ARTICLE IN PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

The Science of the Total Environment xx (2003) xxx–xxx

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

www.elsevier.com/locate/scitotenv

Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada

Glen R. Boyd[a,*], Helge Reemtsma[a], Deborah A. Grimm[b], Siddhartha Mitra[c]

[a]*Department of Civil and Environmental Engineering, Tulane University, New Orleans, LA 70118, USA*
[b]*Coordinated Instrumentation Facility, Tulane University, New Orleans, LA 70118, USA*
[c]*Department of Geological Sciences and Environmental Studies, Binghamton University, Binghamton, NY 13902, USA*

Received 9 March 2002; accepted 2 January 2003

Abstract

A newly developed analytical method was used to measure concentrations of nine pharmaceuticals and personal care products (PPCPs) in samples from two surface water bodies, a sewage treatment plant effluent and various stages of a drinking water treatment plant in Louisiana, USA, and from one surface water body, a drinking water treatment plant and a pilot plant in Ontario, Canada. The analytical method provides for simultaneous extraction and quantification of the following broad range of PPCPs and endocrine-disrupting chemicals: naproxen; ibuprofen; estrone; 17β-estradiol; bisphenol A; clorophene; triclosan; fluoxetine; and clofibric acid. Naproxen was detected in Louisiana sewage treatment plant effluent at 81–106 ng/l and Louisiana and Ontario surface waters at 22–107 ng/l. Triclosan was detected in Louisiana sewage treatment plant effluent at 10–21 ng/l. Of the three surface waters sampled, clofibric acid was detected in Detroit River water at 103 ng/l, but not in Mississippi River or Lake Pontchartrain waters. None of the other target analytes were detected above their method detection limits. Based on results at various stages of treatment, conventional drinking-water treatment processes (coagulation, flocculation and sedimentation) plus continuous addition of powdered activated carbon at a dosage of 2 mg/l did not remove naproxen from Mississippi River waters. However, chlorination, ozonation and dual media filtration processes reduced the concentration of naproxen below detection in Mississippi River and Detroit River waters and reduced clofibric acid in Detroit River waters. Results of this study demonstrate that existing water treatment technologies can effectively remove certain PPCPs. In addition, our study demonstrates the importance of obtaining data on removal mechanisms and byproducts associated with PPCPs and other endocrine-disrupting chemicals in drinking water and sewage treatment processes.

Keywords: Pharmaceuticals; Endocrine disruptors; Sewage treatment plant effluent; Natural waters; Drinking water treatment

1. Introduction

Recent studies indicate the potential widespread occurrence of low-level concentrations (ng–μg/l) of pharmaceuticals, hormones, and other organic sewage contaminants and their metabolites in the aquatic environment (Guillette, 1995; Desbrow et al., 1998; Halling-Sørensen et al., 1998; Ternes, 1998; Daughton and Ternes, 1999; Sedlak et al., 2000; Boyd and Grimm, 2001; Kolpin et al.,

*Corresponding author. Tel.: +1-504-862-3266; fax: +1-504-862-8941.
E-mail address: gboyd@tulane.edu (G.R. Boyd).

0048-9697/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0048-9697(03)00138-4

Table 1
Target analytes

Name	Trade name examples	CAS#	Purity (%)	Stock concentration (mg/l)	Commercial use	Chemical name
Clofibric acid	NA	882-09-7	97.0	10.17	Metabolite of lipid regulator	2-(4-Chlorophenoxy)-2-methyl-propanoic acid
Naproxen	Naprosyn, Aleve	22204-53-1	100.6	10.02	Anti-inflammatory, analgesic	(α*S*)-6-Methoxy-α-methyl-2-naphthaleneacetic acid
Ibuprofen	Advil, Motrin	15687-27-1	99.8	30.08	Anti-inflammatory	α-Methyl-4-(2-methylpropyl) benzene-acetic acid
Acetaminophen	Tylenol	103-90-2	>99.0	100.08	Analgesic	*N*-(4-Hydroxyphenyl)acetamide
Caffeine	Caffeine	58-08-2	>99.9	99.9	Stimulant	3,7-Dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione
Fluoxetine[a]	Prozac	54910-89-3	100.0	357.6	Antidepressant	*N*-Methyl-γ-[4-(trifluoromethyl) phenoxy]benzenepropanamine
Clorophene	Santophen 1	120-32-1	NA	5.08	Disinfectant	4-Chloro-2-(phenylmethyl)phenol
Triclosan	Ster-Zac	3380-34-5	97.0	5.06	Antibacterial, disinfectant	5-Chloro-2-(2,4-dichlorophenoxy) phenol
Bisphenol A	Bisphenol A	80-05-7	>99.0	5.11	Plastics intermediate, fungicide	4,4′-(1-Methylethylidene) bisphenol
Estrone	Estrol, Femidyn	53-16-7	>99.0	10.14	Steroid	3-Hydroxyestra-1,3,5(10)-trien-17-one
17β-Estradiol	Estrace, Estraderm	50-28-2	>98.0	9.99	Steroid	(17β)-Estra-1,3,5(10)-triene-3,17-diol

All chemicals were obtained from Sigma Chemical Corporation (St. Louis, MO). Stock concentrations were prepared in dichloromethane. NA, not available.

[a] Purchased as fluoxetine hydrochloride.

2002). Many of these compounds are suspected or potential endocrine-disrupting chemicals. Pharmaceuticals and personal care products (PPCPs) describe a large class of chemical contaminants that can originate from human usage and excretions, and veterinary applications of a variety of products, such as over-the-counter and prescription medications, and fungicides and disinfectants used for industrial, domestic, agricultural and livestock practices (Daughton and Ternes, 1999). PPCPs and their metabolites are continually introduced into aquatic environs and are prevalent at detectable concentrations (Kolpin et al., 2002), which can affect water quality and ecosystem health and potentially impact drinking water supplies (Roefer et al., 2000; Trussell, 2001). The long-term effects of continuous, low-level exposure to PPCPs and their metabolites are not well understood (Daughton and Ternes, 1999).

Effluents from sewage treatment plants contain a variety of PPCPs (Daughton and Ternes, 1999). Studies have shown that the transformation processes for specific PPCP compounds can vary in a sewage treatment plant, depending on the characteristics of the sewage, weather conditions, and the design and operation of the treatment processes (Ternes, 1998; Johnson and Sumpter, 2001). Upon discharge of treated sewage into a receiving water body, residual PPCPs can be diluted and blended with contaminants from other discharge points, as well as runoff and seepage. These same receiving water bodies also can serve as drinking water sources. Recent studies aimed at investigating drinking-water treatment methods for PPCPs have demonstrated that conventional treatment processes (coagulation, flocculation and sedimentation) are not effective methods, but other treatment processes, such as oxidation with chlorine and ozone, activated carbon and membrane filtration, can be effective in removing antibiotics (Adams et al., 2002) and other selected pharmaceuticals (Ternes et al., 2002).

A list of target analytes representing a variety of PPCPs was developed for this study (Table 1).

To date, there is no universally accepted method for the analysis of PPCPs in aquatic environs. Several analytical approaches have been utilized, including gas chromatography/mass spectrometry (GC/MS), gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS), GC with high-resolution mass spectrometry, liquid chromatography-ultraviolet detection (LC-UV), liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) (Desbrow et al., 1998; Barber et al., 2000; Laganá et al., 2000; Möder et al., 2000; Huang and Sedlak, 2001; Kolpin et al., 2002). The decision to use GC or LC is usually based on the physicochemical characteristics of the target analyte. Many PPCPs contain polar functional groups and/or are thermally labile and do not lend themselves readily to GC analysis. Thus, most GC methods for polar PPCPs must incorporate a derivatization step to overcome these limitations. LC is generally applicable to more polar compounds, in contrast to GC. The use of MS for detection in either case gives a second dimension of information, which can be used to confirm the identity of the targeted compound through its mass spectrum. Nonetheless, PPCPs include a broad range of molecules of differing polarity and functionality, and hence pose a significant analytical challenge.

The objective of this study was to develop a method for quantifying the concentration of a target list of a variety of PPCPs in surface and treated waters of Louisiana, USA and Ontario, Canada. This paper provides a method for analyzing the following diverse list of PPCP compounds in natural and treated water samples: a metabolite (clofibric acid) of a lipid regulator; two analgesics (naproxen and ibuprofen); two steroids (estrone and 17β-estradiol); a chemical intermediate in the synthesis of commercial products (bisphenol A); one disinfectant (clorophene); an antibacterial additive (triclosan); and an antidepressant (fluoxetine). The target list of PPCPs is inclusive of bisphenol A, an intermediate, due to its ubiquitous nature and its endocrine-disrupting potential. The method was used to determine concentrations of the target PPCPs in surface water samples from the Mississippi River and Lake Pontchartrain in Louisiana, and the Detroit River in Ontario. The method was also used to analyze treated water samples from a sewage treatment plant in Louisiana, several stages of drinking water treatment plants in Louisiana and Ontario, and a pilot drinking-water treatment plant in Ontario.

2. Site selection and sampling

Surface water samples were collected from the Mississippi River in New Orleans, Louisiana during September–November 2001 (Fig. 1, Site #1). The Mississippi River extends from northern Minnesota to the Gulf of Mexico and drains 41% of the conterminous United States in an area where 27% of the population resides (Meade, 1996). The mean annual discharge of the lower Mississippi River near New Orleans is 13 500 m^3/s (Meade, 1996). The Mississippi River receives a variety of organic wastes from urban areas, farms, factories and individual households. Approximately 70 US cities rely on the Mississippi River as a source of drinking water. Surface water samples were collected from the Mississippi River at a site outside of direct influence of discharge points of known private or municipal sewage treatment plants.

Surface water samples were also collected on the southern shore of Lake Pontchartrain (Fig. 1, Site #2), which is located within the Lake Pontchartrain estuary in the central Gulf Coast region adjacent to New Orleans, Louisiana. Lake Pontchartrain is influenced by riverine discharges (228 m^3/s) as well as stormwater drainage and freshwater diversion from the Mississippi River through the Bonnet Carre spillway (Flowers and Isphording, 1990; Argyrou et al., 1997). Lake Pontchartrain is not used as a municipal drinking water source.

Sewage plant effluent samples were collected during February and March 2002 from the Jefferson Parish East Bank Wastewater Treatment Plant (Fig. 1, Site #3), which discharges treated sewage effluent into the Mississippi River. The plant is located in metropolitan New Orleans, Louisiana, approximately 5 km west of the city line. The plant uses conventional secondary wastewater treatment and operates at an annual average flow of 125 000 m^3/day. Treated sewage samples were collected prior to chlorination of the effluent (STP1), as shown in Fig. 2a.

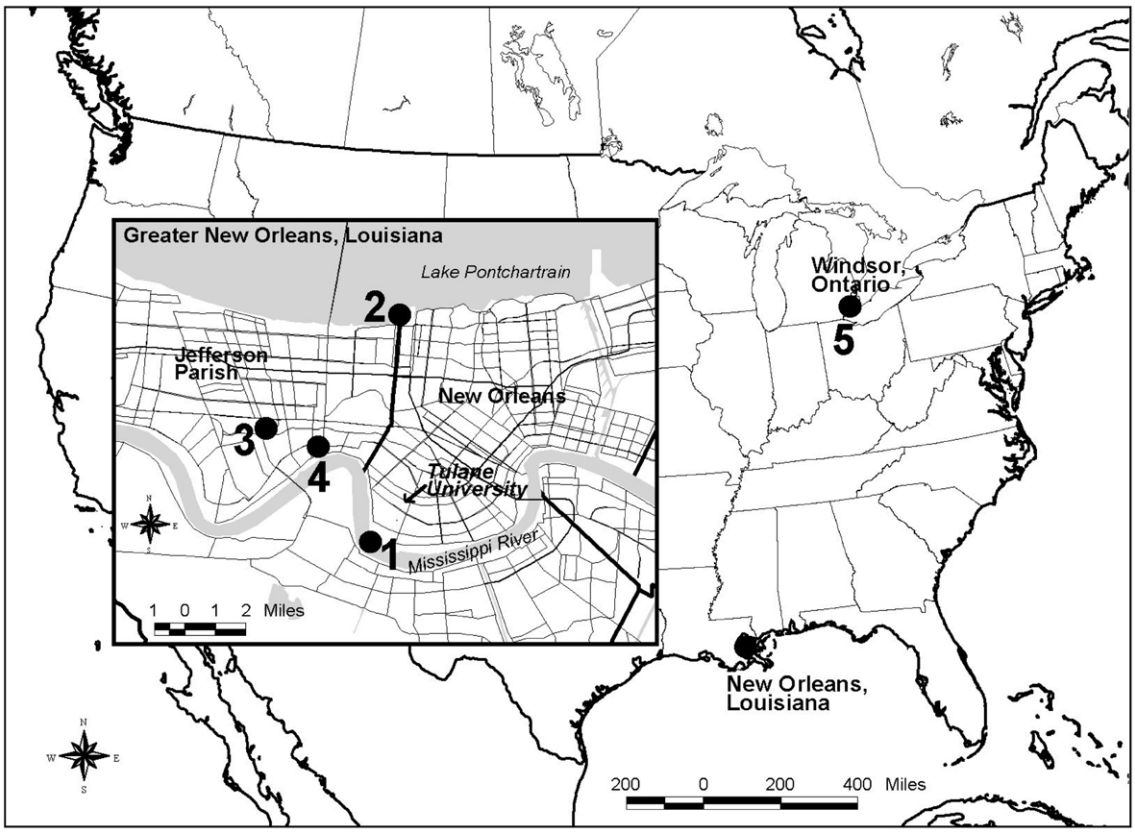

Fig. 1. Sampling sites in greater New Orleans, Louisiana and Windsor, Ontario. Site #1, Mississippi River, Louisiana; Site #2, Lake Pontchartrain, Louisiana; Site #3, Jefferson Parish East Bank Wastewater Treatment Plant, Louisiana; Site #4, Jefferson Parish East Bank Water Treatment Plant, Louisiana; Site #5, A.H. Weeks Water Treatment Plant and ENWIN Pilot Plant, Ontario.

Water samples were collected from various stages of the Jefferson Parish East Bank Water Treatment Plant (Fig. 1, Site #4), which relies on the Mississippi River as its source. The drinking water treatment plant is located approximately 2.5 km west of the New Orleans city line. The plant operates at a maximum flow of 330 000 m^3/day and uses conventional treatment, which includes coagulation (alum and cationic polyelectrolyte polymer), flocculation and sedimentation. The treated water is disinfected by chlorination prior to filtration, and chloramination prior to distribution (Fig. 2b). High-load organic pollutants are removed from the raw water by adding powdered activated carbon (PAC) at a concentration of 2 mg/l. Samples were collected at the plant inlet (JP1), after PAC addition and conventional treatment (JP2), and after chlorination, filtration and storage (JP3), as shown in Fig. 2b.

In Canada, water samples were collected in January 2002 at the A.H. Weeks Water Treatment Plant (Fig. 1, Site #5) in Windsor, Ontario, which relies on the Detroit River as its source. The drinking water treatment plant operates at a maximum flow of 227 000 m^3/day and uses ozonation, conventional treatment (alum and Percol LT22 as coagulants) and chlorination prior to distribution (Fig. 2c). Samples were collected from the Detroit River at the plant inlet (WO1) and after treatment at the plant outlet (WO2), as shown in Fig. 2c. Samples were also collected at the ENWIN Pilot Plant (Fig. 2D), which was located at the same

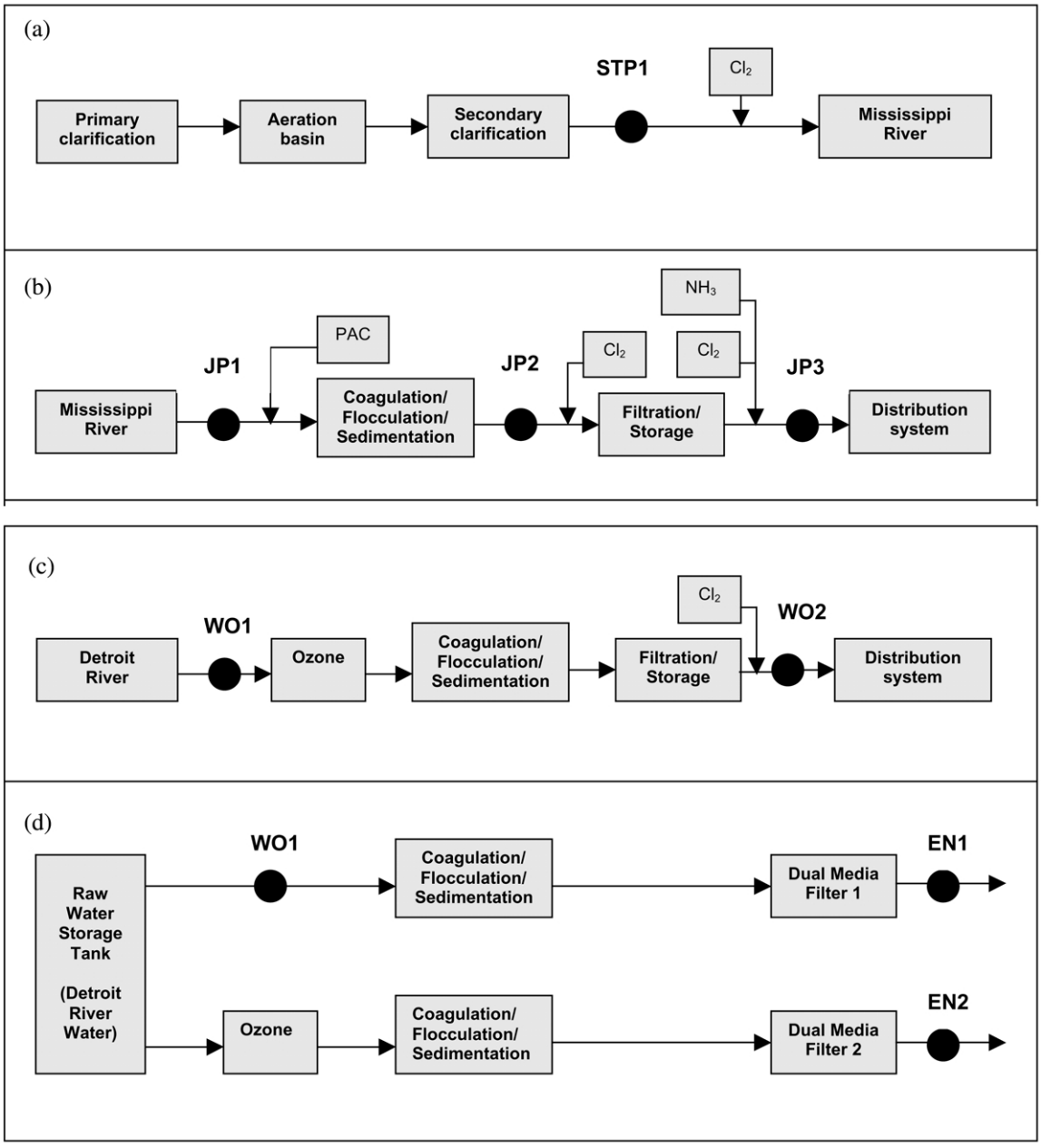

Fig. 2. Process flow diagrams for sewage and drinking water treatment plants in Louisiana and Ontario (● indicates sampling location). (a) Jefferson Parish East Bank Wastewater Treatment Plant, Louisiana (Fig. 1, Site #3); (b) Jefferson Parish East Bank Water Treatment Plant, Louisiana (Fig. 1, Site #4); (c) A.H. Weeks Water Treatment Plant, Ontario (Fig. 1, Site #5); and (d) ENWIN Pilot Plant, Ontario (Fig. 1, Site #5).

site as the A.H. Weeks Water Treatment Plant and used to test treatment processes for the Detroit River water source (WO1). Water samples were collected at the outlet of the pilot plant following conventional treatment and dual media filtration (EN1) and at the outlet of a similar process train preceded by ozonation (EN2), as shown in Fig. 2d.

For all sites, a total of 8 l was collected as grab samples using pre-cleaned 4-l amber glass containers. Louisiana samples were stored on ice during transport to the laboratory and were processed within 7 days. Samples collected from the Canadian plants were acidified prior to shipping and were analyzed immediately upon arrival at Tulane University. A method blank using ultra-pure laboratory water further purified by passing through a SPE disk prior to use (see below) was performed for each batch of samples collected from the sewage and drinking water treatment plants.

3. Analytical methods

A relative response factor (RRF) standard solution of all reference standards was prepared in dichloromethane (DCM) and methanol, with concentrations of each analyte ranging between 5 and 100 mg/l. The RRF standard consisted of the compounds summarized in Table 1 (except fluoxetine hydrochloride, which was prepared separately) and three deuterated surrogate compounds (bisphenol A-d_{14}, estrone-d_4 and acetaminophen-d_4). Fluoxetine was not as stable as our other target analytes; therefore, fluoxetine standards were prepared from the solid material just prior to use. The surrogate standard was prepared in DCM at concentrations between 5 and 100 mg/l prior to adding it to the RRF standard. Phenanthrene-d_{10} (99.3% purity, AccuStandard Inc, New Haven, CT) was chosen as the internal standard, since it was not affected by the derivatization step in the analytical procedure, as discussed below. Phenanthrene-d_{10} was prepared in DCM at a concentration of 495.4 mg/l and added to the sample after the final concentration step and after derivatization.

Sampling bottles and all glassware used for sample collection and preparation were cleaned by washing with soap, soaking in a 5% Contrad solution (Decon Laboratories Inc, Bryn Mawr, PA) and in hydrochloric acid (2 N), and then ashing at 450 °C. All laboratory materials were either made of glass or Teflon to avoid sample contamination. Teflon containers were cleaned in the same manner as glassware, but without ashing. Ultra-pure water was produced in the laboratory by filtering tap water through activated carbon, followed by a mixed-bed deionization tank and ultra-filtration membrane system, and then ultraviolet light exposure (US Filter, Modulab UF/UV, CA, USA). Analysis of ultra-pure water used for spiked recovery experiments and method blanks showed low-level background contamination with bisphenol A. Once this was determined, the procedure was modified to include further purification of the ultra-pure water by passing it through a SPE disk. All solvents were GC grade.

3.1. Solid-phase extraction

The targeted PPCP compounds were isolated from water samples by solid-phase extraction using a polar SDB-XC Empore disk (3M Corporation, St. Paul, MN). Surface water samples, sewage treatment plant effluent samples and untreated drinking water treatment plant samples were pumped through 1.0- and 0.2-μm glass fiber filters (47 mm in diameter, Millipore Corporation, Bedford, MA) to remove particulate matter prior to solid-phase extraction. Pre-filtration was not necessary for water samples collected at the outlet of the drinking water treatment plants. Extraction disks were pre-conditioned with 50 ml of methanol, 50 ml of DCM, 50 ml of methanol and 10 ml of ultra-pure water. If samples were not previously acidified, the pH was adjusted to <2.0 using 12 N HCl prior to spiking with the surrogate standard (0.5 ml/l sample). Samples were then drawn through the extraction disks using vacuum aspiration at an approximate flow rate of 100 ml/min. The disks were then air-dried and the targeted compounds were extracted from the disks by eluting with 50 ml of methanol, 50 ml of DCM and 50 ml of methanol. The extracts were concentrated to an approximate volume of 1 ml using a RapidVap® with mild heat (50 °C) and a gentle stream of nitrogen gas.

The concentrated organic extracts were passed through a column containing 3 g of pre-washed silica gel to remove dissolved interfering compounds (e.g. humic acids) from some samples. The silica gel was then washed with three bed volumes each of DCM and methanol. This clean-up step was added to the analytical procedure after a method revision in the course of this research, and therefore was not applied to all samples. The silica gel-treated samples were carefully evaporated to a volume of 1 ml under the same conditions described previously.

3.2. Derivatization

Derivatization was used to enhance the thermal stability of clofibric acid, which thermally degraded in the GC injection port, and reduce the polarity of specific target analytes (clofibric acid, ibuprofen and naproxen) to facilitate GC analysis. Given the sensitivity of the derivatization reagent [*N*,*O*-bis(trimethylsilyl)-triflouroacetamide in the presence of trimethylchlorosilane; BSTFA; Supelco Inc, Bellefonte, PA] to moisture, and because Na_2SO_4 was not effective at removing traces of water dissolved in methanol, all samples were placed in GC autosampler vials and completely dried under a stream of N_2 prior to derivatization. Derivatization was achieved by dissolving the dried sample residue in 1 ml of BSTFA reagent mixture. The closed vial was then heated at 80 °C for 20 min. Finally, 10 μl of the internal standard (phenanthrene-d_{10}) was added to the sample prior to instrumental analysis.

For the RRF and instrument detection limit experiments, the working standards were carefully dried, dissolved in the BSTFA reagent mixture and derivatized as described here. A 1-ml aliquot of known concentration was prepared for each target compound and analyzed by GC/MS. This same sample was then dried and derivatized as described previously. The derivatized sample was analyzed by GC/MS and the chromatogram was checked for both the non-derivatized and derivatized forms of the analyte. If the derivatization was incomplete, the percentage completion was determined by comparing the peak areas. Caffeine and fluoxetine, lacking the appropriate functional groups, exhibited no response to derivatization. Estrone was derivatized to 84.7% completion. All other analytes were derivatized to 100% completion.

3.3. GC/MS conditions

Samples were analyzed by GC/MS (Agilent 6890 GC and 5972 MSD) under the following conditions. Splitless 2-μl injections were made onto a DB-5MS column (25 m with 0.25-μm film thickness and 0.25 mm i.d.) at a constant flow rate of 1 ml/min. The GC oven was operated from 100 °C (0-min hold) at 5 °C/min to 165 °C (5-min hold), then at 2 °C/min to 175 °C (0-min hold) and at 10 °C/min to 320 °C (5-min hold) for a total run time of 42.5 min. The injector and detector temperatures were 230 and 300 °C, respectively. The MS was operated in +EI mode using selected ion monitoring (SIM) for sensitivity. Table 2 summarizes the SIM conditions.

3.4. Quantification

Quantification of the targeted PPCP compounds was conducted by comparing peak areas of the most intensive ion of each compound with that of the internal standard. Compound identification was confirmed by GC retention time and qualifier ions (usually molecular ion and one or two fragment ions) as shown in Table 2. Baseline interference was observed at or near the retention time of estrone. As part of the method development, ion ratios were monitored, enabling discrimination between interference and the proper response for estrone. In addition, qualifier ions were re-evaluated for the steroid compounds as compared to methods employed by the authors in previous research (Boyd and Grimm, 2001). Before each sequence of samples, response factors were calculated separately from the analysis of the RRF and its dilutions, 1:10, 1:20 and 1:200.

Fig. 3a shows the GC/MS chromatogram of the RRF stock solution containing the target compounds. Fig. 3b shows the chromatogram of a sample collected from the inlet of Jefferson Parish East Bank Water Treatment Plant representing raw Mississippi River water (Fig. 2b, JP1). It identifies

Table 2
Selected ion monitoring (SIM) program for targeted and standard analytes

SIM group	Type	Name	Molecular weight	Retention time (min)	Target ion	Qualifier ion 1	2
1	TGT	Clofibric acid–TMS	286	17.66	128	143	286
1	TGT	Ibuprofen-TMS	278	19.61	263	278	234
2	SS	Acetaminophen-d_4–TMS	227	ND	227	–	–
2	SS	Acetaminophen-d_4–TMS(2)	299	19.79	284	299	–
2	TGT	Acetaminophen–TMS(2)	295	19.83	280	295	206
3	IS	Phenanthrene-d_{10}	188	26.11	188	160	–
3	TGT	Caffeine	194	27.30	194	109	–
3	TGT	Fluoxetine	309	ND	309	104	–
4	TGT	Clorophene–TMS	290	28.39	290	292	275
4	TGT	Naproxen–TMS	302	30.77	243	302	185
5	TGT	Triclosan–TMS	360	31.33	200	360	362
6	SS	Bisphenol A-d_{16}	244	ND	226	–	–
6	SS	Bisphenol A-d_{15}–TMS(1)	315	ND	315	–	–
6	SS	Bisphenol A-d_{14}–TMS(2)	386	32.08	368	386	–
6	TGT	Bisphenol A–TMS(2)	372	32.17	357	372	–
7	SS	Estrone-d_4–TMS	346	36.21	346	220	–
7	TGT	Estrone–TMS	342	36.21	342	327	257
7	TGT	Estrone-d_4	274	ND	274	–	–
8	TGT	17β-Estradiol–TMS(2)	416	36.43	416	285	–

IS, internal standard; ND, not detected; SS, surrogate standard; TGT; targeted analyte; TMS, Trimethylsilyl derivative. Note that (1) or (2) after TMS refers to the mono- or di-derivative, respectively.

the response of the target ion and two qualifier ions of naproxen.

3.5. Limits of detection and determination

The instrument detection limits for all compounds were determined by serial dilution of the RRF and fluoxetine standard solutions. The diluted solutions were prepared by weighing a known amount of working standard into an autosampler crimp-top vial and adding a known mass of BSTFA derivatization reagent and a known amount of internal standard. In this way, the injected mass of each compound could be calculated. Instrument detection limits are reported in Table 3.

3.6. Recoveries

Natural water samples were collected from three surface water bodies, and treated water samples were collected from the effluent of a sewage treatment plant and various stages of treatment from two drinking water treatment plants and a pilot plant. As such, sample matrices were diverse and surrogate standards were added to samples to monitor matrix effects. Spiked recoveries were measured for each compound, including surrogates using ultra-pure laboratory water. Three 1-l laboratory samples were spiked with 1 ml each of a RRF and a fluoxetine standard. The spiked samples were extracted and analyzed using solid-phase extraction, derivatization and GC/MS as described previously. Results were compared to non-extracted RRF and fluoxetine standard solutions. For quantification, the samples and the standard solution were spiked with 10 μl of the internal standard.

Recoveries for most compounds were greater than 47%. Exceptions were acetaminophen and caffeine. Acetaminophen was repeatedly not detected, whereas caffeine exhibited low recovery (2.8%). These low recoveries were attributed to incomplete retention of these compounds on the extraction disk. Recovery rates for the surrogate compounds bisphenol A-d_{14} and estrone-d_4 were greater than 95%. The recovery rate for acetamin-

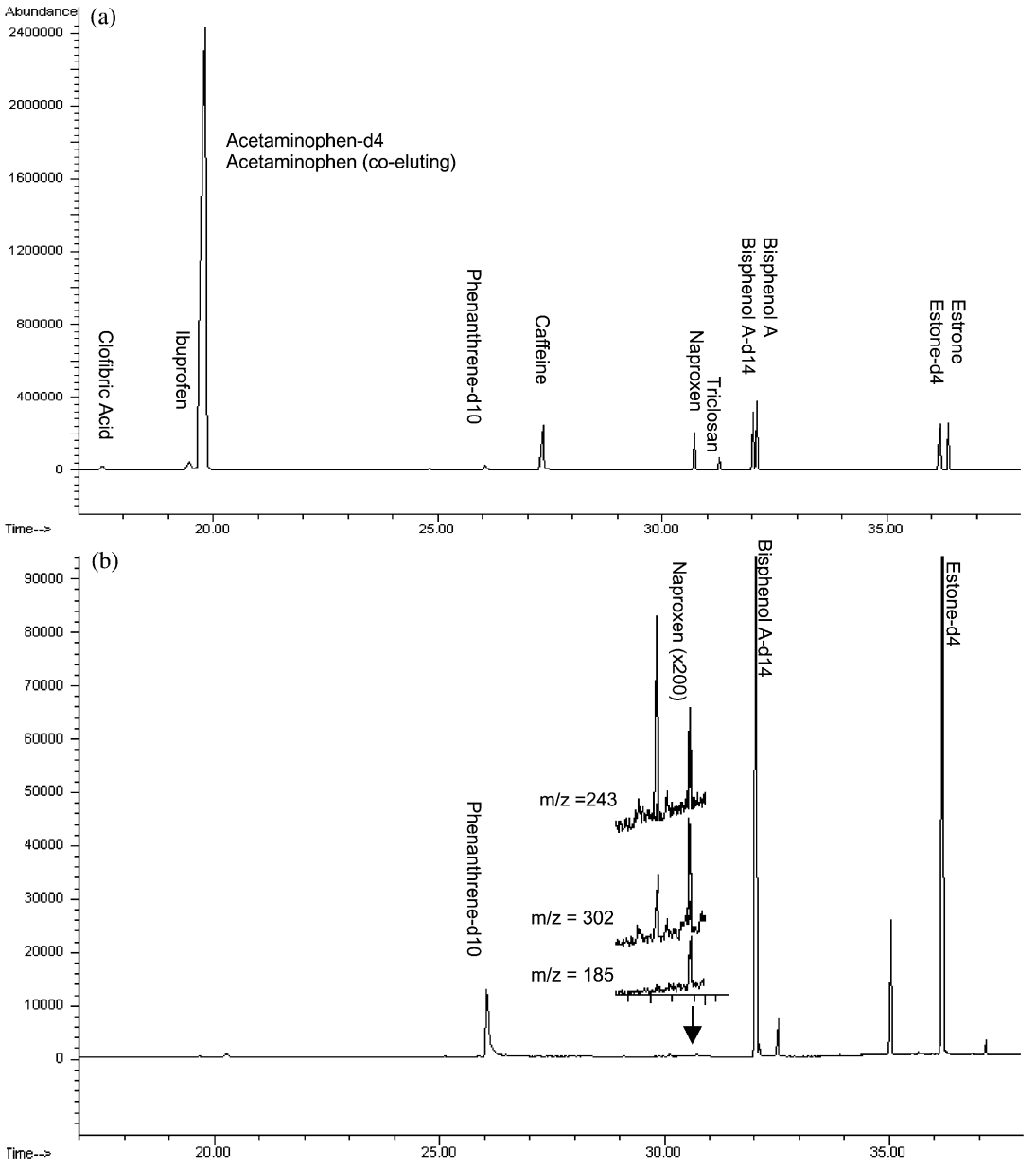

Fig. 3. GC/MS chromatograms: (a) relative response factor standard containing all target compounds (except fluoxetine and chlorophene); (b) inlet sample (JP1) for Jefferson Parish East Bank Water Treatment Plant (Site #4, Fig. 1).

ophen-d_4 could not be determined, as GC/MS analysis repeatedly showed non-detection for this compound. Acetaminophen and caffeine were therefore not included in the final list of target analytes for this method. Recovery rates and method detection limits for the target compounds are summarized in Table 3.

In contrast to spiked recovery experiments with ultra-pure water, for which the recovery was greater than 95% for estrone-d_4 and bisphenol A-d_{14},

recoveries of deuterated compounds were slightly decreased in most surface water samples, which was attributed to the presence of high loads of dissolved organic matter and other matrix complexities. Samples collected from the Louisiana sewage treatment plant and the Louisiana drinking-water treatment plant were filtered through a silica gel column to remove dissolved organic matter. This clean-up step was not applied to samples from the Canadian plants and it had no significant effect on recovery of the target analytes. Water samples that were disinfected with chlorine at the drinking water treatment plants were quenched with 40–50 mg/l of sodium sulfite to avoid reaction of chlorine residuals with the surrogate standard compounds. Samples that were stored in the refrigerator for several days exhibited improved surrogate standard recovery, which was attributed to the dissipation of free chlorine prior to sample analysis.

4. Results and discussion

Sampling results for the nine targeted PPCP compounds are summarized in Table 4 Tables 5–

Table 4
PPCPs in surface waters in Louisiana

PPCP compound	Concentration in surface water (ng/l)			
	Mississippi River		Lake Pontchartrain	
Clofibric acid	ND	ND	ND	ND
Ibuprofen	ND	ND	ND	ND
Fluoxetine	ND	ND	ND	ND
Clorophene	ND	ND	ND	ND
Naproxen	37	39	107	22
Triclosan	ND	ND	ND	ND
Bisphenol A	NQ	NQ	NQ	NQ
Estrone	ND	ND	ND	ND
17β-Estradiol	ND	ND	ND	ND
Bisphenol A-d_{14} (%)[a]	68.0	75.0	67.0	67.0
Estrone-d_4 (%)[a]	103.4	119.4	88.3	124.6
Acetaminophen-d_4 (%)[a]	ND	ND	ND	ND

Samples were collected from the shores of the Mississippi River (Fig. 1, Site #1) and from the shores of Lake Pontchartrain (Fig. 1, Site #2). No silica gel cleanup was used during sample preparation. ND, not detected (see MDLs in Table 3); NQ, not quantified.

[a] Percentage recovery of surrogate standard.

Table 3
Detection limits and percentage recovery

	IDL (ng/l)	Completion of derivatization (%)	Method development			Revised method		
			MDL (ng/l)	Recovery (%)	R.S.D. (%)	MDL (ng/l)	Recovery (%)	R.S.D. (%)
Clofibric acid	3	100	0.6	60.8	12.6	0.8	44.2	26.5
Ibuprofen	13	100[b]	3.5	47.1	26.9	2.6	63.0	12.3
Acetaminophen	45	100	ND	ND	ND	ND	ND	ND
Caffeine	24	0	107.1	2.8	3.6	319.3	0.9	1.1
Fluoxetine	178	0	25.8	86.1	7	25.4	87.7[a]	–
Chlorophene	0.6	100	0.1	71.7	5.9	0.1	108.9[a]	–
Naproxen	3	100	0.4	87.9	2.8	0.4	102.9	17.8
Triclosan	1	100	0.2	53.8	24	0.2	60.1	22.8
Bisphenol A	0.6	100	0.1	99.7	3.5	0.1	95.6	39.5
Estrone	3	84.7	0.4	91.9	5.1	0.3	130.3	22.3
17β-Estradiol	1	100	0.1	90.5	9.1	0.1	117.6	14.8

Method development does not include silica gel clean-up. Revised method includes silica gel clean-up. Completion of derivatization was based on comparison of underivatized peak area and derivatized peak area. IDL, instrument detection limit; MDL, method detection limit; ND, not detected; R.S.D., relative standard deviation. MDL was based on a 2-μl injection from a 1-ml extract of an 8-l sample. Percentage recovery is based on non-extracted RRF and fluoxetine standard.

[a] Due to fast degradation of this compound, the value of only one sample was considered.

[b] Assumed completion. Underivatized ibuprofen did not elute from the GC.

Table 5
PPCPs in sewage treatment plant effluent in Louisiana

PPCP compound	Concentration at STP1 (ng/l)	
Clofibric acid	ND	ND
Ibuprofen	ND	ND
Fluoxetine	ND	ND
Clorophene	ND	ND
Naproxen	106	81
Triclosan	21	10
Bisphenol A	ND	ND
Estrone	ND	ND
17β-Estradiol	ND	ND
Bisphenol A-d_{14} (%)[a]	13.6	13.9
Estrone-d_4 (%)[a]	52.6	28.9
Acetaminophen-d_4 (%)[a]	1.1	1.2

Samples were collected at the Jefferson Parish East Bank Wastewater Treatment Plant (Fig. 1, Site #3). Sampling location is shown in Fig. 2a. Sample preparation included silica gel clean-up. ND, not detected (see MDLs in Table 3).

[a] Percentage recovery of surrogate standard.

7. Results are discussed with regard to occurrence of these nine compounds in surface waters in Louisiana and Ontario, in the effluent of a sewage treatment plant, and during various stages of removal by drinking water treatment processes.

4.1. Surface waters

Results for Louisiana and Ontario surface waters are shown in Tables 4, 6 and 7. Naproxen, which is a common prescription pain reliever, was detected in Mississippi River (Table 4 and JP1 in Table 6), Lake Pontchartrain (Table 4) and Detroit River (WO1 in Table 7) waters at concentrations ranging from 22 to 107 ng/l. These observations are similar to findings reported by Ternes (1998) and Ternes et al. (1999) for German, Canadian and Brazilian surface waters. Clofibric acid, which is a metabolite of the lipid regulator clofibrate (as one of several in this class), was detected in Detroit River water (WO1 in Table 7) at a concentration of 103 ng/l, similar to findings for European surface waters (Stan et al., 1994; Stumpf et al., 1996; Ternes, 1998; Daughton and Ternes, 1999). The absence of clofibric acid in Mississippi River and Lake Pontchartrain waters could be attributed to the declining use of clofibrate in the United States (WHO, 1996).

17β-Estradiol was observed to be below the method detection limit (Table 3) of 0.1 ng/l for all samples collected from surface waters. Other investigators have reported 17β-estradiol in surface

Table 6
PPCPs at Jefferson Parish East Bank drinking water treatment plant in Louisiana, USA

PPCP compound	Concentration at water treatment plant (ng/l)					
	Mississippi R. (JP1)		Precipitator (JP2)		Finished water (JP3)	
Clofibric acid	ND	ND	ND	ND	ND	ND
Ibuprofen	ND	ND	ND	ND	ND	ND
Fluoxetine	ND	ND	ND	ND	ND	ND
Clorophene	ND	ND	ND	ND	ND	ND
Naproxen	64	65	63	68	ND	ND
Triclosan	ND	ND	ND	ND	ND	ND
Bisphenol A	NQ	NQ	NQ	NQ	NQ	ND
Estrone	ND	ND	ND	ND	ND	ND
17β-Estradiol	ND	ND	ND	ND	ND	ND
Bisphenol A-d_{14} (%)[a]	62.8	65.2	46.0	81.3	94.9	18.6
Estrone-d_4 (%)[a]	130.1	68.3	118.1	99.3	106.7	17.7
Acetaminophen-d_4 (%)[a]	0.2	0.2	0.2	ND	0.1	ND

Samples were collected at Jefferson Parish East Bank Water Treatment Plant in Louisiana, USA (Fig. 1, Site #4). Sampling locations at the plant are shown in Fig. 2b. ND, not detected (see MDLs in Table 3); NQ, not quantified.

[a] Percent recovery of surrogate standard. Sample preparation included silica gel clean-up.

Table 7
PPCPs at drinking water treatment plant and pilot plant in Ontario, Canada

PPCP compound	Concentration at water treatment plant (ng/l)			
	Full-scale plant		ENWIN pilot plant	
	Detroit R. water (WO1)	Finished water (WO2)	Filter 1 (EN1)	Filter 2 (EN2)
Clofibric acid	103	ND	ND	ND
Ibuprofen	ND	ND	ND	ND
Fluoxetine	ND	ND	ND	ND
Clorophene	ND	ND	ND	ND
Naproxen	63	ND	ND	ND
Triclosan	ND	ND	ND	ND
Bisphenol A	NQ	NQ	NQ	NQ
Estrone	ND	ND	ND	ND
17β-Estradiol	ND	ND	ND	ND
Bisphenol A-d_{14} (%)[a]	66.7	93.6	80.2	91.5
Estrone-d_4 (%)[a]	77.2	90.7	82.1	74.6
Acetaminophen-d_4 (%)[a]	ND	0.2	ND	ND

Samples were collected at the A.H. Weeks Water Treatment Plant and ENWIN pilot plant in Ontario, Canada (Fig. 1, Site #5). Sampling locations are shown in Fig. 2c,d. Sample preparation did not include silica gel clean-up. ND, not detected (see MDLs in Table 3). NQ, not quantified.

[a] Percentage recovery of surrogate standard.

waters at concentrations ranging from 0.2 to 2.6 ng/l (Snyder et al., 1999; Ternes et al., 1999). More data are therefore needed to determine the occurrence of 17β-estradiol and other PPCPs at lower concentrations in Louisiana and Ontario surface waters.

Ibuprofen, fluoxetine, triclosan, estrone and 17β-estradiol were not detectable in Mississippi River surface waters in our analyses. This observation is consistent with another study, which used multiple analytical techniques to determine PPCP target analytes (Barnes et al., 2002). Detectable but non-quantifiable levels of bisphenol A were found in several of our Mississippi River samples. In contrast, Barnes et al. (2002) were able to detect bisphenol A at a concentration of 60 ng/l in their analysis of Mississippi River surface waters. These contrasting results suggest a need to include bisphenol A as a target analyte in natural water samples.

4.2. Sewage treatment plant effluent

Results for samples collected from the effluent of the Louisiana sewage treatment plant (Table 5) indicate naproxen at concentrations of 81 and 106 ng/l. This sewage treatment plant discharges effluent into the Mississippi River and these naproxen concentrations are approximately 2.5-fold greater than naproxen detected in Mississippi River water. Other investigators (Ternes, 1998; Stumpf et al., 1999) have reported similar findings for naproxen in wastewater effluent, ranging from 20 to 520 ng/l. Results of this study also indicate triclosan in the Louisiana sewage treatment plant effluent at concentrations ranging from 10 to 21 ng/l. Triclosan is added as an antibacterial agent to detergents and it has been reported in sewage treatment plant effluents at concentrations up to 650 ng/l (Paxéus, 1996; Lindström et al., 2002). For this study, samples were collected prior to chlorination of the effluent at the sewage treatment plant. As such, results from this study do not necessarily indicate the quality of the final treated water as discharged into the Mississippi River. Results from this Tulane study also indicate that no other targeted PPCPs were detected in the effluent from the sewage treatment plant.

4.3. Drinking water treatment processes

Samples collected at the inlet of the drinking water treatment plants in Louisiana (JP1 in Table 6) and Ontario (WO1 in Table 7) contained naproxen at concentrations ranging from 63 to 65 ng/l. Samples collected at the precipitator of the Louisiana plant (JP2 in Table 6) exhibited naproxen concentrations of 63–68 ng/l, which indicates that the conventional treatment processes and 2-mg/l PAC addition do not remove naproxen from Mississippi River water. Adams et al. (2002) reported no significant removal of selected antibiotics with alum or ferric salt coagulation. Similarly, Ternes et al. (2002) reported no significant elimination of selected pharmaceuticals using iron chloride coagulation. Adams et al. (2002) also reported 25–50% removal of antibiotics from Missouri River water in batch experiments with a PAC dosage of 5 mg/l, and >90% removal for a PAC dosage of 50 mg/l. For the Louisiana drinking-water treatment plant, routine addition of 2 mg/l of PAC, which is used for the removal of natural organic matter in Mississippi River water, does not appear effective in reducing low-level concentrations of naproxen.

Samples collected after chlorination at the Louisiana drinking water treatment plant (JP3 in Table 6) exhibited non-detectable concentrations of naproxen and all other targeted compounds prior to discharge into the distribution system. A sample collected at the Ontario water plant following ozonation, conventional treatment and chlorination (WO2 in Table 7) exhibited non-detectable concentrations of all the target PPCP compounds. Samples collected from the Ontario pilot plant following conventional treatment plus dual media filtration (EN1 in Table 7) and ozonation (EN2 in Table 7) also exhibited non-detectable concentrations for all of the target PPCP compounds. Ternes et al. (2002) reported variable results in reducing concentrations of selected pharmaceuticals using ozone, and Adams et al. (2002) reported reduction of seven spiked (50 μg/l) antibiotics in distilled water and Missouri River water following laboratory chlorination and ozonation. Results from these studies and our results therefore indicate that oxidation (e.g. chlorination and ozonation) and sorption (dual media) processes may be effective treatments for reducing the concentration of naproxen that was observed in Mississippi River and Detroit River waters. Further research is needed to understand the removal processes and the possible formation of byproducts associated with these and other PPCP compounds.

Most of the water samples collected at the Louisiana and Ontario drinking-water treatment plants exhibited non-quantifiable but detectable concentrations of bisphenol A. These observations may be attributed to low-level contamination of the ultra-pure water used for sample preparation in the laboratory, or possible contamination in the plant (Krishnan et al., 1993). More data are therefore needed to determine if containers and/or chemical conveyor systems contribute to low-level bisphenol A contamination in drinking water treatment and distribution systems.

4.4. Application of method

The analytical method developed for this research is suitable for quantitative determination of nine functionally different PPCP compounds from diverse matrices. The method was successfully applied for the analysis of surface waters, wastewater effluent and treated water samples. Application of this method is limited to analysis of the targeted PPCP compounds only. Additional quantities of these compounds could be present in water samples, either in conjugated or other metabolic forms. Further method development would be required to include other chemical forms (e.g. breakdown products or disinfection byproducts) to the list of targeted compounds developed for this study.

Acknowledgments

The authors wish to acknowledge the generous support of the Center for Bioenvironmental Research at Tulane and Xavier Universities and the Tulane University School of Engineering. The authors are grateful to contributions provided by Wayne Koffskey of the Jefferson Parish Water

Treatment Plant, Ron Huffman of the Jefferson Parish Wastewater Treatment Plant, Saad Jasim and Samar Mazloum of the Windsor Utilities Commission and ENWIN Utilities. The authors also wish to acknowledge assistance by Richard Campanella of the Center for Bioenvironmental Research, contributions by Tulane undergraduate students Apryl Scott and Rhonda Hattar, and support from the Louisiana Alliance for Minority Participation at Tulane University.

References

Adams C, Wang Y, Loftin K, Meyer M. Removal of antibiotics from surface and distilled water in conventional water treatment processes. J Environ Eng 2002;128(3):253–260.

Argyrou ME, Bianchi TS, Lambert CD. Transport and fate of particulate and dissolved organic carbon in the Lake Pontchartrain estuary, Louisiana, USA. Biogeochemistry 1997;38:207–226.

Barber LB, Brown GK, Zaugg SD. Potential endocrine-disrupting organic chemicals in treated municipal wastewater and river water. In: Keith LH, editor. Analysis of environmental endocrine disruptors. ACS Symposium Series, vol. 747. Washington, DC: ACS, 2000. p. 97–123.

Barnes KK, Kolpin DW, Meyer MT, Thurman EM, Furlong ET, Zaugg SD, Barber LB. Water-quality data for pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000. Report 02-94. Iowa City, IA: US Geological Survey, 2002.

Boyd GR, Grimm DA. Occurrence of pharmaceutical contaminants and screening of treatment alternatives for southeastern Louisiana. In: McLachlan JA, Guillette LJ, Iguchi T, Toscano WA, editors. Environmental hormones, the scientific basis of endocrine disruption Ann NY Acad Sci, vol. 948 2001. p. 80–89.

Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 1999;107(Suppl. 6):907–938.

Desbrow C, Routledge EJ, Brighty CG, Sumpter JP, Waldock M. Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening. Environ Sci Technol 1998;32:1549–1558.

Flowers GC, Isphording WC. Environmental sedimentology of the Lake Pontchartrain estuary. Trans Gulf Coast Assoc Geol Soc 1990;11:237–250.

Guillette LH. Endocrine-disrupting environmental contaminants and developmental abnormalities in embryos. Hum Ecol Risk Assess 1995;1(2):25–36.

Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere 1998;36:357–393.

Huang C, Sedlak DL. Analysis of estrogenic hormones in municipal wastewater effluent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry. Environ Toxicol Chem 2001;20:133–139.

Johnson AC, Sumpter JP. Removal of endocrine-disrupting chemicals in activated sludge treatment works. Environ Sci Technol 2001;35:4697–4703.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ Sci Technol 2002;36:1202–1211.

Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 1993;132:2279–2286.

Laganá A, Bacaloni A, Fago G, Marino A. Trace analysis of estrogenic chemicals in sewage effluent using liquid chromatography combined with tandem mass spectrometry. Rapid Commun Mass Spectrom 2000;14:401–407.

Lindström A, Buerge IJ, Poiger T, Bergqvist P-A, Müller MD, Buser H-R. Occurrence and environmental behavior of the bactericide triclosan and its methyl derivative in surface waters and in wastewater. Environ Sci Technol 2002;36:2322–2329.

Meade RH. Contaminants in the Mississippi River, 1987–1992. Report No 1133. Denver, CO: US Geological Survey, 1996.

Möder M, Schrader S, Winkler M, Popp P. Solid-phase microextraction-gas chromatography-mass spectrometry of biologically active substances in water samples. J Chromatogr A 2000;873:95–116.

Paxéus N. Organic pollutants in the effluents of large wastewater treatment plants in Sweden. Water Res 1996;30:1115–1122.

Roefer P, Snyder S, Zegers RE, Rexing DJ, Fronk JL. Endocrine-disrupting chemicals in a source water. J AWWA 2000;92(8):52–58.

Sedlak DL, Gray JL, Pinkston KE. Understanding microcontaminants in recycled water. Environ Sci Technol 2000;34:508A–515A.

Snyder SA, Keith TL, Verbrugge DA, Snyder EM, Gross TS, Kannan K, Giesy JP. Analytical methods for detection of selected estrogenic compounds in aqueous mixtures. Environ Sci Technol 1999;33:2814–2820.

Stan HJ, Heberer T, Linkerhägner M. Vorkommen von Clofibrinsäure im aquatischen System-Führt die therapeutische Anwendung zu einer Belastung von Oberflächen-, Grundund Trinkwasser? Vom Wasser 1994;83:57–68.

Stumpf M, Ternes T, Haberer K, Seel P, Baumann W. Determination of pharmaceuticals in sewage plants and river water. Vom Wasser 1996;86:291–303.

Stumpf M, Ternes TA, Wilken RD, Rodrigues SV, Baumann W. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. Sci Total Environ 1999;225:135–141.

Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. Water Res 1998;32:3245–3260.

Ternes TA, Meisenheimer M, McDowell D, Sacher F, Brauch H-J, Haiste-Gulde B, Preuss G, Wilme U, Zulei-Seibert N. Removal of pharmaceuticals during drinking water treatment. Environ Sci Technol 2002;36:3855–3863.

Ternes TA, Stumpf M, Müller J, Haberer K, Wilken RD, Servos M. Behavior and occurrence of estrogens in municipal sewage treatment plants I. Investigations in Germany, Canada and Brazil. Sci Total Environ 1999;225:81–90.

Trussell RR. Endocrine disruptors and the water industry. J AWWA 2001;93(2):58–65.

World Health Organization. International Agency for Research of Cancer monographs on the evaluation of carcinogenic risks to humans, vol. 66. Lyon, France: IARC, 1996. p. 391–426.