Time Trends of Pharmaceuticals in Wastewater Treatment Plant Effluent with Sources from Pharmaceutical Manufacturing Facilities and Hospitals

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ABSTRACT

Time trends of pharmaceutical concentrations in wastewater treatment plant (WWTP) effluent are currently not well understood, although recent research has generally focused on assessing diurnal pharmaceutical variability. The research presented in this paper makes use of pharmaceutical effluent concentration data collected at four sites in New York state; for three of these sites (NY1, NY2, and NY3), data is sufficient to assess time trends on a seasonal and annual basis. Data from the fourth site (NY9) are sufficient to assess time trends on a diurnal basis. The first plant, NY1, acted as a control for the study with influent only from residential use. The next sites, NY2 and NY3, in addition to receiving residential influent, also receive influent from pharmaceutical manufacturing facilities (PMF). These first three sites have multi-year data sets allowing yearly and seasonal trends to be performed. The last site, NY9 receives influent from a hospital and residential area. All four of these WWTP are small, with flows generally less than 2 million gallons per day (mgd).

Eight compounds were considered in this study: butalbital, diazepam, metaxalone, methadone, oxycodone, phendimetrizine, carisoprodol, and caffeine. The yearly trends ranged over a time span of 8 years in which the effects of plant upgrades and a PMF shutdown were clearly observed. The effect of a plant upgrade at NY1 was seen in the decreasing concentrations of caffeine. Concentrations of diazepam and carisoprodol at NY 2 displayed gradual decreasing trends while butalbital and oxycodone displayed sudden drops in concentration observed in the graphs. These observed concentration decreases are most likely due PMF phase out of different compounds at different speeds as it prepares for closure. Statistical analyses of seasonal trends are not statistically significant for these compounds, except for oxycodone and phendimetrizine which display higher concentrations in the winter and spring. Although it was predicted that the influence of the hospital at NY9 would show diurnal trends, these samples did not show a significant difference in concentration throughout the day. This study concludes that for WWTPs receiving PMF discharges, assessment of pharmaceutical concentrations requires the collection of many samples, multiple times per year, in order to characterize the variability in production at the PMF.

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INTRODUCTION

Emerging contaminants in aquatic environments have recently been an area of growing concern worldwide. Emerging contaminants are chemicals that are not yet well understood by scientists concerning their frequency of occurrence, sources, and risks to ecological and human health (EPA 2013). A recently discovered class of emerging contaminants is organic wastewater compounds which consist of personal care products, household chemicals, and pharmaceuticals. These contaminants enter the environment and waterways from underground septic tanks (Schaider et al. 2014), storm water runoff (Daughton and Ternes 1999), and wastewater treatment plant (WWTP) effluent (Alidina et al. 2014). Many of these organic wastewater compounds exhibit endocrine disrupting properties. This is very concerning for the organisms in streams and waterways that receive effluent from WWTPs. It is believed that these compounds can slowly accumulate in aquatic organisms living in the same environment with multigenerational exposure (Daughton and Ternes 1999). "The possibility for continual but undetectable or unnoticed effects on aquatic organisms is particularly worrisome because effects could accumulate so slowly that major change goes undetected until the cumulative level of these effects finally cascades to irreversible change--change that would otherwise be attributed to natural adaptation or ecologic succession" (Daughton and Ternes 1999). It was also discovered that the presence of antibiotics can have potential effects on microbial communities, changing their composition and sensitivity (Schaider et al. 2014). If these organic wastewater compounds have an effect on aquatic organisms and microbial communities they may also cause changes in humans who have been drinking water with these contaminants. Many places are looking to reuse wastewater for human consumption as water becomes scarcer (Barringer 2012). Human health risks from low levels (parts per trillion or parts per billion) of organic wastewater compounds are a very controversial issue (Schaider et al. 2014). A study performed by Vanderberg et al. (2012) discovered that hormones can display greater effects at lower doses than higher doses when exposed to endocrine disrupting compounds. Another study showed that acetaminophen, a pharmaceutical used as a pain reliever, hinders testosterone production even at very low concentrations (Kristensen et al. 2010). These studies suggest that organic waste water compounds in the environment can raise aquatic and human health concerns. However, the environmental risks from many other compounds and pharmaceuticals are still unknown because

it is difficult to link contaminants to a certain health issue. More research is needed to fully understand organic wastewater fate, transport, and effect on organisms.

This paper will focus on a selection of organic wastewater compounds which are pharmaceuticals. Pharmaceuticals have been discovered by numerous studies to be entering streams and waterways from WWTP effluent (Alidina et al. 2014, Hedgespeth et al. 2012, Alexy et al. 2006). The majority of WWTPs receive influent from residential and commercial waste. It has been determined from many investigations that low levels of pharmaceuticals are being discharged into the environment from the effluent of these WWTPs (Ashton et al. 2004). Recently discovered sources of high pharmaceutical input to the environment are WWTPs that receive influent from pharmaceutical manufacturing facilities (PMF) (Phillips et al. 2010, Larrson et al. 2007) and hospitals (Emmanuel et al. 2005). WWTPs with these inputs are "worst case" scenarios and should be studied more closely. The effects of these pharmaceuticals in the environment are not yet fully understood by scientists, and the discharge of these pharmaceuticals by PMFs and WWTPs are not regulated. This is very concerning for human health in areas where wastewater is reused for drinking water and for aquatic environments receiving WWTP effluent. For now all we can do is monitor their concentrations under different scenarios and time scales.

PMFs were recently discovered to be releasing very high concentrations of pharmaceuticals in comparison to municipal plants serving only residential and other commercial sources (Phillips et al. 2010, Larrson et al. 2007). Studies performed by Phillips and others (2010) and Larrson and others (2007) found that even after PMF discharge undergoes treatment at a WWTP the effluent still contains concerning concentrations of pharmaceuticals. Only three known studies have been done looking at these manufacturing facilities as sources of pharmaceuticals to sewage treatment plants; one in India, one in Taiwan, and one in New York. These studies have found concentrations much higher than what has been found in other studies looking at normal WWTP effluent. In New York, Phillips and others (2010) found that concentrations were found to be as high as 3,800 micrograms per liter (µg/L) of metaxalone, a muscle relaxant. The studies performed by Phillips and others (2010) and Larrson and others (2007) have determined that WWTPs receiving PMF input have concentrations up to 1000 times higher than WWTPs that do not receive this input. Complete data concerning pharmaceuticals

formulated at most PMFs is unavailable to the public. The ability to identify all of the compounds that could potentially be in WWTP effluent is hindered by this lack of data (Phillips et al. 2010). Since it is unknown exactly what compounds these PMFs are discharging researchers only find the select compounds that they are looking for. This is concerning because there can be high concentrations of certain pharmaceuticals in effluent that have not been discovered yet. When studying pharmaceuticals in the environment, manufacturing facilities should be taken into account and studied in further detail.

Another source of pharmaceuticals to the environment are hospitals (Emmanuel et al. 2005, Herberer and Feldmann 2005). Emmanuel and others (2005) and Herberer and Feldmann (2005) found that hospitals can be a source that also discharges higher than normal concentrations into wastewater –positive hospital source studies go back to late 1990a or very early 2000's. Most antibiotics that patients take at hospitals are not fully metabolized by the body and end up being excreted and discharged to WWTPs (Alexy et al. 2006, Kummerer 2001). After undergoing treatment at a WWTP these antibiotics are still not fully degraded and make their way into the environment (Alexy et al. 2006). Hospital discharges are believed to display intraday variability due to certain times of procedures and appointments (Nelson et al. 2011).

When pharmaceuticals go through WWTPs they have variable removal rates. The removal of pharmaceuticals depends on the type of treatment at the plant, solid retention times, and capacity of the plant (Clara et al. 2005, Kasprzyk-Hordern et al. 2009, Sui et al. 2011). There are a few different mechanisms that WWTPs use to reduce contaminants. The activated sludge process is a biological process that uses organisms to reduce the organic content of the sewage. Another treatment method is the trickling filter which allows the sewage to flow downward over a layer of microbial biofilm. Processes used for tertiary treatment include sand filtration and microfiltration. The last step, disinfection, uses chlorination/dechlorination or ultraviolet light to kill microorganisms. One study, done by Phillips et al. (2008), found that the most efficient treatment process in removal efficiency of compounds is activated sludge. However, Stevens (2012) found that many organic waste water compounds are not sufficiently removed by WWTPs. It has also been determined that different compounds have different behaviors in WWTPs (Clara et al. 2005). Some compounds are almost completely removed, while others are not removed at all (Clara et al. 2005, Herberer and Feldmann 2005). It is also believed that

removal efficiency can change depending on the season or temperature (Azzouz and Ballesteros 2013). Azzouz and Ballesteros (2013) found that contaminants were higher in the winter time due to less biodegradation and solar irradiance if the plant is outdoors. One compound that has high removal efficiency in activated sludge treatment is caffeine (Phillips et al. 2008). Caffeine's response to treatment processes can be useful when comparing removal efficiencies between different compounds. Considering these removal rates and efficiencies are very complicated yet necessary to be noted when studying WWTP effluent.

An important part of observing trends in concentrations for any contaminant is the temporal scale used sampling. Concentrations over one day could be very different from the ranges of values seen over a span of years. Many studies interpreting pharmaceutical trends have looked at short-term diurnal variation and seasonal assessments. It has been discovered that , while affected by hydraulic residence times, certain compounds in wastewater effluent exhibit pulses throughout the day, slow daily cycles, or no daily cycle at all (Nelson et al. 2011). This diurnal variability is believed to be caused by influent pulses at times of day when people are most busy, for example in the morning time when people wake up to start their day and use the bathroom. Compound susceptibility to disinfection can also vary throughout the day depending on the temperature and sunlight availability at the WWTP (Nelson et al. 2011). Patterns have also been observed on a day to day basis at hospitals having higher concentrations during the weekdays when appointments are scheduled (Alder et al.2006). Many studies have also found pharmaceutical concentrations to vary seasonally. Higher concentrations have been observed for certain medications used for colds or flu in the winter time due to more pharmaceutical use by humans in the colder months and less degradation by treatment plants (Yu et al. 2013, Hedgespeth et al. 2012). These observed diurnal and seasonal patterns have raised concern about representativeness when sampling for pharmaceutical contamination. Since concentrations can fluctuate depending on the time of day or month of the year, it is very important to take sampling frequency and contaminant pulses into account (Ort et al. 2010). Temporal variability can also be expected to be higher for drugs used for acute conditions and in wastewater effluents from smaller plants that serve few people that may be taking a drug at a given time. A study performed by Ort et al. (2010) only looking at waste water influent suggests that sampling intervals of five minutes or lower may be necessary to account for these quick variations in concentration. There are no known studies that have focused on sampling uncertainty such as

that done by Ort et al. (2010), in wastewater treatment plant effluent. Therefore, when studying effluent samples there is not much information concerning the details of sampling intervals.

Most studies analyzing pharmaceuticals draw their conclusions based on samples collected once or use very short time scales. Many studies such as Hedgespeth et al. (2012), Musolff et al. (2009) and Yu et al. (2013) draw conclusions about seasonal trends based on samples from a period of one year. Other studies performed by Yang et al. (2011), van Nuijs et al. (2009) and Choi et al. (2008) base their conclusions on seasonal trends conducted over only two or three sampling events. Very limited assessments have been done on long term effluent data over many years. Stevens (2012) found that long term data for organic wastewater compounds in WWTP effluent displays important changes that would not be seen in daily or seasonal samples. Stevens (2012) presented that multi-year scales are important when observing effects from large scale changes and management decisions. Stevens (2012) found that effects from treatment plant upgrades, government restrictions, phasing out of compounds by retailers, and a change in consumer product formulation are factors that can explain temporal trends in compound concentrations that are observed. One of the only other studies that observed a data set this large was a study performed by Barber et al. (2012). The study done by Barber et al. (2012) analyzed endocrine disrupting chemicals over a span of twelve years before and after a WWTP upgrade. From this long-term data Barber was able to link decreasing concentrations to treatment plant upgrade. However, the time series of samples Barber et al. (2012) collected was irregular and sporadic with certain years not having any sampling. This study contains data for every year included in the study (except for the pharmaceutical carisoprodol) with multiple samples collected each year. The data set in this study is also unique in that it has a large amount of data for multiple sites, allowing for a comparison among the different locations.

Previous studies

Only a few studies have looked at pharmaceutical manufacturing facilities (PMF's) and hospitals as contributors of pharmaceuticals to waste water treatment plant effluent. One study in particular, Phillips et al. (2010), analyzes the effluent from plants that receive discharge from PMF's in New York (NY). This study will look at three of the same sites and seven of the same

compounds used in Phillips et al. (2010). Phillips et al. (2010) compared the concentrations at these sites receiving PMF discharge with those from a national survey of sites that do not receive this type of discharge. His study found that the sites with PMF input correspond with significantly higher concentrations in WWTP effluent. This study will take a different approach by looking at the trends in pharmaceutical concentration at these sites on different time scales.

Another study performed by Stevens (2012) also includes these same sites in NY that are observed in this study. Stevens (2012) looked at organic wastewater compounds in WWTP effluent in relation to WWTP upgrades and use of consumer products. Stevens found that technology upgrades at treatment plants and use of consumer products affect the concentrations of wastewater compounds in effluent. This study is similar to the study done by Stevens in that it will look at multi-year trends in WWTP effluent. However, this study will focus on a different set of compounds and will observe the influence of PMF operation on effluent concentrations. This study is one of the first to assess data on pharmaceuticals that result from PMF shutdown using multi-year data.

PURPOSE AND SCOPE

The purpose of this study is to evaluate the time trends of pharmaceuticals over varying time scales at select WWTPs. It will determine which time scale is best when analyzing pharmaceutical trends: yearly, seasonal, or hourly. Some sites with "worst case" scenarios (PMF's and hospitals) were chosen so trends can be easily assessed. It will use multi-year and seasonal data to observe variations from PMF input and diurnal data to assess daily changes in hospital input.

This study will look at concentrations of pharmaceuticals in WWTP effluent at four different plants in upstate NY called NY1, NY2, NY3, and NY9. All samples were collected and analyzed by USGS personnel. The concentrations of eight compounds were observed and analyzed; butalbital, diazepam, metaxalone, methadone, oxycodone, phendimetrizine, carisoprodol, and caffeine. These pharmaceuticals were chosen based on their presence at these WWTPs in a previous study by Phillips et al. (2010). The data set analyzed in this study is unique in that it has a very large time scale of thirteen years of pharmaceutical concentrations in

WWTP effluent. It also has the opportunity to assess the effects of a PMF shutdown. While Stevens (2012) used multi-year data to evaluate WWTP upgrades, this study will focus on a different set of compounds more likely to be affected by PMF closure.

METHODS

Site Description

Samples were collected from four different sites in New York; NY1, NY2, NY3, and NY9. These sites are all samples that were collected from WWTP effluent. The owners of these WWTPs prefer that the locations of the plants not be disclosed since these compounds are not regulated. Each WWTP receives different sources of influent. The WWTP at NY1 does not receive influent from a PMF or hospital, its influent is strictly residential and commercial. NY2 receives twenty percent of its total wastewater inflow from a PMF. NY2 also receives discharge from a hospital, however this percentage is unknown. NY3 receives approximately twenty percent of its wastewater inflow from a PMF (a different PMF than site NY2). NY9 receives thirty percent of its influent from a hospital. Since these sites are located in a rural area, water metering is not used. These WWTPs also have different treatment methods and have undergone plant upgrades throughout the time frame of the study (Table 1.) Each WWTP also has a different hydraulic retention time found in Table 1. Upgrades at site NY9 were irrelevant for this study since only data from one day was analyzed.

Table 1: Treatment methods, plant upgrades, and hydraulic retention times at each WWTP. Dates of upgrades and types of upgrades are indicated in each row. (Stevens)

Site	Secondary biological treatment	Tertiary treatment	Disinfection	Upgrades	Hydraulic retention time
NY1	2004-2007: Trickling filter attached media 2007-2014: New trickling filter media and new rotating biological contactors	2004-2008: Sand filtration 2008-2014: Microfiltration	2004-2008: Chlorination/ Dechlorination 2008-2014: Ultraviolet	Yes	5 Hours
NY2	2004-2013: Two stage activated sludge	2004-2009: Sand filtration 2009-2014: Microfiltration	2004-2009: Chlorination/ Dechlorination 2009-2014: Ultraviolet	Yes	19 Hours
NY3	Extended aeration activated sludge	Sand/anthracite microfiltration	Ultraviolet	No	55 Hours
NY9	Activated sludge	Microfiltration	Ultraviolet	(Not relevant for 24-hr data)	22 Hours

These four different sites also had different types of data availability (Table 2). Sites NY1, NY2, and NY3 have long term data available allowing for analysis of long-term time trends and seasonal trends. NY9 only had data from one day, only allowing a diurnal analysis to be performed at this site. Site NY9 was selected to be sampled during the course of one day because it was hypothesized to see fluctuations during the day from hospital input.

Table 2: Availability of data at each site included in the study. Y=Yes, N=No. Note that NY9 has four years of data from 2009-2012, but four years is not considered long enough for this study when analyzing multi-year trends.

	NY1	NY2	NY3	NY9
Long term	Y	Y	Y	N
Seasonal	Y	Y	Y	N
Diurnal	N	N	N	Y

Compounds & samples

Eight compounds were analyzed in this study; butalbital, diazepam, metaxalone, methadone, oxycodone, phendimetrizine, carisoprodol, and caffeine. All compounds are pharmaceuticals except for caffeine which is a widely used additive and stimulant. The use of each pharmaceutical is found in table 3.

Table 3: A list of compounds included in the study and their medical uses.

Compound	Possible application/class	Uses
Pharmaceuticals		
Butalbital	Barbiturate	treat headaches
Carisoprodol	Muscle relaxant	muscle relaxant
Diazepam	Sedative	relieve anxiety, muscle spasms, & seizures
Metaxalone	Muscle relaxant	muscle relaxant
Methadone	Narcotic	relieve moderate-severe pain. prevent withdrawal symptoms in patients addicted to opiate drugs
Oxycodone	Narcotic	relieve moderate-severe pain
Phendimetrizine	Anorectic	treat obesity by decreasing appetite
Other		
Caffeine	N/A	

These compounds were selected based on their presence in the samples at these sites in a previous study done by Phillips et al. (2010). Before the sampling began for the Phillips et al. (2010) study, the USGS National Water Quality Laboratory in Denver performed a full-scan gas chromatography/mass spectrometry breakdown of the composition of effluent at these sites. This test indicated that these seven pharmaceuticals were present at these sites and will be reliable to use throughout the study. Subsequently, a method was developed for these seven compounds, with the method performance data Phillips and others, 2010. Thus, the concentration data presented in this paper are based on the method described in the Phillips and others, 2010 report, and are confirmed for the purposes of this work. The initial work indicated the presence of these compounds, but only quantitative data from validated method are included in this paper. These

seven compounds are also very commonly prescribed pharmaceuticals in the U.S. (Phillips et al. 2010).

Sites NY1, NY2 and NY3 have data spanning over eight years. For all compounds, except carisoprodol, site NY1 had 58 samples, NY2 had 58 samples, NY3 had 65 samples, and NY9 had 26 samples. Carisoprodol had 48 samples for NY1, 49 samples for NY2, 53 samples for NY3, and 26 samples for NY9. The breakdown of the number of samples per year at each site is found in table 4.

Table 4: Number of samples collected each year at each site. Sites NY1, NY2, and NY3 have 2 columns because the compound carisoprodol had a different number of samples at these sites. The total number of samples at each site is found in the bottom row.

	All compounds except carisoprodol	Carisoprodol	All compounds except carisoprodol	Carisoprodol	All compounds except carisoprodol	Carisoprodol	All compounds
	NY1	NY1	NY2	NY2	NY3	NY3	NY9
2004	3	-	2	-	3	-	-
2005	7	-	7	-	8	-	-
2006	6	6	6	6	6	6	-
2007	7	7	7	7	7	7	-
2008	7	7	7	7	7	7	-
2009	9	9	9	9	10	10	7
2010	7	7	7	7	9	9	7
2011	7	7	7	7	9	9	6
2012	4	4	5	5	5	5	6
2013	1	1	1	1	1	1	-
Total	58	48	58	49	65	54	26

Samples at sites NY1, NY2, and NY3 were collected using grab samples and 24-hour composite autosampler samples. Samples were collected by USGS field personnel trained in the sampling of trace organic contaminants. The grab samples were collected during the day time in a 3 liter large-mouth teflon-lined bottle. The 24-hour composite samples were collected using two ISCO automatic samplers, each of which collected twelve samples in one liter glass bottles. These samplers collected a sample once per hour over the course of twenty four hours. Silicone tubing was used in the pump head and distributor arm of the automatic sampler and was disposed of after each sample. All materials in both the grab and 24-hour composite samples were cleaned using the USGS trace-organic protocols (Wilde 2004).

The data analyzed in this study for site NY9 only consists of samples collected over the course of one day. Site NY9 data consists of a grab sample at the beginning of the sampling event, six 4-hour composite samples, and a 24-hour composite sample. The 4-hour composite samples collected effluent water every fifteen minutes over the course of four hours. The 24-hour composite sample took a sample every hour for twenty four hours.

Sample Preparation

Effluent samples were filtered using 0.7 micrometer glass-fiber filters, meaning the concentrations of pharmaceuticals measured was the dissolved concentration. Filtration involved the use of teflon tubing and ceramic-head pumps. Samples were kept below 4 degrees Celsius during collection and filtration and were shipped to the National Water Quality Laboratory (NWQL) in Denver. The NWQL begins sample extraction by vacuum through disposable solid-phase extraction cartridges that contain a polystyrene-divinylbenzene resin (Zaugg et al. 2012). The cartridges are dried with nitrogen gas, then the sorbed compounds are eluted with dichloromethane-diethyl ether. The concentrations of the compounds are determined using capillary-column gas chromatography/mass spectrometry (GC/MS), which uses electron impact ionization (Zaugg et al. 2012). If the results met GC/MS criteria they were reported then quantified using the injection internal standard method using a 5 to 8 point calibration curve. During the course of sampling the calibration curve was extended to better quantify the high concentrations at NY2 and NY3 (Phillips et al. 2010) The maximum concentrations used in the calibration curve for metaxalone was extended from 400 micrograms per liter to 4000 micrograms per liter. The high concentration standard for diazepam was extended from 4 micrograms per liter to 400 micrograms per liter. For all other compounds, the curve started at 40 micrograms per liter and was extended to 400 micrograms per liter. The minimum calibration point was also extended to report low-level concentrations; diazepam extending from 0.04 micrograms per liter to 0.004 micrograms per liter, metaxalone extending from 4 micrograms per liter to 0.4 micrograms per liter, and all other compounds extending from 0.4 micrograms per liter to 0.04 micrograms per liter (Phillips et al. 2010).

Method Performance

A variety of studies were performed to make sure that the analytical method was of high quality. These studies include spikes, a precision study, a method detection limit study, and a holding time study (Phillips et al. 2010). A reagent spike experiment at low and medium concentrations and an effluent spike test at low, medium, and high concentrations was performed to evaluate method performance. The reagent spike experiment consisted of processing reagent water samples with known concentrations of the pharmaceuticals included in the study with environmental samples. These reagent spike samples helped to better understand the precision and sensitivity of each compound as well as the bias in the method used (Phillips et al. 2010). Another spike experiment was taking effluent matrix spikes at sites NY1, NY3, and NY4 (not included in this study). A composite sample was collected at all of these sites and divided into replicate samples. NY3 samples were fortified with high concentration. The results from these spike samples show the percent recoveries were within 60 percent to 130 percent and the relative standard deviations were less than 30 percent. NY1 effluent matrix spikes looked at medium concentrations. The NY1 spikes had relative standard deviations all less than 30 percent and almost all percent recoveries were within the 60 to 130 percent range (except for methadone which was 59 percent). The spikes at site NY4 assessed low analyte concentrations. Almost all percent recoveries were within 60 to 130 percent range (except for oxycodone with 170 percent) and almost all relative standard deviations were less than 30 percent (except for methadone with 31 percent) (Phillips et al. 2010). These spike experiments display that the data for compounds included in this study have acceptable variability and bias with respect to interpreting trends in data in this study where larger variations between plants and times was observed. For this method, concentrations were not adjusted for surrogate recovery.

Blanks & replicates

Blank and replicate samples were collected during this study to assure the quality of the environmental samples collected. Field blanks and equipment blanks were performed using organic-free water from the laboratory. The results from these blanks indicate if any outside or

"blank" contamination is occurring during sample collection and analysis. The total number of field blanks for all pharmaceuticals was 55 samples, while for caffeine there were 49 samples. Carisoprodol, diazepam, and phendimetrizine did not have any detections in the blanks. Butalibital, methadone, oxycodone, and caffeine had detections in less than six percent of blanks. The blank detections for these four compounds were usually below 0.1 microgram per liter, except for oxycodone and methadone which each had one blank detect of 0.73 and 0.19 micrograms per liter respectively. Compounds that had a blank detection in the same week as a sample collected had their samples censored as a non-detect if the concentration was within ten times the blank detection. The censored samples were still used in the results of the study and are qualified as non-detections based on the detection limit for that compound. These detections of concentrations in these blanks were most likely the result of carryover in the lab from samples containing very high concentrations. One compound that did have a high frequency of blank contamination was metaxalone. 7.3 percent of metaxalone blanks were contaminated and usually had a concentration higher than 0.1 micrograms per liter. Most of the higher blank concentrations were before January 1, 2008, therefore all samples with concentrations below 3.0 micrograms per liter before this date were censored as a non-detect. After January 1, 2008 samples were only censored if a blank detection occurred during that week. Besides for this issue with metaxalone, the censoring of data due to blank contamination was minimal for all other compounds (Table 5).

Replicate samples were also collected as a part of quality assurance. These replicates were an extra sample collected the same way as the environmental sample and should produce the same results. Thirty-seven replicate samples were collected and analyzed in order to evaluate sampling and laboratory analysis precision. These thirty-seven samples allowed for 143 concentrations to be compared. The median relative percent differences between the actual sample and the replicate were less than 8 percent. Almost all of the replicate concentrations were less than 20 percent different from each other. There were nine of the replicate samples that had a detection in one of the replicate samples but not in the other. The comparisons between the replicates indicated that the sample concentrations were able to be reproduced with minimal percent difference.

Table 5: Information of blank data. Includes the method detection limit, percent of blanks that had a detection, and a range of the concentrations found in the blanks for each compound.

Compound	Method detection limit, in micrograms per liter	Percent of Blanks with detection	Range in concentrations detected in blanks, in micrograms per liter
Pharmaceuticals			
Butalbital	0.031	1.8	0.051
Carisoprodol	0.041	0	na
Diazepam	0.012	0	na
Metaxalone	0.015	7.3	0.019 - 0.75
Methadone	0.019	1.8	0.19
Oxycodone	0.083	5.5	0.076 - 0.73
Phendimetrazine	0.039	0	na
Other Compounds			
Caffeine	0.06	3.6	0.013-0.02

Methods used for time trend analysis of graphs

The first way that trends were observed was by graphical analysis. All of the graphs were created using Sigma Plot version 12.0. These graphs were made in order to visually assess the trends and changes over time of compound concentration in effluent. This allowed for identification of gradual, short-term, and abrupt changes over time. Time-based trends were displayed with plots of data over time with Lowess Smooth lines (Locally Weighted Scatterplot smoothing). Lowess smooth lines were added into the graphs to better visualize the direction of trends. Both the compound concentration and Lowess Smooth lines were graphed on a log scale. Vertical lines indicating PMF announcement of closure and actual closure were also added to the graphs to see effects of PMF input.

Methods used to assess temporal and seasonal differences in concentrations at WWTPs

The data for pharmaceutical concentrations from sites NY1, NY2, and NY3 were assessed to account for temporal and seasonal variations. This analysis was conducted by using graphical methods which consisted of scatterplots of concentrations with time, boxplots of concentrations for the three time periods, and boxplots of concentrations on a seasonal basis.

The data at sites NY1, NY2, and NY3 were broken up into three different time periods relating to the PMF shutdown at NY2. Although the broken up time periods correspond to the PFF shutdown at NY2, NY1 and NY3 were also analyzed to see if these changes are typical. The first time period, pre-closure, is from 2004 when samples were first collected to 2008 when the PMF plant first announced that it would be closing. The second period, known as the transition period, is from 2008 when the plant announced closure to 2012 when it officially closed. The third period, post-closure, is from 2012 when the PMF closed to 2013 the end of data availability. The yearly data was also broken up into four seasons for a separate analysis. These four seasons consists of winter, spring, summer and fall:

Winter: January, February, March
Spring: April, May, June
Summer: July, August, September
Fall: October, November, December

In order to assess general temporal trends and to assess statistical differences among the time periods and among seasons, two types of statistical analysis were used. The first type of analysis, the LOWESS smoothing procedure, was used to assess general trends in concentrations over time. The LOWESS (locally weighted scatterplot smoothing) smoothing procedure creates a center line that can be fit to a large amount of data (Hensel and Hirsch 1992). In the temporal trends graphs the LOWESS smooth line helps in understanding the overall relationship between time and concentration.

The three time periods and the four seasons were assessed using non-parametric analysis of variance techniques. The Kruskall-Wallace non parametric test was used to evaluate whether different time periods or different seasons have different median concentrations. After this statistical analysis was performed the box plots were able to be labeled indicating if they are statistically similar to each other. The data from each time period and from each season was collapsed into a single box in order to show statistical changes during these specific periods.

The hourly data was assessed by graphical analysis in order to observe diurnal trends. Hourly data was only analyzed for site NY9 and consisted of a small data set ranging over the course of one day. The diurnal graphs plot the concentration vs time of day for the 4-hour composites, grab sample and 24-hour composite. A line was used to connect each concentration

of the four-hour composite in order to better visualize trends throughout the day. Another set of graphs were made to analyze the percent difference between the 4-hour composite, grab sample, and 24-hour composite. Horizontal lines were placed on these graphs at +20 percent and -20 percent to indicate variability.

RESULTS & DISCUSSION

Multi-year overview

Trends in pharmaceutical concentration were assessed through time-series of plots for sites NY1, NY2, and NY3. The 58 samples for NY1, 58 for NY2 and 65 for NY3, were approximately equally distributed over the 8 year time period (2004-2013). General trends are depicted using LOWESS smooth lines (Locally Weighted Scatterplot smoothing). The key beneath each graph shows that the filled in circles are detected concentrations and the open circles are non-detects. The blue and red vertical lines relate to the PMF closure at plant NY2. The blue line at 2008 denotes the announced closure of the PMF discharging to NY2. The red line at 2012 denotes the PMF shutdown. Thus, the period from 2004-2008 indicates concentrations before the announcement of the closure of the PMF discharging to NY2. The 2012-2013 indicates concentrations after the final PMF closure. All graphs for the yearly time trends can be found in appendix 1.

Since several trends were discovered in the multi-year graphs, statistical comparisons were made for concentrations for all seven pharmaceuticals and caffeine for each site among the three periods. A set of box plots for each compound at each site were made and are found in appendix 2. These box plots show the same data set seen in the yearly graphs, but they compact the data from each time period into a single box plot. Although the broken up time periods correspond to the PMF shutdown at NY2, NY1 and NY3 were also analyzed to see if these changes are typical. The letters above each plot tell if the median concentration for a specific compound is statistically different between individual time periods at a particular site. If all of the boxes have the letter “A” then the data in each time period is statistically the same. However, if there is an “A” “B” and “C” then each time period is statistically different. Most of the plots

for each compound at NY1 were statistically similar. The NY2 plots show a similar idea of what the yearly trends graphs presented. NY3 had three compounds that were statistically different (Table 6).

Table 6: Results of the statistical analysis for the 3 period comparison. Green boxes are compounds that were statistically different. An "x" means that the 3 time periods for that compound were not statistically different.

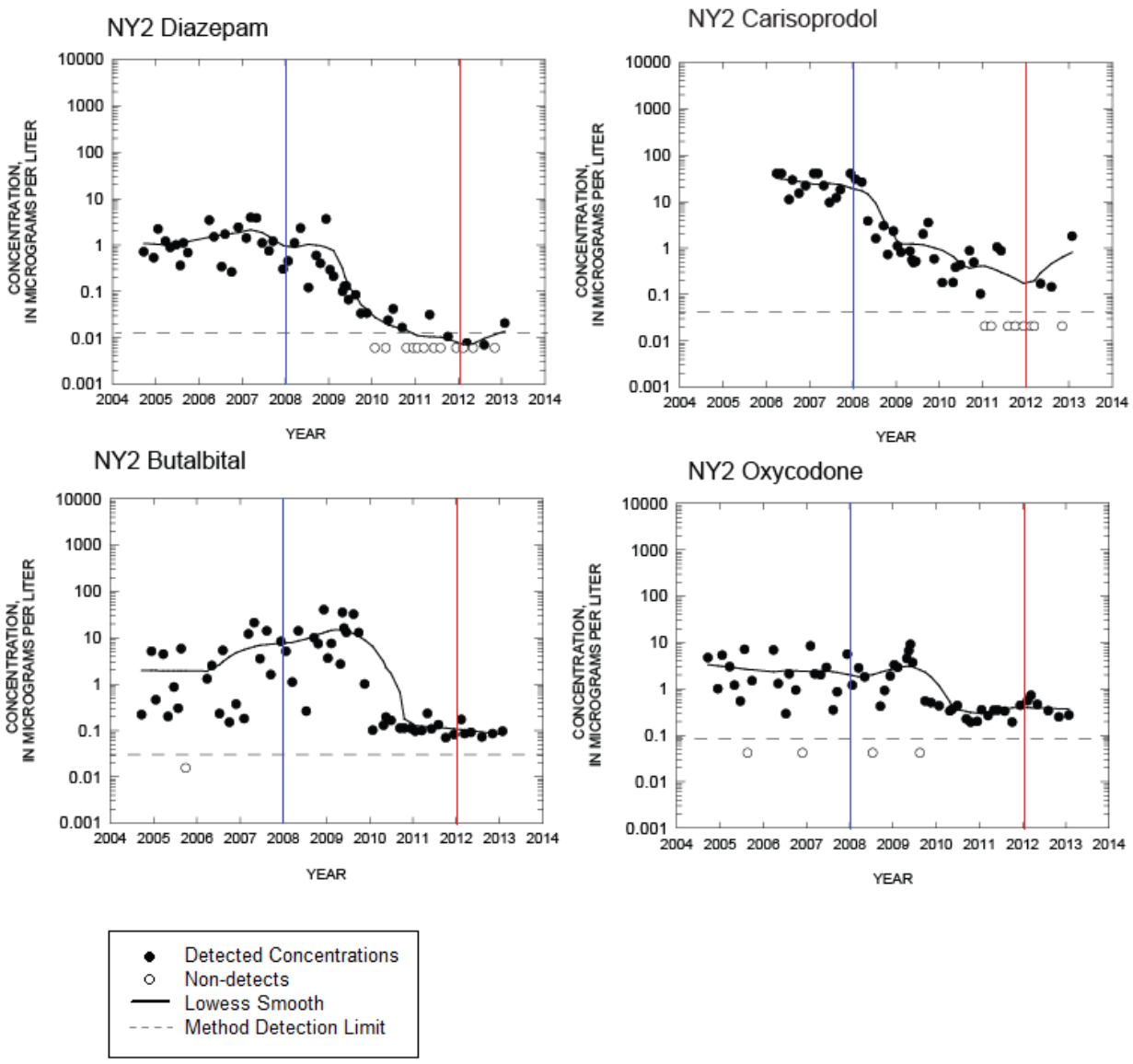
	Butalbital	Diazepam	Metaxalone	Methadone	Oxycodone	Phendimetrizine	Carisoprodol	Caffeine
NY1	x	x	x	x	x	x	x	
NY2			x	x		x		x
NY3		x		x		x	x	x

Multi-year results at NY2

Several compounds for NY2 show a decrease in concentration due to PMF closure starting around 2008 when the closure was first announced. Some of these compounds gradually declined while others had a sudden drop off. Diazepam and Carisoprodol were the only compounds at NY2 that had a gradual decrease in concentration (Figure 1). In the first time period before announcement of PMF shutdown diazepam concentrations remain fairly consistent around 1.5 micrograms per liter. Diazepams maximum concentration of 3.9 micrograms per liter is during this time period. In the second time period concentrations greatly decline over a period of four years. Even though this decline may appear steep these concentrations slowly decrease over many years with no sudden drops in concentration. This trend displays a gradual phase-out of diazepam from announcement of PMF closure to actual PMF closure. After the plant closure in the third time period diazepam reaches its minimum concentration of 0.006 micrograms per liter (its detection limit). Carisoprodol also displayed a gradual phase-out at NY2. Carisoprodol starts out at 40 micrograms per liter in 2006. From 2008 to 2009 it shows the start of a steep decline but then tends to display a more gradual decrease after 2009. Carisoprodol gradually decreases to 0.0205 (its detection limit) micrograms per liter in 2011. Carisoprodol also displays a similar trend to diazepam in the different time period comparison with steady concentrations

pre-closure and decreasing concentrations during the transition period. The PMF shutdown caused the concentrations of carisoprodol to decrease three-fold.

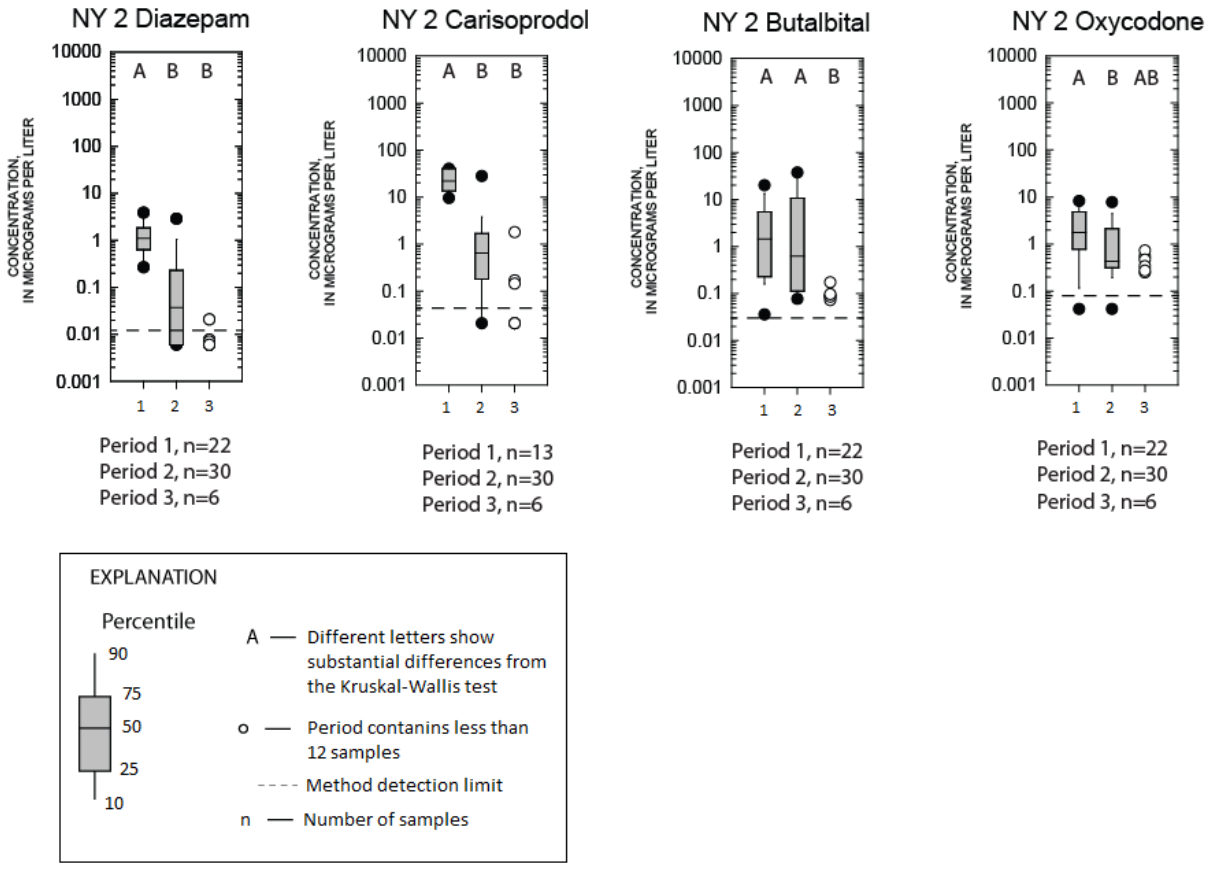
Other compounds at NY2 displayed a sudden drop in concentrations between 2009 and 2010. In 2009 butalbital dropped from 12.8 micrograms per liter to 0.101 micrograms per liter in only four months (Figure 1). Around the same time period in late 2009, oxycodone dropped from 3.7 micrograms per liter to 0.546 micrograms per liter in only three months. Both of these graphs for butalbital and oxycodone display varying concentrations in the pre-closure period, then a sudden drop during the transition period, and a leveling out of concentrations in the post-closure period after PMF closure. These graphs in Figure 1 for butalbital and oxycodone indicate that the PMF suddenly stopped production of these pharmaceuticals during its transition period of closing the facility. When comparing these gradual vs. steep graphs it is evident that the PMF phased out different compounds at different speeds. After PMF shutdown, in the post-closure period most of the concentrations are similar to the concentrations seen at site NY1 with no PMF input to its WWTP. This shows that if a PMF shuts down it is possible for its concentrations to significantly decrease to the concentrations of a normal scenario with no PMF influence.



<u>Figure 1</u>: Multi-year graphs of concentrations from 2004 to 2013 of four pharmaceuticals; diazepam, carisoprodol, butalbital, and oxycodone. All graphs are for site NY2. The graphs plot pharmaceutical concentration vs. year. The black dots are detected sample concentration in micrograms per liter. Open circles are non-detected concentrations. The black line in the graph is a lowess smooth line (Locally Weighted Scatterplot Smoothing). The blue line is the date when the PMF discharging to NY2 first mentioned it will be shutting down. The red line is when this PMF actually shutdown. The dashed line is the method detection limit for each compound.

These four compounds (diazepam, carisoprodol, butalbital, and oxycodone) at site NY2 also had significant differences in the statistical analysis of the three different time periods

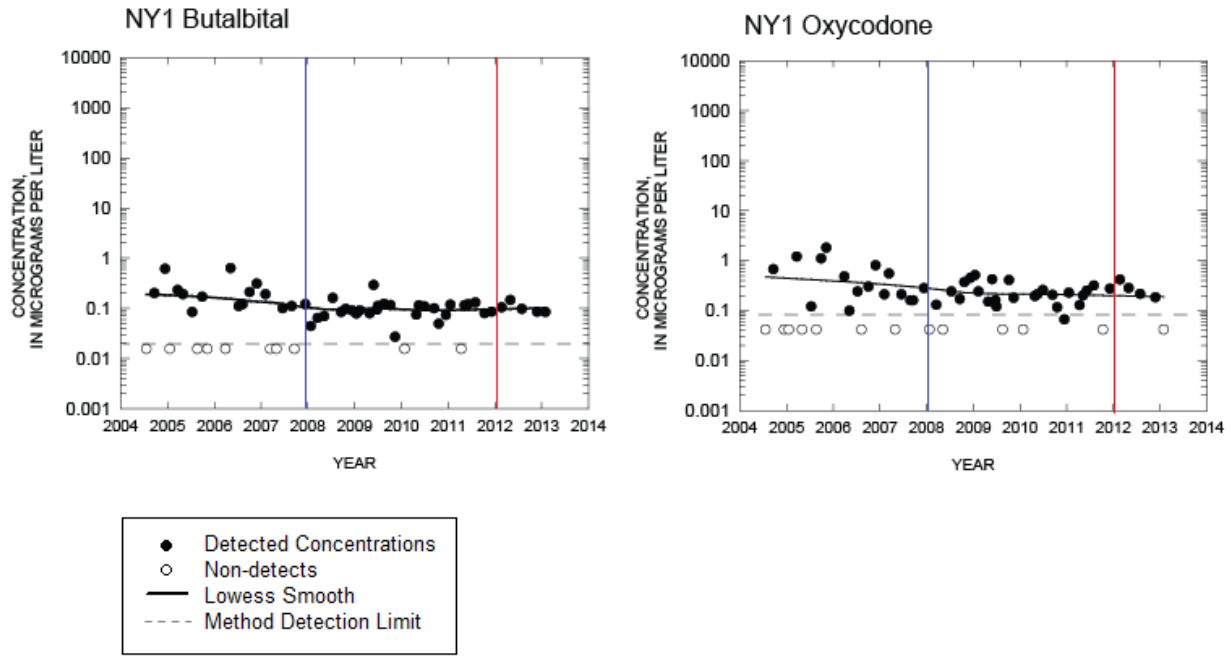
(Figure 2). Diazepam and carisoprodol were statistically different in the second and third period compared to the first. This means that their concentrations greatly decreased from the first period, pre-closure, to the second transition period. Butalbital is statistically the same in the first and second periods and different in the third period indicating a decrease in concentration post-closure. Oxycodone is statistically different in the second period from the first, and the third period is statistically similar to the first and second periods. These box plots in figure 2 further indicate decreasing trends from PMF shutdown.



<u>Figure 2:</u> Three period time comparison of four pharmaceuticals; diazepam, carisoprodol, butalbital, and oxycodone. All box plots are at site NY2. These plots display pharmaceutical concentration vs. time period. The first time period, pre-closure, is from 2004 when samples were first collected to 2008 when the PMF plant first announced that it would be closing. The second period, known as the transition period, is from 2008 when the plant announced closure to 2012 when it officially closed. The third period, post-closure, is from 2012 when the PMF closed to 2013 the end of data availability. An explanation of the box plots is described in the figure.

Multi-year NY1 & NY3

Site NY1 displayed minimal changes in the multi-year graphs due to its lack of PMF input. Only one compound, caffeine, at NY1 was statistically different during the three time periods related to PMF closure at NY2. The first time period, pre-closure, is from 2004 to 2008. The second transition period is from 2008 to 2012. The third period, post-closure, is from 2012 to 2013. For caffeine the first period was statistically different from the second and third periods. This sudden drop in caffeine at this site is most likely due to the WWTP upgrade at NY1 in 2007 (Stevens 2012). The rest of the pharmaceuticals at NY1 displayed stable concentrations throughout the years (Figure 3). Concentrations for the pharmaceuticals for site NY1 generally did not change substantially over the three periods. A few graphs for NY1 stayed consistent (butalbital and oxycodone) indicating that the plant upgrade at NY1 did not increase the treatment plants ability to remove pharmaceuticals. Figure 3 displays that butalbital and oxycodone concentrations do not show any clear trends relating to WWTP upgrade. However, the LOWESS smooth line can indicate that these concentrations slightly decreased continuously over time but not by a large amount.



<u>Figure 3:</u> Multi-year graphs of concentrations of two pharmaceuticals; butalbital and oxycodone. Both graphs are for site NY1. The graphs plot pharmaceutical concentration in micrograms per liter vs. year. The black dots are detected sample concentration in micrograms per liter. Open circles are non-detected concentrations. The black line in the graph is a lowess smooth line (Locally Weighted Scatterplot Smoothing). The blue line is the date when the PMF discharging to NY2 first mentioned it will be shutting down. The red line is when this PMF actually shutdown. Although the blue and red lines correspond to PMF shutdown at NY2, these lines were added to NY1 and NY3 graphs to see if these changes are typical. The dashed line is the method detection limit for each compound.

A few graphs for site NY3 display very high and variable concentrations for a few compounds such as phendimetrizine, butalbital, methadone, and oxycodone. Most of the graphs for NY3 were either consistent or variable because it had no reported change in its WWTP or PMF operations. The concentrations of four pharmaceuticals at NY3 can be observed in Figure 4. Phemdimetrizine, butalbital, and oxycodone exhibit varying concentrations throughout the years with no clear trends seen. These variable concentrations can be a direct result of the PMF manufacturing their pharmaceuticals using "batch" processes. This "batch" process is when a PMF manufactures a particular pharmaceutical in a "campaign". These campaigns continue until enough is manufactured to fulfill the expected sales demand. The campaign can last from a few days up to several months. Once the campaign is over a different pharmaceutical is manufactured

(EPA 1997). Figure 4 displays that while phendimetrizine, butalbital, and oxycodone levels are most likely variable due to such campaigns, methadone stays consistent. These stable methadone concentrations at NY3 indicate that this PMF uses batch processes for select pharmaceuticals.

The only compound that indicated a clear trend at site NY3 was metaxalone (Figure 5). The PMF discharging to the treatment plant at NY3 is believed to have stopped producing metaxalone based on verbal communication given to the USGS from the plant owner. Figure 5 shows that this stop in production most likely started in 2009. Concentrations appear to decrease for a year from 2009 to 2010. The concentrations level out after 2010 and it is unclear what this means in terms of PMF production. This leveling out is interesting because the ending result after production ceased is still higher than the concentrations of metaxalone at both of the other sites NY1 and NY2. Even though production ceased, metaxalone levels could still be high if the PMF is still testing batches of it.

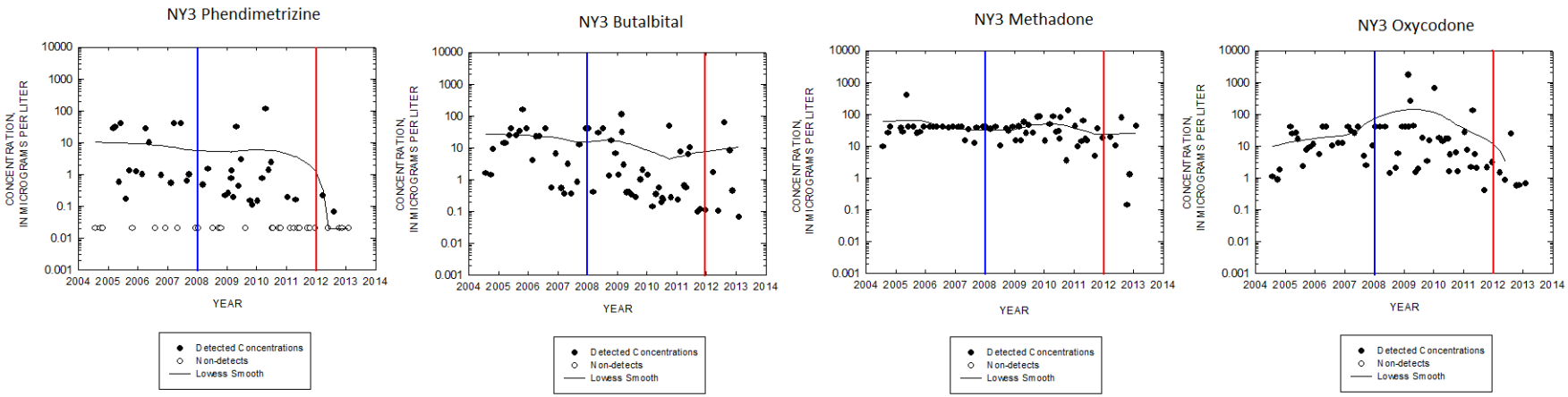
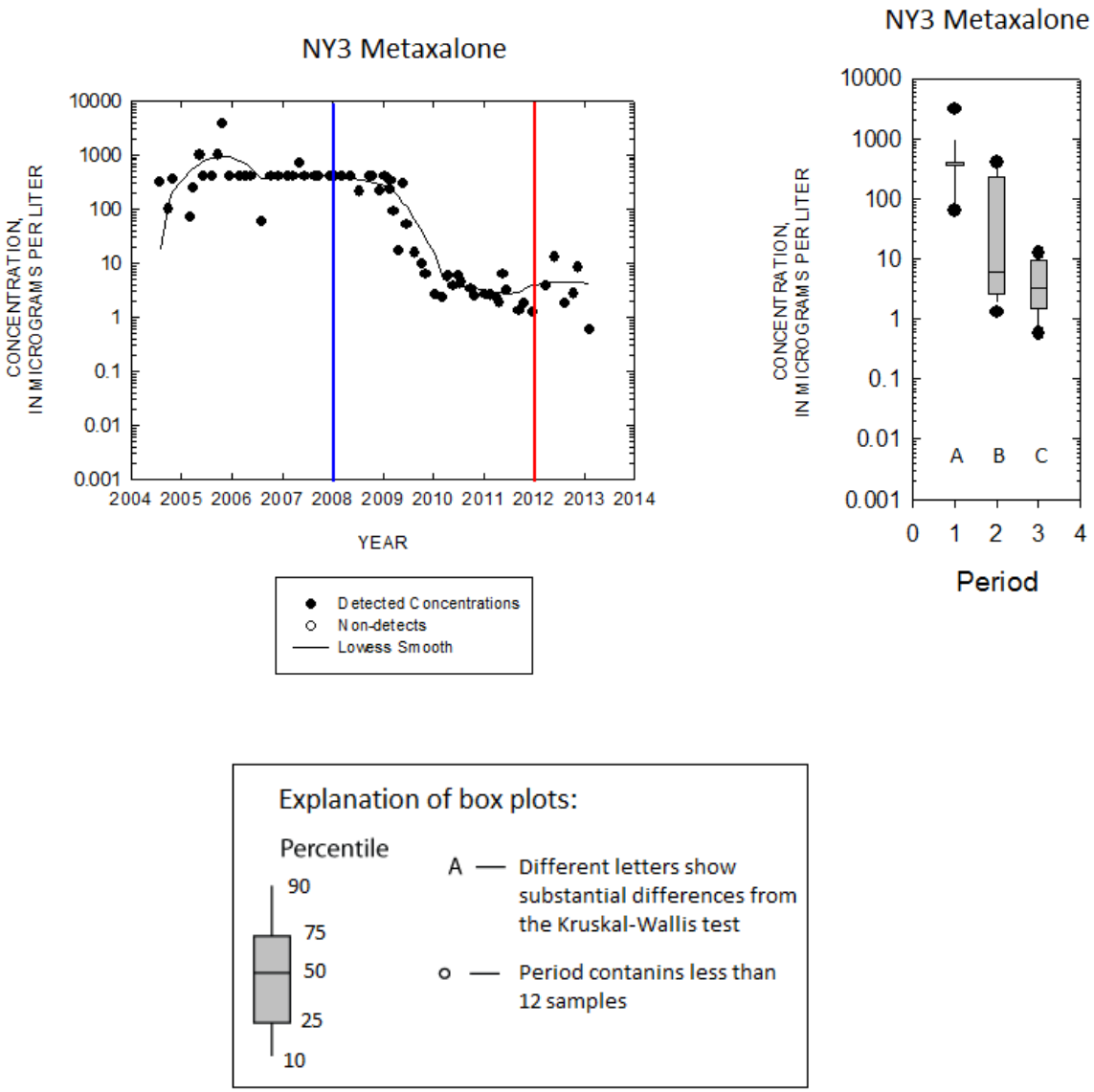


Figure 4: **Multi-year graphs of concentrations of four pharmaceuticals; phendimetrizine, butalbital, methadone, and oxycodone. All graphs are for site NY3. The graphs plot pharmaceutical concentration in micrograms per liter vs. year. The black dots are detected sample concentration in micrograms per liter. Open circles are non-detected concentrations. The black line in the graph is a lowess smooth line (Locally Weighted Scatterplot Smoothing). The blue line is the date when the PMF discharging to NY2 first mentioned it will be shutting down. The red line is when this PMF actually shutdown. Although the blue and red lines correspond to PMF shutdown at NY2, these lines were added to NY1 and NY3 graphs to see if these changes are typical. The dashed line is the method detection limit for each compound.**



<u>Figure 5:</u> Multi-year graph and box plot for the pharmaceutical metaxalone at site NY3. The graphs plot pharmaceutical concentration in micrograms per liter vs. year. In the graph on the left the black dots are detected sample concentration in micrograms per liter. Open circles are non-detected concentrations. The black line in the graph is a lowess smooth line (Locally Weighted Scatterplot Smoothing). The blue line is the date when the PMF discharging to NY2 first mentioned it will be shutting down. The red line is when this PMF actually shutdown. Although the blue and red lines correspond to PMF shutdown at NY2, these lines were added to NY1 and NY3 graphs to see if these changes are typical. The dashed line is the method detection limit for each compound. In the graph on the right, period 1 is from 2004 when samples were first collected to 2008 when the PMF plant first announced that it would be closing. Period 2 is from 2008 when the plant announced closure to 2012 when it officially closed. The third period, post-closure, is from 2012 when the PMF closed to 2013 the end of data availability.

Caffeine

One issue of concern when looking at the effect of PMF shutdown at NY2 was whether a change in plant operation had any effect on declining pharmaceutical concentrations. The compound caffeine was added to this study to better determine what is influencing the change in pharmaceutical concentrations. Caffeine is very sensitive to treatment plant upgrades and was added to this study as a "control". By using caffeine as a control we can better determine the cause of change in concentration seen in the other compounds. If caffeine shows no change then no significant upgrade at the WWTP occurred, allowing us to point the main source of declining concentration to PMF operations or shutdown. Figure 6 shows a comparison of caffeine concentrations among sites NY1, NY2, and NY3. A large decrease in concentration is seen at NY1 starting in 2008. This drop was due to a WWTP upgrade at site NY1 (Stevens 2012). This WWTP upgraded its biological treatment process with a trickling filter media and new rotating biological contactors in the beginning of October 2007 (Stevens 2012). From the graph of NY1 in Figure 6 it is clearly seen how caffeine reacted to this upgrade. The other graphs for NY2 and NY3 show stable concentrations indicating no significant plant upgrade at these sites. This means that any change in concentration at NY2 and NY3 is most likely due to changes in PMF practices or human use.

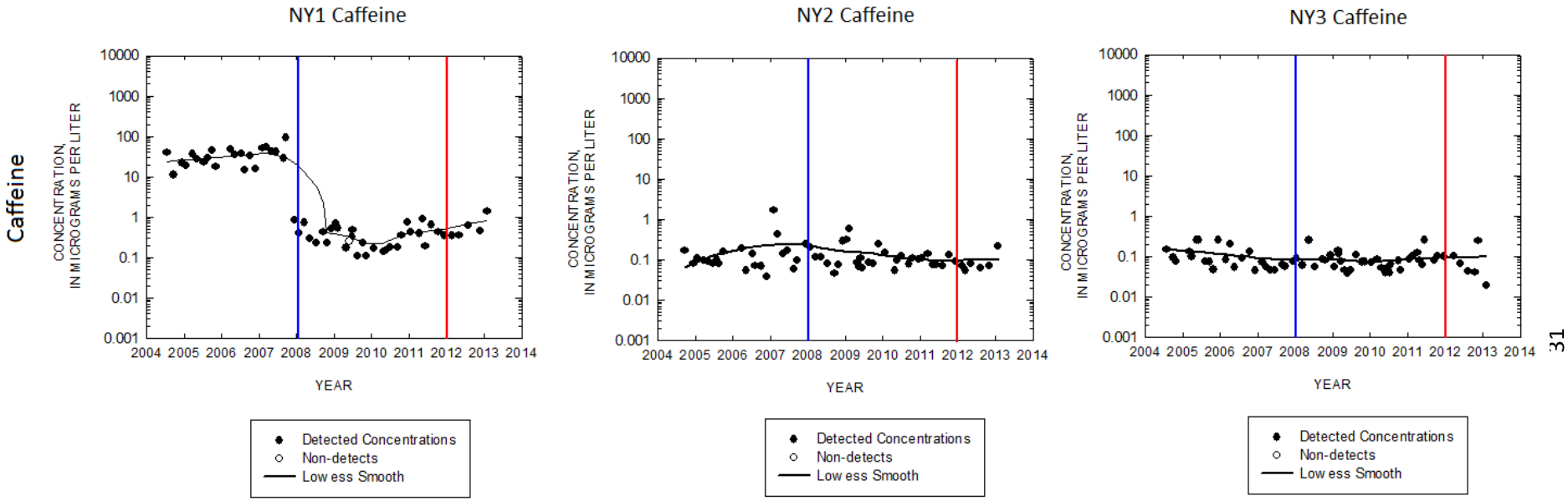


Figure 6: **Multi-year concentrations of caffeine at three different sites, NY1, NY2, and NY3. The site name is found on top of each graph. The graphs display caffeine concentration in micrograms per liter vs. year. The black dots are detected sample concentration in micrograms per liter. Open circles are non-detected concentrations. The black line in the graph is a lowess smooth line (Locally Weighted Scatterplot Smoothing). The blue line is the date when the PMF discharging to NY2 first mentioned it will be shutting down. The red line is when this PMF actually shutdown. Although the blue and red lines correspond to PMF shutdown at NY2, these lines were added to NY1 and NY3 graphs to see if these changes are typical. The dashed line is the method detection limit for each compound.**

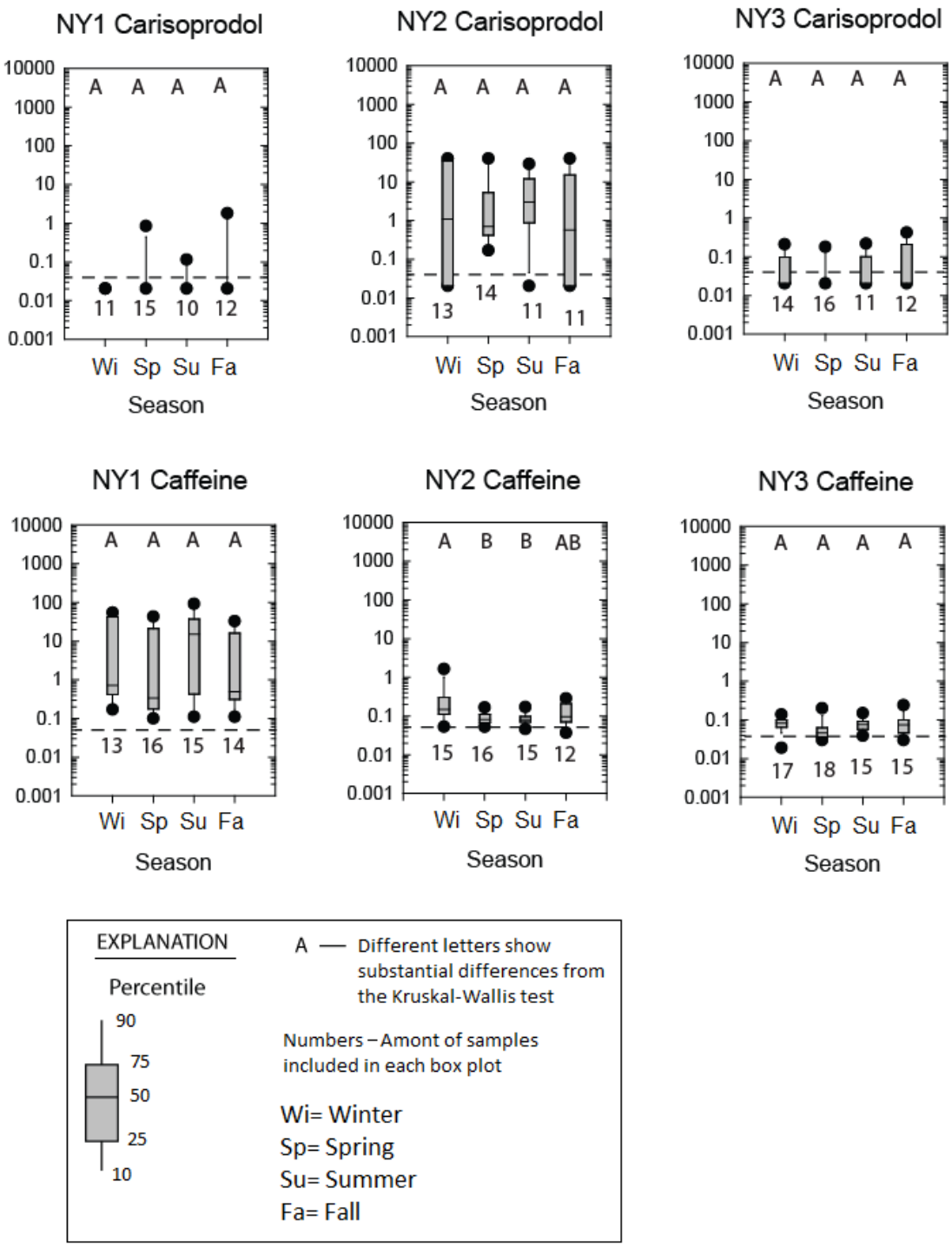
Seasons

This study took all of the same data seen in the above yearly comparisons and divided it by seasons. Box plots were made for sites NY1, NY2 and NY3 and include all eight compounds found in appendix 3. The box plot for each season includes all the data for that season over the eight-year sampling period. A statistical analysis was done on these seasonal plots which represent the letters A, B, and C above each box.

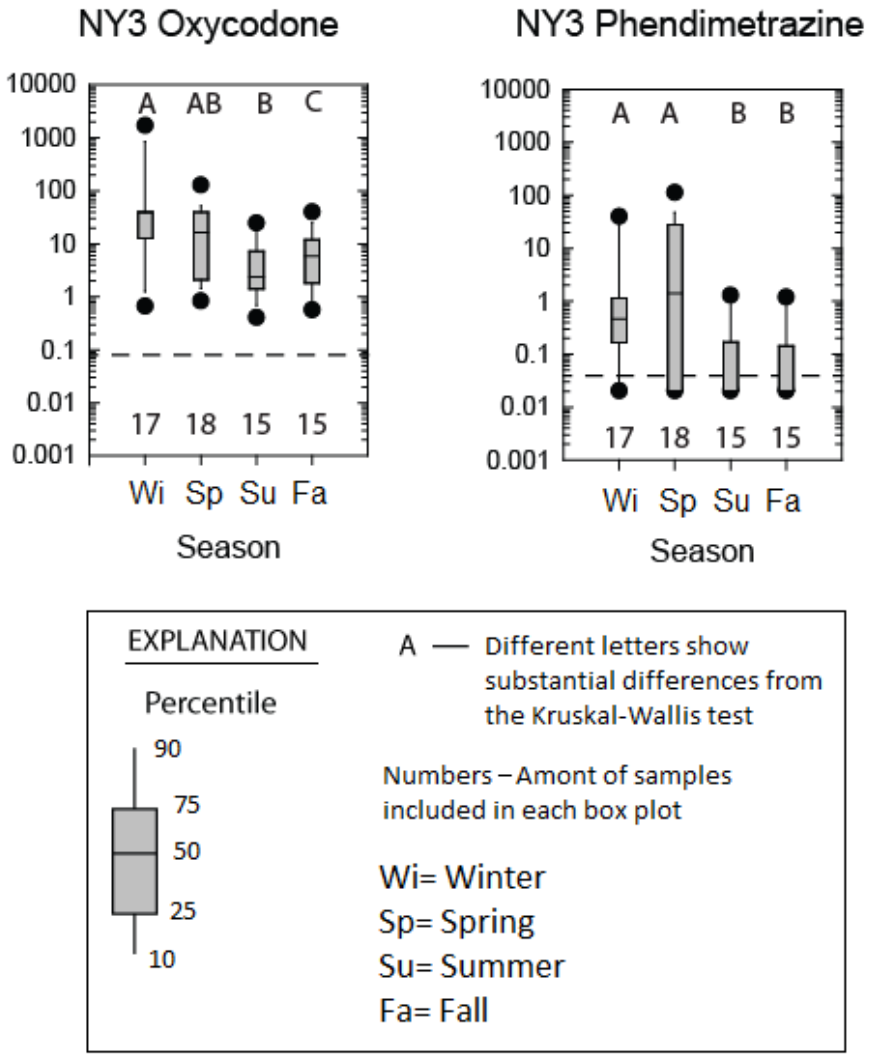
Concentrations of the pharmaceuticals and caffeine did not differ significantly over the four seasons. Almost all of the compounds were found to be statistically the same throughout the different seasons (Figure 7). Stevens (2012) also found that for a different selection of compounds at these same WWTPs the concentrations did not vary much seasonally. The only compounds that displayed seasonal variability in this study were oxycodone and phemdimetrizine at site NY3. This site was the only site that displayed seasonal variation with these two compounds. Oxycodone displayed its highest concentrations in the winter and lowest in the summer at NY3. Oxycodone also had statistically higher concentrations in the spring when compared to the summer season. These differences are seen in Figure 8 with the letters showing the statistical differences in the seasons. Seasonal trends in the concentration of phendimetrizine were observed with high concentrations in the winter and spring and lower concentrations in the summer and fall. The winter and spring were statistically similar, both displaying "A's" in Figure 8 while the summer and fall were statistically similar displaying "B's".

There is no definite known cause for these seasonal differences seen in oxycodone and phendimetrizine at NY3. If the cause of seasonal variation was temperature dependent biodegradation one would expect to see the same differences at the other sites NY1 and NY2 for these compounds. One would also expect caffeine concentrations to show seasonal differences since it is sensitive to treatment processes. Since oxycodone and phendimetrizine only display seasonal variation at site NY3 the cause is most likely coming from a change in NY3's influent during these periods, which would be a change in PMF input. From these graphs it is suspected that the PMF discharging to NY3 formulates oxycodone and phendimetrizine depending on the time of year, or season. This reason cannot be fully proven because the owners of the PMF's do not release this type of information.

The other six compounds at all sites did not show any seasonal trends. There are a few hypothesized reasons as to why no seasonal trends were observed. The first possibility is that the compounds selected for this study are not susceptible to temperature dependent biodegradation. Some studies found seasonal differences because there is less biodegradation and solar irradiance in the winter time for outdoor plants (Azzouz and Ballesteros, 2013). However, the temperature of the plants water is not available for these sites to confirm a seasonal comparison based on temperature. No seasonal variation would also be observed if these pharmaceuticals do not have a certain time of year when they are used most by humans. Antibiotics are believed to show higher concentrations in the winter due to increased human use for colds and flu (Coutu et al. 2013). However it would not be likely to see this change in pharmaceuticals because they show no patterns in seasonal human use. Another idea is removal efficiency is not very dependent on outside temperature. It was not expected to see any variation due to temperature at site NY3 because all of the water at the treatment plant is kept inside at the same temperature year round.



<u>Figure 7:</u> Seasonal box plots of two different pharmaceuticals; carisoprodol and caffeine at sites NY1, NY2, and NY3. The site name is found on top of each box plot. The box plots display concentration in micrograms per liter vs. season. Further explanation of box plots are found in this figure in the explanation box.



<u>Figure 8:</u> Seasonal box plots of two different pharmaceuticals; oxycodone and phendimetrizine. Both box plots are at site NY3. The plots display concentration in micrograms per liter vs. season. Further explanation of box plots are found in the figure.

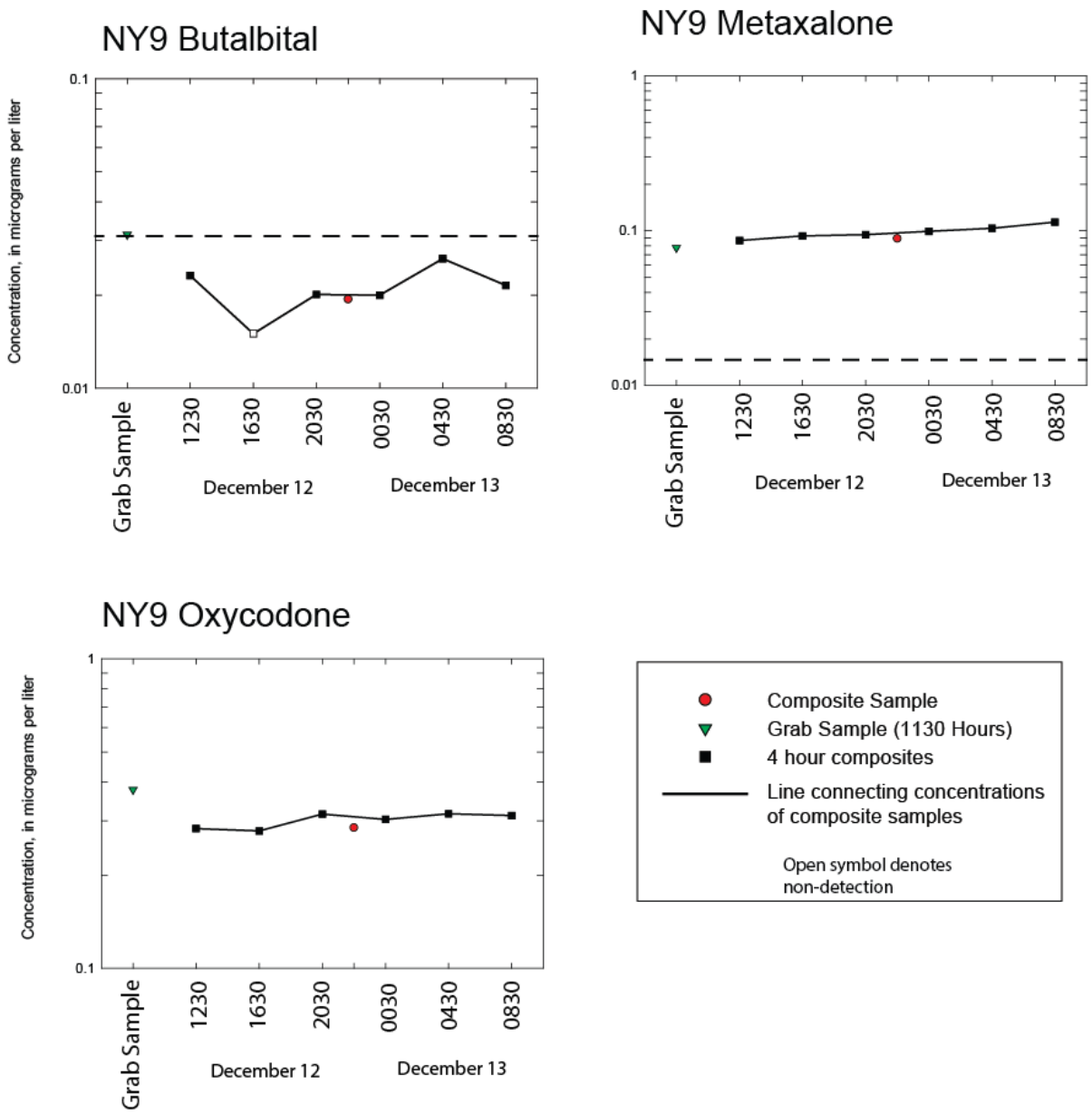
Hourly

Samples were analyzed at site NY9 during the course of one day in December, 2012. This site NY9 was chosen because it receives influent from a hospital. Previous studies have shown that considerable variability in pharmaceutical concentrations can occur due to diurnal

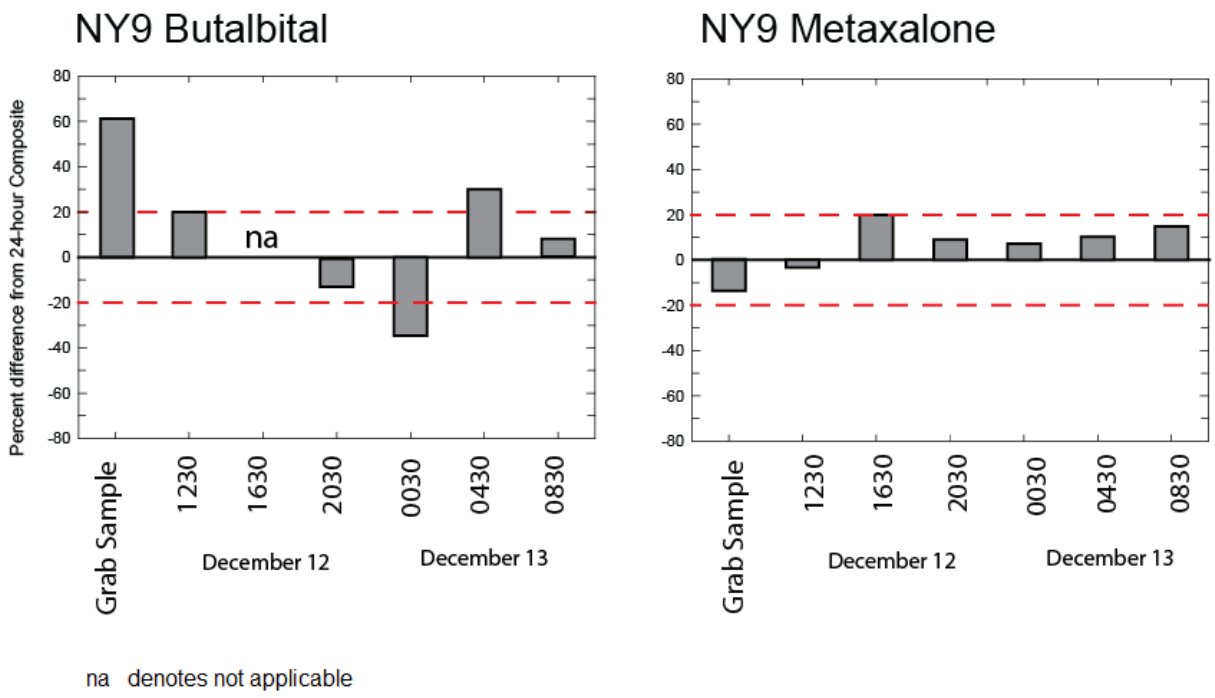
changes in inputs from sources such as hospitals (Nelson et al. 2011). It was hypothesized to see fluctuations during the day at a hospital because of certain times people are given medication or certain times when procedures are performed.

Plots of concentration variability over time for the eight pharmaceuticals and caffeine showed that there was not consistent diurnal fluctuation for all compounds (Figure 9). The most the concentration changed within four hours out of all of the compounds was 0.01 micrograms per liter. This change is insignificant to conclude that the values greatly differed throughout the day. In addition, no trends were observed when comparing the different compounds at certain times of day. For almost all of the compounds (except oxycodone) the 24-hour composite sample was along the line of the four-hour composite samples. This shows that the sample collected representing the entire day is similar to the four-hour composite samples. In a few cases (butalbital, carisoprodol, and oxycodone) the grab sample was higher than the rest of the samples. This could mean that a one-time grab sample could be more concentrated than samples collected over time throughout the day. However, this difference between the grab samples and other samples is also very trivial and the largest difference seen is 0.01 micrograms per liter. Less variability was observed because these samples were effluent and the retention time at this WWTP is approximately one day. All diurnal graphs can be found in appendix 4.

Another set of graphs were made that show the percent difference between the 24-hour composite and the four-hour composites and the grab sample (Figure 10). These graphs, found in appendix 5, have percent difference on the vertical axis and date/time on the horizontal axis. The first bar on the graph (on the left side) is the percent difference between the grab sample and the 24-hour composite. The other bars display the percent difference between the four-hour sample and the 24-hour composite. There are also horizontal lines on the graphs at the 20% and -20% marks what values exceed 20%. Five percent difference graphs were created for compounds butalbital, hydrocodone, metaxalone, oxycodone, and carisoprodol. The greatest differences between the grab and integrated composite sample were observed for butalbital, oxycodone, and carisoprodol. There was minimal percent difference observed between the four hour composites and the 24-hour composite. These percent difference graphs also show no substantial trends or noteworthy differences.



<u>Figure 9:</u> Diurnal graphs for three pharmaceuticals; butalbital, metaxalone, and oxycodone at site NY9. The green triangle represents the concentration of the grab sample. The red square indicates the concentration of the 24-hour composite. The dots are the 4-hour composites connected by a line. These graphs plot concentration vs. time of day. Note that these graphs are not on the same scale because their variation is so small it would not be seen if they were all on the same scale.



<u>Figure 10:</u> Percent difference graphs for two pharmaceuticals; butalbital and metaxalone at site NY9. The first bar on the left side with the red star above it represents the percent difference between the grab sample and the 24-hour composite. The rest of the bars display the percent difference between the 4-hour composites and the 24-hour composite.

SUMMARY & CONCLUSIONS

PMF's are an understudied source of pharmaceuticals to the environment. This study, along with Phillips et al. (2010) and Larrson et al. (2007) displays that WWTP effluent of plants that receive discharge from PMFs have much higher concentrations than WWTPs that do not receive this type of input. The long-term graphs and three period time comparison from this study display the effect of PMF shutdown on concentrations in WWTP effluent. Four of the compounds (diazepam, butalbital, carisoprodol, oxycodone) at site NY2, where the PMF shutdown, display clear decreasing trends as the plant prepared for closure. From these graphs it is suspected that certain pharmaceuticals were slowly phased out, while others had a sudden stop in production. There are no known reasons or information from the PMF at this site as to why the

compounds were phased out on different time scales. Another example of pharmaceutical trends in the yearly analysis was seen in metaxalone at NY3. The decrease in metaxalone at this site is directly related to the verbal communication from the PMF owner that metaxalone production ceased at this plant. However, it is still alarming that the concentrations after production ceased are still much larger than effluent from a normal site that does not receive PMF input. The change in WWTP effluent concentrations after large scale changes from the source of input correlate with what was found in Stevens. Stevens found that large scale marketing changes and consumer use in certain products displayed multi-year trends in WWTP effluent. This study observed trends in pharmaceuticals from large scale changes such as PMF shutdown. In order to observe changes in pharmaceutical concentrations from a large-scale change such as PMF shutdown or ceased production of a type of pharmaceutical multi-year graphs must be used.

For most compounds seasonal changes could not be detected statistically. However, two compounds (oxycodone and phendimetrizine) did display seasonality only at site NY3. Both of these compounds displayed higher concentrations in the winter and spring than the summer and fall. These findings compare to several other studies that have found higher concentrations in the colder months (Azzouz and Ballesteros 2013, Coutu et al. 2013, Hedgespeth et al. 2012, Yu et al. 2013). Even though these two pharmaceuticals displayed seasonal variation, this was not seen in the other pharmaceuticals so a direct correlation of season to pharmaceutical concentration cannot be made. It is unknown why the seasonality for oxycodone and phendimetrizine at NY3 was seen. There could be multiple factors involved in this observation such as PMF production, temperature, or human use of pharmaceuticals.

It has been brought up by several studies, including Nelson et al. 2012 and Ort et al. 2010 that pharmaceutical concentrations can vary throughout the day. The results of the daily analysis at site NY9 in this study did not find any significant changes in concentrations in WWTP effluent throughout the day. The variability was very minimal because these samples were effluent samples, while the concentrations observed by Ort et al. (2010) were influent. Other factors attributing to small variability could include the selection of site, size of WWTP, or detection limit. Further investigations would have to be performed in order to draw any conclusions about hospital input and diurnal variability in pharmaceutical concentrations.

One compound, which was not a pharmaceutical, was considered in the study as a "control" of WWTP upgrades. Caffeine, having a high sensitivity to WWTP upgrades was added so changes in pharmaceutical concentrations can be directly related to PMF shutdown and not treatment plant upgrade. The only site where caffeine displayed decreasing trends was at NY1 which implemented new trickling filter media, new rotating biological contactors, microfiltration, and ultraviolet light during the years 2007 and 2008. Stevens (2012) also found that caffeine displayed a step-decreasing-trend at NY1 corresponding to WWTP upgrade. After the WWTP at NY1 received its upgrade, it efficiently removed caffeine but not butalbital and oxycodone. The results suggest that the plant upgrade at NY1 had no effect on the removal efficiency of these two pharmaceuticals. Site NY2 implemented microfiltration and ultraviolet in 2009, however removal efficiency at this site did not change from these upgrades. Since these upgrades at NY2 did not significantly affect removal efficiency, changes in pharmaceutical concentration were directly related to PMF closure.

The concentrations of pharmaceuticals can be studied using several different time scales. For this study most of the trends were found in the multi-year analysis because of large scale changes from PMF input or changes in WWTP treatment processes. No significant conclusions can be drawn concerning the seasonal and diurnal concentrations because these investigations did not display any substantial trends between the compounds or the sites.

WWTP effluent that receives input from PMFs is an understudied area in the topic of emerging contaminants in the environment. The concentrations of pharmaceuticals being released into the environment from PMFs are alarmingly high. The results from this study can greatly influence large decisions concerning PMFs. Stake holders and politicians may make decisions concerning PMF practices based on the data presented in this study. It could also encourage PMFs to create treatment programs to pre-treat their discharge before it goes to a WWTP. It is important to understand the effects from PMF shutdown or changes in production because these changes are directly related to the concentrations found in environmental samples. These environmental samples of effluent represent what is being put into the receiving surface water body in which organisms and humans rely on. Although direct effects from these emerging contaminants are not immediately seen, if they accumulate in ecosystems, organisms, and humans for many years we do not know what the end result may be.

Sources:

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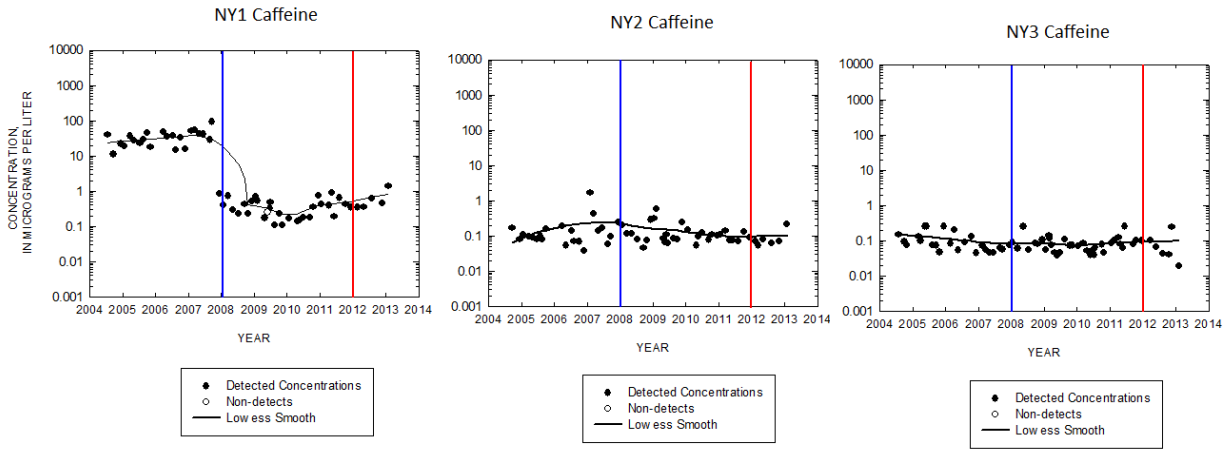
APPENDIX

Appendix 1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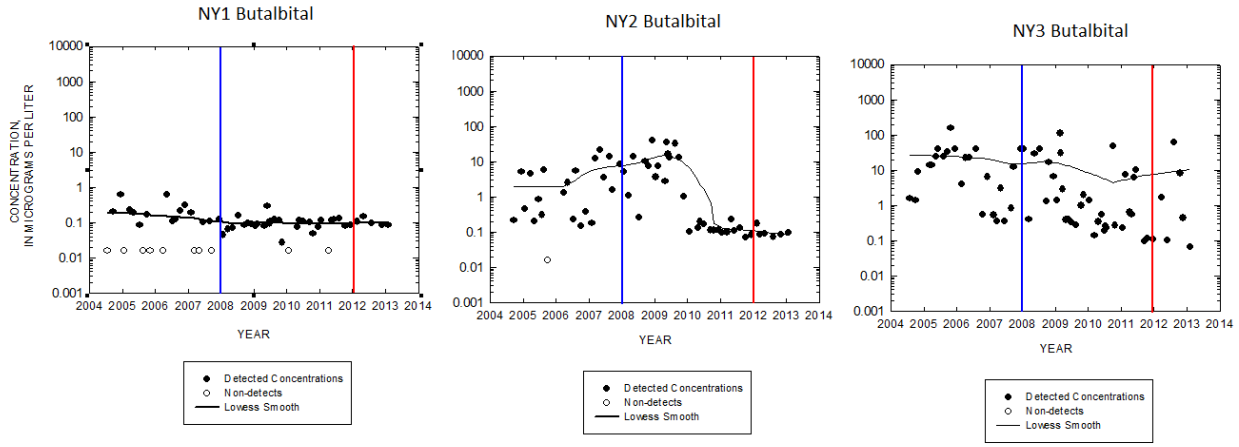
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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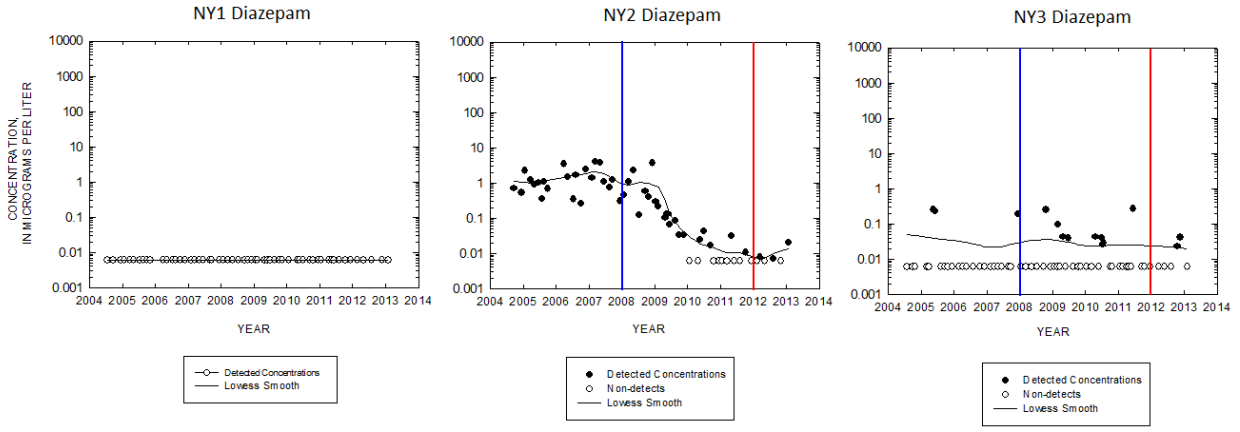
CONCENTRATION IN MICROGRAMS PER LITER
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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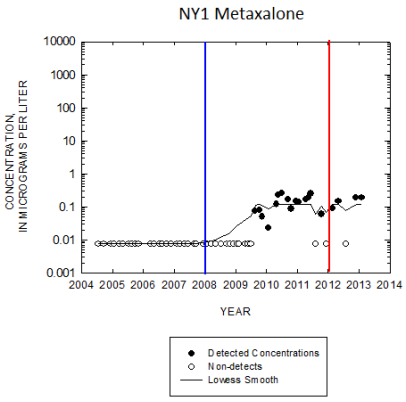
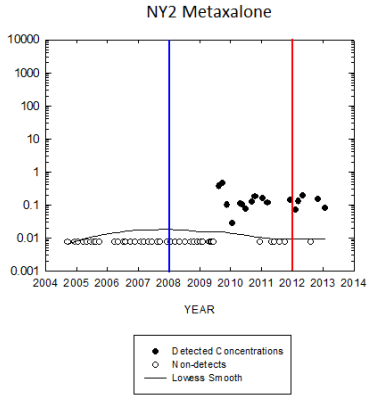
NY3 Diazepam
CONCENTRATION IN MICROGRAMS PER LITER
YEAR
Detected Concentrations
Non-detects
Lowess Smooth


NY1 Metaxalone
CONCENTRATION, IN MICROGRAMS PER LITER
10000
1000
100
10
1
0.1
0.01
0.001
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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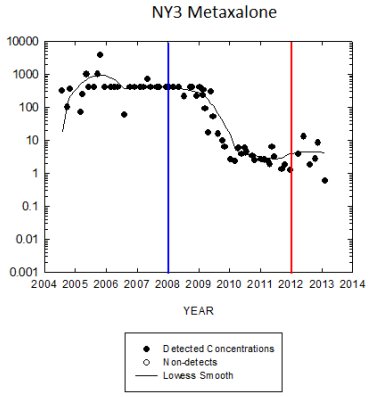
10000
1000
100
10
1
0.1
0.01
0.001
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth


NY3 Metaxalone
10000
1000
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0.1
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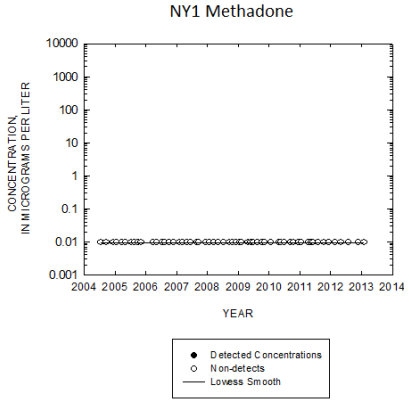
0.001
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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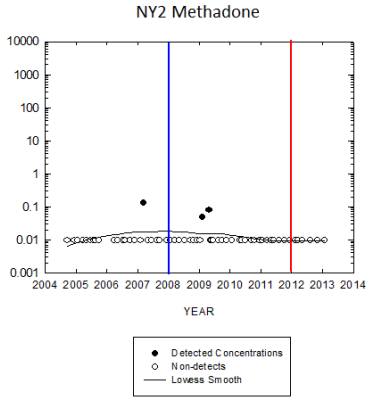
CONCENTRATION, IN MICROGRAMS PER LITER
10000
1000
100
10
1
0.1
0.01
0.001
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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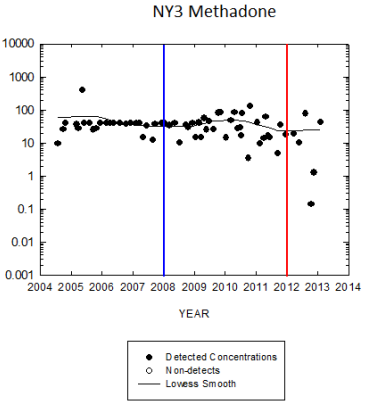
0.001
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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1000
100
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0.01
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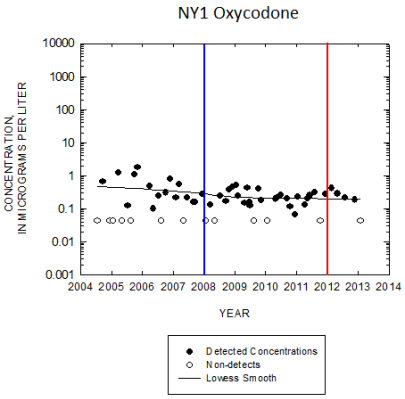
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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CONCENTRATION, IN MICROGRAMS PER LITER
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1000
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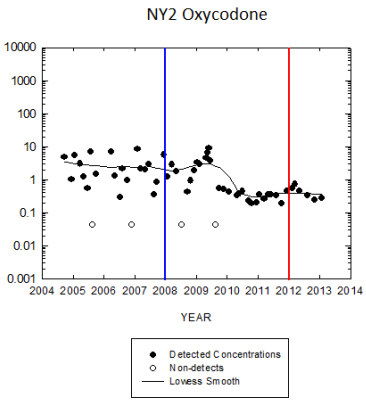
10
1
0.1
0.01
0.001
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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10000
1000
100
10
1
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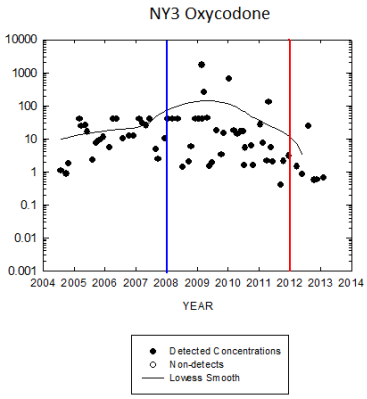
0.01
0.001
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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10000
1000
100
10
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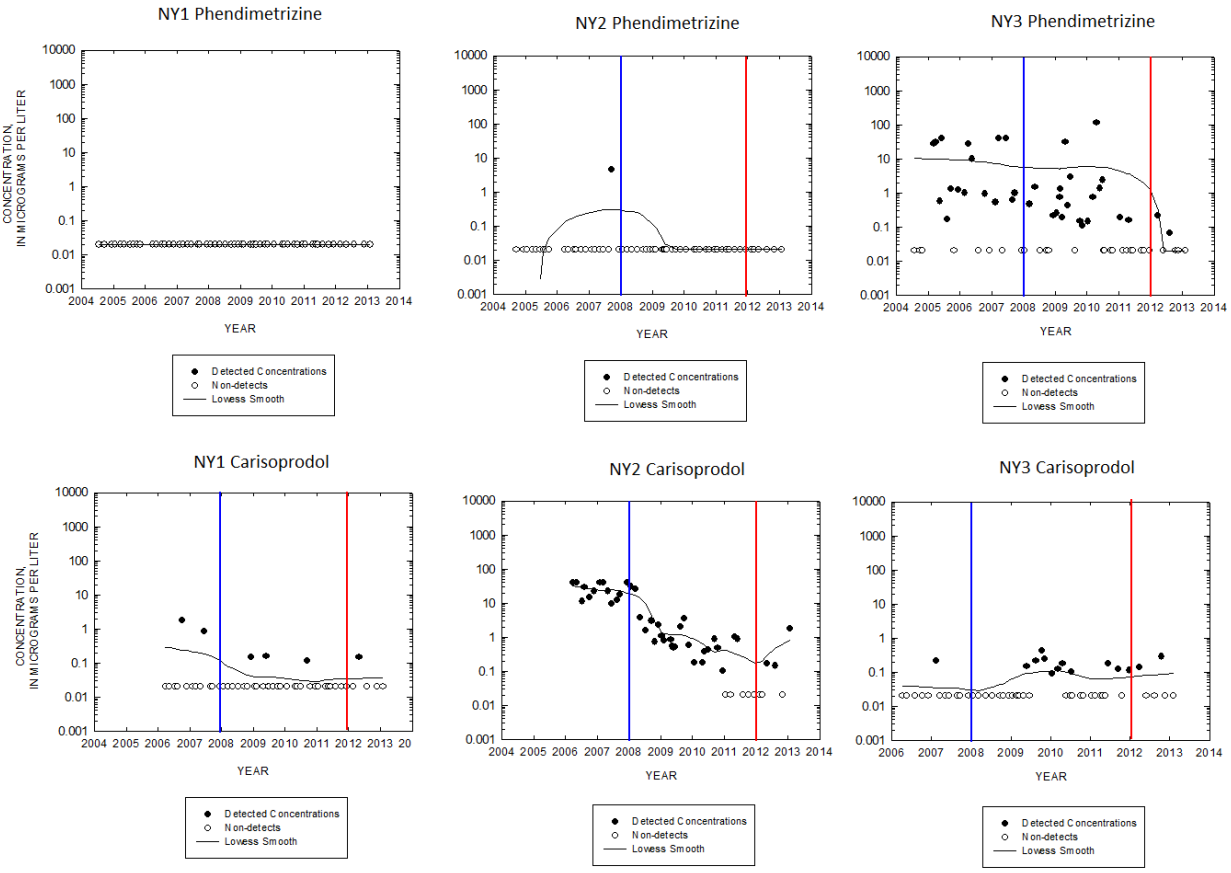
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
YEAR
Detected Concentrations
Non-detects
Lowess Smooth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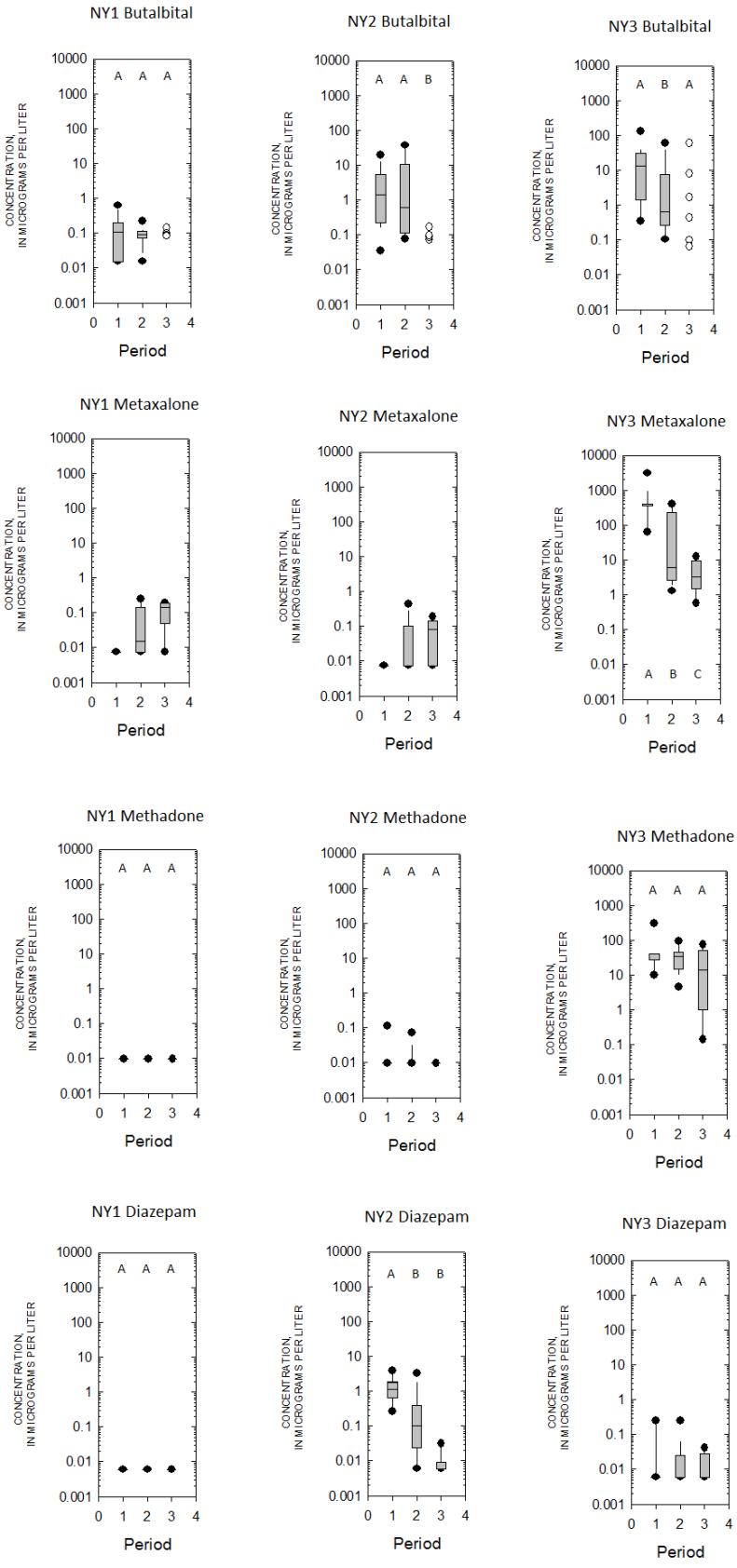
NY2 Phendimetrizine
NY3 Phendimetrizine
NY1 Carisoprodol
NY2 Carisoprodol
NY3 Carisoprodol
CONCENTRATION, IN MICROGRAMS PER LITER
YEAR
Detected Concentrations
Non-detects
Lowess Smooth

Appendix 2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NY3 Butalbital
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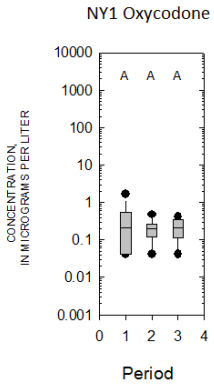
NY2 Metaxalone
NY3 Metaxalone
NY1 Methadone
NY2 Methadone
NY3 Methadone
NY1 Diazepam
NY2 Diazepam
NY3 Diazepam
CONCENTRATION, IN MICROGRAMS PER LITER
Period
A A A
A A B
A B A
A B C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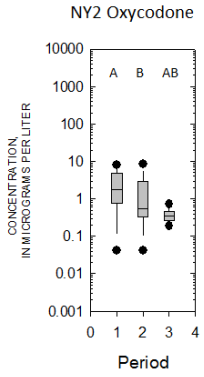
CONCENTRATION, IN MICROGRAMS PER LITER
Period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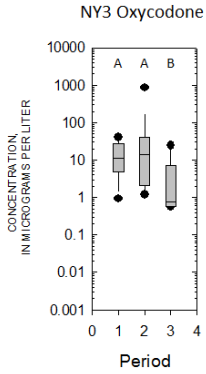
A B AB
CONCENTRATION, IN MICROGRAMS PER LITER
Period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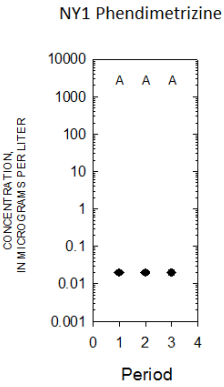
A A B
CONCENTRATION, IN MICROGRAMS PER LITER
Period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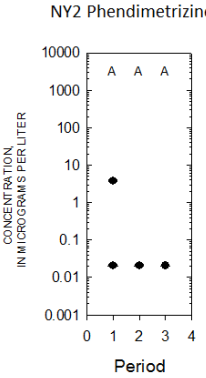
A A A
CONCENTRATION, IN MICROGRAMS PER LITER
Period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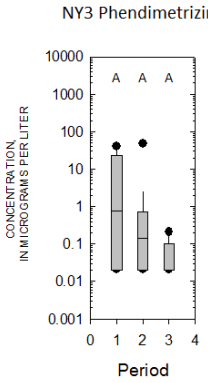
Period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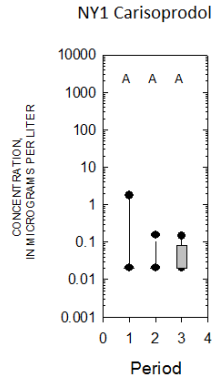
CONCENTRATION, IN MICROGRAMS PER LITER
Period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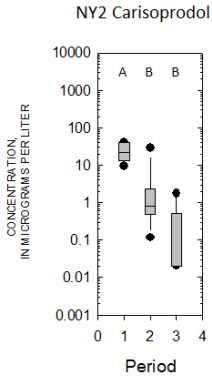
A A A
CONCENTRATION, IN MICROGRAMS PER LITER
Period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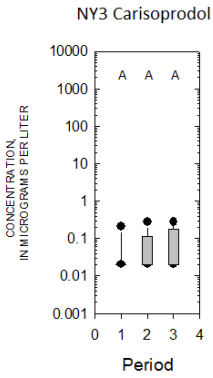
A B B
CONCENTRATION, IN MICROGRAMS PER LITER
Period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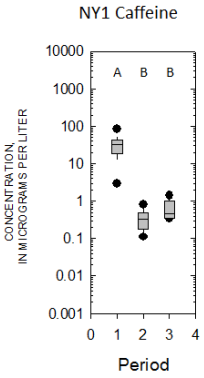
A A A
CONCENTRATION, IN MICROGRAMS PER LITER
Period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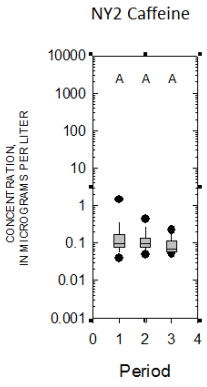
A B B
CONCENTRATION, IN MICROGRAMS PER LITER
Period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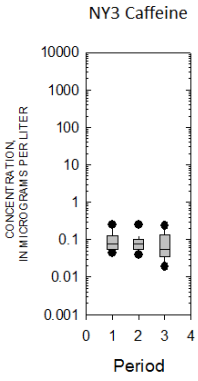
A A A
CONCENTRATION, IN MICROGRAMS PER LITER
Period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CONCENTRATION, IN MICROGRAMS PER LITER
Period

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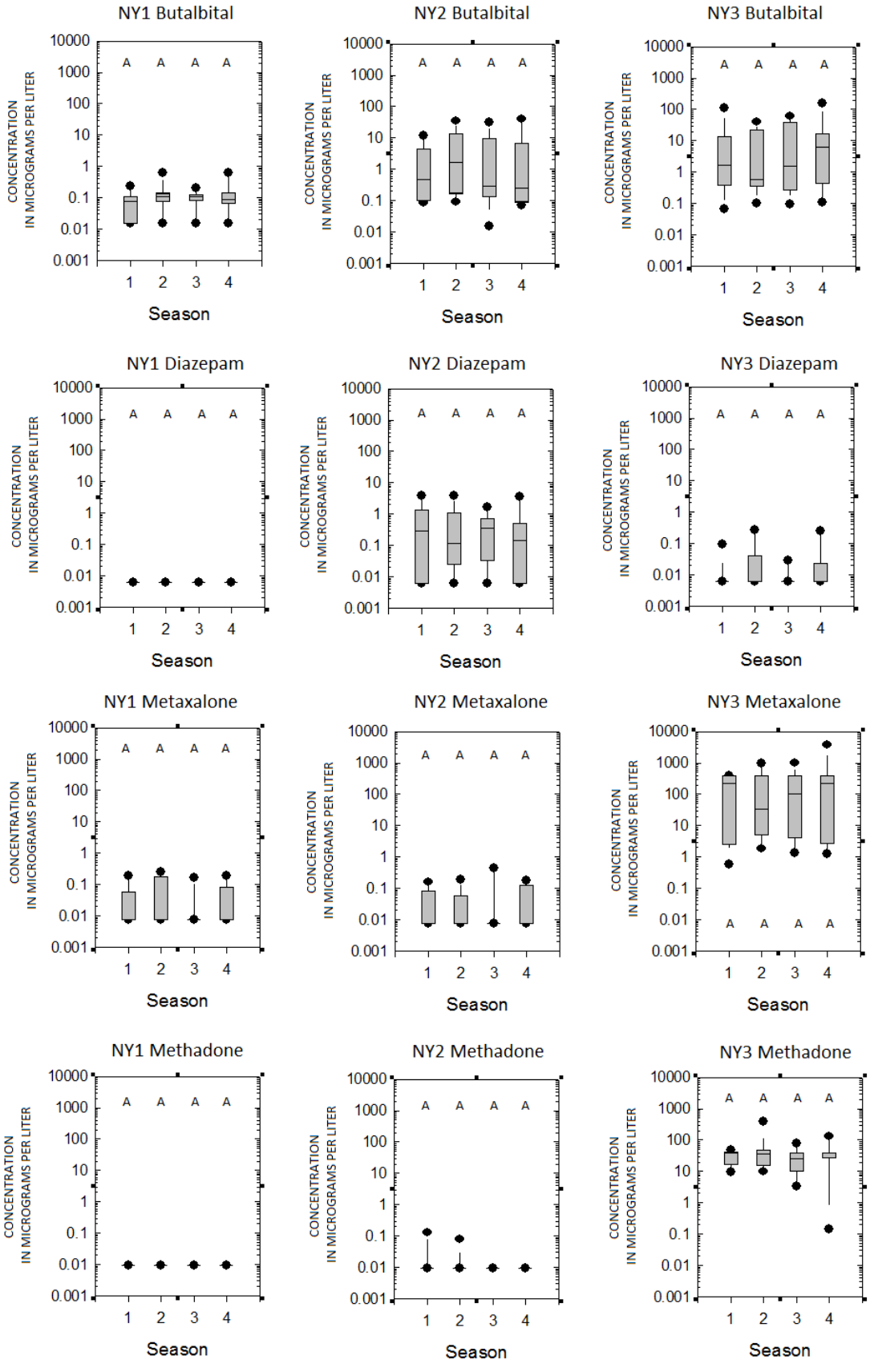
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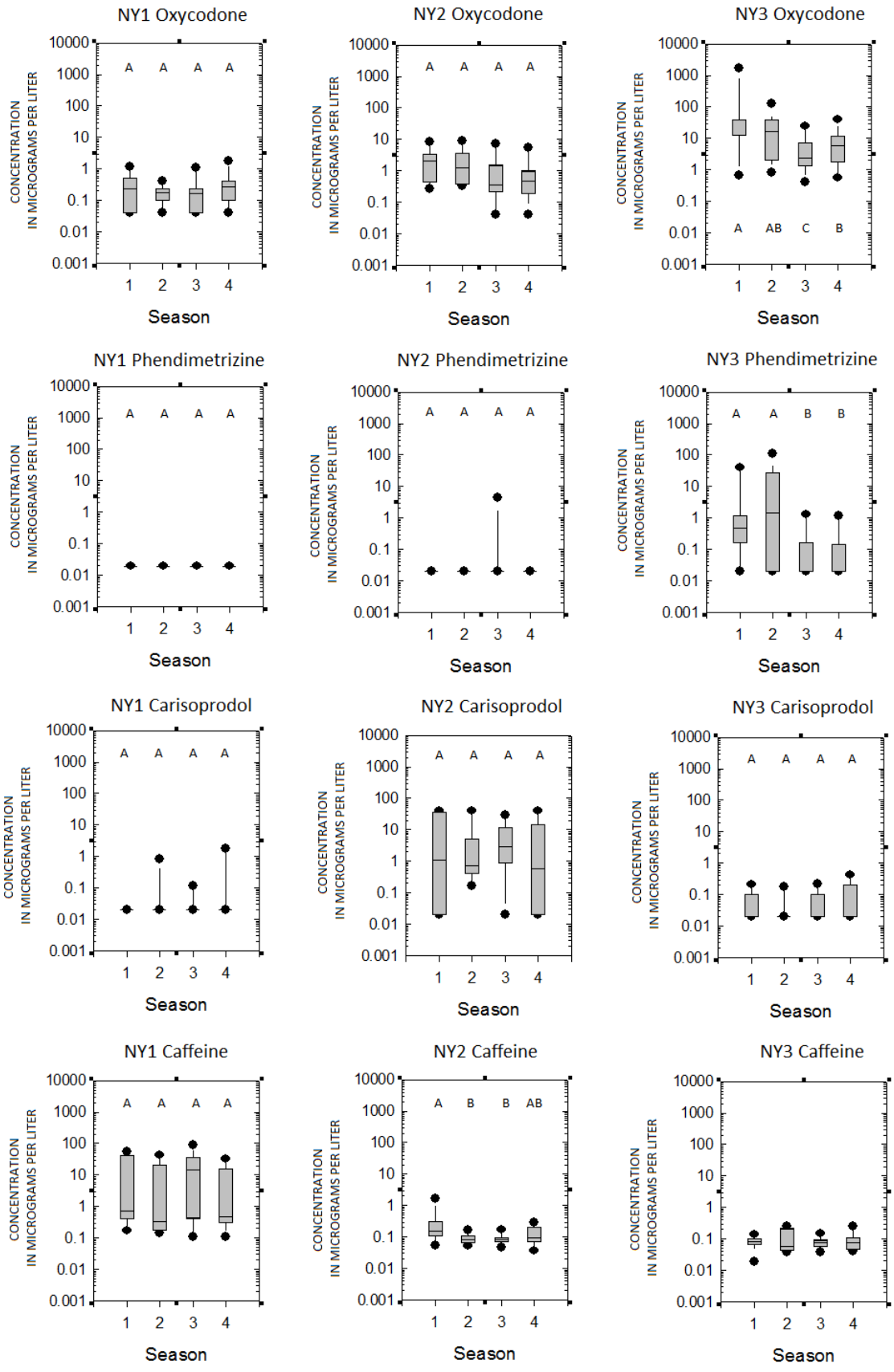
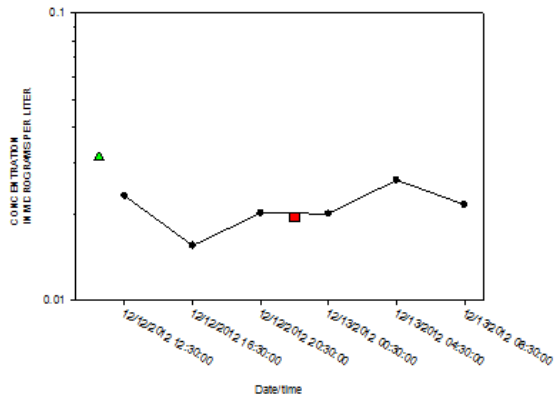
NY2 Butalbital
NY3 Butalbital
NY1 Diazepam
NY2 Diazepam
NY3 Diazepam
NY1 Metaxalone
NY2 Metaxalone
NY3 Metaxalone
NY1 Methadone
NY2 Methadone
NY3 Methadone
CONCENTRATION IN MICROGRAMS PER LITER
Season
10000
1000
100
10
1
0.1
0.01
0.001
1 2 3 4
A A A A


NY1 Oxycodone
NY2 Oxycodone
NY3 Oxycodone
NY1 Phendimetrizine
NY2 Phendimetrizine
NY3 Phendimetrizine
NY1 Carisoprodol
NY2 Carisoprodol
NY3 Carisoprodol
NY1 Caffeine
NY2 Caffeine
NY3 Caffeine
CONCENTRATION IN MICROGRAMS PER LITER
Season
A A A A
A AB C B
A A B B
A B B AB

Appendix 4

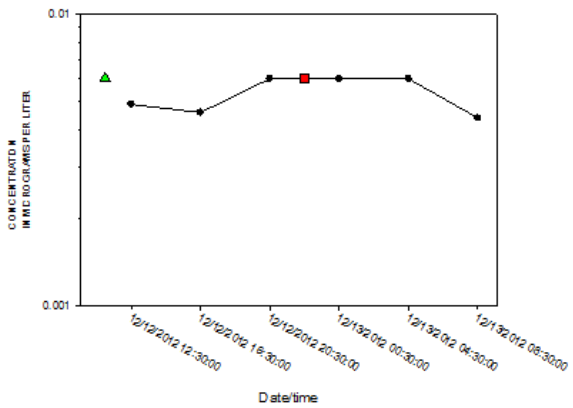
Butalbital

NY9 63768



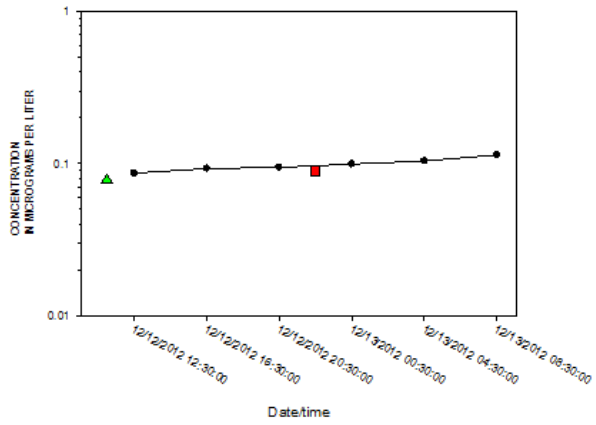
Diazepam

NY9 63770



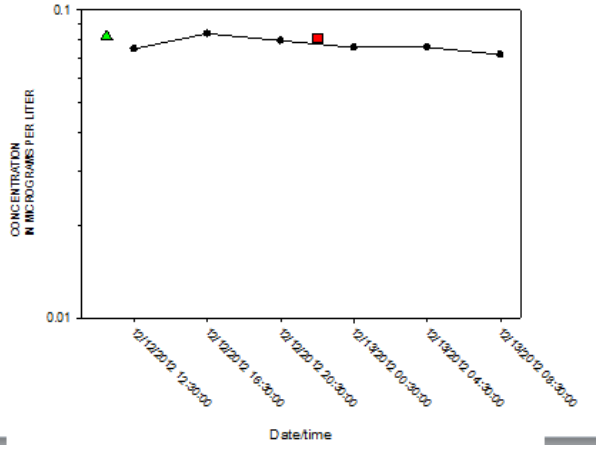
Metaxalone

NY9 63772



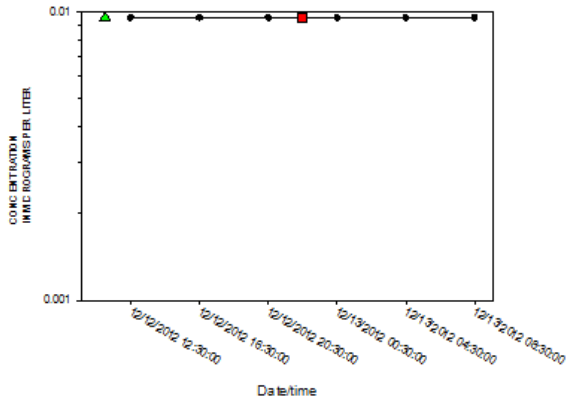
Hydrocodone

NY9 63771



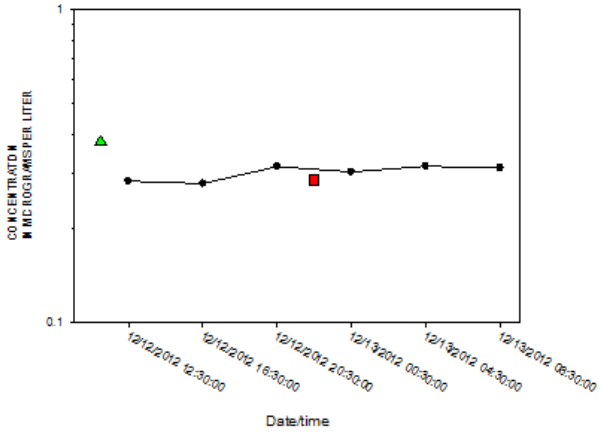
Methadone

NY9 63774



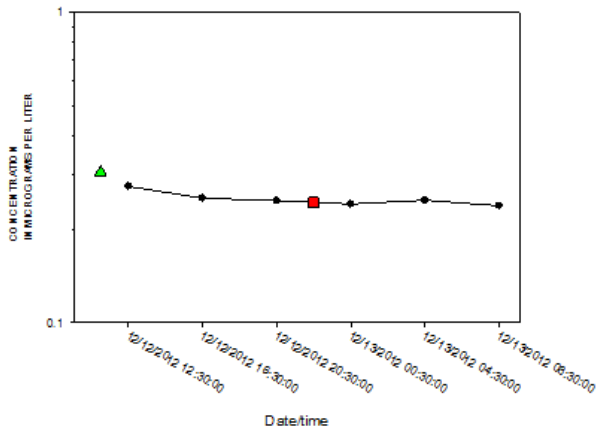
Oxycodone

NY9 63775

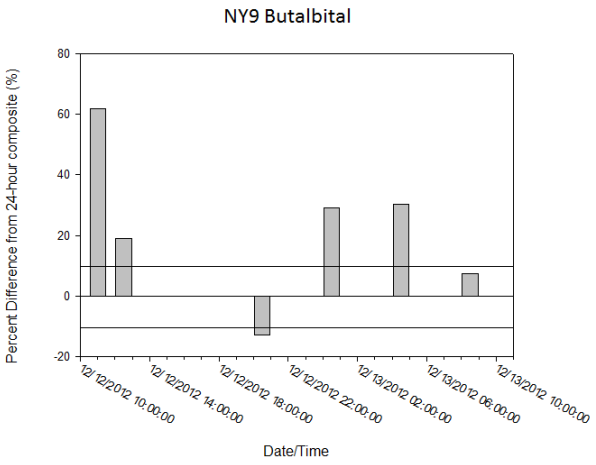


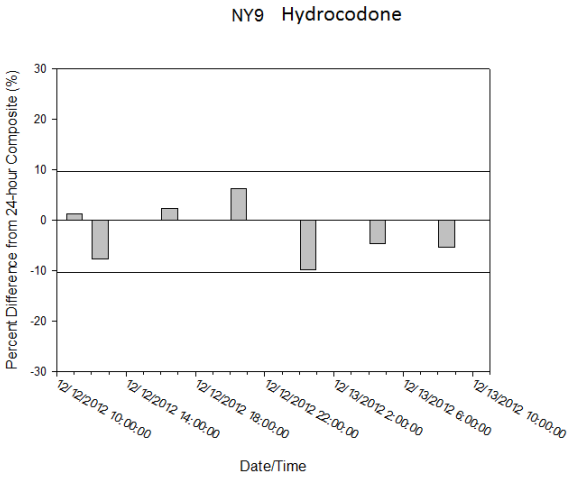
Carisoprodol

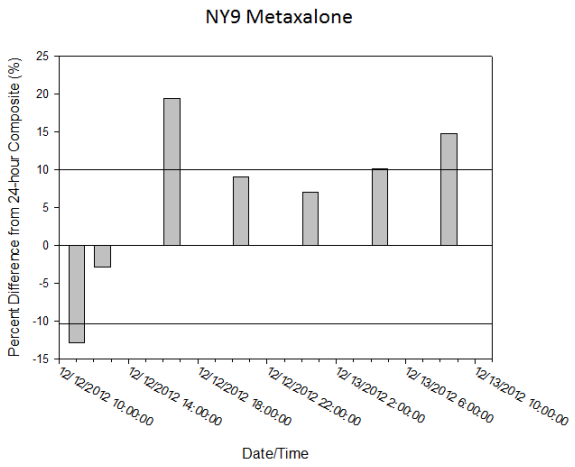
NY9 65180

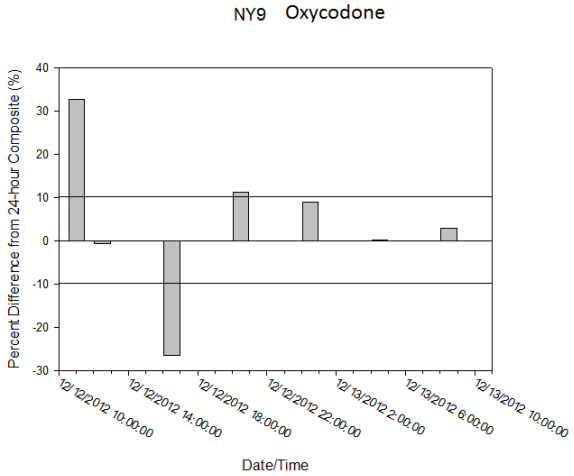


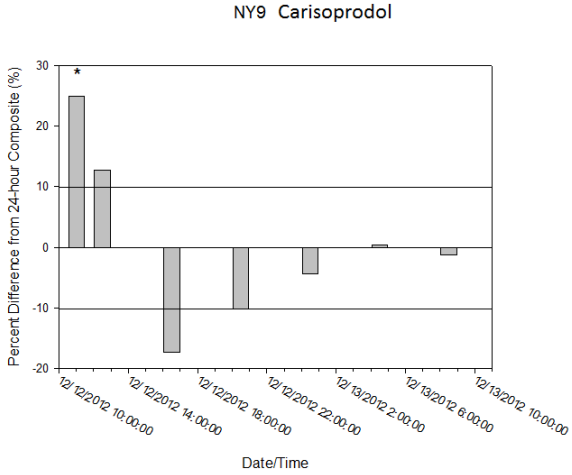
Appendix 5


NY9 Butalbital
Percent Difference from 24-hour composite (%)
80
60
40
20
0
-20
12/12/2012 10:00:00
12/12/2012 14:00:00
12/12/2012 18:00:00
12/12/2012 22:00:00
12/13/2012 02:00:00
12/13/2012 06:00:00
12/13/2012 10:00:00
Date/Time

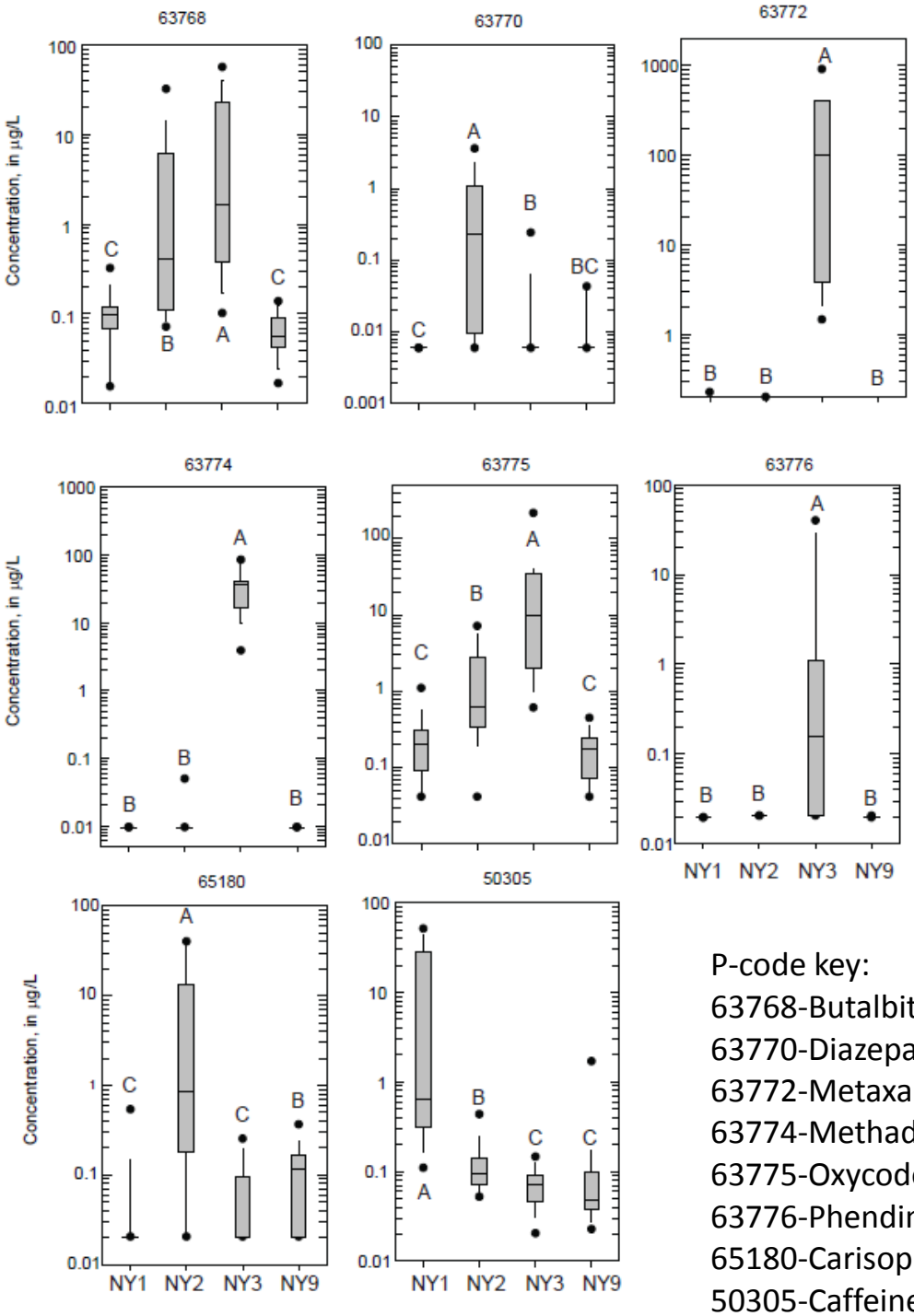

NY9 Hydrocodone
Percent Difference from 24-hour Composite (%)
30
20
10
0
-10
-20
-30
12/12/2012 10:00:00
12/12/2012 14:00:00
12/12/2012 18:00:00
12/12/2012 22:00:00
12/13/2012 2:00:00
12/13/2012 6:00:00
12/13/2012 10:00:00
Date/Time


NY9 Metaxalone
Percent Difference from 24-hour Composite (%)
25
20
15
10
5
0
-5
-10
-15
12/12/2012 10:00:00
12/12/2012 14:00:00
12/12/2012 18:00:00
12/12/2012 22:00:00
12/13/2012 2:00:00
12/13/2012 6:00:00
12/13/2012 10:00:00
Date/Time


NY9 Oxycodone
Percent Difference from 24-hour Composite (%)
40
30
20
10
0
-10
-20
-30
12/12/2012 10:00:00
12/12/2012 14:00:00
12/12/2012 18:00:00
12/12/2012 22:00:00
12/13/2012 2:00:00
12/13/2012 6:00:00
12/13/2012 10:00:00
Date/Time


NY9 Carisoprodol
Percent Difference from 24-hour Composite (%)
*
30
20
10
0
-10
-20
12/12/2012 10:00:00
12/12/2012 14:00:00
12/12/2012 18:00:00
12/12/2012 22:00:00
12/13/2012 2:00:00
12/13/2012 6:00:00
12/13/2012 10:00:00
Date/Time

Appendix 6



P-code key:
63768-Butalbital
63770-Diazepam
63772-Metaxalone
63774-Methadone
63775-Oxycodone
63776-Phendimetrizine
65180-Carisoprodol
50305-Caffeine