

Occurrence of Antimicrobials in the Final Effluents of Wastewater Treatment Plants in Canada

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To investigate the occurrence of antimicrobials in the final effluents from wastewater treatment plants (WWTPs) in Canada, analytical methods were developed or modified from previously described methods using solid-phase extraction followed by liquid chromatography–electrospray ionization tandem mass spectrometry. Thirty-one antimicrobials from the macrolide, quinolone, quinoxaline dioxide, sulfonamide, and tetracycline classes were investigated in the final (treated) effluents from eight WWTPs, located in five Canadian cities. Ciprofloxacin, clarithromycin, erythromycin-H₂O, ofloxacin, sulfamethoxazole, sulfapyridine, and tetracycline were frequently detected in the effluents. The detection of sulfapyridine in effluents is the first report of this compound in environmental samples. Antimicrobials used exclusively for veterinary applications or treatment of livestock, such as carbadox, olaquinox, and chlortetracycline were not detected in the WWTP final effluents. There appear to be differences in the relative concentrations of antimicrobials detected in WWTP final effluents in Canada relative to concentrations reported previously in northern Europe, particularly for quinolone and sulfonamide compounds. These data may reflect differences in prescription patterns in Canada and northern Europe. The antimicrobials frequently detected in WWTP effluents appear to be those prescribed heavily in Canada for medical applications, and these compounds should be considered priority compounds for monitoring in surface water near WWTP discharges. The concentrations of antimicrobials detected in WWTP final effluents did not exceed 1 µg/L; levels that are unlikely to affect the growth and survival of aquatic organisms.

Introduction

The occurrence and biological impacts of pharmaceutically active compounds in the environment is an emerging issue (1, 2). The concern over the release of antimicrobials into the environment is related primarily to the potential for the development of antimicrobial resistance among microorganisms (3, 4). Residues of antimicrobials may also be directly toxic to microorganisms (5). Antimicrobials are used for the therapeutic treatment of bacterial diseases in humans, and

some are also applied to animals such as cattle, swine, poultry, and fish for growth promotion and for disease prophylaxis and treatment. Antimicrobials used to treat humans in hospitals or by prescription are ultimately excreted into domestic sewage and are discharged to wastewater treatment plants (WWTPs). Treatment of raw wastewater (which includes a mix of domestic sewage, industrial wastewater, and stormwater runoff, depending on the WWTPs) may remove a proportion of these compounds, but there is the potential for residues of antimicrobials to be released in treated effluent into the aquatic environment (1).

The classes of antimicrobials included in this study include the macrolides, quinolones, quinoxaline dioxides, sulfonamides, and tetracyclines (Table 1). The lactam class of antimicrobials, including penicillins and cephalosporins, are used for treatment of both humans and animals. However, due to the chemically unstable β-lactam ring, members of the lactam class of antimicrobials readily undergo hydrolysis (6, 7). These compounds were not detected in WWTP effluent, surface water, or groundwater samples in Germany, as reported by Hirsch et al. (8). Trimethoprim is an antimicrobial compound commonly used to treat both humans and animals. However, we previously reported the distribution of trimethoprim in WWTP effluents and adjacent surface water (9). Therefore, penicillins, cephalosporins, and trimethoprim were not included in the present study.

Macrolides are produced by various *Streptomyces* strains and are used for treatment of both humans and animals. Quinolones are used to treat a wide variety of bacterial infections in humans and are also used to treat livestock and fish in the aquaculture industry (10, 11). The class of quinoxaline dioxide antimicrobials includes quinoxin, carbadox, cyadox, and olaquinox. Quinoxin has been removed from the market because of its photoallergic properties (12). Carbadox has been used in the treatment and prevention of porcine infectious diseases and as a growth promoter for swine (13). Olaquinox is used for similar purposes in the swine industry. Sulfonamides have become the most widely used class of antimicrobials in the world since their development in 1968 (14, 15). Sulfonamides are widely used for both humans and livestock. Some sulfonamide residues are of concern because of their potential carcinogenicity. For instance, sulfamethazine is a thyroid carcinogen (15). Since the first member, chlortetracycline, was developed in 1984, eight tetracyclines have been developed for clinical use (16). These compounds are currently used for treatment of livestock and in aquaculture.

In 1980s, Watts et al. (17) reported the presence of several antimicrobials (such as erythromycin, sulfamethoxazole, and tetracycline) in river water samples. Since then, a variety of methods have been developed for the analysis of antimicrobials in environmental samples (18–22). These methods have been used to investigate the occurrence and distribution of antimicrobials in Europe (23, 24). However, except for data on selected antimicrobials as part of a survey of pharmaceuticals and endocrine disruptor substances in surface water in the United States (25), there are few data from North America. In particular, there are no data on the discharges of antimicrobials in domestic sewage in North America. The objective of this study was to obtain data on antimicrobial residues in the final effluents from WWTPs in Canada. These data will direct future studies on the fate of antimicrobials in the aquatic environment, including surface water and groundwater. In this study, 31 antimicrobials belonging to the macrolide, quinolone, quinoxaline dioxide, sulfonamide and tetracycline classes were investigated in

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TABLE 1. Antimicrobials Investigated in the Effluents of WWTPs in Canada

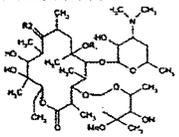
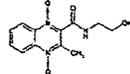
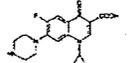
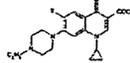
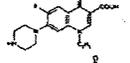
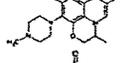
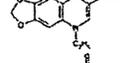
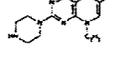
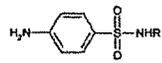
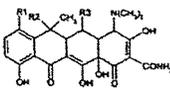
Class	antimicrobial	CASRN*	Structure
	Clarithromycin	81103-11-9	$R_1=CH_3, R_2=O$
	Erythromycin	114-07-8	$R_1=H, R_2=O$
	Roxithromycin	80214-83-1	$R_1=H,$ $R_2=NOCH_2OCH_2CH_2OCH_3$
	Quinoxaline-di-oxides	Carbadox	6804-07-5
	Olaquinox	23696-28-8	
Quinolones	Ciprofloxacin	85721-33-1	
	Enrofloxacin	93106-60-6	
	Norfloxacin	70458-96-7	
	Ofloxacin	82419-36-1	
	Oxolinic acid	14698-29-4	
	Pipemidic acid	51940-44-4	
	Sulfacetamide	144-80-9	
	Sulfachloropyridazine	80-32-0	
	Sulfadiazine	68-35-9	
	Sulfadimethoxine	122-11-2	
	Sulfaguanidine	57-67-0	
	Sulfamerazine	127-79-7	
	Sulfamethazine	57-68-1	
	Sulfamethizole	144-82-1	
	Sulfamethoxazole	723-46-6	
	Sulfamethoxypyridazine	80-35-3	
	Sulfamoxole	729-99-7	
	Sulfapyridine	144-83-2	
	Sulfaquinoxaline	59-99-7	
	Sulfathiazole	72-14-0	
	Sulfisomidin	513-64-0	
Sulfisoxazole	127-69-1		

TABLE 1 (Continued)

Class	antimicrobial	CASRN ^a	Structure		
Tetracyclines		Chlortetracycline	Cl	OH	H
		Doxycycline	H	H	OH
		Oxytetracycline	H	OH	OH
		Tetracycline	H	OH	H

^a Chemical Abstracts Service Registry Number.

TABLE 2. Operational Parameters and Sampling Dates for WWTPs Sampled in Five Canadian Cities in 2002

plant ID	population served	1st treatment		2nd treatment	hydraulic retention (h)	solids retention (d)	design flow (m ³ /d)	disinfection method	sampling date (mm/dd/yy)
A	720 000	+	+		23	na ^a	356 000	UV	10/08/2002
B	180 000	+	+		10	na	97 000	UV	10/08/2002
C	850 000	+		trickling filters, solids contact, secondary clarification	7 ^b	1.6–2.4	580 000 ^b	chlorine, seasonal	10/15/2002
D	575 000	+		none	2 ^c		574 000 ^c	none, deep sea outfall	10/21/2002
H	120 000	+		activated sludge	12–20	4–8	64 000	chlorine, seasonal	05/06/2002
I	630 000	+		activated sludge	12–18	4–6	55 000	chlorine, seasonal	04/24/2002
J	79 000	+		activated sludge	15–22	6–10	64 000	UV, seasonal	07/16/2002
K	123 000	+		none	8–12	-	164 000	Chlorine, seasonal	07/19/2002

^a na, data not available. ^b Average annual flow conditions reported. Peak wet weather HRT = 3.7 d and flow = 1 088 640 m³/d. ^c Average annual flow conditions reported for 2002.

the final effluents of eight WWTPs sampled in 2002 in five Canadian cities. Solid-phase extraction (SPE) methods were developed to extract the antimicrobial compounds from the effluents. Analytical methods based on liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS) were either adapted from previously published methods or (in the case of macrolide, quinolone, and quinoxaline dioxide compounds) were developed for this study.

Experimental Section

Reference Standards. Clarithromycin, roxithromycin, carbadox, norfloxacin, oxolinic acid, piperidic acid, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguandine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypridazine, sulfamoxole, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisoxazole, chlortetracycline, doxycycline, oxytetracycline, and tetracycline were purchased from Sigma (St. Louis, MO). Erythromycin, olaquinox, ciprofloxacin, enrofloxacin, and ofloxacin were purchased from ICN Biomedicals (Aurora, OH). Sulfisomidin was purchased from Sigma-Aldrich Canada (Oakville, ON, Canada).

Anhydro-erythromycin, a major degradation product of erythromycin (26), was not commercially available, so it was generated by acidification transformation from erythromycin using the method described by Sacher et al. (27). All standards were dissolved in methanol and diluted to final stock solutions at a concentration of 20 µg/mL and then stored in a freezer. Working standard solutions were diluted from the stock solutions for sample analysis.

Sample Collection. Samples of the final effluents (7 L from each WWTP) were collected in 2002 as "grab" samples from eight WWTPs in five cities in Canada (the term cities is used for convenience as the WWTPs in the Greater Vancouver Regional District are located in two different

municipalities and serve more than one city), including the Greater Vancouver Regional District in the province of British Columbia ($n = 2$); Calgary in the province of Alberta ($n = 2$); and Burlington, Peterborough, and Windsor in the province of Ontario ($n = 4$). Five WWTPs were located in major cities with populations > 500 000. Detailed information on the WWTPs and the sampling dates are summarized in Table 2. The WWTPs had either primary or secondary treatment processes and used either chlorine or UV disinfection. Note that all WWTPs were given ID codes for the purposes of reporting of the data. All samples were collected in 4-L amber glass bottles that had been prewashed with a sequence of soap and water, distilled water, acetone, and hexane. During collection, the bottles were rinsed with sample three times before a final sample was collected. After collection, samples were either extracted immediately or were shipped to Trent University, where they were stored at 4 °C for a maximum period of 2 d before extraction; that is, 3 d since collection.

Sample Preparation. To remove suspended material, aqueous samples were vacuum filtered through 1.0-µm glass microfiber filters that had been prewashed with hexane/dichloromethane (1:1) in a Soxhlet apparatus for 2 h. After filtration, the aqueous samples were extracted for different classes of antimicrobials with Oasis HLB cartridges (Waters, Oakville, ON, Canada), which are 6-mL/500 mg hydrophilic–lipophilic balance SPE cartridges. Sample volumes of 1 L were chosen based on breakthrough tests using spiked effluent samples. Antimicrobials were extracted using one of the following two SPE methods:

Method 1: Used To Extract Macrolide Antimicrobials. SPE cartridges were preconditioned sequentially with 6 mL of acetone, 6 mL of methanol, and 6 mL of water (pH 6.0). The effluent samples (1 L) were acidified to pH 6.0 with 3.0 M H₂SO₄ and were passed through the cartridges at a rate of approximately 10 mL/min. After passage of the samples, each cartridge was eluted with three 2-mL vol of methanol. The

eluates were collected in a 10-mL test tube and were concentrated with a vacuum centrifuge and then reconstituted to 1.0 mL with methanol.

Method 2: Used To Extract quinolone, Quinolone Dioxide, Sulfonamide, and Tetracycline Antimicrobials. The SPE extraction procedure was adapted from a previously described method (28) where the chelating agent, disodium ethylenediamine tetraacetate (Na_2EDTA), was added to samples to improve recovery efficiency. Briefly, the SPE cartridges were preconditioned sequentially with 6 mL of acetone, 6 mL of methanol and 6 mL of 50 mM Na_2EDTA (pH 3.0). The effluent samples (1 L) were acidified to pH 3.0 with 3.0 M H_2SO_4 , followed by addition of Na_2EDTA (0.5 g). Samples were then passed through the SPE cartridges at a rate of approximately 10 mL/min. After passage of the samples, each cartridge was eluted with three 2-mL volumes of methanol. The eluates were collected in a 10-mL test tube, concentrated with a vacuum centrifuge, and then reconstituted to 1.0 mL with 20% aqueous methanol.

Analytical Methods. Chromatographic separation of analytes was conducted using an Alliance 2690 liquid chromatograph (Waters, Milford, MA). The flow rate was 0.2 mL/min at room temperature, and the injection volume was 20 μL . Mass spectrometry was performed using a Quattro LC tandem quadrupole mass spectrometer (Micromass, Manchester, U.K.) equipped with a Z-Spray electrospray ionization (ESI) source and operated in positive-ion mode. Nitrogen was used as the drying and nebulizing gas at flow rates of 500 and 70 L/h, respectively. The collision-induced dissociation was carried out using 1.0×10^{-3} mbar argon in a hexapole collision cell. MassLynx v 3.5 software was applied for data acquisition and processing. The mass spectrometer was operated in selected reaction monitoring (SRM) mode with unit resolution on both of the first and second analyzers. A dwell time of 200 ms per ion pair was used, and the inter-channel delay was 10 ms. Table 3 summarizes the optimized ESI-MS/MS conditions for analysis of antimicrobials.

Method 1 for Macrolide Antimicrobials. The three macrolides were separated with a Genesis C_{18} column (2.1 \times 50 mm, 3 μm) (Jones Chromatography Lt., Hengoed, Mid Glamorgan, U.K.). Acetonitrile (A) and 20 mM aqueous ammonium acetate (0.05% formic acid, pH 5) (B) were used as mobile-phase solvents. The gradient was increased from 35 to 75% A in 5 min, then ramped to 100% in 2 min, and held at 100% A for 2 min. The source and desolvation temperatures were 90 and 350 $^\circ\text{C}$, respectively.

Method 2 for Quinolone and Quinoxaline Dioxide Antimicrobials. The eight analytes were separated with a Genesis C_{18} column (2.1 \times 150 mm, 3 μm) (Jones Chromatography Lt., Hengoed, Mid Glamorgan, U.K.). Acetonitrile (A) and 20 mM aqueous ammonium acetate (0.1% formic acid, pH 4.0) (B) were used as mobile-phase solvents. The same mobile-phase solvents as in method 1 were used in this method. The gradient was increased from 12–55% A in 8 min to 100% in 2 min, and then held for 2 min at 100% A. The source and desolvation temperatures were 90 and 350 $^\circ\text{C}$, respectively.

Method 3 for Sulfonamide Antimicrobials. The 16 sulfonamide compounds were separated with the same column and mobile-phase solvents as in method 2. The gradient was increased from 18 to 32% A in 14 min, then ramped to 100% in 1 min, and held at 100% A for 2 min. The source and desolvation temperatures were 90 and 380 $^\circ\text{C}$, respectively.

Method 4 for Tetracycline Antimicrobials. The chromatographic separation of the four tetracycline analytes was conducted on the same column as used in method 1. Acetonitrile (A) and 20 mM aqueous ammonium acetate (0.1% formic acid and 4 mM oxalic acid) (B) were used as mobile-phase solvents. The gradient was increased from 25 to 35%

TABLE 3. Optimal ESI-MS/MS Conditions for Analysis of Antimicrobials in WWTP Effluents

analyte	precursor ion, $[\text{M} + \text{H}]^+$ (m/z)	product ion (m/z)	capillary voltage (kV)	cone voltage (kV)	collision energy (eV)
clarithromycin	748	158	4.0	30	28
erythromycin- H_2O	716	158	4.0	30	31
roxithromycin	837	158	4.0	30	35
carbadox	263	231	3.0	40	12
olaquinox	264	143	3.0	25	30
ciprofloxacin	332	314	3.0	30	20
enrofloxacin	360	342	3.0	38	21
norfloxacin	320	302	3.0	40	21
ofloxacin	362	344	3.0	42	20
oxolinic acid	262	244	3.0	35	18
pipemidic acid	304	217	3.0	40	22
sulfacetamide	215	156	4.0	25	11
sulfachloropyridazine	285	156	4.0	30	16
sulfadiazine	251	156	4.0	25	18
sulfadimethoxine	311	156	4.0	35	22
sulfaguanidine	215	156	4.0	25	11
sulfamerazine	265	156	4.0	30	20
sulfamethazine	279	186	4.0	30	21
sulfamethizole	271	156	4.0	35	17
sulfamethoxazole	254	156	4.0	30	17
sulfamethoxy-pyridazine	281	156	4.0	35	20
sulfamoxole	268	156	4.0	25	16
sulfapyridine	250	156	4.0	30	20
sulfaquinoxaline	301	156	4.0	25	18
sulfathiazole	256	156	4.0	30	17
sulfisomidin	279	124	4.0	38	24
sulfisoxazole	268	156	4.0	25	16
chlortetracycline	479	444	3.5	30	26
doxycycline	445	428	3.5	30	20
oxytetracycline	461	426	3.5	25	19
tetracycline	445	410	3.5	32	19

A in 6 min, then ramped to 100% in 2 min, and held at 100% for 2 min. The source and desolvation temperatures were optimized at 90 and 380 $^\circ\text{C}$, respectively.

Quantification. Quantitative analysis of the antimicrobials was performed using LC-ESI-MS/MS with selected reaction monitoring (SRM). The optimal conditions for MS/MS analysis of the compounds and the precursor, $[\text{M} + \text{H}]^+$, and product ions monitored in SRM mode are summarized in Table 3. Electrospray ionization tandem mass spectrometry is susceptible to suppression or enhancement of ion signals as a result of matrix effects induced by sample co-extractives. In the absence of stable isotope-labeled surrogate standards for quantitation, we prepared a series of standard solutions ($n=5$) by spiking the analytes into each of the filtered effluent samples under investigation and these samples were extracted by SPE and analyzed by LC-ESI-MS/MS. Analytical data from the spiked samples were used to construct standard calibration curves for quantifying the analytes in unspiked samples. Unspiked samples of each final effluent were analyzed in triplicate. These calibration curves compensated for both variations in the SPE recoveries and matrix effects that can either suppress or enhance signals with LC-ESI-MS/MS analytical instrumentation (29).

Recovery experiments with spiked samples of effluent collected from plant J on April 21, 2002, were performed to determine the precision and accuracy of the method. The method detection limit was 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. Table 4 lists recoveries and their relative standard deviations (RSDs) as well as method detection limits (MDLs) of the antimicrobials in STP effluent. Note that limits of detection (LOD) and limits of quantitation (LOQ) for the analytes will vary from sample to sample of sewage because of the complexity of the sample

TABLE 4. Percent Recoveries (\pm RSD) and Method Detection Limits (MDL) for Antimicrobials Spiked into WWTP Effluent^a

antimicrobial	% recovery (\pm RSD)	MDL (μ g/L)	antimicrobial	% recovery (\pm RSD)	MDL (μ g/L)
sulfacetamide	82 (8)	0.004	clarithromycin	73 (9)	0.001
sulfachloropyridazine	77 (7)	0.001	Erythromycin-H ₂ O	78 (8)	0.001
sulfadiazine	76 (8)	0.003	roxithromycin	87 (6)	0.001
sulfadimethoxine	78 (7)	0.001	carbadox	83 (8)	0.005
sulfaguandinine	72 (6)	0.005	olaquinox	78 (7)	0.006
sulfamerazine	79 (8)	0.003	ciprofloxacin	92 (5)	0.001
sulfamethazine	81 (7)	0.001	enrofloxacin	88 (6)	0.008
sulfamethizole	78 (10)	0.002	norfloxacin	96 (9)	0.005
sulfamethoxazole	89 (8)	0.001	ofloxacin	95 (9)	0.002
sulfamethoxy-pyridazine	75 (10)	0.001	oxolinic acid	86 (5)	0.005
sulfamoxole	80 (6)	0.001	pipemidic acid	85 (7)	0.007
sulfapyridine	90 (8)	0.001	chlortetracycline	85 (10)	0.004
sulfaquinoxaline	80 (5)	0.001	doxycycline	99 (7)	0.002
sulfathiazole	74 (6)	0.004	oxytetracycline	81 (6)	0.006
sulfisomidin	83 (9)	0.003	tetracycline	79 (8)	0.002
sulfisoxazole	80 (7)	0.001			

^a Final effluent collected from Plant J in April, 2002. Recoveries are the average of triplicate analyses of fortified concentrations of 0.2 and 1.0 μ g/L

matrix. The method of calibration described above compensates for these variations in detection limits.

Results and Discussion

Extraction. Tetracyclines tend to form strong complexes with multivalent cations and bind to protein and silanol groups (30). Chelating agents such as EDTA, oxalic acid, and citric acid are usually applied to decrease the tendency for tetracyclines to bind to cations in the matrix (31). In our study, Na₂EDTA was used as a chelating agent to extract tetracyclines together with quinolone, quinoxaline dioxide, and sulfonamide antimicrobials in one SPE process. The recoveries of quinolone antimicrobials were also improved with the addition of Na₂EDTA (28).

Analytical Methods. Time-scheduled chromatograms of standards (left panel) and examples of WWTP effluent samples (right panel) of antimicrobials are illustrated in Figures 1 and 2. The majority of the macrolide, quinolone, and tetracycline analytes were detected in sewage effluents (Figure 1), and a few of the 16 sulfonamide analytes were detected (Figure 2).

When tetracyclines are separated by liquid chromatography, oxalic acid is usually added to the mobile phase to improve resolution and peak shape (32, 33). Unfortunately, nonvolatile oxalic acid may accumulate in the ESI source when LC-ESI-MS/MS techniques are used. In LC-MS applications with an atmospheric pressure chemical ionization (APCI) source, an elevated probe temperature has been used to reduce this accumulation (33, 34) since oxalic acid decomposes to carbon dioxide and water above 200 °C. However, this technique can be applied to an electrospray ionization source with off-axis or orthogonal spray sampling configuration to reduce the buildup of residues from nonvolatile mobile-phase buffers (20). Therefore, electrospray ionization was applied in this study, and the ESI was operated at a relatively high temperature (380 °C) when tetracyclines were analyzed.

Occurrence of Antimicrobials. (I) Macrolides. Next to the penicillins, macrolides are the second most frequently prescribed class of antimicrobials in Canada, particularly clarithromycin, followed by azithromycin and erythromycin (35). In this study, erythromycin was detected in all of the WWTP final effluents examined (Table 5), while clarithromycin and roxithromycin were detected in six of the eight WWTPs. Erythromycin is readily dehydrated by loss of one water molecule and its dehydration product has been detected predominantly in the environment (8). Therefore,

the concentrations of erythromycin were reported in this study as its dehydration product, erythromycin-H₂O.

In Germany, macrolides have been detected in all WWTP effluents investigated, and erythromycin-H₂O and roxithromycin were detected at higher concentrations than reported here. However, clarithromycin was reported at similar concentrations to those measured in this study, with a median concentration of 0.14 μ g/L and a maximum concentration of 0.26 μ g/L in the German study (8). Roxithromycin and erythromycin-H₂O were detected in WWTP effluents in Germany at median concentrations of 0.68 and 2.5 μ g/L and maximum concentrations of 1.00 and 6.0 μ g/L, respectively (8). The lower median concentrations detected in WWTPs effluents in Canada as compared with those in Germany probably reflect differences in prescription patterns for macrolide antimicrobials in the two countries. Unfortunately, only the relative prescription rates of antimicrobials are available in Canada, so it is not possible to confirm this hypothesis.

A survey of streams in the United States conducted in 1999–2000 showed that the frequencies of detection of erythromycin-H₂O and roxithromycin were 21.5% and 4.8%, but clarithromycin was not included in the study (25). Erythromycin-H₂O was detected at a concentration of 0.049 μ g/L in a groundwater sample in Germany (27).

(II) Quinolones. Although quinolones are prescribed less often than macrolides, these compounds are still the fourth most prescribed class of antimicrobials in Canada (35). In particular, ciprofloxacin has dominated the Canadian and global quinolone markets since its entry in the late 1980s. Ciprofloxacin, listed as number 32 among the top prescribed medications in 2001, represented about 50% of the prescriptions of quinolones in Canada in 2000 and 2001 (35). Levofloxacin, norfloxacin, and ofloxacin are also frequently prescribed in Canada.

Reflecting the relative rates of prescription of quinolone antimicrobials in Canada, ciprofloxacin, norfloxacin, and ofloxacin were detected in WWTP effluents, with ciprofloxacin and ofloxacin most frequently detected (Table 5). Ciprofloxacin was detected even though it has been shown to be readily biodegradable in activated sludge (36). Ciprofloxacin is also a biologically active metabolite derived by de-ethylation from enrofloxacin (37, 38). However, the ciprofloxacin detected in the effluents is most probably from its direct therapeutic usage rather than from the degradation of enrofloxacin, because enrofloxacin was not detected in any of the effluents and is not a major quinolone antimicrobial

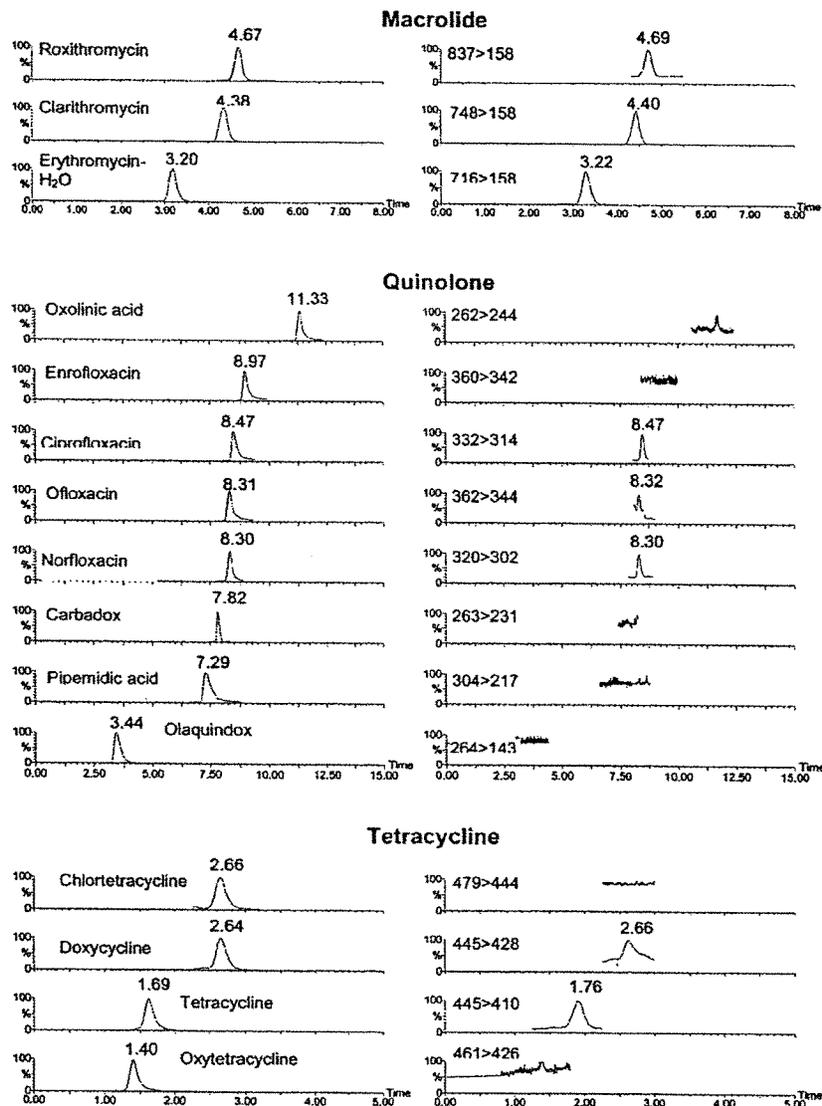


FIGURE 1. Time-scheduled SRM chromatograms of standards (left panels) and samples (right panels) of macrolides, quinolones, and tetracyclines.

prescribed in Canada (35). Oxolinic acid and pipemidic acid were not detected in any of the effluents either (Table 5), probably due to their low therapeutic usage.

Ciprofloxacin and norfloxacin were detected at concentrations of 0.045–0.108 and 0.048–0.120 $\mu\text{g/L}$, respectively, in sewage effluents in Switzerland (21). Norfloxacin was detected at similar concentrations in the present study from Canada, but ciprofloxacin was detected at higher concentrations. This may be due to different prescription rates for quinolones in Canada and Switzerland. For example, each of ciprofloxacin and norfloxacin contributes to 42–48% of the total domestic consumption of quinolones in Switzerland. However, in Canada, ciprofloxacin is much more highly prescribed than norfloxacin (35).

Campagnolo et al. (39) investigated antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. They did not detect ciprofloxacin or norfloxacin. An investigation of pharmaceuticals, including some quinolones in rivers and streams in the United States, showed that ciprofloxacin and norfloxacin were detected at very low frequencies (2.6 and 0.9%

respectively) and that enrofloxacin and sarafloxacin were not detected (25).

(III) Quinoxaline Dioxides. Carbadox and olaquinox are mainly used as growth promoters for animals rather than as human medicines. Carbadox was approved in the 1970s for use in Canada and the United States to promote growth in swine as well as to prevent and treat dysentery and other conditions. However, Health Canada announced the cease sale order in August of 2001 after receiving reports of misuse and accidental contamination. Along with carbadox and olaquinox, several other antimicrobials (e.g., chlortetracycline, penicillin, sulfamethazine, salinomycin, and tylosin) are commonly used for growth promotion of animals in Canada. Carbadox and olaquinox were not detected in any of the effluents in this study, although they are heavily used as growth promoters for animals. Carbadox was not detected in streams in the United States (25), possibly because this compound is rapidly degraded (40).

(IV) Sulfonamides. Sulfonamides are the most frequently prescribed antimicrobials for use in humans. This class is also used in animals (primarily as sulfamethazine,

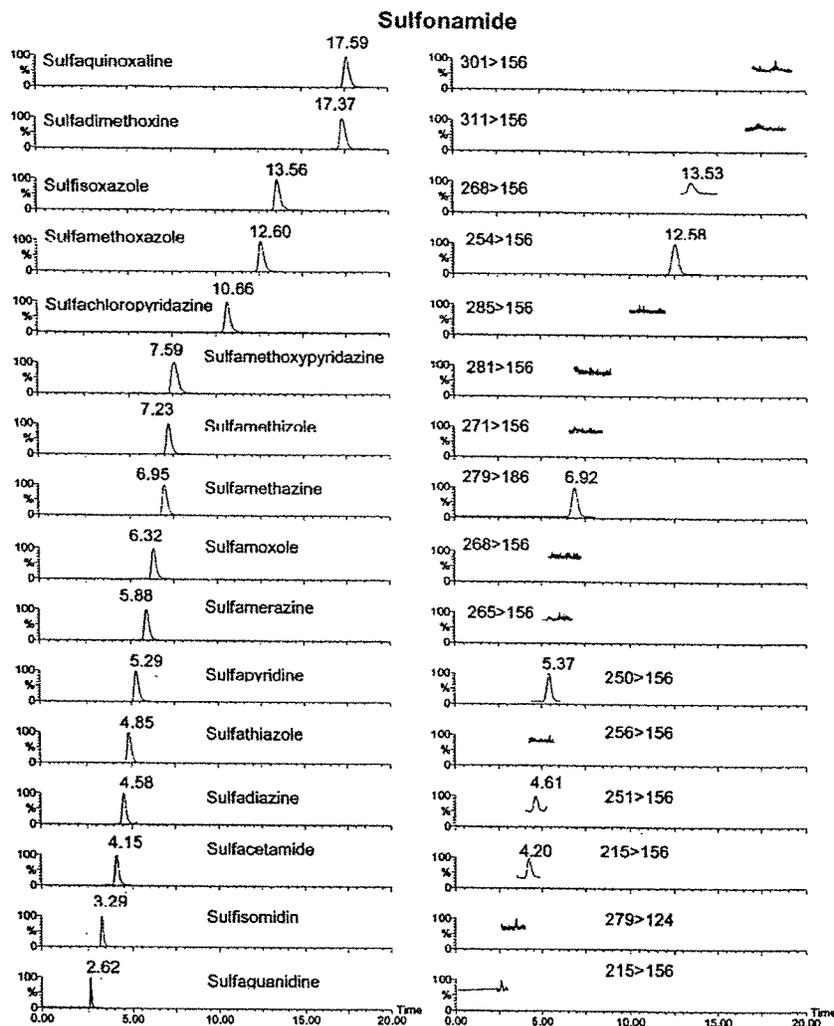


FIGURE 2. Time-scheduled SRM chromatograms of standards (left panels) and a sample (right panels) of sulfonamides.

and sulfaquinoxaline, which are licensed for use as medicating feed ingredients in Canada). Sulfonamides have a high potential to resist degradation and are hydrophilic enough to be transferred into the aquatic environment (41). Holm et al. (42) reported detecting sulfanilamide, sulfaguanidine, sulfadiazine, sulfadimidine, and sulfamethizol in groundwater downgradient of a landfill in Denmark.

In this study, sulfacetamide, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfapyridine, and sulfisoxazole were detected in at least one of the WWTP effluents examined (Table 5). In particular, sulfamethoxazole and sulfapyridine were detected in all effluents. Sulfamethoxazole has been detected frequently in sewage effluents in Germany, at a median concentration of 0.4 $\mu\text{g/L}$ and a maximum concentration of 2.0 $\mu\text{g/L}$ (8), which are generally higher than the concentrations detected in this study in Canada. In the same study in Germany, sulfamethazine was not detected in any effluents, compared with detection in only one effluent of eight reported in this study. Thirteen sulfonamides were investigated in municipal wastewater in Germany in another study by Hartig et al. (43). Sulfamethizole, sulfadiazine, and sulfamethoxazole were detected at concentrations of 0.006, 0.061, and 1.500 $\mu\text{g/L}$, respectively, in the secondary effluent of a WWTP in Berlin, which are all higher than concentrations measured in the present study on Canadian WWTP effluents.

Sulfadimethoxine, sulfamethazine, sulfamethizole, and sulfamethoxazole were detected in streams in the United States, and the frequency of detection for sulfamethoxazole was as high as 19% (25). Sulfamethoxazole was detected at concentrations of 0.030–0.085 $\mu\text{g/L}$ in surface water in Germany (43). Sulfamethoxazole is also the most frequently detected sulfonamide in groundwater and has been detected at concentrations up to 0.22 and 0.41 $\mu\text{g/L}$ in the United States (22) and in Germany (27), respectively. The high usage of the combination of sulfamethoxazole and trimethoprim contributes to the frequent detection of sulfamethoxazole. Trimethoprim is also one of the most frequently detected neutral drugs in the environment (9).

Next to sulfamethoxazole, sulfapyridine was the other most frequently detected sulfonamide in this study, but it has not been investigated previously in environmental samples, including WWTP final effluents. Sulfapyridine is used to control dermatitis herpetiformis (Dühring's disease), but it is relatively ineffective for other kinds of bacterial infections (14). In addition, sulfapyridine is a major metabolite of sulfasalazine, which is commonly used in the treatment of rheumatoid arthritis and inflammatory bowel disease (44). Sulfasalazine is a conjugate of 5-aminosalicylic acid and sulfapyridine linked by an azo bond, and sulfasalazine is metabolized by the bacterial azoreductases enzymes in the colon, reducing the azo bond and releasing these two

TABLE 5. Summary of Analytical Results for Antimicrobials in the Final (Treated) Effluents from Eight WWTPs in Five Canadian Cities

antimicrobial	no. > MDL ^{a,b}	median ($\mu\text{g/L}$)	maximum ($\mu\text{g/L}$)
Macrolides			
clarithromycin	6	0.087	0.536
Erythromycin-H ₂ O	8	0.080	0.838
roxithromycin	6	0.008	0.018
Quinolones			
ciprofloxacin	7	0.118	0.400
norfloxacin	4	0.050	0.112
ofloxacin	8	0.094	0.506
Sulfonamides			
sulfacetamide	3	0.064	0.151
sulfadiazine	1	0.019	0.019
sulfamethazine	1	0.363	0.363
sulfamethoxazole	8	0.243	0.871
sulfapyridine	8	0.081	0.228
sulfisoxazole	5	0.019	0.034
Tetracyclines			
doxycycline	2	0.038	0.046
tetracycline	7	0.151	0.977

^a Method detection limit. ^b The following antimicrobials were not detected: enrofloxacin, oxolinic acid, piperimidic acid, carbadox, olaquinoxidox, sulfachloropyridazine, sulfadimethoxine, sulfaguandine, sulfamerazine, sulfamethizole, sulfamethoxypropyridazine, sulfamoxole, sulfaminoxaline, sulfathiazole, sulfisomidine, chlortetracycline, oxytetracycline.

components (45). Further studies should focus on tracing the sources and environmental fate of this compound.

Sulfadiazine, sulfamerazine, and sulfamethoxazole have been detected in wastewater from swine operations at concentrations of 76, 77, and 69 $\mu\text{g/L}$ (46). However, these sulfonamides, except for sulfamethoxazole, were not frequently detected in the WWTP effluents from this study.

(V) **Tetracyclines.** Tetracyclines are rapidly metabolized and moreover form relatively stable complexes with metal cations (47). However, in this study, doxycycline and tetracycline were detected in the WWTP effluents, with tetracycline having the highest frequency of detection (Table 5). Chlortetracycline and oxytetracycline were not detected (Table 5). Surprisingly, none of the four tetracyclines investigated here were detected in WWTP effluents in Germany (8).

Chlortetracycline and oxytetracycline are mainly used as growth promoters for livestock. They are two of the 10 antimicrobials licensed as growth promoters for livestock in the United States (48), explaining their detection at high concentrations in wastewater lagoons on swine farms (39). Chlortetracycline, oxytetracycline, and tetracycline were detected at very low frequencies (1.2–2.4%) in streams in the United States using one analytical method and were not detected with another analytical method (25).

This study revealed the presence of several classes of antimicrobials in treated effluent discharged from the eight Canadian WWTPs studied. The proportions of different classes of antimicrobials prescribed for humans in Canada are in the following order: broad spectrum penicillins (32%), macrolides (24%), cephalosporins (16%), quinolones (11%), trimethoprim combinations (9%), and tetracyclines (8%) (35). Sulfonamides were not included in the above statistical information although they are an important class of antimicrobials. Penicillins and cephalosporins were not included in the present study because it was presumed that they degrade rapidly in WWTPs (8). Our previous studies have shown that trimethoprim is commonly detected in WWTP effluents and adjacent receiving waters in Canada (9).

Compounds from the macrolide, quinolone, and tetracycline classes were detected in all of the WWTPs sampled, reflecting their importance as antimicrobials prescribed for humans. These WWTPs were sampled in different months, from April to November 2002. Seasonal variations occur in the prescription of antimicrobials, with more prescriptions in the winter and fewer in the summer (35). Therefore, seasonal changes in consumption may have affected the occurrence of antimicrobials in this survey of Canadian WWTPs. However, WWTP effluents are constantly changing in composition in response to temporal changes in loading rates. Since no untreated (raw) effluents were sampled from the WWTPs, it was not possible to estimate the extent of removal of the antimicrobials by sewage treatment. Our previous studies have shown that pharmaceuticals are poorly removed in Canadian WWTPs with hydraulic retention times <12 h (49).

The antimicrobials detected in this study reflected human usage rather than the treatment of animals. For example, antimicrobials heavily used for veterinary applications (such as carbadox, olaquinoxidox, chlortetracycline, etc.) were not detected in the effluents. However, when monitoring surface waters and groundwater, it will be necessary to include antimicrobials used in livestock and veterinary applications since agricultural runoff may be a significant source.

Potential Impacts of Antimicrobials. The most frequently detected antimicrobials in WWTP effluents sampled in Canada included ciprofloxacin, clarithromycin, erythromycin-H₂O, ofloxacin, sulfamethoxazole, sulfapyridine, and tetracycline. The concentrations of these compounds in WWTP final effluents did not exceed 0.9 $\mu\text{g/L}$. Using a dilution factor of 1:10 recommended by the U.S. Federal Drug Administration for estimating the maximum expected concentrations in surface water from effluent data (50), the maximum concentrations of antimicrobials expected in surface water near Canadian WWTPs would be <0.09 $\mu\text{g/L}$. These estimates are consistent with our preliminary data on the concentrations of antimicrobials in samples of surface water collected near WWTPs in the lower Great Lakes region, where the highest concentration detected was 0.099 $\mu\text{g/L}$ of sulfamethoxazole (51).

The lethal concentrations (i.e., LC₅₀ values) of antimicrobials to fish and aquatic invertebrates are usually in the high milligrams per liter range (52, 53), and sublethal effects (i.e., reduced reproduction) occur in aquatic invertebrates exposed to low milligrams per liter concentrations of antimicrobials (54). Therefore, antimicrobial compounds are unlikely to induce acute toxicity in aquatic animals near sewage discharges. However, antimicrobials induce toxic effects in aquatic plants and microorganisms at micrograms per liter concentrations (5, 55, 56). For instance, the EC₁₀ values for reductions in wet weight, frond number, and chlorophyll *a* in duckweed, *Lemna gibba* exposed to sulfamethoxazole were reported as 17, 11, and 36 $\mu\text{g/L}$, respectively (55). Kümmerer et al. (56) showed that ciprofloxacin and ofloxacin induced 50% growth inhibition of the Gram-negative bacterium, *Pseudomonas putida*, at concentrations of 80 and 10 $\mu\text{g/L}$, respectively. The data presented in this study indicate that the concentrations of antimicrobials that occur in the final effluents of WWTPs and adjacent surface waters in Canada are unlikely to be high enough to impact the growth and survival of plants or bacteria. However, it cannot be ruled out that chronic exposure of bacteria and other microorganisms to antimicrobials will contribute to the development of antibiotic resistance in the environment (3).

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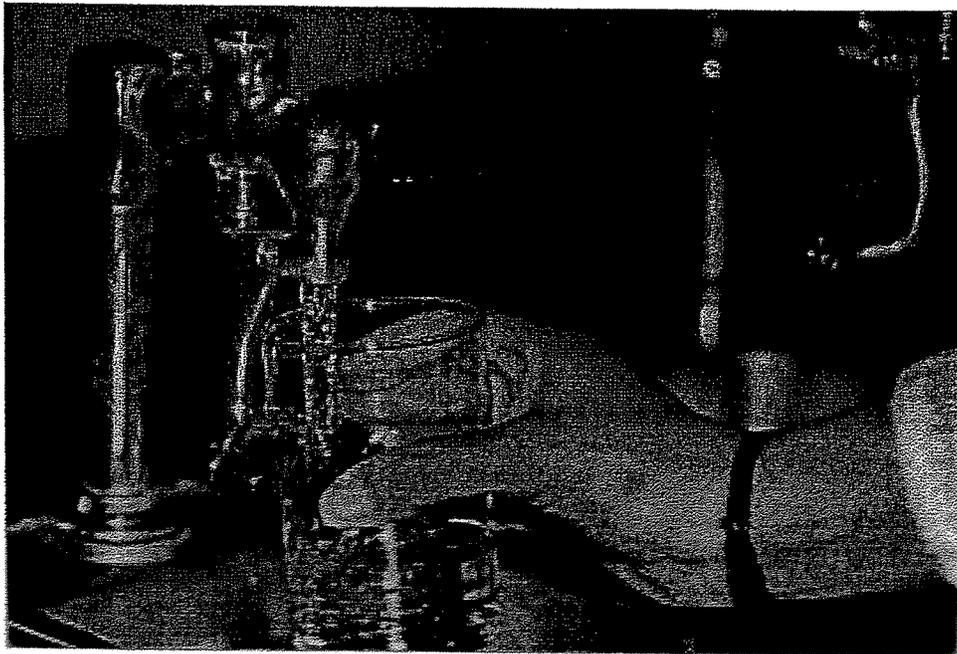
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Removal of Endocrine Disruptor Chemicals Using Drinking Water Treatment Processes



Removal of Endocrine Disruptor Chemicals Using Drinking Water Treatment Processes

I. Introduction

The purpose of this document is to provide a description of methods for the removal of endocrine disruptor chemicals (EDCs) from drinking water. Many of the potential EDCs may be present in surface waters or groundwaters. A number of drinking water treatment processes are available and may be used to remove many of the potential EDCs. This document presents treatment processes for large municipalities as well as small communities to remove specific EDCs from drinking water. References are provided with links to retrieve documents via the Internet, where available.

II. Background

A growing body of scientific research indicates that man-made industrial chemicals and pesticides may interfere with the normal functioning of human and wildlife endocrine systems. A hormone is defined as any substance in the body that is produced by one organ then carried by the bloodstream to have an effect in another organ. The primary function of hormones, or the endocrine system, is to maintain a stable environment within the body; this is often referred to as homeostasis. The endocrine system also controls reproduction and growth. Recently, public concern has focused on the possible hormonal effects of some environmental pollutants on wildlife and humans. These chemicals, referred to collectively as endocrine disruptors, comprise a wide range of substances including pesticides (methoxychlor), surfactants (nonylphenol), plasticizers (diethylphthalate), and organohalogens (PCBs and dioxin). Many industrial chemicals and pesticides have undergone extensive toxicological testing; however, since the purpose of this testing was not to find some subtle endocrine effects these potential effects may not have been revealed. The persistence of some pesticides in the aquatic environment may pose a threat to the human population, especially if such substances occur in the nation's drink-

ing water sources. As a result of this growing concern, the 1996 Safe Drinking Water Act (SDWA) Amendments and the Food Quality Protection Act require EPA to develop a screening and testing program to determine which chemical substances have possible endocrine disrupting effects in humans.

A. Endocrine Disruptor Chemicals

The term "endocrine disruptors" is used to describe substances that are not produced in the body but act by mimicking or antagonizing natural hormones. It is thought that EDCs may be responsible for some reproductive problems in both women and men as well as for the increases in the frequency of certain types of cancer. EDCs have also been linked to developmental deficiencies and learning disabilities in children. Because hormone receptor systems are similar in humans and animals, effects observed in wildlife species raise concerns of potential human health effects. During fetal development and early childhood, low-dose exposure to EDCs may have profound effects not observed in adults such as reduced mental capacity and genital malformations. Evaluating potential low-dose effects of environmental estrogenic compounds has been identified as a major research priority.

III. Descriptions of Specific EDCs

In this section, the potential EDCs are grouped by chemical class. Descriptions of the EDCs provide the Chemical Abstract Registry Number, a brief description of the chemical, its major uses, the major human exposure routes, health effects, water solubility, environmental persistence, occurrence/detection in water sources, drinking water standards, and statutes that regulate the substance in water. The best available technology (BAT) as determined by laboratory testing for removal of specific EDCs from water is indicated when this has been determined. In this document the term "BAT" is NOT used in a regulatory context. That is to say, we do not intend to suggest that the reader is obligated to use a particular technology as a regulatory requirement.

A. Pesticide Residues

A number of pesticides have been implicated as endocrine disruptors, primarily in aquatic and wildlife species. Agricultural runoff is responsible for the presence of most pesticides found in surface waters. The pesticide concentrations in surface waters tend to be highest after the first storm following application. Pesticides may also enter source water from accidental spills, in wastewater discharges, or as runoff from urban and suburban areas. Because pesticides are known to be potentially highly toxic

compounds, the maximum contaminant level (MCL) has been established for each of these substances. These limits were originally established on the basis of known toxicologic effects; however, in the future the MCLs may be set at even lower concentrations if adverse endocrine effects are detected due to their presence. Again, this document does not infer that the reader is obligated to attain an MCL, rather this information is presented to demonstrate how future research on EDCs may eventually impact some MCLs.

DDT

DDT [CASRN - 50-29-3] is an organochlorine insecticide used mainly to control mosquito-borne malaria. It is the common name of the technical product that is a mixture of three isomers of DDT and contains 65 to 80% p,p'-DDT. It is very soluble in fats and most organic solvents and practically insoluble in water. In the U.S., DDT is currently used only for public health emergencies as an insecticide under Public Health Service supervision and by the USDA or military for health quarantine. EPA banned use of DDT in food in 1972 and use in nonfoods in 1988. At present no U.S. companies are producing DDT. The primary supporting evidence for adverse health effects in humans comes from an epidemiological study performed by Rogan in North Carolina in which blood levels of DDE (a metabolite of DDT) were determined in pregnant women. Once the blood levels were determined for each woman, neurologic testing was then performed on the infants that were born from these pregnancies. A very strong correlation was found linking increased blood levels of DDE with poor performance of the neurologic tests by these infants (Rogan, 1986). Strong correlation of maternal serum levels of DDE, a metabolite of DDT, with defects in muscular tone and hyporeflexia was observed in their children. More convincing evidence of endocrine effects has been observed in an ecological setting. The initial reports were of egg shell thinning in bald eagles as well as vitellogenin (a protein that is normally only produced in the livers of female amphibians and fish) production in male African clawed frogs (Palmer and Palmer, 1995). Primary exposure routes for humans are inhalation, ingestion, and dermal contact.

In spite of the 1972 ban of DDT in the U.S., human exposure to DDT is potentially high due to its prior extensive use and the persistence of DDT and its metabolites in the environment. DDT has been detected in air, rain, soil, water, animal and plant tissues, food, and the work environment. Break-down products in the soil environment are DDE and DDD, which are also highly persistent. Due to its extremely low solubility in water, DDT is mainly retained by soils and soil fractions with higher proportions of soil organic matter. While it is generally immobile or only very slightly mobile, DDT may

leach into groundwater over long periods of time. DDT may reach surface waters primarily by runoff, atmospheric transport, drift, or by direct application. DDT has been widely detected in ambient surface water sampling in the U.S. at a median level of one nanogram/L (part per trillion). DDT is regulated by EPA under the Clean Water Act (CWA). Effluent discharge guidelines and water quality criteria have been set under the CWA.

Endosulfan

Endosulfan [CASRN - 115-29-7] is a chlorinated hydrocarbon insecticide which acts as a poison for a wide variety of insects and mites on contact. Although it may be used as a wood preservative, it is used primarily on a wide variety of food crops, including tea, coffee, fruits, and vegetables, as well as on rice, cereals, maize, sorghum, or other grains. Human exposure to endosulfan is primarily through breathing air, drinking water, eating food, or working where endosulfan is used. Exposure to endosulfan mainly affects the central nervous system. The effects of long-term/low-dose exposure are unknown. The most convincing evidence of endocrine effects in mammals is taken from laboratory animal studies in which doses of 5 mg/kg/day resulted in reduced sperm counts and altered testicular enzyme levels in male rats (Sinha, 1995).

Endosulfan has been found in at least 143 of the 1,416 National Priorities List sites identified by the EPA. Although not easily dissolved in water, when released to water, endosulfan isomers hydrolyze readily in alkaline conditions and more slowly in acidic conditions. Endosulfan has been detected at levels of 0.2 to 0.8 µg/L in groundwater, surface water, rain, snow, and sediment samples. Large amounts of endosulfan can be found in surface water near areas of application. The EPA recommends that the amount of endosulfan in lakes, rivers, and streams should not be more than 74 ppb. Humans can become exposed to endosulfan by drinking water contaminated with it.

Methoxychlor

Methoxychlor [CASRN - 72-43-5] is an organochlorine insecticide that is effective against a wide range of pests encountered in agriculture, households, and ornamental plants. It is registered for use on fruits, vegetables, and forage crops. The use of methoxychlor has increased significantly since DDT was banned in 1972. It is similar in structure to DDT, but it has a relatively low toxicity and relatively low persistence in biological systems. Methoxychlor is not highly soluble in water. Methoxychlor is highly toxic to fish and aquatic invertebrates. Levels of methoxychlor can accumulate in algae, bacteria, snails, clams, and some fish, but it is usually transformed into other substances and rapidly released from their bodies. The most

probable routes of exposure for humans are inhalation or dermal contact during home use, and ingestion of food or drinking water contaminated with methoxychlor. Short-term exposure above the MCL causes central nervous system depression, diarrhea, and damage to liver, kidney, and heart tissue. Evidence suggests that high doses of technical methoxychlor or its metabolites may have estrogenic effects.

The risk of human exposure via groundwater should be slight, but it may be greater if application rates are very high, or if the water table is very shallow. At present the strongest evidence of endocrine effects due to methoxychlor is taken from laboratory studies in which the relatively low dose of 0.5 µg/kg/day caused reduced fertility in mice (Welch, 1969).

In an EPA pilot groundwater survey, methoxychlor was found in a number of wells in New Jersey and at extremely low concentrations in water from the Niagara River, the James River, and an unnamed Lake Michigan tributary. Methoxychlor will most likely reach surface waters via runoff. Methoxychlor was detected in drinking water supplies in rural South Carolina. EPA set a limit of methoxychlor in drinking water at 0.04 ppm. EPA advises that children should not drink water containing more than 0.05 ppm for more than one day and that adults should not drink water containing more than 0.2 ppm for longer periods of time.

B. Highly Chlorinated Compounds

Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls [CASRN - 1336-36-3] are a group of manufactured organic compounds that include 209 different chemical forms known as congeners. This high number of many different chemical forms is possible because from one to ten chlorine atoms can attach to the carbon atoms that make up the basic chemical structure of this family of compounds. PCBs are thermally stable, resistant to oxidation, acids, bases, and other chemical agents. PCBs tend to be more soluble in lipid-based solvents than in water; however, among the 209 congeners there is a wide range of water solubility and lipid solubility with the lesser chlorinated congeners being more water soluble. In the environment, PCBs can be contaminated with dibenzofurans, dioxins, and polychlorinated naphthalenes. Since 1974, all PCB manufacturing has been banned and previous use in electrical capacitors and transformers has been greatly reduced. Because of their chemical-resistant properties, PCBs have persisted in the environ-

ment in large quantities despite the manufacturing ban. The primary routes of potential human exposure to PCBs are ingestion of food and water as well as through dermal contact. There is extensive human data which show a strong association of low birth weights and shortened gestation with PCB exposure in humans (Taylor, 1987 and Patandin, 1998). In addition, extensive neurologic testing of children who experienced exposure to PCBs prior to birth revealed impaired motor function and learning disorders (Jacobsen, 1996). Studies have indicated that PCBs concentrate in human breast milk.

PCB releases from prior industrial uses and the persistence of the compounds in the environment have resulted in widespread water and soil contamination. They have been found in at least 383 of the 1,430 National Priorities List sites identified by the EPA. The PCBs with a high degree of chlorination are resistant to biodegradation and appear to be degraded very slowly in the environment. PCB concentrations in water are higher for the lower chlorinated PCBs because of their greater water solubility. PCBs have been found in runoff, sediments, soil, creek water, leachate, in an underground oil-water layer, and in pond effluents. Concentrations in these locations have ranged from 4 to 440,000 µg/L. In water, small amounts of PCBs may remain dissolved, but most adhere to organic particles and sediments. PCBs in water bioaccumulate in fish and marine mammals and can reach levels several orders of magnitude higher than levels found in the water. EPA regulates PCBs under the CWA and has established water quality criteria and toxic pollutant effluent standards. Based on the carcinogenicity of PCBs, EPA published a MCL Goal for PCBs at zero and the MCL of 0.5 µg/L (0.5 ppb) under the SDWA.

Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin TCDD)

Dioxin is considered an EDC on the basis of its effects that occur during pregnancy which result in many malformations observed in the offspring of many species including humans. Dioxin [CASRN - 1746-01-6] is a contaminant formed during the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), an herbicidal compound that comprised about 50% of the defoliant Agent Orange, and 2,4,5-T derivatives, as well as other chemicals synthesized using 2,4,5-trichlorophenol. Dioxins may also be formed during incineration of chlorinated industrial compounds such as plastic and medical waste. Dioxin is one of the most acutely toxic compounds synthesized by modern chemistry. TCDD is the most toxic member of the 75 dioxins that exist and is the one most studied. It is almost insoluble in water. TCDD is stable in water, dimethylsulfoxide, 95% ethanol, or acetone. It can undergo a slow photochemical and bacterial degradation, though normally it is extremely stable. Dioxin is degraded when

heated in excess of 500°C or when exposed to ultraviolet radiation under specific conditions. TCDD has no known commercial applications but is used as a research chemical. TCDD has been found in at least 91 of 1,467 National Priorities List sites identified by the EPA. Dioxins are widespread environmental contaminants. They bioaccumulate throughout the food web because of their lipophilic properties and slow metabolic destruction. The primary source of dioxin exposure to humans is from food.

Furan

Furan [CASRN - 110-00-9] is classified as a cyclic, dienic ether; it is a colorless, flammable liquid. It is insoluble in water, but is soluble in alcohol, ether, and most common organic solvents. Furan is used primarily as an intermediate in the synthesis and production of other organic compounds, including agricultural chemicals (insecticides), stabilizers, and pharmaceuticals. The primary route of potential human exposure to furan is inhalation.

Furan was detected in 1 of 63 industrial effluents at a concentration of less than 10 µg/L. Furan was detected in a creek in the Niagara River watershed and in the Niagara River.

C. Alkylphenols and Alkylphenol Ethoxylates

Nonylphenol (NP) [CASRN - 25154-52-3]/[84852-15-3] and octylphenol are the largest volume alkylphenol products manufactured in the U.S. Alkylphenols (APs) such as nonylphenol and octylphenol are mainly used to make alkylphenol ethoxylate (APE) surfactants. These surfactants are the primary active ingredients in industrial chemicals that are used as cleaning and sanitizing agents. Nonylphenol ethoxylates (NPE) account for approximately 80% of total APE use with total U.S. production exceeding 500 million pounds per year. Alkylphenols are also used as plasticizers, in the preparation of phenolic resins, polymers, heat stabilizers, antioxidants, and curing agents. APEs do not break down completely in sewage treatment plants or in the environment. The most widely used NPEs have nine- or ten-member carbon chains attached to the ethoxylate group. Thus, the great majority of NPEs in use are easily dissolved in water. Human exposure to APs and APEs may occur through contaminated drinking water that has been extracted from polluted waters. At present there is no conclusive evidence that APs or APEs cause adverse health effects in humans; however, there are many reports of alkylphenols causing production of a female-associated liver protein, vitellogenin, in male fish (Jobling, 1995).

Investigations of NP levels in rivers have found values varying between 2 µg/L in the Delaware River in Philadelphia to 1000 µg/L in the

Rhine, and 1000 µg/L in a tributary of the Savannah River. Drinking water is frequently taken from rivers and can easily become contaminated with alkylphenols. Analysis of many drinking water samples in the U.S. has found an overall average concentration of alkylphenolic compounds of 1 µg/L. Studies in the U.S. show NPE removal from wastewater ranging from 92 to 99% with minor seasonal variations. NPE concentrations in discharges after treatment are reportedly low, varying between 50 and 200 ppb. Draft EPA water quality guidelines for nonylphenol in freshwater are 6.6 ppb water (four-day average) and 25 ppb (one-hour average), and in saltwater, they are 1.6 ppb (four-day average) and 6.2 ppb (one-day average).

D. Plastic Additives

Bisphenol A

Bisphenol A [CASRN - 80-05-7] is an industrial chemical used to synthesize epoxy resins or polycarbonate plastic. Human exposure to the potential endocrine disrupting effects of bisphenol A may occur when this chemical leaches out of the plastic due to incomplete polymerization, or breakdown of the polymer upon heating. Polycarbonates are commonly used for food and drink packaging materials and infants are the subgroup of the population that is most highly exposed to this compound. Bisphenol A is also used in plastic dental fillings.

Bisphenol A is a solid which has low volatility at ambient temperatures. It has a water solubility of 120-300 mg/L. Its water solubility increases with alkaline pH values. Releases of bisphenol A into the environment are mainly in wastewater from plastics-producing industrial plants and from landfill sites that contain large quantities of plastics. Bisphenol A does not bioaccumulate in aquatic organisms to any appreciable extent. If released into acclimated water, bisphenol A would biodegrade. In untreated water, bisphenol A may biodegrade after a sufficient adaptation period, it may adsorb extensively to suspended solids and sediments, or it may break down upon exposure to light.

Diethyl Phthalate (DEP)

Diethyl Phthalate [CASRN - 84-66-2] is a synthetic substance that is commonly used to increase the flexibility of plastics used to make toothbrushes, automobile parts, tools, toys, and food packaging. It is also used in cosmetics, insecticides, and aspirin. DEP can be released fairly easily from these products since it is not part of the polymer. Plastic materials containing DEP in waste disposal sites constitute the major reservoir of DEP in the environment. If released to water, DEP is expected to undergo aerobic biodegradation. Humans are exposed to DEP through consumer

products and plastics, contaminated air, or contaminated drinking water and foods.

There is evidence which shows a strong correlation with impaired reproductive performance in multigeneration studies in rodents (Wine, 1997); however, endocrine effects associated with DEP exposure in humans have not been reported.

DEP has accumulated and persisted in the sediments of the Chesapeake Bay for over a century. DEP has been detected in surface water samples from Lake Ponchartrain and the lower Tennessee River, as well as other industrial river basins. Surface water samples collected along the length of the Mississippi River contained DEP in significant concentrations. DEP has been detected in groundwater in New York State public water system wells, near a solid waste landfill site in Norman, OK, and at sites in Fort Devens, MA, Boulder, CO, Lubbock, TX, and Phoenix, AZ. DEP has been identified in drinking water in the following cities: Miami, Philadelphia, Seattle, Lawrence, New York City, and New Orleans.

Di(2-ethylhexyl) Phthalate (DEHP)

Di(2-ethylhexyl) Phthalate [CASRN - 117-81-7] is a manufactured chemical that is used primarily as one of several plasticizers in polyvinyl chloride (PVC) resins that make plastics more flexible. It is the most commonly used of a group of related chemicals called phthalates or phthalic acid esters. DEHP is also used in inks, pesticides, cosmetics, and vacuum pump oil. DEHP is everywhere in the environment because of its use in plastics in large quantities, but it evaporates into air and dissolves in water at very low rates. The primary routes of potential human exposure to DEHP are inhalation, ingestion, and dermal contact in occupational settings and from air, from consumption of drinking water, food, and food wrapped in PVC. It is easily dissolved in body fluids such as saliva and plasma. DEHP is biodegradable, but it tends to partition into sediment where it is relatively persistent. It also tends to bioconcentrate in aquatic organisms. Because of its low vapor pressure, human exposure to DEHP in either water or air appears to be minimal.

DEHP has been detected frequently in surface water, groundwater, and finished drinking water in the U.S. at concentrations in the low ppb range. Groundwater in the vicinity of hazardous waste sites may be contaminated with DEHP. EPA regulates DEHP under the CWA and the SDWAA. DEHP is included on lists of chemicals for which water quality criteria have been established under the CWA. EPA classifies DEHP as a water priority pollutant and has set the MCL Goal at zero. EPA has set the MCL at six parts DEHP per billion parts of drinking water (six ppb).

IV. Water Treatments for EDC Removal

Water suppliers use a variety of treatment processes to remove contaminants from drinking water. Individual processes may be arranged as series of processes applied in a sequence. Water utilities select a treatment train that is most appropriate for the contaminants found in the source water. The most commonly used processes include flocculation, sedimentation, filtration, and disinfection for surface water. Some treatment trains also include ion exchange and adsorption. These conventional processes are inefficient for substantially reducing certain pesticide concentrations and other EDCs.

The processes described later in this section can be used for removal of EDCs as specified, either individually or as a class of compounds. The feasibility of using the various techniques will depend on the size of the system and the cost effectiveness. The two major concerns regarding technologies for small systems are affordability and technical complexity (which determine the needed skills for the system operators).

A. Water Treatment Techniques

Activated Carbon (Granular and Powdered)

Activated carbon is similar to charcoal in composition, but its surface has been altered to enhance its sorption properties. Activated carbon is made from a variety of materials including wood, coal, peat, sawdust, bone, and petroleum distillates. For use in drinking water treatment plants activated carbon produced from wood and coal is most commonly used. The base carbon material is dehydrated then carbonized through slow heating in the absence of air. It is then activated by oxidation at high temperatures (200 to 1000°C), resulting in a highly porous, high surface area per unit mass material. The activation process is considered a two-step procedure in which amorphous material is burned off and pore size is increased. Typically, GACs have surface areas ranging from 500 to 1400 square meters/gram.

GAC treatment removes contaminants via the physical and chemical process of sorption. The contaminants accumulate within the pores and the greatest efficiency is attained when the pore size is only slightly larger than the material being adsorbed. Removal efficiencies for many organic contaminants are good to excellent. Water quality parameters such as dissolved organic matter, pH, and temperature can significantly affect the removal efficiency of GAC. However, for GAC treatment of drinking water it is necessary to reduce the total organic carbon (TOC) of the treated water through the preliminary steps of coagulation/filtration before treat-

ment with GAC. Its removal efficiencies change drastically once the bed nears exhaustion, as contaminant breakthrough occurs. GAC beds can be reactivated by removing the granular carbon from the water treatment chambers, drying the material then placing it in large furnaces that heat the material to 1200 to 1400°F. This heating process removes any residual of contaminants from the pores and again enlarges the pore size. This feature and the high temperatures needed to attain reactivation should be kept in mind when considering claims of some manufacturers that flushing point-of-use (POU) GAC filters with hot water will reactivate units or increase operating efficiency. The increased temperatures that are reached with hot water DO NOT in any manner achieve reactivation.

The performance of GAC for specific contaminants is determined in the laboratory by trial runs and is performed one chemical at a time. The following text is presented to provide the reader with a basic understanding of how the relative capacity of activated carbon to remove a chemical from water (a liquid phase) was determined. Data are gathered within a laboratory setting and determined on the basis of one chemical at a time. This document is not intended to equip the reader to perform laboratory-scale studies to derive values for specific compounds that may be of interest to them. The Freundlich equation can be used to indicate the efficiency of GAC/PAC treatment. The Freundlich equation is expressed as:

$$Q_e = K \times C_e^n$$

where Q_e is the equilibrium capacity of the carbon for the target compound, ($\mu\text{g/g}$), C_e is the equilibrium liquid-phase concentration of the target compound ($\mu\text{g/L}$), and K and $1/n$ are the Freundlich coefficients in $(\mu\text{g/g})(\text{L}/\mu\text{g})^{1/n}$ and dimension-less units, respectively. The K values that are determined for each chemical are a means of expressing the "ability" of a particular GAC to remove a chemical.

Typically when K values that are greater than 200 are attained the process is considered to be economically feasible. In addition, the process of GAC can be fine tuned, that is, certain basic parameters such as pH, temperature or choice of carbon source can be altered to increase efficiency of the process when certain critical contaminants such as pesticides must be removed.

Maintenance--Careful monitoring and testing are required to ensure that all contaminants are removed. The carbon media must be replaced regularly. The replacement intervals depend on the type of contaminant, concentration, rate of water usage, and the type of carbon used in the system. There is potential for bacterial growth on the adsorbed organic chemicals; routine maintenance must be performed. When POU devices are used for compliance for small systems, programs for long-term operation, maintenance, and monitoring must be provided by the water utility.

Powdered activated carbon (PAC) also functions by adsorption of contaminants from water onto a solid phase material, in this case powdered carbon. PAC differs from GAC in that the powdered carbon is added to the water in a large tank, a period of time is provided for adsorption of the contaminants to occur, then the powdered carbon is later removed in a filtration process. This process also differs from GAC in that PAC needs to be added continually to the process; however, the process is less expensive and less technically demanding but it is more labor intensive. PAC is more adaptable to short-term applications rather than as a continual use process. For contaminants such as pesticides which are mostly used during a six-week period in late spring and summer, PAC may be a particularly useful choice. The water being treated comes into contact with much less carbon material per unit volume treated, so the process is not as efficient as GAC.

GAC is the BAT for removal of all of the selected EDCs that are discussed in this document. However, since other technologies are used in the multistep process of drinking water treatment, a brief discussion is included for those processes that enhance the performance of GAC.

Coagulation/Filtration

Coagulation/Filtration processes involve the addition of chemicals like iron salts, aluminum salts, with and without anionic, cationic, or anionic-cationic polymers that coagulate and destabilize particles suspended in the water. The suspended particles are ultimately removed via clarification and/or filtration. Conventional filtration includes pretreatment steps of chemical coagulation, rapid mixing, and flocculation, followed by floc removal via sedimentation or flotation. After clarification, the water is filtered using common filter media such as sand, dual-media, and tri-media. Direct filtration has several effective variations, but all include a pretreatment of chemical coagulation, followed by rapid mixing. The water is filtered through dual- or mixed-media using pressure or gravity filtration units.

Lime Softening

In the lime-softening (LS) process, the pH of the water being treated is raised sufficiently to precipitate calcium carbonate and, if necessary, magnesium hydroxide to reduce water hardness. The chemical groups that contain most of the EDCs are not affected by LS.

Point-of-Use/Point-of-Entry Treatments

The SDWA identifies both point-of-entry (POE) and POU treatment units as options for compliance technologies for small systems. A POU treatment device treats only the water at a particular tap or faucet, resulting in other taps in the facility serving untreated water. POU devices are typically installed at the kitchen tap. POU devices are listed as compliance technologies for inorganic contaminants, synthetic organic contaminants, and radionuclides. POU devices are not listed for volatile organic contaminants because they do not address all routes of exposure. POE treatment units treat all of the water entering a facility (household or other building), resulting in treated water from all taps. POE devices are still considered emerging technologies because of waste disposal and cost considerations.

POE and POU treatment units often use the same technological concepts as those used in central treatment processes, but on a much smaller scale. Technologies that are amenable to the POU and POE scale treatment include activated alumina, GAC, reverse osmosis, ion exchange, and air stripping.

When POU and POE units are used by a public water system to comply with the National Primary Drinking Water Regulations (NPDWRs), the SDWA requires that the units be owned, controlled, and maintained by the public water system or by a person under contract with the public water system. This is to ensure that the units are properly operated and maintained to comply with the MCL or treatment techniques. This will also ensure that the units are equipped with the required mechanical warnings to automatically alert the customers to the occurrence of operational problems.

B. Discussion of Water Treatment Techniques for Specific EDC Removal

The EDCs addressed in this document that are included in the NPDWRs as drinking water contaminants are methoxychlor, DDT and DDE, endosulfan, PCBs, DEP, and DEHP. The EDCs in this section are grouped by chemical class. Removal techniques for the EDCs not listed in the NPDWRs will be based on removal of similar contaminants that are listed.

The treatment processes are described with considerations of advantages, limitations, and special considerations. The actual choice of a process to include in a treatment train will ultimately depend on the source water quality, the nature of the contaminant to be removed, the required quality of the finished water, and the size of the drinking water system.

Methoxychlor

The BAT for removal of methoxychlor from drinking water is GAC. Steiner and Singley (1979) have tested a wide range of water treatment processes and found GAC to be the most efficient for removal of methoxychlor. They found that over a broad range of concentrations (ranging from 1 mg/mL to 25 mg/mL) the GAC process could remove sufficient quantities of methoxychlor so that the finished water met MCL requirements which is 0.1 mg/mL.

Endosulfan

The BAT for removal of endosulfan from drinking water is GAC. In the Dobbs and Cohen report "Carbon Adsorption for Toxic Organics," EPA/600/8-80/023, the following K values, as determined by the Freundlich equation and actual test were determined: alpha-endosulfan-6135, beta-endosulfan-1990, endosulfan sulfate-2548. For small system compliance, GAC, POU-GAC, and PAC can be used to remove endosulfan from drinking water supplies. Please see Table 1.

DDT

The BAT for removal of DDT from drinking water is GAC. In the Dobbs and Cohen report "Carbon Adsorption for Toxic Organics," EPA/600/8-80/023, the following K values, as determined by the Freundlich equation and actual test were determined: DDT has a K value of 10,449 $\mu\text{g/g (L/\mu\text{g})}^{1/n}$ which is sufficiently above the cutoff point of 200 $\mu\text{g/g (L/\mu\text{g})}^{1/n}$ to be judged an effective treatment method and DDE (a DDT metabolite with endocrine activity) of 18,000 $\mu\text{g/g (L/\mu\text{g})}^{1/n}$.

Diethyl Phthalate

The BAT for removal of diethyl phthalate from drinking water is GAC. In the Dobbs and Cohen report "Carbon Adsorption for Toxic Organics," EPA/600/8-80/023, the following K value, as determined by the Freundlich equation and actual test for diethyl phthalate yielded a K value of 17,037 $\mu\text{g/g (L/\mu\text{g})}^{1/n}$.

Di-(2ethylhexyl) Phthalate (DEHP)

The BAT for removal of DEHP from drinking water is GAC. In the Dobbs and Cohen report "Carbon Adsorption for Toxic Organics," EPA/600/8-80/023, the following K value, as determined by the Freundlich equation and the test was determined. DEHP has a K value of $8,308 \mu\text{g/g (L/\mu\text{g})}^{1/n}$ which is one of the highest values established among the 130 compounds that they tested; GAC is very effective for the removal of DEHP from drinking water.

PCBs

In the Dobbs and Cohen report two studies were reported for PCB-1221 and PCB-1232. The K value determined for PCB-1221 was $1,922 \mu\text{g/g (L/\mu\text{g})}^{1/n}$ and the K value for PCB-1232 was $4,067 \mu\text{g/g (L/\mu\text{g})}^{1/n}$. Both mixtures are among the lesser chlorinated groups containing 21 and 32% chlorine, respectively. Relative to other PCB mixtures they are more hydrophilic and hence would have lower K values than the commercial PCB mixtures, Aroclor 1242, 1248, 1254, and 1260. The most troublesome PCB environmental mixtures tend to be derivatives of this later group of compounds; therefore, GAC should be a very effective method for removal of environmental PCB compounds from drinking water.

Dioxin

Dioxin is not water soluble, hence it is not likely to be present in untreated drinking water unless it would be attached to sediment in raw water. Because most conventional water treatment methodologies such as coagulation-sedimentation and filtration are effective in removing sediment, it is likely that these processes would be very effective in the removal of the contaminant, dioxin.

Alkylphenols and Alkylphenol Ethoxylates

GAC is best used for removal of these contaminants from drinking water. Previous laboratory-scale testing for removal of nonylphenol with GAC has yielded K values of 19,406 at a water pH of 7.0. For consistency of removal of synthetic organic chemicals, GAC, POU-GAC, and PAC are recommended for small system compliance. GAC devices include pour-through for treating small volumes, faucet-mounted for POU, in-line for treating large volumes at several faucets, and high volume commercial units for treating community water supply systems. Careful selection of the type of

carbon is based on the specific contaminants in the water and the manufacturer's recommendations. Site-specific conditions may affect the percentage removal using these techniques, including the presence of "competing" contaminants. Source water-specific testing will be needed to ensure adequate removal. For GAC, surface waters may require pre-filtration. PAC is most applicable to those systems that already have a process train including mixing basins, precipitation or sedimentation, and filtration.

Table 1. Isotherm Constants for Selected EDCs

Chemical	Isotherm Constants (K value)	1/N	Calculated Value $\mu\text{g/gm (L}/\mu\text{g)}^{1/N*}$
Alpha-endosulfan	194	.50	6,135
Beta-endosulfan	615	.83	1,990
Endosulfan sulfate	686	.81	2,548
DDT	332	.50	10,499
DDE	232	.37	18,000
Diethyl phthalate (DEP)	110	.27	17,037
Diethylhexyl phthalate (DEHP)	11,300	1.50	8,308
PCB-1221	242	.70	1,922
PCB-1232	630	.73	4,067
Nonylphenol	250	.37	19,406

*Any value above 200 is considered to be economically feasible.



ACTON BOARD OF HEALTH

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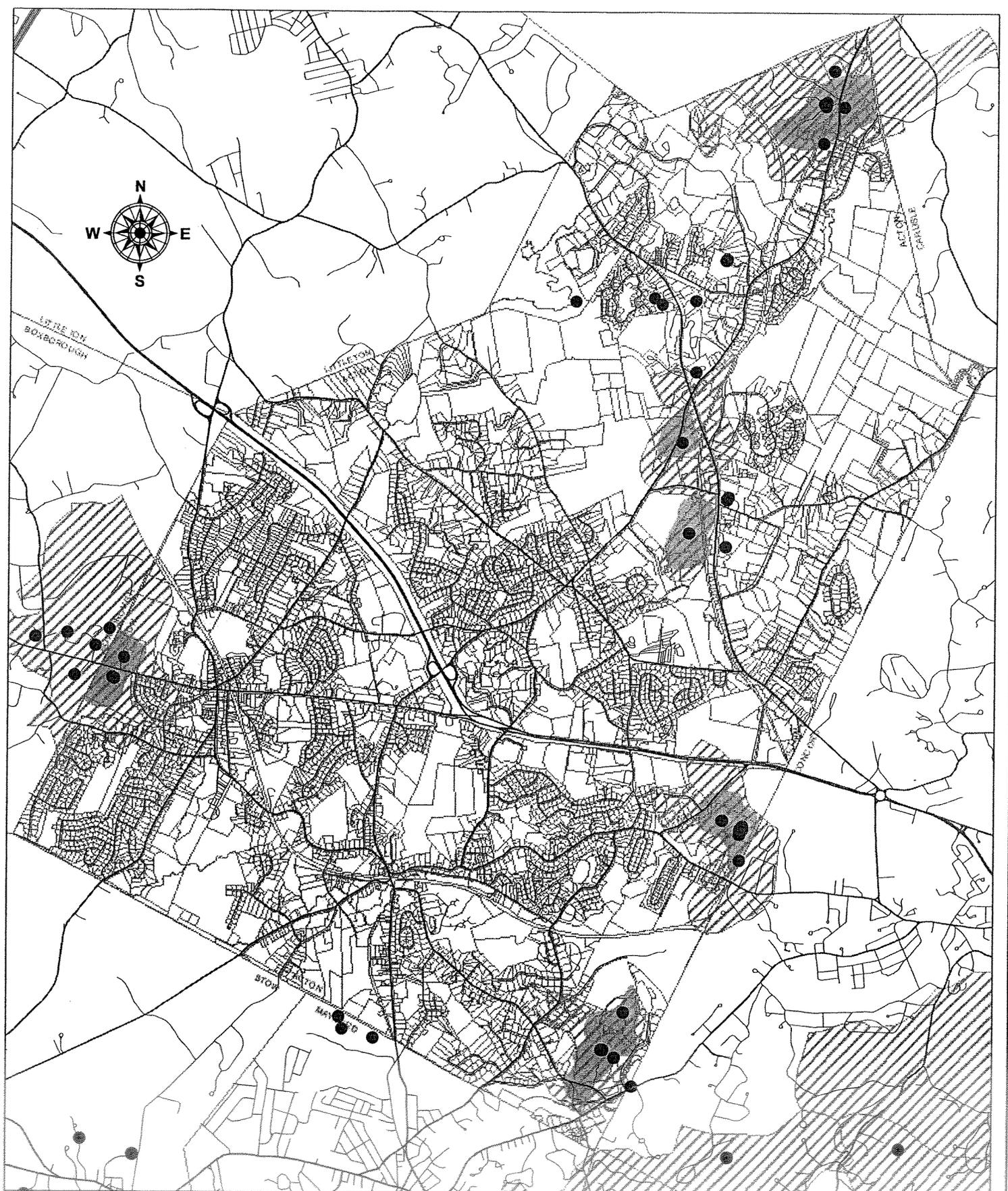
Town of Acton
Comprehensive Water Resources Management Plan
Citizens Advisory Committee
Indirect Potable Reuse Working Group

Meeting #3
7/20/2005
Acton Town Hall, Room 121

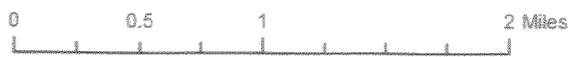
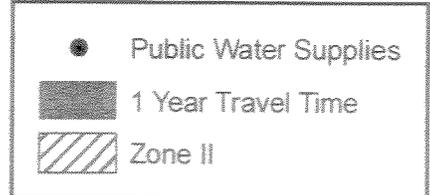
Call to Order 730pm

- I. Introductions
- II. Minutes from 6/30/05
- III. Update on Reuse Activities
- IV. Review of articles from 6/30/05 meeting
- V. Review of new Articles
 - a. Discussion of the four major topics
 - 1) Emerging contaminants – detection and removal
 - 2) The timing of the implementation of the project and coincidence with regulatory, treatment technology, and political timelines
 - 3) Source reduction efforts for water use and pollutant removal
 - 4) Centralized IPR versus Decentralized IPR
- VI. Future meeting dates, sites, and topics

Adjourn by 845pm



**Figure 1:
Aquifer Resource Areas
Acton, MA**



APPENDIX B

INFORMATION ON JOHNS HOPKINS STUDY



November 29, 2004

Dear Collaborators:

Thank you for waiting so patiently on news from us. We are happy to report that a large number of collaborators have come forward to participate in our study. An overview of the current coverage of the U.S. is provided in the attached map that shows volunteers from municipal water treatment utilities (blue) as well as volunteers from the Groundwater Foundation (green). For updates and maps showing complete coverage, visit Dr. Halden's personal webpage.

(<http://www.jhsph.edu/dept/ehs/faculty/halden/home/Nationwide%20Study.htm>), accessible through his faculty webpage (<http://www.jhsph.edu/Dept/EHS/Halden>) by clicking on the link: "For more information visit my personal web page." On the map you will notice that we have excellent coverage in the East and West but still need volunteers in the South and Midwest. If you have colleagues in these areas of poor coverage, please forward our information to them.

On August 25, our research group presented some of our data from our local WWTP at the 228th National Meeting of the American Chemical Society, which included the Second National Symposium on the Environmental Chemistry of Pharmaceuticals and Personal Care Products. The seminar was taped and is available on the Internet at: <http://www.tntech.edu/wrc/PPCPWebcast/Heidler/Heidler.html>. Additional coverage of our research can be found at: http://www.jhsph.edu/dept/ehs/faculty/halden/Different_Studies.htm and <http://www.scienceupdate.com/index.cfm> (archived under "September 30, 2004").

We are now asking for your assistance in the first phase of our nationwide survey of surface waters and wastewater treatment systems. Please confirm your availability during the months of December to February by email (jheidler@jhsph.edu) and we will send you the required sampling materials, including a trip blank, gloves and bottles, and a pre-paid FedEx air bill for returning the samples. In the package, you will find instructions for the collection samples. Essentially, we would like you to provide us with the following:

1. Raw wastewater (after mechanical screening but prior to settling)

- 2 x 250 mL 24-hour composite sample (ideal) **OR**
- 2 x 250 mL grab samples, ideally taken during high-flow and low-flow situations.

2. Treated wastewater (effluent)

- 2 x 500 mL 24-hour composite sample (ideal) **OR**
- 2 x 500 mL grab sample

3. Biosolids

- 1 x 250 ml primary sludge **AND**
- 1 x 250 mL excess (wasted) activated sludge **AND**
- 1 x 250mL pre-digested sludge (primary/activated mix) **AND**
- 1 x 250 mL digested, dewatered sludge (processed sludge)

4. Effluent-receiving streams

- a. 2 x 500 mL of effluent-receiving stream upstream of effluent input
- b. 2 x 500 mL of effluent-receiving stream downstream of effluent input
(Taken ~0.5 miles downstream of WWTP inputs to allow for mixing)

Please record the sampling day, time and location, including the names of the streets at the nearest intersection. If you have access to a global position system (GPS) unit, please log in the sampling locations and forward the information to us. Additionally please provide us with the following information on the plant:

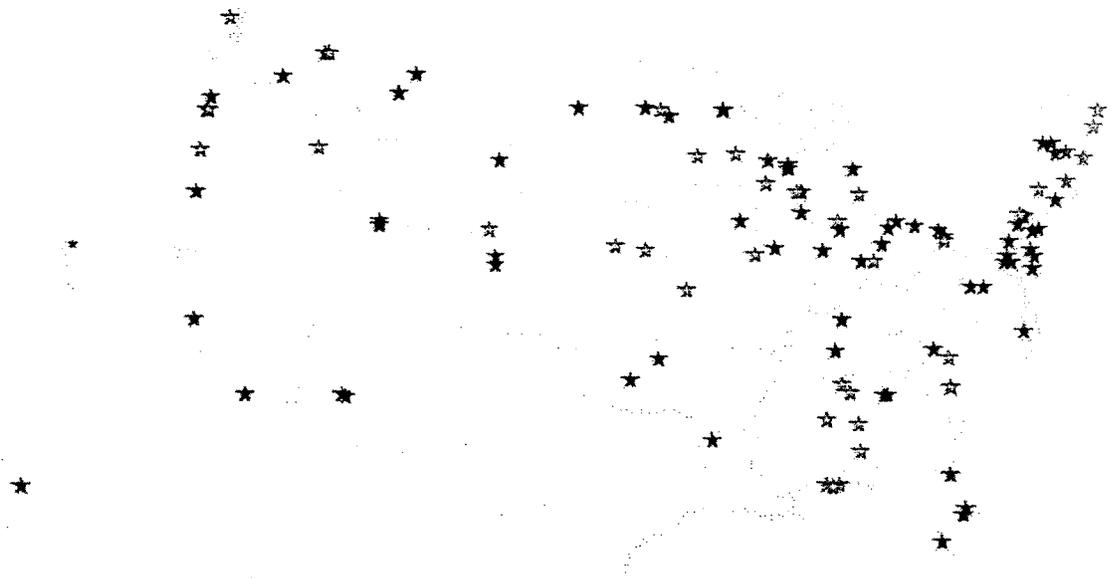
- Description of plant (activated sludge, secondary, tertiary, or trickling filter, etc)
- Capacity (MGD)
- B.O.D. for influent and effluent
- Amount of suspended solids for sampling days
- Flow data for the sampling days

Thank you very much for your support of the JHU Center for Water and Health Nationwide Survey of Pharmaceuticals and Personal Care Products (PPCPs) in U.S. water resources.

Sincerely,

Jochen Heidler and Rolf Halden

Network of collaborators to date



Brent Reagor

From: Jochen Heidler [jheidler@jhsph.edu]
Sent: Monday, November 29, 2004 10:03 AM
To: Jochen Heidler
Subject: Johns Hopkins PPCP Study Update

Dear Collaborators,

thank you very much for your interest in participating at the Johns Hopkins University (JHU) research project on the fate and transport of pharmaceuticals and personal care products (PPCPs) in the environment.

Attached you will find information about the status of our project and detailed sampling instruction.

Please confirm your participation in order to send you the sampling materials.

Again, thank you very much.

Sincerely,

Jochen Heidler and Rolf Halden

Jochen Heidler
Ph.D. Student
Johns Hopkins University
Bloomberg School of Public Health
Department of Environmental Health Sciences
615 N. Wolfe Street / W6704
Baltimore, MD 21205
410-955-8692
jheidler@jhsph.edu

Brent Reagor

From: Jochen Heidler [jheidler@jhsph.edu]
Sent: Tuesday, June 07, 2005 9:49 AM
To: Brent Reagor
Subject: Johns Hopkins Sampling Materials

Dear Mr. Reagor,
I'm finally ready to send your sampling kit by the end of this week.
Again, I apologize for the delay in our study.
Please confirm your availability in order to receive your sampling kit.

Best regards,

Jochen Heidler

Jochen Heidler
Ph.D. Student
Johns Hopkins University
Bloomberg School of Public Health
Department of Environmental Health Sciences
615 N. Wolfe Street / W6704
Baltimore, MD 21205
410-502-2620
jheidler@jhsph.edu

Brent Reagor

From: Jochen Heidler [jheidler@jhsph.edu]
Sent: Friday, June 24, 2005 3:44 PM
To: Brent Reagor
Subject: RE: JSPH Study Samples

Your samples arrived today.
We will inform you as soon as we will have some data from our analysis of your samples.

Thanks for providing these samples for us.

Best regards,

Jochen Heidler

Jochen Heidler
Ph.D. Student
Johns Hopkins University
Bloomberg School of Public Health
Department of Environmental Health Sciences
615 N. Wolfe Street / W6704
Baltimore, MD 21205
410-502-2620
jheidler@jhsph.edu

From: Brent Reagor [mailto:breagor@acton-ma.gov]
Sent: Thu 6/23/2005 12:40 PM
To: Jochen Heidler
Subject: JSPH Study Samples

The samples left my office this afternoon. Tracking numbers:

844998071876 -- influent and biosolids
851851586510 -- effluent and receiving waters

If there are any problems, please let me know.

--Brent

Brent L. Reagor, R.S.
Acton Board of Health
472 Main Street
Acton, MA 01720
P -- (978) 264-9634
F -- (978) 264-9630

Sampling kit for
Raw wastewater (influent)
And
Biosolids.

Dear Collaborator,

Enclosed in your sampling kit you will find 6 x 250 ml sample bottles, biohazard bags, gloves, stickers for labeling, gel packs (for cooling) of the bottles and a prepaid FedEx air bill.

Detailed sampling instructions:

Raw wastewater (influent):

1. Sampling should be done at a location after mechanical screening but prior to settling. (If no such location is accessible, sample prior to the screen.)
2. Please provide us with the following: 2 x 250 mL 24-hour composite influent sample (ideal) **OR** 2 x 250 mL grab influent samples.
Important: Do not overfill the bottles to avoid rupture during subsequent freezing.
3. Collect the grab samples on two consecutive weekdays (e.g., Mo&Tu or Th&Fr).
4. Wear gloves during sampling to avoid both contact with bacteria and contamination of your samples with personal care products.
5. Please record on the bottles using the provided stickers the day, time, flowrate, your name and the location.
6. Make sure that the bottle lid is screwed on tightly
7. Freeze the bottles overnight together with the gel packs.
8. Put bottles into the biohazard plastic bags.
9. Pack the bottles into the same box they arrived in, add the biosolids samples.
10. Remove the paper on the side of the shipping box that covers the “diagnostic specimen” sticker.
11. Send the box back to us using the pre-paid FedEx air bill.

Biosolids:

Please provide us with the following:

1 x 250 ml primary sludge **AND**

1 x 250 mL excess (wasted) activated sludge **AND**

1 x 250mL pre-digested sludge (primary/activated mix) **AND**

1 x 250 mL digested, dewatered sludge (processed sludge)

Important: Do not overfill the bottles to avoid rupture during subsequent freezing.

1. Wear gloves during sampling to avoid both contact with bacteria and contamination of your samples with personal care products.
2. Please record on the bottles using the provided stickers the day, time, your name, the location, and the average mass of suspended solids produced per month if available.
3. Make sure that the bottle lid is screwed on tightly
4. Freeze the bottles overnight together with the gel packs.
5. Put the bottles into the biohazard plastic bags.
6. Pack the bottles into the same box they arrived in with the influent samples
7. Remove the paper on the side of the shipping box that covers the “diagnostic specimen” sticker
8. Send the box back to us using the pre-paid FedEx air bill.

Thank you very much,

Jochen Heidler and Rolf Halden

Sampling kit for
Treated wastewater (effluent)
And
Effluent-receiving stream samples

Dear Collaborator,

Enclosed in your sampling kit you will find 6 x 500 ml sample bottles, one trip blank as our control, gloves, stickers for labeling of the bottles and a prepaid FedEx air bill.

Detailed sampling instructions:

Treated wastewater (effluent):

1. Sampling should be done at a location directly prior to the discharge of the effluent into surface waters.
2. Please provide us with 2 x 500 mL 24-hour composite effluent samples (ideal)
OR
2 x 500 mL grab effluent samples from two consecutive days.
3. Collect the grab samples on two consecutive weekdays (e.g., Mo&Tu or Th&Fr).
4. Wear gloves during sampling to avoid both contact with bacteria and contamination of your samples with personal care products.
5. Using the provided stickers, please record on the bottles the day, time, flow rate, your name and the location.
6. Make sure that the bottle lid is screwed on tightly.
7. Pack the bottles into the same box they arrived in together with the stream samples and send it back to us using the pre-paid FedEx air bill.

Effluent-receiving stream samples:

1. Sampling should be done at normal river height. Don't sample after heavy rain to avoid dilution effects and data bias.
2. Please provide us with the following: **2 x 500 ml water samples from upstream** of your local wastewater treatment plant, and **2 x 500 ml from downstream** of your local wastewater treatment plant (~500-1000 ft downstream of discharge location; record approximate location).
3. Wear gloves during sampling to avoid contact with bacteria and contaminations of your samples with personal care products.
4. Collect duplicate samples upstream and downstream from the riverbanks where the flow velocity is similar to that of the stream. Avoid slow-moving and stagnant water.
5. Please record on the bottles using the provided stickers the day, time and sampling location in relation to the wastewater discharge pipe. Please include the names of the streets at the nearest intersection.
6. Make sure that the bottle lid is screwed on tightly
7. If you have access to a global position system (GPS) unit, please log in the sampling locations and forward the information to us.
8. Pack the bottles into the same box they arrived in with the effluent samples and send it back to us using the pre-paid FedEx air bill.

Thank you very much,

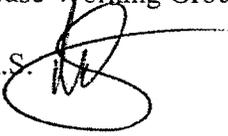
Jochen Heidler and Rolf Halden



MEMORANDUM

Acton Board of Health - Telephone (978) 264-9634

TO: Indirect Potable Reuse Working Group

FROM: Brent L. Reagor, R.S. 

RE: Meeting #3
7/20/2005

DATE: July 12, 2005

Enclosed with this memo you will find the packet for the next meeting. Contents are as follows:

- 1) Agenda
- 2) 6/30/2005 minutes
- 3) Article summations for the previous packet's articles
- 4) Article summation for 2 articles in this packet
- 5) The two articles for which the summation is included
- 6) A series of fact sheets and short easy to understand pieces as requested by the group

Even though I have included summations, please read through the articles as there is a significant amount of information which cannot be properly summarized.

If you have any questions, or cannot make the meeting, please let me know.



INDIRECT POTABLE REUSE WORKING GROUP

Acton Board of Health - Telephone (978) 264-9634

Meeting Minutes

6/30/2005 Meeting

Room 126

Acton Town Hall

Attendees: *Brent Reagor, Acton Health Department (BR)
*Greta Eckhardt, Acton Resident, AWD Land-Water Use Committee (GE)
*Eric Hilfer, Acton Resident, ACES, CAC (EH)
*Art Gagne, Acton Resident, CAC (AG)
*Joanne Bissetta, Acton Resident, BOH (JB)
Mary Michelman, Acton Resident, ACES (MM)

*IPR Working Group Member

The meeting was called to order at 7:32pm

The group reviewed the minutes from the previous meeting. Minor changes were made to the discussion on reuse and its impact on local hydrologic loss, along with a change in phrasing for one of the three possible answers the group may issue in its final report.

Discussion of the minutes spurred discussion of the title of the group. MM states we should change the title, AG and GE both stated that the most important title was the title of the final report. AG stated that if people do not understand what the title means, one of the hurdles we must overcome is education about the definition of indirect potable reuse.

The group discussed the issue of local hydrologic impacts related to a centralized IPR discharge. MM stated she would like to see more about this issue, but stated that an IPR discharge at the High Street wellfields may have a beneficial impact of mounding the groundwater and creating a hydrologic gradient, thereby preventing significant intrusion of contaminant plumes.

BR updated the group about the Johns Hopkins School of Public Health (JHSPH) study. The samples had been sent to Baltimore for analysis. He has also been asked to join the statewide Task Force that has been seated to author Water Reuse regulations for the Commonwealth. He also stated that the Metropolitan Area Planning Council (MAPC) is looking at all forms of water reuse, including greywater, stormwater, and wastewater along the lines of the Massachusetts Water Policy, and that MADEP is in the process of hiring a Watershed Outreach Coordinator to encourage reuse.

The group began a discussion of the four articles sent out with the packets. BR gave a short introduction of each article. GE stated she was surprised by two things: 1) the prevalence of caffeine, and the fact that the USGS study had positive results in every sample analyzed. AG stated that he believes the discovery of emerging contaminants in effluent will always be a continuum as new analytical methods are developed and new compounds are created. MM stated there is a lag time between production of new compounds and development of revised analytical methods and the presence of no data does not mean it is not harmful.

AG stated that the group is not conversant in the topics discussed in the scientific articles. EH stated the results from the JHSPH study will be of some help. AG would like to see more fact sheets and FAQ documents. GE would like to see guiding questions or points to consider sent out with the articles, prior to the meetings. BR agreed to do this for the current articles and any future research.

GE asked what would be considered the major classes of emerging compounds would be. BR stated, as he sees it, they are: Endocrine disruptors/mimics, Pharmaceutical compounds and their metabolites and by-products, and Personal care products and their by-products. However, compounds may be members of more than one class. AG stated that medicine disposal practices (i.e. flushing unused medications) may lead to detection of these contaminants at higher levels. BR stated that the State of Maine has developed a public relations campaign to discourage people from flushing unused medications for just that reason.

MM stated she was intrigued about research into the effects of wastewater treatment processes on the compounds in question. BR stated he would make sure to include information on that in a future packet. AG cautioned that with the continuum of discovery in science, Acton should be careful not to develop the "guinea pig" mentality. GE asked about heavy metals and pesticides in WWTF effluent. BR stated that these must come from an industrial source, and there are no such sources currently connected or planned to be connected to the sewer system.

The group settled on July 20 and August 18 as the next two meeting dates.

The meeting adjourned at 8:54pm.

Respectfully Submitted,

Brent L. Reagor



INDIRECT POTABLE REUSE WORKING GROUP

Acton Board of Health - Telephone (978) 264-9634

Article Summation – Packet #2

Article #1:

“Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada”. The Science of the Total Environment. v. 311, 2003, pgs 135-149.

Key Points

- Normal drinking water treatment processes when combined with chlorination and/or ozonation are effective at removing Naproxen and Triclosan present in surface water sources
- PPCPs include a broad range of dissimilar molecules, which present a challenge in selecting an analytical method
- Naproxen and Triclosan survive through normal wastewater treatment processes

Article #2

“Occurrence of Antimicrobials in the Final Effluents of Wastewater Treatment Plants in Canada”. Environmental Science and Technology. v. 38 n. 13, 2004, pgs 3533-3541.

Key Points

- Frequency of antibiotic prescription is related to the prevalence of antibiotics in wastewater treatment plant effluents
- Penicillin and cephalosporin degrade quickly during the wastewater treatment process
- Wastewater treatment plants with hydraulic retention times of less than 12 hours are poor at removing pharmaceuticals
- Detection of these compounds at the less than 1 microgram/liter (part per billion) is not enough to cause acute exposure effects on plants, animals, or bacteria, but this study does not take into account chronic exposure effects

Article #3

Removal of Endocrine Disruptor Chemicals Using Drinking Water Treatment Processes. EPA-625-R-00-015, USEPA, March, 2001.

Key Points

- The use of granular and powdered activated carbon is the most common and well-accepted treatment process for removal of the endocrine disruptors listed within this document
- GAC is usually installed along with other, more common, water treatment technologies to complete the treatment train associated with drinking water treatment

Article #4

"Pharmaceuticals, Hormones, and other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance". Environmental Science and Technology. v. 36, n. 6, 2002, pgs. 1202-1211.

Key Points

- This study was directly focused on surveillance in surface waters and did not look at wastewater treatment facilities or discharges specifically. Therefore, the source of the compounds detected could include runoff from residential, industrial, or agricultural operations; wastewater treatment facility discharges; industrial operations/discharges; or other means
- One or more of the 95 compounds selected for this surveillance study were detected in 80% of the 139 streams sampled throughout 1999-2000
- Nonprescription drugs were found with greater frequency than any of the prescription drug classes
- Multiple samples had more than 1 compound detected
- This study did not evaluate the preference of some compounds for adsorption to sediment and their presence outside the water column



INDIRECT POTABLE REUSE WORKING GROUP

Acton Board of Health - Telephone (978) 264-9634

PLEASE TRY TO READ THE ARTICLES USING THESE KEY POINTS AS A GUIDE. THERE IS STILL VALUABLE INFORMATION IN THE ARTICLES.

Article Summation – Packet #3

Article #1

“Evaluation of the Fate of Synthetic and Natural Hormones in a Full Municipal Wastewater Treatment Plant”. Proceedings of the 2004 WEF Annual Conference. New Orleans, LA October 4-6, 2004.

Key Points

- The plant at which this study was conducted is similar in design and function, though it is design to treat over 4 million gallons per day, where the Acton facility is only permitted for 0.29 million gallons per day
- The compounds in this study are excreted in an inactive state, but are degraded by microbes present in feces and wastewater to release the active estrogen compounds into the waste stream
- The three hormones selected for this study experienced removal rates by standard wastewater treatment practices anywhere from 76.4% to 93.2%
- These hormones have an affinity for adsorption onto suspended particulate matter, which therefore leads to greater sequestration in the treatment process as the sludge was removed
- Fate of estrogen compounds in UV disinfection treatment units requires further study as a slight increase was seen in estrogen compound concentration after UV disinfection

Article #2

"EDCs in Wastewater: What's the Next Step?". Proceedings of the 2004 WEF Annual Conference. New Orleans, LA October 4-6, 2004.

Key Points

- SRT = Sludge retention time
- HRT = Hydraulic retention time
- AOP = Activated oxygen processes
- NF/RO = Nanofiltration/Reverse Osmosis
- The longer it takes to process the wastewater through the treatment plant, the higher the level of biodegradation of endocrine disruptors
- Higher percentages of EDC removal will lead to increased sludge disposal costs, as the compounds must go somewhere
- The hazardous forms of endocrine disruptors are formed when their parent compounds, which are not necessarily hazardous, are partially broken down during through contact with wastewater and treatment processes.
- Certain treatment processes (activated sludge-type) seem to be more effective at removal of endocrine disruptors
- Processes that use membranes to filter wastewater are named based upon the size of the pores in the membrane ranging from standard Microfiltration, to Ultrafiltration, Nanofiltration, and Reverse Osmosis. These technologies hold promise in EDC removal as they retain the particulate matter of increasingly smaller sizes, which many EDCs are attracted to
- Determining which technologies and at which level to employ the selected technology(s) will be a site-specific decision based upon the EDC characteristics of the raw wastewater and the space and money available for wastewater treatment
- Activated carbon, which is currently used by the Acton Water District at certain wells to remove VOCs from the water supply, is being studied as a possible treatment process for EDC removal
- Significantly more health effects from EDCs have been demonstrated in wildlife than in humans

EVALUATION OF THE FATE OF SYNTHETIC AND NATURAL HORMONES IN A FULL MUNICIPAL WASTEWATER TREATMENT PLANT

Nazim Cicek *, Kathleen Londry, Jan A. Oleszkiewicz, Yoomin Lee

*Department of Biosystem Engineering, University of Manitoba
Winnipeg, MB, Canada. R3T 5V6

ABSTRACT

The impact of a full-scale municipal wastewater treatment plant (WWTP), and each of the treatment units within the stream, on the removal of endocrine-disrupting compounds was evaluated by tracking three estrogenic compounds: 17- β -estradiol (E2, natural); estrone (E1, natural, metabolite of E2); and 17- α -ethinylestradiol (EE2, synthetic). The overall performance of the WWTP compared well with other plants, as 90.5% removal of E1+E2, and 76.4% removal of EE2 were observed. The activated sludge units reduced the concentration of E1+E2, and EE2 in the liquid phase by 88.2% and 44.6%, respectively. Additional removal of soluble phase estrogens (68% and 62% for E1+E2 and EE2, respectively) was observed in the equalization basin prior to UV disinfection. Although not statistically significant, the UV treatment process appeared to result in a slight increase in soluble phase estrogens. The aqueous phase of the tertiary lagoon sludge contained higher levels of estrogens compared to the lagoon influent. This was attributed to the possible de-sorption of particulate matter-bound estrogens during storage in the lagoon.

KEYWORDS

Estrogens, endocrine disrupting compounds, hormones, municipal wastewater treatment plant, activated sludge treatment, effluent

INTRODUCTION

There is a growing concern about the impact of natural and synthetic hormones on the safety of freshwater supplies. Hormones such as estrogens have been shown to be released from a wide variety of wastewater treatment plants (WWTPs), and although they are present in very low concentrations (ng/L), these amounts can be sufficient to disrupt endocrine systems of aquatic species such as fish (Johnson and Sumpter, 2001). EDCs released in domestic sewage treatment plant effluents are causing male fish, living immediately downstream of discharge, to be feminized through the development of unusual testes, production of an egg protein precursor normally found only in females, depressed circulating sex hormone levels, and reduced gonad sizes (Desbrow, et al., 1998; Purdom, et al., 1994; Snyder, et al., 2001). Estrogenic compounds include 17- β -estradiol (E2) which is the natural estrogen, and 17- α -ethinylestradiol (EE2) which is a synthetic estrogen and the main component of birth control pills, both of which are excreted in human urine and feces (Desbrow, 1998). In addition, E2 is transformed biologically and abiotically into a metabolite, estrone (E1) which is weakly estrogenic but is est-

importance than E2 and EE2 because of its relatively lower estrogenicity (Johnson, 2001). These compounds are excreted as inactive conjugates, but microbes in feces and wastewater readily deconjugate these compounds, thereby releasing the actively estrogenic forms either in the collection systems or within the WWTP (Desbrow, 1998). Public awareness of the existence of endocrine-disrupting compounds (EDCs) in WWTPs is growing and municipalities worldwide are anticipating future requirements for removal of EDCs in treatment plants.

Surveys of domestic WWTP in various cities in Europe, North and South America reveal that a wide range of concentrations of estrogens are present in WWTP effluents (Baronti, et al., 2000, Belfroid, et al., 1999, Desbrow, et al., 1998, Kolpin et al., 2002, Snyder, et al., 2001, Ternes, et al., 1999). Typical values are in the low ng/L range for E2 or less than ng/L range for EE2, which is at or close to the limit of detection even with the most sensitive techniques. The removal of estrogens in WWTP and their transport out into the environment has been shown to depend on the design and operational characteristics of the treatment plant (Lee et al. 2004), yet little is known about the potential to increase estrogen removal, or the key processes or parameters to increase net removal of estrogens (Johnson et al. 2000).

Most research that has been done in WWTPs is with activated sludge processes and suggests 64-88% removal efficiency for E2 (Baronti, et al., 2000, Johnson, et al., 2000, Nasu, et al., 2001, Ternes, et al., 1999). The synthetic estrogen, EE2 appears to be removed less than the natural E2, which is consistent with its more stable chemical structure. In a study recently conducted on 18 WWTPs across Canada, a wide range of removal efficiencies were observed for E1, E2, and EE2. These range from 15% to 98% for E1, 9% to 99% for E2, and -637 to 80% for EE2 (Conor Pacific, 1999). The substantial variability across wastewater treatment plants along with reports of increasing levels of EE2 underlines the complexity of EDC behavior in such environments.

Assessing the fate of EDCs requires a comprehensive and structured sampling plan in order to determine the removal rates and processes in each unit operation of a WWTP. Very little is known about the impact of each wastewater treatment unit within WWTPs on the fate of hormones, or the factors that could assist in their removal and thereby mitigate their environmental impact. Municipal treatment plants, such as the one found in Brandon, Manitoba, Canada, offer an excellent model system in which to study the fate of these compounds, from their introduction to the plant from human waste, through the various treatment options, to the final effluent and sludge. With this as a focus, the objective of the present research study was to evaluate the overall effect of the WWTP on the concentrations of E2, E1, and EE2 and identify the impact of each treatment unit process on the removal and overall fate of E1, E2 and EE2. The compounds of interest were analyzed in both aqueous and particulate phases. Attempts were made to isolate and quantify estrogens from wastewater sludge to construct a unit treatment based mass balance.

METHODOLOGY

WWTP Sampling

Eight different sample types were collected from the WWTP at Brandon in either grab (sample locations 1, 2, 3, and 4) or 24hr composite (sample locations 5, 6, 7, and 8) fashion (Figure 1). The plant is centered around two non-nitrifying aerobic sequencing batch reactors (SBRs) with a total hydraulic retention time (HRT) of approximately 6 hours and solids retention time (SRT) of less than 1.2 days. General characteristics of the incoming wastewater on the days of sampling are presented in Table 1.

Figure 1 – Layout of wastewater treatment plant and sampling locations

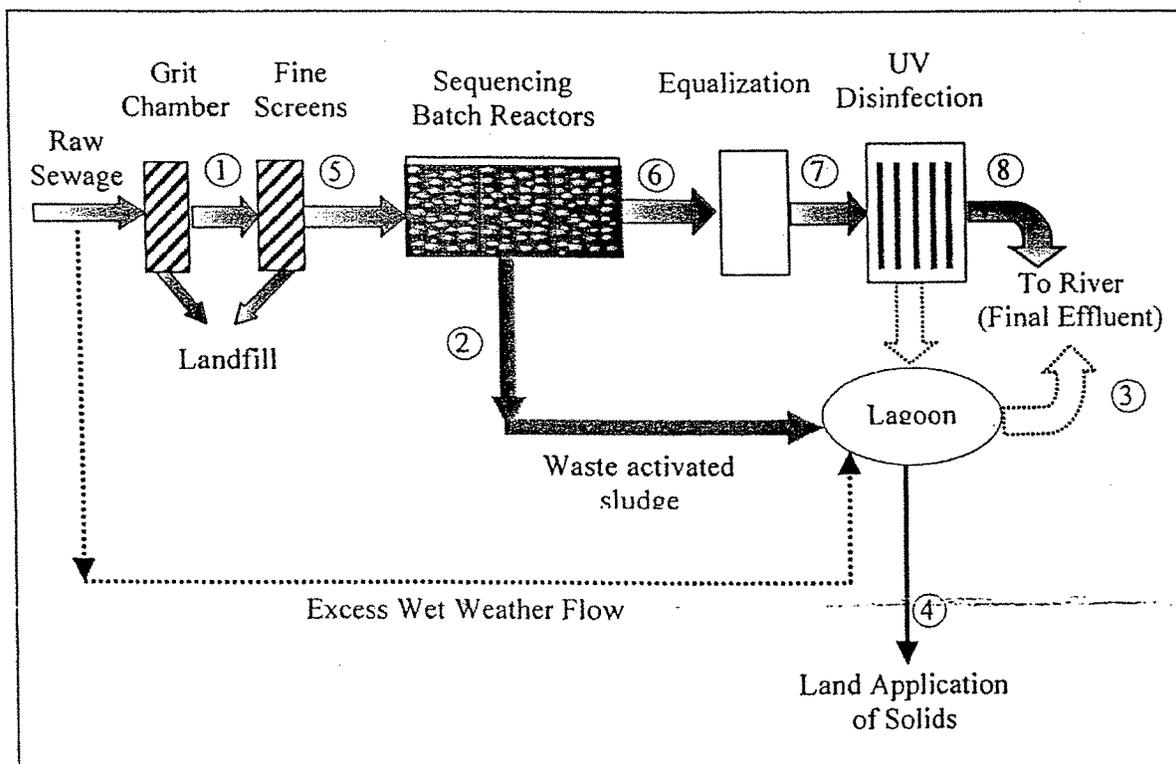


Table 1 - General raw wastewater characteristics for the sampling period

Date	Daily Influent Flow (m ³ /d)	pH	TSS (mg/l)	COD (mg/l)	NH ₃ (mg/l)	Wastewater Temp. (°C)
21-May	16,622	7.57	177	410	22.50	12.4

All containers used in this study were glass and were acid washed and rinsed with 50% methanol to prevent adsorption of estrogens (unless otherwise stated). In addition, methanol (20 ml, HPLC grade) was added to the 4L collection bottles to help reduce loss of estrogens onto glass surfaces. Four composite samplers (sampling for 24 hours, at 330 ml per hour) were used simultaneously during sampling. Water in each carboy was dispensed into smaller (4 L) bottles and transported in coolers packed with ice to the University of Manitoba.

Sample Preparation and Analysis

Liquid samples (1, 3, 5, 6, 7, 8 + supernatant from 2, 4). Each of the eight WWTP samples was filtered at least four times as replicate sub-samples. Each 1L sub-sample was sequentially filtered through 2.5 µm then 0.7 µm GF/C (glass fiber) filters. The filtrate was refrigerated for less than 24 hrs before solid phase extraction (SPE) was performed. SPE cartridges (LC-18, 6 mL, 0.5 g, Supelco) were pre-conditioned with 5 mL acetone then 10 mL Milli-Q water. Samples were filtered at < 20 min L⁻¹; then estrogens were eluted with acetone (4 x 3 ml). All samples were stored frozen (-20°C) for as little time as possible between treatment steps. As a prelude to the testing of the WWTP, the procedures were tested by spiking estrogens (from a stock solution containing E1, E2, and EE2 at 100 ng/mL in acetone) directly into water to final concentrations of 1, 10 or 100 ng/L for each estrogen. Recoveries from the entire procedure averaged 82%.

The GF/C filters were combined and extracted with acetone by accelerated solvent extraction (ASE). An ASE 400 instrument (Dionex) was used to extract filters or sludges from 11 ml cells (filled with Ottawa Sand, Fisher Scientific) with acetone (HPLC grade, Fisher Scientific) at 2000 psi and 100°C (1 cycle, 5 min heat, 5 min static, 60% flush, 90 sec purge). In a test of the extraction procedure, filters were directly spiked with 0, 1, 10, or 100 ng each of E1, E2, and EE2, extracted, and analyzed. Average recoveries were similar for all three estrogens at approximately 79%.

Sludge samples (2, 4) Sludge samples could not be filtered directly, so they were first centrifuged (10 000 x g, 15 min) in acid-washed and methanol-rinsed plastic centrifuge bottles. The supernatant was decanted and pooled in graduated cylinders, then filtered and analyzed as described for the liquid samples. The pellets were transferred to glass vials, frozen, and lyophilized. The wet and dry weights were recorded, and the dried sludge was extracted with acetone on ASE as above. Some sludge samples were spiked with estrogens (1, 10, 100 ng E1, E2, EE2) prior to centrifuging, to test recovery.

Sample processing. Acetone extracts (from SPE or ASE extractions) were concentrated under a stream of N₂ at 37°C. Each extract was applied to a new silica gel column (1 g silica gel (baked 150°C 8h then deactivated with 15 µl H₂O) suspended in 5 ml hexanes:acetone (65:35) in a pipette with a glass wool plug. Estrogens were eluted with 5 ml hexanes:acetone, and the eluent was concentrated under N₂ at 37°C. To remove particulates (including silica gel) the sample was filtered through a 0.2 µm PTFE filter into a glass vial with Teflon-lined cap.

Samples were derivatized with 100 µl MSTFA (N-Methyl-N-(trimethyl-silyl) trifluoroacetamide, Sigma) and 10 µl pyridine for 2 h at 65°C, then dried under N₂, and re-suspended in 100-500 µl hexanes. Blanks and standards (100 ng each of E1, E2, EE2) were prepared with each set of samples derivatized. Samples were analyzed within 10 days of derivatization.

Analysis. The TMS-derivatives of E1, E2 and EE2 were analyzed by gas chromatography-mass spectrometry (GC-MS-MS). A Varian 3800 GC with a Saturn 2000 mass spectrometer was used with a DB-5ms column (30 m x 0.25 mm x 0.25 µm) with a 1 m x 0.53 mm precolumn. Samples of 2-4 µl were injected in splitless mode at 80°C and the injector was heated to 250°C at 200°C/min. The oven temperature program was 80°C for 1.5 min, increased to 180°C at 50°C/min, then increased to 300°C at 20°C/min and held for 5 min. The MS had a transfer line at 250°C, EI ion source of 70 eV, and ion trap temperature of 200°C. The MS-MS was performed for E1 using a precursor ion of 342 and quantifying using the daughter ions 244, 245 and 257. The MS-MS was performed for E2 using a precursor ion of 416 and quantifying using the daughter ions 285 and 326. The MS-MS for EE2 used a precursor ion of 425 and quantifying using the daughter ions 193, 231, and 407. Estrogens were quantified by comparison of peak areas to standard calibration curves generated daily using standards of 10-200 pg E2 and EE2, and confirmed with check standards and blanks.

RESULTS AND DISCUSSION

Overall Reduction of Estrogens in the WWTP

In the analysis of the data, special consideration was given to the relationship between E2 and E1, as oxidation of E2 to E1 occurs quickly yet reversibly. Thus E1 and E2 were examined both individually and as a paired set. Table 2 summarizes the overall removal of E1, E2, and EE2 in the wastewater treatment plant. The influent values for E1 and E2 compare well with previously reported studies involving wastewater treatment plants in Germany, Brazil and Italy (Baronti et al. 2000, Ternes et al., 1999). Effluent concentrations for E1 and E2 are generally lower than those reported in British wastewater treatment plant discharges (Desbrow et al. 1998), but within the ranges reported elsewhere. On the other hand, EE2 concentrations in the influent and effluent appear higher than those reported in studies conducted in Europe and Brazil, but are comparable with results reported in other Canadian wastewater treatment plants (Baronti et al. 2000, Ternes et al., 1999, Lee et al. 2004). This could be attributed to societal similarities in terms of the use of the synthetic hormone EE2 in Canadian cities, as opposed to differences in this regard with other parts of the world. The overall estrogen removal performance of the plant compared well with other plants (Lee et al. 2004). As expected, E1 and E2 were removed to a greater extent than EE2

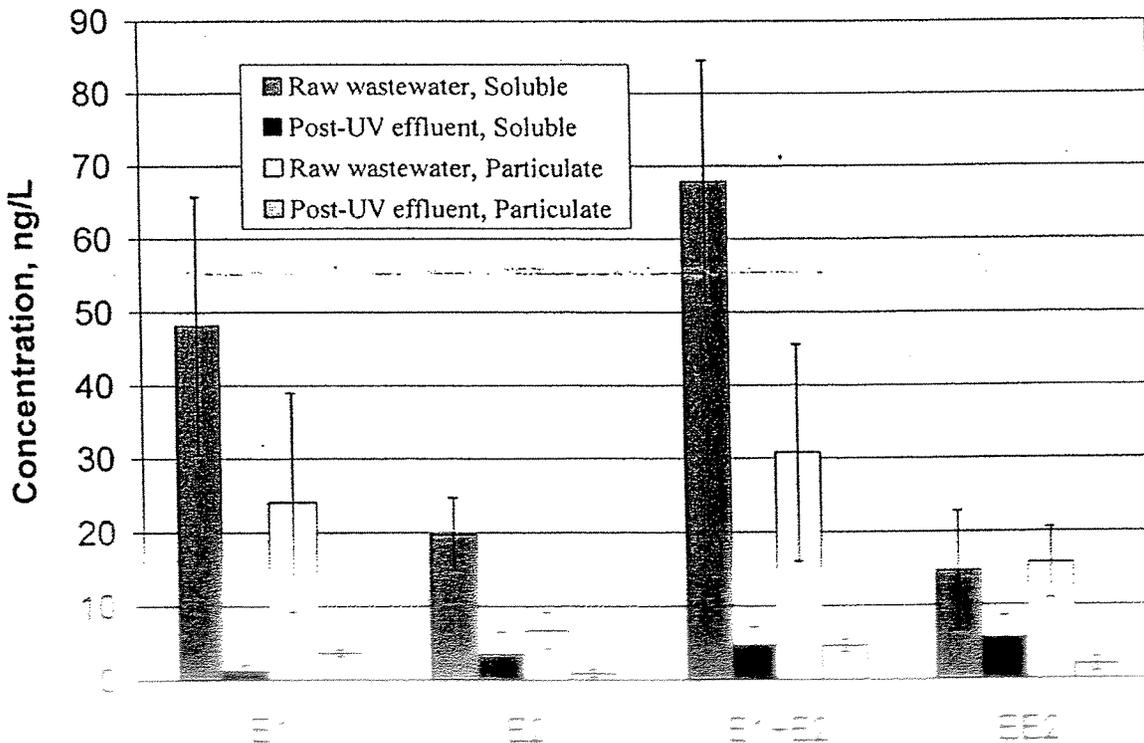
(90.5% versus 76.4%, respectively), which is also in agreement with previous research elsewhere (Baronti, et al., 2000, Johnson, et al., 2000, Nasu, et al., 2001, Ternes, et al., 1999). Nevertheless, EE2 removal was greater than reported in most plants across Canada (Conner Pacific, 1999).

Table 2 - Overall reduction of selected Estrogens in the WWTP

Estrogen	Raw wastewater (Soluble + Particulate) ng/L	Post-UV Effluent (Soluble + Particulate) ng/L	Overall Reduction %
E1	72.26	4.91	93.2
E2	26.45	4.43	83.3
E1+E2	98.71	9.34	90.5
EE2	30.42	7.63	76.4

As apparent in Figure 2, a larger fraction of EE2 (approximately 52 %) entered the plant in particulate form as E1 and E2 (33% and 25%, respectively). This was expected, since EE2 has a higher affinity for the solids phase, which would also indicate that much of the EE2 removal could be attributed to adsorption to suspended solids within the plant. The effluent discharged to the receiving river contained similar amounts of estrogens in aqueous and particulate bound phases. This is significant, as solid bound estrogens are often ignored in research studies involving the analysis of wastewater treatment plant effluents.

Figure 2 – Comparative reduction of estrogens in the particulate and soluble phase

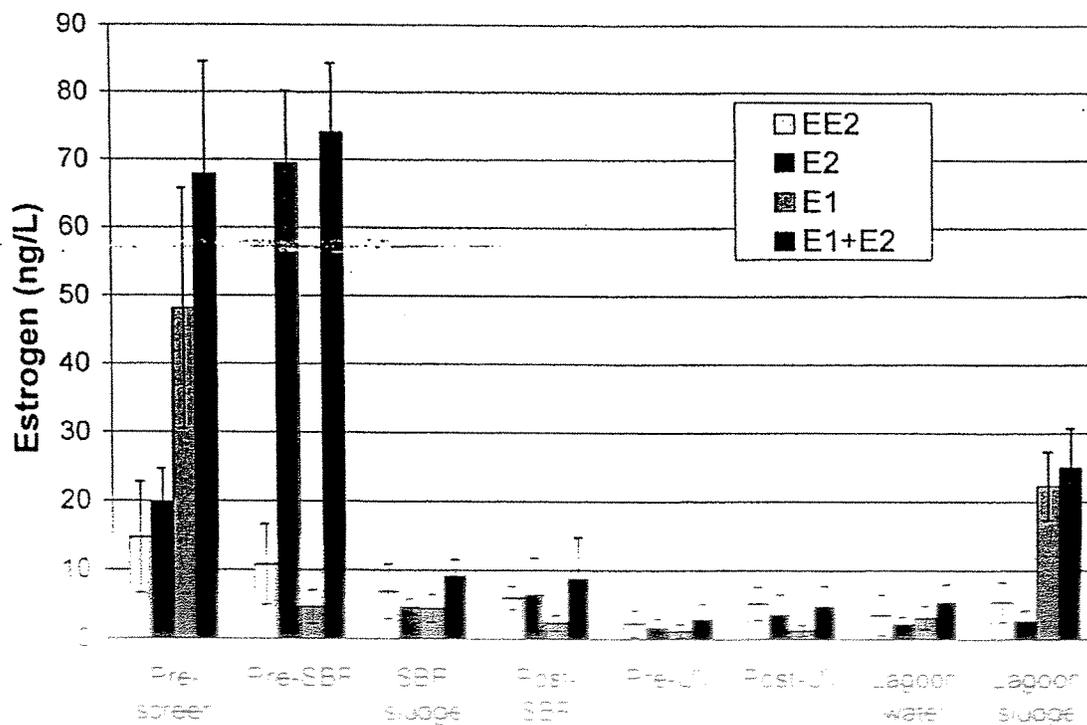


It is also possible to evaluate the overall plant performance in terms of the relative potencies of E1, E2, and EE2. Studies conducted on in-vivo VTG (an egg protein precursor) response in trout established a measure of 17 β -estradiol equivalency, which can be used to sum up the overall impact of each endocrine disrupting compound within a sample (Johnson et al., 2001). Using these multipliers, it was determined that the total removal of 17 β -estradiol equivalency within the plant for all three hormones amounted to 77.4 %. Of the total estrogen potency leaving the plant and entering the lagoon prior to the receiving river, the majority (71.4%) was in aqueous form. Considering that previous reports on the impact of E2 and EE2 exposure of fish under laboratory conditions indicate that as low as 2ng/L would induce measurable change in fish reproduction (Snyder et al., 2003), the values reported in this study reaffirm the possibility of adverse effects on wildlife in the immediate vicinity of the outflow. The impact would be amplified during low river flow periods of the year where dilution effects are suppressed.

Fate of Estrogens Within Each Treatment Unit

Soluble phase concentrations of E1, E2, E1+E2, and EE2 throughout the WWTP are presented in Figure 3. EE2 concentrations were consistently lower than E1+E2 throughout the plant, with most variability observed with E1. Although E1+E2 concentrations remained similar between the influent to the fine screen and the influent to the SBR, the reversible transformation of E2 to E1 is apparent in Figure 3.

Figure 3 – Soluble phase concentrations of E1, E2, and EE2 at various sampling points in the WWTP



The activated sludge unit operations, consisting of two parallel sequencing batch reactors (SBRs), were effective in reducing E1+E2, and EE2 in the liquid phase by 88.2% and 44.6%, respectively (Figure 3). Considering the relatively low HRT and SRT of the SBR units (6 hrs and 1.2 days, respectively), observed removal rates for E1+E2 are close to the higher limit of those previously reported (Lee et al. 2004). The aqueous phases of the waste activated sludge and the SBR effluent appear to be very similar in terms of estrogen distribution, indicating no additional sorption/de-sorption occurring in the sludge.

An equalization basin, which operates at an overall hydraulic retention time of approximately 4 hours, holds the SBR effluent prior to UV disinfection. Some particulate matter settling takes place in this basin which necessitates solids clean-out twice a week. Additional reductions of soluble phase estrogens (68% and 62% for E1+E2 and EE2, respectively) were observed in this basin which could be attributed to additional biological degradation or adsorption/settling. In essence, the equalization basin in this plant behaved similar to a post-secondary clarifier which is still biologically active and provides turbidity removal prior to UV disinfection.

The UV disinfection process in the plant consisted of a medium-pressure, high intensity, flow-through system with an average HRT between 9-12 seconds. Although not statistically significant, the UV process appeared to result in a slight increase in soluble phase estrogens. This could be attributed to UV induced break-down of particulates and consequent release of solid-bound estrogens to the aqueous phase. It is important to note, however, that uncertainties remain with regards to the exact impact of UV treatment on soluble phase estrogens. This was emphasized in reviewed research conducted on the removal E1, E2, and EE2 during lab-scale ultraviolet disinfection studies (Birkett and Lester, 2003). In that study, two separate doses were investigated (32 mWscm^{-2} for 19 seconds and 145 mWscm^{-2} for 20 seconds) with multiple replicates, and in each case some results showed removal occurred while others showed an increase in concentration during the process. Further testing is still required in order to determine the fate of estrogens during UV disinfection processes.

The lagoon process in the plant served several purposes. It acted as the sole treatment process during excess wet weather flow periods, where raw wastewater was directed to the lagoon prior to discharge into the river. When the UV disinfection unit was ineffective due to excess turbidity or not in operation during maintenance and part replacement, the lagoon acted as a final disinfection step prior to river discharge. Finally waste activated sludge was stored in the lagoon prior to bi-annual pumping and application to agricultural land of the sludge sediment (Figure 1). The aqueous phase of the lagoon solids contained higher levels of hormones relative to the lagoon influent. This could be attributed to the possible de-sorption of particulate based hormones from the waste activated sludge while residing in the lagoon. The fact that EE2 did not follow this trend as closely as E1 and E2 supports the de-sorption argument, as this behavior is consistent with the higher affinity of EE2 to organic solids.

One major possible removal process for estrogens in a WWTP is adsorption to particulate matter. The soluble, liquid-phase concentrations of E1, E2, and EE2 were successfully traced within each treatment unit process in the WWTP, but quantification of the solid-phase bound estrogens proved much more difficult. Solids trapped on filters during processing of liquid samples had good recoveries of estrogens. In the sludge stream, raw wastewater effluent after the SBR

effluent after the UV system). Reduction of estrogens in the solid phase within the treatment plant (Figure 2) indicates that biodegradation is probably taking place, and removal of estrogens is not simply due to adsorption. However, no estrogens were detected in the pelleted material from the return activated sludge or the lagoon sludge. Upon further investigation with spiked estrogens, it was discovered that these sludges had a large capacity for adsorption of estrogens, and that the extraction of estrogens from such materials is extremely difficult. This remains one of the main challenges for determining the fate of estrogens in WWTP treatments.

It was not possible to construct a true mass balance of E1, E2, and EE2 in the municipal wastewater treatment plant due to the analytical problems related to sludge analysis. Uncertainties remain with respect to the actual fate of the estrogens within each treatment unit, whether they are being biodegraded or bound to the solid phase. The results suggest that upon land application of the lagoon sludge, one can expect some de-sorption and mobilization of E1. The release and relative effects of estrogens in land applied agricultural wastes and municipal biosolids remain topics of active investigation. Some recent work by Collucci et al. (2001a, 2001b) suggest that in well aerated soils estrogens (E1, E2, EE2) are broken down within several weeks of land application.

CONCLUSIONS

The fate of three selected estrogenic compounds: 17- β -estradiol (E2, natural); estrone (E1, natural, metabolite of E2); and 17- α -ethinylestradiol (EE2, synthetic) were evaluated across each treatment unit within a full-scale municipal wastewater treatment plant. The overall performance of the WWTP compared well with previously reported studies in Canada and elsewhere, as 90.5% removal of E1+E2, and 76.4% removal of EE2 were observed. A larger fraction of EE2 (approximately 52 %) entered the plant in particulate form than E1 and E2 (33% and 25%, respectively). Particulate bound estrogens were as prevalent in the discharged effluent as aqueous phase estrogens. Aerobic sequencing batch reactor units reduced the concentration of E1+E2, and EE2 in the liquid phase by 88.2% and 44.6%, respectively. Additional removal of soluble phase estrogens (68% and 62% for E1+E2 and EE2, respectively) was observed in the equalization basin prior to UV disinfection. The UV treatment process appeared to result in a slight increase in soluble phase estrogens. The aqueous phase of the tertiary lagoon sludge contained higher levels of estrogens compared to the lagoon influent, which was attributed to the possible de-sorption of particulate matter-bound estrogens during storage in the lagoon.

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