

# Do Pharmaceuticals, Pathogens, and Other Organic Waste Water Compounds Persist When Waste Water Is Used for Recharge?

by Gail E. Cordy, Norma L. Duran, Herman Bouwer, Robert C. Rice, Edward T. Furlong, Steven D. Zaugg, Michael T. Meyer, Larry B. Barber, and Dana W. Kolpin

## Abstract

A proof-of-concept experiment was devised to determine if pharmaceuticals and other organic waste water compounds (OWCs), as well as pathogens, found in treated effluent could be transported through a 2.4 m soil column and, thus, potentially reach ground water under recharge conditions similar to those in arid or semiarid climates. Treated effluent was applied at the top of the 2.4 m long, 32.5 cm diameter soil column over 23 days. Samples of the column inflow were collected from the effluent storage tank at the beginning ( $T_{\text{begin}}$ ) and end ( $T_{\text{end}}$ ) of the experiment, and a sample of the soil column drainage at the base of the column ( $B_{\text{end}}$ ) was collected at the end of the experiment. Samples were analyzed for 131 OWCs including veterinary and human antibiotics, other prescription and nonprescription drugs, widely used household and industrial chemicals, and steroids and reproductive hormones, as well as the pathogens *Salmonella* and *Legionella*. Analytical results for the two effluent samples taken at the beginning ( $T_{\text{begin}}$ ) and end ( $T_{\text{end}}$ ) of the experiment indicate that the number of OWCs detected in the column inflow decreased by 25% (eight compounds) and the total concentration of OWCs decreased by 46% while the effluent was in the storage tank during the 23-day experiment. After percolating through the soil column, an additional 18 compounds detected in  $T_{\text{end}}$  (67% of OWCs) were no longer detected in the effluent ( $B_{\text{end}}$ ) and the total concentration of OWCs decreased by more than 70%. These compounds may have been subject to transformation (biotic and abiotic), adsorption, and (or) volatilization in the storage tank and during travel through the soil column. Eight compounds—carbamazepine; sulfamethoxazole; benzophenone; 5-methyl-1H-benzotriazole; N, N-diethyltoluamide; tributylphosphate; tri(2-chloroethyl) phosphate; and cholesterol—were detected in all three samples indicating they have the potential to reach ground water under recharge conditions similar to those in arid and semiarid climates. Results from real-time polymerase chain reactions demonstrated the presence of *Legionella* in all three samples. *Salmonella* was detected only in  $T_{\text{begin}}$ , suggesting that the bacteria died off in the effluent storage tank over the period of the experiment. This proof-of-concept experiment demonstrates that, under recharge conditions similar to those in arid or semiarid climates, some pharmaceuticals, pathogens, and other OWCs can persist in treated effluent after soil-aquifer treatment.

## Introduction

Research has shown that a variety of organic compounds (OWCs) including veterinary and human antibiotics, other prescription and nonprescription drugs, widely used household and industrial chemicals including personal care products and products of oil use and combustion, and steroids and reproductive hormones (Ternes 1998; Daughton and Ternes 1999; Heberer et al. 2001; Kolpin et al. 2002), as well as bacterial, viral, and protozoan pathogens (Toze 1999), can sur-

vive conventional waste water treatment and persist in the aquatic environment. When released into the environment in treated waste water, some OWCs can be adsorbed to sediments (Furlong et al. 2003), transformed into other compounds by biotic (Sedlak and Fono 2003) and abiotic process, volatilized, or degraded by photolysis (Buser et al. 1998; Latch et al. 2003). With the increasing use of treated effluent for irrigation and ground water recharge by soil-aquifer treatment (SAT) in the arid and semiarid Southwest, other parts of the United States, and the world (Bouwer 2002), there are concerns that these practices may introduce OWCs and pathogens into the ground water (Bouwer 2000; Drewes and Shore 2001). The potential for introduction of OWCs and pathogens into ground water in areas of dry

climate is of particular concern because of the potential for contamination of aquifers that are sole sources of drinking water for cities.

Limited data exist describing the persistence and fate of specific OWCs and pathogens when waste water is used for ground water recharge by SAT. In a recent study of ground water quality near surface spreading basins where treated effluent is recharged near Phoenix and Tucson, Arizona, Drewes et al. (2003) reported that the antiepileptic drugs carbamazepine and primidone persisted in ground water more than eight years after the initial introduction of the treated effluent as artificial recharge.

To determine the types of compounds and pathogens that can persist when treated effluent is used for ground water recharge, the U.S. Water Conservation Laboratory (USWCL) and the U.S. Geological Survey (USGS) conducted a proof-of-concept experiment in which treated sewage effluent was passed through a 2.4 m long soil column under recharge conditions similar to those that occur in arid or semiarid climates. Although it was beyond the scope of this experiment to determine the ultimate fate of individual compounds within the soil column, the results presented here can be used to identify the OWCs and pathogens that could be expected to persist in the subsurface during recharge and possibly reach ground water.

### Experiment Design

A 2.4 m long, 32.5 cm diameter vertical stainless steel column was hand-packed with Mohall-Laveen sandy loam soil from an area northwest of Phoenix that had no known history of cultivation or irrigation. The soil was passed through a 2 mm sieve, dried, and uniformly packed in 20 kg layers, each 15.7 cm thick. The saturated hydraulic conductivity of the soil, determined with a laboratory constant-head permeameter test, was 280 mm/day. The total volume of packed soil in the column was 171,825 cm<sup>3</sup>, with a bulk density of 1.63 gm/cm<sup>3</sup>, a porosity of 0.38, and a pore volume of 65,293 cm<sup>3</sup>. The top of the soil was 15 cm below the top of the steel column. A perforated stainless steel tube, extending horizontally from a 5 cm thick sand layer at the bottom of the column, allowed for free drainage at the base of the soil column. A 200 L insulated storage tank holding the treated effluent was suspended on a platform above the soil column (Figure 1). The column was set up in a greenhouse at the USWCL in Phoenix.

This experiment was designed to approximate recharge conditions similar to those of a wetting cycle in a recharge spreading basin where the basin is flooded with effluent that infiltrates into the soil over days or weeks, eventually recharging the ground water. To simulate recharge, a float valve maintained a constant effluent depth of 10 cm at the top of the soil column from May 29 through June 20, 2001 (23 days).

Secondarily treated effluent from a 17.5 million gal/day (66.2 million L/day) municipal waste water treatment facility (WTF), serving 120,000 to 150,000 residents near Phoenix, was used for the experiment. Prior to this experiment, the column was preconditioned to remove readily leachable compounds and to establish microbial communi-

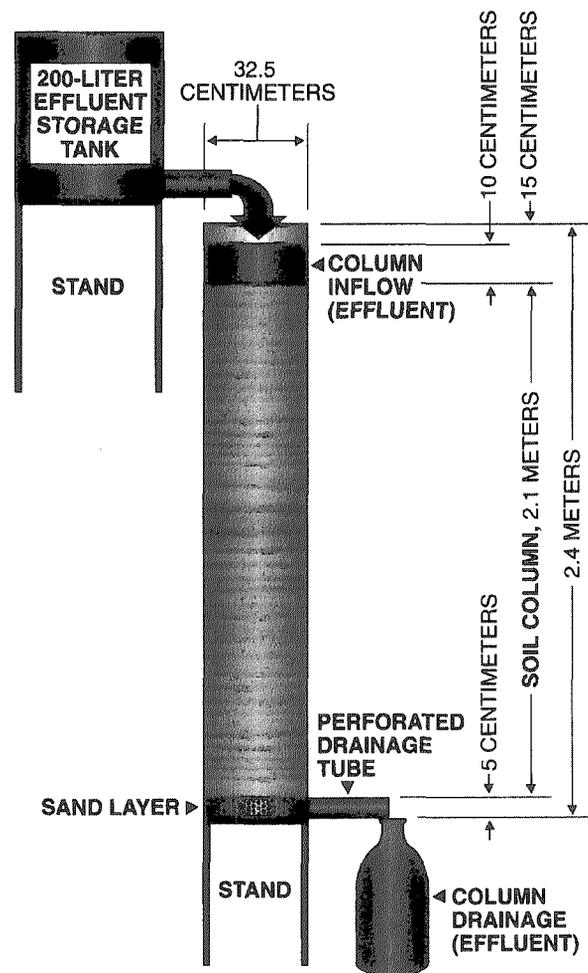


Figure 1. Setup for column experiment.

ties by applying 174 L of effluent from the WTF to the column. After two weeks of continuous effluent application, the soil column was allowed to drain for 60 days prior to the proof-of-concept experiment.

On May 29, 2001, ~200 L of treated effluent was collected from the WTF outflow and transported in a plastic barrel to the USWCL. The effluent was pumped into the storage tank (Figure 1), which remained covered throughout the experiment to minimize evaporation and ensure an adequate supply of effluent for the duration of the experiment. Prior to beginning the recharge of the column, a sample of the effluent in the storage tank was collected to determine the occurrence and concentrations of OWCs and selected pathogens (sample  $T_{begin}$ ). From May 29 through June 20, effluent was supplied at the top of the soil column (column inflow). After nearly two pore volumes of effluent (123 L) had passed through the column, the column drainage was collected over two days for the sample  $B_{end}$ . The two-pore volume end point for the experiment was selected to ensure that (1) any effluent remaining in the column from the preconditioning had been flushed out before the column drainage sample was collected, and (2) the experiment could be completed with a single tank of effluent. The experiment was completed on June 20, and an additional sample of the effluent remaining in the storage tank was collected (sample  $T_{end}$ ) for comparison with  $T_{begin}$  to determine if the occurrence and concentrations of

OWCs and pathogens in the column inflow had changed over the 23 days of the experiment.

Air temperatures measured hourly outside, indoors, and at the top of the column typically ranged from highs of 40° to 45°C during the day to lows of ~20° to 24°C at night. The ambient air temperatures noted during this experiment are considered comparable to those that would be expected at effluent recharge sites in central and southern Arizona, as well as other parts of the Southwest, during the summer.

Measurements of effluent infiltration rate were determined from column drainage. At least once daily, specific conductance and temperature of the column inflow (effluent in the storage tank) and the drainage were measured. In addition, daily samples of the column inflow and the drainage were collected for total organic carbon (TOC) and ultraviolet light absorbance (UV<sub>254</sub>) analyses to determine if the organic constituents in the effluent were changing in quantity and (or) character over the period of the experiment. The TOC and UV<sub>254</sub> were analyzed using methods and equipment described by Barber et al. (2001).

### Sample and Analytical Methods

The three samples for this experiment were collected in baked 10 L clear glass bottles. Immediately after collection, the bulk sample was split using a Teflon® cone splitter (Wilde et al. 1999) and distributed evenly among 1 L baked amber bottles. The samples for chemical analysis were chilled to 4°C and sent by overnight mail to USGS research laboratories in Denver, Colorado, and Ocala, Florida. Researchers at these laboratories analyzed samples for a total of 131 compounds including veterinary and human antibiotics (29 compounds), other prescription and nonprescription drugs (23 compounds), widely used household and industrial chemicals including personal care products and oil use and combustion products (57 compounds), and steroids and reproductive hormones (22 compounds), using a variety of experimental methods developed for the USGS Toxic Substances Hydrology Program. An overview of each of the analytical methods used in this experiment is in Kolpin et al. (2002); however, the numbers and types of compounds analyzed by each method were changed slightly from those detailed in Kolpin et al. (2002) because of laboratory methods refinement. Six compounds (sulfamethoxazole, trimethoprim, caffeine, cotinine, cholesterol, and 3-beta-coprostanol) were analyzed by more than one method with different reporting limits.

Laboratory reporting limits for the compounds analyzed are listed in µg/L in Table 1. Compounds that were not detected are listed as less than the reporting limit (< RL). The RL is equivalent to the lowest concentration standard that can be reliably quantified; however, many of the household and industrial chemicals were detected at concentrations < RL or the lowest calibration standard, although greater error is associated with these values. Quantifiable concentrations < RL are flagged as estimated (e). Concentrations of compounds were also estimated if the compound routinely showed laboratory spike (quality assurance sample) recoveries of < 60% or the reference standard was prepared with technical-grade mixtures.

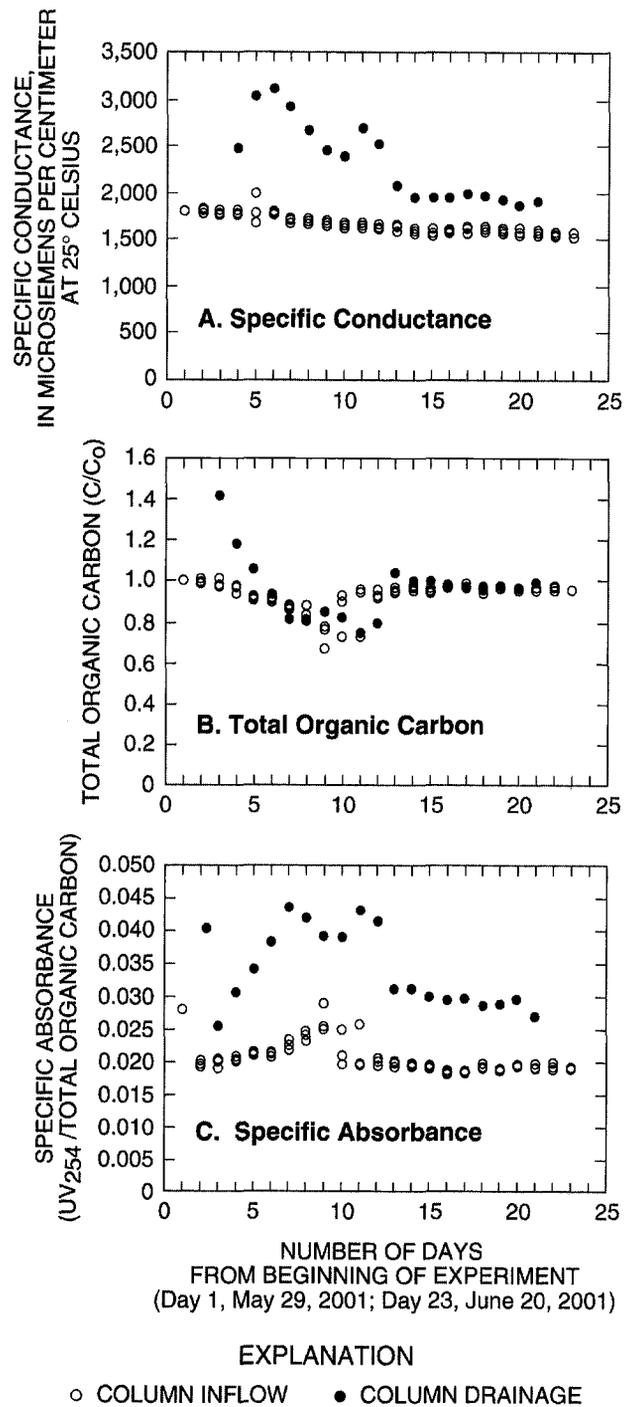


Figure 2. Graphs showing (a) specific conductance, (b) total organic carbon, and (c) specific absorbance for column inflow and column drainage during the 23-day soil-column experiment.

Laboratory blanks were used to assess potential sample contamination; however, blank contamination was not subtracted from results in Table 1. Instead, environmental sample concentrations are footnoted in Table 1, and the blank concentration is given in the table footnotes. Another footnote used in Table 1 is d, indicating a compound concentration that exceeded the highest point on the calibration curve.

*Legionella* and *Salmonella* were targeted for microbial analysis. *Legionella*, a nonenteric pathogen, was chosen

**Table 1**  
**Summary of Analytical Results for Soil-Column Experiment**

<b>Chemical</b>	<b>Reporting Limit (µg/L)</b>	<b>T<sub>begin</sub> 5/29/2001 (µg/L)</b>	<b>T<sub>end</sub> 6/20/2001 (µg/L)</b>	<b>B<sub>end</sub> 6/20/2001 (µg/L)</b>
<b>Veterinary and Human Antibiotics</b>				
azithromycin (3)	ND	< RL	< RL	< RL
carbadox (1)	0.05	< RL	< RL	< RL
chlortetracycline (1)	0.02	< RL	< RL	< RL
ciprofloxacin (1)	0.01	< RL	< RL	< RL
clarithromycin (3)	ND	< RL	< RL	< RL
demeclocycline (1)	0.02	< RL	< RL	< RL
doxycycline (1)	0.05	< RL	< RL	< RL
enrofloxacin (1)	0.01	< RL	< RL	< RL
erythromycin (3)	ND	< RL	< RL	< RL
erythromycin-H <sub>2</sub> O (metabolite) (1)	0.02	0.07	0.05	< RL
lincomycin (1)	0.01	< RL	< RL	< RL
methotrexate (1)	0.02	< RL	< RL	< RL
minocycline (1)	0.02	< RL	< RL	< RL
norfloxacin (1)	0.01	< RL	< RL	< RL
oxytetracycline (1)	0.05	< RL	< RL	< RL
roxarsone (1)	0.5	< RL	< RL	< RL
roxithromycin (1)	0.01	< RL	< RL	< RL
sarafloxacin (1)	0.01	< RL	< RL	< RL
sulfachloropyridazine (1)	0.05	< RL	< RL	< RL
sulfadimethoxine (1)	0.01	< RL	< RL	< RL
sulfamerazine (1)	0.02	< RL	< RL	< RL
sulfamethazine (1)	0.01	< RL	< RL	0.01
sulfamethizole (1)	0.05	< RL	< RL	< RL
sulfamethoxazole (1)	0.05	< RL	< RL	0.02 <sup>e</sup>
sulfamethoxazole (3)	0.023	0.239	0.240	< RL
sulfathiazole (1)	0.05	< RL	< RL	< RL
tetracycline (1)	0.02	< RL	< RL	< RL
trimethoprim (1)	0.01	< RL	< RL	< RL
trimethoprim (3)	0.014	0.122	0.082	< RL
tylosin (1)	0.02	< RL	< RL	< RL
virginiamycin (1)	0.1	< RL	< RL	< RL
<b>Prescription Drugs</b>				
carbamazapine (3)	ND	0.170	0.170	0.116
cimetidine (3)	0.007	< RL	< RL	< RL
codeine (3)	0.24	< RL	< RL	< RL
dehydronifedipine (3)	0.01	0.021 <sup>c</sup>	0.017	< RL
digoxigenin (3)	0.008	< RL	< RL	< RL
digoxin (3)	0.26	< RL	< RL	< RL
diltiazem (3)	0.012	< RL	< RL	< RL
diphenhydramine (3)	ND	0.112	0.081	< RL
fluoxetine (3)	0.018	< RL	< RL	< RL
gemfibrozil (3)	0.015	< RL	< RL	< RL
metformin (3)	0.003	< RL	< RL	< RL
paroxetine metabolite (3)	0.26	< RL	< RL	< RL
ranitidine (3)	0.01	< RL	< RL	< RL
salbutamol (3)	0.029	< RL	< RL	< RL
thiabendazole (3)	ND	< RL	< RL	< RL
urobilin (3)	ND	< RL	< RL	< RL
warfarin (3)	0.001	< RL	< RL	< RL
<b>Nonprescription Drugs</b>				
acetaminophen (3)	0.009	< RL	< RL	< RL
caffeine (3)	0.014	2.3 <sup>d</sup>	0.815	< RL
caffeine (4)	< 0.500	1.9	1.0	< RL
cotinine (3)	0.023	0.137	< RL	0.101

**Table 1 (continued)**

<b>Chemical</b>	<b>Reporting Limit (µg/L)</b>	<b>T<sub>begin</sub> 5/29/2001 (µg/L)</b>	<b>T<sub>end</sub> 6/20/2001 (µg/L)</b>	<b>B<sub>end</sub> 6/20/2001 (µg/L)</b>
cotinine (4)	< 1.000	< RL	< RL	0.140 <sup>e</sup>
1,7-dimethylxanthine (3)	0.018	0.993 <sup>f</sup>	0.324	< RL
ibuprofen (3)	0.018	< RL	< RL	< RL
miconazole (3)	ND	0.074	< RL	< RL
<b>Household and Industrial Chemicals</b>				
acetophenone (4)	< 0.500	< RL	< RL	< RL
acetyl hexamethy tetrahydronaphthalene (AHTN) (4)	< 0.500	1.1	0.180 <sup>e</sup>	< RL
anthracene (4)	< 0.500	< RL	< RL	< RL
anthraquinone (4)	< 0.500	0.310 <sup>e</sup>	0.130 <sup>e</sup>	< RL
benzo[a]pyrene (4)	< 0.500	< RL	< RL	< RL
benzophenone (4)	< 0.500	0.280 <sup>e</sup>	0.150 <sup>e</sup>	0.067 <sup>e</sup>
bisphenol A (4)	< 1.000	0.110 <sup>e</sup>	0.180 <sup>e</sup>	< RL
bromacil (4)	< 0.500	< RL	< RL	< RL
bromoform <sup>1</sup> (4)	< 0.500	< RL	< RL	< RL
camphor (4)	< 0.500	< RL	< RL	< RL
carbaryl <sup>1</sup> (4)	< 1.000	< RL	< RL	0.240 <sup>e</sup>
carbazole (4)	< 0.500	< RL	< RL	< RL
chlorpyrifos (4)	< 0.500	< RL	< RL	< RL
cumene (4)	< 0.500	< RL	< RL	< RL
4-cumylphenol (4)	< 1.000	< RL	< RL	< RL
diazinon (4)	< 0.500	0.100 <sup>e</sup>	0.120 <sup>e</sup>	< RL
1,4-dichlorobenzene <sup>1</sup> (4)	< 0.500	< RL	< RL	< RL
dichlorvos <sup>1</sup> (4)	< 1.000	< RL	< RL	< RL
d-limonene <sup>1</sup> (4)	< 0.500	< RL	< RL	< RL
2,6-dimethylnaphthalene (4)	< 0.500	< RL	0.080 <sup>e</sup>	0.076 <sup>e</sup>
ethanol,2-butoxy-phosphate (4)	< 0.500	1.7	0.370 <sup>e</sup>	< RL
ethyl citrate (4)	< 0.500	0.200 <sup>e</sup>	< RL	< RL
fluoranthene (4)	< 0.500	< RL	< RL	< RL
hexahydrohexamethyl cyclopentabenzopyran (HHCB) (4)	< 0.500	0.330 <sup>e</sup>	0.073 <sup>e</sup>	< RL
indole (4)	< 0.500	< RL	0.077 <sup>e</sup>	< RL
isoborneol (4)	< 0.500	< RL	< RL	< RL
isophorone (4)	< 0.500	< RL	< RL	0.120 <sup>e</sup>
isoquinoline (4)	< 0.500	< RL	< RL	< RL
menthol (4)	< 0.500	< RL	< RL	< RL
metalaxyl (4)	< 0.500	0.110 <sup>e</sup>	< RL	< RL
5-methyl-1H-benzotriazole (4)	< 2.000	0.770 <sup>e</sup>	1.6 <sup>e</sup>	0.550 <sup>e</sup>
1-methylnaphthalene (4)	< 0.500	< RL	< RL	< RL
2-methylnaphthalene (4)	< 0.500	< RL	< RL	< RL
methyl salicylate (4)	< 0.500	< RL	< RL	0.019 <sup>e</sup>
metolachlor (4)	< 0.500	< RL	< RL	< RL
naphthalene (4)	< 0.500	< RL	< RL	< RL
N,N-diethyltoluamide (4)	< 0.500	1.4	1.6	2.3
4-n-octylphenol (4)	< 1.000	< RL	< RL	< RL
4-nonylphenol diethoxylate (total) (NPEO2) <sup>2</sup> (4)	< 5.000	8.800 <sup>e</sup>	4.700 <sup>e</sup>	< RL
4-octylphenol monoethoxylate (OPEO1) <sup>2</sup> (4)	< 1.000	< RL	< RL	< RL
4-octylphenol diethoxylate (OPEO2) <sup>2</sup> (4)	< 1.000	< RL	< RL	< RL
para-cresol (4)	< 1.000	0.110 <sup>e</sup>	< RL	< RL
para-nonylphenol (total) <sup>2</sup> (4)	< 5.000	1.300 <sup>e</sup>	< RL	< RL
pentachlorophenol (4)	< 2.000	< RL	< RL	< RL
phenanthrene (4)	< 0.500	< RL	< RL	< RL
phenol <sup>1</sup> (4)	< 0.500	0.670 <sup>e</sup>	< RL	< RL
prometon (4)	< 0.500	< RL	< RL	< RL
pyrene (4)	< 0.500	< RL	< RL	< RL
skatol (4)	< 1.000	< RL	< RL	< RL

**Table 1 (continued)**

Chemical	Reporting Limit ( $\mu\text{g/L}$ )	T <sub>begin</sub> 5/29/2001 ( $\mu\text{g/L}$ )	T <sub>end</sub> 6/20/2001 ( $\mu\text{g/L}$ )	B <sub>end</sub> 6/20/2001 ( $\mu\text{g/L}$ )
3-tert-butyl-4-hydroxy anisole (BHA) <sup>1</sup> (4)	< 5.000	< RL	< RL	< RL
4-tert-octylphenol (4)	< 1.000	< RL	< RL	< RL
tetrachloroethylene <sup>1</sup> (4)	< 0.500	< RL	< RL	< RL
tributylphosphate (4)	< 0.500	0.130 <sup>e</sup>	0.240 <sup>e</sup>	0.070 <sup>e</sup>
tri(2-chloroethyl) phosphate (4)	< 0.500	0.370 <sup>e</sup>	0.680	0.260 <sup>e</sup>
triclosan (4)	< 1.000	0.350 <sup>e</sup>	0.510 <sup>e</sup>	< RL
tri(dichlorisopropyl) phosphate (4)	< 0.500	0.320 <sup>e</sup>	0.750	< RL
triphenyl phosphate (4)	< 0.500	0.084 <sup>e</sup>	0.055 <sup>e</sup>	< RL
<b>Steroids and Reproductive Hormones</b>				
4-androstene-3,17-dione (5)	0.005	< RL	< RL	< RL
beta-sitosterol (4)	< 2.000	0.940 <sup>e</sup>	< RL	< RL
cholesterol (4)	< 2.000	3	0.700 <sup>e</sup>	< RL
cholesterol (5)	0.005	0.663	0.528	0.158
cis-androsterone (5)	0.005	< RL	< RL	< RL
3-beta-coprostanol (4)	< 2.000	1.900 <sup>e</sup>	0.280 <sup>e</sup>	< RL
3-beta-coprostanol (5)	0.005	< RL	< RL	< RL
diethylstilbestrol (5)	0.005	< RL	< RL	< RL
epitestosterone(5)	0.005	< RL	< RL	< RL
equilenin (5)	0.005	< RL	< RL	< RL
equilin (5)	0.005	< RL	< RL	< RL
estriol (5)	0.005	< RL	< RL	< RL
17 $\alpha$ -ethynyl estradiol (5)	0.005	< RL	< RL	< RL
17 $\alpha$ -estradiol (5)	0.005	< RL	< RL	< RL
17 $\beta$ -estradiol (5)	0.005	< RL	< RL	< RL
estrone (5)	0.005	< RL	< RL	< RL
11-ketotestosterone (5)	0.005	< RL	< RL	< RL
mestranol (5)	0.005	< RL	< RL	< RL
19-norethisterone (5)	0.005	< RL	< RL	< RL
progesterone (5)	0.005	< RL	< RL	< RL
stanoalone (5)	0.005	< RL	< RL	< RL
stigmastanol (4)	< 2.000	< RL	< RL	< RL
testosterone (5)	0.005	< RL	< RL	< RL
trenbolone (5)	0.005	< RL	< RL	< RL

(1), (3), (4), (5)—Compound analyzed by method 1, 3, 4, or 5 as described in Kolpin et al. (2002)

<sup>1</sup>Compound concentration estimated—average recovery < 60% in laboratory spike sample

<sup>2</sup>Compound concentration estimated—reference standard prepared from a technical mixture

T<sub>begin</sub>—Column inflow at beginning of experiment

T<sub>end</sub>—Column inflow at end of experiment

B<sub>end</sub>—Column drainage at end of experiment

< RL—Less than reporting limit

ND—Reporting limit not determined

<sup>e</sup>Detected in laboratory blank at 0.004  $\mu\text{g/L}$

<sup>d</sup>Value greater than highest point on calibration curve

<sup>e</sup>Detected, but concentration is less than the reporting limit or lowest calibration standard

<sup>f</sup>Detected in laboratory blank at 0.009  $\mu\text{g/L}$

because it is commonly found in waste water (Atlas 1999). Unlike enteric pathogens, *Legionella* can survive well in biofilms and in protozoa, allowing it to endure extreme ranges of environmental conditions. *Salmonella* was chosen because it is one of the most common enteric pathogens found in waste water (Toze 1999). It can persist for extended periods in nutrient-rich waters (Kampelmacher and Van Noorle Jasen 1976; Claudon et al. 1971). Both of these pathogens are difficult to culture in the laboratory; therefore, samples were analyzed by the culture-independent method of polymerase chain reaction (PCR). PCR is the exponential

amplification of a gene of interest. A fluorescent dye is used to detect real-time DNA amplification. Identification of a particular pathogen is done through the use of primers specific for a gene that is unique to the pathogen of interest. Positive amplification results in identification of a particular pathogen in a sample.

For microbial analysis, 1 L samples were filtered through 0.2  $\mu\text{m}$  filters and stored at  $-20^{\circ}\text{C}$  until ready for DNA extraction at USWCL. Genomic DNA was extracted from microorganisms retained in the filters using the procedure described by Smalla (1995). Chemical and enzymatic cell

lysis was carried out in the filter housing. The lysate was then removed from the filter and transferred to centrifuge tubes for overnight precipitation of the DNA with ammonium acetate and ethanol. The extracted DNA was washed, dried, and resuspended in Tris-EDTA, purified, and quantified by spectral analysis. Real-time PCR analyses were carried out using *Legionella*-specific primers (L5SL9 and L5SR93) (Mahbubani et al. 1990) and *Salmonella*-specific primers (*fim1A* and *fim2A*) (Cohen et al. 1996). The fluorescent dye, SYBR® Green, was used for detection of amplified products. DNA from *Legionella pneumophila* (ATCC #33152) and *Salmonella typhimurium* (ATCC#700720) were used as positive controls; *Escherichia coli* (ATCC #10798) was the negative control in the PCR reactions.

## Results and Discussion

Infiltration rates were ~16.5 cm/day at the beginning of the experiment, decreasing rapidly during the first three days to ~7.5 cm/day on June 3. By the end of the experiment, the infiltration rate had slowed to ~4.5 cm/day. The infiltration rate was limited by the development of a clogging layer at the top of the soil column as is typically observed under field conditions (Rice 1974) and by the relatively low permeability of the compacted soil in the column. Accumulated infiltration over the duration of the experiment totaled ~123 L or an average rate of 5.3 cm/day.

Specific conductance values, TOC concentrations, and specific absorbance ( $UV_{254}/TOC$ ) values for the column inflow and drainage (Figure 2) were used to determine (1) changes in the effluent in the storage tank (column inflow) and (2) if the dynamic processes involving major ions and organic matter in the soil column (biotic and abiotic transformation, adsorption, and desorption) had reached steady state when the sample of column drainage ( $B_{end}$ ) was collected. Sampling the column drainage after steady state is reached provides a sample that represents the bulk effluent characteristics.

The specific conductance of the column inflow (Figure 2a) gradually decreased during the first 13 days of the experiment, whereas the conductance of the column drainage fluctuated substantially during the same period as constituents stored in the column soils were released. Though the specific conductance of the column inflow and drainage continued to decrease during the last 10 days of the experiment, the rate of decrease in specific conductance appeared to stabilize in both during this period (Figure 2a). Stabilization of rate of specific conductance decrease in the inflow and drainage indicates that the physical, chemical, and biological processes taking place in the storage tank and soil column had reached a steady state when the column drainage sample ( $B_{end}$ ) was collected. However, the higher conductance of the column drainage (1950  $\mu S/cm$ ) compared to the inflow (1590  $\mu S/cm$ ) indicates that processes taking place as the effluent traveled through the column (such as biotransformation, adsorption, and desorption) had not reached equilibrium.

The TOC values for column inflow and drainage were compared to the TOC concentration of the effluent at the beginning of the experiment ( $C_0 = 8.87$  mg/L) to determine if the column inflow was degrading during the experiment.

The  $C/C_0$  ratios in both the inflow and drainage declined over days 1 to 8 of the experiment and then fluctuated during days 9 to 13 as the dynamic processes in the storage tank and the soil column each approached steady state (Figure 2b). During the last 10 days of the experiment, the  $C/C_0$  ratios of the column inflow and drainage converge at slightly  $< 1.0$  indicating that the processes affecting TOC in the storage tank and the column had reached steady state when the drainage sample was collected, and that some degradation of the effluent had taken place.

The specific  $UV_{254}$  absorbance ( $SA_{254}$ ) ratio, which for purposes of this study is the ratio of ultraviolet light absorbance ( $UV_{254}$ ) to TOC ( $SA_{254} = UV_{254}/TOC$ ), is a good indicator of organic carbon derived from vascular plant material (Barber et al. 2001). The lower  $SA_{254}$  ratio of the column inflow compared to the column drainage is indicative of the lower concentration of plant material in the inflow (Figure 2c). The  $SA_{254}$  ratio of the effluent increased significantly as the effluent moved through the soil column, indicating displacement of natural organic matter that was sequestered in the column soils and (or) preferential removal of nonplant organics from the effluent. Over the last 10 days of the experiment, the  $SA_{254}$  ratio of both the inflow and drainage generally stabilized as the dynamic processes in both the storage tank and the soil column approached steady state; however, equilibrium of processes within the soil column had not yet been reached.

## Water Chemistry

### Column Inflow ( $T_{begin}$ )

Thirty-three different OWCs were detected in the column inflow sample,  $T_{begin}$ , collected at the beginning of the experiment (Table 1). Eight compounds in sample  $T_{begin}$  had concentrations  $> 1$   $\mu g/L$ : caffeine; acetyl hexamethyl tetrahydronaphthalene (AHTN); ethanol, 2-butoxy-phosphate; N, N-diethyltoluamide; nonylphenol diethoxylate (NPEO2, total); para-nonylphenol (total); cholesterol; and 3-beta-coprostanol (Table 1). The remaining 25 OWCs were detected at concentrations  $< 1$   $\mu g/L$ . The total concentration of all OWCs detected and quantified in this sample was ~26  $\mu g/L$ . (Only one of the concentrations measured was included for compounds detected by more than one method.)

Concentrations of 13 OWCs in  $T_{begin}$  generally equaled or exceeded their respective RLs; however, NPEO2 and phenol concentrations were considered estimated because of reference standard and laboratory spike recovery issues (Table 1). Concentrations for 17 of the 33 compounds detected in sample  $T_{begin}$  were estimated because they were  $< RL$  (Table 1). Reporting limits for three of the compounds detected in  $T_{begin}$  were not determined.

The effluent used for this experiment is similar in terms of numbers and types of compounds to effluent from other waste water treatment plants in Arizona that is used for ground water recharge. For example, 38 OWCs were detected in effluent from the 91st Avenue Wastewater Treatment Plant outfall in Phoenix, and 30 were detected in effluent in Tucson from the Santa Cruz River at Cortaro Road (Barnes et al. 2002) compared to 33 OWCs in  $T_{begin}$ . Thirteen of the 30 most frequently detected OWCs in U.S. streams

(Kolpin et al. 2002) were detected in all three effluents (91st Avenue, Cortaro Road, and this study) including erythromycin-H<sub>2</sub>O; sulfamethoxazole; trimethoprim; caffeine; cotinine; 1,7-dimethylxanthine; bisphenol A; diazinon; 5-methyl-1H-benzotriazole; N,N-diethyltoluamide; NPEO2 (total); tri(2-chloroethyl) phosphate; and triclosan. The Phoenix and Tucson effluent sources are 100% effluent and are used for ground water recharge in spreading basins and wetlands, with some incidental recharge taking place in stream channels where the effluent is discharged.

#### Column Inflow ( $T_{end}$ )

Twenty-seven different OWCs were detected in the column inflow,  $T_{end}$ , at the end of the experiment. Four OWCs in  $T_{end}$  had concentrations  $\geq 1$   $\mu\text{g/L}$  including caffeine; 5-methyl-1H benzotriazole; N,N-diethyltoluamide; and NPEO2 (total). The remaining 23 OWCs were detected at concentrations of  $< 1$   $\mu\text{g/L}$ . The total concentration of all OWCs detected and quantified in this sample was  $\sim 14$   $\mu\text{g/L}$ .

Concentrations of 10 OWCs in  $T_{end}$  generally equaled or exceeded their respective RLs. Concentrations of 15 of the total of 27 OWCs detected in sample  $T_{end}$  were estimated because they were  $< \text{RL}$ . Reporting limits for two of the compounds detected in  $T_{begin}$  were not determined (Table 1).

A comparison of the compounds detected in the column inflow at the beginning ( $T_{begin}$ ) and end ( $T_{end}$ ) of the experiment indicates that 25 of the 33 OWCs detected in  $T_{begin}$  remained at detectable concentrations in the column inflow,  $T_{end}$ , 23 days later. Two of the OWCs detected in  $T_{end}$ , indole and 2,6-dimethylnaphthalene, were not detected in the column inflow ( $T_{begin}$ ) at the beginning of the experiment. Indole is a bacterial degradation product of the antidepressant tryptophan, as well as being a basic building block for many other pharmaceuticals and a fixative for perfumes (Wiley Interscience 2002). The polyaromatic hydrocarbon, 2,6-dimethylnaphthalene, is an indicator of diesel or kerosene, and is a product of fuel oil degradation. This compound has been reported to occur at greater concentrations in the environment than the parent material, naphthalene (Irwin et al. 1997). Because both of these compounds were at concentrations  $< \text{RL}$  in  $T_{end}$ , they may have been present in  $T_{begin}$  at concentrations that were not detectable. Large organic loads in the column inflow ( $T_{begin}$ ) may have interfered with initial detection of these OWCs, small variations in the analytical accuracy may have prevented or contributed to the detection of these OWCs, and (or) over the period of the experiment, indole and 2,6-dimethylnaphthalene concentrations may have increased to detectable levels owing to processes in the storage tank such as biotransformation. Although care was taken in sample collection to prevent external contamination, one or both compounds may represent environmental contamination from the storage tank or from sample collection and exposure to the atmosphere.

The total concentration of OWCs detected and quantified in  $T_{begin}$  (26  $\mu\text{g/L}$ ) decreased by 46% when compared to  $T_{end}$  (14  $\mu\text{g/L}$ ), indicating that some OWCs were degraded by processes in the storage tank and (or) adsorbed to the tank or particles in the tank. Eight compounds detected in effluent from the storage tank at the beginning of the experiment were not detected 23 days later in sample  $T_{end}$  (Table 1).

They included cotinine (method 3), miconazole, ethyl citrate, metalaxyl, para-cresol, para-nonylphenol (total), phenol, and beta-sitosterol. Some of these compounds may have adsorbed to the storage tank containing the effluent or to particles that settled to the bottom of the tank over the period of the experiment. Some may also have been transformed or volatilized and lost during the experiment and, thus, were not at detectable concentrations when  $T_{end}$  was collected. Of the eight, only cotinine was subsequently detected in the column drainage,  $B_{end}$ .

Many of the compounds with concentrations  $> \text{RL}$  in  $T_{begin}$ , (trimethoprim; diphenhydramine; caffeine; 1,7-dimethylxanthine; AHTN; ethanol, 2-butoxy-phosphate; NPEO2; and cholesterol) were detected at lower concentrations in  $T_{end}$ , contributing to the decrease in total concentration of OWCs in the storage tank effluent. The reduction in caffeine concentration by 50% or more from  $T_{begin}$  to  $T_{end}$  suggests that caffeine may not have a high degree of environmental persistence and may not be suitable for use as a chemical indicator of the presence of human sewage in natural waters (Scott et al. 2002).

The prescription drugs sulfamethoxazole (method 3) and carbamazepine, and the insect repellent N, N-diethyltoluamide, showed no significant change in concentration from  $T_{begin}$  to  $T_{end}$ . Studies have shown that sulfamethoxazole and carbamazepine can persist in the environment and reach ground water in a bank filtration setting (Heberer et al. 2001). Sulfamethoxazole was one of the 30 most frequently detected OWCs in targeted U.S. streams (Kolpin et al. 2002). N, N-diethyltoluamide was the third most frequently detected compound in targeted U.S. streams (Kolpin et al. 2002); however, little information is available on its persistence in ground water.

Studies showing that antimicrobial agents like sulfamethoxazole can be excreted unchanged in the urine (Masters et al. 2003) raise questions about the ecological effects of antibiotic residues in municipal waste water, the potential for selection of antibiotic-resistant bacteria, and possible transport to ground water during recharge. The increased use of sulfamethoxazole, particularly in combination with trimethoprim (TMP-SMX), as antimicrobial treatment for urinary infections and as prophylaxis for *Pneumocystis carinii* pneumonia in HIV patients over the past decade (Martin et al. 1999) has resulted in increased antimicrobial resistance (Masters et al. 2003). Furthermore, there have been reports of sulfamethoxazole antimicrobial-resistant genes in environmental isolates of *E. coli* (Zhao et al. 2001; Roe et al. 2003) and *Salmonella* (Gebreyes et al. 2000).

#### Column Drainage ( $B_{end}$ )

Fourteen different OWCs were detected in the column drainage,  $B_{end}$  (Table 1), compared to 33 in the original effluent,  $T_{begin}$ , and 27 in  $T_{end}$ . Only one compound in  $B_{end}$ , N, N-diethyltoluamide, exceeded a concentration of 1  $\mu\text{g/L}$ . The total concentration of all detected and quantified OWCs in this sample was  $\sim 4$   $\mu\text{g/L}$ .

Concentrations of four OWCs in  $B_{end}$  equaled or exceeded their respective RLs—sulfamethazine; cotinine (method 3); N, N-diethyltoluamide; and cholesterol (method 5). Ten of the OWCs in  $B_{end}$  were detected at concentrations

< RL, including cotinine (method 4) (Table 1). The reporting limit for one compound detected in  $B_{end}$  was not determined (Table 1).

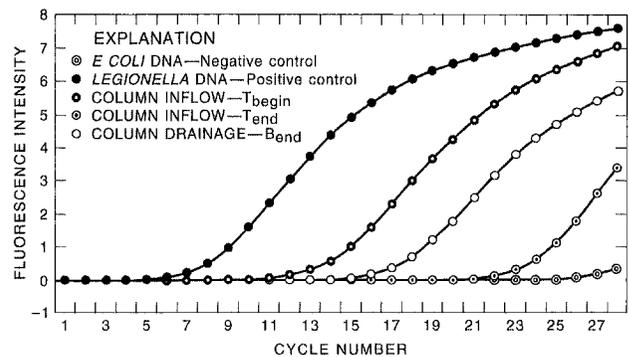
Ten of the 14 OWCs detected in the column drainage were the same compounds detected in one or both of the column inflow samples,  $T_{begin}$  and  $T_{end}$ . One of the 10, sulfamethoxazole, though detected in  $T_{begin}$  and  $T_{end}$  by method 3, was detected only in  $B_{end}$  by method 1. Sulfamethazine, carbaryl, isophorone, and methyl salicylate were detected only in column drainage,  $B_{end}$ . The detection of compounds in the column drainage that were not detected in the column inflow may be the result of compounds being adsorbed in the soil column during the preconditioning and then desorbed and released during this experiment. Other possible explanations could be that large organic loads in the column inflow interfered with detections of these compounds in  $T_{begin}$  and  $T_{end}$ , and (or) small variations in the analytical accuracy may have prevented or contributed to the detection of these OWCs.

The total concentration of OWCs detected and quantified in  $T_{end}$  (14  $\mu\text{g/L}$ ) decreased by more than 70% when compared to  $B_{end}$  (4  $\mu\text{g/L}$ ). Eighteen compounds detected in  $T_{end}$  were not detected in sample  $B_{end}$  (Table 1), indicating that ~67% of the OWCs were attenuated by processes at the top of and within the soil column such as biotic and abiotic transformation, adsorption, and volatilization.

Only eight compounds were common to and persisted in all three samples. These OWCs included carbamazepine; sulfamethoxazole (combining data from methods 1 and 3); benzophenone; 5-methyl-1H benzotriazole; N,N-diethyltoluamide; tributylphosphate; tri(2-chloroethyl) phosphate; and cholesterol (method 5 only) (Table 1). Compounds detected in all three samples, particularly those with concentrations > RL, demonstrated a high degree of persistence and, hence, potential to reach ground water under recharge conditions simulated by this experiment.

### Pathogens

Although the effluent contained a large number of background microflora including heterotrophic bacteria ( $2.0 \times 10^4$  colony-forming units[CFU]/1 mL) and total coliform bacteria ( $1.0 \times 10^3$  CFU/100 mL), DNA for the specific pathogens *Legionella* and *Salmonella* was detected due to high specificity of the PCR primers. The concentrations of total DNA recovered for PCR analysis from  $T_{begin}$ ,  $T_{end}$ , and  $B_{end}$  were 55, 25, and 34  $\mu\text{g/mL}$ , respectively. The lower DNA recovery at  $T_{end}$  (25  $\mu\text{g/mL}$ ) compared to  $T_{begin}$  (55  $\mu\text{g/mL}$ ) suggests a general decrease in microbial density in the column inflow during the experiment. Results from real-time PCR demonstrated the presence of *Legionella* in the column inflow samples,  $T_{begin}$  and  $T_{end}$ , and also in the column drainage,  $B_{end}$  (Figure 3). *Salmonella* was detected in  $T_{begin}$  but not in  $T_{end}$  or  $B_{end}$ , suggesting that the bacteria died off in the storage tank over the period of the experiment. Enzymes responsible for the degradation of DNA are thought to be ubiquitous in most environments as well as in microbial cells (Ogram 1998). Therefore, DNA from dead microbial cells is not likely to persist in sewage effluent, which may explain why *Salmonella* DNA was detected at  $T_{begin}$  but not at  $T_{end}$ . The fact that *Legionella* can survive extreme ranges of envi-



**Figure 3. Real-time PCR amplification results for *Legionella* specific primers. DNA amplification of column inflow ( $T_{begin}$ ,  $T_{end}$ ) and column drainage ( $B_{end}$ ) demonstrated the presence of *Legionella* in all three samples. Amplification of positive controls, but not negative controls, indicates good specificity of the primers.**

ronmental conditions including thermal and chlorine disinfection (Atlas 1999) may have aided in their survival and transport through the soil column. These results suggest that *Legionella* is likely to persist during typical recharge conditions and has the potential to reach the ground water.

### Conclusions

The detection of veterinary and human antibiotics, other prescription and nonprescription drugs, widely used household and industrial chemicals, steroids and reproductive hormones, and pathogens in treated effluent that is used for ground water recharge suggests that this practice may be a potential source of OWCs and pathogens to ground water. This proof-of-concept experiment demonstrated that some OWCs and pathogens might persist during SAT and have the potential to reach ground water when treated effluent is used for ground water recharge under conditions similar to those in arid and semiarid climates.

Analytical results for the two effluent samples taken from the storage tank at the beginning ( $T_{begin}$ ) and end ( $T_{end}$ ) of the experiment indicate that the number of OWCs detected in the column inflow decreased by 25% (eight compounds), and the total concentration of OWCs decreased by 46% while the effluent was in the storage tank during the 23-day experiment. After percolating through the soil column, an additional 18 compounds detected in  $T_{end}$  (67% of OWCs) were no longer detected in the effluent ( $B_{end}$ ), and the total concentration of OWCs decreased by > 70%. These compounds may have been subject to transformation (biotic and abiotic), adsorption, and (or) volatilization in the storage tank and during travel through the soil column.

Eight compounds were detected in all three samples. They included carbamazepine; sulfamethoxazole (from methods 1 and 3 combined); benzophenone; 5-methyl-1H benzotriazole; N,N-diethyltoluamide; tributylphosphate; tri(2-chloroethyl) phosphate; and cholesterol (method 5 only) (Table 1). The persistence of these compounds in effluent in the storage tank and in the column drainage indicates they have the potential to reach ground water under recharge conditions similar to those in arid and semiarid climates. It is

important to note that any compounds sequestered in the soil column have the potential to be remobilized and could still reach ground water in the future. Transport of compounds, even those that appeared less persistent in this experiment, may still occur under field conditions if preferential flow-paths allow recharged effluent to travel more quickly through the subsurface in cracks, root holes, wormholes, and macropores in fine-grained soils (Bouwer 1990).

Application of the results of this study requires consideration of the limitations of the proof-of-concept experiment design. Results for specific conductance, TOC, and SA<sub>254</sub> indicated that although processes in the storage tank and soil column had generally reached a steady state, chemical equilibrium had not been attained. Previous column studies of SAT have shown that repeated wetting and drying cycles over a period of months are necessary to establish steady state, through-column removal efficiencies, and microbial communities (Quanrud et al. 1996; Westerhoff and Pinney 2000). By covering and insulating the effluent storage tank, degradation of the effluent by photolysis, which might occur under natural conditions, was prevented. Finally, depths to ground water in many arid and semiarid areas are much greater than the 2.4 m soil column in this experiment. Because of these limitations, the number of compounds and concentrations reported here might be higher than those that would be detected under recharge conditions at SAT sites in arid and semiarid areas.

Pathogen results indicate that *Legionella* is more likely to persist during recharge and has greater potential to reach ground water than *Salmonella*. The fact that *Legionella* species are found in all stages of waste water treatment, and their numbers do not decline significantly through the treatment process (Palmer et al. 1993) is of concern as water reuse practices continue to increase. Long-term survival of *Legionella* (up to 2.5 years) in ground water has been reported (Paszko-Kolva et al. 1992) as well as the detection of *Legionella* in ground water samples (Riffard et al. 2001). Hence, the fate and transport of *Legionella* under recharge conditions deserves further attention to ensure that recharge with treated waste water and the subsequent use of ground water does not result in a new source of *Legionella* infections. *Legionella* is only one of the many pathogens that can be found in waste water; therefore, proper assessment of water reuse applications and future studies should include the enteric viruses and protozoa, which have a low infectious dose.

As a proof-of-concept, this experiment demonstrates that, under recharge conditions similar to those in arid and semiarid climates, the potential for some pharmaceuticals, pathogens, and other OWCs to be transported into the ground water exists and merits additional research and monitoring to determine the magnitude of potential transport. Results from this study can be used to focus the attention of fate and transport studies from a vast list of OWCs to those compounds and pathogens that are most likely to persist under recharge conditions and are of environmental and public health priority.

Ongoing studies include field-testing the results of this experiment by sampling wells in Arizona where effluent is being recharged or used for irrigation. Because of the persistence of antibiotics in this experiment, future work will also

determine if antibiotic residues in treated sewage effluent can exert selection pressure for the sulfonamide-resistance (*sulI*) gene. This is an important consideration since *sulI* genes are associated with integrons that have the ability to integrate gene cassettes encoding resistance to multiple antimicrobial agents (Fluit and Schmitz 1999) and may, therefore, increase the potential for development of multiple antimicrobial-resistant bacteria in waste water used for recharge or irrigation.

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## Biographical Sketches

**Gail E. Cordy** (USGS, 520 N. Park Ave., Ste. 221, Tucson, AZ 85719; [520] 670-6671, ext. 223; gcordy@usgs.gov) is a supervisory hydrologist and has worked for the USGS since 1984. Her current research interests include determining the potential for ground water contamination by recharge and irrigation with effluent in arid and semiarid climates.

**Norma Duran** (U.S. Environmental Protection Agency, 4050 Rio Bravo, Ste. 100, El Paso, TX 79902; [915] 533-7273, ext. 224; duran.norma@epa.gov) works for the U.S. EPA Border Office, where she coordinates environmental projects along the U.S./Mexico border. Her research interests include the fate and transport of pathogens in the subsurface and in water distribution systems with an emphasis on microbiological safety of water-reuse practices.

**Herman Bouwer** (U.S. Water Conservation Laboratory, U.S. Dept. of Agriculture, 4331 E. Broadway Rd., Phoenix, AZ 85040; [602] 437-1702, ext. 244; hbouwer@uswl.ars.ag.gov) was chief engineer (retired) at the USWCL for more than 42 years, almost all of which was devoted to the study of artificial recharge of ground water and soil-aquifer treatment, especially with sewage effluent. His work has been published in numerous articles in peer-reviewed journals and book chapters.

**Robert C. Rice** (GeoSystems Analysis Inc., 2015 N. Forbes Blvd., Tucson, AZ 85745; [520] 628-9330; riceqhb@netscape.net) spent 33 years at the U.S. Water Conservation Laboratory conducting research on vadose zone transport including hydraulic property evaluation and ground water recharge and waste water renovation by soil-aquifer treatment. He has also conducted research on spatial variability of solute transport and preferential flow phenomenon in the vadose zone as part of best management practice studies related to irrigation and fertilizer applications.

**Edward T. Furlong** (USGS, National Water Quality Laboratory, Denver Federal Center, P.O. Box 25046, MS 407, Lakewood, CO 80225-0046; [303] 236-3945; efurlong@usgs.gov) is a

research chemist who has worked for the last 16 years developing and applying ultratrace analytical techniques to determine the presence, distribution, and environmental chemistry of organic contaminants in aquatic environments. His current research interests encompass the analysis and environmental chemistry of pharmaceuticals, personal care products, and other organic waste water contaminants.

**Steven D. Zaugg** (USGS, National Water Quality Laboratory, Denver Federal Center, P.O. Box 25046, MS 407, Lakewood, CO 80225-0046; [303] 236-3269; sdzaugg@usgs.gov) has worked as an analytical chemist for the USGS since 1987. His current research interests include developing solid-phase extraction techniques and accelerated solvent-extraction techniques for water and sediment analysis of pesticides and emerging contaminants in the environment by gas chromatography/mass spectrometry.

**Michael T. Meyer** (USGS, 4821 Quail Crest Place, Lawrence, KS 66049; [785] 832-3544; mmeyer@usgs.gov) is director of the USGS Kansas District Organic Geochemistry Research Group. The focus of his research is development of analytical methods to study the nature of organic contaminants in surface water and ground water. His primary interest is the study of emerging contaminants such as pesticide degradates and pharmaceutical compounds.

**Larry B. Barber** (USGS, 3215 Marine St., Ste. E-127, Boulder, CO 80303; [303] 541-3039; lbbarber@usgs.gov) is a research geochemist with 20 years of experience at the USGS. His current research interests include the occurrence and fate of waste water derived contaminants in surface and ground water systems.

**Dana W. Kolpin** (USGS, Federal Bldg, Room 269, 400 South Clinton St., Iowa City, IA 52240; [319] 358-3614; dwkolpin@usgs.gov) is a research hydrologist with the USGS. His research includes water quality investigations of ground and surface water at local, regional, and national scales.