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Screening of pharmaceuticals and other pollutants of concern by a combination of chemical and biological methods

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

SCREENING OF PHARMACEUTICALS AND OTHER POLLUTANTS OF
CONCERN BY A COMBINATION OF CHEMICAL AND BIOLOGICAL METHODS

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

in

CHEMISTRY

by

Arlette Azua

2005

To: Dean R. Bruce Dunlap
College of Arts and Sciences

This thesis, written by Arlette Azua, and entitled Screening of Pharmaceuticals and other Pollutants of Concern by a Combination of Chemical and Biological Methods, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Piero R. Gardinali, Major Professor

Date of Defense: March 11, 2005

The thesis of Arlette Azua is approved.

Dean R. Bruce Dunlap
College of Arts and Sciences

Dean Douglas Wartok
University Graduate School

Florida International University, 2005

DEDICATION

I would like to dedicate this thesis to my friends and family, especially to my parents Lourdes Bigotes and Ramon Azua. Without their support and understanding the completion of this work would not be possible.

Quisiera dedicar esta tesis a mi familia y amigos, en especial a mis padres Lourdes Bigotes y Ramon Azua. Sin su apoyo y comprensión la conclusión de este trabajo no hubiese sido posible.

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ABSTRACT OF THE THESIS

SCREENING OF PHARMACEUTICALS AND OTHER POLLUTANTS OF
CONCERN BY A COMBINATION OF CHEMICAL AND BIOLOGICAL METHODS

by

Arlette Azua

Florida International University, 2005

Miami, Florida

Professor Piero R. Gardinali, Major Professor

This study examined the occurrence of pharmaceuticals and personal care products (PPCP's) in surface waters of Florida and their potential to be use as indicators of wastewater contamination. Previous studies have shown that elimination of pharmaceuticals in municipal sewage treatment plants is often incomplete. Aquatic ecosystems are under increased stress from human activities, particularly in heavily populated areas. The purpose of this study was to find an ideal indicator for wastewater. The applied methods, GC/MS and LC/MS, were suitable for the determination of pharmaceuticals and personal care products in aqueous environmental samples to the lower parts-per-trillion (ng/L) level. As a result of this study a snapshot view of the occurrence of pharmaceuticals and personal care products in south Florida was produced. PPCP's were commonly detected in coastal environments of South Florida at relatively low concentrations. In general, PPCP's were higher inside the canals and contained bodies of water than in open water systems. Caffeine was successfully used to describe impacted versus pristine locations. However, no particular correlation was observed among caffeine and other traditional water quality parameters.

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CHAPTER 1

INTRODUCTION

1.1 Background

Pharmaceuticals have become recognized as relevant environmental contaminants in the course of the last decade. Pharmaceuticals and personal care products (PPCP) are a diverse group of chemicals, used internally or externally by humans and domestic animals, including all drugs available by prescription or over-the-counter and constituents of personal care products such as sunscreen agents and insect repellents. Also included are the so-called inert ingredients used in personal care products manufacturing and formulation. Many of these compounds are highly bioactive, most are polar, and all usually occur at trace concentrations when present in the environment (Daughton, 2001).

Normally, drugs are absorbed by the organism and subject to metabolic reactions. However, a significant amount will leave the organism unmetabolized via urine or feces and enter sewage or manure (Hirsch et al. 1999). Some of the pharmaceuticals are excreted by the human body only slightly transformed, often conjugated to polar molecules like glucosonides, which are easily cleaved during sewage treatment (Hererber, 2002). Recent studies show that elimination of pharmaceuticals in municipal sewage treatment plants (STP) is often incomplete (Ternes et al. 1998). This is important because antibiotic residues in the environment are suspected to induce resistances in bacterial strains, causing serious threat for public health. Already, more than 70% of the bacteria are insensitive to at least one antibiotic and many show multiple resistance patterns. Detrimental reproductive system effects have also been documented in the aquatic environment already. These effects are suspected to be caused by man made

estrogen mimics (Hirsh et al., 1998; Hirsh et al., 1999). For example, synthetic estrogens present in birth control pills are excreted after the surplus is not used by the body and these hormones end up in our waters virtually unchanged. Many organisms generally do not have sex differentiation in their early developmental stages therefore, if they encounter trace amounts of female hormones they could become females. When the ratio of females to males changes, population scale changes will occur. It has been hypothesized that the decrease in sperm counts, testicular cancer and other reproductive disorders in humans may be caused by the intake of estrogens via food or drinking water (Sharpe et al. 1993). Aquatic ecosystems are under increased stress from human activities, particularly in heavily populated areas. The reefs may be declining due to high nutrient levels: who is responsible? Risks to public health may also be an issue in these heavily populated areas. Between 70% and 95% of sites studied along the West Coast of Florida to the Florida Keys tested positive for enteric viruses, which include coxsackie viruses, Hepatitis A viruses, and Norwalk virus, which are associated with diarrhea, aseptic meningitis, and myocarditis (Lipp et al., 2001; Porter et al., 1999).

Pharmaceuticals such as growth promoters or feed additives are developed with the intention to perform a biological effect. They are lipophilic, so that it can pass through membranes, and persistent so that they last long enough for the substance to cause a curing effect. Therefore, they have many of the properties needed to bioaccumulate and cause effects in the aquatic ecosystems. Some high volume medical substances are probably released to the environment at the same order of magnitude as other xenobiotics known to be found in nature (Halling-Sorresen, 1998). Even though

all drugs approved by the FDA have undergone pharmacokinetics studies, there still exists a great uncertainty about their fate in the environment (Stumpf et al., 1999).

Figure 1 is a representation of possible pathways of pharmaceuticals into the environment. Highlighted is the portion of the picture applicable to south Florida. Drugs are ingested by the human body and metabolized but a significant amount could get excreted and enter raw sewage. As mentioned before, elimination in sewage treatment plants is often incomplete and consequently the pharmaceuticals could end up in the surface water. Many house in south Florida have septic tanks as their means to treat sewage and therefore there is a direct connection between the raw sewage and ground water.

Figure 1. Possible pathways of pharmaceuticals into the aquatic environment. (Source: Hirsh et al., 1999, pp111).

1.2 Pharmaceuticals and Personal Care Products

A potential chemical marker for domestic wastewater contamination is caffeine (1,3,7-trimethyl xantine). People generally discard hundreds of thousands milligrams of caffeine in the sink per day. A pot of coffee contains about 1500 to 1800 mL, consequently a household can generate hundreds to thousands of mg of caffeine (100 to 500 mg per cup) per day. The global, average consumption is about 70 mg per person per day but varies among different countries. Caffeine is of further importance in pharmaceuticals because it enhances the effect of certain analgesics in cough, cold, and headache medicine. Caffeine is used as a cardiac, cerebral, and respiratory stimulant and as a diuretic. Considering its uptake with beverages and foods, caffeine is probably the most widely consumed drug in the world (Buerger et al., 2003). Caffeine is extensively metabolized by the liver and only about 3% leaves the body unmetabolized (Tang-Liu et al., 1983). The major source of caffeine in domestic wastewater comes from unconsumed coffee, tea, soft drinks, or medication discarded in the sink. This indicator is source-specific and it survives exposure in the marine environment to the extent that it can be found in detectable quantities. Because it is highly soluble (13.5g/l), has a low octanol-water partition coefficient ($\log K_{ow}=0.01$), low volatility, and it is relatively persistent, it can be use as a marker directly related to human activities with no potential biogenic sources (Gardinali et al., 2002).

DEET (N,N-diethyl-meta-toluamide) is the active ingredient in many insect repellent products. It is used to repel biting pests such as mosquitoes and ticks, including ticks that may carry Lyme disease. According to the Environmental Protection Agency (EPA), every year, approximately one-third of the U.S. population is expected to use

DEET. Formulations registered for direct application to human skin contain from 4 to 100% DEET. Except for a few veterinary uses, DEET is registered for human use only, and it is not used on food.

Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) is a broad-spectrum antibacterial and preservative agent that has been used as antibacterial disinfectant for more than 30 years in soaps, creams, toothpastes and plastics in concentrations varying from 0.1 to 0.3% (Sabiliunas et al., 2003). The manufacturer has estimated that 96% of the applications of triclosan involve disposal down residential drains. This compound is relatively non-toxic to higher trophic level species; however, unicellular algae like green algae and cyanobactirium could be vulnerable (Ciba, 1998). Triclosan survives several microbial degradation steps in the wastewater treatment process, and enters the aquatic environment where it is bioavailable to fish (Adolfsson-Erici et al. 2002; Aguera et al. 2003).

Bisphenol A (2,2-bis-(4-hydroxyphenyl) propane) is an industrial chemical used primarily to make polycarbonate plastic and epoxy resins, both of which are used in a wide variety of applications like eyeglass lenses, medical equipment, water bottles, digital media (e.g. CDs and DVDs), among many other applications. Bisphenol A is often contained in environmental water and has been attracting attention as an endocrine disrupter, showing estrogenic activity even at concentrations as low as 1 ng/L level (Von Saal et al, 1998; Bergerton et al., 1999). Nagel et al. (1997) showed that fetal exposure to environmentally relevant parts-per-billion (ppb) levels of Bisphenol A, in the range currently being consumed by people, can alter the adult reproductive system in mice. Although the water solubility of Bisphenol A at room temperature is not high, the

primary sources of Bisphenol A released into the environment are expected to be effluents from facilities that manufacture epoxy and polycarbonate plastics, through its use and handling, permitted discharges, and elution from the products containing it (Suzuki, 2004; Staples et al., 1998).

4n-Nonylphenol is the main toxic metabolite produced by progressive shortening of the ethoxylate chain of nonylphenol ethoxylates, NP n EO, where n is number of ethoxy units. This non-ionic surfactant has a world annual production rate of 500,000 tons in 1996. It is widely used in industrial formulations such as textile, tannery, paper industries, pesticide adjuvants, paint ingredients, personal care products, etc (Diaz et al. 2004). Many scientists have reported this chemical to have an endocrine disrupting effect (Lee et al. 2004; Harries et al. 1997; Jobling et al., 1996).

1.3 Pharmaceuticals and Personal Care Products in the Environment

Garrison et al. from the United States Environmental Protection Agency (EPA) published the first report about the occurrence of pharmaceuticals in the environment in 1976. Since then, the environmental concern of the occurrence of pharmaceuticals and personal care products has increase dramatically. Pharmaceuticals, such as antibiotics and antimicrobials, and personal care products, have been reported all over the world in surface waters (Boyd et al., 2003 and 2004; Cahill et al., 2004; Daughton, 2001; Halling-Sorensen et al., 1998; Hirsch et al., 1998; Hirsch et al., 1999; Kolpin et al., 2002; Kolpin et al., 2004; Stumpf et al. 1999; Ternes et al., 2001; Weigel et al., 2002; Weigel et al., 2004; Zuehlke et al., 2004). Table 1 compiles examples of these pharmaceuticals and personal care products reported by other researchers in the past five years.

Table 1. Example of reported concentrations of Pharmaceuticals and Personal Care products in the literature. n.d= not detected

Compound	Location	Ranges of Concentration	Reference
Caffeine	Switzerland Lakes	<2-164 ng/l	Bueges et al. (2003)
Caffeine	Switzerland Rivers	26-250 ng/l	Bueges et al. (2003)
Caffeine	Mediterranean Sea	<2-5 ng/l	Bueges et al. (2003)
Caffeine	Surface Water of Florida	<4-41.2 ng/l	Gardinali et al. (2002)
Caffeine	Influent	230 µg/l	Hereber et al. (2002)
Caffeine	Effluent	0.18 µg/l	Hereber et al. (2002)
Caffeine	Iowa Stream	0.12-0.5 µg/l	Kolpin et al. (2004)
Caffeine	North Sea	2-16.1 ng/l	Weigel et al. (2001)
Caffeine	US streams	0.014-6.0 µg/l	Kolpin et al. (2002)
4-nonylphenol	Elbe River	1.0-52 ng/l	Heemken et al. (2001)
4-nonylphenol	North Sea	0.3-84 ng/l	Heemken et al. (2001)
4-nonylphenol	Iowa Stream	0.88-5 µg/l	Kolpin et al. (2004)
4-nonylphenol	US streams	0.50-40 µg/l	Kolpin et al. (2002)
Bisphenol A	Chemical Industry	2.5-50 µg/l	Furhacker et al. (2000)
Bisphenol A	Paper production	28-72 µg/l	Furhacker et al. (2000)
Bisphenol A	Influent	10-37 µg/l	Furhacker et al. (2000)
Bisphenol A	Effluent	n.d.-2.5 µg/l	Furhacker et al. (2000)
Bisphenol A	Elbe River	8.9-77.6 ng/l	Heemken et al. (2001)
Bisphenol A	North Sea	n.d.-249 ng/l	Heemken et al. (2001)
Bisphenol A	Iowa Stream	0.088-1.0 µg/l	Kolpin et al. (2004)
Bisphenol A	Orleans Canal	2.2-132 ng/l	Boyd et al (2004)
Bisphenol A	London Canal	1.9-158 ng/l	Boyd et al (2004)
Bisphenol A	Mississippi River	6.0-113 ng/l	Boyd et al (2004)
Bisphenol A	US streams	0.09-12 µg/l	Kolpin et al. (2002)
DEET	Iowa Stream	0.062-0.5 µg/l	Kolpin et al. (2004)
DEET	North Sea	n.d.-0.35 ng/l	Weigel et al. (2001)
DEET	US streams	0.04-1.1 µg/l	Kolpin et al. (2002)
Triclosan	Iowa Stream	0.14-1.0 µg/l	Kolpin et al. (2004)
Triclosan	Fish bile	<0.01-120 mg/Kg fresh wt	Adolfsson et al. (2002)
Triclosan	Milk	<20-300 µg/Kg lipid wt	Adolfsson et al. (2002)
Triclosan	Influent	1.3-37.8 µg/l	Aguera et al. (2003)
Triclosan	Effluent	0.4-22.1 µg/l	Aguera et al. (2003)
Triclosan	Marine Sediments	0.27-130.7 µg/l	Aguera et al. (2003)
Triclosan	Orleans Canal	n.d.-20 ng/l	Boyd et al (2004)
Triclosan	London Canal	2.8-15.1 ng/l	Boyd et al (2004)
Triclosan	Mississippi River	n.d.-8.0 ng/l	Boyd et al (2004)
Triclosan	US streams	0.05-2.3 µg/l	Kolpin et al. (2002)

Kolpin et al. (2002) published a report on pharmaceuticals, hormones and other organic wastewater contamination in United States streams to provide the first nationwide investigation of these compounds in United States. They analyzed 95 organic wastewater contaminants in 139 streams in United States, with 82 of the compounds being found in 80% of the streams studied. The most frequently detected compounds were coprostanol (86%), cholesterol (84%), DEET (74%), caffeine (71%), triclosan (58%), tri(2-chloroethyl) phosphate (58%) and 4-nonylphenol (51%). For this reason, the scope of this research focused on analyzing five of the most widely detected compounds from this list. Table 2 shows the target compound's structures, chemical formulas, molecular weight, chemical name, common name(s), CAS number, and their applications.

1.4 Methods of Assessing Water Quality

Historically, the preferred method to assess water quality has been microbiological analysis of water samples using the "indicator concept." The indicator concept, originally defined to assess drinking water quality, is based on the presence or absence of bacteria or groups of bacteria in a given body of water. These indicators are usually found in the intestines of animals and humans and are shed in feces. The indicator itself is not a pathogen, but its presence indicates the probable presence of pathogenic organisms. Originally, counts of both total and fecal coliforms were made to determine water quality, with intestinal bacteria groups such as the enterococci and species of *Clostridium* adopted in recent years as alternate indicators.

Table 2. Target Compounds.

Structure	Chemical Formula/ MW	Common Name/Chemical Name(s)	CAS No.	Use
$C_8H_{10}O_2N_4$ / 194.2	Caffeine/1,3,7-trimethyl xantine	58-08-2	Stimulant
$C_{12}H_{17}NO$ / 191.3	DEET /N-N-diethyl toluamide	134-62-3	Insect repellent
$C_{15}H_{24}O$ / 220.4	4n-Nonylphenol	104-40-5	Nonionic detergent metabolite
$C_{15}H_{16}O_2$ / 228.3	Bisphenol A/2,2-bis-(4-hydroxyphenyl) propane	80-05-7	Plasticizer

Table 2 continued.

Structure	Chemical Formula/ MW	Common Name/Chemical Name(s)	CAS No.	Use
$C_{12}H_7Cl_3O_2$ / 289.5	Triclosan/5-chloro-2-(2,4 dichlorophenoxy) phenol/ 2,4,4'-trichloro-2'-hydroxydiphenyl ether	3380-34-5	Antimicrobial disinfectant
$C_8H_6D_5N_5Cl$ / 217.7	Atrazine d5/ Deuterated Atrazine		Internal Standard
$C_8H_6Cl_4$ / 242.1	TCMX/Tetrachloro-m-xylene		External Recovery Standard

The United States Environmental Protection Agency (EPA) recommended the use of enterococci, a subgroup of the fecal streptococcus group of bacteria, for monitoring marine microbial water quality. Enterococci are better indicators than Fecal coliforms because they are more directly associated with human rather than with animal fecal matter and survive longer in aquatic environments but it can be found in tropical soils far from human activities (Cabeli, 1983; Hardina et al., 1991). Bacteriophages have also been used as biological tracers in marine environments to study potential sources of fecal pollution, such as septic tanks and wastewater injection wells (Paul et al. 1995).

However, using bacterial indicators to assess water quality presents some problems. For example, the use of fecal coliforms as an indicator of microbial water quality in tropical waters has been questioned because such bacteria survive in sediments and can be found in areas far removed from human activity. Furthermore, the correlation between coliform and human pathogen concentrations has not been established over a wide range of sample locations. Another problem is that many of the media-based detection methods are subject to false positive results. In the marine environment, many native populations of bacteria can grow and mimic the coliforms, that is, they exhibit the same color as coliforms in media-based tests that rely on the color of the colonies to identify the type of bacteria present (Griffin et al., 2001).

Furthermore, chemical indicators such as caffeine and pharmaceuticals have been used as molecular traces of wastewater contamination by researchers (Chen et al., 2002; Standley et al. 2000; Seiler et al., 1999).

1.5. Effects of Septic Systems

Florida has 17 millions residents and Miami Dade County over 2.2 million residents (U.S. Census, 2003). One third of all Florida homes, about 1.6 million households, use septic tanks. A septic tank system serves as an on-site wastewater treatment system in places where public sewers are not available. The septic tank system is an underground system of pipes and tanks designed to treat bathroom, kitchen and laundry wastewater through naturally occurring bacteria and microorganisms. In older homes, a septic system may be a pipe leading from the house to a cesspool. In newer homes, a septic system usually is made of a series of pipes connecting a septic tank, distribution box and a leach, or drainfield.

Crowded areas of septic systems present a high risk of microbial fecal contamination to groundwater (Scalf et al., 1977). Septic tanks installed in Florida before 1983 had less restrictive regulations, and therefore provide little protection for surface and ground water quality (Lipp et al., 2001). Studies in the Florida Keys have shown widespread bacterial and viral contamination of surface waters often associated with septic systems.

Corals in the Florida Keys may be affected by this contamination. Corals and reef environments are under increased stress from anthropogenic activities, particularly those in the vicinity of heavily populated areas such as the Florida Keys. Researchers have noted a loss of diversity in corals and an increase in diseased or damaged corals throughout the Florida Keys National Marine Sanctuary. Reefs in the Florida Keys are experiencing a dramatic increase in the number of places and number of species with coral disease (Griffin et al., 1999; Lipp et al., 2002). A survey from Key Largo to Key

West in 160 stations at 40 randomly chosen sites, there has been a dramatic increase in the number of locations showing signs of disease, 82% of all stations are now affected, which is a 404% increase over 1996 values. The percentage of species affected is 85%, a 218% increase over 1996 values, and the rate of coral mortality have increase, with the deep fore-reef at Carysfort experiencing a 60% reduction of living coral cover during the survey (Porter et al., 1999; Porter et al., 2001) (Figure 2 and 3). Figures 2 shows the dramatic decline in the health of Carysfort reef over a 25 year period and Figure 3 is a picture of a reef infected with black band disease, which is caused by cyanobacterium. Risks to public health may also be an issue in these areas. Areas sampled at Long Key and Marathon have been shown to receive wastewater from septic systems on those respective islands, based on viral tracers studies (Paul et al. 2000).

Figure 2. The health of Carysfort Reef off the coast of Florida declined dramatically in 25 years. The photographs at left show this decline. Coral that was healthy in 1975 are visibly sick by 1985, and dead and broken by 1995 (Source: <http://earthobservatory.nasa.gov>).

Figure 3. Black band disease caused by a cyanobacterium, which destroys the corals delicate living tissue. The disease starts at a point and spreads rapidly outward. It leaves behind dead coral that could become overgrown with algae (Source: <http://sofia.usgs.gov>).

Figure 4 is a schematic representation of the different sewage treatment used in Florida. There are many coastal environments in Florida where the only methods of wastewater disposal are septic tank systems. Other methods include cesspits, shallow injection wells and sewage outfalls. These methods may perhaps impact waters in Florida because the surface water is very close to the disposal systems and therefore, leakage to the surface water might occur. There is mounting evidence that such waste disposal practices are contributing to deterioration of surface water quality in many of Florida's coastal environments. The Florida Keys houses the nation's only living coral reef (Paul et al, 2000). The coral reef ecosystem of the Florida Keys is a very special place of incredible biologic diversity. It attracts residents and visitors from all over the world who enjoy diving, fishing, boating and nature excursions. Tourism and commercial fishing are the basis of the Keys economy. It is also home to many endangered and threatened birds, fish, and other wildlife, which depend upon a wilderness environment to

survive. Preserving Florida's marine environment, the economy, and quality of life are goals to be shared by all.

Figure 4. Schematic representation of sewage treatment in Florida

1.6 Purpose and Scope

This project proposed the development and validation of robust analytical methods for the trace determination of five widely used pharmaceuticals in surface water samples and their application to assess water quality problems in sensitive ecosystems.

The specific objectives of the study were:

- Develop a practical method of extraction and concentration for the five pharmaceuticals at environmentally relevant concentrations applicable to fresh and saltwater samples.

- Develop a method of analysis using Gas Chromatography Mass Spectrometry and Liquid Chromatography Mass Spectrometry for the analysis of the compounds at parts per trillion levels.
- Develop a method of quantitation using internal standards and external standards.
- Correlate the presence or absence of the compounds with other methods of assessment of water quality such as microorganism, nutrients and biological assays such as the yeast estrogen screen (YES).
- Evaluate the potential of the method as an environmental tool to assess water quality problems and infiltration of human waste in the South Florida Ecosystems.
- Use the analytical methods for the assessment of pharmaceuticals and personal care products in surface waters of south Florida and their ability to serve as a wastewater marker.

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals

Water (H_2O), dichloromethane (CH_2Cl_2), hexane (C_6H_{14}) of pesticide grade or equivalent, sodium sulfate (Na_2SO_4), Sodium Chloride (NaCl), and fiberglass, were obtained from Fisher Scientific (Fair Lawn, NJ).

Caffeine, (1,3,7-trimethylxanthine) was purchased from Fisher Scientific (Suwannee, GA, USA). Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether), 4-nonylphenol, Bisphenol A (2,2-Bis(4-hydroxyphenyl) propane), and DEET (N-N-diethyltoluamide) were purchased from Sigma and Aldrich (St. Luis, MO, USA). Deuterated atrazine (Atrazine-d5) used as a surrogate standard was purchased as a certified standard solution from Dr. Ehrenstorfer (Augsburg, Germany). TCMX (Tetrachloro-m-xylene) was use as an external recovery standard and was purchased from Supelco (Saint Luis, MO, USA). Standard stock solutions of all the compounds, TCMX and atrazine-d5 at concentrations of 100ng/ μl in water were prepared and stored in the dark at $-10\text{ }^\circ\text{C}$ for up to 1 year.

All laboratory materials were either made of glass or Teflon to avoid sample contamination. Glassware used throughout the extraction was rinsed with soap and water and combusted at 400°C for six hours. Teflon materials were cleaned in the same manner as glassware, but without combusting, only rinsed with four solvents (methanol, acetone, methylene chloride, and hexane in that order). Ultrapure water used for blanks and spikes was produced in the laboratory using a Milli-Q RG ultrapure water system from Millipore.

2.2 Sampling Strategy and Site Locations

Samples were collected from six locations in south Florida. These locations were chosen because they had documented water quality problems or water quality monitoring was already occurring. Locations that could be directly impacted by human activities were also sampled.

2.2.1. Florida Keys and Little Venice Area

The study area concentrates in South Florida and the Florida Keys, particularly the Little Venice (Marathon Key) area (Figure 5). The Florida Keys extend 150 miles from the southern tip of the state, with the Gulf of Mexico on one side and the Atlantic Ocean on the other. Over the past few decades, the Florida Keys have experienced a sudden increase in population. Once home to approximately 10,000 people who for the most part lived in quiet fishing villages, the Keys are now home to some 85,000 permanent residents, with an additional 2.5 million tourists every year. Most of the Keys do not have modern sewage-treatment plants. Approximately one-third of the Keys' permanent residents are served by a central sewage system. The rest of the residents rely on a system of anywhere from 5,000-10,000 illegal cesspits, 1,000 underground injection control wells and about 24,000 septic tanks. The fate of sewage disposed of by these methods remains largely unknown. Most of the bedrock underlying the Keys is highly porous limestone, the remains of ancient reefs through which contaminated waters can easily flow. Sewage is leaking from malfunctioning cesspits, septic tanks and sewer systems directly through the porous coral foundation of the islands and into the marine ecosystem. The reefs may be declining due to high nutrient levels (Paul et al., 2000).

Little Venice area includes approximately 540 residents with a high density of homes. Inadequate septic tank systems or cesspits serve a large number of homes and the area is known to have water quality problems (Boyer et al., 2004). Dr Joseph Boyer from Southeast Environmental Research Center at FIU monitored nutrient levels and bacterial counts among other water quality assessment parameters in the area for a period of almost two and a half years. For this reason, this project started analyzing caffeine in Little Venice as a way to add a chemical tracer to the study and to attempt to correlate caffeine with the other water quality parameters.

Figure 5. Map of Little Venice Area and Stations

The Little Venice sampling program included ten sampling stations located in four different canals within the Little Venice Service Area (Figure 5). Stations 1 and 3

are located in a connected “U-shaped” canal system located at 112th Street. These canals may receive better tidal flushing than other canals within the Service Area because of their flow-through design and their relatively short length. Also, the canals are surrounded by single-family residences that were built before 1970 and a high percentage of those residences are thought to have no sewage treatment systems. Stations 2, 4, and 5 are located on the canal next to 100th Street and stations 6 and 7 are located on the canal adjacent to 97th Street. Both canals are dead-end canals that are surrounded by single-family houses and mobile homes. Many of these residences are also thought to have poorly functional septic systems or cesspits. Stations 8 and 9 are located next to the 91st Street canal, which is located outside the Little Venice Service Area (Boyer et al., 2004). Station 10 is located about 100 meters offshore.

2.2.2. Miami River and Coconut Grove Marina

The Miami River (Figure 6) brings water from the Everglades through Tamiami Canal and runs through Downtown Miami, from Miami International Airport to Biscayne Bay. The 5.5-mile river is narrow and polluted, due to its proximity to Metropolitan Miami and the large number of leaky sewage collectors. It flows past boatyards, marinas, public parks, tugboats, shipping terminals, pleasure craft, houseboats, mangroves, historic homes, shippers, restaurants and loading docks through the heart of downtown Miami into Biscayne Bay. The Miami River's 32 private terminals handle more than \$4 billion in cargo a year, making it Florida's fifth largest seaport.

Miami River sampling included six sites along the river and one in Biscayne Bay. MRBP is at Brickell Point, which is located at the river mouth and has tidal exchanges and heavy traffic but no ducking. MRNS is at Norseman, which has heavy traffic, a boat

yard and a large vessel docking. MRMS is in front of Merrill Stevens boat yard, MRSF is next to South Fork, a small tributary with large commercial shipyards. MRYC is next to Yacht Club, and MRCP is in front of Curtis Park.

Figure 6. Miami River map and Sampling sites

Coconut Grove is located within the City of Miami along Biscayne Bay, just five miles south of downtown Miami. Local area is about 0.4 square miles and Coconut Grove population is about 17,800 people (US Census, 2003). The coastal area near Coconut Grove Marina contains a group of seven marinas with 582 slips. Figure 7 shows the samples collected in Biscayne Bay and Coconut Grove Marina. One sample was

collected in Biscayne Bay Coconut Grove Marina (BBCGM) and six other samples were collected along six channels in Biscayne Bay. The channels keep going away from land as their number increased, in other words, CHANNEL 1 is closer to land and CHANNEL 6 is the furthest away from land.

Figure 7. Map of Coconut Grove Marina

2.2.3. C-1 Canal

Black Creek Canal (C-1 canal, Figure 8) is located in Cutler Ridge, southeastern Miami-Dade County. The 9.3 miles of navigable Black Creek canals were constructed in the mid-1900s as part of a large flood control project. The main canal ranges from 40 to

over 150 feet wide, and averages about 12 feet deep. The lower portion south of Old Cutler Road is primarily agricultural. The canal runs through an agricultural and an urban area before intersecting Biscayne Bay at Black Point Marina. The canal runs past 'Mt. Trash-More', one of the largest municipal landfills in South Florida. Data collected from this canal provides information about the occurrence of pharmaceuticals and personal care products in agricultural areas and newly developed residential areas as well as the concentrations of pharmaceuticals and personal care products leaching from the landfill.

Figure 8. C1 Canal map and Sampling sites

Sampling along C1 Canal was done in 14 sites (Figure 8). Site 1 is located in a mainly agricultural area. Sites 2, 3, 4, 5, and 6 pass through a recently developed residential area served by septic tanks. Site 7 is next to an apartment building complex, 8 and 14 are next to Mt. Trash-More Landfill and 9, and 13 are collected along the canal passed the landfill, all the way to Black Point Marina. Sites 10, 11, and 12 were collected inside the marina.

2.2.4. Key Largo Harbor

Figure 9. Key Largo Harbor map and Sampling sites

Key Largo, the largest island of the Florida Keys, is known as the diving capital of the world. It has close to 12,000 residents and about 5,000 homes. Key Largo Harbor

(Figure 9) is located near the most populated area of Key Largo with many Class V disposal wells and hundreds of septic tank systems. Key Largo Harbor is located along winding canals, which are about 25 ft deep and are cut into the permeable limestone and have restricted water flows. The canals are surrounded by residential areas as well as hotels, with most of the waterfront properties served by old and aging septic tanks. Key Largo Harbor sampling included eight sites in two transects (Figure 7). Sites 1, 3 and 6 are at the channel ends, while Sites 2, 4, 5, and 7 are along the main channel. Site 8 is located offshore the canal system.

2.2.5. Looe Key (Underwater Music Festival)

Figure 10. Looe Key Map

Looe Key (Figure 10) is a pristine offshore reef located approximately five nautical miles south of Big Torch Key and one of the most popular diving spot in the Lower Florida Keys. It is a popular destination for scuba diving, and snorkeling. Every July, Looe Key Reef Marine Sanctuary houses the Annual Underwater Music Festival, which consists of six hours of specially selected, pre-recorded music to be broadcast by US1 Radio via Lubell Underwater speakers. More than 400 divers and snorkelers attend this event every year in Looe Key Reef (Source: www.florida-keys.fl.us). Sampling during this event was done to provide data about the occurrence of pharmaceuticals and personal care products in a reef area away from land. The presence of so many people is expected to increase the occurrence of some pharmaceuticals during this event.

Forty samples were collected in Looe Key in 2003. Six samples were collected before the event started, twenty-five while the event was taking place, three samples after the event and six samples were collected inside the marinas. The samples collected inside the marina were used to compare the concentrations of caffeine in the reef versus the concentrations inside a marina. Greater contamination is expected to be found at the marina than in a reef five nautical miles away from shore. In 2004, forty samples were also collected. The first 10 samples were collected the day before the Underwater Music Festival (July 9, 2004). Samples 11 through 30 were collected the day of the event (July 10, 2004) and samples 31 through 40 were collected the day after the event (July 11, 2004). Some samples were taken at the surface and some at the bottom to compare concentrations of contaminants at varying depths. The samples were collected all around the reef.

2.2.6 South West Coast of Florida

Figure 11. Map of west coast sampling sites

The west coast of Florida has one of the nation's fastest growing populations. The sampled region in west coast of Florida included three counties; Sarasota County, Charlotte County and Lee County. This region has a population of about one million people (US Census, 2003). In Sarasota most of the population lives close to the coast. The bulk of the population of Charlotte County is in the eastern portion of the county near Charlotte Harbor and the Peace River. The largest portion of the population in Lee County is located in Cape Coral and Fort Myers (Florida Netlink, 2005).

Twenty-eight samples were collected along the southwest Florida estuaries (Figure 11). Three samples were collected in Fort Myers Yacht Base (FMYB), FMYB-01 and FMYB-02 are located inside the marina and FMYB-03 is located outside the marina (at the entrance). Three samples were collected in Liberty Yacht Club (LYC), and two in Peppertree Point Marina (PPM), at both entrances of the “Y” shaped marina. Two samples were collected in Tarpon Point Marina (TPM), TPM-01 is inside the marina and TPM-02 is at the exit of the marina. Four samples were collected in Gasparilla Marina (GM), with sample GM-01 being the most distant site from the entrance of the marina and GM-04 at the exit of the marina. Three samples were collected at Palm Island Marina (PIM), with PIM-01 being, once again, the most distant site from the entrance of the marina and PIM-03 at the entrance of the marina.

Three samples were collected at Charlotte Harbor Marina Base (CHMB), which is a “Y” shaped marina. Sample CHMB-01 and CHMB-03 were collected at the end of both forks of the channels and CHMB-02 was collected at the entrance of the marina. Four samples were collected at Marina Jacks (MJ), with samples MJ-01 and MJ-03 being the most distant sites from the entrance of the marina, and MJ-04 was collected at the entrance. Four samples were collected at Longboat Key Moorings (LKM), which is a very long channel of marinas with many boats. Once again, LKM-01 is the most remote inside the marina and LKM-04 is located at the entrance of the marina.

2.3 Sampling

Water quality monitoring for nutrients and bacterial was already taking place in Little Venice. One-liter samples were collected in each Little Venice station every month

in amber bottles. Samples collected for nutrient analysis and bacteriological analysis were collected in separate bottles and analyzed by Dr. Joseph Boyer from Southeast Environmental Research Center (SERC) in Florida International University (FIU).

Key Largo Harbor, Miami River and Coconut Grove Marina, C1 Canal, Looe Key, and West Coast had random samplings because regular water quality monitoring was not taking place in these locations. Sampling bottles and all glassware used for sample collection and preparation were cleaned by washing with soap, and then combusting at 450 °C for 6 hours. Environmental descriptors such as pH, salinity, dissolve oxygen, surface water temperature, and site depth were also collected as part of the sampling protocol. Upon collection, all samples were kept in ice and shipped to the laboratory where they were kept at 4°C until analysis. To minimize contamination of samples, use of personal care items such as insect repellents, caffeinated products, pharmaceuticals and tobacco were not allowed to be use during sample collection and processing.

2.4 Extraction

2.4.1 Liquid-Liquid Extraction

In liquid-liquid extraction, a one liter sample of water is put in a separatory funnel were 100 µl of 1.00 ng/ml spiking solution containing all the target compounds are added to spikes and 100 µl of 1.000 ng/ml of Atrazine-d5 are added to all samples as internal standard. Then, 20 g of NaCl are added to increase the ionic strength of the solution to force the compounds into the organic layer, and consequently, increase the recoveries. This is followed by 1.00 ml of 1M K_2PO_4 buffer to bring the water to pH~8.1. Samples

are then extracted three times with 50 ml methylene chloride and vigorous shaking. Extracts are passed through a column of combusted anhydrous sodium sulfate to dry and collected in a 250 ml flat bottom round flask. The samples are then concentrated in a water bath to about 1 ml of hexane. 100 μ l of 1.000 ng/ml of TCMX is added as a recovery standard and the samples are analyzed using GC/MS. For the LC/MS analysis, the samples are brought to dryness using a stream of purified nitrogen flow and reconstituted with 500 μ l 50:50 MeOH:H₂O.

2.4.2 Solid Phase Extraction

Solid phase extraction (SPE) is the leading technology for the extensive extraction of organic compounds from aqueous samples. The commercial availability of a multitude of broad spectrum and chemical class-specific solid phases has led to a broadening in the types of organic compounds that have been extracted from and measured in aqueous samples in recent years. The result has been an increase in the measurements of both polar and nonpolar current-use chemicals of emerging interest in the aquatic environment (Koester, 2003). Many pharmaceuticals and personal care products have been extracted using this method instead of liquid-liquid extraction (Aguera et al. 2003; Baynes et al., 2002; Cahill et al., 2004; Chen et al., 2002; Diaz et al. 2004; Kolpin et al., 2002; Ferrer et al., 2001; Lindsey et al. 2001; Patsias et al. 2000; Weigel et al. 2001 and 2004)

Analytes were extracted using a reverse phase cartridge, 225mg/6 μ m divinylbenzene/N-vinylpyrrolidone copolymer (Oasis HLB Plus) from Waters (Millford, MA). Cartridges were preconditioned with 5 mL of MeOH and 5 mL of distilled water. One-liter water samples were passed through the cartridges at 10 mL/min. After isolation,

the analytes were eluted using 10 mL of methylene chloride into a concentration tube. Samples were concentrated to 500 μ L and analyzed using GC/MS or LC/MS.

Recoveries of caffeine using this method were as high as 96%. The method uses much less solvent compared to Liquid-Liquid extraction but the method consumes too much time because the extraction of the compounds from the liquid has to be done at 10 ml/min. Only four samples could be extracted at the same time, taking almost two hours for one-liter sample to pass through the column. With liquid-liquid extraction, twelve samples were extracted in a period of four hours. For this reason, the method using liquid-liquid extraction was preferred until the lab is equipped with a pump that can extract more samples at the same time.

2.5 Instrumental Analysis

The target compounds can be analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) analysis (Adolfsson-Erici et al. 2002; Bester, 2003; Soliman et al. 2004) or Liquid Chromatography-Mass Spectrometry analysis (LC-MS) (Abu-Qare et al. 2001; Baynes et al. 2002; Cahill et al. 2004; Gardinali et al. 2002; Kanazawa et al. 2000; Lindsey et al. 2001; Loffeler et al. 2003; Quintana et al. 2004; Zuehlke et al. 2004). The decision to use GC or LC is usually based on the physicochemical characteristics of the target compounds. Many pharmaceuticals and personal care products contain polar groups and are therefore, not readily to be analyzed by GC (Boyd et al. 2003). A derivatization step is usually required for the analysis of polar compounds using GC-MS. Most of the derivatization processes are not straightforward and sometimes required handling of hazardous chemicals. Since HPLC analysis does not require to derivatize the analyte, it is a good alternative to GC analysis (Patsias et al., 2000). For this reasons the

project started using liquid-liquid extraction followed by analysis using liquid chromatography mass spectrometry until a method using gas chromatography mass spectrometry was developed, which in fact, did not need extra derivatization step.

2.5.1 Liquid Chromatography Mass Spectrometry (LC-MS)

The LC-MS instrument used was a Finnigan navigator aQa LC-MS in positive ion atmospheric pressure chemical ionization (APCI+) for Caffeine, DEET, and Atrazine D5 and negative ion atmospheric chemical ionization for Bisphenol A, Triclosan and 4n-Nonylphenol. Selected ion monitoring (SIM) of $[M+H]^+$ and the $[M-H]^-$ pseudo-molecular ions was used. The corona pin voltage was set at 4.5kv, the fragmentation cone voltage at 10V, and the source temperature at 350°C. For the HPLC separation, a Luna C-18 reverse phase column (150 x 4.6 mm, 5m, Phenomenex) was used with a linear gradient from 30:70 MeOH/H₂O to 100% MeOH for 15 minutes. The flow rate was set at 1.0 ml/min.

2.5.2 Gas Chromatography Mass Spectrometry (GC-MS)

The GC-MS analytical system included a temperature programmable gas chromatograph (Finnigan Trace GC Ultra). The injection port was designed for split or splitless injection and analyses were conducted in the splitless mode. A 30-m long x 0.32mm I.D. fused silica capillary column with a 0.25 DB-5 MS bonded phase (Agilent) was used. The autosampler was capable of making up to 4 μ L injections. Representative aliquots of the sample extracts are injected into the capillary column of the gas chromatograph using the following conditions:

Injector Temp:	280°C
Transfer Line Temp:	280°C
Initial Oven Temp:	90°C
Initial Hold Time:	1 min.
Ramp Rate:	30°C/min
Temperature:	200°C
Hold Time:	1 min.
Final Ramp Rate:	20°C/min
Final Temperature:	280°C
Final Hold Time:	1 min.
Total Run Time:	11.5 minutes
Carrier gas:	Helium
Head Pressure:	15 psi

The mass spectrometer (Finnigan DSQ) operates at 70 eV electron energy in the electron impact ionization mode and is tuned to maximize the sensitivity of the instrument based on manufacturer specifications. The mass analyzer (150°C) and ionization source (250°C) are optimized for the detection of the target analytes. The effluent from the GC capillary column is routed directly into the ion source of the mass spectrometer. The MS is operated in the selected ion monitoring (SIM) mode using appropriate windows to include the quantitation and confirmation masses for each compound in Table 3. For all compounds detected at a concentration above the practical quantitation limit (PQL) the confirmation ion(s) is (are) checked to confirm its presence. Figure 12 is a typical GC/MS chromatogram for a spiked laboratory blank (LBS) showing chromatography and retention times for all target compounds. Total Ion Chromatograms (TIC) of an actual sample and mass spectra of all compounds are shown in Appendix II.

Figure 12. Typical GC/MS chromatogram for a spiked laboratory blank (LBS) sample at 100 ng/L with all the compounds

2.6. Quantitation and Confirmation

Quantification of the targeted compounds was conducted by comparing peak areas of the most intensive ion of each compound with that of the internal standard. Compound identification was confirmed by GC retention time and qualifier ions (usually molecular ion and one or two fragment ions). As part of the method development, ion ratios were monitored, enabling discrimination between interference and the proper response. The data was analyzed using a plot of the area of the most abundant ion divided by the area of the Atrazine-d5 ion versus the concentration of caffeine divided by the concentration of Atrazine-d5. This plot generated a slope that was used in equation 1. The data for this plot was obtained from a set of seven calibration solutions with the compounds concentrations running from 400-250 $\text{pg}/\mu\text{l}$. This calibration solutions were

run at the beginning and at the end of each run to make sure the instrument was stable all throughout the run.

The concentration of the compounds were calculated using the following formula

$$[Compound](ng/L) = \left(\frac{AreaCompound}{AreaAd5} \cdot \frac{[Ad5]}{RRF} \right) * \frac{1}{SampleVol(L)} \quad (eq1)$$

A relative response factor is calculated using the following formula

$$RRF = \frac{AreaCompound}{AreaAd5} \cdot \frac{ConcentrationAd5}{ConcentrationCompound} \quad (eq2)$$

This relative response factor needs to be fairly constant for all samples and it is performed to make sure the instrument is working properly. Table 3 is a summary of the criteria used for the GC/MS method.

Table 3. Retention times and reference assignment for the compounds targeted in this method.

Compound	t _r	Reference Surrogate	Quantitation Ion	Confirmation Ion(s)	Target Relative Abundances (%)
GC Int. Stnd					
TCMX	6.38	N/A	244	242	78.3
Surrogate					
D5-Atrazine	7.01	TCMX	220	222/205	32.1/148.0
Target Compounds					
DEET	6.03	Atrazine-d5	119	190/191	65.3/38.7
Caffeine	7.65	Atrazine-d5	194	109/195	33.5/10.0
4n-nonylphenol	7.73	Atrazine-d5	107	220/108/221	22.0/9.3/3.9
Bisphenol A	9.37	Atrazine-d5	213	228/214/119	30.0/15.1/12.2
Triclosan	9.08	Atrazine-d5	288	290/292	96.8/30.1

Method interferences caused by contaminants in solvents, reagents, glassware, and other sample processing hardware could lead to false positives in GC/MS detection. All materials used in this method are routinely demonstrated to be free from interferences

by processing procedural blanks (one blank per 20 samples or each batch, whichever is more frequent).

Figure 13. Compound's calibration curves showing $r^2 > 0.99$

A seven-point first-order calibration curve is established to demonstrate the linearity of the detector. The recommended standard concentrations are approximately 2.5, 10, 20, 50, 100, 200, and 400 pg/ μ L. The acceptance criteria for the calibration curve requires that the calibration curve must have a correlation coefficient, $r^2 > 0.99$ for a 1st degree fit of the data. Figure 13 is a plot of the concentration of caffeine divided by the

concentration of the internal standard versus area of caffeine divided by area of internal standard to be used as the calibration curves. The linear regressions had a r^2 value greater than 0.99 for all compounds.

Figure 14. Recoveries for all target compounds.

Recoveries of the compounds at four spiking levels are presented in Table 4. Caffeine recoveries were in the range of 55 to 75%. Bisphenol A, 4n-Nonylphenol, and Triclosan had recoveries in the range of 70 to 95%. DEET had a higher recovery, in the range of 90 to 100%. Figure 14 is a plot of spiked concentrations at four levels (10, 20, 50, 100 ppt) versus the percent recovery. The error bars represent the standard deviation for the recovered concentration (n=3). These plots showed to be linear in the range of the

four spiking levels (10, 20, 50, 100 ppt) for Caffeine, DEET, 4n-nonylphenol and Triclosan. Bisphenol A showed lower recoveries at higher spiked concentrations.

Initial method performance based on statistical determination of method detection limit (MDL's) by multiplying the standard deviation by 3 produced minimum detection limits in the sub-nanogram per liter (ppt) range as follows: Caffeine 0.5 ng/L, DEET 0.5 ng/L, Triclosan 0.9 ng/L, Bisphenol A 1.0 ng/L, and 4n nonylphenol 0.6 ng/L. Practical quantitation limits (PQL) were based on the extraction of a 1-liter water sample and the lowest calibration solution is 2.5 ng/L for all compounds.

Table 4. Recoveries of Compounds at 4 Spiking Levels (10, 20, 50, and 100 ppt)

Target Compound	Recovery Range (%)	Average Recovery
Caffeine	55-75	67.3%
DEET	90-100	93.8%
4n-Nonylphenol	70-95	86.5%
Bisphenol A	70-80	76.5%
Triclosan	70-85	79.1%

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Little Venice

3.1.1. Caffeine in Little Venice

Canals in Little Venice are clearly impacted by land use and associated septic tank systems as well as their physical design. The presence of enterococci above the Environmental Protection Agency's recommended levels is a relatively strong indicator of sewage input to the canals (Boyer et al, 2004). For this reason, this was the first location in Florida to be analyzed for caffeine in this project to find the ideal indicator for sewage contamination in coastal environments.

Table 5 is a representation of the data collected for the Little Venice samples from November 2001 to December 2003. The limit of detection for Caffeine is 4.0 ng/L for LC/MS and 2.5 ng/L for GC/MS. Based on the vast collection of data, it was determined that caffeine concentration from BDL to 15 ng/L are not environmentally significant. Any concentration in the range of 15 to 40 ng/L may have an environmental significance and the concentrations higher than 40 ng/L reflect impacted waters. Table 6 presents a summary of the data collected for all the pharmaceuticals in Little Venice from August 2003 to December 2003. The practical quantitation limit for all pharmaceuticals was 2.5 ng/L.

Stations located at the end of the canal system are referred to as the "head", stations at the entrance of the canal are referred to as the "mouth" and stations offshore are located outside the canal systems.

Table 5. Caffeine data collected in Little Venice in ng/L concentration. NA=Not Available, , 15-40, >40

Site	Collection Date					
	Nov, 01	Dec, 01	Jan, 02	Mar, 02	May, 02	Jun, 02
LV-01	103			25.0		
LV-02	46.6				15.1	
LV-03	78.0			103		
LV-04			20.5	58.8		34.5
LV-05	15.0	71.8			15.8	18.7
LV-06	NA	43.2			18.0	33.5
LV-07						
LV-08						NA
LV-09	81.6					
LV-10		19.1		44.3	25.8	50.4
	Jul, 02	Aug, 02	Sep, 02	Oct, 02	Dec, 02	Jan, 03
LV-01	15.3	84.4		15.8	26.5	
LV-02				NA		
LV-03	124	39.2			20.6	15.9
LV-04		119				19.8
LV-05		57.6				19.4
LV-06		56.9				
LV-07		55.5			17.7	15.9
LV-08	26.2	74.3		NA		16.1
LV-09	17.1	203		46.2		17.5
LV-10					24.7	15.3
	Feb, 03	Mar, 03	Apr, 03	May, 03	Jun, 03	Jul, 03
LV-01	35.2	19.2	NA	22.5	14.8	30.4
LV-02	18.3	16.4	NA	27.4		
LV-03	29.1		39.5	27.9	71.9	41.0
LV-04	32.1	37.2	22.4	18.9	16.6	
LV-05		41.1	85.6	NA		13.1
LV-06		NA	42.9		32.8	NA
LV-07	10.0	NA	24.7	15.0	10.6	21.8
LV-08	NA	16.2	24.7	NA	38.0	
LV-09	16.5	23.3	52.3	71.3	59.1	
LV-10		30.6	27.2	16.0		

Table 6. Compounds Concentration in Little Venice (ND=Not Detected, BLD=Below Detection Limit), , 15-40, >40)

Site	Caffeine	DEET	4n-Nnylphenol	Bisphenol A	Triclosan
Aug, 03					
LV-01	15.0	19.1	ND	BDL	BDL
LV-02		4.26	ND	3.65	2.39
LV-03	20.6	12.3	ND	82.4	43.7
LV-04	30.6	5.60	ND	74.5	3.43
LV-05		5.40	ND	9.57	8.04
LV-06	15.8	5.03	ND	21.6	7.29
LV-07	16.2	4.79	ND	32.5	4.39
LV-08	15.7	6.23	ND	6.36	84.4
LV-09	15.5	12.2	ND	9.46	5.16
LV-10		2.10	ND	3.76	8.97
Sep, 03					
LV-01	45.4	9.50	ND	7.35	5.78
LV-02	16.1	6.66	ND	4.85	3.21
LV-03		34.6	ND	6.92	BDL
LV-04	37.7	6.23	ND	9.72	2.27
LV-05		4.38	ND	8.01	BDL
LV-06	39.7	3.70	ND	7.34	1.68
LV-07		2.80	ND	ND	ND
LV-08		8.28	ND	4.46	1.97
LV-09		10.3	ND	5.35	2.33
LV-10		3.22	ND	3.27	2.29
Oct, 03					
LV-01		5.17	ND	9.27	3.66
LV-02	17.4	2.23	ND	11.2	5.40
LV-03		3.46	ND	3.97	2.39
LV-04		2.50	ND	6.33	2.30
LV-05		BDL	ND	10.1	3.44
LV-06		BDL	ND	5.45	4.02
LV-07		BDL	ND	4.43	2.99
LV-08		BDL	ND	5.03	3.71
LV-09		BDL	ND	4.92	4.57
LV-10		BDL	ND	3.51	3.53
Dec, 03					
LV-03	17.8	128	ND	64.0	BDL
LV-06		3.76	ND	16.4	2.84
LV-09		5.35	ND	14.2	BDL
LV-10		7.11	ND	10.3	3.00

Caffeine was detected in Little Venice in concentrations ranging from 4 to 203 ppt. The method used was LLE followed by analysis by LC-MS or GC-MS, whichever was available. The distributions of water quality variables are reported in vertical box plots. This plot is a powerful statistical representation and shows the median, range, and data distribution. Vertical box plot shows the median, 10th, 25th, 75th, and 90th percentiles as vertical boxes with error bars. The center horizontal line of each box is the median of the data, the top and the bottom of the box are the 25th and 75th percentiles and the end of the whiskers are the 10th and 90th percentiles.

Figure 15 and 16 are vertical box plots of the concentrations of caffeine and other water quality assessment parameters arranged by stations. Figure 15 indicated that the highest concentrations of caffeine were found in Stations LV-03, LV-04 and LV09. Stations LV-03 and LV-09 are located at the end (head) of the canals and station LV-04 is located at the entrance (mouth) of the canal and are the most impacted sites in the canal systems with regards to caffeine. Stations LV-05 and LV-07 are also located at the head of the canals but they do not show particularly higher concentrations when compared to sites in the mouth of the canals. Site LV-03 is located at the head of a “U” shaped canal that receives better tidal flush than the other dead end canals within the area because of its design and short length. For this reason station LV-03 was expected to have lower concentrations of caffeine, nutrients, and bacterial compared to the other stations located at the head of the other dead end canals. Station LV-04 shows caffeine concentrations in the range of 5.65 to 199 ppt. These concentrations are similar to concentrations detected in LV-03, which was unexpected since LV-04 is located at the mouth of a canal. Figure 16 shows the concentrations of fecal coliform, enterococci, ammonium, and total

phosphorous. A comparison with bacterial counts and nutrients graphs shows that station LV-03 had the highest concentrations of these parameters as well.

Figure 15. Occurrence of Caffeine in Little Venice by Stations

Figure 16. Occurrence of Other Water quality markers in Little Venice by Station

A vertical box plot of the monthly review of caffeine concentrations is shown in Figure 17. This plot shows no apparent trend among the different months of the year. November 2001 and August 2002 showed the highest concentrations of caffeine. Figure 18 shows the monthly occurrence of caffeine in stations LV-03, LV-09, and LV-04. Comparison with Figure 17 shows that they follow similar trends. Figure 19 is a plot of the monthly occurrence of fecal coliform, enterococci, ammonium and total phosphorous. No particular trend is observed among these parameters either. November 2001 and August 2002 did not show any particularly higher concentrations for any of these parameters. Fecal coliform and enterococci had higher counts in July 2003 but caffeine concentrations were below 40 ppt (environmentally significant but not impacted waters), with station LV-03 having the highest concentration at 41.0 ppt. Monthly correlation with nutrients and bacterial counts did not show any particular trend. These results seem to indicate the complexity of water circulation in the canal system.

Table 7 is a representation of the principal component analysis multivariate correlation matrix for caffeine versus all other water quality parameters. Multivariate looks at how multiple variables relate to each other and how points fit that relationship. This platform helps see correlations between two or more responses, looks for points that are outliers, and examines principal components to look for factors. A correlation table is a matrix of correlation coefficients that summarizes the strength of the linear relationships between each pair of responses. Ranges of caffeine between below detection limit to 15 ppt were excluded from the analysis due to the fact that they may contain larger analytical errors. Caffeine did not strongly correlate with any of the parameters and had slight positive correlations ($R^2 > 0.3$) with seven parameters;

Ammonium NH_4^+ (0.53), total phosphorous (0.46), total organic carbon (0.40), and silica $\text{Si}(\text{OH})_4$ (0.38), salinity at the surface and at the bottom (0.38 and 0.36 respectively), and temperature (0.30). Caffeine had slightly negative correlation with dissolved oxygen at the surface and at the bottom (-0.38 and -0.33 respectively) and percent saturation at the surface and at the bottom (-0.39 and -0.33 respectively). Enterococci are a group of fecal streptococci and they serve as better indicators of sewage contamination than fecal coliform. For this reason, Enterococci as well as Fecal coliform were expected to have a strong correlation with caffeine but both parameters had extremely poor correlations (-0.06 and -0.09 respectively).

Figure 20 is vertical box plot of the grouped occurrence of caffeine in the stations located at head, the mouth of the canals and the stations offshore. Occurrence of caffeine was widespread but more frequent in canals and enclosed waterways than in open waters. Caffeine concentrations at the head and at the mouth of the canals are similar. Stations offshore showed generally lower concentrations of caffeine than those inside the canals. Figure 21 is a plot of the occurrence of fecal coliform, enterococci, ammonium, and total phosphorous at the head, at the mouth of the canals and offshore. This data compares to that of caffeine. Fecal coliform, enterococci, ammonium, and total phosphorous were higher at the head of the canal than at the mouth. Stations offshore also showed significantly lower levels for the four parameters. These results were expected because the heads of the canals receive less tidal flush than the mouths of the canals, therefore contaminants tend to spend more time and accumulate. Stations offshore are exposed to constant mixing with cleaner water and therefore have lower concentrations of contaminants.

Figure 17. Monthly Occurrence of Caffeine in Little Venice

Figure 18. Monthly occurrence of caffeine in LV-03, LV09 and LV-04

Figure 19. Monthly occurrence of other water quality markers in Little Venice

Table 7. Multivariate correlation matrix for caffeine other water quality parameters

	NO _x	NO ₃	NO ₂	NH ₄	TN	DIN	TON	TP	SRP	CHLA	TOC	SI(OH) ₄	SAL-S	SAL-B	TEMP-S	TEMP-B	DO-S	DO-B
NO _x		1.00	0.31	-0.18	0.16	0.97	-0.12	-0.03	0.77	-0.19	-0.46	0.29	-0.31	-0.03	0.13	0.19	-0.27	-0.26
NO ₃	1.00		0.29	-0.18	0.15	0.97	-0.13	-0.03	0.77	-0.20	-0.45	0.28	-0.32	-0.04	0.12	0.18	-0.26	-0.25
NO ₂	0.31	0.29		0.19	0.38	0.36	0.28	0.10	0.25	0.06	-0.31	0.43	0.18	0.33	0.44	0.46	-0.45	-0.48
NH ₄	-0.18	-0.18	0.19		0.16	0.07	0.14	0.31	-0.09	-0.02	0.26	0.29	0.39	0.37	0.45	0.39	-0.36	-0.36
TN	0.16	0.15	0.38	0.16		0.21	0.96	0.09	0.09	0.08	0.07	0.33	0.41	0.54	0.40	0.39	-0.43	-0.33
DIN	0.97	0.97	0.36	0.07	0.21		-0.08	0.04	0.76	-0.20	-0.40	0.37	-0.22	0.06	0.24	0.29	-0.36	-0.35
TON	-0.12	-0.13	0.28	0.14	0.96	-0.08		0.08	-0.13	0.13	0.18	0.23	0.48	0.53	0.34	0.31	-0.33	-0.23
TP	-0.03	-0.03	0.10	0.31	0.09	0.04	0.08		0.06	0.48	0.45	0.65	0.08	0.17	0.20	0.03	-0.32	-0.55
SRP	0.77	0.77	0.25	-0.09	0.09	0.76	-0.13	0.06		-0.13	-0.26	0.20	-0.28	0.01	-0.20	-0.16	-0.04	-0.04
CHLA	-0.19	-0.20	0.06	-0.02	0.08	-0.20	0.13	0.48	-0.13		0.33	0.19	0.04	0.05	0.23	0.09	-0.22	-0.48
TOC	-0.46	-0.45	-0.31	0.26	0.07	-0.40	0.18	0.45	-0.26	0.33		0.16	0.46	0.36	0.10	-0.08	-0.17	-0.18
SI(OH) ₄	0.29	0.28	0.43	0.29	0.33	0.37	0.23	0.65	0.20	0.19	0.16		0.22	0.37	0.55	0.46	-0.73	-0.77
SAL-S	-0.31	-0.32	0.18	0.39	0.41	-0.22	0.48	0.08	-0.28	0.04	0.46	0.22		0.90	0.41	0.31	-0.47	-0.30
SAL-B	-0.03	-0.04	0.33	0.37	0.54	0.06	0.53	0.17	0.01	0.05	0.36	0.37	0.90		0.47	0.39	-0.54	-0.43
TEMP-S	0.13	0.12	0.44	0.45	0.40	0.24	0.34	0.20	-0.20	0.23	0.10	0.55	0.41	0.47		0.97	-0.83	-0.81
TEMP-B	0.19	0.18	0.46	0.39	0.39	0.29	0.31	0.03	-0.16	0.09	-0.08	0.46	0.31	0.39	0.97		-0.77	-0.70
DO-S	-0.27	-0.26	-0.45	-0.36	-0.43	-0.36	-0.33	-0.32	-0.04	-0.22	-0.17	-0.73	-0.47	-0.54	-0.83	-0.77		0.86
DO-B	-0.26	-0.25	-0.48	-0.36	-0.33	-0.35	-0.23	-0.55	-0.04	-0.48	-0.18	-0.77	-0.30	-0.43	-0.81	-0.70	0.86	
DO-S X	0.26	0.26	0.37	0.31	0.37	0.34	0.28	0.18	-0.04	0.09	0.02	0.60	0.32	0.39	0.80	0.79	-0.89	-0.75
DO-B X	0.20	0.20	0.28	0.44	0.14	0.32	0.05	0.27	0.03	0.27	0.08	0.50	0.23	0.29	0.68	0.66	-0.75	-0.77
TN-EX	0.14	0.13	0.38	0.11	0.80	0.17	0.77	0.08	-0.10	0.02	-0.02	0.41	0.38	0.44	0.59	0.59	-0.61	-0.52
TP-EX	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TN:TP	0.03	0.02	0.16	0.02	0.74	0.03	0.75	-0.51	-0.04	-0.18	-0.12	-0.21	0.37	0.38	0.11	0.17	-0.07	0.13
TS	-0.13	-0.12	-0.44	-0.45	-0.41	-0.24	-0.34	-0.20	0.20	-0.23	-0.10	-0.55	-0.41	-0.47	-1.00	-0.97	0.83	0.81
TS_B	-0.19	-0.18	-0.46	-0.38	-0.39	-0.29	-0.31	-0.03	0.16	-0.09	0.08	-0.45	-0.31	-0.39	-0.97	-1.00	0.77	0.70
%SAT-S	-0.29	-0.28	-0.44	-0.34	-0.43	-0.37	-0.32	-0.35	-0.08	-0.21	-0.19	-0.75	-0.45	-0.53	-0.78	-0.72	1.00	0.84
%SAT_B	-0.27	-0.26	-0.47	-0.34	-0.31	-0.35	-0.21	-0.59	-0.07	-0.50	-0.19	-0.79	-0.28	-0.41	-0.76	-0.64	0.84	1.00
SIGT-S	-0.41	-0.40	-0.23	-0.04	0.00	-0.42	0.13	-0.11	-0.08	-0.17	0.33	-0.31	0.56	0.41	-0.53	-0.59	0.33	0.45
SIGT-B	-0.20	-0.20	-0.11	-0.01	0.13	-0.20	0.19	0.14	0.15	-0.04	0.40	-0.08	0.53	0.54	-0.45	-0.56	0.21	0.24
DSIGT	0.49	0.49	0.26	0.06	0.27	0.51	0.12	0.56	0.51	0.30	0.10	0.53	-0.13	0.24	0.24	0.13	-0.28	-0.50
FC	0.39	0.39	0.20	-0.16	0.09	0.35	-0.01	0.02	0.09	-0.19	-0.18	0.15	-0.05	0.06	0.24	0.26	-0.25	-0.29
EC	0.15	0.15	0.24	-0.10	0.01	0.13	-0.03	0.07	-0.02	-0.04	-0.06	0.27	0.10	0.07	0.12	0.10	-0.23	-0.26
Caffeine	-0.03	-0.03	0.02	0.53	0.15	0.10	0.12	0.46	-0.10	-0.02	0.40	0.38	0.36	0.35	0.30	0.17	-0.38	-0.33

Table 7 Continued.

	DO-S X	DO-B X	TN-EX	TP-EX	TN:TP	TS	TS_B	%SAT-S	%SAT_ B	SIGT-S	SIGT-B	DSIGT	FC	EC	Caffeine
NOX	0.26	0.20	0.14	0.00	0.03	-0.13	-0.19	-0.29	-0.27	-0.41	-0.20	0.49	0.39	0.15	-0.03
NO3	0.26	0.20	0.13	0.00	0.02	-0.12	-0.18	-0.28	-0.26	-0.40	-0.20	0.49	0.39	0.15	-0.03
NO2	0.37	0.28	0.38	0.00	0.16	-0.44	-0.46	-0.44	-0.47	-0.23	-0.11	0.26	0.20	0.24	0.02
NH4	0.31	0.44	0.11	0.00	0.02	-0.45	-0.38	-0.34	-0.34	-0.04	-0.01	0.06	-0.16	-0.10	0.53
TN	0.37	0.14	0.80	0.00	0.74	-0.41	-0.39	-0.43	-0.31	0.00	0.13	0.27	0.09	0.01	0.15
DIN	0.34	0.32	0.17	0.00	0.03	-0.24	-0.29	-0.37	-0.35	-0.42	-0.20	0.51	0.35	0.13	0.10
TON	0.28	0.05	0.77	0.00	0.75	-0.34	-0.31	-0.32	-0.21	0.13	0.19	0.12	-0.01	-0.03	0.12
TP	0.18	0.27	0.08	0.00	-0.51	-0.20	-0.03	-0.35	-0.59	-0.11	0.14	0.56	0.02	0.07	0.46
SRP	-0.04	0.03	-0.10	0.00	-0.04	0.20	0.16	-0.08	-0.07	-0.08	0.15	0.51	0.09	-0.02	-0.10
CHLA	0.09	0.27	0.02	0.00	-0.18	-0.23	-0.09	-0.21	-0.50	-0.17	-0.04	0.30	-0.19	-0.04	-0.02
TOC	0.02	0.08	-0.02	0.00	-0.12	-0.10	0.08	-0.19	-0.19	0.33	0.40	0.10	-0.18	-0.06	0.40
Si(OH)4	0.60	0.50	0.41	0.00	-0.21	-0.55	-0.45	-0.75	-0.79	-0.31	-0.08	0.53	0.15	0.27	0.38
SAL-S	0.32	0.23	0.38	0.00	0.37	-0.41	-0.31	-0.45	-0.28	0.56	0.53	-0.13	-0.05	0.10	0.36
SAL-B	0.39	0.29	0.44	0.00	0.38	-0.47	-0.39	-0.53	-0.41	0.41	0.54	0.24	0.06	0.07	0.35
TEMP-S	0.80	0.68	0.59	0.00	0.11	-1.00	-0.97	-0.78	-0.76	-0.53	-0.45	0.24	0.24	0.12	0.30
TEMP-B	0.79	0.66	0.59	0.00	0.17	-0.97	-1.00	-0.72	-0.64	-0.59	-0.56	0.13	0.26	0.10	0.17
DO-S	-0.89	-0.75	-0.61	0.00	-0.07	0.83	0.77	1.00	0.84	0.33	0.21	-0.28	-0.25	-0.23	-0.38
DO-B	-0.75	-0.77	-0.52	0.00	0.13	0.81	0.70	0.84	1.00	0.45	0.24	-0.50	-0.29	-0.26	-0.33
DO-S X		0.81	0.60	0.00	0.09	-0.80	-0.79	-0.87	-0.73	-0.44	-0.37	0.21	0.31	0.16	0.23
DO-B X	0.81		0.36	0.00	-0.12	-0.68	-0.66	-0.74	-0.75	-0.40	-0.33	0.20	0.21	0.11	0.19
TN-EX	0.60	0.36		0.00	0.55	-0.59	-0.59	-0.59	-0.49	-0.19	-0.15	0.12	0.34	0.18	0.21
TP-EX	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TN:TP	0.09	-0.12	0.55	0.00		-0.11	-0.18	-0.05	0.15	0.24	0.17	-0.17	-0.04	-0.07	-0.05
TS	-0.80	-0.68	-0.59	0.00	-0.11		0.97	0.78	0.76	0.53	0.45	-0.24	-0.24	-0.12	-0.30
TS_B	-0.79	-0.66	-0.59	0.00	-0.18	0.97		0.72	0.65	0.59	0.56	-0.13	-0.26	-0.10	-0.17
%SAT-S	-0.87	-0.74	-0.59	0.00	-0.05	0.78	0.72		0.83	0.29	0.18	-0.29	-0.24	-0.23	-0.39
%SAT_B	-0.73	-0.75	-0.49	0.00	0.15	0.76	0.65	0.83		0.43	0.21	-0.52	-0.27	-0.26	-0.33
SIGT-S	-0.44	-0.40	-0.19	0.00	0.24	0.53	0.59	0.29	0.43		0.90	-0.34	-0.25	-0.01	0.06
SIGT-B	-0.37	-0.33	-0.15	0.00	0.17	0.45	0.56	0.18	0.21	0.90		0.11	-0.18	-0.01	0.18
DSIGT	0.21	0.20	0.12	0.00	-0.17	-0.24	-0.13	-0.29	-0.52	-0.34	0.11		0.19	-0.01	0.26
FC	0.31	0.21	0.34	0.00	-0.04	-0.24	-0.26	-0.24	-0.27	-0.25	-0.18	0.19		0.65	-0.09
EC	0.16	0.11	0.18	0.00	-0.07	-0.12	-0.10	-0.23	-0.26	-0.01	-0.01	-0.01	0.65		-0.06
Caffeine	0.23	0.19	0.21	0.00	-0.05	-0.30	-0.17	-0.39	-0.33	0.06	0.18	0.26	-0.09	-0.06

Figure 20. Occurrence of Caffeine by Location in the Canals in Little Venice

Figure 21. Occurrence of other water quality markers by location in the canals in Little Venice

3.1.2. Pharmaceuticals in Little Venice

Samples collected in Little Venice from August 2003 to December 2003 were analyzed for other highly used pharmaceuticals and personal care products such as Triclosan, DEET, Bisphenol A and 4n-Nonylphenol. The data generated during this period is presented in Table 5.

Figure 22 shows box plots of the occurrence of pharmaceuticals and personal care products in Little Venice for the four sampling events during 2003. With the exception of 4n-nonylphenol, all pharmaceuticals and personal care products were found in surface waters from Little Venice. Caffeine concentrations ranged from 4.78 to 45.4 ppt during these sampling periods. DEET was found in Little Venice in concentrations ranging from BDL to 128.1 ppt. Triclosan and Bisphenol A were present in concentrations ranging from ND-84.4 ppt and ND-82.4 ppt respectively. 4n-Nonylphenol was not detected in the period sampled in Little Venice. Table 8 represents the multivariate correlation matrix for the pharmaceuticals and personal care products showing that no strong correlations were found among any of the compounds. A small correlation ($R^2 > 0.3$) was found among Caffeine and Bisphenol A (0.3), and Bisphenol A and DEET (0.45).

Table 8. Multivariate correlation matrix for pharmaceuticals and personal care products in Little Venice

	Caffeine	DEET	4n-Nonylphenol	Bisphenol A	Triclosan
Caffeine		0.11	0.00	0.30	0.08
DEET	0.11		0.00	0.45	-0.04
4n-Nonylphenol	0.00	0.00		0.00	0.00
Bisphenol A	0.30	0.45	0.00		0.21
Triclosan	0.08	-0.04	0.00	0.21	

Figure 23 is a vertical box plots for the concentration distribution of caffeine, DEET, Bisphenol A and Triclosan for each station during the sampling period. Caffeine showed the highest concentrations in Stations LV-01, LV-04, and LV-06 during these months. These three stations are located at the mouth of the canals; therefore these stations were expected to have lower concentrations than the ones inside the canals. Station LV-03, once again, seems to be the most affected with DEET, Bisphenol A, and Triclosan. During this sampling period, caffeine was not higher in station LV-03. These data demonstrate the variability of results from month to month.

Figure 22. Occurrence of pharmaceuticals in Little Venice

Figure 23. Occurrence of pharmaceuticals in Little Venice

Figure 24. Occurrence of Pharmaceuticals and Personal Care Products by locations in Little Venice

Figure 24 shows the occurrence of pharmaceuticals and personal care products inside the canals systems versus the stations offshore. In general, concentrations of pharmaceuticals and personal care products were higher inside the canals and lower in the stations offshore. This compares to the nutrients and bacterial data presented in Figure 21.

3.1.3. Caffeine vs. Rainfall in Little Venice

Boyd, R.G et al. (2004) correlated rainfall with concentrations of pharmaceuticals and personal care products in Orleans and London canals. Their data showed increasing concentration of contaminants with increasing periods of increased rainfall, which was attributed to cross flows with sanitary sewers in the aging New Orleans sewer system. In contrast, data collected in 23 Iowa streams located upstream and downstream by Kolpin et al. (2004) also showed that urban contributions of wastewater contaminants varied with stream flows. The results from this paper showed that concentrations of wastewater contaminants in streams decreased as stream flow increased from low, to normal, to high flows conditions. Many compounds were only detected during low-flow conditions and had significant higher concentrations upstream than downstream.

Paul et al. (2000) used two bacteriophages, the marine bacteriophage ϕ HSIC and the *Salmonella* phage PRD1, as tracers in injection well and septic tank studies in Saddlebunch Keys of the Lower Florida Keys and in septic tank studies in Boot Key Harbor, Marathon, of the Middle Keys. Both phage tracers were detected at all stations sampled in Marathon Key. The station that was located at the major channel of drainage of Boot Key Harbor showed a correlation with tracers and rain events. An increase in rain events created a higher rate of flow and greater volume of water moving through the

channel. This explained the early appearance of the tracer at the station located at the major channel of drainage compared to other stations. Also, this rainfall event apparently helped flush the tracer to other stations as well.

These three papers suggest that concentrations of contaminants and tracers could be correlated with rainfall, with an increase in concentrations as rainfall events increase due to the “first flush” effect. Also, concentrations of contaminants decrease as they get more diluted due to higher flows of water. The first flush effect simply explains that pollutants that had accumulated on paved surfaces in dry weather are quickly washed off during the beginning of a storm, showing higher concentrations of pollutants. The amount of pollutants available for wash off will deplete, and consequently the concentration of pollutants will decrease as the rainfall event progresses. However, linking rainfall events with surface water movement could be difficult since the tidal effects may be bigger than the rainfall effects.

Since abundant data was collected in Little Venice monthly as well as by stations, an attempt to correlate rainfall with caffeine concentrations was made. Figure 25-A is a vertical box plot which plots the median, 10th, 25th, 75th, and 90th percentiles as vertical boxes with error bars for the monthly occurrence of caffeine concentrations in Little Venice and Figure 25- B is a plot of the average daily rainfall in Marathon Key from November 2001 to December 2004. A comparison of these plots shows a slight trend between rainfall and caffeine concentration in Little Venice. With the exception of a few outlier points in the monthly concentration of caffeine, caffeine concentrations tend to be higher during wet season (July to October) and lower during dry season (November to March). Table 9 shows multivariate correlation matrix for the concentration of caffeine

versus rainfall for all stations in Little Venice. The table shows no statistical correlation between caffeine concentration and rainfall events for any of the sites sampled. But in general, concentrations were lower and less variable for November to March (dry season) than for April to October (wet season).

Figure 25. A. Monthly Occurrence of Caffeine in Little Venice. B. Daily rainfall in Marathon Key

Table 9. Multivariate correlation matrix for caffeine concentrations by stations and rainfall in Little Venice

	Date	Temp ave	LV-01	LV-02	LV-03	LV-04	LV-05	LV-06	LV-07	LV-08	LV-09	LV-10	Precipita- tion (in)
Date		0.36	0.05	0.54	-0.19	-0.11	-0.38	0.08	-0.02	0.02	-0.04	-0.39	0.08
Temp ave	0.36		0.39	0.30	0.29	0.42	-0.03	0.39	0.26	0.54	0.45	-0.40	-0.38
LV-01	0.05	0.39		0.10	0.07	0.90	0.37	0.66	0.80	0.75	0.84	-0.14	-0.18
LV-02	0.54	0.30	0.10		-0.25	-0.14	0.11	0.31	0.06	0.02	-0.02	-0.06	-0.01
LV-03	-0.19	0.29	0.07	-0.25		0.14	-0.28	-0.22	-0.06	0.29	0.11	0.24	-0.17
LV-04	-0.11	0.42	0.90	-0.14	0.14		0.42	0.55	0.78	0.74	0.84	-0.02	-0.10
LV-05	-0.38	-0.03	0.37	0.11	-0.28	0.42		0.74	0.53	0.42	0.50	-0.07	-0.01
LV-06	0.08	0.39	0.66	0.31	-0.22	0.55	0.74		0.58	0.64	0.68	-0.31	-0.16
LV-07	-0.02	0.26	0.80	0.06	-0.06	0.78	0.53	0.58		0.86	0.93	-0.27	0.03
LV-08	0.02	0.54	0.75	0.02	0.29	0.74	0.42	0.64	0.86		0.96	-0.36	-0.07
LV-09	-0.04	0.45	0.84	-0.02	0.11	0.84	0.50	0.68	0.93	0.96		-0.32	-0.08
LV-10	-0.39	-0.40	-0.14	-0.06	0.24	-0.02	-0.07	-0.31	-0.27	-0.36	-0.32		0.00
Precipitation (in)	0.08	-0.38	-0.18	-0.01	-0.17	-0.10	-0.01	-0.16	0.03	-0.07	-0.08	0.00	

3.2. Key Largo Harbor Results

Key Largo Harbor is an area of intersecting canals surrounded by residential areas with waterfront properties. The first samples analyzed for caffeine were collected on February 2003. All forty-six samples tested positive with concentrations ranging from right below the quantitation limits (only one sample) to as high as 809 ppt. The average concentration was 75.9 ppt and the median 52.3 ppt. The average and the median concentrations were above the 40 ppt criteria, indicating that many samples (63%) had concentrations high enough to be considered impacted waters. Three samples showed very high concentrations of caffeine; KLH2001 (809 ppt), KLH2006 (388 ppt), and KLH2007 (108 ppt). These samples were analyzed for estrogen equivalence (YES Bioassay).

Variations of the Yeast estrogen screen assay (YES assay) system were originally described by Routledge and Sumpter (1996). It uses a recombinant yeast strain (*Saccharomyces cerevisiae*) that has had the human estrogen receptor (hER) stably integrated into the yeast. The yeast cells also contain expression plasmids that contain both estrogen response elements (ERE) and the reporter gene *lac-Z* which encodes the enzyme β -galactosidase. When an active ligand interacts with the ER, gene transcription of the reporter gene is initiated, and the induced activity of β -galactosidase is detected when it causes the metabolism of chlorophenol red- β -D-galactopyranoside (CPRG) into a red product. Detection of the CPRG is easily done with a spectrophotometer. This system is design to detect compounds in the water extracts with the ability to bind and activate the human estrogen receptor implying that compounds in the extract have the potential to mimic the biological activities of estrogen. Several studies have used the YES

assay to study estrogen-like activity in environmental extracts of surface waters (Garcia-Reyero, 2001 and Murk et al., 2002). A summary of the results is presented in Table 10. No correlation was seen between caffeine and estrogen equivalence, but with only three samples, it was difficult to make any correlation. The expectation was that caffeine concentration could be a good indicator for other estrogen active compounds present in human derived wastewater. However, up to date we have been unable to establish a strong link between the two parameters.

Table 10. Summary of data for Key Largo Harbor on February 2003

Max conc.	809.4	
Min conc.	0.7	
Average	75.9	
Median	52.3	
Below Detection Limit	1 (2%)	
Below 15 ppt	6 (13%)	Not Environmentally significant
Below 40 ppt	11 (23%)	Environmentally significant
Above 40 ppt	30 (63%)	Impacted waters
	[Caffeine] ng/L	[Estrogen Equivalence] pg/L
KLH 2001	809	35.79±7
KLH 2006	388	28.95±5
KLH 2007	108	40.73±9

Results for the 2003 data indicated that large areas of Key Largo Harbor had caffeine in high concentrations. Fifty-six samples collected in Key Largo Harbor on February 2004 were analyzed for caffeine, DEET and Triclosan. Figure 26 is a vertical box plot of the occurrence of caffeine, DEET and Triclosan. Caffeine was the most frequently detected compound, with concentrations ranging from below detection limit to 104 ppt. Only four (7%) samples were above 40 ppt. These results are very different from the previous year, where 63% of the samples were above 40 ppt. DEET was

detected in the area of Key Largo Harbor in 35 out of 56 (62%) samples collected with concentrations in the range of not detected to 11.7 ppt. Triclosan was detected in 16 out of 56 (29%) samples with concentrations ranging from not detected to 9.72 ppt.

Figure 26. Occurrence of Caffeine, DEET and Triclosan in Key Largo Harbor on February 2003

Figure 27 is a vertical box plot of the occurrence of pharmaceuticals and personal care products in Key Largo Harbor on five monthly sampling trips from February 2004 to August 2004. Caffeine concentrations were in the range of 4.74 to 89.5 ppt. In this sampling period caffeine was detected in concentrations from below detection limit to 15

ppt in 11 out of 35 samples (31%), from 15-40 ppt in 13 out of 35 samples (37%) and concentrations above 40 ppt were present in 9 out of 35 samples (26%). Caffeine has been detected in Boston Harbor seawater with concentrations ranging from 140 to 1600 ng/L, and in Massachusetts Bay seawater at concentrations from 5.2 to 71 ng/L (Siegener et al. 2002).

Figure 27. Occurrence of Pharmaceuticals and Personal Care Products in Key Largo Harbor from February 2004 to August 2004

Caffeine concentrations in Key Largo Harbor are comparable to those in Massachusetts Bay, while Boston Harbor had considerably higher concentrations of caffeine than Key Largo Harbor. DEET, Bisphenol A and Triclosan were detected at

concentrations ranging from below detection limit to 39.9, 58.0, and 16.7 respectively. 4n-Nonylphenol was not detected in any of the samples.

Table 11 is a multivariate correlation matrix for pharmaceuticals and personal care products were it could be seen that the compounds did not have any correlation among each other. Figure 28 is a Key Largo Harbor map showing the distribution of pharmaceuticals and personal care products on August 2004. Table 12 presents this data as well.

Table 11. Multivariate correlation matrix for pharmaceuticals and personal care products in Key Largo Harbor

	Caffeine	DEET	Bisphenol A	Triclosan
Caffeine		0.03	0.08	0.03
DEET	0.03		0.06	-0.25
Bisphenol A	0.08	0.06		0.21
Triclosan	0.03	-0.25	0.21	

Samples collected in August 2004 were also analyzed for fifteen hormones and steroids by (Singh et al. Unpublished results). The samples were positive for four hormones and steroids; Equiline, coprostanol, estrone, and β -estradiol. Figure 29 is a box plot of the distribution of the concentrations of the pharmaceuticals, personal care products, steroids and hormones detected in Key Largo Harbor in August 2004. DEET showed the highest concentrations, in the range of 11.7 to 40.0 ppt. Caffeine concentrations were in the range of 5.71-24.7, with only two sampling sites (KLH-03 and KLH-08) with low concentrations (below 15 ppt). The other sites showed environmentally significant concentrations but not impacted waters. Bisphenol A and Triclosan had concentration ranging from 7.06 to 18.0 and BDL to 9.29 ppt respectively (Table 12). Compared to Little Venice, Miami River, C1 canal and Palm Island Marina in

the west coast of Florida, these levels are quite low but all the samples were above the practical quantitation limit, with the exception of one sample, KLH-03, that had detectable levels of Triclosan but below the quantitation limit. Equiline concentrations were in the range of 1.27-5.51, Estrone 1.28-5.25, coprostanol 0.84-2.54, and β -estradiol 0.31-1.84 ppt.

Figure 28. Key Largo Harbor Map with distribution of concentrations of pharmaceuticals and personal care products on August 2004

Table 12. Concentrations of pharmaceuticals in Key Largo Harbor on August 2003 in ng/L. ND=Not Detected, BLD=below detection limit

Site	N (Latitude)	W (Longitude)	Caffeine	DEET	4n- nonylphenol	Bisphenol A	Triclosan
KLH-01	25.09612	-80.43604	22.4	32.3	ND	13.6	3.56
KLH-02	25.00610	-80.42999	14.2	39.9	ND	12.0	3.04
KLH-03	25.09423	-80.43536	8.67	19.9	ND	7.69	BDL
KLH-04	25.09342	-80.43027	21.3	21.9	ND	7.06	4.55
KLH-05	25.09210	-80.43562	12.1	16.4	ND	6.16	3.63
KLH-06	25.09227	-80.43028	18.0	21.5	ND	10.1	4.49
KLH-07	25.09103	-80.43081	24.7	22.2	ND	8.73	9.27
KLH-08	25.08914	-80.43067	5.71	11.7	ND	18.0	4.02

Figure 29. Occurrence of pharmaceuticals, personal care products, hormones and steroids in Key Largo Harbor on August 2004

Figure 30 is a scatter line plot of the concentrations of pharmaceuticals and personal care products in each sampling site. No particular trend is apparent among caffeine and the pharmaceuticals. Stations KLH-01, KLH-03, and KLH-06 were expected to have higher concentrations of compounds due to their locations at the ends of the canals. Station KLH-03 did not follow the expected trend. In general, pharmaceuticals and personal care products were higher inside the canal system than in open bay areas. Station KLH-08, which is located outside the canal system, showed very low concentrations of caffeine, DEET, and Triclosan. Figure 31 is a scatter plot of the concentrations of caffeine and the hormones in each site. Some trends could be observed in this plot. Caffeine is high in KLH-01, which is the most distant site from the mouth of the canal system. Concentrations decrease for station KLH-02, which is located at the end of the transect that receives more water circulation. Concentration decreases at station KLH-03, increases at station KLH-04 and decreases at station KLH-05. Concentrations increase again for stations KLH-06 and KLH-07 and decrease in station KLH-08, which is located at the entrance of the canal system. This same trend is observed for the hormones and steroids, except for station KLH-07, which has similar concentration as KLH-06 while caffeine increases from site KLH-06 to KLH-07. The hormones and steroids also showed lower concentrations outside the canal system (KLH-08) and higher concentrations inside, with KLH-01 having the highest concentrations.

Table 13 is a presentation of the multivariate correlation matrix for pharmaceuticals, personal care products, hormones and steroids in Key Largo Harbor on August 2004. This table reflects what is observed in Figures 30 and 31. Caffeine correlated positively with DEET (0.42), Triclosan (0.63), Equiline (0.75) and coprostanol

(0.44). Caffeine also correlated weakly with Bisphenol A and Estrone (-0.30 and 0.35 respectively). The strongest correlation was found between Estrone and Estradiol (0.97), which is expected due to their common source.

Table 13. Multivariate correlation matrix for pharmaceuticals, personal care products, hormones and steroids in Key Largo Harbor

	Bisphenol				Copro			
	DEET	Caffeine	A	Triclosan	Equiline	Estrone	stanol	Estradiol
DEET		0.42	0.05	-0.13	0.58	0.52	0.68	0.57
Caffeine	0.42		-0.30	0.63	0.75	0.35	0.44	0.37
Bisphenol A	0.05	-0.30		-0.10	0.17	0.55	0.13	0.47
Triclosan	-0.13	0.63	-0.10		0.16	-0.15	-0.25	-0.13
Equiline	0.58	0.75	0.17	0.16		0.87	0.81	0.86
Estrone	0.52	0.35	0.55	-0.15	0.87		0.83	0.97
Coprostanol	0.68	0.44	0.13	-0.25	0.81	0.83		0.87
Estradiol	0.57	0.37	0.47	-0.13	0.86	0.97	0.87

Figure 30. Concentrations of pharmaceuticals and personal care products by sites

Figure 31. Concentration of caffeine, hormones and steroids by sites

3.3. Looe Key (Underwater Music Festival)

Caffeine was analyzed during the Underwater Music Festival in Looe Key Reef in 2003 and in 2004 for all the pharmaceuticals. Figure 32 is a vertical box plot of the occurrence of caffeine the day before the Underwater Music Festival, during the event, and the day after the event in 2003. Six samples were also collected inside the canals leading to a marina onshore for comparison purposes.

Figure 32. Caffeine concentrations before, during, after the UMF and inside the marina in 2003

In 2003 the concentrations of caffeine ranged from below detection limit to 38.5 ng/L. Concentrations of caffeine were higher the day before the event, decreased during the event, and increased again after the event. Most of the people are expected during the event, but the reef had many people the day of the event and they start leaving after the event. Concentrations were higher at the reef than inside the canal system with the private marinas. In 2004, more samples were collected during the same event and analyzed for all the compounds. A summary of the data is represented in Table 15.

Figure 33 is a vertical box plot for the distribution of the pharmaceuticals and personal care products in Looe Key in 2004. Caffeine concentrations ranged from BDL-30.6 ng/L, which was comparable to the previous year. DEET concentrations ranged from BDL-16.8 ng/L. Bisphenol A and 4n-Nonylphenol were not detected. Triclosan was detected in only one sample at very low concentration (3.60 ppt). These concentrations are quite low, with only four samples (10%) having caffeine concentrations higher than 15 ppt (environmentally significant concentrations) and no samples had concentrations higher than 40 ppt (impacted waters).

Figure 34 is a vertical box plot of the concentrations of caffeine and DEET in samples taken at the surface and at the bottom during the underwater music festival to compare concentrations of contaminants in the water closer to the reef. Caffeine shows slightly higher concentrations at the surface than at the bottom while DEET have slightly higher concentrations at bottom. Despite the fact that there is a difference between surface and bottom, not enough data has been collected to accurately assess any trends for the individual compounds. However, is worth notifying that caffeine and DEET have different mechanisms of release to the environment.

Figure 35 is a plot of the concentrations of the compounds by samples. Samples 1 through 10 were collected the day before the UMF, 11 through 20 during UMF, 21 through 30 the same day of UMF but after the event was over, and 31 through 40 were collected the day after the UMF. No particular trend is observed between Caffeine and DEET among the samples. Bisphenol A, 4n-Nonylphenol and Triclosan were not detected.

Figure 36 and 37 are vertical box plots for the distribution of Caffeine and DEET respectively before, during and after UMF. Comparable to the previous year, concentrations of caffeine were higher the day before the event and concentrations decreased while the event was taking place and increased slightly the day after the event for the two years sampled. Concentrations of DEET were lower the day before the event, higher on the day of the event, and decreased again the day after the event. This trend was expected for DEET because many formulations for sun-block lotions contain DEET to protect for insect bits as well as for the radiation of the sun. The occurrence of DEET in the environment comes from direct contact of consumers applying DEET with the environment. Therefore, the concentrations of DEET are expected to be higher during an event that attracts 400 to 600 people, with many of them wearing sun-block lotions that might contain the chemical.

Figure 38 is a vertical box plot of the occurrence of estrone, equiline, cholesterol, (Singh et al., Unpublished results) as well as estrogen equivalence before, during and after the same event. Except for equiline, the hormones and steroids show similar trends as caffeine. Estrone, cholesterol, and estrogen equivalence were higher the day before the UMF and decreased while the event was taking place. Estrone kept decreasing the day after the event while cholesterol and estrogen equivalence showed a slight increase in concentration the day after the event. Equiline was lower the day before the event, higher during the event and decreased again after the event.

Table 14. Multivariate correlation matrix for pharmaceuticals, personal care products, hormones and steroids in Looe Key on 2004.

	Date	Caffeine	DEET	Triclosan	Estrone	Equiline	Cholesterol	Estrogen Equivalence
Date		-0.52	-0.16	-0.23	-0.85	-0.05	-0.72	-0.30
Caffeine	-0.52		0.05	-0.09	0.64	-0.10	0.62	0.28
DEET	-0.16	0.05		0.14	0.02	0.18	0.15	-0.03
Triclosan	-0.23	-0.09	0.14		0.19	0.05	0.04	0.06
Estrone	-0.85	0.64	0.02	0.19		-0.10	0.70	0.36
Equiline	-0.05	-0.10	0.18	0.05	-0.10		0.02	0.00
Cholesterol	-0.72	0.62	0.15	0.04	0.70	0.02		0.30
Estrogen Equivalence	-0.30	0.28	-0.03	0.06	0.36	0.00	0.30	

Table 14 is a multivariate correlation matrix for pharmaceuticals, personal care products, hormones and steroids in Looe Key on 2004. Caffeine showed a negative correlation with date (-0.52), indicating that it decreased from July 9th to July 11th. Estrone and Cholesterol had a negative correlation with date (-0.85 and -0.72) and this decreasing trend may also be observed in Figure 38. Caffeine also correlated with Estrone and cholesterol (0.64 and 0.62 respectively). DEET and Triclosan did not show any strong correlations with any of the other compounds. Estrogen Equivalence did not show any strong correlation with any of the pharmaceuticals and personal care products. It weakly correlated with Estrone and Cholesterol (0.36 and 0.30 respectively) and correlated negatively with date (-0.30).

Table 15. Pharmaceutical concentration in ng/L in Looe Key during the Underwater Music Festival in 2004 (ND=Not Detected, BDL=Below Detection Limit)

		N (Latitude)	W (Longitude)	Caffeine	DEET	4n- nonylphenol	Bisphenol A	Triclosan
Before UMF 07-09-04	LK04-01	24.54468	-81.40949	16.1	8.42	ND	ND	BDL
	LK04-02	24.54502	-81.40842	16.4	BDL	ND	ND	BDL
	LK04-03	24.54561	-81.40708	18.7	BDL	ND	ND	BDL
	LK04-04	24.54594	-81.40652	12.8	2.90	ND	ND	BDL
	LK04-05	24.54603	-81.40552	12.3	BDL	ND	ND	BDL
	LK04-06	24.54644	-81.80364	5.72	BDL	ND	ND	BDL
	LK04-07	24.54655	-81.40180	2.47	4.92	ND	ND	3.60
	LK04-08	24.54816	-81.40668	3.91	BDL	ND	ND	BDL
	LK04-09	24.54815	-81.40612	29.4	BDL	ND	ND	BDL
	LK04-10	24.54785	-81.40714	9.99	BDL	ND	ND	BDL
During UMF 07-10-04	LK04-11	24.54481	-81.41098	6.80	9.39	ND	ND	BDL
	LK04-12	24.54500	-81.41031	BDL	BDL	ND	ND	BDL
	LK04-13	24.54436	-81.40984	BDL	BDL	ND	ND	BDL
	LK04-14	24.54479	-81.40942	BDL	BDL	ND	ND	BDL
	LK04-15	24.54512	-81.40780	4.36	8.44	ND	ND	BDL
	LK04-16	24.54547	-81.40670	3.78	9.33	ND	ND	BDL
	LK04-17	24.54556	-81.40559	4.36	6.47	ND	ND	BDL
	LK04-19	24.54633	-81.40319	BDL	BDL	ND	ND	BDL
	LK04-20	24.54810	-81.40612	2.64	4.48	ND	ND	BDL
	LK04-21	24.54794	-81.40816	3.06	5.99	ND	ND	BDL
After UMF 07-10-04	LK04-22	24.54513	-81.41122	3.54	BDL	ND	ND	BDL
	LK04-23	24.54450	-81.41042	6.66	13.1	ND	ND	BDL
	LK04-24	24.54492	-81.40925	BDL	BDL	ND	ND	BDL
	LK04-25	24.54509	-81.40805	BDL	BDL	ND	ND	BDL
	LK04-26	24.54524	-81.40675	4.22	BDL	ND	ND	BDL
	LK04-27	24.54563	-81.40562	BDL	3.92	ND	ND	BDL
	LK04-28	24.54619	-81.40349	3.78	BDL	ND	ND	BDL
	LK04-29	24.54677	-81.40307	BDL	4.30	ND	ND	BDL
	LK04-30	24.54840	-81.40661	BDL	BDL	ND	ND	BDL
	LK04-31	24.54516	-81.41119	3.36	7.37	ND	ND	BDL
Day After UMF 07-11	LK04-33	24.54492	-81.40929	BDL	4.00	ND	ND	BDL
	LK04-34	24.54508	-81.40834	9.14	BDL	ND	ND	BDL
	LK04-35	24.54529	-81.40744	3.21	BDL	ND	ND	BDL
	LK04-36	24.54588	-81.40639	6.42	BDL	ND	ND	BDL
	LK04-37	24.54602	-81.40534	2.88	2.06	ND	ND	BDL
	LK04-38	24.54645	-81.40310	4.34	BDL	ND	ND	BDL
	LK04-39	24.54819	-81.40601	2.77	BDL	ND	ND	BDL
	LK04-40	24.54783	-81.40725	3.30	BDL	ND	ND	BDL

Figure 33. Occurrence of pharmaceuticals and personal care products in UMF in 2004

Figure 34. Surface vs. bottom analysis for the concentrations of caffeine and DEET

Pharmaceuticals and Personal Care Products in Looe Key in 2004

Figure 35. Concentrations of pharmaceuticals and personal care products by samples during UMF in 2004

Figure 36. Caffeine occurrence before, during and after the UMF in 2004

Figure 37. DEET occurrence before, during and after the UMF in 2004

Figure 38. Occurrence of hormones and steroids before, during and after the UMF in 2004

3.4. Miami River and Coconut Grove Marina

Miami River and Coconut Grove Marina sampling took place on October 2004. Table 18 and 19 show the data collected for the pharmaceuticals and personal care products in Miami River and Coconut Grove Marina respectively. Caffeine concentrations in Miami River ranged from 18.4 to 47.2 ppt. DEET concentrations ranged from below detection limit to 24.1 ppt and Bisphenol A concentrations were in the range of not detected to 78.9 ppt. Triclosan and 4n-Nonylphenol were not detected. Figure 41 is a scatter line plot for the concentrations of the pharmaceuticals and personal care products in Miami River. The stations are arranged in order from inside the river to the mouth of the river, with MRCP being the most distant site from the mouth of the river and MRBP is located at the mouth of the river. Caffeine concentrations did not follow any particular trends with the distance to the bay. Station MRNS had the highest concentration of caffeine. Station MRBP located at the mouth of the river, had lower concentrations of caffeine. The other compounds, DEET and Bisphenol A did not show any appreciable spatial trends along the river either. Biscayne Bay Coconut Grove Marina had undetected levels of all compounds except for DEET, which had a low concentration (15.6 ppt). In summary, concentrations of pharmaceuticals and personal care products were higher inside the river than in open waters. Figure 39 is a map of Miami River with distribution of concentrations of pharmaceuticals and personal care products in each site.

Figure 39. Miami River map with distribution of concentrations of compounds in ng/l. C=Caffeine, D=DEET, B=Bisphenol A

Miami River samples were also analyzed for 15 hormones and tested positive for three; coprostanol, estrone, and equiline (Sing et al., Unpublished results). Figure 42 is a scatter line plot of caffeine concentrations and the hormones in each Miami River site. The hormones follow similar trends along the river but no trend was observed with the caffeine data. Also, no spatial trends were observed with the hormone data. Unlike caffeine, hormones and steroids show higher concentrations at the mouth of the river. Table 16 shows the multivariate correlation matrix for pharmaceuticals, personal care products, hormones and steroids in Miami River. Caffeine shows a weak negative correlation with coprostanol (-0.36). DEET and coprostanol show the strongest negative

correlation (-0.89). Equiline correlates negatively with DEET (-0.55) and positively with Bisphenol A, coprostanol, and estrone (0.83, 0.68, and 0.82 respectively).

Table 16. Multivariate correlation matrix for pharmaceuticals, personal care products, hormones and steroids in Miami River.

	Caffeine	DEET	Bisphenol A	Coprostanol	Estrone	Equiline
Caffeine		0.20	0.21	-0.36	-0.27	-0.12
DEET	0.20		-0.14	-0.89	-0.14	-0.55
Bisphenol A	0.21	-0.14		0.35	0.83	0.83
Coprostanol	-0.36	-0.89	0.35		0.34	0.68
Estrone	-0.27	-0.14	0.83	0.34		0.82
Equiline	-0.12	-0.55	0.83	0.68	0.82	

Figure 40 is a map of Biscayne Bay showing distribution of pharmaceuticals and personal care products for all stations. Figure 43 is a scatter line plot of the concentrations of pharmaceuticals and personal care products for the seven stations in Coconut Grove Marina and surrounding areas. Figure 44 is vertical box plot for the distribution of caffeine and DEET. Stations along the Coconut Grove Marina showed low concentrations of caffeine and DEET, ranging from BDL-10.6 ng/L and 3.27-8.19 ng/L respectively (Table 18). Triclosan and 4n-Nonylphenol were not detected and Bisphenol A data was not available. These concentrations are very low and expected for an open-bay water sample. Oddly, station BBCGM located at Coconut Grove Marina had undetectable levels of caffeine, Bisphenol A, and Triclosan, and levels of DEET comparable to those in Miami River. These results were not expected because this station is located next to the metropolitan area of Coconut Grove and the marina is loaded with boats. The concentrations in Coconut Grove surrounding areas were on average four fold lower than inside the river. This data is consistent with a previous work by Gardinali et

al. (2002), who found concentrations of caffeine in the Miami River and Biscayne Bay ranging from 4 to 41 ppt. The levels of caffeine in open bay areas (Biscayne Bay) were on average about four times lower than the levels inside the river as well. Concentrations of DEET tend to decrease from Channel 1 to Channel 6, with Channel 1 being closer to land and Channel 6 the most distant from land.

**Figure 40. Coconut Grove Marina and surrounding areas with distribution of concentrations in ng/l.
C=Caffeine and D=DEET**

Table 17 is the multivariate correlation matrix for caffeine and DEET in Biscayne Bay. DEET shows a strong negative correlation with sampling sites, indicating that the concentration of DEET decreases with distance from land. Caffeine and DEET had a correlation of -0.50 . Excluding Site BBCGM from the multivariable analysis produces a

different result, with caffeine has a very strong negative correlation with site (-0.94) and DEET a negative correlation as well (-0.70). Caffeine and DEET had a positive correlation of 0.67.

Table 17. Multivariate correlation matrix for pharmaceuticals and personal care products in Biscayne Bay.

	Site	Caffeine	DEET
Site		-0.04	-0.79
Caffeine	-0.04		-0.50
DEET	-0.79	-0.50	
With BBCGM excluded			
	Site	Caffeine	DEET
Site		-0.94	-0.70
Caffeine	-0.94		0.67
DEET	-0.70	0.67	

Table 18. Miami River Data for Pharmaceuticals, concentration in ng/L (ND=Not Detected, BLD=Below Detection Limit)

	N	W			4n-	Bisphenol	
Site	Latitude	Longitude	Caffeine	DEET	Nonylphenol	A	Triclosan
MRYC	25.79826	-80.24819	22.1	11.7	ND	17.6	ND
MRCP	25.78464	-80.22868	21.7	24.1	ND	27.8	ND
MRNS	25.78625	-80.22409	47.2	12.5	ND	45.4	ND
MRMS	25.78300	-80.21569	40.8	16.0	ND	29.9	ND
MRSF	25.77993	-80.20975	30.8	14.5	ND	78.9	ND
MRBP	25.76988	-80.18964	18.4	BDL	ND	42.4	ND

Table 19. Coconut Grove Marina Data for Pharmaceutical, concentration in ng/L (ND=Not Detected, N/A= Not Available)

	N	W			4n-	Bisphenol	
Site	Latitude	Longitude	Caffeine	DEET	nonylphenol	A	Triclosan
BBCGM	25.72669	-80.23173	ND	15.6	ND	ND	ND
CHANNEL 1	25.72770	-80.23119	10.4	5.99	ND	N/A	ND
CHANNEL 2	25.72652	-80.23048	10.6	8.19	ND	N/A	ND
CHANNEL 3	25.72669	-80.22900	8.92	3.70	ND	N/A	ND
CHANNEL 4	25.72560	-80.22770	5.62	6.00	ND	N/A	ND
CHANNEL 5	25.72425	-80.22642	4.61	3.30	ND	N/A	ND
CHANNEL 6	25.72915	-80.22753	4.95	3.27	ND	N/A	ND

Figure 41. Concentration of pharmaceuticals and personal care products in Miami River

Figure 42. Concentration of caffeine, hormones and steroids in Miami River by stations

Figure 43. Concentration of pharmaceuticals and personal care products in Coconut Grove Marina

Figure 44. Occurrence of pharmaceuticals and personal care products in Coconut Grove Marina

3.5. C1 Canal

Black Creek Canal is located in southeastern Miami-Dade County. The canal runs through an agricultural and urban area before intersecting Biscayne Bay at Black Point Marina. The canal runs past 'Mt. Trash-More', one of the largest municipal landfills in South Florida. This canal was sampled to provide information about the occurrence of pharmaceuticals and personal care products in agricultural areas and newly developed residential areas as well as the concentrations of pharmaceuticals and personal care products leaching from the landfill. The results from this study will provide information about areas directly impacted by human activities.

Figure 45. Occurrence of pharmaceuticals and personal care products in C1 canal

Table 20, 21 and 22 have all the data collected for the pharmaceuticals in C1 canal for the three sampling events and Figure 45 shows occurrence and distribution of pharmaceuticals and personal care products in the C1 canal. DEET was detected in the canal in concentrations ranging from below detection limit to 28.9 ppt. Site 1, which is located in an agricultural area has a very low level (below quantitation limits) of DEET. As seen in Table 23, some small positive correlation may be observed between caffeine and DEET in the sampling events (0.30), as caffeine concentration increases, DEET tends to increase also. Triclosan was detected in concentrations ranging from not detected to 18.1 but no apparent trend in sites is observed. Triclosan was detected in three out of fourteen samples (21%) for the first sampling event and in six out of fourteen samples (43%) for the second sampling event and six out of twelve samples (50%) for the third sampling event. Levels of Triclosan were very low in all sampling events and did not follow any spatial trend along the canal. Bisphenol A was not detected in the first two sampling trips, but it was detected in four samples (33%) in the last sampling trip. The concentrations were in the range of 28.2 to 120 ppt, and were all detected in sites downstream from the landfill. 4n-Nonylphenol was not detected in any of the samples. Yamamoto et al. (2000) found concentrations of Bisphenol A ranging from 1.3 to 17,200 $\mu\text{g/L}$ (ppb) with a median concentration of 269 $\mu\text{g/L}$ in hazardous waste landfill leachates in Japan. These concentrations are substantially higher compared to the concentrations found in C1 canal, with the higher concentration in C1 canal (120 ppt) being lower than the lower concentration in the published paper (130 ppt). The source of Bisphenol A in landfills may be coming from the waste plastics in the landfill.

Figure 46 is a map of C1 canal showing the distribution of concentrations along the canal as well as the locations of the agricultural area, the residential area and the landfill. Concentrations of caffeine in C1 canal were low, ranging from not detected to 24.9 ppt. Caffeine was not present in the agricultural or the residential area of the canal. Concentrations of caffeine start increasing as the landfill is approached (SITE 8). These results indicate that sources of caffeine in C1 Canal may come from the landfill, the wastewater treatment plant in the area or the Black Point Marina, but not from the agricultural or the newly developed residential area.

Figure 46. Map of C1 canal with the distribution of concentrations in ng/L of pharmaceuticals and personal care products along the canal. C=Caffeine, D=DEET, B=Bisphenol A, T=Triclosan.

Figure 47, 49, and 51 are scatter line plots of the concentration of pharmaceuticals and personal care products in C1 canal on October 2004, December 2004, and January 2005 respectively. The sites are arranged downstream along the canal. Figure 47 shows that caffeine concentration increased downstream from the landfill, and decreased at the exit of the marina. DEET showed increasing trends all throughout the canal. Figure 49 shows concentrations of caffeine and DEET upstream from the landfill, starting to increase at SITE 6. Figure 51 shows concentrations of caffeine, DEET, Bisphenol A and Triclosan increasing downstream from the landfill.

Estrone and coprostanol were also detected in C1 Canal (Singh et al., Unpublished results). Figures 48, 50, and 52 are scatter line plots of caffeine, estrone and coprostanol for the three sampling events. Hormones and steroids did not show any particular correlations with caffeine, their correlation values were 0.07 for estrone, -0.05 for coprostanol, and -0.15 for cholesterol (Table 20). Hormones and steroids tend to increase in concentration downstream from the landfill as well.

Table 23 shows the multivariate correlation matrix for pharmaceuticals, personal care products, hormones and steroids in C1 canal. Caffeine did not correlate strongly with any of the compounds and it shows a weak positive correlation with DEET (0.30). DEET correlated with Bisphenol A (0.60) and weakly correlated with Estrone (0.30). DEET's correlation with sites and date was weak (0.35 and 0.37 respectively), but indicates that downstream DEET concentrations tends to increase, and DEET's concentrations also increased from month to months in the three sampling periods. Triclosan correlated positively with Bisphenol A (0.49). Cholesterol showed the highest correlation with date, indicating that the concentrations of cholesterol tended to decrease

over the three sampling periods. Also, cholesterol correlated positively with coprostanol (0.42).

Table 20. Pharmaceutical Data for C1 Canal in ng/L concentration on 10/06/04 (ND=Not Detected, BDL=Below Detection Limit, N/A=Not Available)

10/06/04	N Latitude	W Longitude	Caffeine	DEET	4n- Nonylphenol	Bisphenol A	Triclosan
SITE1	25.64036	-80.22751	ND	BDL	ND	ND	ND
SITE2	25.62593	-80.42697	ND	10.8	ND	ND	ND
SITE3	25.61877	-80.42154	ND	BDL	ND	ND	8.11
SITE4	25.59668	-80.41283	ND	4.82	ND	ND	ND
SITE5	25.58076	-80.40373	ND	5.68	ND	ND	ND
SITE6	25.57495	-80.39704	ND	3.87	ND	ND	ND
SITE7	25.56593	-80.36118	ND	5.12	ND	ND	ND
SITE14	25.54848	-80.34734	8.37	15.6	ND	ND	ND
SITE8	25.54673	-80.34586	5.71	BDL	ND	ND	ND
SITE9	25.54305	-80.33033	ND	2.16	ND	ND	ND
SITE13	25.54139	-80.32887	BDL	13.6	ND	ND	ND
SITE10	25.53873	-80.32702	6.42	23.4	ND	ND	ND
SITE11	25.53562	-80.32550	BDL	BDL	ND	ND	6.39
SITE12	25.55922	-80.35952	BDL	BDL	ND	ND	18.1

Table 21. Pharmaceutical Data for C1 Canal in ng/L concentration on 12/08/04 (ND=Not Detected, BDL=Below Detection Limit, N/A=Not Available)

12/08/04	N Latitude	W Longitude	Caffeine	DEET	4n- Nonylphenol	Bisphenol A	Triclosan
SITE1	25.64036	-80.22751	ND	4.05	ND	ND	BDL
SITE2	25.62593	-80.42697	ND	8.63	ND	ND	ND
SITE3	25.61877	-80.42154	ND	3.75	ND	ND	ND
SITE4	25.59668	-80.41283	ND	9.42	ND	ND	4.56
SITE5	25.58076	-80.40373	ND	16.6	ND	ND	3.69
SITE6	25.57495	-80.39704	23.3	17.8	ND	ND	3.79
SITE7	25.56593	-80.36118	3.03	27.3	ND	ND	BDL
SITE14	25.54848	-80.34734	ND	7.04	ND	ND	ND
SITE8	25.54673	-80.34586	3.94	26.6	ND	ND	2.61
SITE13	25.54305	-80.33033	2.95	6.53	ND	ND	ND
SITE9	25.54139	-80.32887	BDL	6.99	ND	ND	ND
SITE10	25.53873	-80.32702	BDL	5.80	ND	ND	4.26
SITE11	25.53562	-80.32550	12.4	9.31	ND	ND	6.51
SITE12	25.55922	-80.35952	BDL	8.21	ND	ND	ND

Table 22. Pharmaceutical Data for C1 Canal in ng/L concentration on 01/12/05 (ND=Not Detected, BDL=Below Detection Limit, N/A=Not Available)

01/12/05	N Latitude	W Longitude	Caffeine	DEET	4n- nonylphenol	Bisphenol A	Triclosan
SITE1	25.64036	-80.22751	ND	1.74	ND	ND	ND
SITE2	25.62593	-80.42697	ND	1.75	ND	ND	ND
SITE3	25.61877	-80.42154	ND	4.46	ND	ND	ND
SITE4	25.59668	-80.41283	N/A	N/A	N/A	N/A	N/A
SITE5	25.58076	-80.40373	ND	5.03	ND	ND	ND
SITE6	25.57495	-80.39704	ND	2.14	ND	ND	ND
SITE7	25.56593	-80.36118	ND	8.72	ND	ND	ND
SITE14	25.54848	-80.34734	ND	9.78	ND	28.2	ND
SITE8	25.54673	-80.34586	1.88	24.8	ND	ND	13.1
SITE9	25.54305	-80.33033	ND	13.7	ND	ND	6.26
SITE13	25.54139	-80.32887	N/A	N/A	N/A	N/A	N/A
SITE10	25.53873	-80.32702	ND	36.2	ND	67.4	12.5
SITE11	25.53562	-80.32550	3.64	28.8	ND	120	16.3
SITE12	25.55922	-80.35952	13.8	35.0	ND	60.4	6.99

Table 23. Multivariate correlation matrix for pharmaceuticals, personal care products, hormones and steroids in C1 canal.

	Site	Date	Caffeine	DEET	Bisphenol A	Triclosan	Estrone	Coprosta nol	Choleste rol
Site		0.02	0.09	0.35	0.29	0.16	0.28	0.14	0.21
Date	0.02		0.08	0.37	0.36	0.19	-0.19	0.42	-0.85
Caffeine	0.09	0.08		0.30	0.11	0.27	0.07	-0.05	-0.15
DEET	0.35	0.37	0.30		0.60	0.29	0.30	0.22	-0.26
Bisphenol A	0.29	0.36	0.11	0.60		0.49	0.17	0.14	-0.13
Triclosan	0.16	0.19	0.27	0.29	0.49		0.22	0.17	-0.01
Estrone	0.28	-0.19	0.07	0.30	0.17	0.22		0.15	0.12
Coprostanol	0.14	0.42	-0.05	0.22	0.14	0.17	0.15		-0.29
Cholesterol	0.21	-0.85	-0.15	-0.26	-0.13	-0.01	0.12	-0.29	

In summary, concentrations of pharmaceuticals, personal care products, hormones and steroids were very low in C1 canal and more frequent downstream from the landfill. In January 2005, Bisphenol A showed high concentrations in samples downstream from the landfill. The agricultural and residential area of the canal had either no detection or extremely low concentrations of all compounds, indicating that contamination of the

canal could originate from the landfill, the wastewater treatment plant located in the vicinity or the marinas but not from the agricultural area nor the newly developed residential area.

3.6. West Coast of Florida

Sampling on nine marinas was done in the west coast of Florida in January 2005. Results for the twenty-eight samples are reported on Table 25 and are plotted as a vertical box plot in Figure 54. Concentrations of caffeine were low, in the range of below detection limit to 13.8 ppt, with only two samples with concentrations of 34.0 and 42.3 ppt. DEET concentrations were also low, from below detection limit to 13.6 ppt. Only five samples had triclosan at very low concentrations (3.70-7.79 ppt). Bisphenol A concentration ranged from not detected to 390 ppt.

Figure 53 is a map of the west coast of Florida with the concentration distribution of pharmaceuticals and personal care products. Palm Island Marina (PIM) showed elevated levels for all pharmaceuticals and personal care products. Bisphenol A had concentrations of 309, 156, and 103 ppt on stations PIM-01, PIM-02, and PIM-03 respectively. PIM-01 is the most remote site inside the marina and PIM-03 is located at the entrance of the marina. Concentrations of all pharmaceuticals and personal care products are higher in PIM-01, and significantly lower in PIM-03. Except for Liberty Yacht Club (LYC), all marinas had higher concentrations of pharmaceuticals and personal care products at the sites inside the marinas than at the connections with open bay waters.

Figure 47. Concentration of pharmaceuticals and personal care products along C1 canal on October 2004

Figure 48. Concentration of caffeine, hormones and steroids along C1 canal on October 2004

Figure 49. Concentration of pharmaceuticals and personal care products along C1 canal on December 2004

Figure 50. Concentration of caffeine, hormones and steroids along C1 canal on December 2004

Figure 51. Concentration of pharmaceuticals and personal care products along C1 canal on January 2005

Figure 52. Concentration of caffeine, hormones and steroids along C1 canal on January 2005

Figure 53. West coast map with distribution of concentrations in ng/l of pharmaceuticals and personal care products. C=Caffeine, D=DEET, B=Bisphenol A, and T=Triclosan.

Table 24 show the multivariate correlation matrix for pharmaceuticals and personal care products at the west coast. The site number is indicative of concentrations according to location inside the marinas. Caffeine shows positive correlation with DEET, Bisphenol A and Triclosan (0.59, 0.75 and 0.53 respectively), indicating that as caffeine concentration increases, the other compounds tend to increase too. The highest correlation was observed between DEET and Bisphenol A (0.86). The negative correlation of site number and the compounds indicate that concentrations of compounds tend to decrease as samples move from inside the marinas to the entrance of the marina.

Table 24. Multivariate correlation matrix for pharmaceuticals and personal care products in the west coast of Florida.

	Caffeine	DEET	Bisphenol A	Triclosan	Site #
Caffeine		0.59	0.75	0.53	-0.19
DEET	0.59		0.86	0.41	-0.37
Bisphenol A	0.75	0.86		0.53	-0.25
Triclosan	0.53	0.41	0.53		-0.37
Site #	-0.19	-0.37	-0.25	-0.37	

Table 25. Concentration of pharmaceuticals in West coast of Florida January 2005 in ng/L. ND=Not Detected, BLD=below detection limit

SITE	N (Latitude)	W (Longitude)	Caffeine	DEET	4n- nonylphenol	Bisphenol A	Triclosan
FMYB-01	26.64689	-81.86981	8.38	3.14	ND	ND	4.18
FMYB-02	26.64683	-81.87068	3.81	BDL	ND	25.8	ND
FMYB-03	26.64905	-81.87190	3.93	3.32	ND	22.2	ND
LYC-01	26.53188	-81.93984	BDL	BDL	ND	8.63	7.79
LYC-02	26.53362	-81.93859	BDL	2.22	ND	16.8	ND
LYC-03	26.53605	-81.93790	2.96	BDL	ND	ND	ND
PPM-01	26.52648	-81.95407	4.24	2.47	ND	13.8	ND
PPM-02	26.55255	-81.95590	BDL	BDL	ND	ND	ND
TPM-01	26.53854	-82.00043	5.67	BDL	ND	ND	ND
TPM-02	26.53911	-81.99946	3.27	BDL	ND	14.8	ND
GM-01	26.83539	-82.26083	BDL	4.81	ND	11.4	ND
GM-02	26.83475	-82.26023	BDL	3.71	ND	ND	ND
GM-03	26.83396	-82.25918	5.08	4.25	ND	27.3	ND
GM-04	26.83357	-82.26093	BDL	2.94	ND	ND	ND
PIM-01	26.86960	-82.31039	34.0	13.6	ND	390	6.19
PIM-02	26.87051	-82.31049	42.3	3.96	ND	156	5.40
PIM-03	26.87111	-82.31222	6.11	2.90	ND	103	ND
CHMB-01	26.87525	-82.31286	5.98	3.10	ND	84.1	ND
CHMB-02	26.87510	-82.31407	5.10	BDL	ND	27.9	3.70
CHMB-03	26.87442	-82.31407	6.57	BDL	ND	ND	ND
MJ-01	27.33381	-82.54597	13.8	6.45	ND	44.5	ND
MJ-02	27.33438	-82.54773	4.43	BDL	ND	ND	ND
MJ-03	27.33232	-82.54454	7.44	BDL	ND	ND	ND
MJ-04	27.33246	-82.54633	7.08	BDL	ND	ND	ND
LKM-01	27.36878	-82.61869	11.4	2.87	ND	ND	ND
LKM-02	27.37017	-82.61822	12.9	BDL	ND	ND	ND
LKM-03	27.37177	-82.61085	12.0	BDL	ND	ND	ND
LKM-04	27.37298	-82.61716	5.54	BDL	ND	ND	ND

Figure 54. Occurrence of pharmaceuticals and personal care products in the west coast of Florida on January 2005

Figure 55 is a scatter line plot of the concentrations of the pharmaceuticals and personal care products at each site. Bisphenol A was plotted in another axis to visualize the lower concentrations better.

Occurrence of Pharmaceuticals in the South West Coast of Florida

Figure 55. Concentration of pharmaceuticals and personal care products in different marinas of west coast of Florida on January 2005

CHAPTER 4

CONCLUSIONS

The result of this study produced a snapshot view of the occurrence of pharmaceuticals and personal care products in South Florida. The purpose of this study was to find an ideal indicator for waste water, to provide background information on the general status of South Florida coastal environments with respect to contaminants and to evaluate which areas of the region are most likely impacted by anthropogenic activities. Pharmaceuticals and Personal Care Products were detected in coastal environments of Florida as well as in the Miami River.

The analytical methods discussed were successfully used for the determination of pharmaceuticals in aqueous matrices. Surface waters from south Florida were extracted and analyzed for several pharmaceuticals and personal care products in order to establish occurrence and fate of these compounds. Many of the investigated compounds were detected in surface water from the coast of Florida as well as in a river in Miami (Miami River). Therefore, the applied methods, which included GC/MS and LC/MS, were appropriate for the determination of pharmaceuticals and personal care products in aqueous environmental samples down to the lower parts-per-trillion (ng/L) level.

Based on the vast collection of data, it was determined that caffeine concentrations in the range of BDL to 15 ng/L are not environmentally significant. Any concentration in the range of 15 to 40 ng/L may be environmentally significant and the concentrations higher than 40 ng/L reflect impacted waters. Since caffeine is not toxic to organisms, the criteria established here does not imply risk but provides a trigger for further action.

Caffeine was detected in Little Venice in concentrations ranging from 4 to 203 ppt. Locations in Little Venice did not show any particular trend in caffeine concentrations and nor any apparent temporal trend was observed among the different months of the year. Occurrence of caffeine was widespread and more frequent in canals and enclosed waterways than in open bay waters. Monthly correlation with nutrients and bacterial counts did not show any particular trend. These results indicate the complexity of water circulation in the canal system. DEET was found in Little Venice in concentrations ranging from BDL to 128.1 ppt. Triclosan and Bisphenol A were present in concentrations ranging from ND-84.4 ppt and ND-82.4 ppt respectively. 4n-Nonylphenol was not detected in the period sampled in Little Venice. An attempt to correlate rainfall with caffeine concentrations was made. No statistical correlation between rainfall and caffeine concentration was observed in Little Venice, but with the exception of a few outlier points in the monthly concentration of caffeine, caffeine concentrations tend to be higher during the wet season (July to October) and lower during the dry season (November to March).

Key Largo Harbor's 2003 data indicated that caffeine was present in the samples. Samples collected in Key Largo Harbor on February 2004 were analyzed for caffeine, DEET and Triclosan. Caffeine was the most frequently detected compound, with concentrations ranging from below detection limit to 104 ppt. Only four (7%) samples were above 40 ppt. These results are very different from the previous year, where 63% of the samples were above 40 ppt. DEET was detected in 35 out of 56 (62%) samples collected and triclosan was detected in 16 out of 56 (29%) samples. In general, and in accordance with the general water circulation trends in Key Largo Harbor,

pharmaceuticals, personal care products, hormones and steroids were higher inside the canal system than outside. No particular trend was observed among caffeine and the pharmaceuticals. With the exception of one site (KLH07), caffeine, hormones, and steroids showed similar patterns in concentrations along the canals in Key Largo Harbor.

Samples collected during the Underwater Music Festival in 2003 and 2004 had concentrations of caffeine in the range from below detection limit to 38.5 ng/L and 30.6 ng/L respectively. Concentrations of caffeine were higher the day before the event, decreased during the event, and increased again after the event for both years sampled. Concentrations were higher at the reef than inside the canal system with the private marinas. DEET concentrations ranged from BDL-16.8 ng/L and Triclosan, Bisphenol A and 4n-Nonylphenol were not detected. Comparable to caffeine trends, estrone, cholesterol, and estrogen equivalence (YES) concentrations were higher the day before the UMF and decreased while the event was taking place. Even though estrogen equivalence had a weak statistical correlation with caffeine (0.28), it followed similar trends before, during and after the underwater music festival as caffeine and could be used as an alternative tracer measurement of torrential biological responses.

Caffeine concentrations in Miami River ranged from 18.4 to 47.2 ppt. DEET concentrations ranged from below detection limit to 24.1 ppt and Bisphenol A concentration were in the range of not detected to 78.9 ppt. Triclosan and 4n-Nonylphenol were not detected. Pharmaceuticals and personal care product concentrations did not follow any particular trends with the distance to the bay but they were higher inside the river than in open waters. The hormones and steroids followed similar trends along the river but no correlations were observed with the caffeine data.

Stations along the Coconut Grove Marina showed low concentrations of caffeine and DEET, ranging from 4.61-10.6 ng/L and 3.27-8.19 ng/L respectively. These concentrations were very low compared to the Miami River concentrations but well within the expected levels for open-bay water samples.

Concentrations of caffeine in C1 canal ranged from not detected to 24.9 ppt. Caffeine was not detected in the agricultural or the residential area of the canal. Detection of caffeine increased in frequency and concentration as sampling proceeded near the landfill and the wastewater treatment plant. These results indicate that sources of caffeine in C1 Canal may come from the landfill, the wastewater treatment plant in the area and or the Black Point Marina, but not from the agricultural or the newly developed residential areas. DEET was detected in the canal in concentrations ranging from below detection limit to 28.9 ppt. Hormones and steroids did not show any particular correlations with caffeine, but they tend to increase in concentration downstream from the landfill too. In summary, concentrations of pharmaceuticals, personal care products, hormones and steroids were very low in C1 canal and more frequently detected downstream from the landfill and the water treatment plant.

Concentrations of caffeine in the west coast of Florida were low, in the range of below detection limit to 13.8 ppt with only two samples with concentrations of 34.0 and 42.3 ppt. DEET concentrations were also low, from below detection limit to 13.6 ppt. Only five samples had triclosan at very low concentrations (3.70-7.79 ppt). Palm Island Marina (PIM) showed elevated levels of all pharmaceuticals and personal care products, with the highest concentration of Bisphenol A of 390 ppt detected in south Florida. Except for Liberty Yacht Club (LYC), all nine marinas had higher concentrations of

pharmaceuticals and personal care products at the sites inside the marinas than at the connections with the open bay waters.

CHAPTER 5

FUTURE WORK

Many pharmaceuticals and personal care products undergo incomplete biological degradation. Therefore, these compounds are only eliminated to some extent when passing through water treatment plants or septic tanks and end up in receiving waters. Source control and distribution seems to be the most permanent, economical solution for most compounds (Ternes et al. 2004).

Ternes et al. (2004) proposed four measures to decrease pharmaceuticals in the environment. Separate treatment of hospital wastewater and adding information about the environmental impacts of a pharmaceutical to its packaging could significantly reduce their presence in the environment. The disposal of pharmaceuticals and personal care products should be controlled and supervised. For example, as general practice, expired or surplus products should be collected and incinerated or perhaps be reused under controlled conditions. Pharmaceuticals are excreted to a great extent in urine. Separation and exclusive treatment of urine can drastically reduce the loading of the wastewater and can allow recycling of the nutrients.

Nevertheless, these measures require political decisions, approval from the general population, money for new infrastructure, and decades for their implementation. Therefore, in the short term, a load reduction is easier to achieve within the wastewater treatment system (Ternes et al, 2004; Larsen et al., 2004). For now, The Little Venice Service area will receive a low-pressure, vacuum wastewater collection system that will transfer wastewater to a central treatment plant. The treatment plant will produce effluent that meets or exceeds the current advanced wastewater treatment standards of 5:5:3:1

(BOD5:TSS:TN:TP) and will use Class V injection well for disposal of treated wastewater. Central collection and treatment of wastewater will remove a substantial portion of nutrient loading into the canals by removing the current sources of wastewater, which include malfunctioning septic tanks and cesspits (Boyer et al, 2004).

Water samples in Florida should be monitored for pharmaceuticals and personal care products as they are collected and treated. In particular samples need to be collected at Little Venice, to monitor for signs of improvement or changes provided by the completion of water quality improvement project. Other locations of Florida should also be sampled to monitor for signs of persistent deteriorations of bodies of waters do to infiltration of wastewater as urban development and new encroachment are established.

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APPENDICES

APPENDIX I. Chemical Structure of Pharmaceuticals and Personal Care Products

Caffeine

Triclosan

Atrazine d5

TCMX

DEET

4n-nonylphenol

Bisphenol
A

APPENDIX II. Chromatogram of an Actual Sample and Mass Spectral Confirmation of Pharmaceuticals and Personal Care Products

Figure A1. Total Ion Chromatogram of an actual sample.

Figure A2. GC-MS Spectra of Caffeine

Figure A2. GC-MS Spectra of DEET

Figure A3. GC-MS Spectra of 4n-nonylphenol

Figure A4. GC-MS Spectra of Bisphenol A

Figure A5. GC-MS Spectra of Triclosan

Figure A6. GC-MS Spectra of TCMX

Figure A7. GC-MS Spectra of Atrazine D5