SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 28, No. 12, pp. 2587–2597, 2009
© 2009 SETAC
Printed in the USA
0730-7268/09 $12.00 + .00

Pharmaceuticals and Personal Care Products in the Environment

OCCURRENCE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN FISH: RESULTS OF A NATIONAL PILOT STUDY IN THE UNITED STATES

ALEJANDRO J. RAMIREZ,†§ RICHARD A. BRAIN,‡§ SASCHA USENKO,‡§ MOHAMMAD A. MOTTALEB,†§ JOHN G. O'DONNELL,|| LEANNE L. STAHL,# JOHN B. WATHEN,# BLAINE D. SNYDER,|| JENNIFER L. PITT,|| PILAR PEREZ-HURTADO,† LAURA L. DOBBINS,‡§ BRYAN W. BROOKS,‡§ and C. KEVIN CHAMBLISS*†§

†Department of Chemistry and Biochemistry, ‡Department of Environmental Science, §Center for Reservoir and Aquatic Systems Research, Baylor University, One Bear Place 97348, Waco, Texas 76798, USA

||Tetra Tech, Center for Ecological Sciences, 400 Red Brook Boulevard, Suite 200, Owings Mills, Maryland 21117, USA

#U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology (4305T), 1200 Pennsylvania Avenue, Northwest, Washington, DC 20460

(*Received* 5 *November* 2008; *Accepted* 18 *February* 2009)

Abstract—Pharmaceuticals and personal care products are being increasingly reported in a variety of biological matrices, including fish tissue; however, screening studies have presently not encompassed broad geographical areas. A national pilot study was initiated in the United States to assess the accumulation of pharmaceuticals and personal care products in fish sampled from five effluent-dominated rivers that receive direct discharge from wastewater treatment facilities in Chicago, Illinois; Dallas, Texas; Orlando, Florida; Phoenix, Arizona; and West Chester, Pennsylvania, USA. Fish were also collected from the Gila River, New Mexico, USA, as a reference condition expected to be minimally impacted by anthropogenic influence. High performance liquid chromatography–tandem mass spectrometry analysis of pharmaceuticals revealed the presence of norfluoxetine, sertraline, diphenhydramine, diltiazem, and carbamazepine at nanogram-per-gram concentrations in fillet composites from effluent-dominated sampling locations; the additional presence of fluoxetine and gemfibrozil was confirmed in liver tissue. Sertraline was detected at concentrations as high as 19 and 545 ng/g in fillet and liver tissue, respectively. Gas chromatography–tandem mass spectrometry analysis of personal care products in fillet composites revealed the presence of galaxolide and tonalide at maximum concentrations of 2,100 and 290 ng/g, respectively, and trace levels of triclosan. In general, more pharmaceuticals were detected at higher concentrations and with greater frequency in liver than in fillet tissues. Higher lipid content in liver tissue could not account for this discrepancy as no significant positive correlations were found between accumulated pharmaceutical concentrations and lipid content for either tissue type from any sampling site. In contrast, accumulation of the personal care products galaxolide and tonalide was significantly related to lipid content. Results suggest that the detection of pharmaceuticals and personal care products was dependent on the degree of wastewater treatment employed.

Keywords—Pharmaceuticals Personal care products Accumulation Fish tissue Effluent-dominated sites

INTRODUCTION

The presence of pharmaceuticals and personal care products (PPCPs) in the environment has increasingly received attention from scientific, regulatory, and public sectors [1,2]. International efforts are examining environmental occurrence, fate, effects, ecological and human health risks, and risk management approaches for these substances. Although the majority of information on environmental occurrence of PPCPs is available from effluent discharges and surface waters, an increasing body of literature indicates that select pharmaceuticals [3–9] and personal care products (PCPs) [10–17] accumulate in wild-caught fish populations at nanogram-per-gram concentrations. However, environmental analytical chemistry efforts to examine PPCPs in fish tissue have previously focused on specific chemicals or chemical classes at single study sites.

Brooks et al. [3] provided the first report of human pharmaceuticals accumulating in liver, brain, and muscle tissues of three fish species collected in Pecan Creek, a lower order effluent-dominated stream in north central Texas, USA. Using forensic toxicology methods including gas chromatography–mass spectrometry, distribution of the antidepressants fluoxetine and sertraline and their respective primary metabolites norfluoxetine and desmethylsertraline were determined at highest levels (low nanograms per gram) in liver and brain tissues relative to lateral fillets, which generally paralleled disposition of these compounds in mammals [3]. Ramirez et al. [8] significantly advanced this initial study by developing a fish tissue screening method with high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) for a larger list of select pharmaceuticals reported to partition appreciably to sediments [18] and biosolids [19]. Ramirez et al. [8] further provided a novel report of accumulation of diphenhydramine, diltiazem, and carbamazepine in fish collected from Pecan Creek [8]. More recent studies in the laboratory and field have provided independent validation of findings in the Brooks et al. [3] and Ramirez et al. [8] studies [5,7,20–22] and identified additional compounds in fish blood [4], suggesting that further studies of pharmaceutical bioaccumulation in aquatic ecosystems are necessary.

Select PCPs have similarly been detected in effluent-dominated streams. For example, Mottaleb et al. [6] developed a gas chromatography–tandem mass spectrometry (GC-MS/MS) screening method for fish tissue fillets sampled from Pecan Creek that detected several compounds, including the

* To whom correspondence may be addressed (kevin_chambliss@baylor.edu).
Published on the Web 3/25/2009.

fragrances galaxolide and tonalide at concentrations up to 970 and 97 ng/g, respectively. Comparable values for these compounds and others have also been quantified in fish tissue from rivers receiving discharge from heavily urbanized and industrialized areas in Germany [23]. Detections have also been identified in Swiss rivers [11,24], and PCP residues have further been characterized in fish tissue sampled from fish farms [12]. Investigations have also identified PCPs in large lentic systems including Lake Michigan [16] and other Michigan waters [13] and even in more remote areas such as alpine lakes in Switzerland [17]. Terrestrial bioaccumulation of these PPCPs is also possible as indicated by a recent study identifying accumulation to similar levels in earthworms exposed to biosolid-amended soils [25].

The most significant entry route for human pharmaceuticals into the aquatic environment is the point-source release from wastewater treatment plants (WWTPs) [2]. Although WWTPs are capable of removing a large proportion of pharmaceuticals through various treatment processes [26], not all compounds are eliminated completely, with removal efficiencies varying according to the wastewater treatment processes employed at individual facilities, resulting in potential discharge to receiving waters [27]. Consequently, when selecting sampling locations for field assessments of PPCP exposure, influence of municipal effluent discharges on in-stream hydrology is a critical consideration. Effluent-dominated systems generally represent worst-case scenarios for PPCP exposure to aquatic life and human populations consuming fish tissue [28]. Under circumstances where environmental dissipation rates (half-lives) are exceeded by introduction rates from effluent loadings, but longer than hydrologic retention times, PPCPs can take on "pseudopersistent" exposure characteristics [29]. Consequently, effluent-dominated streams present particular potential for accumulation of these compounds in aquatic life because effective exposure duration is increased [1]. As a result, organisms residing in these aquatic systems receive continuous exposures to wastewater-derived contaminants over their entire life cycle [28]. Such exposure scenarios are not represented in standardized toxicity tests used to assess ecological risk of PPCPs to aquatic life [30].

Information on PPCPs in fish to date indicate that an additional understanding of PPCP accumulation in aquatic life at a broad scale is necessary to support future efforts characterizing ecological and human health risks of PPCPs in the environment. As a consequence, the U.S. Environmental Protection Agency's (U.S. EPA's) Office of Science and Technology (within the Office of Water), in conjunction with Tetra Tech and Baylor University, designed a pilot study to advance the science related to detecting PPCPs in the environment by investigating the occurrence of PPCP chemicals in fish tissue from locations distributed across the United States. All aspects of the pilot study were executed according to publically accessible quality assurance project plans (www.epa.gov/waterscience/ppcp/studies/fish-tissue.html). The targeted study design involved sampling and analysis of fish from five effluent-dominated rivers in various parts of the country and from one reference location in New Mexico. Fish sampling, handling, and processing followed procedures employed during the U.S. EPA's National Study of Chemical Residues in Lake Fish Tissue (www.epa.gov/waterscience/fish/study), and analytical methods for PPCPs followed those previously developed by the project team [6,8]. Conceptually similar to a previous stream study by Kolpin et al. [31], this is the first study of PPCPs accumulating in fish tissue that provides results for locations distributed widely throughout the country.

MATERIALS AND METHODS

Study site selection

Site selection criteria were applied to identify target areas where PPCPs were expected to occur, specifically, effluent-dominated river segments near WWTP discharges, WWTP discharges subject to different levels of treatment, urban or suburban areas with a large percentage of elderly residents, and cities with higher median incomes (as a surrogate for prescription sales). Site selection was also contingent upon the availability of sufficient numbers and sizes of resident fish for analysis. Based on these criteria six geographical locations in various parts of the United States were selected as suitable sampling sites (Fig. 1): five effluent-dominated rivers receiving discharge from WWTPs located in Chicago, Illinois; Dallas, Texas; Orlando, Florida; Phoenix, Arizona; and West Chester, Pennsylvania; and one reference site on the Gila River, New Mexico. The reference site was expected to be removed from anthropogenic point sources; therefore, no accumulation of PPCPs was expected in fish collected from this site. Information on WWTP design capacity, average discharge, and in-stream waste concentration is presented in Table 1, along with demographic data pertinent to each location.

Fish sampling and preservation

A total of 18 to 24 adult fish of the same resident species were collected from each sampling location during late summer and fall of 2006 (Table 2), since lipid content is usually highest and water levels lowest at that time. Fish sampled at each site were divided into six composites, each containing three or four adult fish of similar size. Each sample consisted of adult-sized fish that are typically consumed by wildlife and humans. Fish were selected and sorted to adhere to the U.S. EPA's 75% length rule (i.e., all specimens in a composite needed to be within 75% of the length of the largest fish) [32]. Fish were collected by backpack or boat electrofisher and were identified by experienced fisheries biologists. Total body mass (in grams) and length (in millimeters) were measured on site, and each fish selected for the composite sample was individually wrapped in aluminum foil and placed in food-grade polyethylene bags. Samples were frozen on dry ice and shipped to the analytical laboratory via next-day air delivery. Upon receipt, all samples were catalogued and stored at $-20^{\circ}C$ prior to dissection and homogenization.

Preparation of composite tissue samples

Samples were composited using the batch method, in which like tissue from all individual specimens comprising a composite were homogenized together, regardless of each individual's respective portion. Each fish was rinsed with distilled water and scaled. Fillet (including skin and belly flap) and liver tissues were dissected independently. Each fillet was cut into approximately 2.5-cm cubes using stainless steel scissors. Individual cubes were combined and frozen (at $-20^{\circ}C$) prior to being ground to a fine powder using a high-speed blender. Successive addition of small amounts of dry ice during grinding was critical in maintaining the consistency of

Fig. 1. Geographical locations of fish sampling sites in the United States.

frozen tissues and greatly simplified this aspect of sample preparation. The ground sample was then divided into quarters, opposite quarters were combined, and the resulting halves were mixed together by hand. After applying this procedure to at least three fish from one site, portions were combined again by dividing them into quarters and mixing halves to prepare a composite. Liver samples did not require the preparation of frozen cubes or the use of a high-speed blender for homogenization. Instead, livers from specimens in each composite (at least three livers per composite) were combined in a clean glass container and homogenized using a Tissuemiser (Fisher Scientific) set to rotate at 30,000 rpm.

Table 1. Wastewater treatment plant information for facility discharges in the United States

Location	Treatment	Receiving water	Population	Design capacity (MGD)[a]	Existing flow (MGD)	Effluent (%)	65 and older (%)	Median income
Phoenix, Arizona	Advanced treatment I with nutrient removal[b]	Salt River	1,418,041	165	153	100	8.1	$41,207
Orlando, Florida	Advanced treatment II with nutrient removal[c]	Little Econlockhatchee River	442,542	40	36	64	11.3	$35,732
Chicago, Illinois	Advanced treatment I with nutrient removal[b]	North Shore Channel	5,376,741	333	234	100[d]	10.3	$38,625
West Chester, Pennsylvania	Advanced treatment I with nutrient removal[b]	Taylor Run	17,701	1.8	1.3	36–86	9.0	$37,803
Dallas, Texas	Advanced treatment II with nutrient removal[c]	Trinity River	3,500,000	175	152	100[d]	8.1	$43,324

[a] Million gallons per day.
[b] Advanced treatment I. Wastewater discharged after receiving biological treatment, physical or chemical treatment, or both. A wastewater treatment plant with a concentration of biochemical oxygen demand (BOD5; the amount of dissolved oxygen consumed in 5 d by biological processes breaking down organic matter) greater than or equal to 10 mg/L but less than 20 mg/L (30 d average) in its National Pollutant Discharge Elimination System (NPDES) permit is considered to be providing advanced treatment I.
[c] Advanced treatment II. Wastewater discharged after receiving biological treatment, physical or chemical treatment, or both. A wastewater treatment plant with a BOD5 concentration less than 10 mg/L (based on 30-d averages) in its NPDES permit is considered to be providing advanced treatment II. To further clarify the treatment level, the state should indicate whether the facility currently uses any processes to remove nutrients (nitrogen or phosphorus) from its effluent. Note that the addition of nutrient removal is considered to be an improvement in effluent quality (e.g., secondary effluent with nutrient removal represents higher quality effluent than secondary effluent without nutrient removal.
[d] Flow is primarily made up of effluent discharged from multiple facilities.

Table 2. Biological and physiological information of sampled fish at respective geographical locations in the United States[a]

Location	Species	Date	No. fish	Length (cm)	Weight (kg)	Composite lipid % (mean ± SD)[b]	
						Fillet	Liver
Gila Wilderness Area, New Mexico	Sonora sucker	11/2006	24	33–34	0.7–0.9	4.9 ± 1.6	4.9 ± 2.5
Chicago, Illinois	Largemouth bass	09/2006	24	27–36	0.4–0.9	2.3 ± 0.6	2.2 ± 0.4
Phoenix, Arizona	Common carp	11/2006	18	35–55	0.8–2.0	3.9 ± 0.8	11.6 ± 2.1
Orlando, Florida	Bowfin	10/2006	17	34–44	0.4–2.2	1.0 ± 0.7	2.9 ± 1.6
West Chester, Pennsylvania	White sucker	08/2006	24	29–36	0.3–0.6	1.9 ± 0.4	4.7 ± 0.9
Dallas, Texas	Smallmouth buffalo	10/2006	18	43–60	1.4–4.6	2.2 ± 1.1	8.1 ± 2.7

[a] Each independent composite ($n = 6$) contained at least three similarly sized individual fish, with the exception of the Orlando, Florida, location.
[b] SD = standard deviation.

Determination of lipid content

Gravimetric lipid determinations were performed on three replicate aliquots (~2 g) of each fillet composite using the procedure described in Mottaleb et al. [6]. This procedure was modified slightly for liver specimens. Approximately 2 g of liver tissue was combined with 15 ml of 1:1 dichloromethane-hexane and approximately 2 g solid Na_2SO_4 in a borosilicate glass vial. Samples were sonicated for 30 min in an ultrasonic water bath at room temperature. The vials were subsequently placed in an incubator for 24 h at 35°C and agitated by gentle end-over-end rotation. Following extraction, samples were centrifuged at 16,000 rpm for 40 min at 4°C to ensure complete phase disengagement. The supernatant was collected by filtration, and the solid residue was washed with an additional 15 ml of 1:1 dichloromethane:hexane that was also passed through the filter and collected in a preweighed test tube. The solvent was evaporated and residues were dried to constant weight in a vacuum oven at 40°C. Due to limited sample mass, triplicate measurements were made for only one liver composite from each sampling site. All other determinations were based on a single measurement.

CHEMICALS

All chemicals were reagent grade or better, obtained from commercial vendors, and used as received. 7-Aminoflunitrazepam-d_7, fluoxetine-d_6, acetaminophen-d_4, diphenhydramine-d_3, fluoxetine, norfluoxetine, sertraline, codeine, diphenhydramine, propranolol, and ibuprofen were purchased as certified analytical standards from Cerilliant. Atenolol and mirex were also purchased from Cerilliant. Benzophenone, 4-methylbenxylidine camphor, *m*-toluamide, *p*-octylphenol, galaxolide, tonalide, musk xylene, musk ketone, triclosan, octocrylene, *p-n*-nonylphenol, pentachloronitrobenzene, benzophenone-d_{10}, phenanthrene-d_{10}, meclofenamic acid, 1,7 dimethylxanthine, acetaminophen, caffeine, miconazole, carbamazepine, erythromycin, gemfibrozil, trimethoprim, diltiazem, cimetidine, warfarin, thiabendazole, sulfamethoxazole, lincomycin, metoprolol, tylosin, and clofibric acid were purchased in highest available purity from Sigma-Aldrich. Carbamazepine-d_{10}, *p*-*N*-nonylphenol-$^{13}C_6$, and ibuprofen-$^{13}C_3$ were purchased from Cambridge Isotopes Laboratories. Celestolide was obtained from Cambridge Corporation. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide was obtained from VWR Scientifics.

Analysis of PPCPs in fish tissue

All fillet composites were screened for 36 target chemicals: 24 pharmaceuticals and 12 PCPs; liver specimens were screened for pharmaceuticals only. Pharmaceuticals were analyzed using HPLC-MS/MS. Unless detailed later, methodology employed was identical to that reported previously by Ramirez et al. [8]. Each analytical sample (1.0-g fillet or 0.5-g liver) was spiked with four surrogates: acetaminophen-d_4 (500 ng), diphenhydramine-d_3 (10 ng), carbamazepine-d_{10} (40 ng), and ibuprofen-$^{13}C_3$ (840 ng). Eight ml of extraction solvent (a 1:1, v/v, mixture of 0.1 M aqueous acetic acid and methanol) was added, and the sample was sonicated for 15 min at 25°C. The sample was subsequently centrifuged, and the decanted supernatant was evaporated to dryness under a stream of nitrogen. A constant amount of the internal standards 7-aminoflunitrazepam-d_7 (100 ng), fluoxetine-d_6 (585 ng), and meclofenamic acid (995 ng) was added (half of these amounts were added to liver samples), and the residue was reconstituted in mobile phase (1 ml for fillet and 0.5 ml for liver composites). It is important to point out that fluoxetine-d_6 replaced 7-aminoflunitrazepam-d_7 as the internal standard for quantification of fluoxetine, norfluoxetine, and sertraline. Similarly, 7-aminoflunitrazepam-d_7 replaced meclofenamic acid as the internal standard for quantification of ibuprofen. Prior to analysis, each sample was manually shaken, sonicated for 1 min, and filtered using a 0.2-μm syringe filter. We analyzed PCPs using GC-MS/MS. Sample preparation protocols and GC-MS/MS instrumental settings utilized have been detailed in Mottaleb et al. [6]. Briefly, 1.0-g fillet composites were extracted with 10 ml of acetone, and extracts were subjected to successive silica-gel and gel-permeation chromatography cleaning steps prior to derivatization and analysis.

Quantification and reporting limits

To minimize potential matrix effects in HPLC-MS/MS analyses, calibration standards were prepared by adding target pharmaceuticals and related surrogates to equally massed aliquots of a single fillet or liver composite derived from the New Mexico reference site. Fortified tissues were carried through the entire sample preparation protocol prior to analysis [8]. In contrast, GC-MS/MS calibration standards were prepared by dissolving target PCPs and related surrogates in acetone, and these samples were not extracted prior to derivatization and analysis. Calibration curves were constructed for each compound from a minimum of five points by plotting the ratio of observed peak areas for the analyte and internal standard versus analyte concentration. Calibration data were subjected to independent linear regressions that were forced through the origin, and the resulting equations were used to calculate analyte and surrogate concentrations in all

subsequent analyses. The criterion imposed for acceptable initial calibration required that the relative standard deviation of independent relative response factors observed for each analyte be less than 30%.

Experimentally derived method detection limits (MDLs) for target analytes in fillet tissue were reported in previous work [6,8], and it was initially assumed that these values would be appropriate for establishing detection and quantitation thresholds for the present study. However, evaluation of matrix spike recoveries revealed that matrix interference encountered in the analysis of liver samples was more pronounced than that observed for fillet tissue. Consequently, independent MDLs for target pharmaceuticals in liver were determined using a liver composite from the New Mexico reference site and an identical approach to that employed previously for fillet specimens.

In the present paper, MDL signifies the lowest concentration of analyte that can be reported in a defined matrix with 99% confidence that the concentration is nonzero [33]. It is generally assumed that experimentally derived MDLs for a defined matrix are appropriate reporting metrics for identical analyses conducted on samples of similar type (e.g., analysis of fish fillets from different locations). Accordingly, MDLs determined using tissue derived from the New Mexico reference site were utilized as global quantification or reporting limits in the present study. However, select compounds were identified below MDL in samples collected from alternative locations. Analyte identification was confirmed by comparing retention times and relative ion abundance ratios (two independent MS/MS transitions were typically monitored for each compound) with those observed for fortified tissues derived from the New Mexico site. The criteria imposed for positive identification were matching retention time and 80% agreement in relative ion abundance ratios. Qualifier transitions monitored for fluoxetine and gemfibrozil were m/z 310 > 44 and m/z 249 > 127, respectively. All other monitored transitions for detected analytes are reported in Mottaleb et al. and Ramirez et al. [6,8].

Quality control and quality assurance

The following quality control criteria were applied throughout the study to monitor performance of the analytical methods. All environmental samples were grouped into batches of less than 20 samples. Each analytical batch contained one blank (composite tissue from the New Mexico site spiked with surrogates only), a minimum of one continuing calibration verification sample (CCV) and two laboratory control samples (LCS1 and LCS2). Laboratory control sample 1 was a low-level control sample spiked with analyte concentrations near the MDL, while LCS2 was spiked with analyte concentrations that were approximately 20 to 50 × MDL (i.e., in the middle to upper third of the calibrated range). Composite samples from no more than two sites were also included in each batch, and one composite sample from each site was randomly selected and used to prepare two identical matrix spikes (i.e., one matrix spike–matrix spike duplicate pair). Spike concentrations employed in the preparation of matrix spikes were equivalent to concentrations added to LCS2. The CCVs for HPLC-MS/MS analyses were prepared using tissue from the New Mexico site and were extracted prior to analysis, consistent with the matrix-matched calibration approach. In contrast, CCV standards for GC-MS/MS analyses were not subject to extraction and prepared in acetone.

Table 3. Quality assurance and quality control data summary for the overall performance of employed HPLC-MS/MS and GC-MS/MS analytical methods; a range of 60 to 150% was considered acceptable[a,b]

	Fillet (%)		Liver (%)	
	Mean	SD	Mean	SD
Pharmaceuticals (HPLC-MS/MS)				
Surrogates				
Acetaminophen-d_4	96	7	140	25
Diphenhydramine-d_3	95	10	92	11
Carbamazepine-d_{10}	91	10	99	12
Ibuprofen-$^{13}C_3$	92	12	105	17
Control Samples				
LCS1	103	8	111	10
LCS2	101	8	105	10
Matrix spikes	*	*	*	*
Personal care products (GC-MS/MS)				
Surrogates				
Benzophenone-d_{10}	120	24	—	—
p-n-Nonylphenol-$^{13}C_6$	160	38	—	—
Pentachloronitrobenzene	136	15	—	—
Control samples				
LCS1	87	27	—	—
LCS2	84	16	—	—
Matrix spikes	93	17	—	—

[a] See text for explanation of acronyms.
[b] * = variable; see text.

No target pharmaceuticals were detected in blank samples analyzed via HPLC-MS/MS. In contrast, analytical responses equivalent to analyte concentrations near respective MDLs were observed at retention times corresponding to benzophenone and octocrylene in one or more blanks analyzed via GC-MS/MS. An analytical response of similar magnitude was also observed at the benzophenone retention time in all unfortified tissues from effluent-dominated sampling locations. Excluding data below the benzophenone MDL (16 ng/g), a mean (n = 28) analytical response of 23 ng/g was observed in tissue from all six sites with 35% relative standard deviation. While these data may indicate ubiquitous occurrence of benzophenone in fish tissue, an equally compelling argument may be made for ubiquitous interference from a nontarget component of the sample matrix [6]. Accordingly, results for benzophenone were considered inconclusive and are not presented or discussed further.

Instrument calibration was evaluated on a continuing basis by monitoring the percent difference between the relative response factors calculated for each analyte in CCV samples and the corresponding average relative response factors from initial calibration with an acceptability criterion for continued analysis of ±20%. Method performance was monitored by evaluating surrogate recoveries in all samples and analyte recoveries in control samples (LCS1 and LCS2) and matrix spikes. Data summarizing overall performance of the employed analytical methods are given in Table 3. In general, recoveries ranging from 60 to 150% were considered acceptable.

Surrogate data in Table 3 suggest that sample preparation protocols were controlled and reproducible for both HPLC-MS/MS and GC-MS/MS analyses. Although high bias was observed for acetaminophen-d_4 in liver tissue, this result was strongly influenced by tissue originating from the Texas sampling site. In these samples, acetaminophen-d_4 recoveries ranged from 131 to 234%. However, acceptable recoveries

Table 4. Matrix spike recovery data for pharmaceutical analytes detected in liver tissue that exceeded defined acceptability limits (60–150%)[a]

	Location (USA, %)					
Analyte	IL	NM	AZ	FL	PA	TX
Fluoxetine	144	105	271	349	362	335
Norfluoxetine	92	115	197	350	247	398
Sertraline	96	120	172	407	473	584
Gemfibrozil	172	106	246	527	218	166

[a] All fillet data were within defined limits.

were observed for acetaminophen-d_4 in CCV and LCSs and for unlabeled acetaminophen in matrix spike samples from this batch. Thus, it is possible that acetaminophen-d_4 experienced unique matrix interference in samples derived from this site. It is likely that a significant matrix influence was also responsible for the more general high bias observed for surrogate recoveries in GC-MS/MS analyses of PCPs, as mean recoveries ($\pm$ standard deviation) for unlabeled benzophenone and *p-N*-nonylphenol in matrix spike samples (averaged over all analytical batches) were 94 $\pm$ 15% and 77 $\pm$ 12%, respectively. The larger standard deviation observed for *p-N*-nonylphenol-$^{13}C_6$ relative to the other two GC-MS/MS surrogates reflects a stronger sampling site influence on the degree of matrix interference observed for this analyte. Laboratory control samples also suggested excellent performance for an overwhelming majority of target analytes, although it should be noted that mean recoveries observed for octylphenol in LCS1 and LCS2 and for musk ketone in LCS1 fell below the acceptable range (54, 49, and 47%, respectively).

No significant matrix influence was identified by matrix spike samples in GC-MS/MS analyses. In contrast, significant matrix effects were observed for select pharmaceuticals in HPLC-MS/MS analyses, consistent with previous observations [8]. With the exception of ibuprofen, observed recoveries for all analytes eluting beyond 30 min fell outside of control limits (60–150%) in one or more matrix spike samples. In general, matrix effects were more pronounced in liver extracts than in extracts derived from fillet tissue. Signal enhancement relative to the New Mexico reference condition was the effect observed for the majority of influenced analytes, resulting in recoveries that were inflated by a factor of approximately 2 to 6. Suppression was also observed in some cases (e.g., tylosin in both fillet and liver samples from the Illinois site and warfarin in the fillet sample from Arizona). Interestingly, gemfibrozil response was suppressed in the fillet sample from Illinois but enhanced to varying degrees in liver extracts. These findings clearly demonstrate that extracts derived from fish sampled at different locations exerted variable influence on the analytical response of affected analytes, even though extracts were derived from a single biological tissue. Matrix spike recoveries that fell outside of previously noted acceptability limits (60–150%) are given in Table 4 for pharmaceuticals that were detected in liver tissue at concentrations exceeding MDL.

Regression analyses

Lipid content versus analyte concentration data for all compounds detected in fillet and liver tissues were modeled in Sigmaplot 9.0 (SPSS) using the linear regression equation $y = y_o + ax$, where y is the dependent variable, y_o is the y-intercept, a is the slope, and x is the independent variable; p values were calculated for tests of significant regression.

RESULTS

Analytical concentrations of pharmaceuticals detected in fish fillet and liver tissues are given in Tables 5 and 6, respectively. We only analyzed PCPs in fish fillets, and these data are also presented in Table 5. Among the 24 pharmaceuticals analyzed, 5 were detected in fillets and 7 in livers; only 2 PCPs were detected in fillets out of 12 analyzed. No PPCPs were detected in any tissue composite (fillet or liver) above MDLs at the reference site (Gila River, NM, USA). Galaxolide and tonalide PCPs were detected at every effluent-dominated site in fish fillets, with maximum concentrations ranging from 300 to 2,100 ng/g and 21 to 290 ng/g, respectively. Triclosan was also detected, although concentrations were below the respective MDL. Among pharmaceuticals, diphenhydramine was most frequently detected in fish fillets, identified at three sites, followed by norfluoxetine, sertraline, diltiazem, and carbamazepine at three, two, two, and one site, respectively. Among fish liver composites, norfluoxetine was most frequently detected, identified in samples from all five sites, followed by sertraline, diphenhydramine, fluoxetine, diltiazem, gemfibrozil, and carbamazepine at five, four, three, three, two, and one site, respectively. Frequency of detection among all six composites at a given site for fillet and liver tissues is given in Tables 5 and 6, respectively. Sertraline consistently displayed the greatest maximum concentration among pharmaceuticals in both fillet and liver tissues, up to 19 and 545 ng/g, respectively.

Seventeen of the 24 pharmaceuticals and 10 of the 12 PCPs were not detected in any of the fillet or liver samples from the five effluent-dominated sites. These compounds, along with their associated MDLs and octanol–water partition coefficients (log K_{OW}) are outlined in Table 7. Although class representation in the screening initiative was unequal, detected pharmaceuticals belonged to antidepressants, antihistamines, antihypertensions, antilipemics, and antiseizures. No compounds belonging to antispasmodic, analgesic, stimulant, antibiotic, antifungal, and anticoagulant classes were detected in any sample above MDLs. Both PCP compounds detected above respective MDLs were fragrances. No compounds belonging to ultraviolet filter, insect repellant, nitro musk, or surfactant classes were detected in any sample above MDLs, though again representation was unequal among classes.

The total number of compounds detected, the concentration of compounds detected, and the frequency of detection were higher in liver tissue than fillets. Seven pharmaceuticals were detected in liver composites versus five for fillets. At least two of these compounds were detected in liver samples from all five effluent-dominated sites, versus only three sites for fillets. Comparisons of PCP detection, concentration, and frequency between liver and fillet composite samples were not possible due to matrix effects encountered for liver samples. For each sampling site, the concentration of each compound detected above the MDL was plotted against fillet lipid content for PPCPs, and liver lipid content for pharmaceuticals. Representative examples are provided in Figure 2 and mean lipid content is given in Table 2. Since accumulation is expected to depend on the aqueous concentration of individual contaminants, which likely differs from site to site, it was not considered appropriate to pool the data. As a result, only individual sites were investigated for potential correlations.

Table 5. Pharmaceutical and personal care product results for fish tissues (fillets) obtained from independent composite samples for each site[a]

			Tissue concentration (ng/g)														
			Chicago			Dallas			Orlando			Phoenix			West Chester		
Compound	MDL	Log K_{OW}[b]	Freq (n/6)[c]	Mean	Max	Freq (n/6)	Mean	Max	Freq (n/6)	Mean	Max	Freq (n/6)	Mean	Max	Freq (n/6)	Mean	Max
Antidepressant																	
Norfluoxetine	2.9	4.36	2/6	3.2	3.2	0/6	*	*	0/6	*	*	4/6	4.0	4.8	6/6	3.9	5.0
Sertraline	3.6	4.81	0/6	*	*	0/6	*	*	0/6	*	*	6/6	5.0	6.5	6/6	11	19
Antihistamine																	
Diphenhydramine	0.05	3.66	6/6	1.4	1.7	0/6	*	*	0/6	*	*	6/6	1.2	1.4	6/6	1.7	2.5
Antihypertension																	
Diltiazem	0.12	3.63	5/6	0.13	0.2	0/6	*	*	0/6	*	*	0/6	*	*	3/6	0.15	0.2
Antiseizure																	
Carbamazepine	0.54	2.67	6/6	2.3	3.1	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*
Antimicrobial																	
Triclosan	38	5.17	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*
Fragrance																	
Galaxolide	12	6.23	6/6	1,300	1,800	6/6	800	1,800	5/6	100	300	6/6	1,800	2,100	6/6	1,800	2,000
Tonalide	13	6.37	6/6	160	230	6/6	70	150	1/6	NA	21	6/6	240	290	6/6	60	70

[a] MDL = method detection limit; K_{OW} = octanol–water partition coefficient; Freq = frequency; Max = maximum; * = values < MDL; ND = not detected; NA = not applicable.
[b] All log K_{OW} values were calculated using SciFinder (Chemical Abstracts Service).
[c] The number of independent composite samples (n) used to calculate each mean is given by the frequency. Results ≤ MDL were excluded from this calculation.

Linear regressions revealed no significant ($\alpha = 0.05$) concentration–lipid content relationships for any pharmaceutical compound detected for either fillet or liver tissues. However, both galaxolide and tonalide PCPs (fragrances) demonstrated significant positive linear regressions: galaxolide at Orlando, Florida ($p = 0.0191$), and Dallas, Texas ($p = 0.0048$), and tonalide at Chicago, Illinois ($p = 0.0436$), and Dallas, Texas ($p = 0.0024$). Therefore, based on the analytical data there appears to be no clear association between accumulation of detected pharmaceuticals and lipid content, though relationships exist for detected PCPs. No other PCPs were detected above the MDL; consequently, PCP data is limited to these two examples.

Examination of Tables 1, 5, and 6 suggests that relationships may vary depending on the degree of wastewater treatment, demographics, and PPCP accumulation data among the different effluent-dominated sampling locations. Sites with more advanced wastewater treatment (Dallas, TX and Orlando, FL) tended to demonstrate fewer detections at lower concentrations and lower frequencies than sites with less advanced treatment (Chicago, IL, Phoenix, AZ, and West Chester, PA). No clear relationships were established among population, age, and income.

DISCUSSION

Recent advances in analytical techniques have facilitated the detection of multiple pharmaceutical residues in fish tissue matrices [3–9,20,34]. Several of the pharmaceuticals detected, including diphenhydramine, diltiazem, carbamazepine, and norfluoxetine, have been quantified in similar tissues previ-

Table 6. Pharmaceutical results for fish livers obtained from independent composite samples for each site[a]

			Tissue concentration (ng/g)														
			Chicago			Dallas			Orlando			Phoenix			West Chester		
Compound	MDL	Log K_{OW}[b]	Freq (n/6)[c]	Mean	Max	Freq (n/6)	Mean	Max	Freq (n/6)	Mean	Max	Freq (n/6)	Mean	Max	Freq (n/6)	Mean	Max
Antidepressant																	
Fluoxetine	12	4.09	3/6	19	23	0/6	*	*	0/6	*	*	0/6	ND	ND	6/6	70	80
Norfluoxetine	15	4.36	6/6	73	130	4/6	37	48	5/6	57	78	5/6	33	44	6/6	38	48
Sertraline	17	4.81	6/6	84	149	4/6	27	28	1/6	NA	21	6/6	71	105	6/6	381	545
Antihistamine																	
Diphenhydramine	0.26	3.66	6/6	7	10	5/6	0.5	0.9	0/6	*	*	6/6	7	11.1	6/6	10	11
Antihypertension																	
Diltiazem	0.26	3.63	6/6	0.7	0.9	0/6	*	*	0/6	*	*	4/6	0.3	0.4	6/6	0.7	0.8
Antilipemic																	
Gemfibrozil	25	4.77	0/6	ND	ND	0/6	ND	ND	0/6	ND	ND	6/6	70	90	2/6	27.1	27.3
Antiseizure																	
Carbamazepine	1.9	2.67	6/6	6	8	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*

[a] MDL = method detection limit; K_{OW} = octanol–water partition coefficient; Freq = frequency; Max = maximum; * = values < MDL; ND = not detected; NA = not applicable.
[b] All log K_{OW} values were calculated using SciFinder (Chemical Abstracts Service).
[c] The number of independent composite samples (n) used to calculate each mean is given by the frequency. Results ≤ MDL were excluded from this calculation.

Table 7. Pharmaceuticals and personal care products not detected in fish fillet or liver samples[a]

Analyte	Class	Log K_{OW}[b]	MDL (ng/g)	
			Fillet	Liver
Pharmaceuticals				
1,7-Dimethylxanthine	Antispasmodic	−0.63	1.1	5.8
Acetaminophen	Analgesic	0.34	4.4	34
Atenolol	Antihypertension	0.097	1.5	13
Caffeine	Stimulant	−0.13	3.9	25
Cimetidine	Antiacid reflux	0.072	1.0	5.2
Codeine	Analgesic	1.20	6.1	31
Erythromycin	Antibiotic	2.83	6.4	43
Ibuprofen	Analgesic	3.72	46	173
Lincomycin	Antibiotic	0.91	5.5	56
Metoprolol	Antihypertension	1.79	2.5	8.9
Miconazole	Antifungal	5.93	11	NA
Propranolol	Antihypertension	3.10	1.1	3.8
Sulfamethoxazole	Antibiotic	0.89	2.3	14
Thiabendazole	Antifungal	2.47	2.6	7.8
Trimethoprim	Antibiotic	0.79	2.2	8.0
Tylosin	Antibiotic	3.41	5.0	35
Warfarin	Anticoagulant	3.42	0.9	2.7
Personal care products				
4-Methylbenzylidene-camphor	Ultraviolet filter	4.95	120	—
Celestolide	Fragrance	5.51	18	—
m-Toluamide	Insect repellant	1.20	5.1	—
Musk ketone	Nitro musk	3.86	321	—
Musk xylene	Nitro musk	3.83	397	—
Nonylphenol	Surfactant	NA	9.7	—
Octocrylene	Ultraviolet filter	7.53	36	
Octylphenol	Surfactant	NA	8.2	—

[a] MDL = method detection limit; K_{OW} = octanol–water partition coefficient; NA = not applicable.
[b] All log K_{OW} values were calculated using SciFinder (Chemical Abstracts Service).

ously, as reported in Ramirez et al. [8], the study upon which the pharmaceutical analytical methodology for the present study is based. These compounds were detected in fish fillet tissue collected at the effluent-dominated stream Pecan Creek (Denton, TX), at concentrations ranging from 0.66 to 1.32, 0.11 to 0.27, 0.83 to 1.44, and 3.49 to 5.14 ng/g, respectively, demonstrating good agreement with the concentrations reported in Table 5. An earlier analysis of fish tissue sampled from Pecan Creek (Denton, TX) revealed several antidepressants in liver and muscle [3], including fluoxetine (1.34 and 0.11 ng/g, respectively), norfluoxetine (10.27 and 1.07 ng/g, respectively), and sertraline (3.59 and 0.34 ng/g, respectively). Those values were also consistent with results reported in Tables 5 and 6, respectively. Similarly, fluoxetine and norfluoxetine have been quantified in several species of fish collected at Hamilton Harbor (ON, Canada) ranging from 0.14 to 1.02 and 0.15 to 1.08 ng/g, respectively. The antilipemic gemfibrozil has been detected previously in plasma of laboratory exposed fish at 210 mg/L [4]; however, the present study represents the first quantified accumulation in wild fish tissue (liver). Although species and geographical differences exist among reported values for each compound, the range of concentrations quantified in fish tissue appears to be consistent among sampling sites. Furthermore, although the selection of PPCPs for analytical screening is at the discretion of the analyst, the identity of detected compounds also appears to be consistent with previous studies, particularly among effluent-dominated ecosystems.

Detection of PCPs has similarly benefitted from advances in analytical techniques, and a variety of these compounds have been quantified in fish tissue matrices [10–17]. Two of the PCPs detected in the present study (galaxolide and tonalide) have also been previously quantified in fish fillets sampled from Pecan Creek (Denton, TX) by Mottaleb et al. [6], in addition to other compounds. Analysis of fish tissue fillets at this site revealed concentrations of galaxolide and tonalide ranging from 234 to 970 ng/g and 26 to 97 ng/g, respectively [6], which is slightly lower yet similar to the values reported in Table 5, again demonstrating good agreement for effluent-dominated ecosystems. Similarly, galaxolide and tonalide have been detected in bream sampled in German rivers with mean site concentrations of 491 and 50 ng/g, respectively, based on wet weight [23]. Lower concentrations of galaxolide and tonalide were found in trout sampled from Danish fish farms at average concentrations ranging from 5.87 to 8.54 and 2.24 to 2.70 ng/g, respectively [12]. Both of these compounds have also been detected in liver tissue of hammerhead sharks at concentrations from 16 to 48 ng/g and less than 9.1 ng/g, respectively [14]. Analysis of Lake Michigan fish tissue standard reference material demonstrated the presence of galaxolide at 1.12 ng/g [16], which has even been found in several species of trout sampled from remote alpine lakes in Switzerland [17]. Triclosan was also identified in fillet tissue from all five effluent-dominated sites, although concentrations were below the corresponding MDL. However, its presence is not surprising given that Mottaleb et al. [6] have quantified concentrations of this compound up to 31 ng/g in fillet tissue collected from Pecan Creek (Denton, TX) and the metabolite methyl-triclosan has been detected in fish from Swiss lakes at concentrations up to 35 ng/g wet weight [24].

The present study encompassed a large geographical distribution of sampling sites and included a large and diverse subset of screened PPCPs. However, of the 24 pharmaceuticals and 12 PCPs analyzed, 71 and 83%, respectively, were not

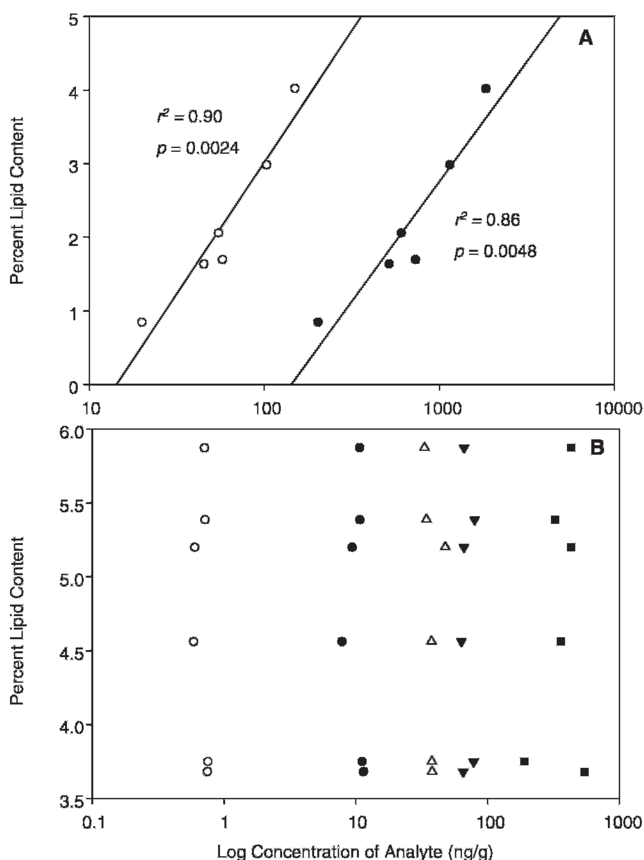

Fig. 2. Plots of personal care product concentrations (● galaxolide and ○ tonalide) versus lipid content in fish tissue fillets obtained for composites collected from Dallas, Texas, USA (**A**), and pharmaceutical concentrations (● diphenhydramine, ○ diltiazem, ▼ fluoxetine, △ norfluoxetine, ■ sertraline) versus lipid content in fish livers obtained for composites collected from West Chester, Pennsylvania, USA (**B**). The r^2 is the adjusted coefficient of determination, and p values correspond to tests of significant regression regarding the slope value.

detected. Results obtained for pharmaceuticals are largely consistent with the literature, where screened compounds have been detected previously in similar matrices and at similar concentrations; thus, the proportion of nondetects is not surprising. However, a number of PCPs that were screened in this investigation but were not detected above corresponding MDLs have been detected elsewhere. For example, analysis of fish tissues from Pecan Creek (Denton, TX) revealed the presence of benzophenone (37–90 ng/g) and triclosan (17–31 ng/g) [6], both of which were not detected for any of the five effluent-dominated sites. Nearly all PCPs that were screened but not detected above respective MDLs in the present study have been detected in other environmental fish tissue samples [11–14]. This is primarily due to differences in analytical sensitivity between studies, as in certain cases the values reported for PCPs detected elsewhere were below the corresponding MDLs for target analytes monitored by GC-MS/MS in the present study.

Comparison of liver and fillet tissue results for all effluent-dominated sites indicates greater numbers, concentrations, and frequencies of compounds detected in livers versus fillets. Considering that the liver is the primary site of metabolism for xenobiotics in fish, as in humans, this result is logical. Furthermore, since lipid content was significantly higher in livers versus fillets, and basing accumulation assumptions on prevailing ideology regarding log K_{OW} as a predictor, the observed trends may also have been forecast. However, analysis of percent lipid versus analyte concentration plots for all composites collected at each sampling site suggest no relationship between these two variables for any detected pharmaceutical. Traditional analysis of chemical residues in fish tissue suggests that hydrophobic organic contaminants will concentrate in tissues with high lipid content [35–38]. Such rationalization is based on a collection of studies demonstrating a correlation between experimentally derived bioconcentration factors and analyte-specific physicochemical properties, most commonly the log K_{OW}. Mackay [39] suggests a directly proportional relationship between bioconcentration factor and log K_{OW} provided that the dominant concentrating phase has similar solute interaction characteristics to octanol. Bertelsen et al. [40] found that *n*-octanol was a good nonpolar lipid surrogate for predicting chemical partitioning of six moderately hydrophobic compounds with K_{OW}s ranging from 1.46 to 4.04 in blood and tissue for four species of fish. However, log K_{OW} alone accounted for less than 50% of combined dataset variation, where results for lean tissue differed from those expected for chemicals partitioning to lipid only [40]. This suggests that partitioning to nonlipid–nonwater cellular constituents can contribute substantially to chemical accumulation [40], which may not accurately be predicted based solely on log K_{OW} for compounds that do not readily partition to lipid.

In contrast to pharmaceuticals, the PCPs galaxolide and tonalide each demonstrated significant positive correlations between lipid content and analyte concentration at two of five effluent-dominated sites, respectively. This finding confirms previous observations [6] and suggests that the traditional contention regarding log K_{OW} as a predictor for analyte lipid partitioning appears to be appropriate for musk fragrances, based on the two compounds detected in the present study. The contrasting results between pharmaceuticals and PCPs is confounding, however, given that the log K_{OW}s encountered for detected pharmaceuticals are between 2.67 and 4.81. Although it is impossible to make broad generalizations concerning relationships between fish tissue lipid content and accumulation of pharmaceuticals versus PCPs, evident trends can be attributed to differing physiochemical properties, particularly pK_a. Six of the seven pharmaceuticals detected in fish tissue were all ionizable compounds. Diltiazem, diphenhydramine, fluoxetine, gemfibrozil, norfluoxetine, and sertraline have respective pK_as of 8.94, 8.76, 10.1, 4.75, 9.05, and 9.47, whereas neither galaxolide nor tonalide are readily ionizable. Compounds that display ionizable functional groups do not correlate well with tissue lipid content, whereas those that are not readily ionizable show good positive correlations. This finding is not surprising given that traditional lipid partitioning assumptions were developed from analysis of neutral, lipophilic persistent organic pollutants, such as organochlorines and polychlorinated biphenyls [39,41]. The polarity of ionizable compounds, including many pharmaceuticals and select PCPs, will vary with site-specific receiving system pH and is subject to change in different biological compartments, thus modifying their expected and actual accumulation, partitioning behavior, and toxicity [42]. Metabolism of pharmaceuticals may also drastically alter environmental fate [43]. As evidenced by the detection of norfluoxetine (a metabolite of fluoxetine), future tissue screening efforts should also consider analyses of metabolic products corresponding to targeted parent compounds, though such analyses

would be subject to standard availability and method performance limitations. Improved future models are likely to include considerations of contaminant ionization state, differential metabolic and partitioning processes in vivo, and receptor-binding interactions. These factors may in part explain much of the variability observed in analyte concentrations among independent samples from the same site and among different biological tissues in the present study. In the context of the present discussion, basing accumulation assumptions for pharmaceuticals solely on log K_{OW} may lead to erroneous and inaccurate partitioning estimates on a site-specific basis.

CONCLUSIONS

The comparatively greater occurrence of PPCPs encountered in effluent-dominated rivers identified in the present study and previous research [3,6,8] is not surprising given that these water resources represent worst-case scenarios for studying PPCPs and other organic wastewater contaminants [28]. The underlying assumption is that occurrence data are derived primarily from bioconcentration across biological membranes. As discussed earlier, PPCPs can adopt a pseudopersistent exposure nature given their continuous introduction from WWTP effluent [2], thus providing a continuous exposure scenario for organisms residing in these aquatic systems over their entire life cycle. Despite the capacity to remove a large proportion of PPCPs through various treatment processes [26], WWTPs do not eliminate all compounds completely [27]. Furthermore, human pharmaceuticals are typically excreted unchanged or only slightly transformed, mostly conjugated to polar molecules (e.g., as glucuronides), which can easily be cleaved during sewage treatment back to the parent compound [27,44,45]. Therefore, the degree and nature of treatment processes has a substantial influence over the removal efficiency of pharmaceuticals from wastewater discharge [46,47]. As a result, exposure, and consequently tissue accumulation, would be expectedly higher in organisms residing in water resources receiving discharge from WWTPs employing less advanced versus more advanced treatment. In the present study, fish tissue analyses from the two sampling sites receiving more advanced treatment (Dallas, TX, and Orlando, FL) showed lower overall concentrations of PPCPs, fewer compounds detected, and lower frequency of detection compared to the other three sampling sites (Phoenix, AZ, Chicago, IL, and West Chester, PA), which employed less advanced treatment according to National Pollutant Discharge Elimination System data (Table 1). Furthermore, the Orlando, Florida, WWTP facility diverts 20 million of the 46 million gallons per day capacity through a constructed wetland discharging into the St. John's River, further reducing the load discharged into the Little Econlockhatchee River (www.cityoforlando.net/public_works/wastewater/downloads/ib.pdf), which is only 64% effluent. Although other demographics such as population, percentage of population age 65 and older, and median income were considered to have potential influence on exposure or accumulation, no relationships were apparent in the present study, based on the limited number of sites evaluated. The sampling sites with the greatest proportion of individuals 65 and older (Orlando, FL) and with the highest median income (Dallas, TX) were consistently the two sites demonstrating the lowest detection of PPCPs. Furthermore, WWTP capacity is typically scaled accordingly with population. Although these relationships are reasonable, their influence on PPCP exposure, accumulation, or both in fish tissue appears to be mitigated by advanced wastewater treatment. Observed variability among sites may also reflect differences in bioavailability of contaminants, potentially contributed by site-specific pH influences on ionization states of target analytes [42], but assessment of this factor was beyond the scope of the present study. Further efforts are warranted to examine these factors on a site-specific basis.

Acknowledgement—The present study was funded by the U.S. Environmental Protection Agency under EPA contract EP-C-04-030. The manuscript has been subjected to review and approved for publication by U.S. EPA's Office of Science and Technology (OST) within the Office of Water. Approval does not signify that the contents reflect the views of the U.S. EPA, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The authors sincerely thank D. Keehner, S. Rudzinski, E. King (U.S. EPA/OST), and J. Hochheimer (Tetra Tech) for their leadership and management support. Gratitude is also expressed to the following professionals who assisted with sample collection activities: C. Barbour, C. Gallardo, and H. Latimer (Tetra Tech); G. Schiffmiller and S. Stringer (New Mexico Environment Department); E. Murphy and T. Nettesheim (Great Lakes National Program Office); and the Metropolitan Water Reclamation District of Greater Chicago.

REFERENCES

1. Ankley GT, Brooks BW, Hugget DB, Sumpter JS. 2007. Repeating history: Pharmaceuticals in the environment. *Environ Sci Technol* 41:8211–8217.
2. Daughton CG, Ternes TA. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environ Health Perspect* 107:907–938.
3. Brooks BW, Chambliss CK, Stanley JK, Ramirez AJ, Banks KE, Johnson RD, Lewis RJ. 2005. Determination of select antidipressants in fish from an effluent-dominated stream. *Environ Toxicol Chem* 24:464–469.
4. Brown JN, Paxéus N, Förlin L, Larsson JDG. 2007. Variations in bioconcentration of human pharmaceuticals from sewage effluents into fish blood plasma. *Environ Toxicol Pharmacol* 24: 267–274.
5. Chu S, Metcalfe CD. 2007. Analysis of paroxetine, fluoxetine, and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography–tandem mass spectrometry. *J Chromatogr A* 1163:112–118.
6. Mottaleb MA, Usenko S, O'Donnell JG, Ramirez AJ, Brooks BW, Chambliss CK. 2009. Gas chromatography–mass spectrometry screening methods for select UV-filters, synthetic musks, alkylphenols, an antimicrobial agent, and an insect repellent in fish. *J Chromatogr A* 1216:815–823.
7. Nakamura Y, Yamamoto H, Sekizawa J, Kondo T, Hirai N, Tatarazako N. 2008. The effects of pH on fluoxetine in Japanese medaka (*Oryzias latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish. *Chemosphere* 70:865–873.
8. Ramirez AJ, Mottaleb MA, Brooks BW, Chambliss CK. 2007. Analysis of pharmaceuticals in fish using liquid chromatography–tandem mass spectrometry. *Anal Chem* 79:3155–3163.
9. Wen Y, Wang Y, Feng Y-Q. 2006. Simultaneous residue monitoring of four tetracycline antibiotics in fish muscle by in-tube solid-phase microextraction coupled with high-performance liquid chromatography. *Talanta* 70:153–159.
10. Balmer ME, Buser H-R, Müller MD, Poiger T. 2005. Occurrence of some organic UV-filters in wastewater, in surface waters, and in fish from Swiss lakes. *Environ Sci Technol* 39:953–962.
11. Buser H-R, Balmer ME, Schmid P, Kohler M. 2006. Occurrence of UV-filters 4-methylbenzylidene camphor and octocrylene in fish from various Swiss rivers with inputs from wastewater treatment plants. *Environ Sci Technol* 40:1427–1431.
12. Duedahl-Olesen L, Cederberg T, Pedersen KH, Højgård A. 2005. Synthetic musk fragrances in trout from Danish fish farms and human milk. *Chemosphere* 61:422–431.
13. Keith TL, Snyder SA, Naylor CG, Staples CA, Summer C, Kannan K, Giesy JP. 2001. Identification and quantitation of

nonylphenol ethoxylates and nonylphenol in fish tissues from Michigan. *Environ Sci Technol* 35:10–13.
14. Nakata H. 2005. Occurrence of synthetic musk fragrances in marine mammals and sharks from Japanese coastal waters. *Environ Sci Technol* 39:3430–3434.
15. Okumura T, Nishikawa Y. 1996. Gas chromatography–mass spectrometry determination of triclosans in water, sediment and fish samples via methylation with diazomethane. *Anal Chim Acta* 325:175–184.
16. Peck AM, Kucklick JR, Schantz MM. 2007. Synthetic musk fragrances in environmental standard reference materials. *Anal Bioanal Chem* 387:2381–2388.
17. Schmid P, Martin Kohler M, Gujer E, Zennegg M, Lanfranchi M. 2007. Persistent organic pollutants, brominated flame retardants and synthetic musks in fish from remote alpine lakes in Switzerland. *Chemosphere* 67:S16–S21.
18. Burkhardt MR, ReVello RC, Smith SG, Zaugg SD. 2005. Pressurized liquid extraction using water/isopropanol coupled with solid-phase extraction cleanup for industrial and anthropogenic waste-indicator compounds in sediment. *Anal Chim Acta* 534:89–100.
19. Kinney CA, Furlong ET, Zaugg SD, Burkhardt MR, Werner SL. 2006. Survey of organic wastewater contaminants in biosolids destined for land application. *Environ Sci Technol* 40:7207–7215.
20. Paterson G, Metcalfe CD. 2008. Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (*Oryzias latipes*). *Chemosphere* 74:125–130.
21. Schultz M, Furlong ET, Kolpin DW, Werner SL, Shoenfuss H, Barber LB. 2008. Occurrence and distribution of antidepressant pharmaceuticals in two U.S. streams, sediments and fish brain tissue exposed to wastewater effluent. *Abstracts,* 5th World Congress, Society of Environmental Toxicology and Chemistry, Sydney, Australia, August 2–6, No. 3.
22. Zhou SN, Oakes KD, Servos MR, Pawliszyn J. 2008. Application of solid-phase microextraction for in vivo laboratory and field sampling of pharmaceuticals in fish. *Environ Sci Technol* 42:6073–6079.
23. Rüdel H, Böhmer W, Schröter-Kermani C. 2006. Retrospective monitoring of synthetic musk compounds in aquatic biota from German rivers and coastal areas. *J Environ Monit* 8:812–823.
24. Balmer ME, Poiger T, Droz C, Romanin K, Bergqvist P-A, Müller MD, Buser H-A. 2004. Occurrence of methyl triclosan, a transformation product of the bacericide triclosan, in fish and from various lakes in Switzerland. *Environ Sci Technol* 38:390–395.
25. Kinney CA, Furlong ET, Kolpin DW, Burkhardt MR, Zaugg SD, Werner SL, Bossio JP, Benotti MJ. 2008. Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolid or swine manure. *Environ Sci Technol* 42:1863–1870.
26. Boyd G, Reemtsam H, Grimm D, Mitra S. 2003. Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada. *Sci Total Environ* 311:135–149.
27. Heberer T. 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: A review of recent research data. *Toxicol Lett* 131:5–17.
28. Brooks BW, Riley TM, Taylor RD. 2006. Water quality of effluent-dominated stream ecosystems: Ecotoxicological, hydrological, and management considerations. *Hydrobiologia* 556:365–379.
29. Daughton CG. 2002. Environmental stewardship and drugs as pollutants. *Lancet* 360:1035–1036.
30. Brooks BW, Foran CM, Richards SM, Weston J, Turner PK, Stanley JK, Solomon KR, Slattery M, La Point TW. 2003. Aquatic ecotoxicology of fluoxetine. *Toxicol Lett* 142:169–183.
31. Kolpin DK, Furlong ET, Meyer MT, Thurman ME, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ Sci Technol* 36:1202–1211.
32. U.S. Environmental Protection Agency. 2000. Guidance for assessing chemical contaminant data for use in fish advisories, Vol 1—Fish sampling and analysis, 3rd ed. EPA 823/B-00/007. Final Report. Office of Water, Washington, DC.
33. Code of Federal Regulations. 1986. Definition and procedure for the determination of the method detection limit. Title 40, Section 136, Appendix B.
34. Mimeault C, Woodhouse AJ, Miao X-S, Metcalfe CD, Moon TW, Trudeau VL. 2005. The human lipid regulator, gemfibrozil bioconcentrates and reduces testosterone in the goldfish, *Carassius auratus. Aquat Toxicol* 73:44–54.
35. Barber LB, Keefe SH, Antweiler RC, Taylor HE, Wass RD. 2006. Accumulation of contaminants in fish from wastewater treatment wetlands. *Environ Sci Technol* 40:603–611.
36. Reinert RE. 1969. Insecticides and the Great Lakes. *Limnos* 2:3–9.
37. Reinert RE, Stewart D, Seagran HL. 1972. Effects of dressing and cooking on DDT concentrations in certain fish from Lake Michigan. *J Fish Res Board Can* 29:525–529.
38. Skea JC, Simonin HA, Harris EJ, Jacking S, Spagnoli JJ, Symula J, Colquhoun JR. 1979. Reducing levels of mirex, Arochlor 1254, and DDE by trimming and cooking Lake Ontario brown trout (*Salmo trutta* Linnaeus) and smallmouth bass (*Micropterus dolomieui* Lacepede). *J Gt Lakes Res* 5:153–159.
39. Mackay D. 1988. Correlation of bioconcentration factors. *Environ Sci Technol* 16:274–278.
40. Bertelsen SL, Hoffman AD, Gallinant CA, Elomen CM, Nichols JW. 1998. Evaluation of log K_{OW} and tissue lipid content as predictors of chemical partitioning to fish tissues. *Environ Toxicol Chem* 17:1447–1455.
41. Halmelink JL, Spacie A. 1977. Fish and chemicals: The process of accumulation. *Annu Rev Pharmacol Toxicol* 17:167–177.
42. Valenti TW, Perez-Hurtado P, Chambliss CK, Brooks B. 2009. Aquatic toxicity of sertraline to *Pimephales promelas* at environmentally relevant surface water pH. *Environ Toxicol Chem* 28: 2685–2694.
43. Nikolaou A, Meric S, Fatta D. 2007. Occurrence patterns of pharmaceuticals in water and wastewater environments. *Anal Bioanal Chem* 387:1225–1234.
44. Ternes T. 2001. Pharmaceuticals and metabolites as contaminants of the aquatic environment. In Daughton CG, Jones-Lepp TL, eds, *Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues*. American Chemical Society, Washington, DC, p 396.
45. Richardson ML, Bowron JM. 1985. Review: The fate of pharmaceutical chemicals in the aquatic environment. *J Pharmacol* 37:1–12.
46. Kim SD, Cho J, Kim K, Vanderford BJ, Snyder SA. 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. *Water Res* 41:1013–1021.
47. Okuda T, Kobayashi Y, Nagao R, Yamashita N, Tanaka H, Tanaka S, Fujii S, Konishi C, Houwa I. 2008. Removal efficiency of 66 pharmaceuticals during wastewater treatment process in Japan. *Water Sci Technol* 57:65–71.