EMERGING ORGANIC CONTAMINANTS IN SURFACE AND GROUND WATERS OF NEW YORK

By

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Abstract

Emerging Organic Contaminants in Surface and Ground Waters of New York

By

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The first study was about monitor estrogens (estrone, 17α-estradiol, 17β-estradiol, and estriol) in three headwater streams within a concentrated animal feed operation (CAFO) site on a monthly base for a year. In general, estrogen concentrations in the streams are low (<1 ng/l), and appeared to increase in spring, likely due to the mobilization of estrogens from soils upon snow melting/precipitation. Estrogens were detected in the streams during dry periods, indicating the contribution of estrogens from groundwater. The low concentrations of estrogens in stream water were probably the result of the long residence time (~8 months) of the manure in the lagoons where the majority of the estrogens were degraded during storage.

The second study was designed to distinguish between unsewered areas and septic systems application as two possible sources of nitrogen to coastal groundwater by analyzing groundwater samples for pharmaceutical residuals. Groundwater samples were taken through piezometers at shoreline sites in unsewered areas in Northport Harbor and in sewered areas adjacent to Manhasset Bay, both in western Long Island Sound. The frequent detection of the anticonvulsant compound carbamazepine in groundwater samples of Northport (unsewered), together with the fact that few pesticides associated with lawn applications were detected, suggest that wastewater input and atmospheric input are the likely sources of nitrogen in Northport groundwater. High concentrations of nitrogen were also detected in Manhasset (unsewered) groundwater. The low detection frequency of carbamazepine, however suggests that the sewer system effectively intercept nitrogen from wastewater there.

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Chapter 1

Introduction

Since the world population has significantly increased in the last 20 years, the demand of water is increasing. In many areas, ground water and stream water are used as drinking water sources (*1*). These precious water resources, however, are often contaminated by various endocrine disrupting compounds (*2*).

Endocrine disrupting compounds (EDCs) are chemicals with potential adverse effects on the reproductive biology of aquatic organisms (fish, frogs, turtles etc.), humans and wildlife by disrupting their normal endocrine function (*3*). The US Environmental Protection Agency (EPA) defines an EDC as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (*3*). Many pharmaceutical compounds, personal care products, herbicides and insecticides, and steroid hormones are potential EDCs.

Humans and farm animals can excrete a large amount of steroid hormones, including estrogens such as estrone (E1), 17α-estradiol (E2α), 17β-estradiol (E2β), and estriol (E3), androgens such as testosterone, and progestagens such as progesterone. Studies have indicated that women can excrete in urine around 7 μg of E1, 2.4 μg of E2β, and 4.6 μg of E3 per day (*4*). In feces, women can excrete around 0.5 μg of E1, 0.4 μg of E2β, and 1.25 μg of E3 per day (*5*). Pregnant women can excrete 100 μg natural estrogens per day (*6*). Males also produce and excrete estrogens up to 25 μg per day (*6*). For dairy farm, cows can excrete 51 μg E1/cow/day and 148 μg E2β/cow/day in feces, and 787 μg E1/cow/day and 236 μg E2β/cow/day in urine (*7*). The range for cycling (non-pregnant) pigs in feces is from 4 to 20 μg E1/head/day, and up to 3

µg E2β/head/day. Values for pregnant sows in feces range from 16–80 E1 µg/head/day, and from 16 to 40 µg E2β/head/day (*7*). Such large amounts of estrogens can be introduced to stream water, wastewater treatment plants (WWTPs), and groundwater.

Pharmaceuticals, herbicides, insecticides, personal care products, lipid regulators, and β-blockers etc. act as another group of contamination in the natural aquatic environment. Waste streams from hospitals and, WWTPs have been identified as major contributors to environmental contamination with human-derived medications (*8*). Ubiquitous occurrences of these compounds have been reported for rivers, lakes, and reservoirs around the world (*3,9-11*). Some of those compounds degraded naturally through hydrolysis (*12*), oxidation (*13*), direct or indirect UV light (*14,15*), but many of them are very persistent in the environment. Carbamazepine, diazepam, diclofenac and clofibric acid are the most persistent compounds in the environment (*16,17*). The most frequently detected compounds were sulfamethoxazole, caffeine, acetaminophen, and ibuprofen, followed closely by cephalexin, ofloxacin, and diclofenac with the concentration in the environment as high as 0.4µg/l (*15*). Clofibric acid also acts as a lipid regulator which is taken up by plant cuticle (*18*). Cutin, a polymeric lipid, which plays another role in the fate of up taking organic pollutants, is a major sorption medium due to its large mass fraction and liquid-like nature (*19*). Gemofibrozil and bezafibrate, two most frequently detected lipid regulators, have reported concentrations up to 2 µg/l (*18*). Herbicides, insecticides and fungicides are widely used in farms and lawns and have been found in lake, river and marsh. The degradation of herbicides and insecticides has also been examined (*20*).

Many analytical methods have been developed to analyze these organic pollutants in the aquatic environment. Gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS) play a dominant role in analyzing steroid

hormones, herbicides, insecticides and pharmaceutical compounds. Steroid hormones were analyzed by GC/MS as early as 1992 (*21*). Solid phase extraction (SPE) system was used to extract estrogens from the aquatic phase and concentrated 1000 times. Estrogens were analyzed by GC/MS without any derivatization procedure for years with poor detection limits. BSTFA [N,O-bis(trimethylsilyl)fluoroacetamide] and DMF (dimethylformamide) were then used as derivatization reagents and improved the detection limit to 10 μg/l (*22*). As the typical estrogen concentration in the aquatic environment is only a few ng/l, a new derivatization method using pentafluorobenzyl bromide (PFBBr) and *N*-trimethylsilylimidazole (TMSI) as derivatization reagents has been introduced to lower the detection limit to less than 1 ng/l (*23*). Very recently, LC/MS and liquid chromatography/tandem mass spectrometry (LC/MS/MS) with different ionization techniques (electrospray (ESI) and atmospheric pressure chemical ionization (APCI)), ionization modes (negative ion (NI) and positive ion (PI)) and monitoring modes (selected ion monitoring (SIM) and selected reaction monitoring (SRM)) are generally employed (*24*,*25*). Based on sensitivity and selectivity, LC/ESI/MS/MS is the general method to determine estrogens in the NI mode and progestogens in the PI mode with detection limits as low as 0.6 ng/l (*26*). Without derivatization procedures, instrumental detection limits achieved by LC/ESI/MS in the SIM mode and by LC/ESI/MS/MS in the SRM are comparable, but the latter has higher selectivity and avoids false peak retention time determination in the analysis of real samples (*25*). When dansyl chloride was introduced as a derivatization reagent, the detection limit is down to 0.2 pg/l (*27*).

Pharmaceutical compounds, herbicides and insecticides are usually analyzed by LC/MS/MS and analytical methods have been well established without derivatization procedures

(*18,28,29*). Two SRM transitions are typically monitored to identify and quantify the compounds with detection limit of lower than 1 ng/l.

The major theme of my research was to develop analytical methods to examine the occurrence of emerging organic pollutants in the environment. A GC/MS method was developed to examine estrogen concentrations in an organic concentrated animal feed operation (CAFO) in upstate New York (Chapter 2). An LC/MS/MS method was also developed to explore the potential of using organic tracers to identify sources of nitrogen in groundwater of western Long Island (Chapter 3).

Chapter 2

Estrogens in Streams Associated With an "Organic" Concentrated Animal Feeding Operation in Upstate New York

Abstract

Estrogens (estrone, 17α-estradiol, 17β-estradiol, and estriol) in three headwater streams within a concentrated animal feed operation (CAFO) site were monitored on a monthly base for a year (Nov. 2006 – Oct. 2007). The CAFO has about 800 dairy cows confined in two barns, two concrete waste storage lagoons, and about 3,200 acres of agriculture land that receives all the manure generated from the CAFO. This CAFO is certified as "organic" (no growth promoters administrated) and uses many "Whole Farm Planning" practices (e.g., 12-month-capacity storage lagoons). In general, estrogen concentrations in the streams are low (<1 ng/l), and appeared to increase in spring, likely due to the mobilization of estrogens from soils upon snow melting/precipitation. Estrogens were detected in the streams during dry periods, indicating the contribution of estrogens from groundwater. The low concentrations of estrogens in stream water were probably the result of the long residence time (~8 months) of the manure in the lagoons where the majority of the estrogens were degraded during storage. An analysis of liquid manure at the beginning of manure application season (after ~ 8 months storage) showed that over 97% of the estrogens potentially excreted by the cows was degraded. Moreover, about 90% of the estrogens in the liquid manure were associated with particulates larger than 0.7 μm. Batch experiments with spiked deuterium-labeled 17β-estradiol-16,16,17-d_3 (d_3-E2β) in the liquid manure demonstrated sorption of d_3-E2β onto particulates in the liquid manure, and rapid degradation of d_3-E2β in the aqueous phase and on particulates of the liquid manure.

2.1 Introduction

The presence of steroidal hormones in the environment has become a concern since low concentrations (e.g., a few ng/l) of such hormones in water may have adverse effects on the reproductive biology of aquatic organisms (fish, frogs, turtles, etc.) by disrupting their normal endocrine function (*30-35*). For example, when exposed to low concentrations (30 ng/l) of 17β-estradiol (E2β) or estrone (E1), male fathead minnows started the synthesis of vitellogenin (a female specific egg yolk precursor protein) and showed abnormal testicular growth (*32*).

Farm animals can excrete large amounts of steroid hormones (e.g., estrogens such as 17α-estradiol (E2α), E2β, estriol (E3) and E1, androgens such as testosterone, and progestagens such as progesterone). Some species can produce up to a few milligrams of hormones per animal per day, and the estimated overall hormone excretion in the U.S. is over 330 tons per year (*36,37*). These natural hormones are concentrated in animal manure and may be released to the environment through overflow or leakage from storage structures or land application, potentially contaminating surface and ground waters (*38-46*). A typical concentrated animal feed operation (CAFO) confines a large number of animals (e.g., hundreds to thousands of cattle or pigs) into several large buildings and generates a tremendous amount of manure, which is often applied to nearby agriculture fields. The high manure to land ratio often leads to the practice of applying manure at disposal rates rather than agronomic rates (i.e., rates based on current soil nutrient levels and plant needs) (*47*), further increasing the potential of hormone contamination in surface and ground waters.

There are about 650 CAFOs (mostly dairy farms) in the State of New York, most of which are located in upstate New York (*48*) where glacial outwash is abundant in stream and river valleys (*49*). The water tables in stream valleys are very shallow, typically ranging from 5 ft

to 30 ft below ground surface (*50*). The high permeability of the glacial outwash and the shallow water tables in these areas make the groundwater vulnerable to contamination. Many of the farms are also connected to headwater streams, which may be contaminated by animal steroid hormones. For example, a few studies suggest that estrogenic activities in headwater streams affected by livestock farms may reach levels that could cause endocrine disruptions in some aquatic organisms (*46,51*).

While many studies have examined hormonal activity in rivers receiving effluent from sewage treatment plants, few studies have focused on hormone contamination in streams associated with livestock farms, especially associated with CAFOs. The primary objective of this study was, therefore, to examine the occurrence of manure-borne estrogens in a few headwater streams associated with a CAFO in upstate New York. This CAFO is the only one in this region of New York that is certified as "organic", which means that no growth promoters are administrated in the CAFO. This farm also adopts many Whole Farm Planning (WTP) practices, including two 12-month-capacity concrete manure storage lagoons. Such an "organic" CAFO with many WTP practices is of a particular interest because the occurrence of hormones in such a site could be used as a baseline for comparison with traditional CAFOs and may be used as a model for more environmentally friendly yet exceedingly profitable feeding operations.

Estrogen levels in three headwater streams within the CAFO were monitored for a year (Nov. 2006 – Oct. 2007). A liquid manure sample from a storage lagoon was also analyzed for estrogens. Batch experiments with spiked deuterium-labeled 17β-estradiol-16,16,17-d_3 (d_3-E2β) in the liquid manure were conducted to examine the kinetics of estrogen sorption/degradation.

2.2 Materials and Methods

2.2.1 Study Site

The study site (Eklund Farm) is located in the northwest part of the Catskill/Delaware watersheds in upstate New York (Fig. 2.1). The farm has about 800 dairy cows confined in a few barns, two concrete waste storage lagoons with a total capacity of 3 million gallons, and about 3,200 acres of agriculture land that receives all the manure generated from the CAFO. Land application of manure typically starts in April and continues through August, with an application rate of about 4000 gallons per acre. There are 6 headwater streams in the farm, most of which drain to the west branch of the Delaware River. This study focused on 3 streams (see locations in Fig. 2.1) that receive about half of the drainage from the farm. Stream 1 and stream 2 join together to form stream 3. The coordinates of the 3 sampling sites are listed in Table A1 in Appendix.

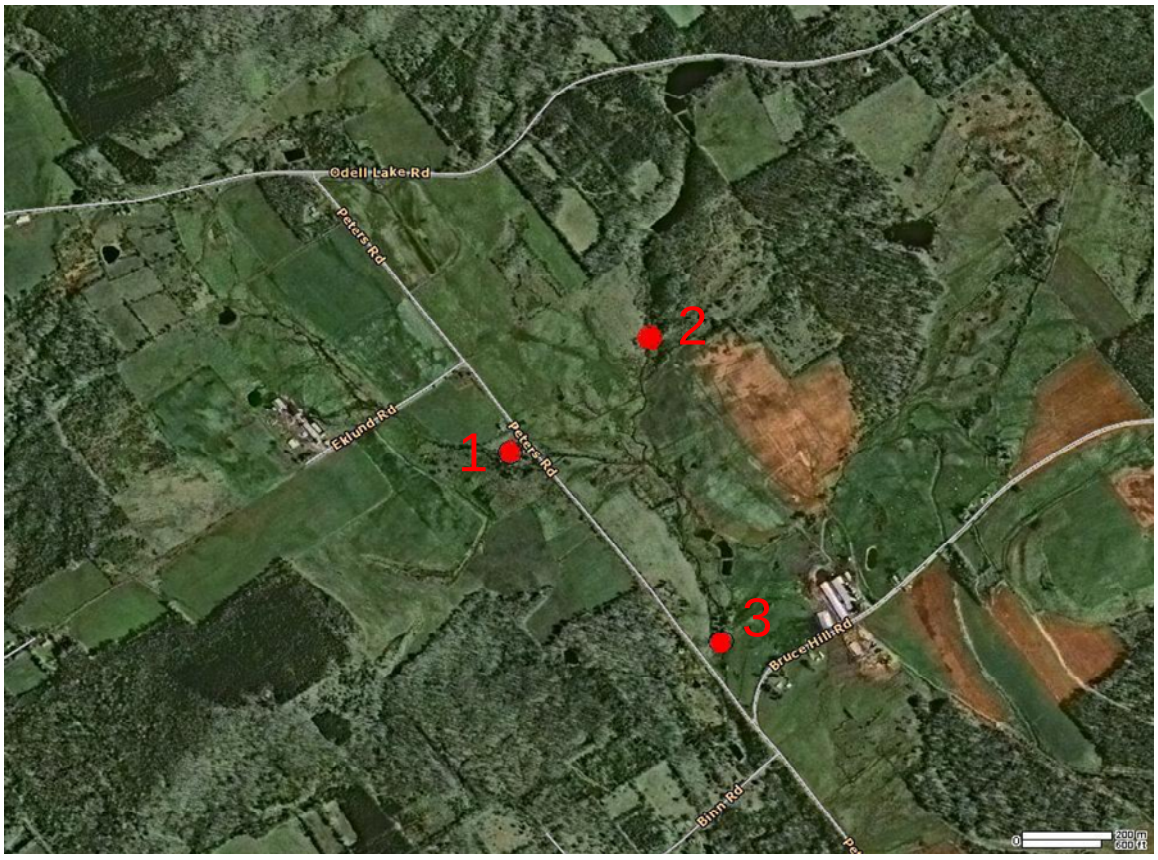


Figure 2.1 Sampling sites in Eklund Farm.

2.2.2 Field Sample Collection

Stream water samples were collected at the three sites on a monthly basis from Nov. 2006 to October 2007 except for Dec. and Feb. when cold weather and snow prevented access to the streams. Stream water was collected directly (i.e., grab samples) using 1l pre-cleaned, certified amber glass bottles with Teflon lined caps. Three bottles of water samples were collected at site 1 and 2, and 6-9 bottles were collected at site 3 for replications. The sample collection time and water temperature information is listed in Tables A1 and A2 in the Appendix.

A liquid manure sample was collected in mid-April when the land application of liquid manure at the farm just started. Three liters of liquid manure were collected from one of the two storage lagoons via a high capacity pump which was used to pump liquid manure from the lagoon to trucks. According to the farmer, the liquid manure was circulated within the lagoon (well-mixed) via a mixer before pumped out for land application.

All samples were kept in a cooler with ice in the field, and brought back to the lab on the same day. The liquid samples were typically filtered within 24 hours and extracted within 72 hours.

2.2.3. Stream Gauging

Stream discharges (flows) were determined by measuring the velocity of the stream water at a series of locations across the width of the stream (segments) using a Price type current meter. The cross-sectional area of each segment was determined by the width and depth of the segment. The total discharge was then calculated by summarizing the discharges at the segments. All discharge data are listed in Table A4 in the Appendix.

2.2.4. Laboratory Sorption/Degradation Experiments

Two aliquots of the liquid manure (100 ml each) were transferred into 250-ml media bottles, one was autoclaved (121 °C, 12.3 psi for 20 min), and both were spiked with d_3-E2β (final concentration of 25 ng/ml) after the bottles reached room temperature. A small fraction of the sample (10 ml) was taken out at different time intervals, filtered, and both the filtrate and the filter were analyzed for estrogens (see detailed methods below).

2.2.4 Sample Preparation

2.2.4.1 Filtration of liquid samples

Stream water samples were adjusted to pH of 6.5-7.0 using 1M HCl and pressure filtered through 142 mm GF/F precleaned glass fiber filters using a 142 mm stainless-steel pressure filter holder (Cole-Parmer, Vernon Hills, IL) and a piston metering pump with a ceramic-head (Fluid metering, Syosset, NY). Teflon tubing was used throughout the filtration device to minimize sorption of hormones. Each filer was rinsed with ~0.1 l acetone, ~0.5 l ultra-pure Milli-Q (Millipore) water, and ~ 0.5 l sample. Two liters of filtered sample were collected and spiked with d_3-E2β (final concentration of 0.2 ng/l). One aliquot of the site 3 samples was also spiked with the 4 naturally occurring estrogens (final concentration of 0.2 ng/l) to give accurate retention times during analysis (see section 2.4.2 below). For liquid manure samples, only 10 ml of each sample (spiked with d_3-E2β to a final concentration of 10 ng/ml) was filtered (using a vacuum filtration device) due to a high particle load. The filtrate was extracted by solid phase extraction (SPE) system, and the filters with the captured particulates were subsequently extracted via an accelerated solvent extraction (ASE) system (Dionex, Sunnyvale, CA), described below.

2.2.4.2 Accelerated solvent extraction

Filters with particulates were extracted with an ASE system following the method of Hooijerink et al. (*52*) with some slight modifications. A 34-ml stainless steel ASE extraction cell was filled from bottom to top with a glass fiber filter (Dionex), 5 g of cleaned florisil, 10 g of anhydrous sodium sulfate, the filter with particulates, and a second glass fiber filter. The void space on the top (if any) was then filled with diatomaceous earth (Dionex). The extraction solvent (acetonitrile) flowed through the extraction cell from top to bottom. The samples were extracted under 1500 psi at 50 °C with a static time of 5 min for 2 cycles. Flushing volume and purge time were 60% and 100s, respectively. The eluate (~60 ml) was concentrated to 1 ml using a TurboVap (Zymark®, Hopkinton, MA) before derivatization. The dry weight of the particulates was not determined, and the concentration was simply reported as the mass of estrogen per volume of liquid manure filtered.

2.2.4.3 Solid phase extraction (SPE)

Forty-seven mm C18 SPE discs (Empore, 3M, St. Paul, MN) were used to vacuum extract hormones from aqueous samples. The discs were first rinsed with 20 ml acetone and dried under vacuum. They were then pre-conditioned with 25 ml methanol followed by two rinses of 20 ml ultra-pure water each according to the manufacturer's protocol. Before the ultra-pure water totally went through the disk, 2 l of stream water sample was added and vacuum was adjusted to control the extraction flow at ~ 50 ml/min. For liquid manure samples the volume was 10 ml and the extraction flow was much slower. After the entire sample passed, the disk was dried in air for 30 min under 35 inch vacuum. The disk was then transferred to a clean set of vacuum extraction device and eluted with acetone (6 ml each for 3 times). The eluate was evaporated to 1 ml under a gentle stream of nitrogen before derivatization, described below.

2.2.4.4 Derivatization

Estrogens were derivatized with pentafluorobenzyl-bromide (PFBBr) and N-trimethylsilylimidzole (TMSI) according to published methods (*23,53*). The chemical reaction of estrogens (using E2 as an example) with PFBBr and TMSI is illustrated in Figure A1 in the Appendix. One hundred μl of 10% (w/w) K_2CO_3 solution and 10 μl PFBBr were added to 1 ml eluate (acetone or acetonitrile) and kept at 60°C for 2 hrs. After cooling, the solution was blown down to about 100 μl with nitrogen to remove solvents. Hexane (1 ml) was added and the vial was shaken well before 100 μl ultra-pure water was added. The vial was shaken again, and the upper layer (organic phase) was taken out and transferred to a 2nd 2-ml vial and ~50 mg anhydrous sodium sulfate was added (more anhydrous sodium sulfate would be added if water residual was observed on the wall of the vial). After shaking, hexane was carefully taken out from the sodium sulfate and transferred to a 3rd 2-ml vial. The extract was blown to dry in nitrogen and 100 μl TMSI was added. The vial was rolled so that the TMSI can contact the entire inside glass surfaces. After 45 min at room temperature, 1 ml hexane was added, and the vial was shaken before 100 μl Mili-Q water was added. After continuous shaking for ~5 min, the solution became clear, and the upper organic layer was transferred to a 4th 2-ml vial. For stream water samples, the extract was blown to dryness and resuspended in 200 μl hexane for analysis, described below. For liquid manure samples, the extract was blown to 1 ml and then analyzed.

2.2.5 Sample Analysis

All estrogens were analyzed using gas chromatography-mass spectrometry (GC-MS, Thermo Trace GC and DSQ MS, Thermo Finnigan) with negative ion chemical ionization (NICI) and selected ion monitoring (SIM). An HP-5MS capillary column (30m × 0.25mm i.d. × 0.25μm film thickness) was used for separation. Helium was used as the carrier gas (1.5 ml/min), whereas methane was used as the reagent gas (2.0 ml/min). The GC oven temperature was held

at 150°C for 1 min, increased to 310°C at a rate of 15°C/min, and held at 310°C for 5 min. The temperatures of the injector, the transfer line, and the ion source were 220°C, 300°C, and 240°C, respectively. Two μl of sample was injected splitless (split opened after 1.5 min), and 3 injections were carried out for each sample. $[M\text{-}PFB]^-$ ions (masses of 269, 343, 343, 431, and 346 for E1, α-E2, β-E2, E3 and D-β-E2, respectively) were monitored for all compounds (*23*). To increase the sensitivity, only one mass was monitored during an entire GC/MS run.

2.2.6 Method Detection Limit (MDL) Estimation

A low concentration standard with estrogens (2 ng/ml in methanol) was prepared and derivatized, as described above. The standard was then analyzed for multiple times (n=13) and the standard deviation of the analyses was determined. The MDL for the standard was then calculated by multiplying the standard deviation by the Student's t-value (11 degrees of freedom), according to ref (*54*). The MDL for the stream water samples and the liquid manure was then estimated based on the MDL of the standard and the concentration factor. The underlying assumption was that the SPE extraction was 100% efficient for estrogens in stream water samples and liquid manure samples, and the matrix effects were small.

2.3. Results and Discussion

2.3.1 Sensitivity, Reproducibility, and Recovery

The MDL values of the estrogens in stream water and liquid manure were tabulated in Table 2.1. Very low MDL values were achieved for stream water samples mainly due to the high concentration factor (2 l down to 0.2 ml, a factor of 10,000). In contrast, MDL values for liquid manure were much higher, due to the small concentration factor (10 ml down to 1 ml, a factor of 10). The standard deviation of the analysis of stream water samples (based on triplicates)

averaged 11%, 7.8% and 8.8% for E2α, E2β, and E3, indicating excellent reproducibility of the analytical method. Complete recovery of the surrogate standard (d_3-E2β) in stream water samples (105±19%, n=36) was also achieved (Table A5 in Appendix). The recovery of d_3-E2β in the liquid manure sample was close to 90%. The MDL for d_3-E2β in real stream water (based on the 36 spiked samples) was estimated to be 0.046 ng/l, about 50% higher than the MDL estimated based on a clean standard. Since the matrix in stream samples at different seasons may vary and the stream water contained some estrogens, it would be difficult to spike estrogens to the real samples obtain true MDL values. For the liquid manure samples it would be impossible to spike the liquid manure to obtain true MDL values as the spiked estrogens would be quickly degraded, as discussed in Section 2.3.4.

Table 2.1. Method detection limits (ng/l) of the estrogens in stream water and liquid manure.

	E1	E2α	E2β	E3	d_3-E2β
Stream water	0.061	0.036	0.026	0.036	0.030
Liquid manure	61.1	35.9	26.4	35.8	30.3

2.3.2 Estrogens in Stream Water

Concentrations of E2α, E2β, and E3 at the three stream sites are presented in Figure 2.2 and Table A6 in the Appendix. Of the three estrogens, E2β has the highest detection frequencies, followed by E2α and E3. E1 was always below detection limit in all stream samples collected and therefore was not plotted. Estrogens were present consistently in the streams in the spring months (March to June) and sporadically in other seasons, and even in the spring the estrogen concentrations were fairly low (< 1 ng/l, Figure 2.2). Snow usually starts to melt and the frozen ground starts to thaw in the study area in March. As a result, there is a large amount of runoff (overland flow, interflow and groundwater flow) towards the streams (as evidenced by the stream discharges presented in Figure 2.3), moving some estrogens from soils to the streams.

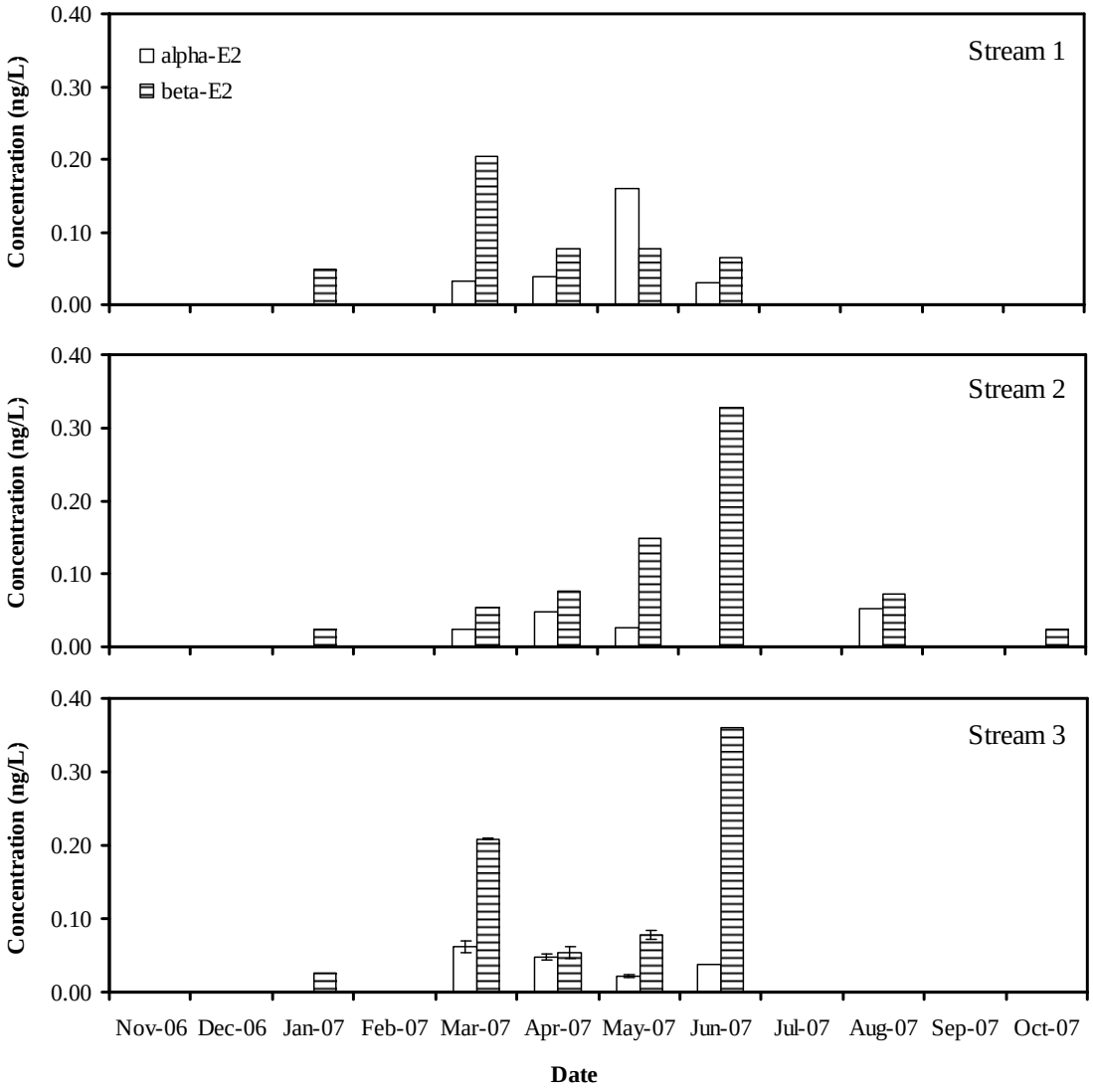


Figure 2.2. Estrogen concentrations in three streams from Nov 2006 to Oct 2007.

Land application of liquid manure usually starts in mid-April (and lasts till August), and there was a general trend of increasing estrogen concentrations in the streams from April to June (Figure 2.2). The stream discharges decreased significantly in May and June, suggesting less runoff (and less leaching of estrogens from soils) to the streams from the watershed. However, smaller discharges also led to lesser dilution, and hence it was still possible to observe increased estrogen concentrations during this period. There were few detections of estrogen in the streams

during summer and fall, presumably due to the fast degradation of estrogens in manure and soils at high temperatures and due to the low runoff to the streams (see Figure 2.3 for stream discharges). Our laboratory tests indicated that estrogens were rapidly degraded in liquid manure (see Section 2.3.4 below). During winter months, the study area is usually covered by snow and the ground is typically frozen and as such little surface water and groundwater flow towards the streams. Therefore, little estrogens would be expected in the streams during winter. The unseasonably warm temperature in January 2007, however, resulted in early snow melting and the detection of estrogens in the streams.

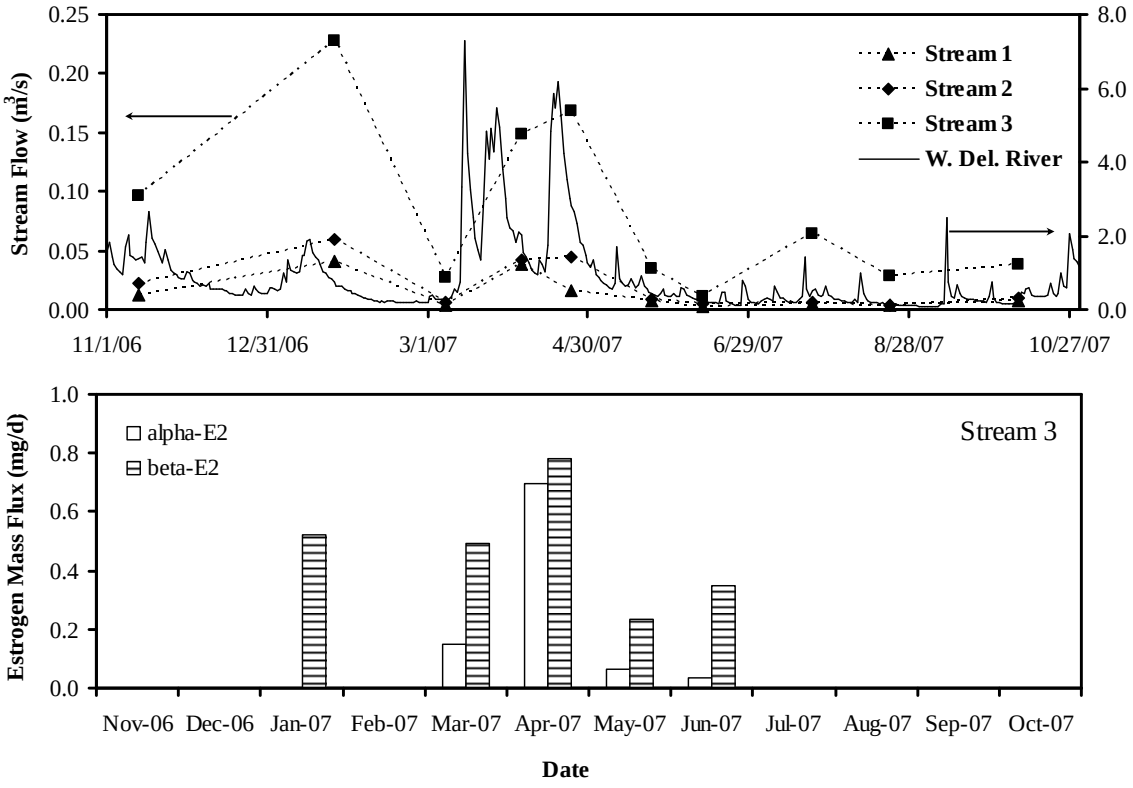


Figure 2.3. Steam flows at 3 sampling sites and the West Delaware River (upper panel) and estrogen mass flux in stream 3 (lower panel).

It is noted that there was no precipitation during and a few days prior to the sampling events from May to August. In other words, groundwater was the major contributor to stream

discharges during those sampling events. The detection of estrogens in the streams in May, June, and August therefore indicated the contribution of estrogens from groundwater.

The mass fluxes (mass per time, calculated by multiplying concentrations and stream discharges) of the three estrogens in Stream 3 are presented in Figure 2.3. Higher mass fluxes were observed in the unseasonably warm January of 2007 and the spring months, due to higher stream discharges and/or higher estrogen concentrations during these months. The estimated yearly input of the estrogens to Stream 3 (which captures about one half of the runoff from the farm) was 129 mg. Assuming each cow produces about 1 g estrogens per year (*37*,*55*), the total estimated amount of estrogens produced by the 800 or so cows in the CAFO would be around 800 g per year. Obviously, only a tiny fraction (0.016%) of the estrogens potentially excreted by the cows ended up in the stream. As discussed below, most of the estrogens are assumed to be degraded during storage in the lagoons.

2.3.3 Estrogens in Liquid Manure

Estrogen concentrations in the liquid manure were 475, 98, 104, and 657 ng/l for E1, E2α, E2β, and E3, respectively (Table 2.2). These values are similar to the concentrations observed by others in dairy farm lagoons (*43*,*56*). By April when field application of the liquid manure starts, the manure has been stored in the lagoons for about 8 months and the lagoons are at about 75% capacity (3 million gallons total). Using the concentrations measured in the lagoon sample (1334 mg/l) and the volume of the liquid manure at that time, the total estimated amount of estrogens in the liquid manure was 11.6 g. The total potential amount of estrogen excreted by the cows in 8 months would be around 367 g (*55*). Therefore, about 97% of the estrogens may have been degraded. Our lab experiments did demonstrate such rapid degradation (Section 2.3.4 below).

Table 2.2. Estrogen concentrations (ng/l) in liquid manure.

	E1	E2α	E2β	E3	Total
Liquid manure-filtrate	BDL	BDL	BDL	BDL	
Liquid manure-particulates	475	98	104	657	1334

It is interesting that all these estrogens were exclusively detected on the particulates (captured by a 0.7 μm filter), not in the aqueous phase. Unconjugated estrogens are moderately hydrophobic ($\log K_{ow}$ of 2.6-4.0) (*37*) and are expected to be associated with colloidal particles in solutions with high organic carbon contents. For instance, one study revealed that up to 60% of E2β can be associated with aqueous particulates in biological wastewater systems (*57*). The non-detection of estrogens in the aqueous phase of the liquid manure was probably due to the significant sorption onto particulates and the relatively poor detection limit of liquid manure samples (61.1, 35.9, 26.4, 35.8, and 30.3 ng/l for E1, E2α, E2β, and E3, respectively). If we assume that concentrations near these detection limits were present in the aqueous phase, then the particulate-bound estrogens would account for about 90% of the total estrogens in the liquid manure (*56*). Hutchins et al. (*56*) observed estrogen concentrations of a few hundreds to a few thousands ng/l in the "aqueous phase" (passing through 1.2 μm filters) of CAFO lagoon samples but pointed out that much of the estrogens could be associated with colloidal materials. Our result, in conjugation with the study of Hutchins et al (*56*), suggests that much of the estrogens in liquid manures would be associated with colloidal materials of a size of 0.7 to 1.2 μm present in the liquid manures. Since colloids of this size range are the most mobile ones in porous media (*58*), future studies on the fate and transport and risk assessment of steroid hormones from animal wastes need to consider colloid-facilitated transport of hormones.

2.3.4. Sorption and Degradation of d_3-E2β in Liquid Manure

The sorption and degradation kinetics of d_3-E2β in liquid manure is presented in Figure 2.4. For the non-sterile sample, the amount detected in the aqueous phase and on the particulates after 0.3 hr accounted for 71% and 20% of the total spiked concentration (25 ng/ml), respectively (Figure 2.4), suggesting about 20% sorption. After about 3 hours, the amount in the aqueous phase and on the particulates decreased to 24% and 5%, respectively, indicating rapid degradation or non-extractable sorption. More than 90% of the spiked amount dissipated after about 100 hours (Figure 2.4). For the autoclaved sample, about 85% and 15% of the total spiked concentration was recovered from the aqueous phase and the particulates, respectively, after about 0.3 hr. The concentrations continued to drop in both the aqueous and particulate phases during the entire duration of the experiment, albeit the rate of drop was significantly less than that of the non-sterile sample. Degradation of estrogen in autoclaved soils was also observed by others (*59*), and was attributed to incomplete sterilization and/or abiotic degradation (*37*,*59*).

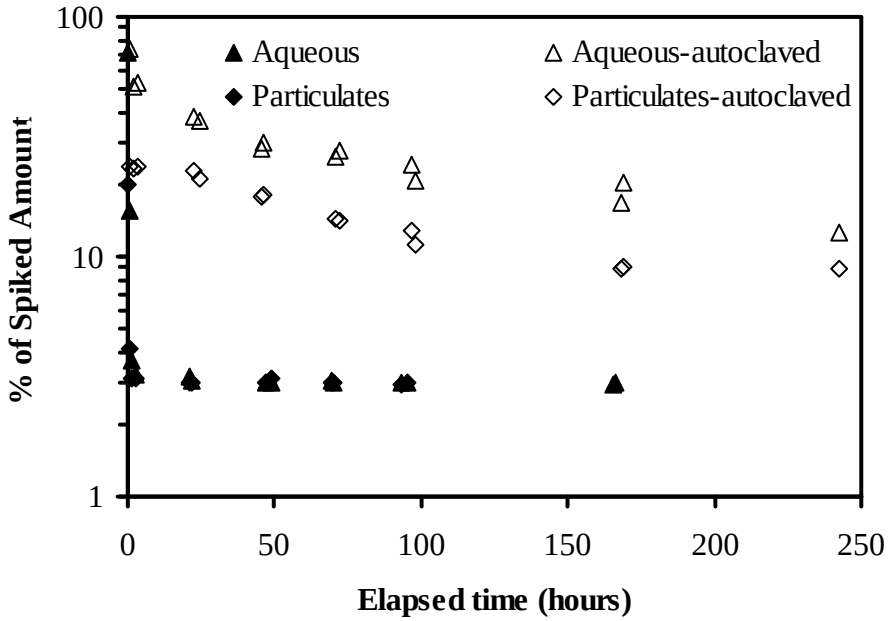


Figure 2.4. Sorption and degradation of d_3-E2β in liquid manure.

2.4 Conclusions

Low concentrations of estrogens (< 1 ng/l) were detected in the headstreams of the "organic" CAFO, with E2β has the highest detection frequencies, followed by E2α and E3. E1 was always below the detection limit. Estrogens were present consistently in the streams in spring months (likely due to the mobilization of estrogens from soils upon snow melting/precipitation) and sporadically in other seasons. Estrogens were detected in the streams during dry periods, indicating the contribution of estrogens from groundwater. The analysis of liquid manure at the beginning of manure application season (after ~ 8 months storage) showed that over 97% of the estrogens potentially excreted by the cows were degraded. Moreover, about 90% of the estrogens in the liquid manure were associated with particulates larger than 0.7 μm. Batch experiments with spiked d_3-E2β in the liquid manure demonstrated sorption of d_3-E2β onto particulates in the liquid manure, and rapid degradation of d_3-E2β in the aqueous phase and on the particulates of the liquid manure. It is concluded that the low estrogen concentrations in the streams were probably due to the long residence time (~8 months) of the manure in the lagoons (i.e., majority of the estrogens were degraded during storage), and/or strong retention of estrogens in soils after land application of the manure. The strong association of estrogens with particulates in liquid manure suggests that future studies on the fate and transport and risk assessment of steroid hormones from animal wastes need to consider colloid-facilitated transport of hormones.

Chapter 3

Use of Pharmaceuticals and Pesticides to Constrain Nutrient Sources in Coastal Groundwater of Northwestern Long Island, New York

Abstract

In developed, non-agricultural, unsewered areas, septic systems and fertilizer application to lawns and gardens represent two major sources of nitrogen to coastal groundwater, in addition to atmospheric input. This study was designed to distinguish between these two possible nitrogen sources by analyzing groundwater samples for pharmaceutical residuals, because fertilizers do not contain any of these pharmaceuticals, but domestic wastewater can. In addition, several herbicides and insecticides used in lawn treatment were analyzed as indicators of nitrogen delivery to ground water from fertilizers. Groundwater samples were taken through piezometers at shoreline sites in unsewered areas in Northport Harbor and in sewered areas adjacent to Manhasset Bay, both in western Long Island Sound. Excessive nitrogen loading has led to reduced dissolved oxygen concentrations in the sound, and the groundwater contribution to the nitrogen budget is very poorly constrained. The frequent detection of the anticonvulsant compound carbamazepine in groundwater samples of Northport (unsewered), together with the fact that few pesticides associated with lawn applications were detected, suggest that wastewater input and atmospheric input are the likely sources of nitrogen in Northport groundwater. High concentrations of nitrogen were also detected in Manhasset (unsewered) groundwater. The low detection frequency of carbamazepine, however suggests that the sewer system effectively intercept nitrogen from wastewater there. The likely source of nitrogen in Manhasset groundwater would be atmospheric input (e.g., automobile exhausts) as this area is densely populated, and possibly lawn fertilizers.

3.1 Introduction

Long Island Sound (LIS) is an estuary bounded by the coast of Connecticut to the north and Long Island (New York) to the south (Figure 3.1) and is one of the largest estuarine systems on the Atlantic coast of the U.S. (*60*). Western LIS suffers summer hypoxia (reduced concentration of dissolved oxygen in water leading to stress and death in aquatic organisms) due to excessive nitrogen loading to the sound (*61*,*62*). The relative importance of various nitrogen sources (e.g., direct atmospheric deposition, riverine input, wastewater treatment plant input, and groundwater input) to the sound, however, is poorly understood. The discharge of nitrogen through groundwater could be particularly significant in unsewered regions of Long Island, where domestic waste is discharged directly into groundwater through septic systems. In addition to the septic systems, fertilizer application to lawns and gardens in this developed, non-agricultural region may also represent a major source of nitrogen input to groundwater.

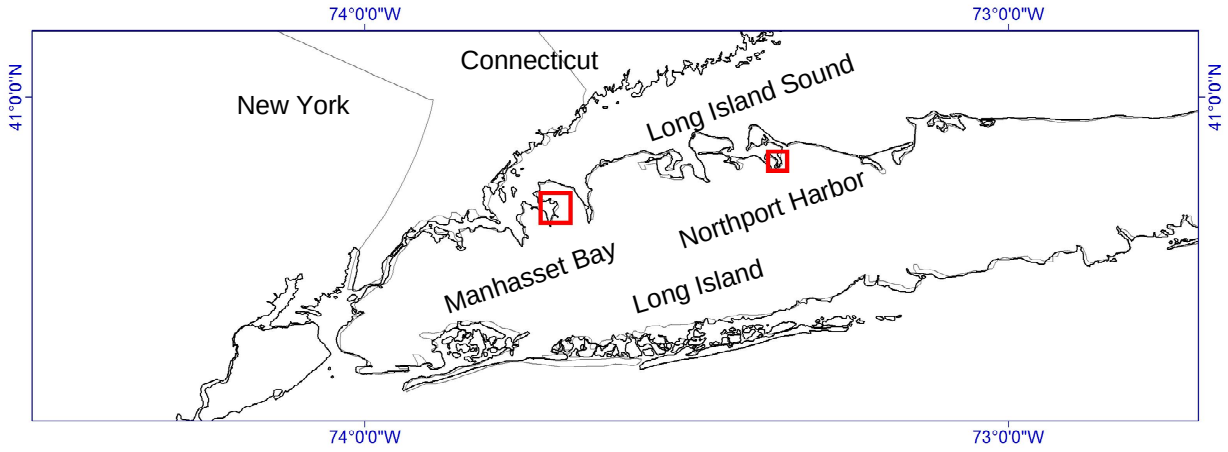


Figure 3.1. Map of western Long Island. Sampling areas are indicated by red rectangles.

Knowledge of the relative contributions of wastewater and fertilizer nitrogen to groundwater would better inform management decisions regarding mitigation strategies (e.g., installing sewers and wastewater treatment plants vs. restricting fertilizer usage). Differentiating

these two nitrogen sources, however, is not trivial. Stable nitrogen isotopes ($\delta^{15}N$) have frequently been used to identify different sources of NO_3^- in groundwater (*63-67*), but mixing of distinct NO_3^- sources and denitrification in the subsurface may mask the original $\delta^{15}N$ signature (*68*). As such, analyzing $\delta^{15}N$ alone often does not lead to conclusive identification of NO_3^- sources. The coupling of ^{15}N with a second stable isotope (e.g., $\delta^{18}O$ of NO_3 or ^{11}B) generally provides more conclusive information on NO_3^- sources in water (*69,70*). Nevertheless, sample preparation for isotope analysis is often complex, labor intensive, and expensive (*68*).

A potential alternative (or complementary) means of differentiating the two nitrogen sources (wastewater vs. fertilizer) is to examine nitrogen concentrations and speciation in groundwater together with organic tracers found only in human sewage (e.g., pharmaceutical residuals) or associated with lawn fertilizers (e.g., herbicides/insecticides used in lawn treatment). For instance, carbamazepine, an anticonvulsant (antiepileptic) and mood-stabilizing drug, is barely removed during wastewater treatment (*71*), river-bank filtration (*72*), and groundwater recharge (*73*) processes, and may be stable in groundwater for decades (*73*). Carbamazepine has been detected in shallow groundwater in Long Island, New York (in 26% of 61 wells tested;) (*74*) and elsewhere, and has been proposed as a possible anthropogenic marker in the aquatic environment (*75*) and as an indicator for sewer leakage (*76*).

Over two dozen pesticides are commonly applied to U.S. lawns and golf courses (*77*). Some of the pesticides (e.g., prometon, halofenozide, and imidacloprid) have relatively long half-lives (hundreds of days) and relatively low K_{oc} values (< 500 cm^3/g) (*77*) and therefore have the potential to reach groundwater. A review of water quality data indicated that 21 pesticides were detected in groundwater near golf courses with 5 of them exceeding maximum allowable

concentrations (*78*). Many commercially available fertilizers contain insecticides or herbicides and therefore it may be possible to use these as indicators for fertilizers.

The primary goal of this work was to examine the potential of using organic tracers (pharmaceutical residuals and pesticides) to distinguish nitrogen associated with septic systems from nitrogen associated with lawn fertilizers. As a first step, the concentrations of nitrogen and a few pharmaceuticals and pesticides were measured in groundwater of unsewered areas adjacent to Northport Harbor and sewered areas surrounding Manhasset Bay, both in western Long Island, New York (Figure 3.1). The distribution patterns of nitrate and the organic tracers in the two different areas, as well as the correlations between total dissolved inorganic nitrogen (TDN) and the organic tracers at each area, were examined.

The secondary goal of this work was to develop a direct injection liquid chromatography tandem-mass spectrometry (LC/MS/MS) method for quantitative analysis of a few selected pharmaceuticals and pesticides in groundwater. Analyzing these trace organic compounds in water usually involves a solid phase extraction (SPE) step where a large volume (e.g., 1 l) of water is passed through an SPE disc or cartridge (e.g., C-18) and the organic compounds retained on the disc or cartridge are then eluted with an organic solvent (*79,80*). The SPE step allows a large concentration factor (e.g., from 1 l to 1 ml) but is time-consuming and expensive (a few dollars per disc or cartridge). Moreover, the SPE step may lead to some loss of the analytes (incomplete recovery), and/or potential cross-contamination if the filtration device is not cleaned thoroughly. Direct injection of water samples into the LC column would avoid such potential problems associated with SPE, save time, reduce cost, and increase the overall robustness of analysis if the compounds of interest could be detected and quantified without pre-concentration using the LC/MS/MS method.

3.2 Materials and Methods

3.2.1 Study Sites

The study sites, Manhasset Bay and Northport Harbor, are located along the north side of western Long Island, New York (Figure 3.1). Samples from Manhasset Bay were taken along the sewered section of the bay shoreline. Northport Harbor was considered unsewered because sewers, which were built in the 1930's, serve only a small section of the town. The population densities for Manhasset and Northport are 2,800/km^2 and 1,200/km^2, respectively.

3.2.2. Field Sample Collection

Ground water samples were collected in spring and fall of 2008 along the bay shorelines at depths of 0.3-4.6 m below the ground surface. A stainless steel drive point piezometer with Teflon tubing was driven into the aquifer using a slide hammer, and groundwater (2 l per site) was pumped out with a peristaltic pump into pre-cleaned, certified amber glass bottles with Teflon-lined caps. The samples collected in spring were pre-concentrated using SPE discs prior to LC/MS/MS analysis, whereas the fall samples were analyzed via direct injection LC/MS/MS, described below. One field equipment blank was collected per trip. The coordinates of the sampling sites are listed in Table A5 in the Appendix. All samples were kept in a cooler with ice in the field and typically filtered within 24 hours; the spring 2008 samples were extracted within 72 hours.

3.2.3 Sample Preparation

3.2.3.1 Filtration of Ground Water Samples

Groundwater samples were adjusted to pH of 8.5 using 1 M HCl and pressure filtered through 142-mm GF/F (0.7µm pore size) precleaned glass fiber filters using a stainless-steel pressure filter holder (Cole-Parmer, Vernon Hills, IL) and a piston metering pump with a

ceramic-head (Fluid metering, Syosset, NY). Teflon tubing was used in the filtration device to minimize sorption of compounds. Each filer was rinsed with ~0.1 l methanol, ~0.5 l ultra-pure Milli-Q (Millipore) water, and ~ 0.5 l sample. Filtered samples (1 l each) were then spiked with 30 µl of 0.1 µg/ml flurazepam (surrogate standard) for spring samples or 30 µl of 0.01 µg/ml flurazepam for fall samples.

3.2.3.2 Solid Phase Extraction (SPE)

Spring field samples were pre-concentrated with 47-mm C18 SPE discs (Empore, 3M, St. Paul, MN). Each disc was first rinsed with 20 ml acetone and then dried under vacuum. The disc was then pre-conditioned with 25 ml methanol followed by two rinses of 20 ml ultra-pure water according to the manufacturer's protocol. Before the ultra-pure water had completely passed through the disk, 1 l of water sample was added and the vacuum was adjusted to maintain the extraction flow at ~ 50 ml/min. After the entire sample volume had passed through, the disk was dried in air for 30 min under 35 inch vacuum. The disk was then transferred to a clean vacuum extraction device and eluted with methanol (6 ml each, 3 times). The eluate was evaporated to 1 ml under a gentle stream of nitrogen.

3.2.4 Sample Analysis

All samples were analyzed using LC/MS/MS with electron spray ionization (ESI) and multiple reaction monitoring (MRM). A Shimadzu HPLC, consisting of a DGU-20A3 degasser, LC-20AD binary pumps, a SIL-20AC HT autosampler, and a CTO-20AC oven, was used. An Agilent Eclipse Plus C18 (1.8 µm × 4.6 mm × 50 mm) column was used for separation. The temperature of the column oven was kept at 35°C. The mobile phase consisted of water (component A) and methanol (component B) buffered with 0.1% (v/v) formic acid and 4 mM

ammonium formate. The following flowing gradient elution program was used: 5% B for 1min, 95% B from 2 to 4.5 min, and 5% B from 4.5 to 5 min. The flow rate was 0.8 ml/min.

For the spring 2008 samples, 20 µl of each extracted sample was injected through the autosampler, and the needle was automatically rinsed with 0.2 ml methanol between injections. For the fall 2008 samples, a ten-port Valco valve (model EHMA) with a 1 ml stainless steel loop was used for direct injection. For each sample, 4 ml was manually injected into the loop using a syringe while the valve was set at position A (waste). At 0.1 min the valve was automatically switched to position B (column) and at 4.7 min the valve was switched back to position A for the next injection.

Mass spectrometry was performed with an ABI 4000 Q-trap mass spectrometer (Applied Biosystems) with an ESI ion source generator and N300DR nitrogen generator (Peak Scientific, Billerica, MA). Nitrogen was used as both the collision gas and nebulizing gas. The curtain gas, collision gas, and ion source gas 1 and gas 2 were set at 25, 6, 60, and 65 psi, respectively. The nebulizer current was set at 3 mA. The temperature of the interface heater was maintained at 600°C. Quantification of all compounds was made by two multiple reaction monitoring (MRM) transitions. Table A7 in the Appendix shows the MRMs for all compounds and quantitative optimization results.

3.2.5 Method Detection Limit (MDL) Estimation

A groundwater sample (MIZ 5) that was free of the compounds tested was used to determine MDL and recovery. For MDL determination the sample was spiked with the compounds at 0.1 ng/l (10 ng/l for halofenozide), whereas for recovery tests the sample was spiked at 1 ng/l (10 ng/l for halofenozide). The sample was then analyzed multiple times either through direct injection LC/MS/MS or SPE pre-concentration LC/MS/MS. The MDL was then

calculated by multiplying the standard deviation of the multiple analyses by a Student's t-values (*54*).

3.3 Results and Discussion

3.3.1 Sensitivity and Recovery of the Analytical Methods

The sensitivity and recovery of the SPE pre-concentration method and the direct injection method are tabulated in Table 3.1. The MDL for the direction injection method ranged from 0.01 to 0.05 ng/l for all the organic tracers examined except for halofenozide, which had an MDL of 10 ng/l. These MDL values were comparable to those obtained using the SPE pre-concentration method (Table 1). Although the SPE method allowed a large-volume water sample to be concentrated (e.g., from 1 l to 1ml), only 20 μl (equivalent to 20 ml of raw sample) was injected to the LC/MS/MS via an autosampler. In contrast, the direct injection methods allowed 1 ml of the raw water sample to be injected into the LC column, concentrated within the column, and then eluted to the mass spectrometer through gradient elution. Therefore, the total mass injected was 5% of that analyzed using the SPE method. The background noise of the direct injection, however, was much less than with the SPE method because the concentration step also increased the background level. Therefore, the overall sensitivity was about the same for the two methods.

The recoveries for the compound spiked in one groundwater sample (MIZ5) were near 100% (98%-108%, with a mean of 103%) for all the organic tracers tested for the direct injection method, and the standard deviation was equal to or less than 1% (Table 3.1). The excellent mass recovery and small standard deviation of triplicate injections demonstrated the robustness of the direct injection method. The recoveries for the SPE pre-concentration method were also complete (96%-109%, with a mean of 102%) for all the organic tracers, with a slightly higher standard deviation (up to 5.6%, Table 3.1).

Table 3.1. Comparison of the method detection limits of the direct injection method and SPE pre-concentration method.

	Direct Injection			SPE Pre-concentration		
	MDL (ng/l)	Recovery (%)	Standard Deviation (%)	MDL (ng/l)	Recovery (%)	Standard Deviation (%)
Bupropion	0.032	99.5	0.97	0.10	102	0.65
Carbamazepine	0.010	101	1.05	0.016	100	0.77
Citalopram	0.053	105	0.17	0.093	108	0.28
Diazepam	0.018	108	0.35	0.19	101	2.05
Diclofenac	0.0092	102	0.81	0.013	102	2.46
Oxcarbazepine	0.015	99.6	0.98	0.53	96.1	5.62
Promidone	0.0078	104	0.35	0.026	102	0.72
Venlafaxine	0.030	103	0.36	0.066	96.1	3.50
Caffeine	0.033	98.2	0.17	0.030	103	1.16
DEET	0.034	105	1.00	0.034	108	1.72
Oxybenzone	0.027	99.6	0.98	0.12	109	0.64
Clofibric Acid	0.012	102	1.45	0.0064	103	2.03
Halofenozide	10.8	101	7.55	0.14	102	3.40
Imidacloprid	0.012	105	1.08	0.023	102	0.98
Pendimethalin	0.053	98.5	0.62	0.62	101	1.20
Prometon	0.020	105	0.78	0.069	101	3.40
Flurazepam	0.032	103	0.30	0.023	99.4	0.42

The high sensitivity and recovery of the direct injection method would significantly reduce the cost and time of analysis compared to the SPE pre-concentration method. The small

volume needed for the direct injection method (tens of ml) would also save time and sample storage space during field sampling.

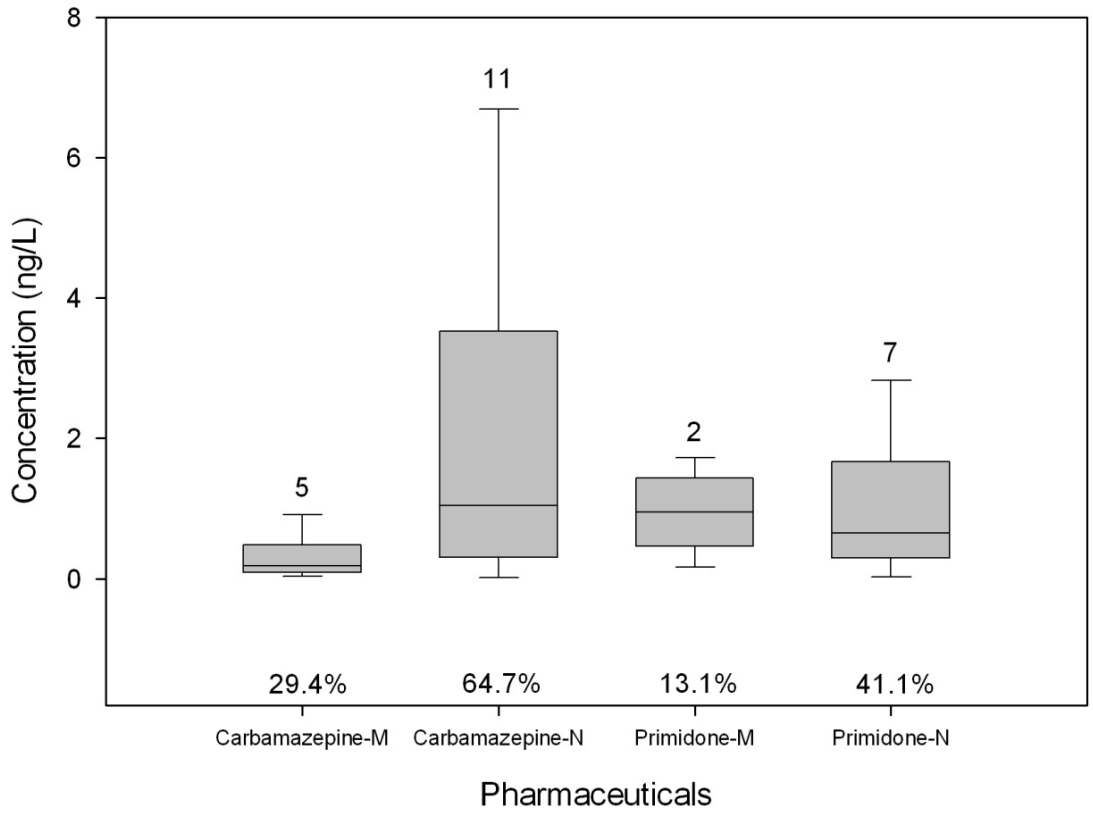
3.3.2 Occurrence of Pharmaceutical Residuals and Pesticides in Groundwater

The anticonvulsant drug carbamazepine was detected in 65% of the samples collected at Northport (mainly unsewered), with a maximum concentration of 6.7 ng/l and a median concentration of 1.0 ng/l (Figure 3.2). In contrast, carbamazepine was only detected in 33% of the samples collected at Manhasset (mainly sewered), with much lower maximum (0.91 ng/l) and median concentrations (0.33 ng/l) with respect to Northport samples (Figure 3.2). Primidone, another anticonvulsant, was found in 41% of the Northport samples in contrast to 13% of the Manhasset samples (Figure 3.2). Clearly, these two anticonvulsants occur in the groundwater of the largely unsewered Northport area more frequently (and at higher concentrations in most cases) than in the mainly sewered Manhasset area. It is important to note, however, that there are small sections of the Manhasset area that are not sewered and that the highest anticonvulsant concentrations measured in Manhasset groundwater appear to be concentrated near an area with approximately 16 unserwered houses (Figure 3.3). Therefore, the presence of anticonvulsants in the groundwater of the Manhasset Bay watershed does not necessary indicate the leakage of sewer lines, but rather direct input from isolated septic systems. Other pharmaceuticals were either not detected or detected at very low frequencies, and therefore are not presented here.

Prometon, an herbicide (weed killer), was found in 29% of the Northport samples with a maximum concentration of 5.0 ng/l and a median of 2.5 ng/l. In contrast, prometon was detected in 87% of the Manhasset samples with a maximum concentration of 65.6 ng/l and a median of 1.9 ng/l (Figure 3.4). Clearly, there is more prometon usage in the Manhasset area than in the

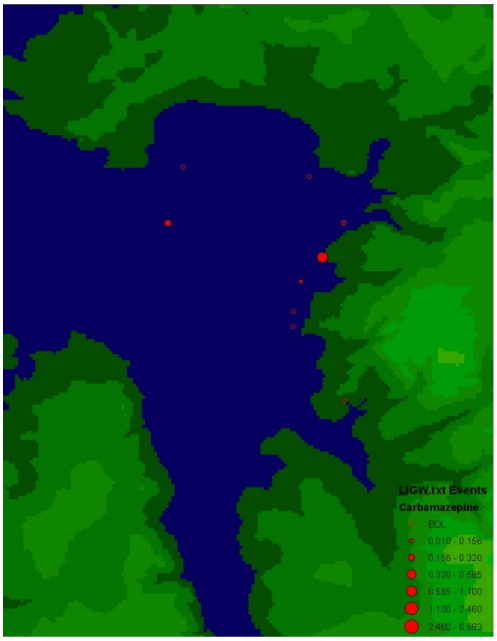
North-port area. This appears to be in accordance with the population densities at these two areas (2,800/km^2 for Manhasset and 1,200/km^2 for Northport). Imidacloprid, an insecticide used in seed treatment, was detected at similar frequencies and median concentrations in both areas. In addition to seed treatment, imidacloprid is also used for pest control, termite control, flea control, and as a systemic insecticide. Therefore, its presence may not necessary reflect lawn usage. Two other lawn-related pesticides, pendimethalin and halofenozide were below detection limits in all samples.

Figure 3.2 Boxplot of carbamazepine and primidone, two anticonvulsants. M =



Manhasset and N = Northport; the number of samples with detections is indicated above each bar, and the percent frequency of detection is shown below each bar.

Manhasset - sewered



Northport - unsewered

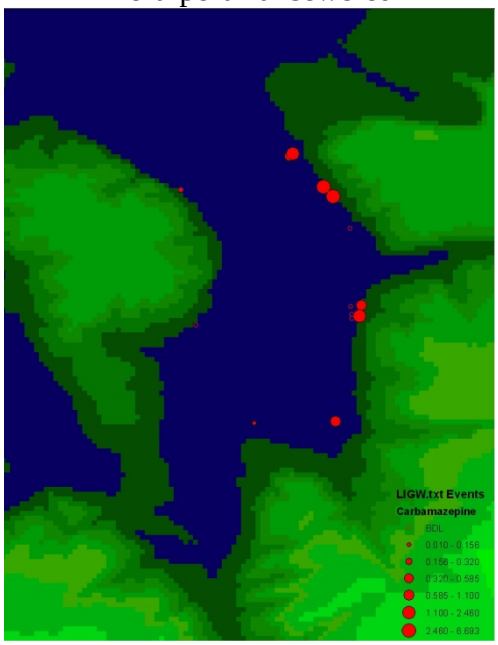


Figure 3.3 Distribution of carbamazepine and primidone.

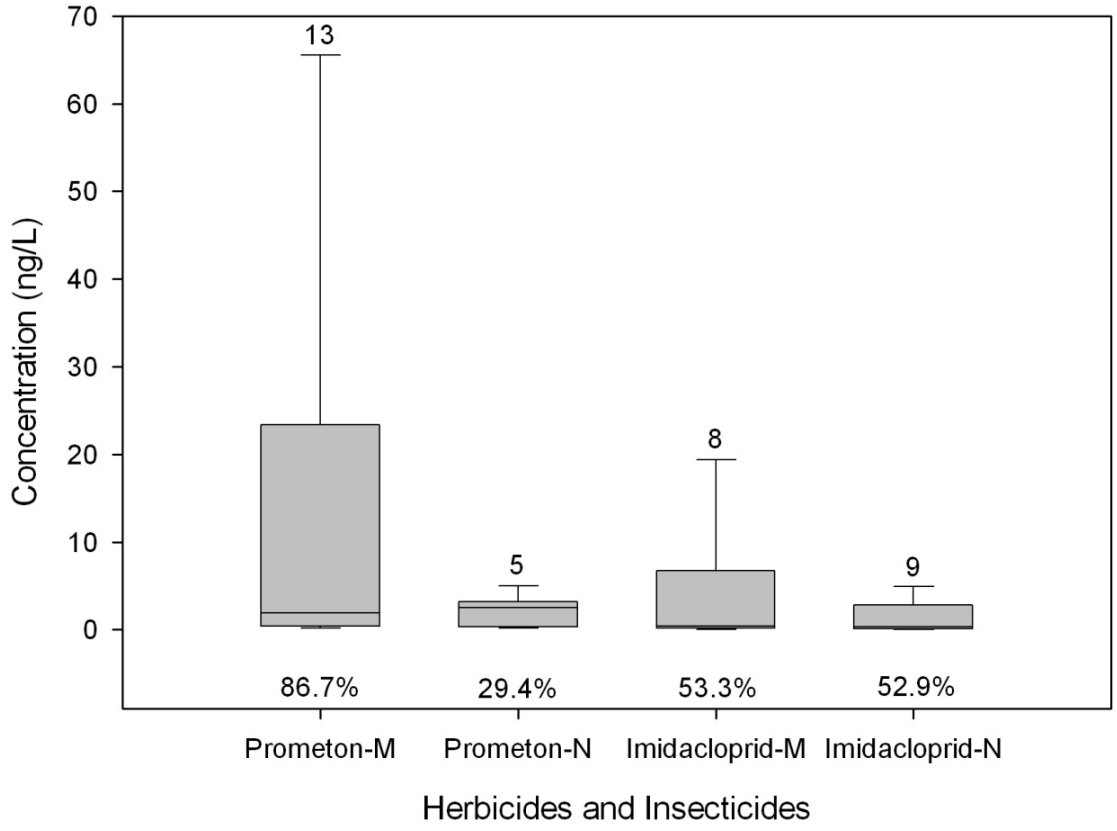


Figure 3.4 Boxplot of herbicides and insecticides. M = Manhasset and N = Northport; the number of samples with detections is indicated above each bar, and the percent frequency of detection is shown below each bar.

3.3.3 Relations to nitrate concentration

Nitrogen species were detected in all 17 samples collected for organic tracer analysis at Northport (mainly unsewered). Concentrations of TDN ranged from 1.0 to 435 μM, with a median value of 235 μM (Figure 3.5). Surprisingly, nitrogen species were also detected in most groundwater samples (13 out of 14) from the Manhasset Bay watershed (mainly sewered) at

comparable TDN concentrations (0.5-367 μM), but with a somewhat lower median value (135 μM, Fig. 3.5).

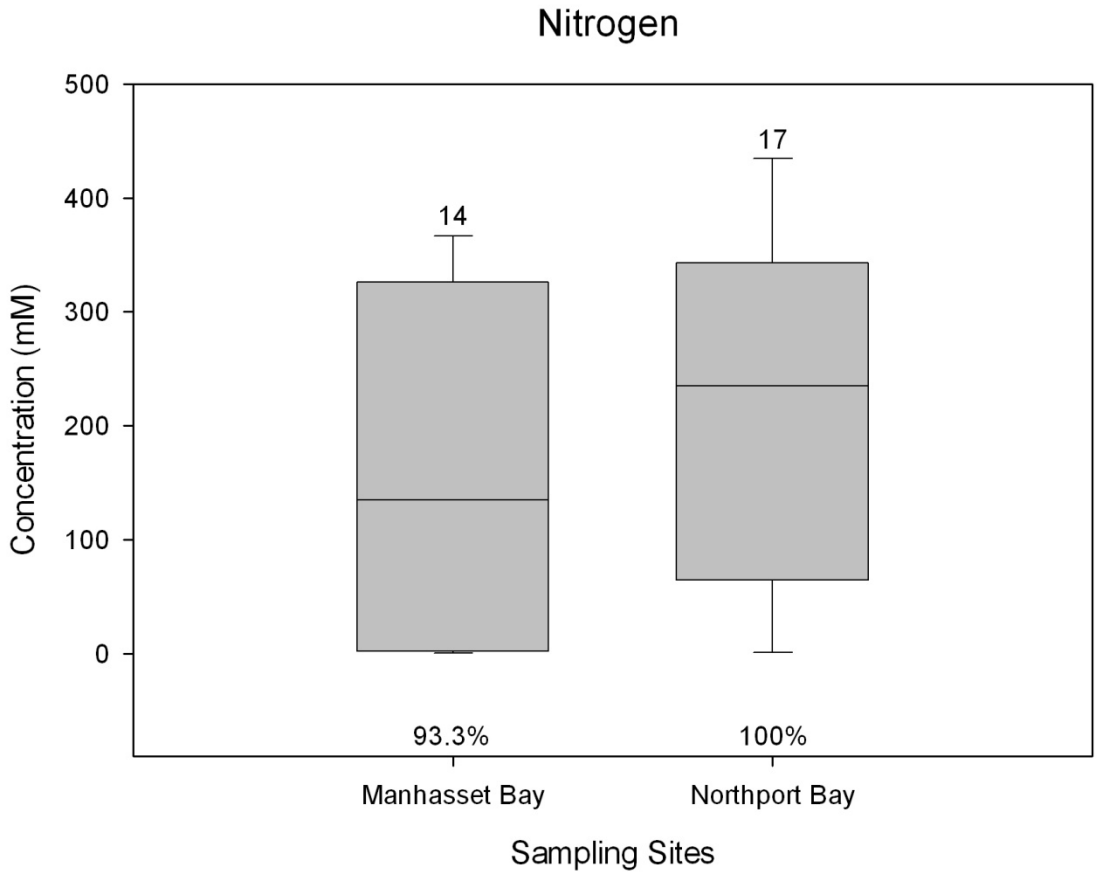


Figure 3.5. Box plots of TDN concentrations. The number of samples with detections if indicated above each bar, and the percent frequency of detection is shown below each bar.

Generally speaking, nitrogen is delivered to groundwater from the atmosphere (from automobile exhaust, for example), from fertilizer applications, and from wastewater. There is some positive cor-relation between carbamazepine and TDN concentrations ($r^2 = 0.52$, Figure 3.6) at Northport, suggesting a significant wastewater source of nitrogen in that area. The lack of detection of herbicides and insecticides at Northport suggests that fertilizer is probably not a

significant nitrogen source. Therefore, sewer input and atmospheric input are likely the main nitrogen sources in Northport groundwater that discharges to Northport Harbor.

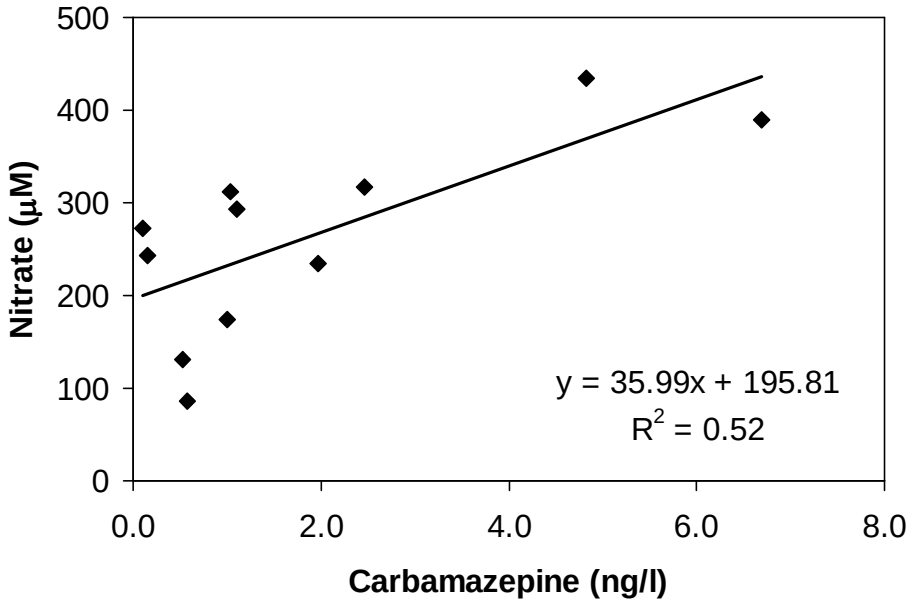


Figure 3.6. Cor-relation between carbamazepine and TDN at Northport (mainly unsewered)

The much lower detection frequency and concentration of carbamazepine around Manhasset Bay indicates that the sewer system here is effective, and that wastewater is not likely a primary source of nitrogen in groundwater in the areas sampled. There is no cor-relation between the frequently detected (87% of samples) herbicide, prometon, and TDN at Manhasset, suggesting that lawn fertilizers are not the likely nitrogen source either. It is possible, however, that prometon usage is independent of fertilizer application, in which case there would not be a strong relationship between TDN and prometon. Therefore, the most likely source of elevated nitrogen in Manhasset groundwater would be atmospheric input, but lawn fertilizers could also be a source.

Management efforts to reduce nitrogen in groundwater of developed areas and adjacent coastal waters such as those studied here might include sewering, reducing atmospheric inputs by reducing automobile emissions, or reducing fertilizer inputs. Impacts of reductions in the sources, however, may take decades to be realized in the coastal groundwater and surface water, because it takes this long for the existing degraded groundwater present in setting such as this to discharge to the ocean.

3.4 Conclusions

A direct injection LC/MS/MS method was developed for analysis of 17 organic tracers (pharmaceuticals and pesticides) in groundwater. The detection limits were typically tens of pg/l (except for the insecticide, halofenozide, which had much higher detection limits), and the recovery was close to 100%. The sensitivity and recovery of the direct injection method, compared to the SPE pre-concentration method, were similar or better; use of direct injection could also significantly reduce the cost and time of analysis. Further, the small volume needed for the direct injection method (tens of ml) would save sample collection time and storage space during field sampling.

The frequent detection of the anticonvulsant, carbamazepine, in groundwater samples of Northport (unsewered), together with the fact that few pesticides associated with lawn fertilizer applications were detected, suggest that wastewater input and atmospheric input are the likely sources of nitrogen in Northport's coastal groundwater. High concentrations of nitrogen were also detected in Manhasset (unsewered) groundwater. The low detection frequency of carbamazepine, however, suggests that the sewer system is effective. The likely sources of nitrogen in Manhasset groundwater would be atmospheric input (e.g., from automobile exhausts), as this area is densely populated, and possibly lawn fertilizers.

Appendix

Materials

All chemicals were used as received unless otherwise specified. Estrone (E1, CAS 53-16-7) 99%, estradiol (E2 β, CAS 50-28-2), 17α-estradiol (E2 α, 57-91-0), estriol (E3, CAS 50-27-1) 99%, 17 α-ethynylestradoil (EE2, CAS 57-63-6) 98%, pentafluorobenzyl-bromide (PFBBr), N-trimethylsilylimidzole (TMSI), 1M hydrochloride acide (HCl), potassium carbonate (K_2CO_3), sodium sulfate anhydrous (10-60 mesh) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (Saint Louis, MO). 17β-estradiol-16, 16, 17-d_3 (d_3-E2β) was purchased from CDN isotopes (Pointe-Claire, Quebec, Canada). Florisil (PR 60-100 mesh) was obtained from Supelco (Bellefonte, PA). All solvents used were pesticide grade. Methanol, acetone, hexane were obtained from Fisher Scientific (Pittsburgh, PA). Water used was deionized water (DW) (Milli-Q gradient system, Millipore, Bedford, MA). Helium (5.0 ultra high purity) and nitrogen (5.0 ultra high purity) were obtained from Welco-CGI (Newark, NJ). Carbamazepine (CAS 298-46-4), primidone (CAS 125-33-7), flurazepam (CAS 17617-23-1 surrogate), halofenozide(CAS 112226-61-6), imidacloprid (CAS 138261-41-3), bupropion (CAS 34841-39-9), citalopram (CAS 59729-33-8), venlafaxine (CAS 93413-69-5), caffeine (CAS 58-08-2), dicrofenac (CAS 15307-86-5), clofibric acid (CAS 882-09-7), oxybenzone (CAS 131-57-7), prometon (CAS 1610-18-0)), diazepam (CAS 439-14-5), DEET (CAS 134-62-3), pendimethalin (CAS 40487-42-1), oxcarbazepine (CAS 28721-07-5) were obtained from Fisher Scientific.

Preparation of stock solution and surrogate spiking solution

E1, E2α, E2β, E3 and d_3- β E2 were purchased as powders. Twenty µg/ml methanol stock solutions of each chemical were prepared by weighting respective amounts of chemicals and dissolving with methanol in volumetric flasks. All stock solutions were stored at 4°C.

A surrogate spiking solution with a concentration of 40ng/ml was prepared by diluting D- β E2 stock solution with methanol in a 4ml glass vial.

Carbamazepine, primidone, halofenozide, imidacloprid, bupropion, citalopram, venlafaxine, caffeine, dicrofenac, clofibric acid, oxybenzone, and oxcarbazepine were purchased as powders. Twenty µg/ml methanol stock solutions of each chemical were prepared by weighting respective amounts of chemicals and dissolving with methanol in volumetric flasks. Diazepam, pendimethalin, prometon and DEET were purchased as liquid solutions with concentration of 1mg/mL. The stock solution was made by taking 0.1mL each of these 4 solutions and diluted, to 100mL methanol (final concentration 1 µg/ml). All stock solutions were stored at 4°C. A surrogate spiking solution (flurazepam) with a concentration of 0.1µg/ml was prepared by diluting this surrogate standard with methanol in a volumetric flask.

Preparation of calibration curves

Six mixed estrogen standards of 0.2ng/ml, 1ng/ml, 3ng/ml, 5ng/ml, 10ng/ml 100ng/ml were prepared, derivatized (steps in 4.2.4) individually and the final volume was 1ml. No more dilution was made after the dirivatization.

Seven standard calibration solutions with concentrations in the range of 0.001- 1000ng/l (direct injection method) and 0.001 - 100ng/ml (SPE pre-concentration method) of each individual standard were prepared by diluting the respective stock standard solution with Mili-Q water and methanol, respectively, in volumetric flasks.

Preparation of potassium carbonate solution

Three grams potassium carbonate was measured, transferred to 40mL plastic tube and dissolved with 30g Mili-Q water.

Preparation of GF/F glass filter

Pieces of GF/F glass filters were wrapped into aluminum foil and baked at 300°C for one hour.

Preparation of anhydrous sodium sulfate

Sodium sulfate was precleaned with methylene chloride, dried in the hood for 30min, and kept in the oven at 60 °C for 1h. It was further purified by heating at 400°C for 4h in a muffle furnace. The sodium sulfate was cooled in a desiccator (*81*).

Preparation of Florisil

Florisil was precleaned by acetone, dried in the hood for 30min, and kept in the oven at 60 °C for 1h. It was further purified by heating at 130°C 2h. The florisil was cooled in a desiccators (*81*).

TABLE A1. Coordinates of three sampling sites in Eklund Farm.

Sampling Sites	Latitude (N)	Longitude (W)
Stream 1	42.4116	-74.66018
Stream 2	42.41457	-74.65673
Stream 3		

TABLE A2. Date and time of stream sample collection.

Time	Stream 1 (ºC)	Stream 2 (ºC)	Stream 3 (ºC)
Nov. 13th 2006	11:10	12:00	13:00
Jan. 16th 2007	13:30	13:00	14:00
Mar. 8th 2007	13:30	12:42	14:25
Apr. 5th 2007	13:00	12:20	13:50
Apr 24th 2007	13:30	12:55	14:25
May 24th 2007	13:20	12:40	14:15
Jun 12th 2007	13:45	13:10	14:25
Jul. 23rd 2007	13:30	12:40	14:20
Aug. 21st 2007	13:50	13:10	14:25

TABLE A3. Stream water temperatures at time of collection

Time	Stream 1 (ºC)	Stream 2 (ºC)	Stream 3 (ºC)	Air (ºC)
Nov. 13th 2006	7	7	7	7
Jan. 16th 2007	1	1	2	-5
Mar. 8th 2007	1	3	1	4
Apr. 5th 2007	3	5	4	3
Apr 24th 2007	12	12	14	15
May 24th 2007	15	16	19	28
Jun 12th 2007	15	15	15	20
Jul. 23rd 2007	14	13.5	14	15
Aug. 21st 2007	12	13	13.5	14

TABLE A4. D_3-E2β recovery in stream samples.

ISO recovery	Stream 1	Stream 2	Stream 3 triplicate	Stream 3 triplicate	Stream 3 triplicate
11/1/2006	74.0%	100%	96.0%		
1/25/2007	106%	100%	104%		
3/8/2007	123%	87.0%	104%	94.0%	67.0%
4/5/2007	123%	108%	94.1%	68.0%	97.5%
4/24/2007	108%	92.5%	112%	95.6%	
5/24/2007	119%	124%	109%		
6/12/2007	134%	130%	129%	127%	
7/23/2007	87.3%	121%	186%	135%	
8/21/2007	101%	94.5%	95.0		
10/18/2007	99.0%	99.0%	150%		

TABLE A5. Coordinates of Long Island sampling sites

Sample Name	longitude	latitude
ND2	-73.353410	40.902140
ND6	-73.356950	40.906310
ND8	-73.353310	40.896940
ND10	-73.353330	40.897170
ND11	-73.362310	40.896560
ND12	-73.353400	40.897630
ND13	-73.352780	40.897700
ND14	-73.352880	40.897080
ND16	-73.363180	40.904350
ND17	-73.363180	40.904350
ND19	-73.354260	40.891000
ND21	-73.354960	40.904540
ND23	-73.354390	40.903960
ND33	-73.358950	40.890870
MD1	-73.704900	40.825130
MD4	-73.702240	40.830230
MD6	-73.704990	40.823720
MD7	-73.715330	40.838670
MD9	-73.703460	40.837800
MDF1	-73.700215	40.833446
MDF2	-73.700215	40.833446
MDF3	-73.700251	40.833413
MDF5	-73.700163	40.833466
MDF6	-73.700065	40.816729
MDF8	-73.716728	40.833402
MDF9	-73.70424	40.82793
MIZ5	-73.35683	40.90648
MIZ6	-73.35683	40.90648
MIZ7	-73.35683	40.90648
NIY6	-73.35674	40.90643
NIY7	-73.35674	40.90643
NIY8	-73.35674	40.90643

TABLE A6. MRM transitions for analyzed compounds and quantitative optimization results in LC/MS/MS.

Name	Q1/Q3	DP	CE	CXP
Carbamazepine	237.1/194.1	76	29	12
	237.1/193.2	76	47	12
Primidone	219.13/162.1	61	19	16
	219.13/106.1	61	29	20
Flurazepam	388.15/315	61	33	22
	388.15/317	61	29	20
Halofenozide	331.13/275.1	41	13	6
	331.13/139.1	41	35	8
Imidacloprid	256.1/209.1	66	25	12
	256.1/175	66	25	10
Bupropion	240.1/184	56	19	12
	240.1/131.1	56	39	6
Citalopram	325.17/109.1	66	37	6
	325.17/262.1	66	29	18
Venlafaxine	278.19/58.1	56	39	10
	278.19/121.1	56	43	6
Caffeine	195.11/138.1	66	27	8
	195.11/110	66	33	22
Oxybenzone	229.4/151.1	61	28	10
	229.4/105.1	61	28	8
Prometon	226.1/142.1	56	33	8
	226.1/86	56	39	4
Diazepam	285/193	86	43	12
	285/154.1	86	39	8
DEET	192.12/118.9	86	26	8
Pendimethalin	282/212	41	15	12
	282/194	41	27	12
Oxcarbazepine	253/180	66	43	10
	253/208	66	29	12
Diclofenac	293.83/249.8	-55	-16	-15
	293.83/214.1	-55	-28	-13
Clofibric Acid	212.88/126.9	-55	-20	-7
	212.88/84.9	-55	-14	-3

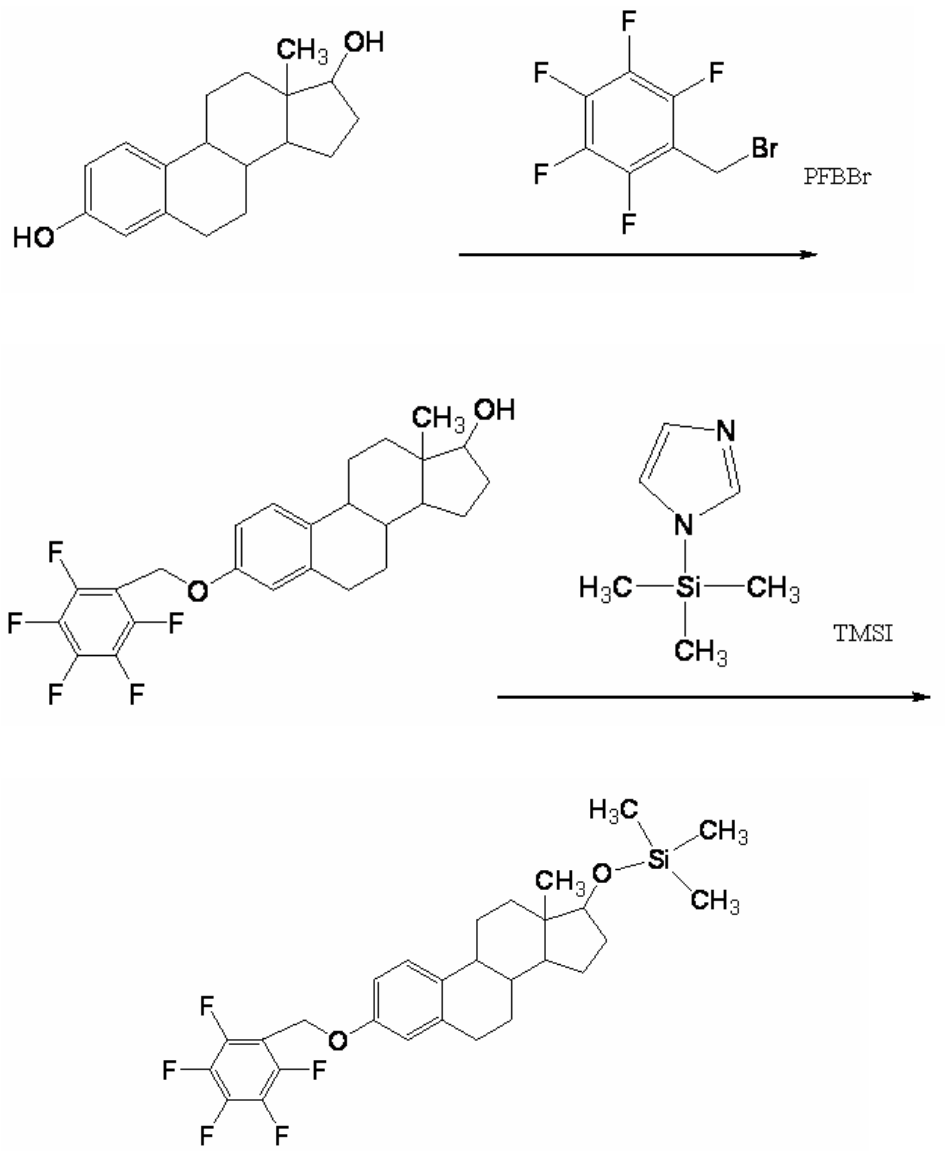


Figure A1. Derivatization reaction of E2 with PFBBr and TMSI.

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