

DISTRIBUTION OF ACIDIC AND NEUTRAL DRUGS IN SURFACE WATERS NEAR SEWAGE TREATMENT PLANTS IN THE LOWER GREAT LAKES, CANADA

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(Received 18 December 2002; Accepted 13 May 2003)

Abstract—Prescription and nonprescription drugs have been detected in rivers and streams in Europe and the United States. Sewage treatment plants (STPs) are an important source of these contaminants, but few data exist on the spatial distribution of drugs in surface waters near STPs. Samples of surface water were collected in the summer and fall of 2000 at open-water sites in the lower Great Lakes (Lake Ontario and Lake Erie), at sites near the two STPs for the city of Windsor (ON, Canada), and at sites in Hamilton Harbour (ON, Canada), an embayment of western Lake Ontario that receives discharges from several STPs. In a follow-up study in the summer of 2002, samples of surface water and final effluent from adjacent STPs were collected from sites in Hamilton Harbour and Windsor. In addition, surface water and STP effluent samples were collected in Peterborough (ON, Canada). All samples of surface water and STP effluents were analyzed for selected acidic and neutral drugs. In the survey of Hamilton Harbour and Windsor conducted in 2000, acidic drugs and the antiepileptic drug carbamazepine were detected at ng/L concentrations at sites that were up to 500 m away from the STP, but the hydrological conditions of the receiving waters strongly influenced the spatial distribution of these compounds. Drugs were not detected at open-water locations in western Lake Erie or in the Niagara River near the municipality of Niagara-on-the-Lake (ON, Canada). However, clofibric acid, ketoprofen, fenoprofen, and carbamazepine were detected in samples collected in the summer of 2000 at sites in Lake Ontario and at a site in the Niagara River (Fort Erie, ON, Canada) that were relatively remote from STP discharges. Follow-up studies in the summer of 2002 indicated that concentrations of acidic and neutral drugs in surface waters near the point of sewage discharge into the Little River (ON, Canada) STP were approximately equal to the concentrations in the final effluent from the STP. Caffeine and cotinine, a metabolite of nicotine, were generally present in STP effluents and surface waters contaminated by drugs. The antidepressant fluoxetine and the antibiotic trimethoprom were also detected in most STP effluents and some surface water samples. For the first time, the lipid regulating drug atorvastatin was detected in samples of STP effluent and surface water.

Keywords—Pharmaceuticals Sewage Carbamazepine Fluoxetine Atorvastatin

INTRODUCTION

Prescription and nonprescription drugs are used extensively to treat diseases in both humans and domestic animals. Prescription pharmaceutical drugs are produced and used by human populations in quantities that exceed hundreds of tons [1], and quantities of nonprescription drugs probably exceed this total by an order of magnitude [2]. According to Halling-Sorensen et al. [2], 30 to 90% of the dose of a drug administered to humans or animals is excreted in urine and feces as an active substance. In urban environments, these compounds make their way in sewage to municipal sewage treatment plants (STPs), where the potential exists for discharge in effluents or application onto agricultural land with biosolids as soil amendment. In rural environments, drugs used by humans or administered to livestock and poultry have the potential to enter the aquatic environment through runoff into surface water or through contamination of groundwater. Whether these agents have the potential to impact humans or aquatic organisms is currently an area of investigation. However, contamination by pharmaceuticals is an emerging environmental issue that may require legislative intervention.

Much of the recent literature on the concentrations of drugs in STP effluents and surface waters is based on European studies [1–7]. However, we surveyed effluents from STPs in 14 Canadian cities and detected several analgesic/anti-inflam-

matory drugs (ibuprofen and naproxen) and the antiepileptic drug carbamazepine at $\mu\text{g/L}$ concentrations [8]. We also detected the lipid-regulating drugs bezafibrate and gemfibrozil in some effluent samples [8]. In Germany, prescription and nonprescription drugs have been identified in rivers and streams near discharges from STPs [1–7]. Persistent compounds, such as the metabolite of the lipid-regulating drug clofibrate (clofibric acid) and the analgesic diclofenac, have been detected in Europe in surface waters far from STP sources [9,10]. In the United States, a variety of prescription and nonprescription drugs were detected in 139 streams surveyed across 30 states [11]. However, these residues in surface water could have originated from a variety of sources, including domestic sewage and runoff from intensive agricultural operations.

Currently, no data exist describing the spatial distribution of drugs near STP sources. Thus, it is not clear whether contamination is localized to areas a few meters from STP discharges or whether these compounds are distributed widely in surface waters, potentially contaminating sources of drinking water or impacting aquatic ecosystems. In addition, knowledge of the distribution of drugs near STPs is required to assess exposures of aquatic organisms and humans to drugs for environmental risk assessment purposes. Currently, the U.S. Food and Drug Administration requires environmental risk assessments of new pharmaceuticals if predicted introduction concentrations are estimated to be greater than $1 \mu\text{g/L}$ [12]. To estimate a predicted introduction concentration, a default

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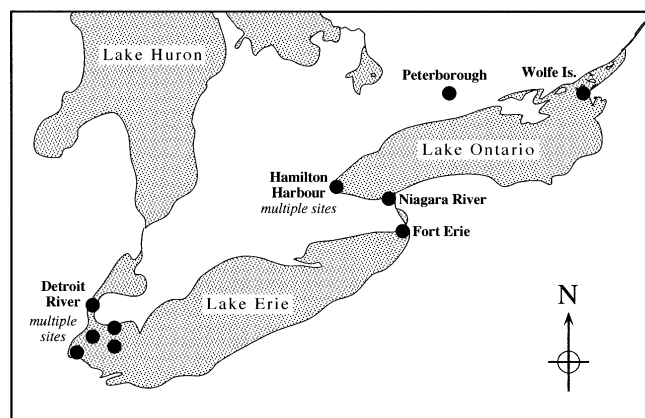


Fig. 1. Locations in the lower Great Lakes region of Canada sampled for acidic and neutral drugs. Sites in Hamilton Harbour (ON, Canada) and the Detroit River (Canada) were sampled in both 2000 and 2002. Note that the Peterborough location was sampled only in 2002.

dilution factor can be applied to predict the concentrations of drugs in surface waters from estimated concentrations in STP effluents. This approach is thought to be appropriate since 77% of permitted effluent discharges in the United States receive greater than 10-fold dilution at annual mean flow rates [13].

Our study objective was to evaluate the concentrations and distribution of selected acidic and neutral drugs in surface waters near STPs. The lower Great Lakes region, including Lake Ontario, Lake Erie, and the rivers that connect these lakes (Niagara River, Canada; Detroit River, Canada), were selected as the study area since this area is the most densely populated region of Canada and large numbers of municipalities discharge treated sewage into these surface waters. In the summer and fall of 2000, only surface waters were sampled, but in a follow-up study in the summer of 2002, final effluents from STPs discharging into these locations were also sampled. In the initial survey, extracts prepared from samples of surface water were analyzed for a range of acidic drugs and only one neutral drug, carbamazepine. In the subsequent survey, the number of acidic and neutral drug analytes in surface water and effluent samples was expanded. Concentrations of drugs were compared to two marker compounds for human excretion: caffeine and the metabolite of nicotine, cotinine.

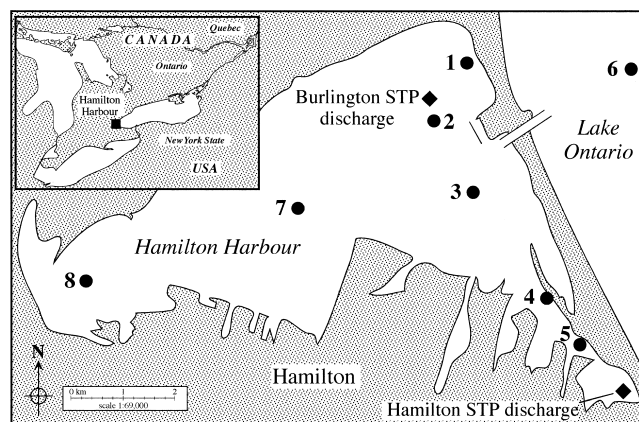


Fig. 2. Sites in Hamilton Harbour (ON, Canada) sampled for acidic drugs and carbamazepine in the summer (July) and fall (October) of 2000. Site 2 was also sampled in the summer of 2002. The diamond symbols indicate the locations of the points of discharge of the sewage treatment plants for the cities of Burlington and Hamilton (Canada). Note that station 6 was located outside the harbor in western Lake Ontario.

MATERIALS AND METHODS

Sample collection

In the initial survey of surface waters in 2000, grab samples (4 L) were collected by hand at the water surface. No replicates were analyzed for these samples. Samples were collected from several sites in Lake Ontario, western Lake Erie, and the Niagara River and Detroit River (Fig. 1). Samples were collected in July and October 2000 from eight sites in the Hamilton Harbour region, at two sites in the Niagara River (Niagara-on-the-Lake and Fort Erie), and at a site in eastern Lake Ontario (Wolfe Island). In Hamilton Harbour, sample sites were distributed in north-south and east-west transects within the harbor, and samples were also collected at a site located nearby in western Lake Ontario (Fig. 2). Hamilton Harbour is a highly urbanized and industrialized embayment of western Lake Ontario that receives discharges at its eastern end from STPs for the cities of Burlington and Hamilton (Fig. 2) and may be influenced by STPs for two other smaller municipalities (Dundas, Waterdown). The operational data for the Burlington and Hamilton STPs are summarized in Table 1.

In August 2000, surface water samples were collected at

Table 1. Operational parameters for the sewage treatment plants (STPs) in Ontario (Canada) impacting the surface water sites sampled during the 2000 survey. During the 2002 survey, effluent samples were collected from the STPs indicated (*) and adjacent surface waters

Location	Population served	Primary treatment	Secondary treatment	Retention time (h)		Design flow (m ³ /d)	Disinfection method	Discharge
				Hydraulic	Solids			
*Little River (Windsor, ON, Canada)	79,000	+	Activated sludge	15–22	6–10	73 × 10 ³	Ultraviolet, seasonal	Direct into Little River
*West Windsor (Windsor, ON, Canada)	123,000	+	Clarifiers only	8–12	—	155 × 10 ³	Chlorine, seasonal	Direct into Detroit River (Canada)
*Burlington (Skyway) (ON, Canada)	120,000	+	Activated sludge	12–20	4–8	93 × 10 ³	Chlorine, seasonal	Diffuser in Hamilton Harbour (ON, Canada)
Hamilton (ON, Canada)	300,000	+	Activated sludge	10–15	2–4	409 × 10 ³	Chlorine, all year	Diffuser in Hamilton Harbour
Peterborough (ON, Canada)	63,000	+	Activated sludge	12–18	4–6	55 × 10 ³	Chlorine, seasonal	Diffuser in Otonabee River (ON, Canada)

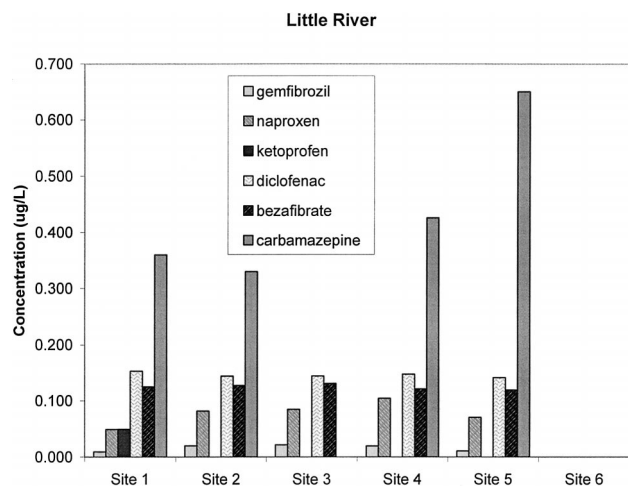


Fig. 3. Concentrations ($\mu\text{g/L}$) of acidic drugs and carbamazepine in samples collected in the summer of 2000 at a point adjacent to the sewage treatment plant (STP) of Little River (Windsor, ON, Canada; site 1) and at five sites (sites 2–6) in the Little River at 100-m intervals downstream of the STP. Note that site 6 is located at the confluence of the Little River with the Detroit River (Canada). Samples were not analyzed as replicates.

four sites in western Lake Erie (Fig. 1). Samples were also collected in August at sites downstream of two STPs in the city of Windsor (ON, Canada). At the Little River STP and the West Windsor (Windsor, ON, Canada) STP (Fig. 3), samples were collected at six sites at 100-m intervals downstream of the points of discharge. The former STP discharges effluents into the Little River, which is a small channel that empties into the Detroit River approximately 600 m downstream of the STP discharge. The operational data for the Little River and West Windsor STPs in Windsor are summarized in Table 1.

In June and July 2002, grab samples of surface water were collected from Hamilton Harbour, the Detroit River, and the Little River (Windsor) at sites immediately adjacent to discharges from the Burlington STP, West Windsor STP, and Little River STP, respectively. On the same day, grab samples were also collected from the final effluents of these STPs. During this survey, a grab sample of final effluent was also collected from the STP for the municipality of Peterborough (ON, Canada; Fig. 1), and a grab sample of surface water was collected from the Otonabee River (ON, Canada) immediately adjacent to the point of discharge of the Peterborough STP. The parameters for the operation of the Peterborough STP are summarized in Table 1. In all samples collected in 2002, triplicate analyses were conducted.

Grab samples of surface water and STP effluent were placed in solvent-washed, glass sample containers. Samples were immediately transported unrefrigerated to the laboratory and within 1 h of collection and were stored in the dark under refrigerated conditions. Within 24 h of collection, samples were split into aliquots and extracted.

Sample preparation

Samples analyzed for acidic drugs were extracted and prepared for analysis as described previously [8,14]. Briefly, samples were filtered and then extracted with preconditioned solid-phase extraction (SPE) cartridges consisting of 0.5 g of LiChrolut 100 RP-18 (40–63 μm) solid-phase material (Supelco, Toronto, ON, Canada) manually packed into 6-ml polypropylene cartridges (Supelco). Samples of water (2 L) were

filtered through prewashed glass-fiber filters (1.0- μm nominal mesh). The pH of the samples was then adjusted to 2.0 with 3.5 M H_2SO_4 . Samples were aspirated through SPE cartridges at a rate of approximately 10 ml/min. After extraction of the samples, the cartridges were dried under vacuum for less than 1 min and then extracted by eluting with three successive 3-ml aliquots of methanol. Each aliquot of methanol was eluted through the cartridge for a minimum of 10 min. During the follow-up survey in 2002, separate samples of surface water and STP final effluent were prepared for analysis of the lipid-regulating drug atorvastatin. As previously described [15], after sample filtration, the pH of the sample was adjusted to 4.0, and the samples were aspirated through an HLB Oasis[®] SPE cartridge (Waters, Manchester, UK) at a rate of approximately 10 ml/min. The sample bottles were rinsed with 10 ml of pH 4.0 distilled water, and the rinses were passed through the cartridges. The cartridges were then dried under vacuum for less than 1 min and then eluted with three successive 3-ml aliquots of methanol.

During the initial survey, samples were filtered and extracted for neutral drugs with preconditioned Supelclean LC-18 SPE cartridges (Supelco), as described previously [8]. Samples of water (2 L) were filtered through 1.0- μm nominal mesh glass-fiber filters, and the pH of the sample was adjusted to 7.5 with 1 M NaOH. Samples were aspirated through the SPE cartridges at a rate of approximately 20 ml/min. After passage of the sample through the cartridge and aspiration to partial dryness, the SPE cartridges were dried for 1 h under a stream of nitrogen and were then extracted by eluting with four successive 1-ml aliquots of methanol. In the follow-up study, samples were extracted for neutral drugs using HLB Oasis SPE cartridges. The cartridges were preconditioned with 6 ml acetone, 6 ml methanol, and 6 ml high-performance liquid chromatography (HPLC)-grade water adjusted to pH 7.5. Prior to extraction, the samples were filtered as described previously, and the pH was adjusted to 7.5 with 1.0 M NaOH. The samples were then passed through the cartridges at a flow rate of approximately 10 ml/min. The sample bottles were rinsed with 10 ml of pH 7.5 distilled water, and the rinses were passed through the cartridges. The cartridges were then dried under vacuum for less than 1 min and then eluted with three successive 3-ml aliquots of methanol.

During the initial survey in 2000, extracts prepared for acidic drugs were methylated with methanol in the presence of barium trifluoride (BF_3) reagent as described previously [8] in preparation for analysis by gas chromatography/mass spectrometry (GC-MS). Meclofenamic acid (100 μl of 10 $\mu\text{g/ml}$ stock in methanol) was added as an internal standard to the extracts before methylation. After methylation, the sample was evaporated and solvent exchanged into isoctane. As explained previously [8], acetylsalicylic acid and indomethacin degrade during this methylation procedure and could not be analyzed in this survey. In the follow-up survey in 2002, the extracts prepared for acidic drugs were not derivatized, and they were analyzed directly by liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS).

Analysis

Table 2 summarizes the acidic drug analytes in both the initial and the follow-up surveys conducted in 2000 and 2002. Derivatized samples prepared during the initial survey (2000) were analyzed for acidic drugs by gas chromatography-mass spectrometry in selected-ion mode (GC-MS-SIM) as described

Table 2. Acidic and neutral drugs analyzed in surveys of surface water and sewage treatment plant (STP) effluents conducted in the summer and fall of 2000 and in the summer of 2002

Compound	Use	2000 ^a	2002 ^b
Atorvastatin	Lipid regulator		✓
Gemfibrozil	Lipid regulator	✓	✓
Clofibric acid	Lipid regulator (active metabolite)	✓	✓
Bezafibrate	Lipid regulator	✓	✓
Ibuprofen	Analgesic/anti-inflammatory	✓	✓
Fenoprofen	Analgesic/anti-inflammatory	✓	✓
Ketoprofen	Analgesic/anti-inflammatory	✓	✓
Diclofenac	Analgesic/anti-inflammatory	✓	✓
Naproxen	Analgesic/anti-inflammatory	✓	✓
Indomethacin	Analgesic/anti-inflammatory	✓	✓
Caffeine	Marker compound		✓
Cotinine	Marker compound		✓
Carbamazepine	Antiepileptic, psychiatric drug	✓	✓
Fluoxetine	Antidepressant		✓
Norfluoxetine	Metabolite of fluoxetine		✓
Trimethoprim	Antibiotic		✓
Pentoxifylline	Vasodilator		✓
Cyclophosphamide	Antineoplastic		✓

^a Only surface water sample.^b Surface water and STP final effluent sampled.

previously [8]. Analysis was conducted with a Hewlett-Packard Model 5890 Series II gas chromatograph (Avondale, PA, USA) equipped with a 30-m DB-5 MS column and a Model 5971A mass selective detector. Selected ions were monitored at m/z 152, 120 for salicylic acid, m/z 139, m/z 130, 128 for clofibric acid, m/z 143, 83 for gemfibrozil, m/z 221, 120 for bezafibrate, m/z 161, 119 for ibuprofen, m/z 197, 103 for fenoprofen, m/z 209, 105 for ketoprofen, m/z 214, 107 for diclofenac, m/z 185, 141 for naproxen, and m/z 242, 178 for meclofenamic acid (internal standard).

In the follow-up survey, acidic drugs were analyzed directly by LC-ESI-MS/MS, as described by Miao et al. [14]. All analyses were carried out with a Waters 2690 HPLC coupled with a Micromass Quattro LC-ESI-MS/MS operated in negative-ion mode with selected reaction monitoring (SRM). Ions were monitored at m/z 213 > 127 for clofibric acid, 249 > 121 for gemfibrozil, 360 > 274 for bezafibrate, 205 > 161 for ibuprofen, 241 > 197 for fenoprofen, 253 > 209 for ketoprofen, 294 > 250 for diclofenac, 229 > 170 for naproxen, and 356 > 312 for indomethacin.

The lipid-regulating drug atorvastatin was analyzed separately by microbore LC-ESI-MS/MS, as described previously [15]. Briefly, chromatographic separation was achieved using a Waters microbore octadecylsilane column (YMC ODS-AQ; 100 × 1.0-mm i.d., 3- μ m particle size, 120 Å). Analyses were carried out with a micromass LC-ESI-MS/MS operated in positive-ion mode with SRM set at m/z 559 > 440.

Neutral drugs were analyzed with the same Micromass LC-ESI-MS/MS system operated in positive-ion mode. In the initial survey (2000), carbamazepine was the only neutral drug analyzed, but in the follow-up survey (2002), the number of analytes was increased to include seven more neutral compounds (Table 2). Prior to analysis, dihydrocarbamazepine was added as an internal standard. Analytes were separated chromatographically with a Genesis C18 column (150 × 2.1-mm i.d., 4- μ m particle size) purchased from Jones Chromatography (Hengoed, UK). The mobile phase used in the chromatographic separation consisted of a binary mixture of solvent A

(acetonitrile) and solvent B (20 mM ammonium acetate and 0.1% formic acid) at a flow rate of 0.2 ml/min. The gradient was operated from 20 to 100% eluent A for 8 min, then held at 100% eluent A for 5 min. Methanol was used as the washing solution for the autosampler. The solvents were degassed by an in-line degasser. A volume of 20 μ l was injected, and the LC effluent was directed to the MS without splitting.

For LC-ESI-MS/MS analyses of neutral drugs, instrument control, data acquisition, and evaluation were done with Masslynx NT software (Ver 3.4, Waters). The capillary and cone voltages were operated at 3.0 kV and 30 V, respectively. The temperatures of the electrospray source and nebulizing gas were 80 and 300°C, respectively. Nitrogen was used as both nebulizing gas and desolvation gas at flow rates of 70 and 600 L/h, respectively. Ultra-high-purity argon at a pressure of 2.0×10^{-3} mbar was used as collision gas in the hexapole collision cell. Selected reaction monitoring was used to monitor the $[M+H]^+$ ion and product ions. In the initial survey, the ions monitored and collision energies used to generate product ions (in parentheses) were m/z 237 > 194 (20 eV) for carbamazepine and m/z 239 > 194 (35 eV) for the internal standard, dihydrocarbamazepine. In the follow-up survey, these ions were monitored, and additional ions were monitored in positive-ion mode for SRM channels at m/z 195 > 138 (25 eV) for caffeine, 177 > 80 (25 eV) for cotinine, 261 > 140 (25 eV) for cyclophosphamide, 310 > 148 (15 eV) for fluoxetine, 296 > 134 (11 eV) for norfluoxetine, 279 > 181 (30 eV) for pentoxifylline, and 291 > 123 (28 eV) for trimethoprim.

Quantitation

Coextractives in water and effluent samples cause matrix-induced effects that influence the sensitivity of LC-ESI-MS/MS detection of drugs in sample extracts. Therefore, calibration standards dissolved directly in solvents are not appropriate for quantifying drugs. Samples were quantified against calibration curves prepared by analysis of standards of neutral or acidic drugs spiked into samples of final effluent or surface water, as appropriate. In the initial survey (2000), the standards were spiked into surface water samples collected in the Detroit River at site 5 downstream of the West Windsor STP. It is recognized that this method has limitations because different samples could vary in their matrix effects. Therefore, in the follow-up survey (2002), a calibration curve was prepared for each individual sample by spiking the sample with different dilutions of drugs.

Aliquots of the STP effluent or surface water samples were spiked with various amounts of stock solutions of drug analytes to yield final concentrations of 0 (blank), 10, 50, 100, 500, and 1000 ng/L. These samples were extracted and analyzed using the methods described previously to give a calibration curve over the range of concentrations of drugs detected in water. Regressions were calculated for these calibration curves in which correlation coefficients varied between 0.77 and 0.94. Detection limits were estimated as the second-lowest point on the linear portion of the calibration curves. Detection limits for drugs analyzed in the 2000 survey are listed in Table 3. The detection limits for drugs analyzed in the 2002 survey were specific to the sample type (STP, surface water) and to the sampling site. However, limits of detection varied between 1 and 10 ng/L in surface water samples and 5 and 20 ng/L in STP effluents. The detection limits were defined as the lowest concentration of an analyte that yielded an ion signal with a signal-to-noise ratio of 3:1 in the sample matrix.

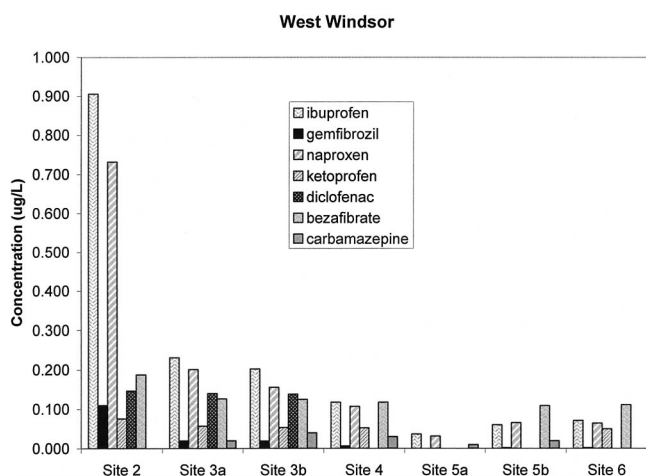


Fig. 4. Concentrations ($\mu\text{g/L}$) of acidic drugs and carbamazepine in samples collected in the summer of 2000 at a site 100 m from the discharge of the sewage treatment plant (STP) of West Windsor (ON, Canada; site 2) and at four sites (sites 3–6) in the Detroit River (Canada) at 100-m intervals downstream of the STP. Note that there are replicate samples (a and b) at sites 3 and 5.

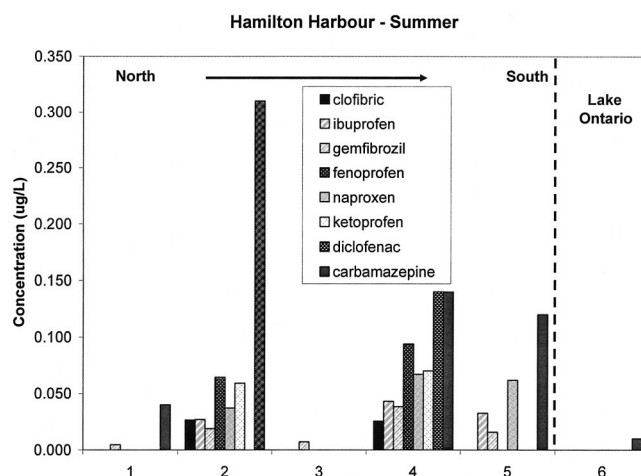


Fig. 5. Concentrations ($\mu\text{g/L}$) of acidic drugs and carbamazepine in samples collected in the summer of 2000 in Hamilton Harbour (ON, Canada) on a north-south transect (stations 1–5). Station 6 was located in western Lake Ontario (Canada) outside the harbor. Data for stations 7 and 8 along an east-west transect are not included in this figure. Samples were not analyzed as replicates.

RESULTS AND DISCUSSION

In samples collected in August 2000 at a site immediately adjacent to the Little River STP discharge in Windsor (site 1), several drugs were detected at concentrations between 0.01 and 0.35 $\mu\text{g/L}$, including carbamazepine, naproxen, diclofenac, bezafibrate, gemfibrozil, and ketoprofen (Fig. 3). Except for ketoprofen, concentrations of these drugs remained relatively constant at sites 2 through 5, located at 100-m intervals downstream of the STP. The drug present in the highest concentrations in the Little River was the antiepileptic carbamazepine, at concentration as high as 0.65 $\mu\text{g/L}$ in a grab sample collected 400 m from the point of STP discharge (site 5). The Little River is a small tributary that discharges into the Detroit River approximately 500 m downstream of the STP (site 6), where concentrations of all drugs fell to below detection limits (Fig. 3). High concentrations of drugs in the Little River system are a result of the high contribution of sewage effluents to the total flow of this small river. Ternes [1] detected acidic drugs at comparable concentrations in the River Mainz in Germany, a small river that receives direct discharges from an STP.

In samples collected in August 2000 near the West Windsor STP (Fig. 4), ibuprofen, ketoprofen, naproxen, diclofenac, gemfibrozil, bezafibrate, and carbamazepine were detected in

a sample collected in the Detroit River 100 m downstream of the effluent discharge (site 2). No data were available at site 1, immediately adjacent to the STP discharge. Within 400 m of the STP discharge (site 5), the high flow of the Detroit River quickly diluted the drugs to concentrations near or below detection limits. It is interesting to note that ibuprofen was detected at a relatively high concentration in the Detroit River downstream of the West Windsor STP (Fig. 4), but this non-prescription drug was not detected downstream of the Little River STP (Fig. 3) despite these STPs being located in the same city. Similar concentrations of gemfibrozil and ibuprofen have been detected in streams in the United States [11].

Drugs were detected at concentrations up to 0.31 $\mu\text{g/L}$ in surface waters collected in July 2000 (Fig. 5) and in October 2000 at sites within Hamilton Harbour. Samples collected along a north-south transect in the Harbour showed the influence of the discharges from the STPs for the cities of Burlington and Hamilton. Carbamazepine was the most prevalent drug in samples collected from Hamilton Harbour in both summer (Fig. 5) and fall. At sampling stations 7 and 8 in the western part of in Hamilton Harbour, located >1,000 m from the Burlington and Hamilton STP outflows, some acidic drugs (ibuprofen, ketoprofen, gemfibrozil, clofibric acid, naproxen)

Table 3. Number of sampling sites (n), and the maximum and median concentrations ($\mu\text{g/L}$) of acidic drugs and carbamazepine in surface water samples collected during the 2000 survey in the regions of the Detroit River (Canada) and Hamilton Harbour (ON, Canada) and at other sites in eastern Lake Ontario (Wolfe Island), western Lake Ontario outside Hamilton Harbour (station 6), the Niagara River (Niagara-on-the-Lake, Fort Erie), and western Lake Erie. See Figure 1 for site locations. No. ND refers to the number of sites where drugs were not detected in samples

Drug	Detection limit (ng/L)	n	Detroit River				Hamilton Harbour				Other sites			
			Max.	Median	No. ND	n	Max.	Median	No. ND	n	Max.	Median	No. ND	
Clofibric acid	5	13	0.175	0.059	7	14	0.101	0.077	10	17	0.015	0.015	15	
Ibuprofen	5	13	0.790	0.141	8	14	0.093	0.064	12	17	—	—	17	
Gemfibrozil	5	13	0.112	0.066	7	14	0.067	0.012	8	17	—	—	17	
Fenoprofen	5	13	—	—	13	14	0.064	0.045	12	17	0.059	0.059	16	
Naproxen	5	13	0.551	0.207	4	14	0.139	0.094	11	17	—	—	17	
Ketoprofen	5	13	0.017	0.012	11	14	0.047	0.031	11	17	0.050	0.050	16	
Diclofenac	5	13	0.042	0.026	11	14	0.194	0.194	13	17	—	—	17	
Bezafibrate	10	13	0.200	0.052	3	14	—	—	14	17	—	—	17	
Carbamazepine	1	11	0.650	0.185	3	14	0.310	0.120	5	15	0.020	0.020	14	

Table 4. Means and standard deviations about the mean (in parentheses) of concentrations (μL) from three replicate analyses of neutral and acidic drugs in sewage treatment plant (STP) effluents conducted during the 2002 survey. ND = not detected

Compound	Peterborough (ON, Canada) STP	Burlington (ON, Canada) STP	Little River (Windsor, ON, Canada) STP	West Windsor (Windsor, ON, Canada) STP
Caffeine	0.022 (± 0.001)	0.022 (± 0.001)	0.028 (± 0.002)	0.677 (± 0.027)
Carbamazepine	0.126 (± 0.003)	0.064 (± 0.005)	0.112 (± 0.005)	0.007 (± 0.001)
Cotinine	0.039 (± 0.001)	ND	0.022 (± 0.001)	0.058 (± 0.003)
Cyclophosphamide	0.004 (± 0.001)	ND	0.008 (± 0.001)	ND
Fluoxetine	0.050 (± 0.005)	0.038 (± 0.003)	0.099 (± 0.007)	ND
Norfluoxetine	ND	ND	ND	ND
Pentoxifylline	0.007 (± 0.001)	0.011 (± 0.001)	0.011 (± 0.001)	0.005 (± 0.001)
Trimethoprim	0.071 (± 0.005)	0.084 (± 0.008)	0.194 (± 0.006)	0.009 (± 0.001)
Bezafibrate	0.259 (± 0.013)	0.012 (± 0.001)	0.082 (± 0.003)	0.065 (± 0.003)
Clofibric acid	0.044 (± 0.002)	0.002 (± 0.001)	ND	ND
Gemfibrozil	1.493 (± 0.111)	0.005 (± 0.001)	0.012 (± 0.001)	0.043 (± 0.003)
Diclofenac	0.359 (± 0.026)	0.005 (± 0.001)	0.088 (± 0.007)	0.063 (± 0.005)
Fenoprofen	0.405 (± 0.038)	0.062 (± 0.004)	0.075 (± 0.002)	ND
Ketoprofen	ND	0.013 (± 0.001)	ND	ND
Ibuprofen	1.885 (± 0.166)	0.094 (± 0.007)	0.079 (± 0.002)	0.167 (± 0.007)
Indomethacin	0.378 (± 0.015)	0.010 (± 0.001)	0.021 (± 0.002)	ND
Naproxen	0.524 (± 0.524)	0.041 (± 0.001)	0.021 (± 0.002)	0.168 (± 0.005)
Atorvastatin	0.022 (± 0.001)	0.044 (± 0.002)	0.019 (± 0.002)	ND

were detected at concentrations below 10 ng/L. Carbamazepine was detected in the summer at Hamilton Harbour stations 7 and 8 at concentrations of 0.17 ng/L and 0.14 $\mu\text{g/L}$, respectively. It cannot be ruled out that these sites in western Hamilton Harbour were impacted by sewage discharges from small municipalities that surround the western part of the harbor. Unlike an earlier survey of acidic drugs in STPs in Canada where clofibric acid was not detected in effluents [8], this metabolite of the lipid regulator clofibrate was detected at low concentrations in some surface water samples from Hamilton Harbour. Concentrations of all acidic drugs were below detection limits at a site in western Lake Ontario (station 6), which is located a few hundred meters from the shipping channel that connects Hamilton Harbour to the lake. However, carbamazepine was detected at a concentration of 0.02 $\mu\text{g/L}$ at this Lake Ontario site in the summer of 2000 (Fig. 5).

When the data on concentrations of acidic drugs and carbamazepine are organized according to sampling location (Table 3), it becomes obvious that drugs were generally present only in surface water samples collected at sites near STPs for the city of Windsor and at sites in Hamilton Harbour. In surface water samples collected at a site in the Niagara River near the municipality of Niagara-on-the-Lake and at four sites in western Lake Erie, concentrations of neutral and acidic drugs were below the limits of detection. As mentioned previously, carbamazepine was detected in surface water sampled outside Hamilton Harbour in western Lake Ontario (site 6). Clofibric acid was detected in the Niagara River near the municipality of Fort Erie and at Wolfe Island in eastern Lake Ontario in summer samples at concentrations of 0.009 and 0.015 $\mu\text{g/L}$, respectively (Table 3). Fenoprofen was also detected in the summer at Fort Erie at a concentration of 0.059 $\mu\text{g/L}$, and ketoprofen was detected in the summer at Wolfe Island at a concentration of 0.050 $\mu\text{g/L}$ (Table 3). Pharmaceuticals may have been detected at Fort Erie because of discharges from the STP for Buffalo (NY, USA), which is located on the opposite side of the river from the sampling location. No obvious explanation exists for why clofibric acid and ketoprofen were detected in samples collected at Wolfe Island since no STPs

are in the vicinity. More sampling is required to confirm these findings.

The concentrations of acidic and neutral drugs in STP effluents and adjacent surface waters from the 2002 survey are summarized in Tables 4 and 5, respectively. The concentrations of acidic drugs in STP final effluents were generally consistent with concentrations that we previously reported for these drugs in the effluents from 14 Canadian STPs [8]. However, unlike the previous survey [8], clofibric acid and diclofenac were detected at low concentrations in two of four and four of four STP effluents, respectively. This may reflect greater sensitivity with the LC-ESI-MS-MS analytical technique in comparison to the earlier GC-MS analyses of methylated analytes. Indomethacin was also detected at concentrations as high as 0.378 $\mu\text{g/L}$ (Peterborough STP). It was not possible to analyze this nonprescription analgesic in our earlier studies because of degradation during the methylation step prior to GC-MS analysis. All acidic drug analytes, except ketoprofen, were detected in the effluents from a least two of the four STPs surveyed. Only ibuprofen and gemfibrozil were detected in STP effluents at concentrations that exceeded 1 $\mu\text{g/L}$, and these higher concentrations were observed only in the effluent of the Peterborough STP (Table 4).

Among the data for acidic drugs in surface waters near the STPs (Table 5), most drugs were present in samples collected from the Little River (Windsor) site and the Hamilton Harbour (station 2) site. Figure 6 illustrates total ion chromatograms for acidic drugs in the spiked sample matrix and in the sample (unspiked) of a surface water sample collected in the Little River. Concentrations of acidic drugs were all below detection limits in the samples from the Detroit River and the Otonabee River. These data reflect the high degree of dilution that occurs in the Detroit River and Otonabee River hydrologic systems.

Concentrations of the neutral drug carbamazepine in STP effluents (Table 4) were consistent with concentrations that we previously reported for Canadian STP effluents [8]. We also reported recently that several metabolites of carbamazepine are present in STP effluents [16]. Unlike our previous study of STP effluents [8], cyclophosphamide was detected at low

concentrations (<0.01 µg/L) in the final effluents from two of four STPs (Table 4). The trends for neutral drugs in surface water samples were consistent with the data for acidic drugs; that is, they were not detected in surface water samples from the high-flow areas of the Detroit River and the Otonabee River (Table 5). However, carbamazepine was detected in all surface water samples regardless of the hydrologic system.

The data set for the 2002 survey includes several drugs that have not been routinely reported in other studies of STP effluents. Webb [17] recently identified fluoxetine as having potential for effects in the aquatic environment because it is a highly prescribed antidepressant. This compound was detected in final effluents from three of the four STPs surveyed at concentrations as high as 0.099 µg/L (Table 4) and was detected at the surface water sampling sites in Hamilton Harbour and Little River (Table 5). Kolpin et al. [11] detected fluoxetine at a concentration of 0.012 µg/L in one sample during a survey of streams in the United States. Even though fluoxetine is rapidly metabolized to the active compound norfluoxetine and is primarily excreted in this form [18], norfluoxetine was not detected in either STP effluents (Table 4) or in surface water (Table 5). Future studies should investigate whether norfluoxetine is present in a conjugated form in STP effluents. The neutral antibiotic trimethoprim was also detected in all STP effluents (Table 4) and in surface water samples from Hamilton Harbour and the Little River (Table 5). The maximum trimethoprim concentration of 0.134 µg/L in the Little River sample is below the maximum concentration of 0.71 µg/L reported in a survey of U.S. streams [11].

Although caffeine is a widely used stimulant that is present in high concentrations in coffee and other beverages and foods [19], the concentrations detected in STP effluents did not exceed 1 µg/L (Table 4). Only about 3% of ingested caffeine is excreted unmetabolized in the urine [20], and STP treatment may remove a high proportion of the remainder. However, caffeine was present in all surface water samples near STPs (Table 5) and so may be a good marker of discharges of domestic effluents into receiving waters. Cotinine is the major metabolite of nicotine and is a marker of smoking status and exposure to tobacco smoke [21]. This compound was present in the effluents of three of four STPs and associated surface

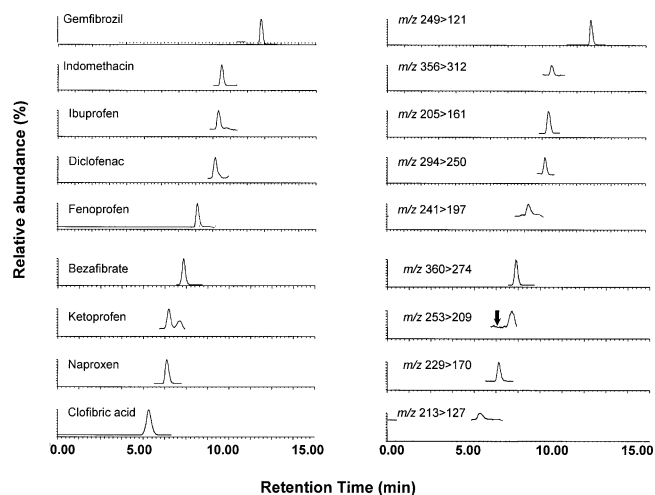


Fig. 6. Selected reaction monitoring (SRM) chromatograms generated by liquid chromatography tandem mass spectrometry analysis of acidic drugs in the spiked sample matrix (left panels) and the sample of surface water (right panels) collected from the Little River (Windsor, ON, Canada) in 2002 downstream from the Little River sewage treatment plant. The ions monitored in SRM are shown in the right panel. In relation to the chromatogram for ketoprofen, an interference peak at 7.11 min was observed with channel m/z 253>209. The concentration of ketoprofen was below detection limits in the surface water sample, as indicated by the arrow in the right panel.

waters (Table 4), so this compound may also be a good marker of effluent discharges. In comparison to previously published data on the maximum concentrations of these compounds in streams in the United States [11], concentrations of caffeine detected in surface waters in this study were two orders of magnitude lower, and concentrations of cotinine were one order of magnitude lower (Table 5).

The lipid-regulating agent atorvastatin is one of the most commonly prescribed drugs in the United States [22], and it is the most highly prescribed drug for control of blood lipids in Canada [23]. This compound was detected at concentrations that were below 0.05 µg/L in the effluents from three of the four STPs sampled (Table 4) and was also detected in surface waters from Hamilton Harbour and Little River (Table 5).

Table 5. Means and standard deviations about the mean (in parentheses) of concentrations (µg/L) from three replicate analyses of neutral and acidic drugs in surface waters adjacent to discharges of effluents from sewage treatment plant (STP) effluents conducted during the 2002 survey. ND = not detected

Compound	Otonabee River	Hamilton Harbour	Little River	Detroit River
Caffeine	0.014 (± 0.001)	0.033 (± 0.002)	0.017 (± 0.007)	0.046 (± 0.003)
Carbamazepine	0.002 (± 0.001)	0.023 (± 0.002)	0.080 (± 0.005)	0.004 (± 0.001)
Cotinine	0.004 (± 0.001)	ND	0.014 (± 0.001)	0.013 (± 0.001)
Cyclophosphamide	ND	ND	0.005 (± 0.001)	ND
Fluoxetine	ND	0.013 (± 0.001)	0.046 (± 0.004)	ND
Norfluoxetine	ND	ND	ND	ND
Pentoxifylline	ND	0.008 (± 0.001)	0.009 (± 0.001)	ND
Trimethoprim	ND	0.043 (± 0.004)	0.134 (± 0.008)	ND
Bezafibrate	ND	0.010 (± 0.005)	0.137 (± 0.001)	ND
Clofibric acid	ND	0.001 (± <0.001)	0.003 (± <0.001)	ND
Gemfibrozil	ND	0.038 (± 0.001)	0.034 (± 0.003)	0.002 (± <0.001)
Diclofenac	ND	0.018 (± 0.001)	0.050 (± 0.003)	ND
Fenoprofen	ND	0.142 (± 0.008)	0.132 (± 0.001)	ND
Ketoprofen	ND	ND	ND	ND
Ibuprofen	ND	0.027 (± 0.002)	0.008 (± 0.001)	ND
Indomethacin	ND	0.005 (± 0.001)	0.018 (± 0.001)	ND
Naproxen	ND	0.039 (± 0.002)	0.073 (± 0.005)	ND
Atorvastatin	ND	0.010 (± 0.001)	0.015 (± 0.001)	ND

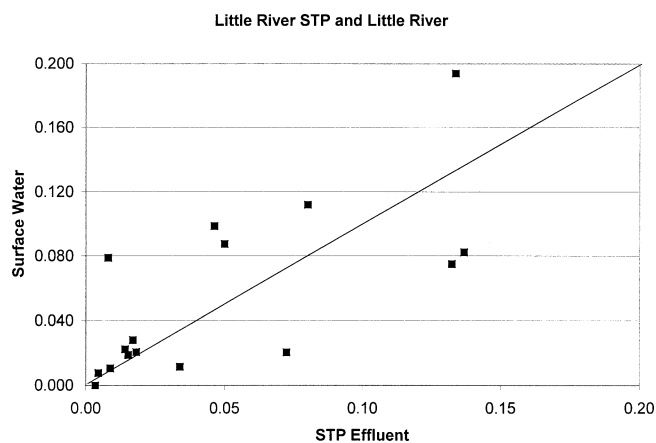


Fig. 7. Concentrations of neutral and acidic drugs ($\mu\text{g/L}$) in the final effluent of the Little River (Windsor, ON, Canada) sewage treatment plant (STP) plotted against the concentrations of these analytes ($\mu\text{g/L}$) in the Little River immediately adjacent to the STP discharge (2002 survey). The trend line marks a 1:1 ratio between concentrations in effluent and surface water.

These data and data from our other studies [15,23] are the only published data on the environmental distribution of this compound. The statin class of lipid regulators is prescribed in Canada to a greater extent than fibrate lipid regulating drugs (<http://www.imshealthcanada.com>). However, it is interesting to note that gemfibrozil and bezafibrate, the most commonly prescribed fibrate drugs in Canada, were present at higher concentrations in the surface water samples than atorvastatin (Table 5). More data are needed to determine the environmental distribution of other lipid regulators from the statin class [23].

Figure 7 illustrates the concentrations of all analytes in the final effluent from the Little River STP (Windsor) plotted against the concentrations at the surface water sampling site in the Little River immediately adjacent to STP discharge. The trend line for a 1:1 ratio between these values falls within the data distribution, indicating that virtually no dilution of drugs occurred in this low-flow system. A similar plot for analyte concentrations in the effluent from the Burlington STP and in surface water at the discharge site in Hamilton Harbour (site 2) shows that data are generally distributed about the trend line for a 1:10 ratio, with the exception of the data point for fenoprofen, which is closer to a 1:1 ratio (Fig. 8). The concentrations of neutral and acidic drugs in surface water samples from the Detroit River near the West Windsor STP and from the Otonabee River near the Peterborough STP were generally below detection limits, with the exception of carbamazepine, caffeine, and cotinine. Drugs are present in low concentrations in these systems because of the high degree of dilution. The data from the Little River indicate that the current recommendation from the Food and Drug Administration in the United States of a 10-fold dilution factor to predict the concentrations of drugs in surface waters from estimated concentrations in STP effluents ([12]; <http://www.fda.gov/cder/guidance/1730fnl.pdf>) is not a conservative estimate. However, our data are consistent with the conclusion by Dorn et al. [13] that this dilution factor is representative of effluent discharges under the majority of hydrologic conditions.

Drugs have been detected at concentrations below $1 \mu\text{g/L}$ in northern Europe in samples of surface water collected near STPs [1,4,6,7]. Ibuprofen, gemfibrozil, and fluoxetine, as well as several other prescription and nonprescription drugs, have

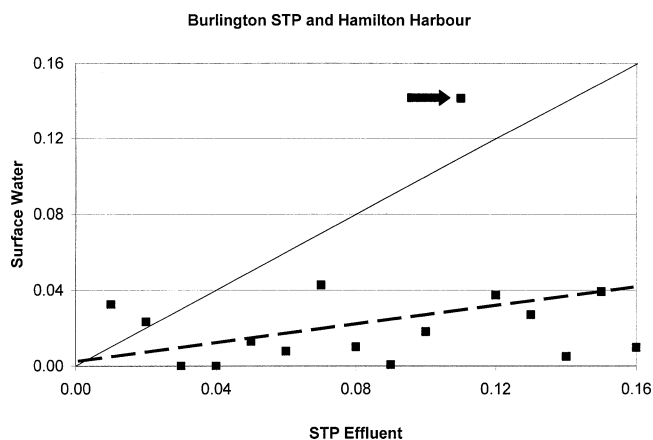


Fig. 8. Concentrations of neutral and acidic drugs ($\mu\text{g/L}$) in the final effluent of the Burlington (ON, Canada) sewage treatment plant (STP) plotted against the concentrations of these analytes ($\mu\text{g/L}$) in Hamilton Harbour (ON, Canada) immediately adjacent to the STP discharge (2002 survey). The solid trend line marks a 1:10 ratio, and the dashed line marks a 1:1 ratio between concentrations in effluent and surface water. The arrow points at the data point for fenoprofen.

also been detected at concentrations below $1 \mu\text{g/L}$ in streams in the USA [11]. The data from this study indicate that pharmaceuticals may be present at concentrations below $1 \mu\text{g/L}$ in surface water close to the point of discharge from STPs, especially in locations where hydrologic conditions produce little dilution of STP effluent. Drugs have been detected at very low concentrations ($<0.01 \mu\text{g/L}$) in northern Europe in samples of surface water at locations remote from STPs, including ibuprofen [5], clofibric acid, [9], and diclofenac [10]. This study also showed that some drugs (carbamazepine, ketoprofen, fenoprofen, clofibric acid) can be present in surface waters in the Great Lakes region of Canada at concentrations below $0.01 \mu\text{g/L}$. More work is required to determine what hydrologic conditions and characteristics of drugs lead to the detection of these compounds at locations remote from STP point sources. This study also showed that a dilution factor of 10 may be suitable for estimating concentration of drugs in surface waters near STP discharges in the majority of cases, but this factor is not conservative for all hydrologic conditions.

Acknowledgement—This work was supported by the Toxic Substances Research Initiative of Health Canada/Environment Canada and by the Natural Sciences and Engineering Research Council of Canada through the Strategic Grants program. Shaun O'Toole provided technical assistance in the preparation of samples for analysis. Lindsay Odum and John Rimaldi of the University of Mississippi provided norfluoquine. We thank research staff at the Great Lakes Institute of Environmental Research at the University of Windsor and at the Canada Centre for Inland Waters (Burlington, ON, Canada) for their help in collecting samples. Tony Ho of the Ontario Ministry of the Environment provided information on the operational characteristics of sewage treatment plants. We also thank Ed Sverko and Josey Grabuski of the National Laboratory for Environmental Testing (Burlington, ON, Canada) for extracting samples collected in 2000 from Hamilton Harbour, Lake Ontario, and Lake Erie.

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