)	19.0	1.74	20.4	1.62	19.1	1.73	27.2	1.22
Return activated sludge (plant 1)	16.3	N/A	17.5	N/A	16.4	N/A	23.3	N/A
Return activated sludge (plant 2)	8.8	N/A	9.4	N/A	8.8	N/A	12.6	N/A
Secondary clarifiers (1-4)	17.7	4.55	18.9	4.24	17.8	4.53	25.3	3.18
Secondary clarifiers (5-6)	19.0	4.22	20.4	3.94	19.1	4.20	27.2	2.96
Waste activated sludge (plant 1)	1.1	N/A	1.2	N/A	1.1	N/A	1.6	N/A
Waste activated sludge (plant 2)	.5	N/A	.6	N/A	.5	N/A	.8	N/A
Chlorine contact tanks (1-4)	20.93	.50	22.43	.46	21.03	.50	29.9	.35
Total	83.7	7.05 ¹ 6.71 ²	89.7	6.58 ¹ 6.26 ²	84.1	7.02 ¹ 6.68 ²	119.6	4.94 ¹ 4.70 ²

¹Plant 1 total.

²Plant 2 total.

An important concept in understanding these data is the difference between the MDL and the minimum reporting level (MRL). The MDL is defined as the minimum concentration of a constituent that can be determined and reported with 99-percent confidence that the value is greater than zero (U.S. Environmental Protection Agency, 1997). The MRL is defined as the lowest measured concentration of a compound that may be reliably reported by a certain analytical method (Timme, 1995).

The MRL is the lowest concentration that the NWQL calibrates to for a specific analysis, and values below this level are routinely reported but must meet specific qualitative criteria (retention times, quantification and qualification ions, and ion ratios) for the analysis in question. These values, however, are qualified with an "e," indicating they are considered estimated (S.D. Zaugg, U.S. Geological Survey, written commun., 2006).

Dry-Season Low-Inflow Constituents

The dry-season, low-inflow sampling event was conducted during March 2-3, 2004, with a measured average inflow rate of 83.7 Mgal/d. During this period, 26 constituents were detected and constituent percent transformations in concentration were determined (table 7). One constituent percent transformation in concentration at the effluent pump and two at the reuse train were not determined because of censored data below the MDL. The percent transformed in concentration at the effluent pump and reuse train for 13 and 12 constituents, respectively, were estimated because one or more data values were below the MRLs. The transformations in concentration for constituents ranged from -100 to 180 percent at the effluent pump and from -100 to 85 percent at the reuse train. The diltiazem and diphenhydramine pharmaceutical compounds showed positive transformations in concentration at the effluent pump and reuse train, respectively. One pharmaceutical compound (codeine) and one macrolide antibiotic compound (trimethoprim) showed a positive percent transformation in concentration only at the effluent pump. Two "known" endocrine disrupting compounds—17 beta-estradiol (E2) and diethoxynonylphenol—and four "suspected" endocrine disrupting compounds—1,4-dichlorobenzene, benzophenone, tris(2-chloroethyl) phosphate, and tris(dichloroisopropyl) phosphate—were detected at the effluent pump and reuse train. Of the 25 compounds detected at the effluent pump, 9 compounds had transformations in concentration of -90 percent or greater and 15 compounds had transformations in concentration of -80 percent or greater. Of the 24 compounds detected at the reuse train, 7 compounds had transformations in concentration of -90 percent or greater and 14 compounds had transformations in concentration of -80 percent or greater. Anomalous positive percent transformations in concentration for selected compounds (codeine, diltiazem, diphenhydramine, and trimethoprim) at the effluent pump and (or) reuse train may result from solubility changes caused

by matrix conditions. Additionally, selected compounds are sensitive and easily ionizable in the LC/MS mode and give a strong signal at the detector when interferences are minimal. When the extract complexity increases as in sewage influent, increased competition for ionization in the LC/MS source results in a weaker signal, and thus, a detected lower concentration (J.D. Cahill, U.S. Geological Survey, written commun., 2005).

Wet-Season Average-Inflow Constituents

The wet-season, average-inflow sampling event was conducted during July 20-21, 2004, with a measured average inflow rate of 89.7 Mgal/d. During this period, 27 constituents were detected and constituent percent transformations in concentration were determined (table 8). Five constituent percent transformations in concentration at the effluent pump and one at the reuse train were not determined because of censored data below the MDL. The percent transformations in concentration for 6 and 11 constituents were estimated at the effluent pump and reuse train, respectively, because one or more data values were below the MRLs. These transformations in concentration ranged from -100 to 1,609 percent at the effluent pump and from -100 to 832 percent at the reuse train. Erythromycin, anhydro-erythromycin, diltiazem, diphenhydramine, and tylosin all showed positive percent transformations in concentration at the effluent pump. The same constituents as well as phenathrene showed positive changes at the reuse train. Erythromycin and tylosin are macrolide antibiotics, anhydro-erythromycin is a macrolide degradation product, and diltiazem and diphenhydramine are pharmaceutical compounds. One "known" endocrine disrupting compound, 17 beta-estradiol (E2), and one "suspected" endocrine disrupting compound, benzophenone, were detected at both the effluent pump and reuse train. Of the 22 compounds detected at the effluent pump, 8 compounds had transformations in concentration of -90 percent or greater and 10 compounds had percent transformations of -80 percent or greater. Of the 26 compounds detected at the reuse train, 10 compounds had transformations in concentration of -90 percent or greater and 11 compounds had transformations in concentration of -80 percent or greater.

Constituents Detected during High-Rate Disinfection/Chlorine Dioxide and Ultraviolet Pilot Tests

The Miami-Dade Water and Sewer Authority conducted tests that evaluated alternative disinfection strategies to compare HRD/CLO₂ and ultraviolet irradiation in the removal of pathogens to the current high-level disinfection procedure with chlorination used at the SDWWTP. Because HRD/CLO₂ and ultraviolet irradiation have been known to selectively transform and remove emerging contaminants (Snyder and others, 2003), assessing these strategies was considered important.

Table 7. Constituents detected during the dry-season, low-flow sampling event of March 2-3, 2004, percent transformations in concentrations at the effluent pump and reuse train, and endocrine disruptor status.

[C, unable to compute percent transformed due to censored data; e, estimated due to one or more data values below the minimum reporting level. Positive percent transformed in red]

Compound	Percent transformed at effluent pump	Percent transformed at reuse train	Endocrine disruptor status
1,4-Dichlorobenzene	-80e	-83e	Suspected
1,7-dimethylxanthine	-97	-98	
17 beta-estradiol (E2)	-95	-79	Known
3 beta-coprostanol	-91e	C	
4-Nonylphenol	-97e	-97e	
Acetaminophen	-100	C	
Acetophenone	-85e	-85e	
Acetyl-hexamethyl-tetrahydro-naphthalene (AHTN)	-56	-63	
Benzophenone	-88e	-88e	Suspected
Caffeine	-97e	-98e	
Carbamazepine	-27	-23	
Cholesterol	-90e	-90e	
Codeine	12	-49	
Cotinine	-89e	-90e	
Diethoxynonylphenol	C	-85	Known
Diltiazem	180	32	
Diphenhydramine	150	85	
Indole	-10	-6	
Isoborneol	-76e	-82e	
Metformin	-8	-32	
Monoethoxyoctylphenol	-100	-100	
N,N-diethyl- <i>meta</i> -toluamide (DEET)	-88e	-88e	
Trimethoprim	8	-1	
Tris(2-butoxyethyl) phosphate	-98e	-98e	
Tris(2-chloroethyl) phosphate	-80e	-80e	Suspected
Tris(dichloroisopropyl) phosphate	-70e	-72e	Suspected

The existing high-level disinfection procedure includes sand filtration followed by chlorination. During these tests, unfiltered secondary effluent was diverted from one of the injection wells and conveyed independently to the HRD/CLO₂ unit and ultraviolet pilot unit. High total suspended-solids concentrations were achieved during these tests by seeding the influent with return activated sludge to attain a total suspended-solids concentration of 40 mg/L, which is favorable for pathogen adsorption to suspended material. Composite and

discrete samples were collected at the HRD/CLO₂ and ultraviolet pilot units in the tests performed during the wet season on October 5 and 20, 2004, at average inflow rates of 84.1 and 119.6 Mgal/d and durations of 3 and 6 hours, respectively.

Table 8. Constituents detected during the wet-season, average-flow sampling event of July 20-21, 2004, percent transformations in concentrations at the effluent pump and reuse train, and endocrine disruptor status.

[C, unable to compute percent transformed due to censored data; e, estimated due to one or more data values below the minimum reporting level. Positive percent transformed in red]

Compound	Percent transformed at effluent pump	Percent transformed at reuse train	Endocrine disruptor status
1,7-dimethylxanthine	-98	-97	
17 beta-estradiol (E2)	-56	-93	Known
3 beta-coprostanol	C	-94e	
Acetyl-hexamethy-tetrahydro-naphthalene (AHTN)	-86	-56e	
Anhydro-erythromycin	11	38	
Benzophenone	-91e	-73e	Suspected
Caffeine	-99e	-98e	
Camphor	C	-97e	
Carbamazapine	-42	-13	
Cholesterol	C	-92e	
Ciprofloxacin	-57	-94	
Codeine	-13	-74	
Cotinine	-99	-98	
Diltiazem	1,609	801	
Diphenhydramine	911	832	
Erythromycin	87	130	
Estrone (E1)	-100	-100	
Hexahydrohexamethyl cyclopentabenzopyran	C	-63e	
Menthol	-99e	C	
N,N-diethyl- <i>meta</i> -toluamide (DEET)	-98e	-96e	
Ofloxacin	-62	-75	
Phenanthrene	C	25e	
Sulfamethoxazole	-85	-78	
Triethyl citrate	-75e	-50e	
Trimethoprim	-37	-29	
Tris(2-butoxyethyl) phosphate	-96e	-83e	
Tylosin	125	216	

October 5th Sampling Event

During the October 5 sampling event, 29 constituents were detected and percent transformations in concentration were determined (table 9). There were no censored data values for any of the percent transformations in concentration determined at the effluent pump; however, censored data prevented the determination of six constituent percent transformations in concentration at the reuse train, seven at the ClO₂ unit, and four at the ultraviolet pilot unit. Additionally, eight constituent

percent transformations in concentration were estimated for the same constituents at all four locations in the waste stream.

Ranges of transformations in concentration for constituents were -100 to -37 percent at the effluent pump, -100 to -62 percent at the reuse train, -100 to -56 percent at the ClO₂ unit, and -100 to -40 percent at the ultraviolet pilot unit. Two "known" endocrine disruptor compounds, 17 beta-estradiol (E2) and diethoxynonylphenol, and one "suspected" endocrine disruptor compound, benzophenone, were detected.

Table 9. Constituents detected during the high-rate disinfection sampling event of October 5, 2004, percent transformations in concentrations at selected locations, and endocrine disruptor status.

[C, unable to compute percent transformed due to censored data; e, estimated due to one or more data values below the minimum reporting level; UV, ultraviolet]

Compound	Percent concentration transformation				Endocrine disruptor status
	Effluent pump	Reuse train	Chlorine dioxide unit	UV pilot unit	
1,7-dimethylxanthine	-95	-97	-99	-95	
17 beta-estradiol (E2)	-41	-93	-94	-57	Known
3 beta-coprostanol	-96e	-98e	-96e	-98e	
4-Nonylphenol	-96e	-96e	-97e	-97e	
Acetyl-hexamethyl-tetrahydro naphthalene (AHTN)	-66	-69	-71	-69	
Anhydro-tetracycline	-74	C	C	C	
Benzophenone	-88e	-88e	-79e	-88e	Suspected
Caffeine	-97	-99	-98	-98	
Carbamazapine	-85	-94	-56	-54	
Cholesterol	-96e	-99e	-97e	-97e	
Ciprofloxacin	-84	C	C	-92	
Codeine	-37	-76	-64	-40	
Cotinine	-99	-100	-98	-95	
Diethoxynonylphenol	-86	-86	-82	-89	Known
Doxycycline	-53	C	C	C	
Erythromycin	-73	C	C	-81	
Estrone (E1)	-100	-100	-100	-100	
Hexahydrohexamethyl cyclopentabenzopyran N,N-diethyl- <i>meta</i> -toluamide (DEET)	-72e	-80e	-80e	-88e	
Ofloxacin	-66	-94	-92	-72	
Phenol	-98	-99	-99	-98	
Sulfamethoxazole	-92	-91	C	-96	
Tetracycline	-68	C	C	C	
Triethyl citrate	-74e	-74e	-68e	-74e	
Trimethoprim	-56	-73	-60	-44	
Triphenyl phosphate	-71e	-86e	-86e	-86e	
Tris(2-butoxyethyl) phosphate	-96	-96	-96	-96	
Tris(dichloroisopropyl) phosphate	-62	-62	-62	-69	
Tylosin	-87	C	C	C	

Results indicate that 29, 23, 22, and 25 compounds were detected at the effluent pump, reuse train, CLO₂ unit, and ultraviolet pilot unit, respectively. Of the compounds detected, 11 compounds had transformations in concentration of -90 percent or greater and 16 compounds had transformations in concentration of -80 percent or greater at the effluent pump; 14 compounds had transformations in concentration of -90 percent or greater and 18 compounds had transformations in concentration of -80 percent or greater at the reuse train;

12 compounds had transformations in concentration of -90 percent or greater and 15 compounds had transformations in concentration of -80 percent or greater at the CLO₂ unit; and 12 compounds had transformations in concentration of -90 percent or greater and 17 compounds had transformations in concentration of -80 percent or greater at the ultraviolet pilot unit.

The use of CLO₂ as a disinfectant can result in oxidation of selected emerging contaminants such as herbicides,

pesticides, and phenylarsenine oxides and is generally a stronger and faster oxidant than chlorine. Ultraviolet irradiation has been used widely for microbial disinfection and may also be of use in treatment of selected emerging contaminants, especially some endocrine disrupting compounds and pharmaceuticals and personal care products (Snyder and others, 2003). A comparison of selected constituent percent transformations determined at the effluent pump and reuse train with those determined at the CLO₂ and ultraviolet units indicates that there were no additional compounds transformed as a result of either the CLO₂ or ultraviolet processes. Several antibiotic results were censored (anhydro-tetracycline, ciprofloxacin, doxycycline, erythromycin, sulfamethoxazole, tetracycline, and tylosin), and consequently, some percent transformations could not be determined for these compounds at the CLO₂ or ultraviolet pilot units. One constituent (carbamazepine) showed a generally lower percent transformation at both the CLO₂ and ultraviolet pilot units than at the effluent pump and reuse train. Two constituents (17 beta-estradiol (E2) and ofloxacin) showed lower percent transformations at the reuse train and CLO₂ units than at the ultraviolet pilot unit but were higher at the effluent pump. Most constituents showed comparable transformations at all four locations in the waste stream.

October 20th Sampling Event

During the October 20 sampling event, 35 constituents were detected and percent transformations in concentration were determined (table 10). Censored data prevented the determination of four constituent percent transformations at the effluent pump, six at the reuse train, five at the CLO₂ unit, and six at the ultraviolet pilot unit. Additionally, 14 constituent percent transformations in concentration were estimated for the same constituents at all four locations in the waste stream.

Ranges of transformations in concentration were -100 to -4 percent at the effluent pump, -100 to 17 percent at the reuse train, -100 to -40 percent at the CLO₂ unit, and -100 to -14 percent at the ultraviolet pilot unit. A positive 17-percent transformation in concentration was determined for phenanthrene at the reuse train. Phenanthrene sources include explosives, tar, diesel fuel, and crude oil. Four "suspected" endocrine disrupting compounds—1,4-dichlorobenzene, benzophenone, tris(2-chloroethyl) phosphate, and tris(dichloroisopropyl) phosphate—and two "known" endocrine disrupting compounds—17 beta-estradiol (E2) and diethoxynonylphenol—were detected and percent transformations in concentration were determined during this sampling.

Results indicate that 31, 29, 30, and 29 compounds were detected at the effluent pump, reuse train, CLO₂ unit, and ultraviolet pilot unit, respectively. Of the compounds detected, eight compounds had transformations in concentration of -90 percent or greater and 12 compounds had transformations in concentration of -80 percent or greater at the effluent pump; 6 compounds had transformations in concentration of -90 percent or greater and 12 compounds had transformations

in concentration of -80 percent or greater at the reuse train; 12 compounds had transformations in concentration of -90 percent or greater and 15 compounds had transformations in concentration of -80 percent or greater at the CLO₂ unit; and 7 compounds had transformations in concentration of -90 percent or greater and 14 compounds had transformations in concentration of -80 percent or greater at the ultraviolet pilot unit.

Comparison of the percent transformations at the CLO₂ and ultraviolet pilot units with those at the reuse train and effluent pump indicated that there were no additional compounds transformed by these processes other than those already transformed at the effluent pump and reuse train. Several constituents (anthracene, carbazole, naphthalene, phenanthrene, phenol, and sulfamethoxazole) could not be compared because of censored data values at either the CLO₂ and ultraviolet pilot units. Several constituents (codeine, diltiazem, and trimethoprim) showed higher percent transformations at the CLO₂ unit than that at the other three locations; however, most constituents showed comparable percent transformations at all four locations in the waste stream.

Concentration and Distribution of Constituents Detected in Composite Samples

During this study, composite samples were collected from both influent and effluent for detection of 129 different constituents, including 65 organic wastewater compounds, 24 pharmaceutical compounds, 37 antibiotic compounds, and 3 hormones. The concentrations and ranges of constituents detected in influent and effluent are shown as boxplots in figures 3 to 6. In several instances, multiple detection limits were used by the laboratories because of matrix effects. The boxplots are censored at the highest detection limit; constituents were not included for which all data exist only below the highest detection limit. The two divisions within each boxplot represent the lowest and highest values within the interquartile range (25th to 75th percentile). Upper and lower outside values represent values greater than 1.5 times the interquartile range, and upper and lower detached values represent data values greater than 3 times the interquartile range.

Constituents Detected in Influent

Constituents detected in influent consisted of 38 organic wastewater compounds, 13 pharmaceutical compounds, 12 antibiotic compounds, and 2 hormones. Among the organic wastewater compounds (fig. 3), phenanthrene and indole showed the greatest concentration ranges (0.1 to 96 µg/L and 0.2 to 91.7 µg/L, respectively) and the highest concentrations. Phenanthrene is a combustion product used in the manufacture of explosives and is a component of tar, diesel fuel, and crude oil. Indole is a pesticide inert ingredient and also is used as a fragrance in coffee. Triethyl citrate, found in cosmetics and pharmaceuticals, showed the smallest concentration range (1.1 to 1.9 µg/L).

Table 10. Constituents detected during the high-rate disinfection sampling event of October 20, 2004, percent transformations in concentrations at selected locations, and endocrine disruptor status.

[C, unable to compute percent transformed due to censored data; e, estimated due to one or more data values below the minimum reporting level; Positive percent transformed in red]

Compound	Percent transformed at effluent pump	Percent transformed at reuse train	Percent transformed at chlorine dioxide unit	Percent transformed at ultraviolet pilot unit	Endocrine disruptor status
1,4-Dichlorobenzene	-83e	-83(e)	-83e	-83e	Suspected
1,7-dimethylxanthine	-98	-98	C	-96	
17 beta-estradiol (E2)	-65	-86	-98	-82	Known
4-Nonylphenol	-98e	-96e	-99e	-99e	
Acetophenone	-75e	-88e	-75e	-75e	
Acetyl-hexamethyl-tetrahydro-naphthalene	-49e	-53e	-55e	-45e	
Anhydro-erythromycin	-70	-73	-77	-72	
Anthracene	C	-50	C	C	
Benzophenone	-85e	-77e	-85e	-85e	Suspected
Caffeine	-98	-98	-98	-98	
Carbamazepine	-55	-73	-74	-51	
Carbazole	C	-75	C	C	
Ciprofloxacin	-74	C	-93	-89	
Codeine	-52	C	-74	-53	
Cotinine	-90	-90	-90	-90	
Diethoxynonylphenol	-63e	-63e	-69e	-69e	Known
Diltiazem	-4	C	-54	-38	
Diphenhydramine	-29	-62	-60	-14	
Erythromycin	-76	C	-90	-78	
Estrone (E1)	-100	-100	-100	-100	
Hexahydrohexamethyl cyclopentabenzopyran	-76e	-76e	-76e	-82e	
N,N-diethyl- <i>meta</i> -toluamide (DEET)	-96e	-99e	-99e	-99e	
Naphthalene	-92	C	C	C	
Ofloxacin	-68	-79	-91	-78	
Phenanthrene	C	17	C	C	
Phenol	C	C	-96	C	
Sulfamethoxazole	-89	-60	-87	C	
Tetrachloroethylene	-60e	-80e	-60e	-80e	
Tetracycline	-85	-88	-97	-88	
Triethyl citrate	-55e	-55e	-55e	-55e	
Trimethoprim	-29	-35	-61	-59	
Triphenyl phosphate	-50e	-50e	-50e	-50e	
Tris(2-butoxyethyl) phosphate	-90e	-85e	-92e	-92e	
Tris(2-chloroethyl) phosphate	-50e	-50e	-50e	-50e	Suspected
Tris(dichloroisopropyl) phosphate	-40e	-40e	-40e	-40e	Suspected

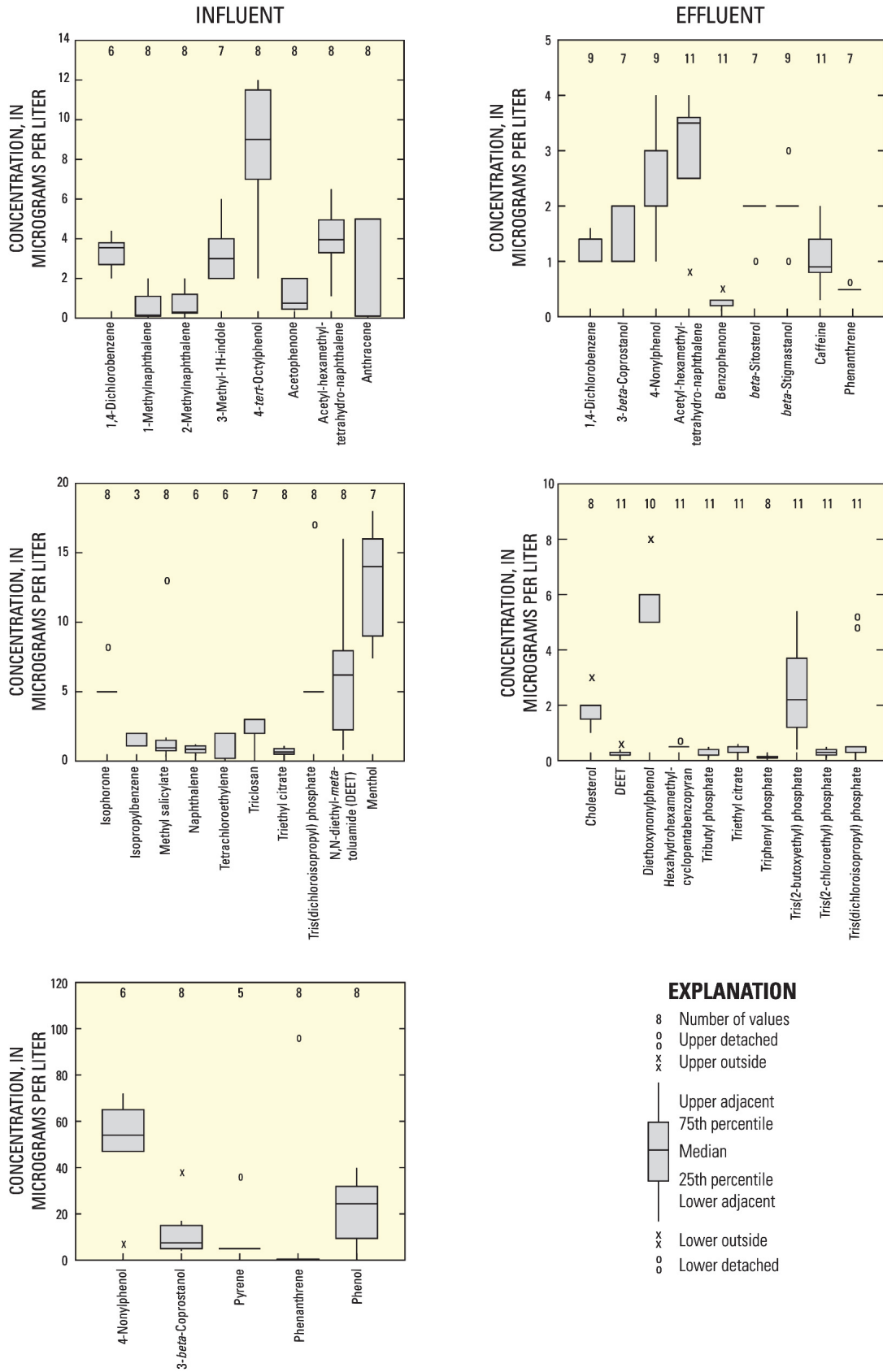


Figure 3. Constituent concentrations and ranges of organic wastewater compounds in influent and effluent.

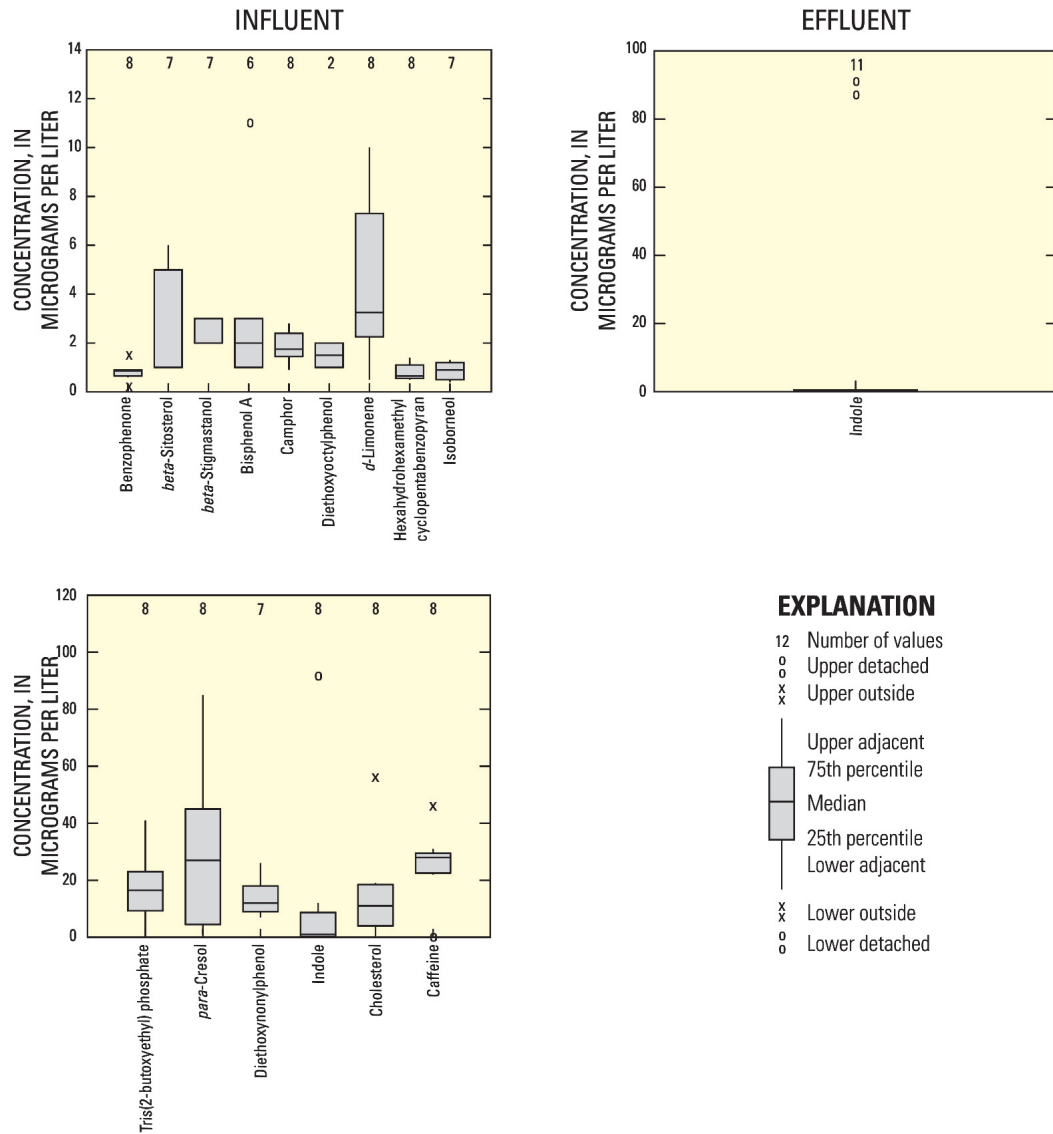


Figure 3. Constituent concentrations and ranges of organic wastewater compounds in influent and effluent.—Continued

Among the pharmaceutical compounds (fig. 4), acetaminophen showed the greatest concentration range (0.04 to 32.5 $\mu\text{g/L}$) and highest concentration. Warfarin showed the smallest concentration range from 0.012 (MRL) to 0.015 $\mu\text{g/L}$.

Among the antibiotic compounds (fig. 5), sulfamethoxazole (a sulfonamide) showed the greatest concentration range (less than 0.05 to 1.97 $\mu\text{g/L}$) and highest concentration. Sulfathiazole, also a sulfonamide, showed the smallest concentration range (less than 0.005 to 0.05 $\mu\text{g/L}$).

Two hormones were detected in influent (fig. 6). Estrone (E1) and 17 beta-estradiol (E2) ranged in concentration from 0.07 to 0.10 $\mu\text{g/L}$ and 13 to 62 ng/L , respectively.

Constituents Detected in Effluent

Constituents detected in effluent consisted of 20 organic wastewater compounds, 11 pharmaceutical compounds, 8 antibiotic compounds, and 1 hormone. Among the organic wastewater compounds (fig. 3), indole showed the greatest concentration range (less than 0.5 to 90.9 $\mu\text{g/L}$) and highest concentration. Triphenyl phosphate, used as a plasticizer and found in resin, wax, and roofing paper, showed the smallest concentration range (0.1 to 0.2 $\mu\text{g/L}$).

Among the pharmaceutical compounds (fig. 4), dehydronifedipine showed the greatest concentration range (0.06 to 1.56 $\mu\text{g/L}$) and highest concentration. Warfarin showed the smallest concentration range from 0.012 (MRL) to 0.12 $\mu\text{g/L}$.

Among the antibiotic compounds (fig. 5), anhydro-erythromycin (a macrolide degradation product) showed the greatest range from 0.1 to 2.0 $\mu\text{g/L}$. Sulfadiazine (a sulfonamide) and tetracycline showed the lowest concentration ranges from less than 0.05 to 0.053 $\mu\text{g/L}$ and less than 0.10 to 0.13 $\mu\text{g/L}$, respectively.

Only one hormone, 17 beta-estradiol (E2), was detected in effluent. This hormone ranged in concentration from 2.03 to 56 ng/L .

Comparison of Analytical Results from Composite and Discrete Samples

A matched pair statistical approach using the Wilcoxon signed-ranks test was used to determine whether any statistically significant differences exist at the 95-percent confidence level (two-sided p-value less than 0.025) between the concentrations detected in composite and discrete samples. Of the organic wastewater compounds, only benzo[*a*]pyrene showed a statistically significant difference, with an estimated Wilcoxon test statistic of 2.41 and a p-value of 0.02 (table 11). This compound is a regulated polycyclic aromatic hydrocarbon used in cancer research. Many of the results were considered "estimated" because the values were below the MRL, but above the MDL.

There were no statistically significant differences for pharmaceutical compounds and hormones; however, two antibiotic compounds (lincomycin and oxolinic acid) showed statistically significant differences (table 12). Lincomycin (a macrolide) and oxolinic acid (a quinoline) each had a Wilcoxon test statistic of 2.40 and a p-value of 0.016. Of the 83 constituents detected from all constituent classes, only 3.5 percent showed any statistically significant differences between composite and discrete concentrations at the 95-percent confidence level. These results can probably be attributed to the high degree of mixing that occurs in the waste stream during the treatment process.

Quality Assurance

Quality assurance, an integral part of this study, included four field blanks, four equipment blanks, one duplicate sample, and two matrix spikes that were collected during sampling events. Additionally, surrogates were added by the NWQL to assess analytical procedures. Field blanks and equipment blanks were collected using organic-free water from the NWQL. Duplicate and matrix spike samples were collected as discrete samples from the grit chamber at inflow site 1; the matrix samples were spiked at the NWQL. All quality-assurance samples were processed in the same manner as the environmental samples, chilled to 4 °C, and shipped to the NWQL for analysis.

Analytical Results of Field Blanks, Equipment Blanks, and Duplicate Samples

Analytical results of field and equipment blanks and duplicate samples were determined for 63 organic wastewater compounds (table 13). Phenol had maximum concentrations of 1.2 $\mu\text{g/L}$ for field blanks and 1.0 $\mu\text{g/L}$ for equipment blanks that were greater than the MRL. Three constituents had concentrations or detected values that were less than the MRL, including acetophenone (0.1 $\mu\text{g/L}$ for field and equipment blanks), DEET (0.1 $\mu\text{g/L}$ for equipment blank), and naphthalene (0.08 $\mu\text{g/L}$ estimated for equipment blank).

One duplicate sample was collected and analyzed for the 63 organic wastewater constituents (table 13). The relative percentage differences (RPDs) for 62 (98 percent) of these compounds were estimated or not determined. Censored data prohibited the determination of RPDs for 39 compounds (62 percent). The RPDs were estimated for 16 compounds (25 percent) because of data below the MRLs, and no RPDs were determined for 7 compounds (11 percent) because of matrix interference. Of the RPDs determined, values ranged from 0.0 to 97. Tetrachloroethene, tris(2-chloroethyl) phosphate, and tris(dichloroisopropyl) phosphate all had the lowest RPD of 0.0, and caffeine had the highest RPD of 97.

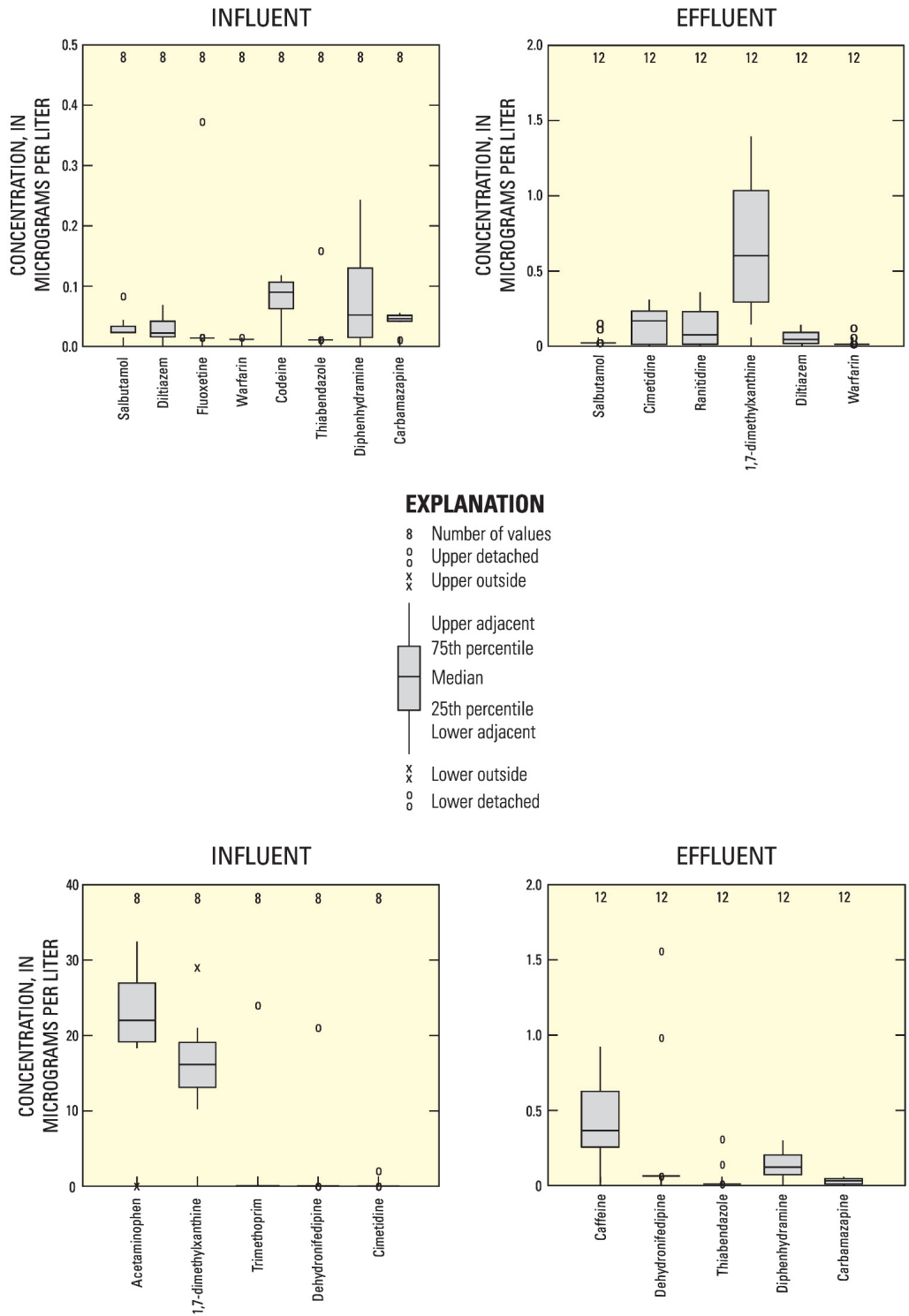


Figure 4. Constituent concentrations and ranges of pharmaceutical compounds in influent and effluent.

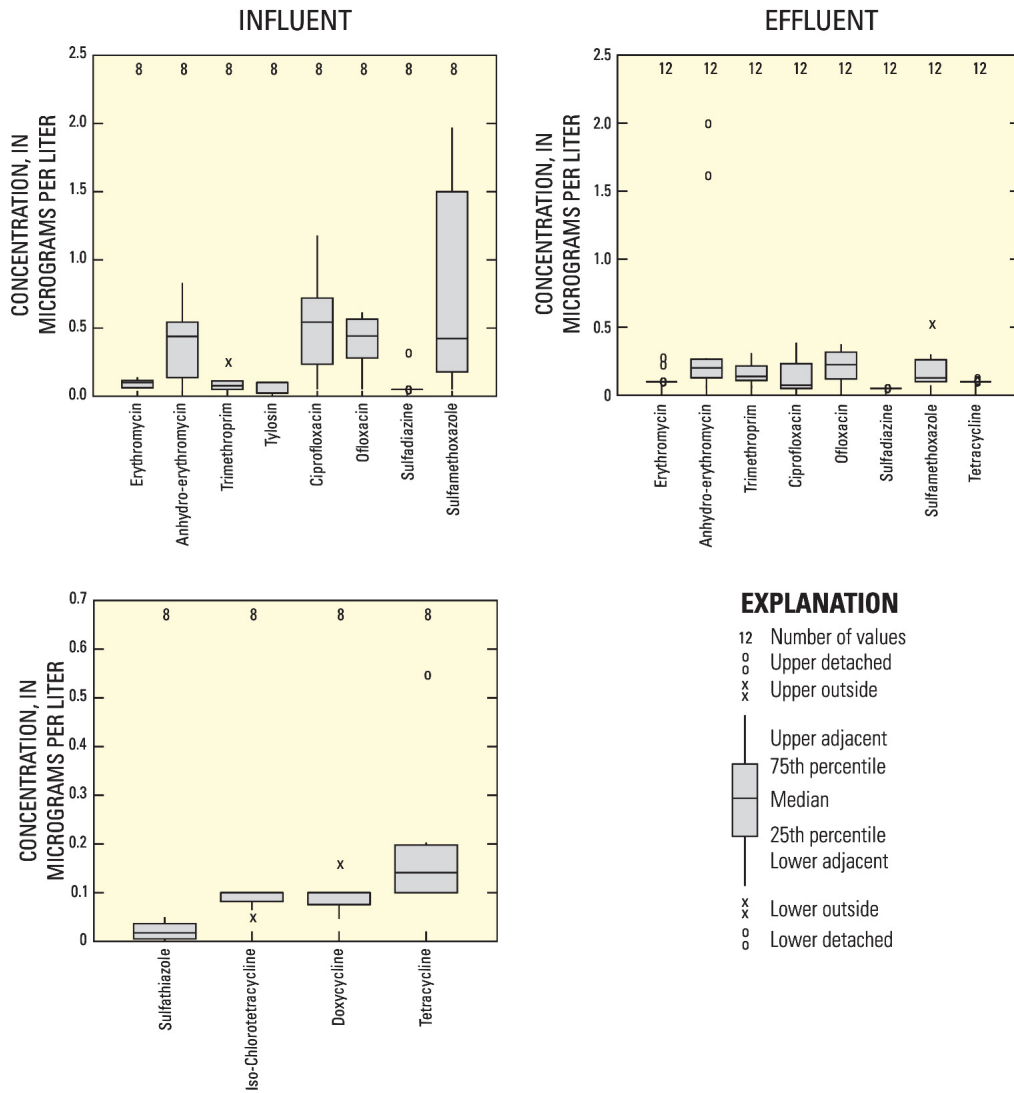
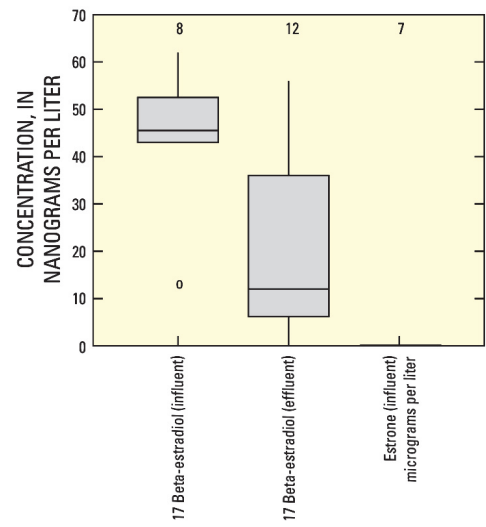


Figure 5. Constituent concentrations and ranges of antibiotic compounds in influent and effluent.



EXPLANATION

- 12 Number of values
- o Upper detached
- x Upper outside
- Upper adjacent
- 75th percentile
- Median
- 25th percentile
- Lower adjacent
- x Lower outside
- o Lower detached

Figure 6. Constituent concentrations and ranges of hormones in influent and effluent.

Table 11. Statistical comparison of composite and discrete samples for organic wastewater compounds using the Wilcoxon signed-ranks test.

[Statistically significant if p-value (shown in red) is less than 0.025; e, estimated due to one or more values below the minimum reporting level]

Compound	Wilcoxon test statistic	p-value	Compound	Wilcoxon test statistic	p-value
1,4-Dichlorobenzene	-1.23e	0.22e	Diethoxyoctylphenol	.75e	.45e
1-Methylnaphthalene	.63e	.53e	<i>d</i> -Limonene	-.84e	.40e
2,6-Dimethylnaphthalene	.93	.35	Fluoranthene	.91e	.36e
2-Methylnaphthalene	1.35e	.18e	Hexahydrohexamethyl cyclo-pentabenzopyran	-.72e	.47e
3 beta-coprostanol	-.72e	.47e	Indole	-1.74e	.08e
3-Methyl-1H-indole	-.82	.41	Isoborneol	-.35	.73
3- <i>tert</i> -Butyl-4-hydroxyanisole	-.92	.36	Isophorone	.92	.36
4-Cumylphenol	.93e	.35e	Isopropylbenzene	.91	.36
4-Nonylphenol	-1.61	.11	Isoquinoline	.94	.35
4-Octylphenol	.94e	.35e	Menthol	-.03(e)	.98e
4- <i>tert</i> -Octylphenol	-.53e	.60e	Metalaxyl	.94	.35
9,10-Anthraquinone	.0	1.0	Methyl salicylate	-.72	.47
Acetophenone	.13e	.89e	Metolachlor	.94	.35
Acetyl hexamethyl tetrahydro naphthalene	-.52	.60	Monoethoxyoctylphenol	1.21e	.22e
Anthracene	.94e	.35e	N,N-diethyl- <i>meta</i> -toluamide (DEET)	-1.18e	.24e
Benzo[<i>a</i>]pyrene	2.41e	.02e	Naphthalene	-.90e	.37e
Benzophenone	-.52e	.60e	<i>para</i> -Cresol	-.36e	.72e
<i>beta</i> -Sitosterol	-.64e	.52e	Phenanthrene	1.08e	.28e
<i>beta</i> -Stigmastanol	1.35e	.18e	Phenol	-.81e	.42e
Bisphenol A	.38	.70	Prometon	.94	.35
Bromacil	.94	.35	Pyrene	.90e	.37e
Caffeine	-.23e	.82e	Tetrachloroethylene	.46e	.64e
Camphor	-.92	.36	Tribromomethane	.93	.35
Carbaryl	.92	.36	Tributyl phosphate	-.46e	.64e
Carbazole	1.37e	.17e	Triclosan	.86	.39
Chlorpyrifos	.94	.35	Triethyl citrate	-.40e	.69e
Cholesterol	-.92e	.36e	Triphenyl phosphate	-1.18e	.24e
Cotinine	.35e	.72e	Tris(2-chloroethyl) phosphate	.60e	.55e
Diazinon	-.46	.64	Tris(dichloroisopropyl) phosphate	-.53e	.59e
Diethoxynonylphenol	-0.76e	0.44e			

Table 12. Statistical comparison of detected composite and discrete samples for pharmaceutical compounds, antibiotic compounds, and hormones using the Wilcoxon signed-ranks test.

[Statistically significant if p-value (shown in red) is less than .025]

Compound	Wilcoxon test statistic	p-value
Pharmaceutical Compounds		
1,7-dimethylxanthine	1.47	0.14
Carbamazapine	-1.14	.26
Codeine	-.90	.36
Diltiazem	-.08	.93
Diphenhydramine	1.16	.24
Metformin	.00	1.0
Trimethoprim	-.13	.89
Antibiotic Compounds		
Anhydro-erythromycin	1.28	.20
Ciprofloxacin	2.01	.04
Clinafloxacin	-1.96	.05
Doxycycline	.54	.59
Erythromycin	1.69	.09
Lincomycin	-2.40	.016
Ofloxacin	1.14	.25
Oxolinic acid	2.40	.016
Sulfadiazine	1.96	.05
Sulfadimethoxine	-1.37	.17
Sulfamethoxazole	1.17	.24
Sulfathiazole	-1.50	.13
Tetracycline	.52	.60
Trimethoprim	1.47	.14
Tylosin	-1.69	.09
Hormones		
17 beta-estradiol (E2)	1.39	.16
Estrone (E1)	.11	.90

Table 13. Analytical results of field blanks, equipment blanks, and relative percentage differences (RPD) in duplicate samples for organic wastewater compounds.

[Values shown in micrograms per liter. <, less than the value; C, unable to compute RPD due to censored data; e, estimated due to one or more values below the minimum reporting level; µg/L, micrograms per liter; ND, not determined due to matrix interference]

Compound	Field blank range	Equipment blank range	Duplicate RPD	Minimum reporting level
1 4-Octylphenol	<1	<1	C	1.0
1,4-Dichlorobenzene	<.5	<.5	62e	.5
1-Methylnaphthalene	<.5	<.5	C	.5
2,6-Dimethylnaphthalene	<.5	<.5	C	.5
2-Methylnaphthalene	<.5	<.5	C	.5
3 beta-coprostanol	<2	<2	86e	2.0
3-Methyl-1H-indole	<1	<1	C	1.0
3- <i>tert</i> -Butyl-4-hydroxyanisole	<5	<5	C	5.0
4-Cumylphenol	<1	<1	C	1.0
4-Nonylphenol	<5	<5 – 1e	95e	5.0
4- <i>tert</i> -Octylphenol	<1	<1	ND	1.0
5-Methyl-1H-benzotriazole	<2	<2	C	2.0
Acetophenone	<.5 – .1e	<.5 – .1e	60e	.5
Acetyl-hexamethyl-tetrahydro-naphthalene (AHTN)	<.5	<.5	5.8	.5
Anthracene	<.5	<.5	C	.5
Anthraquinone	<.5	<.5	C	.5
Benzo[<i>a</i>]pyrene	<.5	<.5	C	.5
Benzophenone	<.5	<.5	75e	.5
<i>beta</i> -Sitosterol	<2	<2	ND	2.0
<i>beta</i> -Stigmastanol	<2	<2	ND	2.0
Bisphenol A	<1	<1	ND	1.0
Bromacil	<.5	<.5	C	.5
Caffeine	<.5	<.5	97e	.5
Camphor	<.5	<.5	C	.5
Carbaryl	<1	<1	C	1.0
Carbazole	<.5	<.5	C	.5
Chlorpyrifos	<.5	<.5	C	.5
Cholesterol	<2	<2	88e	2.0
Cotinine	<1	<1	81e	1.0
Diazinon	<.5	<.5	C	.5
Dichlorvos	<1	<1	C	1.0
Diethoxynonylphenol	<5	<5	C	5.0
Diethoxyoctylphenol	<1	<1	C	1.0
<i>d</i> -Limonene	<.5	<.5	C	.5
Fluoranthene	<.5	<.5	C	.5
Hexahydrohexamethyl cyclopentabenzopyran	<.5	<.5	33e	.5
Indole	<.5	<.5	C	0.5
Isoborneol	<.5	<.5	C	.5

Table 13. Analytical results of field blanks, equipment blanks, and relative percentage differences (RPD) in duplicate samples for organic wastewater compounds.—Continued

[Values shown in micrograms per liter. <, less than the value; C, unable to compute RPD due to censored data; e, estimated due to one or more values below the minimum reporting level; µg/L, micrograms per liter; ND, not determined due to matrix interference]

Compound	Field blank range	Equipment blank range	Duplicate RPD	Minimum reporting level
Isophorone	<.5	<.5	C	.5
Isopropylbenzene	<.5	<.5	C	.5
Isoquinoline	<.5	<.5	C	.5
Menthol	<.5	<.5	C	.5
Metalaxyl	<.5	<.5	C	.5
Methyl salicylate	<.5	<.5	C	.5
Metolachlor	<.5	<.5	C	.5
Monoethoxyoctylphenol	<1	<1	ND	1.0
N,N-diethyl- <i>meta</i> -toluamide (DEET)	<.5	.5 – 0.1e	78e	.5
Naphthalene	<.5	<.5 – .08e	ND	.5
<i>para</i> -Cresol	<1	<1	C	1.0
Pentachlorophenol	<2	<2	C	2.0
Phenanthrene	<.5	<.5	C	.5
Phenol	<.5 – 1.2	<.5 – 1.0	C	.5
Prometon	<.5	<.5	C	.5
Pyrene	<.5	<.5	C	.5
Tetrachloroethylene	<.5	<.5	0.0e	.5
Tribromomethane	<.5	<.5	C	
Tributyl phosphate	<.5	<.5	C	.5
Triclosan	<1	<1	ND	1.0
Triethyl citrate	<.5	<.5	37e	.5
Triphenyl phosphate	<.5	<.5	50e	.5
Tris(2-butoxyethyl) phosphate	<.5	<.5	66e	.5
Tris(2-chloroethyl) phosphate,	<.5	<.5	0.0e	.5
Tris(dichloroisopropyl) phosphate	<.5	<.5	0.0e	.5

Analytical results of field and equipment blanks were determined for 20 pharmaceutical compounds (table 14). For these results, nondetects were reported by the laboratory as less than the MRLs, and detected values were reported as the actual numerical value, which may be less than the MRL. Three constituents had concentrations or detected values that were greater than the MRL, including 1,7-dimethylxanthrine (0.144 µg/L for field and equipment blanks), diphenhydramine (0.029 µg/L for field blank), and fluoxetine (0.051 µg/L for field blank). Four constituents had concentrations or detected values that were less than the MRL, including acetaminophen (0.011 µg/L for equipment blank), diltiazem (0.0044 µg/L for field blank), thiabendazole (0.0058 for field blank), and trimethoprim (0.011 µg/L for field blank).

Analytical results of field and equipment blanks were determined for 45 antibiotic compounds and 3 hormones (table 15). Among the antibiotic compounds, only lincomycin (as high as 0.037 µg/L), ofloxacin (as high as 0.008 µg/L), and tetracycline (0.021 µg/L) had detected values greater than the MRLs for equipment blanks. The hormone 17 beta-estradiol (E2) had detected values (less than 2 to 1.99 ng/L for field blank and less than 2 to 8.54 ng/L for equipment blank) greater than the MRL. None of the antibiotic compounds and hormones had detected values less than the MRL.

Table 14. Analytical results of field and equipment blanks for pharmaceutical compounds.

[Values in micrograms per liter; <, less than the value]

Compound	Field blank range	Equipment blank range	Provisional minimum reporting level
1,7-dimethylxanthine	0.144	0.144	0.140
Acetaminophen	<.036	<.036 – .011	.036
Azithromycin	<.004	<.004	.004
Carbamazepine	<.011	<.011	.011
Cimetidine	<.012	<.012	.012
Codeine	<.015	<.015	.015
Dehydronifedipine	<.015	<.015	.015
Diltiazem	<.016 – .0044	<.016	.016
Diphenhydramine	<.015 – .029	<.015	.015
Erythromycin	<.009	<.009	.009
Fluoxetine	<.014 – .051	<.014	.014
Gemfibrozil	<.013	<.013	.013
Ibuprofen	<.042	<.042	.042
Miconazole	<.018	<.018	.018
Ranitidine	<.013	<.013	.013
Salbutamol	<.023	<.023	.023
Sulfamethoxazole	<.064	<.064	.064
Thiabendazole	<.011 – .0058	<.011	.011
Trimethoprim	<.013 – .011	<.013	.013
Warfarin	<.012	<.012	.012

Table 15. Analytical results of field and equipment blanks for antibiotic compounds and hormones.

[Results in micrograms per liter, except for 17 beta-estradiol which is in nanograms per liter. Hormones are in red.

<, less than the value]

Compound	Field blank range	Equipment blank range	Minimum reporting level
17 beta-estradiol (E2)	<2 – 1.99	<2 – 8.54	2.0
Amoxicillin	<.010	<.010	.010
Ampicillin	<.010	<.010	.010
Anhydro-chlorotetracycline	<.010	<.010	.010
Anhydro-erythromycin	<.005	<.005	.005
Anhydro-tetracycline	<.010	<.010	.010
Cefotaxime	<.010	<.010	.010
Chlorotetracycline	<.010	<.010	.010
Ciprofloxacin	<.005	<.005	.005
Clinafloxacin	<.005	<.005	.005
Cloxacillin	<.010	<.010	.010
Demeclocycline	<.010	<.010	.010
Doxycycline	<.010	<.010	.010
Epi-anhydro-chlorotetracycline	<.010	<.010	.010

Table 15. Analytical results of field and equipment blanks for antibiotic compounds and hormones.—Continued

[Results in micrograms per liter, except for 17 beta-estradiol which is in nanograms per liter. Hormones are in red.
<, less than the value]

Compound	Field blank range	Equipment blank range	Minimum reporting level
Epi-anhydro-tetracycline	<.010	<.010	.010
Epi-chlorotetracycline	<.010	<.010	.010
Epi-iso-chlorotetracycline	<.010	<.010	.010
Epi-oxytetracycline	<.010	<.010	.010
Epi-tetracycline	<.010	<.010	.010
Erythromycin	<.005	<.005	.005
Estrone (E1)	<.05	<.05	.05
Ethenyl estradiol (EE2)	<.05	<.05	.05
Flumequin	<.005	<.005	.005
Iso-chlorotetracycline	<.010	<.010	.010
Lincomycin	<.005	<.005 – .037	.005
Lomefloxacin	<.005	<.005	.005
Minocycline	<.010	<.010	.010
Norfloxacin	<.005	<.005	.005
Ofloxacin	<.005	<.005 – .008	.005
Ormetoprim	<.005	<.005	.005
Oxacillin	<.010	<.010	.010
Oxolinic acid	<.005	<.005	.005
Oxytetracycline	<.010	<.010	.010
Penicillin G	<.010	<.010	.010
Penicillin V	<.010	<.010	.010
Roxithromycin	<.005	<.005	.005
Sarafloxacin	<.005	<.005	.005
Sulfachlorpyridazine	<.005	<.005	.005
Sulfadiazine	<.005	<.005	.005
Sulfadimethoxine	<.005	<.005	0.005
Sulfamerazine	<.005	<.005	.005
Sulfamethazine	<.005	<.005	.005
Sulfamethoxazole	<.005	<.005	.005
Sulfathiazole	<.005	<.005	.005
Tetracycline	<.010	<.010 – .021	.010
Trimethoprim	<.005	<.005	.005
Tylosin	<.005	<.005	.005
Virginiamycin	<.005	<.005	.005

Matrix Spike Recoveries

Average matrix spike recoveries and the expected matrix spike recoveries were determined for 55 organic wastewater compounds (table 16). Average recoveries ranged from 0 to -1,188 percent. Average spike recoveries for 22 compounds (40 percent) were outside of the expected spike recovery range for that particular compound. Average recoveries for 16 compounds (29 percent) were considered "estimated" because one or more values were below the MRLs as a result of matrix interference or multiple detection levels. Recoveries could

not be determined for 22 compounds (40 percent) because of a preponderance of censored data values, and two samples showed no recoveries.

An important consideration in matrix spike analysis is that with organic wastewater compounds, the error bars for analytical results may be as high as ± 20 percent above the MRLs (S.D. Zaugg, U.S. Geological Survey, written commun., 2005). If this range is applied to both the spike and the duplicate sample, especially for high compound concentrations, the spike results may not be very informative. Some compounds may also react with the wastewater matrix and

Table 16. Average matrix spike recoveries and expected matrix spike recovery ranges for organic wastewater compounds.

[C, unable to compute percent recovery due to censored data; e, estimated due to one or more data values below the minimum reporting level. Results outside expected recovery ranges are in red]

Compound	Average matrix spike recovery (percent)	Expected matrix spike recovery range (percent)	Compound	Average matrix spike recovery (percent)	Expected matrix spike recovery range (percent)
1,4-Dichlorobenzene	69e	40 - 110	Diethoxynonylphenol	138e	40 - 110
1-Methylnaphthalene	100e	60 - 110	<i>d</i> -Limonene	19e	20 - 110
2,6-Dimethylnaphthalene	C	60 - 110	Fluoranthene	C	60 - 110
2-Methylnaphthalene	106e	60 - 110	Hexahydrohexamethyl cyclopentabenzopyran	100	50 - 110
3 beta-coprostanol	-125e	50 - 110	Indole	200	50 - 110
3-Methyl-1H-indole	0	30 - 110	Isoborneol	106	60 - 110
4-Cumylphenol	C	60 - 110	Isophorone	C	60 - 110
4-Nonylphenol	190e	30 - 100	Isoquinoline	C	60 - 110
4- <i>n</i> -Octylphenol	C	40 - 110	Menthol	-12e	60 - 110
4- <i>tert</i> -Octylphenol	188	60 - 110	Metalaxyl	C	70 - 110
5-Methyl-1H-benzotriazole	C	40 - 110	Methyl salicylate	119	60 - 110
9,10-Anthraquinone	C	70 - 110	Metolachlor	C	70 - 110
Acetophenone	C	50 - 110	Monoethoxyoctylphenol	36e	50 - 110
Acetyl-hexamethyl- tetrahydro-naphthalene	88	50 - 110	N,N-diethyl- <i>meta</i> -tolua- mide (DEET)	156	70 - 110
Anthracene	C	60 - 110	Naphthalene	100	60 - 110
Benzo[<i>a</i>]pyrene	C	50 - 110	<i>para</i> -Cresol	-1,188	60 - 110
Benzophenone	138	60 - 110	Pentachlorophenol	C	30 - 110
<i>beta</i> -Sitosterol	-47e	50 - 110	Phenanthrene	125e	60 - 110
<i>beta</i> -Stigmastanol	C	50 - 110	Phenol	0	60 - 110
Bromacil	C	70 - 110	Prometon	C	70 - 110
Caffeine	313	70 - 110	Tetrachloroethylene	38e	10 - 110
Camphor	144	60 - 110	Tributyl phosphate	C	60 - 110
Carbaryl	C	40 - 110	Triclosan	188	60 - 110
Carbazole	C	70 - 110	Triethyl citrate	150	60 - 110
Chlorpyrifos	C	60 - 110	Triphenyl phosphate	144e	60 - 110
Cholesterol	16e	50 - 110	Tris(2-butoxyethyl) phosphate	94	50 - 110
Cotinine	125e	20 - 110	Tris(dichloroisopropyl) phosphate	119e	70 - 110
Diazinon	C	40 - 110			

result in no recoveries (S.D. Zaugg, U.S. Geological Survey, written commun., 2005). Additionally, if sample matrices have environmental concentrations that are higher than the spiked constituents added to them, achieving analytically acceptable results may not be possible. In general, these analyses should be interpreted with caution.

The enzyme-linked immunosorbent assay (ELISA) tests are generally semiquantitative. To assess potential limitations of the 17 beta-estradiol (E2), estrone (E1), and ethenyl estradiol (EE2) tests, a subset of 123-mL spiked and unspiked samples was analyzed using ELISA and LC/MS. This subset included eight inflow samples, two effluent samples, five reuse train samples, three ultraviolet pilot samples, one CLO₂ plant sample, and one equipment blank sample. A 200- μ L standard mix containing 1.23 ng/ μ L of 17 Beta-estradiol (E2), estrone (E1), and ethenyl estradiol (EE2) was added to the spiked samples; the samples were analyzed using the three ELISA tests. The remaining sample was extracted at the Kansas Water Science Center, Organic Geochemistry Research Laboratory. The sample extracts were then shipped on ice to the Environmental Research Laboratory at the University of Missouri-Rolla for LC/MS analysis.

A comparison was made between the average concentration and relative standard deviation of the spiked samples and the concentration of the spiked samples minus the concentration of the equivalent unspiked samples for the ELISA and the LC/MS analyses (table 17). For 17 beta-estradiol (E2), the average concentration of the spiked samples and the spiked minus the unspiked samples was about twice as high for the ELISA test as for the LC/MS analysis. In general, however, the relative differences in concentration between the ELISA and LC/MS were similar. For the estrone (E1) and ethenyl estradiol (EE2) analyses, the average concentrations for the spiked samples and spiked minus unspiked samples were similar. For the ethenyl estradiol (EE2) ELISA test, there were no detections in any of the samples, this hormone was observed at levels below the detection limits of the ELISA test in the LC/MS analyses. A comparison of the ELISA and LC/MS data indicates that more variation is apparent in the absolute concentration between the 17 beta-estradiol (E2) for the ELISA and LC/MS analyses; however, the relative variation in concentration is similar.

Table 17. Comparison of average spiked sample results and average spiked sample minus unspiked sample results for enzyme-linked immunosorbent assay (ELISA) and liquid chromatography/mass spectrometry (LC/MS) analyses of selected hormones.

[ng/L, nanograms per liter; μ g/L, micrograms per liter]

Statistic	17 beta-estradiol (E2) analysis results (ng/L)		Estrone (E1) analysis results (μ g/L)		Ethenyl estradiol (EE2) analysis results (μ g/L)	
	ELISA	LC/MS	ELISA	LC/MS	ELISA	LC/MS
Average spiked sample \pm relative standard deviation	232 \pm (79.1)	121 \pm (37.7)	0.119 \pm (0.056)	0.165 \pm (0.080)	0.135 \pm (0.021)	0.130 \pm (0.045)
Average spiked - unspiked sample \pm relative standard deviation	205 \pm (83.5)	96 \pm (40.9)	.092 \pm (0.065)	.126 \pm (0.066)	.135 \pm (0.021)	.090 \pm (0.066)

Surrogate Analyses

Another important element in the quality-assurance process is the evaluation of surrogate performance. Surrogate performance was determined at the NWQL for each sample analyzed by the household and organic wastewater compounds method. Surrogates are compounds added to samples to monitor the performance of the analytical processes and extraction steps. Surrogate recovery ranges are typically from 50 to 120 percent. Anomalous surrogate recovery rates could be the result of laboratory error, and as such, could have detrimental effects on the sample analytical results. Surrogate recoveries for four compounds determined during the household and organic wastewater analyses are listed in table 18. All average surrogate recoveries were within acceptable recovery ranges for the specific surrogate in question, indicating acceptable laboratory extraction and processing procedures.

Table 18. Surrogate performance results and expected recovery ranges for selected compounds.

Compound	Average surrogate recovery (percent)	Expected surrogate recovery ranges (percent)
Bisphenol A-d3	99	20 – 120
Caffeine 13C	107	50 – 120
Decafluorobiphenyl	52	40 – 120
Fluoranthene d10	86	50 – 120

Summary

The Comprehensive Everglades Restoration Plan (CERP) has identified highly treated wastewater as a possible water source for the restoration of natural water flows and hydroperiods in selected coastal areas, including the Biscayne Bay coastal wetlands. One source of reclaimed wastewater for the Biscayne Bay coastal wetlands is the South District Wastewater Treatment Plant (SDWWTP) in southeastern Miami-Dade County. The U.S. Geological Survey, in cooperation with the CERP Wastewater Reuse Technology Pilot Project Delivery Team, initiated a study to assess the presence of emerging contaminants of concern in SDWWTP influent and effluent using current (2004) treatment methods.

During 2004, four seasonal sampling events were undertaken to assess constituent concentrations in influent and effluent and transformations in concentration at the effluent pump, reuse train, CLO₂ unit, and ultraviolet irradiation pilot unit in the waste stream at the SDWWTP. Water samples were collected during a dry-season, low-flow event (March 2-3, 2004); an average wet-season event (July 20-21, 2004); and two wet-season high-rate disinfection (HRD) sampling events (October 5 and 20, 2004) for household and industrial (organic) wastewater compounds, pharmaceutical compounds, antibiotic compounds, and hormones. Composite samples were collected for all four of these events, and discrete samples were collected for three of the events as a means of comparison.

Water samples for organic wastewater compounds were analyzed using an approved National Water Quality Laboratory production-line method utilizing polystyrene-divinylbenzene solid-phase extraction (SPE) and capillary-column gas chromatography/mass spectrometry (GC/MS). The analytical methods used for pharmaceutical compounds, antibiotic compounds, and hormones are provisional, however, and incorporate SPE with high-performance liquid chromatography/electrospray-ionization mass spectrometry (HPLC/ESI-MS) and a combined SPE and an enzyme-linked immunosorbent assay (ELISA) method.

For the dry-season, low-flow sampling event conducted during March 2-3, 2004, the average inflow rate was 83.7 Mgal/d. During this period, 26 constituents were detected and constituent percent transformations were determined. One constituent percent transformation in concentration at the effluent pump and two at the reuse train were not determined because of censored data below the method detection limit. Percent transformations for 13 and 12 constituents were estimated at the effluent pump and reuse train, respectively, because of data values below the minimum reporting levels (MRLs). The transformations in concentration for constituents ranged from -100 to 180 percent at the effluent pump and from -100 to 85 percent at the reuse train. Two known "endocrine" disrupting compounds—17 beta-estradiol (E2) and diethoxynonylphenol—and four "suspected" endocrine disrupting compounds—1,4-dichlorobenzene, benzophenone, tris(2-chloroethyl) phosphate, and tris(dichloroisopropyl)

phosphate—were detected at the effluent pump and the reuse train.

For the average wet-season sampling event conducted during July 20-21, 2004, the average inflow rate was 89.7 Mgal/d. During this period, 27 constituents were detected and constituent percent transformations in concentration were determined. Five constituent percent transformations in concentration at the effluent pump and one at the reuse train were not determined because of censored data below the method detection limit. The transformations in concentration for 6 and 11 constituents were estimated at the effluent pump and reuse train, respectively, because one or more data values were below the MRLs. The transformations in concentration ranged from -100 to 1,609 percent at the effluent pump and from -100 to 832 percent at the reuse train. One "known" endocrine disrupting compound, 17 beta-estradiol (E2), and one "suspected" endocrine disrupting compound, benzophenone, were detected at both the effluent pump and reuse train.

Composite 3- and 6-hour samples and discrete samples were collected during the HRD tests during the wet season. The samples were collected at the pilot units during tests performed on October 5 and 20, 2004, at average inflow rates of 84.1 and 119.6 Mgal/d and durations of 3 and 6 hours, respectively. During the October 5 sampling event, 29 constituents were detected and percent transformations in concentration were determined. Ranges of transformations in concentration for constituents at the effluent pump, reuse train, CLO₂ unit, and ultraviolet pilot unit were -100 to -37 percent, -100 to -62 percent, -100 to -56 percent, and -100 to -40 percent, respectively. Two "known" endocrine disruptor compounds, 17 beta-estradiol (E2) and diethoxynonylphenol, and one "suspected" endocrine disruptor compound, benzophenone, were detected. During the October 20 sampling event, percent transformations in concentration for 35 constituents were determined. Ranges of transformations in concentration were -100 to -4 percent, -100 to 17 percent, -100 to -40 percent, and -100 to -14 percent at the effluent pump, reuse train, CLO₂ unit, and ultraviolet pilot unit, respectively. Four "suspected" endocrine disrupting compounds—1,4-dichlorobenzene, benzophenone, tris(2-chloroethyl) phosphate and tris(dichloroisopropyl) phosphate—and two "known" endocrine disrupting compounds—17 beta-estradiol (E2) and diethoxynonylphenol—were detected and constituent percent transformations in concentration were determined during this sampling.

Constituents detected in influent consisted of 38 organic wastewater compounds, 13 pharmaceutical compounds, 12 antibiotic compounds, and 2 hormones. The greatest ranges in concentration and highest concentrations were for indole and phenanthrene (organic wastewater compounds), acetaminophen (pharmaceutical compounds), and sulfamethoxazole (antibiotic compounds). The smallest ranges in concentration and lowest concentrations were warfarin (pharmaceutical compounds) and sulfathiazole (antibiotic compounds). Two hormones, 17 beta-estradiol (E2) and estrone (E1), were detected in influent.

Constituents detected in effluent consisted of 20 organic wastewater compounds, 11 pharmaceutical compounds, 8 antibiotic compounds, and 1 hormone. The greatest ranges in concentration and highest concentrations were for indole (organic wastewater compounds), dehydronifedipine (pharmaceutical compounds), and anhydro-erythromycin (antibiotic compounds). The smallest ranges in concentration and lowest concentrations were for triphenyl phosphate (organic wastewater compounds), warfarin (pharmaceutical compounds), and sulfadiazine and tetracycline (antibiotic compounds). One hormone, 17 beta-estradiol (E2), was detected in effluent with "known" endocrine disrupting potential.

Study results indicate that many constituents detected during the sampling events exhibited transformations in concentration of -80 percent or greater at all locations in the waste stream and some showed transformations in concentration of -90 percent or greater. This finding indicates that conventional wastewater-treatment processes succeed in reducing constituent concentrations for many emerging contaminants. Anomalous increased values for selected constituents may be the result of matrix effects on solubility. Selected compounds are sensitive and easily ionizable in the LC/MS mode and give a strong signal at the detector when interferences are minimal. As the extract complexity increases in sewage influent, increased competition for ionization in the LC/MS source results in a weaker signal and thus, a detected lower concentration.

A statistical approach using the Wilcoxon signed-rank test was used to determine statistically significant differences at the 95-percent confidence level between concentrations from composite and discrete samples. Of the 83 compounds detected from all constituent classes, only 3.5 percent showed any significant differences at the 95-percent confidence level. Benzo[a]pyrene (organic wastewater compounds) and lincomycin and oxolinic acid (antibiotic compounds) were the only constituents that showed statistically significant differences between composite and discrete samples. The absence of any other compounds showing differences may attest to the high degree of mixing that occurs during the wastewater-treatment process.

Quality-assurance samples were collected during this study and included four field blanks, four equipment blanks, one duplicate sample, and two matrix spikes. Analytical results of field and (or) equipment blanks for organic wastewater compounds were greater than the MRL for phenol. Acetophenone, N,N-diethyl-*meta*-toluamide (DEET), and naphthalene had values less than the MRLs. One duplicate sample was collected and analyzed for 63 organic wastewater compounds. The RPDs were not determined for 62 percent of the compounds because of censored data; RPDs were estimated for 25 percent of the compounds because of data below the MRLs; and no RPDs were determined for 11 percent of the compounds because of matrix interference. The RPDs that could be determined ranged from 0.0 to 97.

Quality-assurance samples also were collected for pharmaceutical compounds, antibiotic compounds, and

hormones. Analytical results of field and (or) equipment blanks for pharmaceutical compounds were greater than the MRLs for 1,7-dimethylxanthine, diphenhydramine, and fluoxetine. Acetaminophen, diltiazem, thiabendazole, and trimethoprim had values less than the MRLs. Three antibiotic compounds (lincomycin, ofloxacin, and tetracycline) and the hormone 17 beta-estradiol (E2) had values greater than the MRL. None of the antibiotic compounds and hormones had values less than the MRLs.

Average matrix spike recoveries and the expected matrix spike recoveries were determined for 55 organic wastewater compounds. Average spike recoveries for 40 percent of the compounds were outside of the expected spike recovery range for that particular compound. Average recoveries for 29 percent of the compounds were considered "estimated" because one or more values were below the MRLs as a result of matrix interference or multiple detection levels. Recoveries could not be determined for 40 percent of the compounds because of a preponderance of censored data values, and two samples showed no recoveries.

For the hormones, confirmation of the ELISA results was made by LC/MS analyses. For the hormone 17 beta-estradiol, the average concentration of the spiked samples minus the unspiked samples was about twice as high for the ELISA test as for the LC/MS analyses. The relative differences in concentration between the ELISA test and the LC/MS analyses were similar. For the estrone (E1) and ethenyl estradiol (EE2) analysis, the average concentrations for the spiked samples and the spiked samples minus the unspiked samples were similar.

Surrogate performance was analyzed for selected compounds. Results indicated that decafluorobiphenyl, caffeine C-13, bisphenol A-d3, and fluoranthene d-10 were all within expected surrogate recovery ranges, indicating acceptable laboratory extraction and processing procedures.

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