Sampling and Analysis of Pharmaceutical Contaminants in Lake Pontchartrain and the Mississippi River

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Background

Pharmaceutical contaminants are known to enter the water supply through sewage treatment plants (STP). Other pathways of pharmaceutical contaminants into the environment include agricultural runoff of veterinary drugs and pharmaceutical manufacturing facilities. These contaminants are chemically similar to certain types of pesticides and were first discovered when chemists in Germany and Switzerland stumbled upon clofibric acid in rivers and lakes while analyzing for herbicides in the early 1990s (Raloff, 1998). The clofibric acid resembles a common herbicide called mecoprop, which environmental chemists were screening for at the time. Clofibric acid is a metabolite of clofibrate, a widely used cholesterol-lowering drug. Back in the late 1970s, clofibric acid had also been discovered in the waters near a pharmaceuticals manufacturing facility as well as in a municipal STP in Kansas City, MO (Stan and Heberer). Scientists then began looking for pharmaceutical compounds in water bodies throughout Europe and have discovered many different types of drugs thus far (Ternes, 1998). Pharmaceutical contaminants have also been detected in surface and ground water sources in Denmark, Israel, Brazil, and Canada. United States scientists have been slow to jump on the bandwagon; however, they are slowly beginning studies on the pharmaceuticals in the water supply (AWWARF RFP, 1999).

Most pharmaceutical contaminants are not very water soluble which means that they would be more difficult to treat than other contaminants. The human body is about 70% water, so drugs are formulated to not dissolve easily into the human body. Up to 50-60% of pharmaceuticals are known to be excreted from the human body, which could include the original compound as well as several metabolites of the drug (Raloff, 1998). Concerns have been arising about the pharmaceutical contaminant's effects on small organisms and bacteria in the environment. Additionally, pharmaceuticals are known to be dispensed on the same order of magnitude as pesticides, which are continuously debated and researched in environmental circles.

Locally, the occurrence of pharmaceutical contaminants in our source and treated water supplies in the lower Mississippi River and metropolitan New Orleans area is not well understood. As such, research needs to be conducted to investigate the occurrence of pharmaceutical contaminants in our area, to determine if these contaminants pose environmental and human health risks, and to determine the effectiveness of treatment technologies for low level (ng/L) pharmaceutical contaminants in our drinking water supplies.

Objectives

The objective of this research is to investigate the occurrence of a limited number of pharmaceutical compounds in raw water sources and potable water in the lower Mississippi River and the New Orleans metropolitan area. The three selected pharmaceuticals for this study

are clofibric acid, estrogen, and naproxen. We hope to characterize a trend over time in the different contaminant concentrations at various locations throughout the New Orleans metropolitan area. This data is paramount to an analysis of the pathways that pharmaceutical contaminants are able to enter the environment. Data and information will be used to define and prioritize future research at Tulane on the occurrence, potential public health impacts, environmental impacts, and treatment of pharmaceutical compounds in drinking water.

Materials and Methods

Contaminants

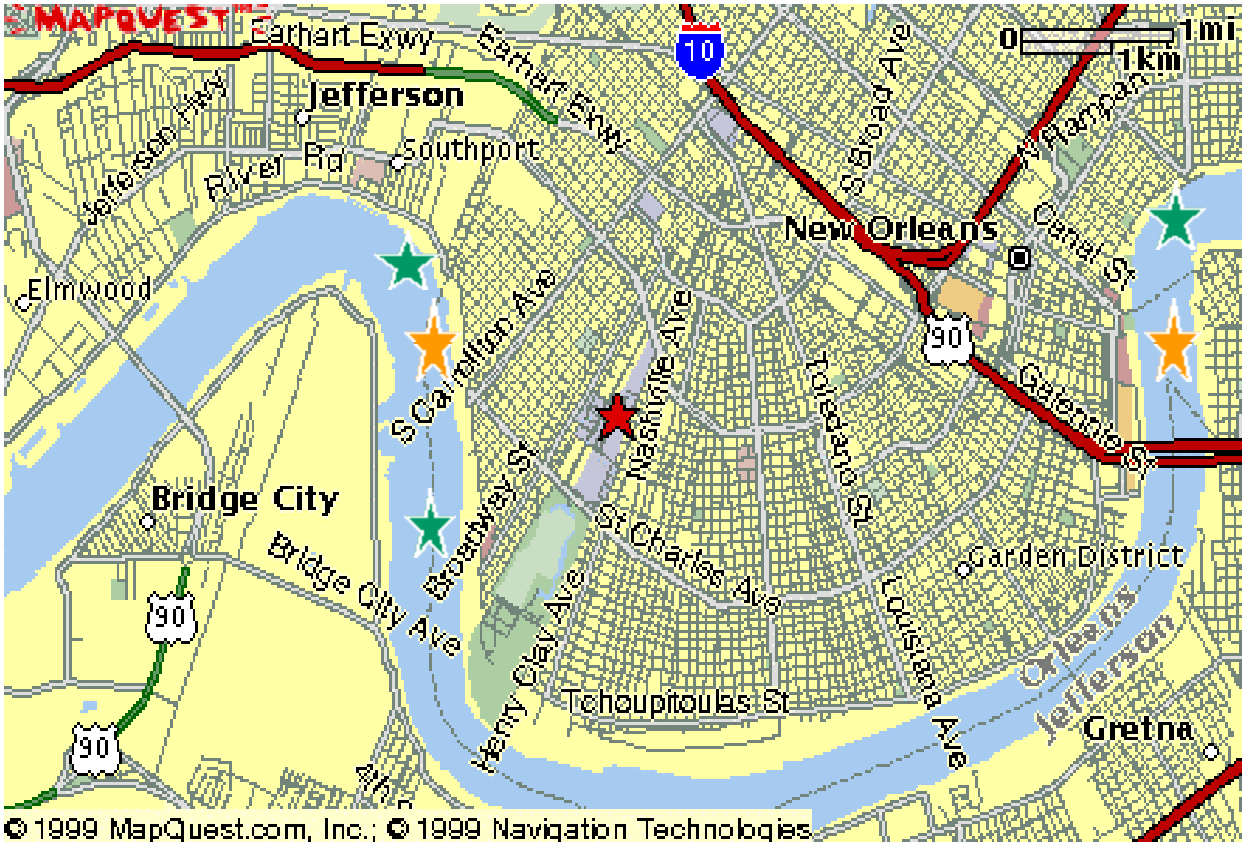
Three pharmaceuticals have been chosen for sampling in the New Orleans area: clofibric acid, estrogen, and naproxen. Each chemical to be analyzed is from a different class of compounds: clofibric acid is a lipid regulator, estrogen is a hormone, and naproxen is an antiphlogistic. Therefore, in choosing these three pharmaceuticals for our study, we are attempting to mirror the broad range of chemicals possible in the water bodies. Clofibric acid was mainly chosen because it has been the most widely studied pharmaceutical compound to date (Ternes, 1998; Buser and Muller, 1998; Hignite and Azarnoff, 1977; Sorenson *et al.*, e1998; Heberer *et al.*, 1997; Stumpf *et al.*, 1999) , thereby giving us information that other researchers have previously established to go by. Our findings can then be "checked" against that of other researchers. Estrogen is a hormone that is widely found throughout water bodies and is a known endocrine disrupter (Shore *et al.*, 1993; Cheek *et al.*, 1998; Ternes *et al.*, 1999). Therefore, there are biological functions that estrogen is known to disturb, which makes estrogen a possibility for ecotoxicological effects. Finally naproxen was chosen to round out the survey because it is compatible with the previous two choices' analyzation procedure (Grimm, 1999). In addition, naproxen is one of the top 200 most prescribed drugs in the United States and the most prescribed antiphlogistic (Rx List—The Top 200). The antiphologistic class of compounds—anti-inflammatory and fever-reducing--are very commonly used, so a representative from this class of drugs is useful in this type of research. Depending upon its degradation in the water bodies, naproxen has a very high chance of being detected in the lower Mississippi River. By assessing the occurrence of these three drugs, a lot of information can be obtained about the area's pharmaceutical contaminants in the water supply.

Sampling Program

In order to determine the occurrence of pharmaceutical compounds in the water bodies surrounding New Orleans, a wide variety of sampling sites were necessary. One of the most commonly analyzed areas for pharmaceuticals is upstream and downstream of the sewage treatment plants (STP), since STPs are usually viewed as a point source of pharmaceutical contamination (Ternes, 1998). Sampling upstream and downstream of the STP discharge into the Mississippi River would enable us to determine the extent of the contamination from that source. Orleans Parish operates two STP, one on the east bank (120 MGD) and the other on the west bank (5 MGD). Three sampling sites in and around the effluents are proposed: one upstream of the east bank effluent, one downstream of the east bank effluent but upstream of the west bank effluent, and the third downstream of the west bank effluent. To assess the ability of the water treatment plant a fourth sample would be taken from tap water in the laboratory. A new sampling site was recently added at the New Orleans Sewage and Water Board Water Treatment Plant. With this site, we can assess the fate of the contaminants between the WTP and

the tap. Figure 1, below, should be referenced to better comprehend the actual samlping sites in Orleans and Jefferson Parish. The smaller stars represent sampling sites, while the larger sites represent either WTP influent or WWTP effluent. Through sampling the Mississippi River, our aim was to gauge the fate of pharmaceuticals in the environment from human sources.

Figure 1--Mississippi River Sampling Sites



The second type of pharmaceutical contamination is through veterinary drugs. Additional sampling sites in Lake Pontchartrain and its tributaries are also proposed. The north end of the lake is known for its agricultural run off. Livestock that may use veterinary pharmaceuticals would secrete them onto the land and that would be run off with the pesticides and herbicides. Since two of the three drugs investigated, estrogen and naproxen, are also used in veterinary medicine, these sites would serve the purpose of giving us an idea how much pharmaceutical contamination is a result of agriculture. One sample each from the two main tributaries of Lake Pontchartrain, the Tangipahoa and Tchefuncta Rivers, were taken near their discharge into the lake. Both rivers bring the large majority of agricultural runoff into Lake Pontchartrain each year. Two more samples would be taken from Lake Pontchartrain, one on the north shore and one on the south shore, to compare to the other samples taken from the agricultural runoff area. See Figure 2, below, for the map representation of these sites. From the Lake Pontchartrain sites, we hope to be able to assess the pathways into the environment for these chemicals as well as to do a preliminary analysis of their fate in the environment.

Figure 2--Lake Pontchartrain Sampling Sites

So far, each site has been sampled every two weeks for fourteen weeks. The study may continue through the summer. If after the three month period a lot of fluctuation is still being been seen at any of the sampling sites (possibly due to change in season, rainfall, etc.), it would be recommended that the sampling period be extended for an additional six months.

One liter samples were collected in amber glass bottles and stored in a cooler filled with ice while in the field. Each sample was then transferred to the refrigerator and stored at 4 degrees Celsius up to seven days or until laboratory extraction was performed.

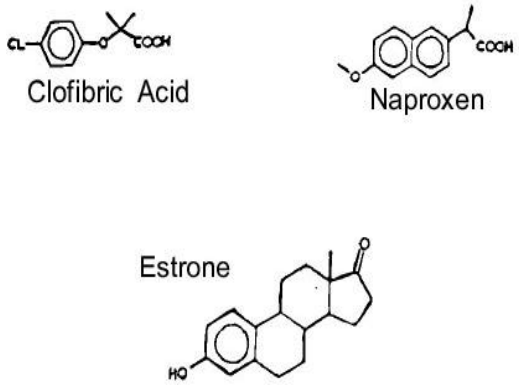
Analytical Methods

Descriptions of sampling and analytical procedures by different researchers were compared to our overall sampling objectives to see which procedures were appropriate for our intended New Orleans area research. The other resource used was a research scientist at the Coordinated Instrumentation Facility (CIF), Dr. Grimm. Dr. Grimm suggested mirroring the procedure described by Buser and Muller (1998) in their research looking for clofibric acid in a large water body. The basic procedure to detect the pharmaceutical compounds consists of using the gas chromatography-mass spectrometer (GC/MS) technology. However, in order to detect the contaminants using the GC/MS, it is necessary to first extract the clofibric acid, estrogen, and naproxen from the impure water sample to prepare the sample for the GC/MS. The extraction was performed using solid phase extraction (SPE).

Hydrophobic adsorbent bio-beads (SM-2 Bio-Beads, Bio-Rad) were saturated with methanol and packed into 1 inch diameter glass columns. The methanol was then allowed to drain through the columns, leaving the beads in place. Glass wool was placed on the top and bottom of the beads in order to keep them in place. Each column was conditioned by rinsing with successive washings of methanol, dichloromethane, and distilled water. Columns were reused and reconditioned for multiple sample runs, depending on the column. Tests were conducted with column rinses to assure that all contaminants were removed from the column through the solvent eluting process.

Before laboratory extraction, each sample was acidified to a pH of 2.0. The samples were then poured through the columns and allowed to fall at a rate of 15 mL/min or less. The water collected at the bottom is wasted and methanol and dichloromethane are poured through the column to elute the organics from the beads. The solvent mixture is collected in small vials at the bottom of the column. Finally, the methanol layer is removed from the solvent mixture and the remaining dichloromethane is evaporated with nitrogen gas down to 0.1 mL. These steps are based on the organic formula of each compound, Figure 3 below.

Figure 3--Organic Contaminant Formulas



In order to promote greater recovery efficiencies for the pharmaceutical contaminants, each sample is methylated with BSTFA. Clofibric acid was found to degrade into a degradation product at the temperature naproxen needs to volatilize. This was a problem with the GC/MS run because only one compound would be able to have high recoveries. Methylating the compounds makes the naproxen more volatile, which allows all compounds to be analyzed in one run. Additionally, the BSTFA was found to solidify when reacting with water. Therefore, an additional analytical step needed to be added to avoid these circumstances. Sodium sulfate is added to the extracted samples before the solvents are evaporated to ensure no remaining water. The samples are then placed in 2 mL vials for analysis and adjusted to 1 mL of the sample. The samples are now ready to run through the GC/MS. For the specific operating procedure for this process, see appendix.

Quality Assurance and Quality Control

In order to ensure the quality of the results, several steps are taken. First, one duplicate sample is taken from at least one site each sampling trip. The contaminant concentrations can then be compared between the two samples for the precision of the study. Additionally, a third sample is also taken from the same site that is spiked with a concentration of each contaminant prior to

extraction. This matrix spike allows us to assess the contaminant extraction efficiency by comparing the final analytical concentration of the spike and the actual sample. An internal standard, 2,3,5-trimethylnapthalene, is used to determine the efficiency of the GC/MS analysis. External GC/MS calibration standards (10 ppm to 0.01 ppm) were also prepared to plot calibration curves for each contaminant on the instrument. These QA/QC steps are necessary to guarantee the adequacy of the study.

Results and Discussion

Table 1, below, is a summary of the maximum concentrations detected at each site from the first set of samples. As previously mentioned, there were some analytical problems with the first set of samples, which are given as not detected (ND) in the appendix. We believe these are a direct result of the analytical problems. The second set of sample analysis proved more effective, but there are still some details that need to be worked on. Also, this table doesn't take into account the recovery efficiency of the solid phase extraction. This is still being calculated and will be made available at a later date. Therefore, the concentrations of each contaminant listed below is less than what we believe is actually in the water bodies throughout the New Orleans Metropolitan area.

Table 1—Preliminary Sample Results

Site	Naproxen (ng/L)	Clofibric Acid (ng/L)	Estrone (ng/L)
River Road & Harvey Ave.	10	20	20
USACE	30	110	150
French Quarter	90	60	30
New Orleans Tap Water	150	70	80
Lake Pont. South Shore	0	20	40
Tchefuncta River	0	20	10
Tangipahoa River	70	50	60
Pass Manchac	40	20	80

Low level concentrations (ng/L) were found at all sites. Estrogen was more widely found than clofibric acid and naproxen, with naproxen the less concentrated contaminant. Reasons for these results could be their environmental fate characteristics. Additionally, the calibration curve for naproxen was found to be somewhat higher than we had wanted because it was not as easily detected by the GC/MS. The one interesting thing to note is that the tap water concentrations were not significantly different from any of the surface water sites and in some cases actually higher than that found at sites. Preliminarily, we can infer that the pharmaceutical contaminants are not removed through the water treatment process. Studies are now undergoing considering the effects of different water treatment processes on clofibric acid.

Each compound labeled N.D.* are those that were solidified when they were methylated. The reason we believe these results are so skewed is because the internal standard, 2,3,5-trimethylnapthalene, was barely detected in these samples also. Therefore, we have no idea whether the compounds occur in those samples, but can only infer that they are present from later sample runs. A printout of the raw data can be found in the appendix. The sites where the

internal standard was significantly low are marked with a star and are assumed to be faulty due to analytical reasons.

Conclusions

At this point, it is too early to make any solid conclusions about this study. So far, it can be stated that clofibric acid, estrogen, and naproxen are not eliminated through conventional water treatment plants. No sites had true non-detects for any contaminant, but naproxen was detected at fewer sites than clofibric acid and estrone, which were detected at all sites. Analytical procedures need to be improved and sampling continued to get more solid results on the presence of naproxen, clofibric acid, and estrone in the water supply. Much more can be done on this project to ensure better results.

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New Orleans Sewage and Water Board: www.swbnola.org

RxList – The Top 200: www.rxlist.com/top200.htm