



PHARMACEUTICALLY ACTIVE COMPOUNDS IN ATLANTIC CANADIAN SEWAGE TREATMENT PLANT EFFLUENTS AND RECEIVING WATERS, AND POTENTIAL FOR ENVIRONMENTAL EFFECTS AS MEASURED BY ACUTE AND CHRONIC AQUATIC TOXICITY

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Abstract—Ten acidic and two neutral pharmaceuticals were detected in the effluents of eight sewage treatment plants (STPs) from across Atlantic Canada. Concentrations varied between nondetectable and 35 $\mu\text{g/L}$. The analgesic, nonsteroidal anti-inflammatory drugs ibuprofen and naproxen were predominant. Carbamazepine, a neutral compound used as an antiepileptic drug, was observed consistently at a median concentration of 79 ng/L. Acetaminophen was found in the effluents of the three largest mechanical STPs at a median concentration of 1.9 $\mu\text{g/L}$, but not in the lagoon treatment systems. The substantially longer hydraulic retention times may have contributed to more effective removal of acetaminophen in the lagoon treatment systems. Drugs generally were not detected at significant concentrations in the larger bodies of receiving water (Saint John River, Hillsborough River, and Bedford Bay, Canada). However, drug residues in the small receiving streams were 15 to 30% of the effluent median concentrations. Six compounds (caffeine, naproxen, salicylic acid, carbamazepine, metoprolol, and sotalol) were found to persist in a small stream for a distance of at least 17 km, suggesting that small stream exposure to pharmaceutically active residues may be relatively greater than that in large bodies of water. Bioassays assessing acute and chronic effects on four organisms were conducted on four high-use drugs: Acetaminophen, ibuprofen, naproxen, and salicylic acid (metabolite of acetyl salicylic acid). Results indicated no negative effects except for the chronic algal (*Selenastrum capricornutum*) growth test on ibuprofen (no-observed-effect concentration, 10 $\mu\text{g/L}$; lowest-observed-effect concentration, 32 $\mu\text{g/L}$). Effects of these four compounds on invertebrates and plants in the receiving environments are unlikely based on the concentrations measured.

Keywords—Pharmaceuticals Drugs Sewage Water Toxicity

INTRODUCTION

Pharmaceuticals play a very important role in health care. The annual production of individual prescription and nonprescription pharmaceutical compounds is estimated between tens of kilograms and thousands of tons worldwide [1]. In Canada alone, approximately 24,000 products, including human pharmaceutical and biological drugs, veterinary drugs, and disinfectants, are registered on Health Canada's Drug Product Database [2] (http://www.hc-sc.gc.ca/dhp-mps/prodpharma/databasdon/index_e.html). In the body, drugs may be metabolized totally or partially and excreted intact or in conjugated form along with metabolites in urine and fecal matter. Domestic waste streams carry these compounds to municipal wastewater treatment plants, private septic systems, or in some cases, directly to receiving water without treatment. Although conventional sewage treatment is effective at removing/degrading some of the more labile compounds, it generally is not effective in removing many drugs and by-products in municipal waste streams [3–6]. Sewage treatment plants (STPs) therefore are considered to be an important and continuous source of drug input to the aquatic environment.

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Excellent reviews concerning the occurrence, fate, and effects of drugs in the environment have been conducted [3,7,8]. Considering the inherent nature, diversity, number, and amounts of therapeutic drugs, the scarcity of information regarding environmental exposure and effects, it is interesting to note that relatively little research has been conducted until recently. Occurrence of pharmaceutical compounds in municipal sewage was first reported during the 1970s in the United States [9,10]. More recently, an extensive study was conducted by the U.S. Geological Survey, in which they reported on pharmaceuticals and other organic contaminants in a survey of 139 streams from across 30 U.S. states during the period from 1999 to 2000 [11]. In Ontario and western Canada, several studies have reported on the occurrence of acidic and neutral drugs in municipal sewage and surface water, showing that the majority of these compounds are removed only partially, or are not at all, by conventional sewage treatment processes [12–15]. In Germany, the occurrence of drugs in STP effluents, surface water, groundwater, and drinking water is a recognized environmental issue [16,17]. In large cities such as Berlin, where the rate of sewage discharge is high compared to receiving stream flow, a potential always exists for potable water contamination, especially when groundwater is the source [18]. Acidic drug residues also have been reported in several STPs, lakes, and rivers in Switzerland, the North Sea, and sewage from a number of urban areas in Greece [19,20].

Information regarding exposure and the impact of phar-

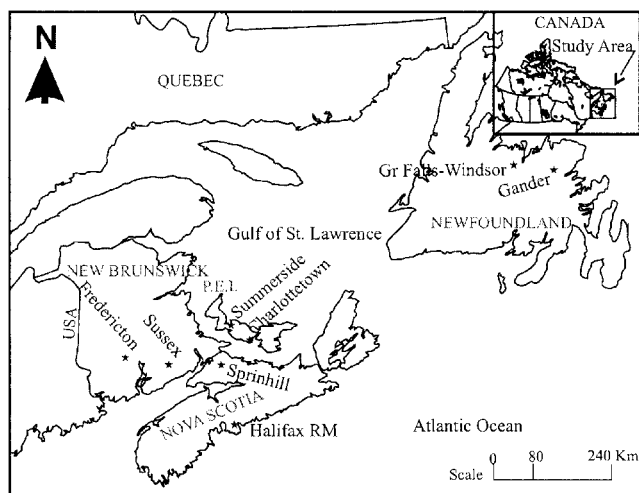


Fig. 1. Location of eight sewage treatment plants sampled for final effluents and receiving waters in Atlantic Canada during 2003. Inset delineates the study area. Gr Falls-Windsor = Grand Falls-Windsor; Halifax RM = Halifax Regional Municipality; P.E.I. = Prince Edward Island.

maceutical residues on the environment is sparse. However, what information is available raises some concern about potential acute and long-term effects of these biologically active compounds. For example, ethinylestradiol, which is used in the manufacture of birth-control pills has been shown to be a potent and complete feminizer in life-cycle studies of fathead minnows and to reduce egg fertilization at environmentally significant concentrations [21]. The same study reported that life-cycle exposure of fathead minnows to the acidic drug indomethacin, an analgesic and nonsteroidal anti-inflammatory drug (NSAID), resulted in decreased gonad size compared with that in control males at a concentration of 360 ng/L, a level similar to what is being discharged in some effluents from Canadian STPs but higher than the level generally found in receiving surface waters [14,22]. Estrogen-like effects have been reported in fish from wildlife populations in rivers downstream of United Kingdom wastewater treatment plants [23]. A report relating the cause of a catastrophic decline of Oriental white-backed vultures in Pakistan to diclofenac residues unquestionably will heighten concern regarding pharmaceuticals in the environment [24]. Diclofenac, an analgesic and NSAID, is used as a veterinary and over-the-counter drug for treating hoofed livestock in Pakistan. Death of vultures was associated with renal failure from feeding on animal carcasses contaminated with diclofenac.

In Atlantic Canada, very little data are available regarding pharmaceuticals in the environment and potential impacts. In the present study, we report on a project implemented in the year 2003 to investigate the occurrence of pharmaceutically active compounds in STP effluents and receiving waters of the four Atlantic Canadian Provinces. Results of acute and chronic toxicity testing performed on a bacterium, two crustaceans, and an alga are presented.

MATERIALS AND METHODS

Sampling sites and sample collection

Two STPs each were selected in the Canadian provinces of New Brunswick (NB), Nova Scotia (NS), Prince Edward Island (PE), and Newfoundland and Labrador (NL), for a total of eight sites (Fig. 1). Information regarding population served,

type of treatment, and description of receiving waters is presented in Table 1. The STPs from the smaller communities of Summerside (St. Eleanors, PE, Canada), Springhill (NS, Canada), Sussex (NB, Canada), and Grand Falls-Windsor (NL, Canada), had secondary or tertiary treatment as the highest treatment. Gander (NL, Canada), although designed for higher treatment, used primary treatment. The population served ranged between 1,800 and 15,000 people, and the average daily flows were between 900 and 12,900 m³/d. The Summerside, Springhill, and Gander STPs discharge effluents into small streams only a few meters in width, whereas Sussex discharges into the Kenebecasis River, a shallow river approximately 10 m in width at the point of discharge. The Grand Falls-Windsor plant discharges directly into the Exploits River, which is approximately 3 m in depth and 60 m in width at the point of discharge. The three larger mechanical STPs serve the cities of Charlottetown (PE, Canada), Fredericton (NB, Canada), and part of the Halifax Regional Municipality (HRM; NS, Canada) and are secondary treatment systems except for Charlottetown, which is primary. Hydraulic retention times of a few hours in these plants typically are much lower than the hydraulic retention times of STPs with lagoon treatment (e.g., 10–40 d) (Table 1). The Charlottetown STP discharges its effluent approximately 600 m from shore on the bottom of the Hillsborough River, a marine tidal system. The Fredericton STP is located in nearby Nashwaaksis (NB, Canada) and also has an underwater discharge outlet into the Saint John River. The HRM (Mill Cove, NS, Canada) STP serves a population of approximately 52,500 in Bedford (NS, Canada) and Lower Sackville (NS, Canada), and its underwater point of discharge is located approximately 200 m from shore in the marine waters of Bedford Bay/Basin.

Sampling stations for receiving waters were established approximately 100 m upstream and 100 m downstream from the STP outfall except for the Fredericton site in the St. John River, which was approximately 200 m upstream and 300 m downstream, just below the confluence with the Nashwaak River, and the HRM (Mill Cove) site, which was 200 m north and 300 m south of the point of discharge. Grab-sample collection of final effluent and surface water was performed in May 2003 and repeated in September 2003. Samples for drug analysis were collected in 1-L amber glass bottles (500 ml, low-density polyethylene, for physical tests) with the aid of an extendable aluminum pole. Samples were placed immediately on ice packs in portable coolers and transported to the laboratory for analysis, where they were stored at 4°C until analysis. The sewage effluent samples were analyzed for the 10 acidic and five neutral drugs listed in Table 2, biochemical oxygen demand (BOD), total organic carbon (TOC), total suspended solids (TSS), specific conductance, pH, and color. Biochemical oxygen demand and TSS were not determined in the surface-water samples. In 2004, five β -blockers (metoprolol, nadolol, pindolol, propranolol, and sotalol) and caffeine were added to the suite of drug analytes determined in samples taken downstream (100 m, 500 m, 3 km, 5 km, and 17 km) of the STP in Gander. The temperature of the final effluent and surface-water samples was recorded. Spatial coordinates (latitude/longitude) were taken with a geographical positioning system to ensure accurate relocation of sampling sites.

Reagents and standards

Laboratory pure water was double-deionized or equivalent. Methanol, *tert*-methyl butyl ether (MTBE), and toluene were

Table 1. Sewage treatment plant (STP) location, sampling information, and plant characteristics

STP ^a	Date of sampling	Population served	ADF ^b (m ³ /d)	Highest treatment ^c	Receiving waters ^d
Halifax RM (NS, Canada) (Mill Cove)	13 May 2003, 1440 h 10 September 2003, 1350 h	52,500	18,900	Secondary (aeration with pure O ₂ , UV, anaerobic sludge digestion); HRT: 4–7 h	Bedford Bay (MW)
Fredericton (NB, Canada) (Nashwaakasis)	12 May 2003, 1450 h 8 September 2003, 1420 h	34,000	18,200	Secondary (activated sludge, UV); HRT: N/A	Saint John River (FW)
Charlottetown (PE, Canada)	5 May 2003, 1340 h 9 September 2003, 0945 h	30,000	21,700	Primary (two-stage anaerobic digestion, Cl disinfection); HRT: 3 h	Hillsborough River (MW)
Grand Falls–Windsor (NL, Canada)	20 May 2003, 1600 h 30 September 2003, 1445 h	15,000	12,900	Secondary (aerated lagoon/WSP); HRT: 9.7–16.5 d	Exploits River (FW)
Gander (NL, Canada) (Beaverwood)	20 May 2003, 1240 h 30 September 2003, 1130 h	6,000	11,400	Primary (hydrodynamic separators, Cl disinfection); HRT: 2–16 h	Headwaters Soulis Pond (FW)
Sussex (NB, Canada)	12 May 2003, 1105 h 8 September 2003, 1000 h	4,293	1,900	Secondary (aerated lagoon and polishing pond); HRT: 28 d	Kenebecasis River (FW)
Springhill (NS, Canada)	13 May 2003, 0945 h 10 September 2003, 0940 h	4,250	2,000	Tertiary (aerated lagoon and polishing pond, filtration/UV); HRT: 48 d	Coal Mine Brook (FW)
Summerside (PE, Canada) (St. Eleanors)	5 May 2003, 0955 h 9 September 2003, 1300 h	1,800	900	Secondary (aerated lagoon and polishing pond, UV); HRT: 40–45 d	Unnamed Brook (FW)

^a In municipalities with more than one STP, the name of the plant sampled is italicized and placed within brackets.

^b ADF = average daily flow.

^c HRT = hydraulic retention time; UV = ultraviolet; WSP = wastewater stabilization pond.

^d FW = freshwater; MW = marine water.

distilled-in-glass grade or equivalent. Hydrochloric acid (HCl), sulfuric acid (H₂SO₄), and sodium hydroxide (NaOH) were certified American Chemical Society grade or equivalent. Analytical drug standards and 1-methyl-3-nitrosoguanidine (MNNG) were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). An individual stock solution (10 ml) for each drug (Table 2) was prepared at a concentration of 1 mg/ml in methanol. An intermediate mixed-standards stock solution was prepared from the individual stock solutions at 50 µg/ml in methanol. Five working standards for acidic drugs were prepared by diluting 25, 50, 100, 250, and 500 µl of the intermediate mixed-standards solution in 1.0 ml of methanol for calibration purposes. For the neutral drugs (Table 2), six working standard solutions were prepared by diluting 25, 50, 100, 250, 500, and 750 µl of the intermediate mixed-standards solution in 2.0 ml of methanol, which also was used for the calibration process. Likewise, individual surrogate solutions of mecoprop (Sigma-Aldrich Canada) and 10,11-dihydrocarbamazepine (Sigma-Aldrich Canada) were prepared at 5.0 and 2.0 µg/ml in methanol, respectively. The former solution was used for acidic drug analyses and the latter for the fall neutral drug sample analyses. Unfortunately, the use of mecoprop as surrogate did not work well in this case, because it was observed in some of the effluent samples. For the acidic drugs, 2,4-D (2,4-dichlorophenoxy acetic acid) (Sigma-Aldrich Canada) was used as internal standard for the spring samples. However, external calibration was used instead, because 2,4-D also was present in samples. Mecoprop and 2,4-D are registered for herbicide use in Canada. Residential weed-control application is the most likely source of the residue concentrations found in the sewage effluent samples. Fluorene-d₁₀ (Caledon Laboratories, Georgetown, ON, Canada) was subsequently used as internal standard for analysis of the fall samples.

Sample preparation and extraction of acidic drugs

The procedure used for the present study was adapted from the published literature, with minor modifications in the filtration of samples [15]. It should be noted that caffeine was added later to the suite of acidic drugs for the analysis of stream-water samples in the year 2004. Samples were stored in a cool room at 4°C and extracted within 48 h of sampling. Samples (1 L) initially were treated by adding 100 µl of the mecoprop surrogate solution. This choice of surrogate turned out to be problematic, because mecoprop was found to be present in some samples. The sample was then filtered under vacuum through a fine, 1.2-µm, glass-fiber filter (Whatman, VWR, Mississauga, ON, Canada), and the pH of the filtrate was adjusted to pH 2 with concentrated HCl. A 6-ml, 200-mg Oasis® HLB (Waters, Millford, MA, USA) solid-phase extraction cartridge was conditioned by elution with 5 ml of methanol and 10 ml of purified water acidified to pH 2 with HCl. The acidified sample filtrate was siphoned through a Visiprep solid-phase extraction vacuum manifold (Sigma-Aldrich Canada) onto the conditioned Oasis HLB cartridge at a rate of 10 ml/min or less. The cartridge was then washed with 2 ml of 25% methanol in water, which was discarded. The acidic drugs (Table 2) were extracted off the cartridge with 5 ml of methanol. The extract was evaporated to dryness under a steady stream of nitrogen, and 2 ml of MTBE were added.

Methylation of acidic drugs

Diazomethane and MNNG are extremely toxic compounds, and appropriate handling and safety precautions must be fol-

Table 2. List of acidic and neutral drugs determined in the final effluents and receiving waters of eight sewage treatment plants from Atlantic Canada in 2003, including pertinent chemical information and principal use/application of individual drug compounds^a

Drug (acronym)	CAS no.	Molecular wt	Log K_{ow}	Application
Acidic compounds				
Bezafibrate (BZF)	41859-67-0	361.8	4.30	Lipid regulator
Clofibric acid (CFA)	882-09-7	214.7	2.84	Metabolite of clofibrate and etofyllin clofibrate
Diclofenac (DCF)	15307-86-5	296.2	4.02	Analgesic, NSAID
Fenoprofen (FNP)	31879-05-7	242.3	3.99 ^b	Analgesic, NSAID
Gemfibrozil (GFB)	25812-30-0	250.4	4.77	Lipid regulator
Ibuprofen (IBP)	15687-27-1	206.3	3.79	Analgesic, NSAID
Indomethacin (IDM)	53-86-1	357.8	4.23	Analgesic, NSAID
Ketoprofen (KTP)	22071-15-4	254.3	3.00	Analgesic, NSAID
Naproxen (NPX)	22204-53-1	230.3	3.10	Analgesic, NSAID
Salicylic acid (SA)	69-72-7	138.1	2.24	Metabolite of ASA (an analgesic, NSAID), food preservative, also a natural plant substance
Neutral compounds				
Acetaminophen (ACT)	103-90-2	151.2	0.49	Analgesic, antipyretic
Carbamazepine (CBM)	298-46-4	236.3	2.25	Antiepileptic, analgesic
Cyclophosphamide (CPM)	50-18-0	261.1	0.97	Antineoplastic
Pentoxifylline (PTX)	5/6/6493	278.3	0.99 ^b	Vasodilator
Phenazone (PNZ)	60-80-0	188.2	0.59	Analgesic

^a ASA = acetylsalicylic acid; CAS = Chemical Abstract Service; K_{ow} = octanol–water partition coefficient; NSAID = nonsteroidal anti-inflammatory drug.

^b Calculated value.

lowed. A diazomethane generator was used with MNNG to produce gaseous diazomethane for the methylation of the organic acids. Approximately 0.1 g of MNNG was added to the impinger tube, followed by 4 ml of water. After assembling the diazomethane generator, the Pasteur pipette (Fisher Scientific, Ottawa, ON, Canada) was immersed into the sample extract. Two milliliters of a 10 N NaOH solution were added to the impinger, and the nitrogen flow was adjusted to maintain gentle bubbling in the sample. Bubbling was continued for 2 min after the straw-yellow color first appeared, indicating that methylation was complete. After an hour, the sample was placed under a nitrogen stream until the yellow color disappeared, indicating that no more diazomethane was left in the sample. Approximately 0.5 ml of toluene was then added to the methylated extract followed by evaporation under a gentle stream of nitrogen to approximately 0.5 ml. The extract was then filtered through a 0.45- μ m Millex-FH filter (Millipore Canada, Nepean, ON), followed by rinsing with toluene, evaporation under a nitrogen stream, addition of 20 μ l of internal standard, and final volume being made up to 1 ml with toluene.

Gas chromatography–mass spectrometry of acidic drugs

An Agilent 6890N gas chromatograph equipped with an automated sample injector and Agilent 5973N mass-selective detector (Agilent Technologies, Palo Alto, CA, USA) was used to analyze the methylated drugs. Separation of compounds was performed on a HP-5 MS (i.e., low-bleed) column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μ m) with the oven temperature program set as follows: Initial temperature of 50°C for 2 min, ramp at 6°C/min to 310°C, with a final hold time of 4.7 min. A 2- μ l injection volume, with pulsed splitless-mode injection, and inlet temperature of 290°C were used. The carrier gas was helium (ultrahigh-purity grade) at an initial flow of 0.6 ml/min.

The methylated drug compounds were analyzed using electron-impact ionization–mode mass spectrometry (MS) at 70 eV. Full-scan MS was performed on all individual compounds, and selected-ion monitoring mode was used for quantitative analysis. Quantifier and qualifier ions for individual drugs are

provided in Table 3. Multipoint calibration was performed using a linear or quadratic curve-fitting model and correlation coefficient of at least 0.995.

Sample preparation and extraction of neutral drugs

A 100- μ l aliquot of the dihydrocarbamazepine surrogate solution was added to each sample before filtration with a 1.2- μ m, glass-fiber filter. Then, 750 μ l of a 40% solution of H₂SO₄ was added to the filtrate. The solid-phase extraction Oasis cartridge was conditioned just before sample extraction by eluting with 3 ml of methanol, 3 ml of 0.5 N HCl, and 3 ml of water. The sample filtrate was passed through the cartridge at a flow rate of 10 ml/min or less, followed by a 1-ml wash with purified laboratory water (discarded). The neutral drugs were eluted from the cartridge with 5 ml of methanol into a 10-ml centrifuge tube. The extract was evaporated down to 1 ml under a nitrogen stream, 0.5 ml of mobile phase A (MP-A; used for high-performance liquid chromatography [HPLC]–MS) added, and volume made up to 2 ml with methanol.

HPLC-MS of neutral drugs

An Agilent 1100 series HPLC equipped with autosampler and Agilent 1100 series mass-selective detector (Model G1946C; Agilent Technologies) were used for the analysis. Separation of the five neutral drugs (Table 2) was achieved on a reverse-phase column (Hewlett-Packard Zorbax Eclipse XDB-C8; length, 150 mm; inner diameter, 4.6 mm; particle size, 5 μ m; Agilent Technologies) using binary gradient elution at a constant temperature of 25°C. The MP-A consisted of 10 mM ammonium formate and 0.3% formic acid in water/methanol (9:1), and mobile phase B (MP-B) consisted of 10 mM ammonium formate and 0.5% formic acid in methanol. The solvent gradient program was set to start with a 91:9 ratio of MP-A to MP-B for the first 5 min, then a 58:42 ratio at 15 min, then 100% MP-B at 20 min, with a final hold for 5 min. The injection volume was 25 μ l, and the column flow rate was 0.6 ml/min. The mass-selective detector was operated on electrospray (API-ES) mode with spray chamber temperature of 300°C, drying gas flow rate of 12.0 L/min, nebulizer pressure

Table 3. Field quality-control results for acidic and neutral drugs, including spiking levels, recoveries, method detection limits (MDLs), and selected mass spectrometry (MS) ions^a

Drug	Spike concn. (ng/L)	Recoveries			MDL (ng/L)	MS Ions	
		Mean (%)	RSD (%)	<i>n</i>		Quant (<i>m/z</i>)	Qual (<i>m/z</i>)
Acidic compounds							
BZF	101	64.0	58.4	6	30	120	220
CAF	27	100	6.0	5	7.6	194	109
CFA	104	106	8.5	6	30	128	130
DCF	97.2	87.2	12.1	6	30	214	242
FNP	99.2	93.9	18.6	6	30	256	257
GFB	121	95.5	33.1	6	30	143	122
IBP	90.2	74.9	21.8	6	30	161	177
IDM	117	51.3	12.8	6	30	139	141
KTP	99.2	96.9	26.4	6	30	209	268
NPX	133	89.4	20.6	6	30	185	244
SA	114	68.4	21.0	6	30	120	152
F-D10						176	174
2,3-D						199	175
Neutral compounds							
ACT	13.3	27.6	18.8	4	10	110	152
CBM	11.8	92.3	1.8	4	20	237	194
CPM	15.4	81.8	11.5	4	20	261	263
PTX	11.9	93.2	1.7	4	20	181	279
PNZ	27.0	109	2.2	4	25	189	190
DH-CBM						239	240

^a Note that MDLs may vary based on sample matrix and efficiency of cleanup procedure. Refer to Table 2 for the list of drug acronyms. CAF = caffeine; 2,3-D = 2,3-dichlorophenoxyacetic acid (surrogate); DH-CBM = dihydrocarbamazepine (surrogate); F-DIO = fluorene-10 d (internal standard); RSD = relative standard deviation; Qual = qualifier; Quant = quantifier.

of 30 psig, capillary voltage of 3,000 V, and positive polarity. The neutral drugs were analyzed individually by full-scan MS in positive-ion mode, and quantitative analyses were performed using selective-ion monitoring mode. The selected ions are illustrated in Table 3.

Sample preparation and extraction of β -blockers

Samples were kept cold and extracted within 48 h of collection. Before extraction, each sample was filtered through a 1.2- μ m, glass-fiber filter in the presence of Celite (VWR, Mississauga, ON, Canada). To an aliquot of 500 ml, the surrogates 10,11-dihydrocarbamazepine and diazepam-*d*₅ (in methanol; CIL, Andover, MA, USA) were added to the sample to make up concentrations of 400 and 200 ng/L, respectively. The sample was subsequently acidified to approximately pH 3 with 1 N HCl. Meanwhile, for each sample, a 6-ml, 150-mg Oasis MCX cartridge (Waters) was conditioned by elution with 6 ml of methanol, followed by 10 ml of water at pH 3. Via a siphon tube, each sample was applied to the MCX cartridge using a Visiprep solid-phase extraction manifold at a flow rate of 10 to 15 ml/min. After extraction, the cartridges were dried for 10 min under vacuum. They were then rinsed with 6 ml of water at pH 3, then eluted with 6 ml of methanol (fraction 1) and 8 ml of a mixture of dichloromethane/2-propanol/ammonium hydroxide (78:20:2, v/v/v; fraction 2). Both fractions were evaporated in a water bath (40–45°C) by a stream of nitrogen just to dryness. Each fraction was redissolved in 1.0 ml of solvent A (see below) for liquid chromatography (LC)/MS/MS analysis. If removal of particulate matter was required, the sample extract was filtered through a 13-mm, 0.45- μ m nylon syringe filter before LC analysis.

LC/MS/MS analysis of β -blockers

Chromatographic separation of the β -blockers metoprolol, nadolol, pindolol, propranolol, and sotalol was achieved by a Finesse Genesis C18 column (length, 150 mm; inner diameter, 2.1 mm; particle size, 4 μ m; Jones Chromatography, Chromatographic Specialties, Brockville, ON, Canada) together with a guard column (Genesis C18; length, 10 mm; inner diameter, 2.1 mm). A Waters 2695 Separations Module was used to generate a gradient profile involving two solvent mixtures—solvent A, which consisted of formic acid/acetonitrile/water (0.1:5:94.9, v/v/v), and solvent B, which consisted of 0.1% (v/v) formic acid in acetonitrile—for optimal separation. The gradient profile was as follows: 0.0 to 1.0 min, 100% solvent A and 0% solvent B, curve 1; at 5.0 min, 98% solvent A and 2% solvent B, curve 1; at 5.1 min, 90% solvent A and 10% solvent B, curve 1; at 15 min, 81% solvent A and 19% solvent B, curve 6; at 20.0 min, 78% solvent A and 22% solvent B, curve 6; at 30 min, 55% solvent A and 45% solvent B, curve 6; at 35 min, 50% solvent A and 50% solvent B, curve 6; at 40 min, 48% solvent A and 52% solvent B, curve 6; at 41.0 min, 0% solvent A and 100% solvent B, curve 6; at 50 min, 0% solvent A and 100% solvent B, curve 1; at 51.0 min, 100% solvent A and 0% solvent B, curve 6; and at 60 min, 100% solvent A and 0% solvent B, curve 1. A constant flow rate of 0.2 ml/min and a sample volume of 10 μ l were used for all injections. Sample extracts were analyzed with a Micromass Quattro Ultima triple-quadrupole mass spectrometer (Manchester, UK) equipped with an electrospray-ionization interface operated in the positive mode. Nitrogen (purity, 99%) was used as the nebulizing and desolvation gas, with typical flow rates of from 150 to 200 and from 500 to 600 L/h, respectively. Source and desolvation temperatures of 120 and 320°C, re-

Table 4. Results for acidic and neutral drugs and physicochemical parameters in the final effluents of sewage treatment plants (STPs) serving eight municipalities in Atlantic Canada^a

STP	Acidic drugs (ng/L)									
	BZF	CFA	DCF	FNP	GFB	IBP	IDM	KTP	NPX	SA
Spring										
Halifax (NS, Canada)	330	ND	140	ND	530	7,000	85	ND	ND	100
Fredericton (NB, Canada)	130	ND	ND	190	940	17,000	ND	79	5,900	1,900
Charlottetown (PE, Canada)	810	38	500	ND	780	11,000	98	ND	9,100	20,000
Grand Falls–Windsor (NL, Canada)	250	ND	IN	ND	210	37	60	52	1,200	170
Sussex (NB, Canada)	310	ND	ND	57	ND	ND	55	ND	850	110
Springhill (NS, Canada)	42	ND	39	ND	180	140	ND	ND	650	70
Summerside (PE, Canada)	460	ND	ND	48	590	810	42	ND	800	750
Gander (NL, Canada)	69	ND	220	ND	110	6,200	40	170	4,000	11,000
Median	280	—	39	15	370	3,500	48	15	1,000	460
Summer										
Halifax (NS, Canada)	68	ND	37	ND	320	52	35	IN	1,700	ND
Fredericton (NB, Canada)	130	ND	87	59	840	3,800	120	IN	3,300	53
Charlottetown (PE, Canada)	IN	ND	IN	140	1,400	22,000	110	280	14,000	35,000
Grand Falls–Windsor (NL, Canada)	440	ND	ND	ND	570	3,800	97	ND	1,700	190
Sussex (NB, Canada)	280	ND	ND	15	240	410	52	ND	860	56
Springhill (NS, Canada)	ND	ND	ND	ND	240	300	ND	ND	220	ND
Summerside (PE, Canada)	64	ND	ND	ND	1,300	690	ND	ND	370	ND
Gander (NL, Canada)	680	IN	ND	IN	620	9,600	310	310	7,600	26,000
Median	130	—	15	15	600	2,200	75	15	1,700	54

^a Sampling was conducted in spring and summer of 2003. For calculation of the median, nondetect (ND) values were replaced with values half the method detection limit. IN = interference, *T* = temperature; SC = specific conductance; TOC = total organic carbon; TSS = total suspended sediments; BOD = biochemical oxygen demand. Refer to Table 2 for the list of drug acronyms.

spectively, were used. The capillary voltage was held at 3,450 V. Cone voltage and collision energy varied with each compound (20–60 V and 8–30 kV, respectively). To establish the mass spectrum and the optimal multiple-reaction monitoring transitions used in the quantitative analysis of the pharmaceuticals, a 10 µg/ml solution of each drug was infused to the analyzer using a Harvard syringe pump (Harvard Apparatus, Holliston, MA, USA) at a flow rate of 10 µl/min.

Physicochemical analysis of water

Standard laboratory water analyses were performed for various parameters according to Environment Canada–approved methods [25]. Specific conductivity and pH were determined simultaneously on a PC Titrate® System (Man-Tech Associated, Tonawanda, NY, USA). The automated apparatus uses a conductance meter with platinum-tip electrodes calibrated against a standard solution of potassium chloride (KCl). Results were reported as µS/cm, corrected to 25°C. Similarly, measurements of pH were conducted on the PC Titrate System, consisting of a pH meter and pH combination-electrode calibrated against standard buffer solutions. Apparent color, which is color originating from dissolved material and suspended matter, also was determined with the PC Titrate System. Color was determined by comparing light absorbance of a sample aliquot against platinum–cobalt color standard solutions. Measurements were reported in Hazen units. Total organic carbon was determined by the high-temperature combustion technique using a Shimadzu TOC analyzer (Model TOC-5050; Mandel Scientific, Guelph, ON, Canada). An aliquot of the sample is acidified with HCl, and the resulting CO₂ is purged with nitrogen gas to remove inorganic carbon. The purged sample is introduced into a high-temperature furnace at 680°C over platinum catalyst, oxidizing organic carbon to CO₂ gas, which is carried directly into a nondispersive infrared detector for quantitation. Results were reported as mg/L of carbon, and the limit of detection was 0.5 mg/L. Total suspended solids was deter-

mined by filtering a water sample through a Millipore 47-mm filter, then weighing the residue/filter (tared) paper after drying to constant weight. Results were reported as mg/L. Biochemical oxygen demand was performed by incubating a sample in an appropriate container at 20°C for a period of 5 d. Dissolved oxygen was measured initially and at the end of the 5-d incubation period. Biochemical oxygen demand was calculated from the initial and final dissolved oxygen and reported as mg/L.

Quality control of chemical analyses

To evaluate possible effects of sample contamination and degradation of analytes during the sample collection process, transport, and storage before laboratory analysis, several blanks and field-spiked samples were prepared using purified laboratory water. The concentration levels spiked into the water and recoveries are presented in Table 3. Study results were not adjusted for method recoveries. In addition, and as part of the internal laboratory quality-control protocol, reagent method blanks, including spiked samples, also were routinely prepared and analyzed within the regular sample stream. With very few exceptions, blanks were found to be below the measurable levels. Recoveries varied between 28% for acetaminophen (ACT) and 109% for phenazone. The low recovery for ACT (28%) and indomethacin (51%) suggests that these analytes may be unstable under the prescribed sample handling or preservation procedures, and means of improving the preservation of samples should be investigated further. However, results are considered to be reproducible with relative standard deviations of 18.8 and 12.8%, respectively. The laboratories conducting the various analyses were accredited by the Standards Council of Canada and the Canadian Association of Environmental Analytical Laboratories under International Organization for Standardization Guide 17025.

Table 4. Extended

Neutral drugs (ng/L)					Physicochemical tests					
ACT	CBM	CPM	PTX	PNZ	T (°C)	pH	SC (µS/cm)	TOC (mg/L)	TSS (mg/L)	BOD (mg/L)
ND	45	IN	IN	ND	11.5	7.1	1,860	8.3	8.5	8.0
4,800	97	ND	IN	ND	9.7	7.1	666	5.7	33	26
3,800	64	IN	IN	ND	—	7.1	1,750	6.9	80	120
ND	ND	ND	IN	ND	10.5	7.2	466	3.8	17	ND
ND	41	ND	IN	ND	9.7	7.3	689	3.0	12	ND
ND	88	ND	IN	ND	8.8	8.6	942	3.7	15	6.0
ND	24	ND	IN	ND	5.0	7.6	720	4.5	43	5.0
3,800	ND	ND	IN	ND	8.0	6.9	408	5.6	100	45
5.0	43	—	—	—	9.7	7.2	705	5.1	25	7.0
ND	130	IN	ND	ND	19.8	6.6	2,070	11.0	74	9.0
27	83	ND	ND	ND	19.1	6.5	599	8.2	10	6.0
9,000	240	ND	ND	ND	18.2	7.4	2,090	6.7	40	37
ND	75	IN	36	ND	—	7.2	425	7.3	27	13
ND	98	ND	ND	ND	18.9	7.5	638	6.8	18	8.0
ND	180	ND	ND	ND	18.2	7.1	557	4.4	2.2	ND
ND	66	ND	ND	ND	17.8	7.2	944	9.9	66	13
5,400	170	ND	ND	ND	—	6.4	345	12.0	150	200
5.0	110	—	—	—	18.6	7.2	619	7.8	34	11

Toxicity testing of pharmaceuticals

The substances tested were ACT (purity, 98–101%; Sigma-Aldrich Canada), ibuprofen (IBP; meets U.S. Pharmacopeia [USP; Rockville, MD, USA] specifications; Sigma-Aldrich Canada), naproxen (NPX; meets USP specifications; Sigma-Aldrich Canada), and salicylic acid (SA; purity, 99.5–100.5%; meets USP specifications; Sigma-Aldrich Canada [Riedel-de Haën]). All substances were tested in multiconcentration tests using, except as outlined below, the following test concentrations: 32, 10, 3.2, 1.0, and 0.1 µg/L, as well as a clean-water control.

Quality control for the toxicity tests consisted of the following measures: All tests were accompanied by reference toxicant tests. The results of these tests were entered into the control chart for each species to ensure that normal operating conditions were maintained and that the population of organisms used in the test was of normal sensitivity. In addition, the Environment Canada test protocols list test-acceptability criteria, such as minimum acceptable survival in the control population (test with *Daphnia magna*) and minimum number of broods of offspring in the control animals (*Ceriodaphnia dubia* test). When these test-acceptability criteria were not met, test data were rejected, and the tests were repeated.

Acute lethality test using *D. magna*

Testing was conducted according to the published Environment Canada biological test method [26]. The test organisms were *D. magna* younger than 24 h of age. Dilutions of the stock sample solution were prepared in culture/dilution water and allowed to equilibrate. Portions of each concentration were then transferred to two glass beakers, one for testing and one for water-quality measurements. Ten *D. magna* neonates were counted and transferred to each test vessel. Test temperature was 20 ± 2°C (i.e., acceptable range). Testing was performed with a 16:8-h light:dark photoperiod. At 48 h, the test was terminated. The number of dead and immobilized organisms was recorded, as were water-quality measurements of each test vessel, including temperature, pH, and dissolved

oxygen. The results are expressed as a 48-h median lethal concentration (i.e., the estimated concentration that would cause a 50% decrease in survival).

Microtox testing using *Vibrio fischeri*

An acute lethality test, this was conducted according to the published, approved Environment Canada test method [27]. This method exposes the bacterium for 15 min to concentrations of the sample; if toxic materials are present, they interfere with the cellular respiration of the organism. This interference is measured as a decrease in light output by the bacterium *V. fischeri* (previously *Photobacterium phosphoreum*). Test temperature was 15 ± 0.3°C (i.e., acceptable range). The results are expressed as the 15-min median inhibition concentration (i.e., the concentration that causes inhibition of light by 50%).

Test of reproduction and survival using *C. dubia*

This test method was conducted by Stantech Consulting (Guelph, ON, Canada) according to published Environment Canada methods [28]. The test organisms were newly born (age, <24 h) microcrustaceans (*C. dubia*). The method exposes these organisms to the sample under controlled conditions for 6 to 8 d, until they have reproduced three times. Test solutions are renewed, and the animals are fed daily. Test temperature was 25 ± 1°C (i.e., acceptable range). If toxic materials are present, they interfere with the reproduction or survival of the organism. This interference is measured as a decrease in number of living offspring and is expressed as the 25% inhibition concentration (IC25; i.e., the concentration that causes inhibition of the number of living offspring by 25%).

Growth inhibition test using the freshwater alga *Selenastrum capricornutum*

A chronic sublethal test, this was conducted by Stantech Consulting according to the published Environment Canada standard method [29]. The test organism is the single-celled, freshwater green alga, formerly *Pseudokirchneriella subcapitata* (*S. capricornutum*). This method exposes the algal cells

to the sample for 3 d under carefully controlled conditions. Test temperature was $24 \pm 2^\circ\text{C}$ (i.e., acceptable range). If toxic materials are present, they interfere with growth of the algae. This interference is measured as a decrease in number of living algal cells, or as a decrease in biomass, compared to the control organisms. The results are expressed as the IC25.

RESULTS AND DISCUSSION

Information regarding human therapeutic drug residues in the Canadian environment is sparse. Pathways, fate, and biological effects in aquatic ecosystems generally are not well understood, making it difficult to carry out risk assessment. Information is required about concentrations in raw and treated sewage effluents, receiving streams, and biological effects [8]. Only recently, results of three studies were published on the occurrence of acidic and neutral drugs in STP effluents and receiving waters from several municipalities in Quebec, Ontario, and western Canada [13–15]. In the present study, we report on the residue concentrations of 15 acidic and neutral drugs in the final effluents of comparatively smaller STPs discharging in fresh and marine waters across Atlantic Canada. The pharmaceutical drugs selected for the present study included commonly prescribed and over-the-counter drugs, such as IBP and NPX, also referred to as NSAIDs, and less common drugs, such as cyclophosphamide, a cancer treatment chemical. Various uses of these drugs are provided in Table 2. Based on exposure data, we proceeded to test four high-use drugs for acute and chronic toxicity.

Drug residue concentrations in STP effluents

In the Atlantic Region, approximately 130 municipalities have a population of 1,000 or more connected to some type of sewage treatment [30] (http://www.ec.gc.ca/water/en/manage/use/e_data.htm). A principal objective of the present study was to select a small number of STPs typical of sewage treatment facilities operating across Atlantic Canada, essentially small-scale lagoon and somewhat larger-capacity mechanical treatment systems (Table 1). Currently, only a handful of tertiary treatment plants operate in the region. The eight selected STPs served municipal populations in the range of 1,800 to 52,500 inhabitants. Being in a coastal region, it was important to include two sites discharging directly into marine waters, Charlottetown and HRM (Mill Cove). Because of resource constraints, a grab-sampling approach was adopted instead of the more time-consuming integrative/composite sampling strategies. This approach is reliable and provides a good snapshot of drug residue concentrations in final effluents of the STP infrastructure of Atlantic Canada.

Drug residue and physicochemical results are presented in Table 4 for the eight STPs sampled in Atlantic Canada during the spring and summer of 2003. Residue concentrations were observed for all acidic and neutral drugs in the STP final effluents with the exception of two compounds, cyclophosphamide and phenazone. The absence of these two compounds concurs with results of surveys conducted in other areas of Canada, where the compounds either were not detected or were observed at concentrations near the limit of detection [13,14]. Cyclophosphamide is a cytostatic drug used frequently in chemotherapy. Residues therefore would be expected to emanate almost exclusively from hospital effluents. In fact, cytostatic drug residues, including cyclophosphamide, have been detected at trace levels (low-ng/L range) in STPs treating hospital effluents [3]. Sales information is not readily accessible for

this drug. However, we assume that the volume must be relatively low. If this is any indication, the total volume of cyclophosphamide used in 1996 in Germany, a country with a higher population than Canada, was only 250 kg [31]. This information would suggest that low use and absence of hospitals in some municipalities, combined with dilution, is the most plausible explanation for why this compound was not detected in final effluents.

Cytostatic drugs remain important chemicals to monitor in the environment, because they are relatively persistent and have well-established carcinogenic, mutagenic, and fetotoxic properties [31]. Ifosfamide is another chemotherapeutic drug very similar to cyclophosphamide that was not incorporated into the 2003 suite of chemicals. The compound was subsequently included in a 2004 survey. Neither drug was detected in any of the effluent and receiving water samples, which included various sites in St. John's Harbor, Newfoundland and Labrador, Canada, Halifax Harbor–Bedford Bay, Pictou Harbor, and the East River of Pictou in Nova Scotia, Canada [32]. The other nondetected drug in the present study, phenazone, has been detected in Europe in sewage, surface water, and groundwater and, in some instances, at trace-level concentrations in a few samples of drinking water [3]. The information also suggests that this drug was not detected in the present study because of the relatively small amounts in use and the dilution to a concentration at which it was not detectable.

Clofibrac acid was detected only once in a Charlottetown sample at a concentration of 37 ng/L, just above the method detection limit (MDL) (Table 4). Clofibrac acid is the active metabolite of the lipid-regulators clofibrate, etofylin clofibrate, and etofibrate [18]. According to studies conducted in Europe, the drug metabolite seems to be fairly persistent, undergoing 0 and 51% removal in STP effluents [16,17]. Considering that the other fibrates lipid regulators in the present study, bezafibrate and gemfibrozil (GFB), were present in most samples at relatively high concentrations, it would seem that the absence of clofibrac acid in effluent samples was related to low use. Just two clofibrate products are currently registered for use in Canada [2]. Similarly, pentoxifylline was only detected in one sample (Grand Falls–Windsor, summer) at a concentration of 36 ng/L. This is comparable to results reported for STP effluents from other Canadian STPs [13,14]. Pentoxifylline is registered for human and veterinary use in Canada and is not considered to be a high-use drug [2,33].

The three drugs observed with the highest median effluent concentrations in the spring samples were IBP (an over-the-counter drug), NPX, and SA (the bioactive metabolite of another common over-the-counter drug, acetyl SA [ASA]). Figure 2 illustrates the spring and summer drug residue profiles with 25th and 75th percentiles. The next two highest median concentrations corresponded to the lipid-regulators GFB and bezafibrate, followed by indomethacin, carbamazepine (CBM), diclofenac, fenoprofen, and ketoprofen. As a result of the substitution of nondetect values by half the MDL, the fenoprofen, ketoprofen, and ACT median concentrations are reported below their respective MDLs. The spring and summer profiles are fairly similar. Overall, the predominant drugs were IBP, NPX, GFB, and bezafibrate, whereas ACT, ketoprofen, fenoprofen, and diclofenac had the lowest median concentrations. Carbamazepine and indomethacin were consistently present at intermediate levels.

The only drug with inconsistent concentrations between sampling periods was SA (Fig. 2). The almost 10-fold differ-

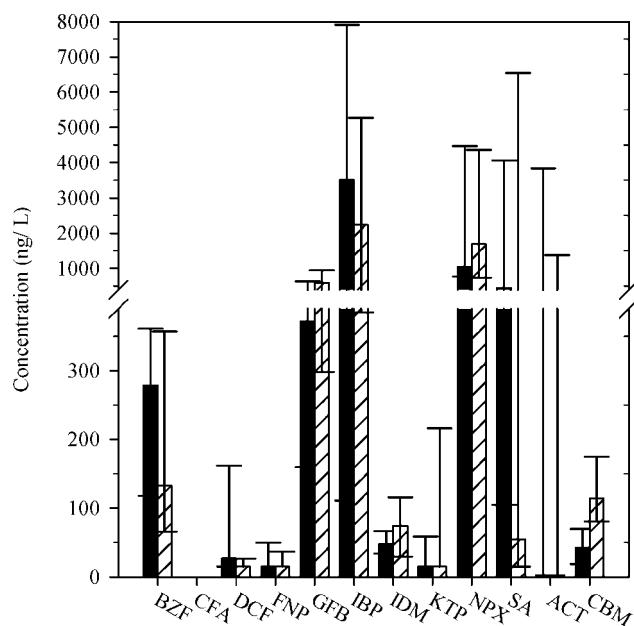


Fig. 2. Concentration profiles of acidic and neutral drug residues in the final effluents of eight sewage treatment plants in Atlantic Canada. Black bars represent median concentration for spring 2003, and hatched bars represent median concentration for summer 2003. Error bars correspond to the 25th and 75th percentiles. ACT = acetaminophen; BZF = bezafibrate; CBM = carbamazepine; CFA = clofibrac acid; DCF = diclofenac; FNP = fenoprofen; GFB = gemfibrozil; IBP = ibuprofen; IDM = indomethacin; KTP = ketoprofen; NPX = naproxen; SA = salicylic acid.

ence in median concentrations between spring (460 ng/L) and summer (54 ng/L) was, however, not statistically significant (Wilcoxon's signed-rank test, $p = 0.844$). There may be a seasonal factor at play here, and we believe that higher summer temperatures, in combination with microbial activity or chemical reactivity, may have contributed to the degradation of SA. The SA concentration, in fact, was negatively correlated (Spearman rank-order correlation coefficient [SROCC],

-0.739 ; $p < 0.05$) with temperature in the lagoon treatment systems (Appendix; <http://XXX>). A negative correlation (-0.700) was also observed for the larger mechanical treatment systems, but it was not significant ($p > 0.05$). The hydraulic retention time of the lagoon treatment systems would be relatively higher in the dry summer period than during the high-flow spring period, thus contributing to conditions amenable to the degradation of SA (e.g., longer exposure to sunlight, higher temperatures, photodegradation, and higher microbial productivity).

Information regarding the stability and removal of SA in sewage is not well understood. In one study, 12% SA was recovered from spiked sewage effluent after storage for only 24 h at 4°C, and 98% of SA was removed from sewage in southern Ontario STPs [15]. Another study in Germany reported the elimination (99%) of high amounts of this metabolite by STPs [16]. In a study consisting of 14 Canadian STPs, approximately 99% of SA present in influent samples was removed, but a high median residue concentration of 3.6 $\mu\text{g/L}$ was still left in the final effluents [13]. Assuming that quality control and expediency in sample analyses were not problematic, the information suggests that SA generally is unstable and may vary considerably depending on the biotic and physicochemical characteristics of the sewage stream, type of treatment, and operational engineering constraints of the STP. Our data show large differences in residue concentrations among the larger treatment plants (HRM [Mill Cove], Fredericton, and Charlottetown) and between the larger (mechanical) plants and the lagoon treatment systems (Table 4 and Fig. 3). On a per-capita basis, the gap between the two types of treatment is relatively small (Fig. 3C and D). In the lagoon treatment effluents, SA correlated ($p < 0.05$) with bezafibrate (SROCC, 0.814), and in the mechanical plant effluents, SA correlated with various other parameters, such as IBP (SROCC, 0.943), ACT (SROCC, 0.841), pH (SROCC, 0.880), and population (SROCC, -0.837). The correlation between drugs may be indicative of common sources or pathways. In addition to SA, NPX (SROCC, -0.956) and ACT (SROCC, -0.849) were

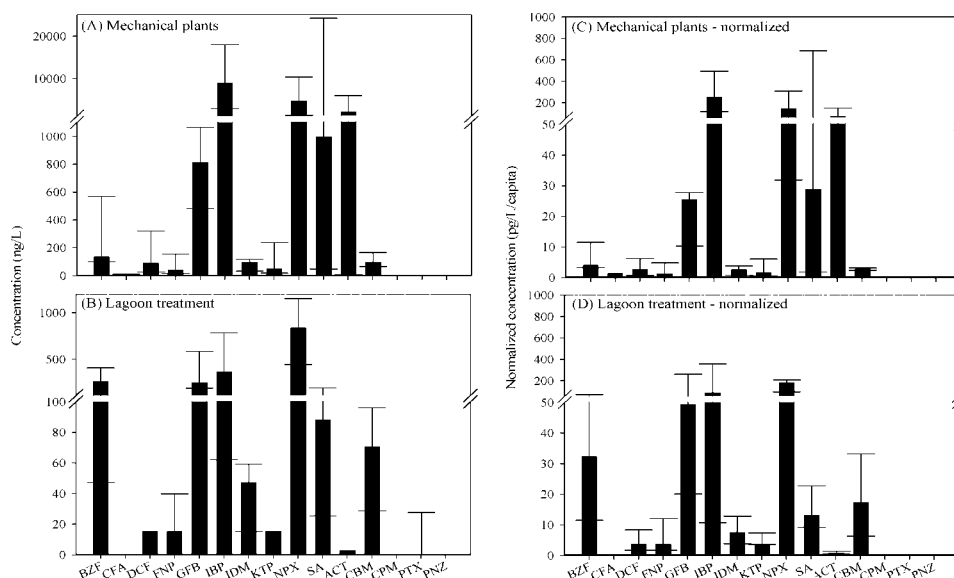


Fig. 3. Median final effluent concentration of acidic and neutral drugs in eight sewage treatment plants (STPs) in Atlantic Canada: (A) mechanical plants, (B) lagoon treatment, (C) mechanical plants normalized on per-capita basis, and (D) lagoon treatment normalized on per-capita basis. Error bars correspond to the 25th and 75th percentiles. Refer to Figure 2 for definitions of first 12 drug acronyms. CPM = cyclophosphamide; PTX = pentoxifylline; PNZ = phenazone.

Table 5. Results for acidic and neutral drugs, and physicochemical parameters in the receiving waters downstream of sewage treatment plants (STPs) serving eight municipalities in Atlantic Canada^a

STP	Acidic drugs (ng/L)									
	BZF	CFA	DCF	FNP	GFB	IBP	IDM	KTP	NPX	SA
Spring										
Halifax (NS, Canada)	ND	ND	ND	ND	ND	85	ND	ND	47	ND
Fredericton (NB, Canada)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Charlottetown (PE, Canada)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Grand Falls–Windsor (NL, Canada)	ND	ND	ND	ND	ND	ND	ND	ND	36	ND
Sussex (NB, Canada)	ND	ND	ND	ND	ND	ND	ND	ND	40	ND
Springhill (NS, Canada)	ND	ND	ND	ND	110	85	ND	ND	390	57
Summerside (PE, Canada)	180	ND	ND	ND	210	210	ND	ND	220	57
Gander (NL, Canada)	ND	ND	89	ND	47	4,200	ND	79	4,500	850
Median	15	—	15	—	15	150	—	15	44	15
Summer										
Halifax (NS, Canada)	ND	ND	ND	ND	ND	ND	ND	ND	51	ND
Fredericton (NB, Canada)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Charlottetown (PE, Canada)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Grand Falls–Windsor (NL, Canada)	ND	ND	ND	ND	ND	95	ND	ND	53	ND
Sussex (NB, Canada)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Springhill (NS, Canada)	ND	ND	ND	ND	310	270	ND	ND	250	ND
Summerside (PE, Canada)	ND	ND	ND	ND	420	75	ND	ND	160	ND
Gander (NL, Canada)	470	IN	ND	IN	580	6,400	150	ND	4,300	17,000
Median	15	—	—	—	15	45	15	—	52	15

^a Sampling was conducted in spring and summer of 2003. For calculation of the median, nondetect (ND) values were replaced with values half the method detection limit. IN = interference, *T* = temperature; SC = specific conductance; TOC = total organic carbon; HU = Hazen units. Refer to Table 2 for the list of drug acronyms.

negatively correlated ($p < 0.05$) with population. We do not have a plausible explanation for this finding.

In comparing final effluent concentration profiles with sales volumes, it is evident for some drugs that no direct correlation exists with amounts dispensed. According to figures for the year 2001, the order of Canadian drug sale volumes was as follows: ACT > ASA > IBP > NPX > CBM > diclofenac > GFB > indomethacin, with annual sales volumes ranging between approximately 1,000 metric tons for ACT down to approximately one metric ton for indomethacin [33]. Our high drug residue concentrations for IBP and NPX seem to agree with the high amounts dispensed, but the present results for ACT and ASA (SA) do not follow the market trend (assuming no major changes in drug sale trends or patterns in 2003 compared to 2001). The presence of various drugs and their metabolites in the untreated waste stream (influent) undoubtedly is influenced mostly by pharmacokinetics; thus, it would be reasonable to expect a direct association with the amounts dispensed. However, as indicated earlier, the efficiency of STPs in removing pharmaceutical residues from waste streams may vary depending on several factors associated with the type and combination of treatment used and resilience to degradation of the drug compounds.

Acetaminophen was not detected in any effluents of the lagoon treatment systems, but high concentrations were found in some of the large mechanical STPs, such as Fredericton and Charlottetown (Table 4 and Fig. 3). The HRM (Mill Cove) STP, which was the largest plant of the group, with secondary treatment, did not have any residues of ACT in its final effluents. The data suggest that some plants, generally those with lagoon treatment and high hydraulic retention times (16.5 – 48 d), were more efficient than others at removing ACT. The rates of elimination for IBF and NPX in other Canadian STPs were reported higher in STPs with hydraulic retention times greater than 12 h [13]. Acetaminophen, like SA, has been reported to degrade in sewage [15,16]. However, in the present

study, anomalously high residue concentrations up to 9.0 µg/L were observed in several mechanical plant effluents, which is not inconsistent with its high sales volume. In comparing normalized (i.e., population) median effluent concentrations of these drugs, there does not appear to be any appreciable variations between the lagoon and mechanical plant treatment systems, with the possible exception of bezafibrate, ACT, and CBM (Figs. 3C and D). We have explained already that ACT is unstable. The differences for bezafibrate and CBM may be attributable to different use patterns or to more efficient removal by a particular treatment system. Carbamazepine was detected in the majority of samples, but at significantly lower concentrations compared with those reported in other Canadian and German STPs. Carbamazepine seems to be persistent in sewage treatment systems [13,18].

The final effluents were analyzed for six physicochemical parameters (Table 4). These parameters are useful in characterizing the quality of the effluent and, to some extent, the general performance of the STP [34]. The temperature of the effluents from the eight STPs varied between 5.0 and 11.5°C, with a median of 9.7°C, for the spring samples and from 17.8 to 19.8°C, with a median of 18.6°C, for the summer samples. Temperature was negatively correlated with SA, and it is believed to contribute, in combination with other factors previously mentioned, to its degradation (Appendix; SETAC Supplemental Data Archive, Item ETC-25-08-003; <http://etc.allenpress.com>). The median observed pH was 7.2 for both the spring and summer effluents, falling within the Canadian Federal Wastewater-Quality Guidelines [35]. The correlation ($p < 0.05$) of pH with IBP (SROCC, 0.880) and SA (SROCC, 0.880) in the mechanical STPs suggests that lower pH may play a role in the removal of these two high-use compounds (Appendix; SETAC Supplemental Data Archive, Item ETC-25-08-003; <http://etc.allenpress.com>). This observation was not apparent in the lagoon treatment systems, probably for the same reasons mentioned above and higher sewage retention

Table 5. Extended

Neutral drugs (ng/L)					Physicochemical tests				
ACT	CBM	CPM	PTX	PNZ	T (°C)	pH	SC (μS/cm)	TOC (mg/L)	Color (HU)
ND	ND	ND	ND	ND	4.9	7.6	40,100	1.7	18
ND	ND	ND	ND	ND	7.3	6.8	34.8	4.2	39
ND	ND	ND	ND	ND	—	8.1	39,900	1.3	22
ND	ND	ND	ND	ND	—	6.9	39.8	4.2	38
ND	ND	ND	ND	ND	6.9	7.3	148	2.3	20
ND	62	ND	IN	ND	7.6	8.0	799	3.0	18
ND	ND	ND	IN	ND	3.0	7.7	569	1.8	32
2,300	ND	ND	IN	ND	7.5	6.8	313	5.1	51
5	10	—	—	—	7.1	7.5	441	2.7	27
ND	ND	ND	ND	ND	11.8	7.7	42,000	3.7	13
ND	ND	ND	ND	ND	18.6	7.3	68.7	3.2	22
33	ND	ND	ND	ND	16.9	7.8	41,100	2.5	12
ND	ND	ND	ND	ND	—	6.9	39.7	4.5	24
ND	ND	ND	ND	ND	16.3	7.5	356	2.5	12
ND	170	ND	ND	ND	17.8	7.3	579	4.1	14
ND	40	ND	ND	ND	11.9	7.4	709	3.9	12
3,600	ND	ND	ND	ND	—	6.7	306	7.5	59
5	10	—	—	—	16.6	7.3	468	3.8	14

time. The median specific conductance was 705 and 619 μS/cm for the spring and summer sampling period, respectively. The consistently higher conductance in the HRM (Mill Cove) and Charlottetown effluents most likely is caused by infiltration of salt water into the sewage stream or industrial inputs.

In the lagoon treatment systems, conductance is negatively correlated ($p < 0.05$) with population (SROCC, -0.830) and average daily flow (SROCC, -0.781) (Appendix; SETAC Supplemental Data Archive, Item ETC-25-08-003; <http://etc.allenpress.com>). A dilution effect, perhaps because of higher gray-water use, larger storm-sewer drainage, or a cleaner (i.e., lower in mineral content) source of water in the more populated areas, may have contributed to this observation. Biochemical oxygen demand generally is associated with TOC and TSS, depending on treatment systems and types/combination of source inputs (e.g., residential, institutional, and industrial) that may affect the composition of a sewage stream. This is clearly apparent from the correlation between these parameters in the lagoon STP effluents (Appendix; SETAC Supplemental Data Archive, Item EIC-25-08-003; <http://etc.allenpress.com>). For example, high concentrations of IBP are observed in lagoon STP effluents that are relatively high in BOD, TOC, and TSS, which is an indication that plants having difficulties in removing BOD and TSS may not be removing IBP effectively. Two studies have reported that Canadian and German STPs removed 87 and 90% of IBP, respectively, from municipal sewage [15,16]. The ramification is that STPs not operating optimally, even on a sporadic basis, may be removing much less than 90% of this high-use drug, thus resulting in the unnecessary discharge of higher residue concentrations to the aquatic environment. In addition to IBP (SROCC, 0.738), GFB (SROCC, 0.857) also is correlated ($p < 0.05$) with TSS, indicating that the two drugs are, in some way, associated with the concentration of suspended particles. We assume that the weakly sorbed (or conjugated) drugs may be stripped from the suspended solids during filtration of the sample or by the sample acidification step of small particles passing through the glass-fiber filter. Reported TSS levels exceed the Canadian

Federal Wastewater-Quality Guideline of 25 mg/L in 50% of samples and the BOD guideline of 20 mg/L in 31% of samples [35].

Drug residue concentrations in receiving waters

Receiving waters were sampled upstream and downstream from the point of discharge of STPs. A few of the high-use NSAIDs were detected in upstream samples, but only at very low frequency and concentration: Summerside (SA, 34 ng/L; IBP, 48 ng/L), Sussex (NPX, 93 ng/L), and Gander (SA, 33 ng/L; ketoprofen, 67 ng/L). The unexpected occurrence of these drug residues may be the result of residential dwellings still not connected to the sewer system, leakage of the lagoon system itself, and the possibility of false detects at levels near the MDL. Salicylic acid is a natural constituent in some plants, so this could be another source of input to streams. Results for acidic and neutral drugs in downstream waters, including physicochemical parameters, are presented for the spring and summer sampling periods in Table 5. In general, the frequency of detection of drugs compared to final effluents is lower, but the high-use drugs IBP and NPX still predominate. It should be noted that the methodologies for the present study generally are not as sensitive as some of the higher-resolution techniques, such as LC/MS/MS, and this most likely is a contributing factor to the lower detection frequency of drugs in receiving streams. Clofibric acid, fenoprofen, cyclophosphamide, pentoxifylline, and phenazone were not detected in any of the samples. This was expected, however, because they either were not observed or were present at very low levels in the STP sources.

The highest median concentration observed was for IBP in the spring, and the second highest was for NPX in the summer (Table 5). Relative to the final effluents, receiving water concentrations were approximately 70 to 85% lower. The median concentrations for the other drugs were below the MDL. The extent of effluent dilution in the receiving stream is very important in terms of assessing exposure. This is highlighted in Figure 4, which illustrates median drug concentrations ob-

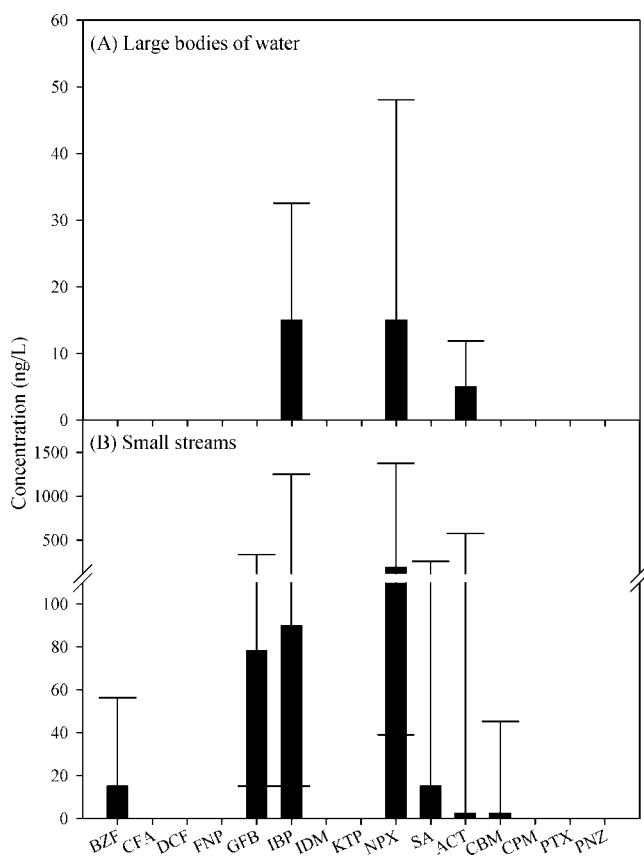


Fig. 4. Median residue concentration of acidic and neutral drugs in receiving waters of eight sewage treatment plants (STPs) in Atlantic Canada: (A) large bodies of water (STPs: Halifax Regional Municipality, Fredericton, and Charlottetown), and (B) small streams (STPs: Grand Falls–Windsor, Gander, Sussex, Springhill, and Summerside). Error bars correspond to 25th and 75th percentiles. Refer to Figures 2 and 3 for definitions of drug acronyms.

served in the large bodies of water versus those in the small streams. Median concentrations (calculated after substitution of ND values by half the MDL) of NPX, IBP, and ACT were observed at levels below the respective MDLs in the large bodies of water (Fig. 4A). In the small streams, several drug compounds were detected, including NPX, IBP, GFB, bezafibrate, SA, ACT, and CBM at median concentrations up to approximately 200 ng/L (Fig. 4B). It is noteworthy that 75th percentile concentrations approached and exceeded 1,000 ng/L for NPX, IBP, and ACT. As mentioned, this is particularly interesting from the point of view of exposure, which would be significantly greater in terms of concentration in the small receiving streams compared to the high dilution volumes of the Saint John and Hillsborough rivers—or, for that matter, Bedford Bay/Basin. The downstream waters were arbitrarily sampled 100 m below the point of effluent discharge, and further dilution combined with degradation would be expected as the effluent plume moves downstream. The half-life of drug residues in the small stream may be affected by various factors, such as natural stream flow (e.g., dilution and mixing), landscape characteristics (e.g., drainage area, soil/sediment type, and groundwater input), and chemical–biological degradation (e.g., photolysis) [5,18]. Drug compounds with relatively high $\log K_{ow}$ (i.e., >3.0–3.5) (Table 2), for example, would have a propensity to adsorb onto organic particles and settle at the bottom of the stream or, possibly, undergo biological uptake into fatty tissue of plants and animals.

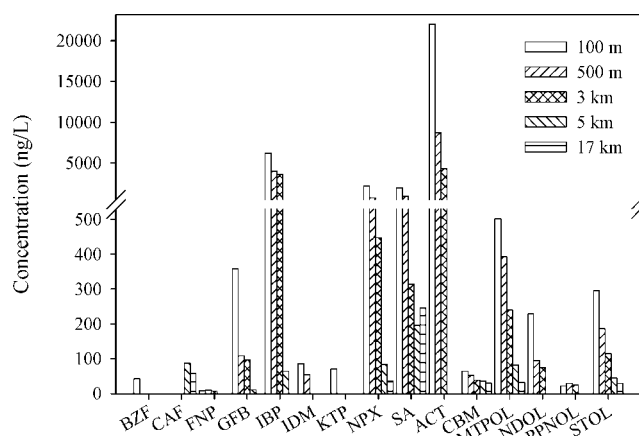


Fig. 5. Concentration gradient of acidic and neutral drug residues along a small stream receiving effluents from the Gander (NL, Canada) sewage treatment plant in July 2004. The stream was sampled at 0.1, 0.5, 3, 5, and 17 km downstream. Several drugs were added to the suite of pharmaceuticals: Caffeine (CAF) and the β -blockers metoprolol (MTPOL), nadolol (NDOL), propranolol (PPNOL), and sotalol (STOL). Refer to Figures 2 and 3 for definitions of acidic and neutral drug acronyms.

In the small stream at Gander, seven drug compounds were detected in both the spring and summer samples. The concentrations in the spring samples ranged between 47 ng/L for GFB and 4,500 ng/L for NPX, and in the summer samples, concentrations ranged between 150 ng/L for indomethacin and 17,000 ng/L for SA. The STP effluent discharge at this site constitutes most of this small stream's flow with little dilution. This is reflected by the slightly lower conductance and TOC results and the comparable pH in the downstream samples as compared to the STP effluents (Table 5). Consequently, the observed drug concentrations in the stream samples were slightly lower than those in the effluent samples (Table 4). It is interesting that SA concentration dropped significantly, from 11,000 ng/L in the spring effluent to 850 ng/L in the downstream sample (factor of 12), whereas it decreased only from 26,000 ng/L to 17,000 ng/L in the summer (factor of 1.5), another indication that SA is unstable.

The receiving stream for the Gander STP was sampled at five sites up to a distance of 17 km in July 2004. The concentration gradient for the acidic and neutral drugs along the stream is illustrated in Figure 5. Caffeine and five β -blockers were added to the existing group of drugs tested. In total, six drugs were found to have persisted in samples as far as 17 km downstream from the STP: Caffeine, NPX, SA, CBM, metoprolol, and sotalol. Individual drug concentrations dropped gradually along the stream course with the exception of CBM, for which the relative rate of decrease was more gradual. This concurs with its relative persistence reported in wastewater [5,16,18]. Caffeine also was detected at 5 and 17 km, although interferences with the analytical procedure prevented its reporting in the three upstream samples. Pindolol (not shown in Fig. 5), another β -blocker, was not detected in any of the samples. This compound would seem to be either highly degradable or a low-use drug, because it was not detected in the 100-m sample. Gemfibrozil and IBP both disappeared between 5 and 17 km, and ACT, with an initial (100-m) concentration of 22,000 ng/L, disappeared abruptly between the 3- and 5-km sampling locations. The present study suggests that various drugs may persist in small streams at significant distances downstream from STP sewage outfalls, that the present results

Table 6. Results of acute and chronic toxicity tests

Test compound	Acute toxicity ($\mu\text{g/L}$)		Chronic toxicity ($\mu\text{g/L}$)	
	<i>Daphnia magna</i> ^a	Bacteria (<i>Vibrio fischeri</i>) ^b	<i>Ceriodaphnia dubia</i> ^c	<i>Selanastrum capricornutum</i> ^d
Acetaminophen	>32	>437	>32	>32
Ibuprofen	>32	>500	>32	>32 ^e
Naproxen	>32	>451	>32	>32
Salicylic acid	>32	>446	>32	>32

^a 48-h concentration causing a 50% decrease in survival.

^b 15-min concentration causing inhibition of light by 50%.

^c 7-d concentration causing inhibition of the number of living offspring by 25%.

^d 72-h concentration causing inhibition of the number of living algal cells by 25%.

^e The ibuprofen 72-h lowest-observed-effect concentration for growth was 32 $\mu\text{g/L}$.

most likely are typical of small STPs using low-level treatment and discharging into low-flow streams, and that the continual replenishment of small streams with contaminated sewage implies the de facto presence and continual exposure of these aquatic ecosystems to pharmaceuticals, the effects of which remain mostly unknown.

Acute and chronic toxicity of high-use drugs

The results of the toxicity tests are summarized in Table 6. For the acute tests using the crustacean *D. magna* and the bacterium *V. fischeri*, no toxic effects were observed, even at the highest concentrations tested (32 and 500 $\mu\text{g/L}$, respectively). In the chronic toxicity tests, results indicate no toxic effects on *C. dubia* survival/reproduction or on algal growth (*S. capricornutum*), even at the highest concentrations tested (32 $\mu\text{g/L}$), with one exception. The IBP algal growth test resulted in a no-observed-effect concentration of 10 $\mu\text{g/L}$ and a lowest-observed-effect concentration of 32 $\mu\text{g/L}$. The effects (23.3% growth inhibition compared with the control population) were not strong enough to calculate an IC25. The toxicity of IBP, NPX, and SA to *D. magna* and several algal species have been reported previously [36–38]. Our toxicity data, presented in Table 6, appear to be in agreement with other reported toxicity values for these compounds. A possible exception is IBP, for which it appears that the algal species we used (*S. capricornutum*) might be more sensitive to IBP than the algal species (*Desmodesmus subspicatus*) used in another study, which reported a 20% effective concentration of 102,700 $\mu\text{g/L}$ [36]. To put these results in perspective, the median (and highest) concentration of each test substance detected in the receiving waters downstream of the eight STPs measured in the present study (based on results for the year 2003) were as follows: ACT, 0.005 (3.6) $\mu\text{g/L}$; IBP, 0.045 (6.4) $\mu\text{g/L}$; NPX, 0.050 (4.5) $\mu\text{g/L}$; and SA, 0.015 (17) $\mu\text{g/L}$. Using the highest concentration of each test substance detected in the receiving waters and the lowest toxic result for the four tests conducted on each test substance, we can calculate conservative safety factors between measured values and toxic effects levels for each test substance as follows: ACT, >9.0; IBP, >5.0; NPX, >7.4; and SA, >1.9. It would be more realistic to use the far lower median values in calculating a safety factor between measured values and toxic effects levels, but the above calculations demonstrate that effects on invertebrates, bacteria, and plants in the receiving environments are unlikely based on the results of the tests used in the present study.

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