Potential Trends in Attention Deficit Hyperactivity Disorder (ADHD) Drug Use on a College Campus: Wastewater Analysis of Amphetamine and Ritalinic Acid

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Attention Deficit Hyperactivity Disorder (ADHD) medication use is on the rise in the United States. The most widely used ADHD medications are the amphetamine-type compounds Adderall (mixed amphetamine salts) and Ritalin (methylphenidate). According to survey data ADHD medications are used as a study drug or "Smart Drug" by students without a prescription on college campuses. Survey data of non-prescribed drug use has limitations with accurate reporting and no empirical data of usage exists in the literature. This study looks for trends in the use of these drugs on a college campus among low-stress and high stress periods. The metabolites of these two drugs, amphetamine and ritalinic acid, are quantified in campus wastewater using solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Trends show a possible increase in amphetamine levels during periods of high stress such as midterms, the last week of classes and finals week over levels from the baseline low stress weeks such as the first week of classes. Both semesters from the 2011-12 academic year were studied and the highest increase over baseline (760%) occurred during finals week of the second semester. Ritalinic acid levels gradually climbed first semester but had no obvious periodic trend second semester.

Keywords:
Sewage analysis
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Highlights

- First evidence-based approach to look for non-prescription abuse of ADHD medication.
- Usage data from nine weeks over two semesters on a college campus.
- Trend found between increased amphetamine use and academically stressful periods.

1. Introduction

The detection, measurement, and fate of pharmaceuticals and personal care products (PPCPs) in

aquatic environments such as surface and wastewater has been the focus of rapidly increasing study over the past two decades (Daughton and Ternes, 1999; Daughton and Ruhoy, 2009). More recently the measure of illicit drugs and their metabolites have also gained focus in aquatic environments (Castiglioni et al., 2011; van Nuijs et al., 2011). Unlike pharmaceuticals whose quantities released into the environment can be better estimated with sales data, illicit drug consumption has, until recently, been estimated with surveys, interviews, medical records, and crime statistics. These general indicators have been shown to significantly under report usage (E. Zuccato et al., 2005). The vast majority of these PPCP and illicit drug studies have included sampling at wastewater treatment plants (WWTPs) involving measurements of either influent or effluent water or often both (Jones-Lepp et al., 2004; Castiglioni et al., 2006a; Castiglioni et al., 2006b; Batt et al., 2008; Gros et al., 2009; Thomas et al., 2012). The monitoring of illicit drugs and their metabolites can be studied to answer human toxicological questions about abuse levels for populations (Van Nuijs et al., 2011). Thus far mostly large populations such as cities and regions have been surveyed with these data. One study moved upstream from a WWTP and sampled wastewater from smaller populations at fitness centers taken directly at their building outlets before entering the municipal sewer system (Fr. Schröder et al., 2010). It has been hypothesized that moving sampling even further upstream could detect illegal drug use in Olympic villages; however, this application has been debated (Katsoyiannis and Jones, 2011; Harman et al., 2011). The debate comes because as this field of sewer epidemiology evolves, merely finding a metabolite of detectable concentration is not sufficient. Reliable and proper sampling and the associated sampling uncertainty has recently become as much of an issue as the detected drug levels (Ort et al., 2010a,b; Mathieu et al., 2011; van Nuijs et al., 2012; Castiglioni et al., 2012; Castiglioni et al., 2013).

While many papers report levels of pharmaceuticals or illicit drugs in the environment, there has been less mention of pharmaceutical overuse and/or non-prescription use. One such non-prescription concern is the use of Attention Deficit Hyperactivity Disorder (ADHD) medication as a “Smart Drug” (Talbot, 2009). These medications come in two general forms, amphetamine containing salts and methylphenidate. The most popular amphetamine type drug is Adderall and to a lesser extent Vyvanse. Methylphenidate is best known as Ritalin but other formulations include Concerta and Focalin. Use of these compounds as “Smart Drugs” (and to a lesser extent for pleasure) has been documented with surveys in the toxicology and drug abuse literature as well as the popular US media (Babcock and Byrne, 2000; Kaye and Darke, 2012; Williams et al., 2004; Trudeau, 2009).

Adderall and Ritalin are used as study aids to enhance executive functions which allow students to focus for long periods of time (Farah et al., 2004). This neurocognitive enhancement has a potential benefit during academically stressful periods such as midterms or final exams. These drugs are reportedly easy to find for college students, either obtaining extra drugs from friends prescribed the drugs (5% of the entering class of 2011 nationwide were diagnosed with the disorder) or by falsely listing ADHD symptoms to a medical provider and obtaining a prescription (Johnson, 2011; Talbot, 2009). A study that used the 2001 College Alcohol Study survey of 119 American 4-year colleges and universities reported approximately 6.9% of the students had non-medical use of either methylphenidate or amphetamine in their lifetime and 2.1% in the past month (McCabe et al., 2005). Survey data of college students found the prevalence rate of non-prescriptive Ritalin and/or Adderall use to range from 0% to 25% for past

year use and 0% to 13% for past month use (Shillington et al., 2006). A 2012 review paper by Kaye reported that earlier studies had shown that Ritalin was the stimulant of choice on college campuses but the report continues by citing college students now misuse amphetamine salts at a higher rate and more than their non-student peers (7.9% versus 5.4% with the trend reversed for methylphenidate (1.7% versus 2.7%) (Kaye and Darke, 2012).

To detect and quantify the use of these drugs in wastewater, the metabolites must be known. Adderall is excreted in the urine as 30-40% intact amphetamine (Adderall Prescribing Information) and Vyvanse (lisdexamfetamine dimesylate) is excreted as 42% amphetamine (Vyvanse Prescribing Information; Krishnan et al., 2008). Ritalin, Ritalin-SR (methylphenidate), Concerta and Focalin (dexmethylphenidate) are excreted as α-phenyl-2-piperidine acetic acid (ritalinic acid) at 86% in adults (we assume our population to be all adults 18+ years of age) (Concerta Prescribing Information, Focalin Prescribing Information, Ritalin Prescribing Information). Previous studies have found these compounds in the raw influent at WWTPs. Amphetamine levels at WWTPs in the US have been shown to range from 80 to 550 ng/L (Chiaia et al., 2008). The only reported study to measure ritalinic acid in wastewater reported "high variability" ranging from <50 to 270 ng/L in effluent samples from Germany (Letzel et al., 2010).

One small population has been sampled on a college campus for seven drugs of abuse (Panawennage et al., 2011). It was noted that the highest level of amphetamine in the wastewater came on a day during finals week but no subsequent analysis was made. To the authors' knowledge, this is the first comprehensive investigation to determine the non-prescription abuse of ADHD medication by sampling wastewater and using an evidence-based approach. This type of wastewater epidemiology requires measurement of very specific sewer systems, upstream from WWTPs. The sampled population in the following study was made up of nearly 500, 18-22 year old men and women sampled from their dormitories at a residential college in the Pacific Northwest. Sampling occurred throughout the academic year and included periods of low and high academic stress. Although the number of individuals in the population was well known, the human marker creatinine was included in the analysis to account for variations in dilution among sampling periods and to estimate sampling uncertainty (Chiaia et al., 2008; Smith-Palmer, 2002; Barr et al., 2005).

2. Methods

2.1 Standards and Reagents

(±)-amphetamine, (±)-amphetamine-D6, (±)-methamphetamine-D9, ritalinic acid, and (±)-threo-ritalinic acid-D10 were purchased from Cerilliant (Round Rock, TX). Creatinine standard and reagent were purchased from EnzoLife Sciences (#ADI-907-030A Farmingdale, NY). SPE conditioning and mobile phase solvents used Milli-Q-purified water (Milford, MA) and HPLC grade ChromaSolv methanol and acetonitrile from Sigma-Aldrich (St. Louis, MO) and ACS grade formic acid, glacial acetic acid and hydrochloric acid were from EMD chemicals (Gibbstown, NJ). The purchased standards were diluted in 0.5% acetic acid.

2.2 Sample Collection

Raw wastewater composite samples were collected for 72-hour periods at 1 hour intervals, (125 mL each draw) with a Teledyne ISCO 6712 portable sampler (Lincoln, NE). An ISCO 2150 continuous wave Doppler flow meter logged instantaneous flow at 15 minute intervals and these data were used to integrate total volume over the 72 hour period. These devices were placed in a manhole in the sanitary system of the four dorms (Schematic shown in Figure S1). This sewer line services these dorms and the sampling site is the last accessible point on campus before the sewer line connects to the municipal sewer. This sanitary line is not connected to surface water lines and is thus not susceptible to high flows from rain events. A subsequent fluorescein dye test was used to determine the length of individual toilet flushes from each of the dorms within this gravity fed system. Residence time between the point of entry and the sampler were 5-7 min and 7-30 min at typical high and low flow rates, respectively. The fluorescent dye persisted for 7-12 minutes at high flow rates and 14-30 min at low flow rates. Table 1 shows the dates, times, total flow volume, and time during the semester for each of the nine sampling events. Initially only four samples were planned each semester but by the spring semester, a fifth sample was included during the high stress of the last week of school. The composite samples were filtered and extracted onto SPE cartridges within 6 hours of the last draw event.

2.3 Creatinine

An Enzo Life Sciences colorimetric detection kit was used for creatinine analysis. This kit is designed for urine samples; however, the kit requires a minimum of 1:20 dilution of urine before testing. This urine dilution factor placed the undiluted concentrations found in the composite campus effluent within the calibration region. The colorimetric test works via a modified Jaffe's reaction with picric acid. The acidified wastewater was filtered through a 0.2 μm syringe filter and the analysis was performed as the kit directed with five replicate aliquots.

2.4 ADHD Metabolites

2.4.1 Solid Phase Extraction

All composite samples were collected in 10 L glass containers. These samples were brought to pH 2 with concentrated hydrochloric acid after collection. The samples were then rough filtered with extra-fast paper followed by glass fiber GF/A. The composite sample was analyzed in triplicate with 100 mL aliquots spiked with 20 ng of methamphetamine-D9 as a method surrogate. Waters (Milford, MA) Oasis MCX 3cc 60mg SPE cartridges were conditioned with 2 mL of methanol then equilibrated with 3 mL ultra-pure water at pH 2. The SPE cartridges were loaded with sample at approximately 5 mL/min. The cartridges were then washed with 2 mL of 2% formic acid followed by 3 mL of methanol before eluting the analytes in 5 mL of 2% NH_4OH in methanol. The eluates were dried under a stream of nitrogen at 30 °C and redissolved in 200 μL of a 0.5% acetic acid solution containing 20 ng of the internal standards amphetamine-D6 and ritalinic acid-D10. These solutions were transferred to autosampler vials containing glass inserts for analysis.

2.4.2 Liquid Chromatography/Tandem Mass Spectrometry

Liquid chromatography was performed on an Agilent (Santa Clara, CA) 1290 UHPLC binary pump system. The column was a Phenomenex Kimetex C-18 (100 mm × 2.10 mm i.d. with 2.6 μm particle size) and thermostatted at 30 °C. Separations were performed with 5 μL injections

and a constant 0.35 mL flow rate over a gradient consisting of (A) 0.5% acetic acid and (B) 100% acetonitrile. Initial conditions were 0% (B) linearly increased to 40% at 5 min, then additionally increased to 100% (B) at 6 min and returned to 0% (B) at 7 min. The column was then allowed to equilibrate for 9 min for a total run time of 16 min. Mass spectrometry was performed on an Agilent 6460 triple quadrupole mass spectrometer with a Jet Stream spray source. The gas temperature was 300 °C at a flow of 3.0 L/min. The nebulizer was set at 45 psi and the sheath gas flow was 5.0 L/min at 250 °C. The capillary potential was set at +3000 V. Conditions for target Selected Reaction Monitoring (SRM) analysis are given in Table S1.

2.4.3 Quantitation

Table 2 shows both the instrumental and method lower limit of quantification (LOQ) and spike recoveries. The instrumental LOQ was determined using the mean and 10 × standard deviation from a "blank" composite urine sample (2 adult males and 2 adult females) diluted with campus tap water to reach the creatinine levels of typical wastewater. These sub-ng/ml LOQs are somewhat of a moot point since all SPE eluants from the composite samples contained nearly 100 ng/ml or more of each analyte. Calibration was achieved with a seven point calibration curve using 0.5, 5, 25, 50, 250, 500, and 2500 pg of each analyte on column and r^2 was typically >0.999. Comparison of calibration curve slopes for all analytes during various instrument runs resulted in % RSD ranging from 2.87-6.38%. Accuracy of standards was checked against the calibration curve routinely during instrumental analyses and ranged from 95.3-100.26%. Method recoveries used Milli-Q water and were surprisingly low compared to other literature recoveries of the same compounds. Low recoveries persisted for samples extracted with SPE followed by the dry down step and with just a spike and dry down sample. If the dry down step was removed and just the internal standard/reconstitution solution was spiked, the recovery was high and reproducible. We cannot account for the reason for the loss at the drying step; however, Table 2 shows that when the metabolites were corrected for loss of surrogate in the spike/recovery experiments the amphetamine and ritalinic acid levels were nearly 100% and the % RSD improved.

2.4.4 Stability in Wastewater

The stability of creatinine was studied over a 72 h period with a grab sample that was spiked with creatinine standard. Since other studies have shown creatinine loss, we spiked our grab sample to obtain a value close to the high end of the calibration curve such that loss could be quantified throughout the 72 h period. (Brewer et al., 2012) The spiked sample was placed in a cool bath maintained at an average temperature of 12.9 °C. During this time, the site where collection occurred was monitored at an average temperature of 11.5 °C (range 10-13 °C) which was insulated from the outside temperature that averaged 5.0 °C (range 3-7 °C). The spiked grab sample was assayed at eight time periods over 72 h with at least 4 replicates at each time point.

The stabilities of the drug metabolites were studied over a 72 h period with two, 3-liter grab samples of raw wastewater. One sample was sterilized by autoclave and both were spiked with 3.0 µg of amphetamine and ritalinic acid (1ppb). The two samples were each kept at roughly 20 °C while eight aliquots were subsampled in triplicate over a 72 h period. The temperature of the bulk composite samples during collection was not recorded; however, the portable sampler was kept under ground and it is unlikely that the temperature was ever as high as 20 °C. Other studies have shown that amphetamine does not degrade in wastewater over 24 h or 72 h periods

at 4 °C, nor at 20 °C up to 12 hours and is even stable at 37 °C for 72 hours and longer in urine (Brewer et al., 2012; Castiglioni et al., 2006b; van Nuijs et al., 2012; Jiménez et al., 2006) Thus the tested temperature acted as a conservative, worst case scenario for the degradation analysis of amphetamine-type compounds.

2.5 Sampled Population

The sampled population in this study was 476 college-aged students in four dormitories at a private, liberal arts, residential college in the Pacific Northwest. Sampling occurred throughout the academic year and included four 72 h periods of low academic stress and five 72 h periods of high academic stress. The number of student ADHD prescriptions was obtained for this population. The office of residential life provided the student health center with a roster of names of those students living in the sampled dormitories. The health center staff was able to compare the medical records of these students and provide a tally of ADHD medication prescriptions for the sampled population. Of the 476 students, there were 15 prescriptions for amphetamine-type medications and 9 methylphenidate prescriptions. These numbers are presumed to be quite accurate although they may be slightly conservative since some students may not report their prescription to the health center. The population size is fixed as all students were residential for both semesters and the facilities were restricted to just residents by card swipe access.

3. Results and Discussion

3.1 Wastewater flow and sampling

Student water use behavior was fairly consistent among each of the sampling periods. Total flow was different among weeks but peak periods were consistent. A comparison of flow data from the nine weeks is shown in Figure S2. Wastewater flow was presumably generated from toilets, showers and clothes washing facilities in the residence halls. Each of the first weeks of school reported the lowest water usage for that semester. This may come from the least amount of clothes washing needed upon their return to the dormitories. If water usage and thus wastewater flow was not consistent across the nine sampled periods, then a week where the absolute concentration of amphetamine or ritalinic acid was low could be from either low usage or high dilution. A human urine marker compound was needed to normalize analyte concentration. Caffeine is often used as a human marker; however, it was assumed that caffeine intake would also fluctuate during times of academic stress and therefore creatinine was determined to be a more suitable marker.

This study was designed to look at trends in ADHD medication use and initially consistent hourly draws were determined to be acceptable. Relatively long sampling intervals have since been questioned by Ort and colleagues (Ort et al., 2010a,b; Mathieu et al., 2011) who have performed rigorous studies to measure and model uncertainty associated with sampling. We realize that hourly, time-proportional sampling is not ideal; however, in 2010 when permission was being obtained and sampling routines were finalized much of the questions involving sampling uncertainty had not yet been standardized. Our one hour sampling frequency was established due to experience with inherent issues sampling raw wastewater at near-source locations. Unlike nearly every other wastewater study that has been performed, where sampling

occurs further downstream at a WWTP, our near-source location has a higher solid content (sanitary tissue, feces, etc..) which has not been broken down with the turbulent travel of the sewer system. These solids can more easily result in temporary clogs of the automated sampler causing "non-detect" events and thus no sample obtained. Larger volume draws (>100ml) by the sampler help alleviate these events. These large volume draws at a higher frequency would have overwhelmed the total volume of the sampler over the 72 hour period and we did not have daily access for shorter collection periods. In light of the non-ideal nature of hourly sampling we have attempted to quantify the uncertainty associated with this sampling mode below.

3.2 Creatinine Analysis

3.2.1 Creatinine Levels

Stability analysis showed that creatinine levels in wastewater stayed constant for the first 48 hours but by 72 hours there was a 17% loss (Figure S3). Since the composite samples were over 72 hours there is no doubt some loss due to degradation; however, all composites are assumed to lose proportionally equal amounts. The results from the creatinine analysis without any correction for stability are shown in Table 3. Average creatinine concentration levels in wastewater were found by colorimetric assay to be between 1.9 and 10.7 mg/L. As seen in Figure 1, the 72 hour period with the lowest creatinine concentration corresponded to the largest total wastewater volume passing the sampler of the nine weeks. The highest creatinine concentration, 10.7 mg/L, came from the period of lowest total volume. This creatinine concentration was more than twice that of another low volume period. We hypothesize that the reason for this high concentration is that the 72 hour period occurred during a week in January where winter storms shut down the academic functions of the campus for two days. Thus the students were mostly confined to their dorms, as was their creatinine excretion. Table 3 shows the calculated average mass of creatinine per person per day. Twenty-four hour average creatinine excretion in the urine is typically between 1000 mg to 3300 mg (Smith-Palmer, 2002). Table 3 shows that creatinine ranges from 390 mg/person/day to 950 mg/person/day among the nine sampling periods. These low values are expected and seem reasonable due both to the degradation of creatinine during storage and because of the students' activities that lead to excretion elsewhere on campus during the day.

3.2.2 Sampling Uncertainty using Creatinine

However, the more interesting aspect of the creatinine numbers is that it gives insight into the uncertainty associated with the study's sampling. The student's schedules are presumed to be quite consistent due to regular class meeting times. Excretion of creatinine at their dorms is also expected to be quite routine with urination coming before and after sleep and at least one other time during the day. This should lead to a fairly constant value for mass of creatinine/person/day being measured among the nine 72 hour sampling periods. These values in Table 3 deviate by 31% which conservatively can be attributed entirely to the uncertainty associated with sampling. This measure of sampling uncertainty was compared to modeled uncertainty calculated by Ort et al. for a similarly gravity fed sewer system (Ort et al., 2010b). In this paper the authors suggest that their model could, "retrospectively assess the sampling uncertainty of past sampling campaigns." Figure 3b from this reference shows time-proportional 24-hour sampling error listed by sampling interval and number of pulses per day. With a 60 minute time interval and roughly

1500 pulses per day (476 students with 3 pulses each) a sampling uncertainty of approximately 29% can be interpolated. This shows that our assumed uncertainty due to measured creatinine variability has excellent agreement with the literature. This figure can then be further used to determine sampling uncertainty for the drug metabolites as described below.

3.3 Stimulant Analysis

3.3.1 LC-MS/MS

Chromatograms for both metabolites and the surrogate in a typical wastewater sample are shown in Figure 2. The retention times from amphetamine, methamphetamine-D9 and ritalinic acid are 2.5 min, 2.7 min and 3.2 min respectively. The ratios of the quantifier to qualifier ions for all analytes in the samples and standards were consistent across the entire study and the %RSD ranged from <1% to 16%. In Figure 2, the amphetamine mass channel for both the quantifier and qualifier ion show other peaks from the matrix but the retention times and peak ratios are not consistent with the amphetamine standard. The other analytes show no other compounds eluting from the column at their mass transitions. These chromatograms show that even though the sampling occurred much further upstream than is typical, such as at a WWTP, the method was able to easily quantitate these ions in this matrix. Table S2 shows the mean SPE eluant concentrations, the recovery of the methamphetamine-D9 surrogate, and the calculated concentrations in raw wastewater adjusted for losses determined by the surrogate. These values show that as far upstream as this site was located, direct measurement of the wastewater (without SPE concentration) was almost possible since the concentrations in raw wastewater were near the edge of the linear calibration.

3.3.2 Stability of metabolites

The stability of the metabolites was calculated by determining the difference between the two spiked samples (autoclave and raw) relative to the spiked autoclave sample. Ritalinic acid appears to be stable at 20 °C for 72 h in the raw wastewater matrix since there was no significant decrease in concentration (Figure S4). Amphetamine appeared to decay linearly over the 72 h period. By the end of the 72 h period, the original spiked raw sample was 62% of the concentration of the sterile autoclaved sample. This decrease affects the absolute concentration of amphetamine but would not have an effect on the trends presented below because a correction factor would be equally applied to all weeks.

3.3.3 Amphetamine Trends

In this study amphetamine levels are assumed to be the metabolite of Adderall (or Vyvanse) and not from illicit sources. This assumption is based on the reported ease with which students can obtain these prescription pills. In order to better decipher the amphetamine origin, an analysis of the stereochemistry would have to be performed and this was beyond the scope of this study (Kasprzyk-Hordern and Baker, 2012). Other “false-positives” could come from methamphetamine use (Khan and Nicell, 2012) but reported use of methamphetamine on colleges campuses is low (Johnston, 2010). The amphetamine concentrations shown in Table S2 have uncertainties that only include instrumental uncertainty. As suggested by Mathieu, the uncertainty associated with each metabolite needs to reflect all of the independent components (Mathieu et al., 2011). A linear uncertainty propagation using relative standard deviations (RSD) was performed which took into account the analytical measurements (metabolite and creatinine),

the flow measurements, and the uncertainty associated with sampling. Tables 3 and 4 show the analytical uncertainties. The flow meter was calibrated before deployment and has an RSD of at most 1%. The sampling uncertainty was estimated using figure 3b from Ort et al. (Ort et al., 2010b). Significantly fewer flushes contained drug metabolites than creatinine as explained in section 3.2.2. For the metabolites, the estimation of daily flushes used the number of student prescriptions for each drug (amphetamine n = 15 and methylphenidate n = 9) and assumed three flushes per student. This is a conservative estimate since as the drugs are used by students without prescriptions the number of flushes increases and the uncertainty decreases. Flushes of 45 and 27 were used to interpolate from figure 3b in Ort et al., 2010b to give 50 and 62 as percent sampling uncertainty for amphetamine and methylphenidate, respectively. Table 4 shows the final values for each metabolite in ng per mg of creatinine with the associated total uncertainty.

The associated uncertainties are quite large and dominated by the time-proportional, 1 hour sampling uncertainty but Figure 3 still shows that some periods of high stress show greater levels of amphetamine than low stress weeks. Figure 3a shows that for the first semester, the largest increase in amphetamine occurs from the first week to midterms with a 3-fold increase. The levels drop back to the levels found during week 1 after midterms but then appear to increase again during finals over initial levels. A more definitive analysis would be possible with a different flow-proportional, high frequency sampling routine. The second semester begins at the same amphetamine level as the week 1 of the first semester and may increase at midterms week but does not appear to decrease after midterms during the second semester. For the second semester an extra sampling period was added during the last week of school which is also anecdotally reported to be high stress. The last week of classes were a 2.5-fold increase over the levels of the first week but again sampling uncertainty obstructs validation of whether this is a significant increase. The largest increase in amphetamine was seen during finals week of the second semester when 560 ng AMP/mg creatinine was measured. This is nearly a factor of 8 times higher than the first week of the semester. These measurements indicate that usage of drugs metabolized to amphetamine (presumably Adderall and Vyvanse) was indeed used at a higher rate during finals week on this college campus. The trends during the two semesters are not identically mirrored; however, it is not assumed that students' schedules or coping mechanisms are identical between semesters. The majority of the students in these residence halls are first-years and their introduction and adjustment to college is in flux the entire academic year.

3.3.4 Ritalin Trends

Ritalin's metabolite appears to have less of a trend with academically stressful periods during the academic year. During the first semester, ritalinic acid measured in the wastewater appears to increase as the semester proceeds to nearly an order of magnitude more during finals week as seen in Figure 3b. This may not be due to a trend but rather low usage by prescribed students during the first week of school and subsequent weeks show more normal use. This is supported by the ritalinic acid levels found during the first week of the second semester where the level is more consistent with subsequent weeks in the first semester. This lack of a trend with academically stressful periods is consistent with the survey results that found that misuse of methylphenidate was decreasing (Kaye and Darke, 2012).

3.4 Dose Analysis

The total calculated amphetamine over 72 hours can be used as a check for the above findings. Adderall capsules range in dosage from 5 - 30 mg total amphetamine and this leads to 1.5 - 9 mg amphetamine excreted (Panawennage et al., 2011). During week one of the first semester the total volume was 126,000 L at a measured concentration of 0.33 μg/L of amphetamine. This leads to 42 mg of total amphetamine over the 72 h period. Fifteen students taking one dose per day for three days would produce between 68 and 405 mg of amphetamine depending on the prescribed dose. However, it was previously discussed that not all of the students' urine would be collected at this site. During the first week of the first semester the value was 390 mg creatinine/person/day which is 12 - 39 % of typical daily creatinine excretion. This leads to the measurable mass of amphetamine being 8 – 158 mg at this site. The 42 mg of amphetamine calculated above is thus reasonable as it falls within this range. Furthermore, the total calculated mass would be closer to the middle of this range if the loss of amphetamine due to stability in the matrix was considered. Ritalin capsules range from 5 to 20 mg of methylphenidate and Concerta ranges from 18 to 54 mg of methylphenidate. With a metabolism of 80% and unmeasurable losses as shown by creatinine levels, 9 students taking one dose per day should eliminate 13 – 1061 mg of ritalinic acid over 72 hours. The low stress, first week concentration of ritalinic acid in wastewater is harder to determine than amphetamine because it varied between the two semesters, 0.16 and 1.5 μg/L of RIT which leads to 20 – 182 total mg when multiplied by total water flow over three days. However, this shows that the measured levels of ritalinic acid are also reasonable.

The y-axis in Figures 3a and 3b show that the concentrations of creatinine-normalized amphetamine and ritalinic acid in the wastewater are similar. However, again it should be noted that methylphenidate is more effectively metabolized to ritalinic acid (68-84%) than amphetamine salts are to amphetamine (30-50%). Thus, although the measured concentrations of the two metabolites in wastewater are similar, the actual number of doses of amphetamine type compounds consumed could be as much as 54% higher than Ritalin (assuming the same mg/dose). If the only students excreting ADHD metabolites during the low stress, first week of school are those prescribed the medication, then the tallied numbers from the health center can be applied to the measured wastewater concentrations. Thus 15 students account for the measured 75 ng AMP/mg creatinine. The 760% increase in use at the end of the second semester then accounts for roughly 110 students using drugs containing amphetamine. This is 24% of the 476 students living in these dormitories which is at the high end of surveyed results of stimulant use among college students (Kaye and Darke, 2012).

4. Conclusions

For the first time, sewage analysis was used to specifically identify trends in ADHD medication metabolites for the purpose of correlating to academic stress. This study was designed to identify and quantitate trends; however, absolute levels of these drug metabolites in the extracted samples were reasonable for amphetamine and ritalinic acid. This study provides the first insight into using sewage epidemiology to specifically investigate this special population for non-prescriptive stimulant use. The sampling regime was not ideal and led to rather large uncertainties but amphetamine levels, normalized for creatinine, appear to be higher during times of high academic stress (especially finals week during the second semester) in comparison to the

first week of school (which had excellent agreement between the two semesters). Ritalinic acid does not appear to be tied as much to academic stress but shows an increasing trend throughout the first semester. For more definitive trends, future work should include higher frequency, flow-based sampling. Survey data suggests that amphetamine salts such as Adderall are the preferred study drug choice among college students. The percent use of amphetamines are in the same range as reported in survey data and the largest increase found in this study is as high as any reported by surveys. These evidence-based data can be tied to self-report data of the same population to provide complementary information. Unlike survey data, no permission has to be obtained from those being measured which is one advantage to this type of study. Recently the ethical issues of this type of analysis were explored but the authors found no major concerns when the analysis was used for public health purposes (Hall et al., 2012). A concurrent survey was performed during the first semester of this study and a comparison will be forthcoming.

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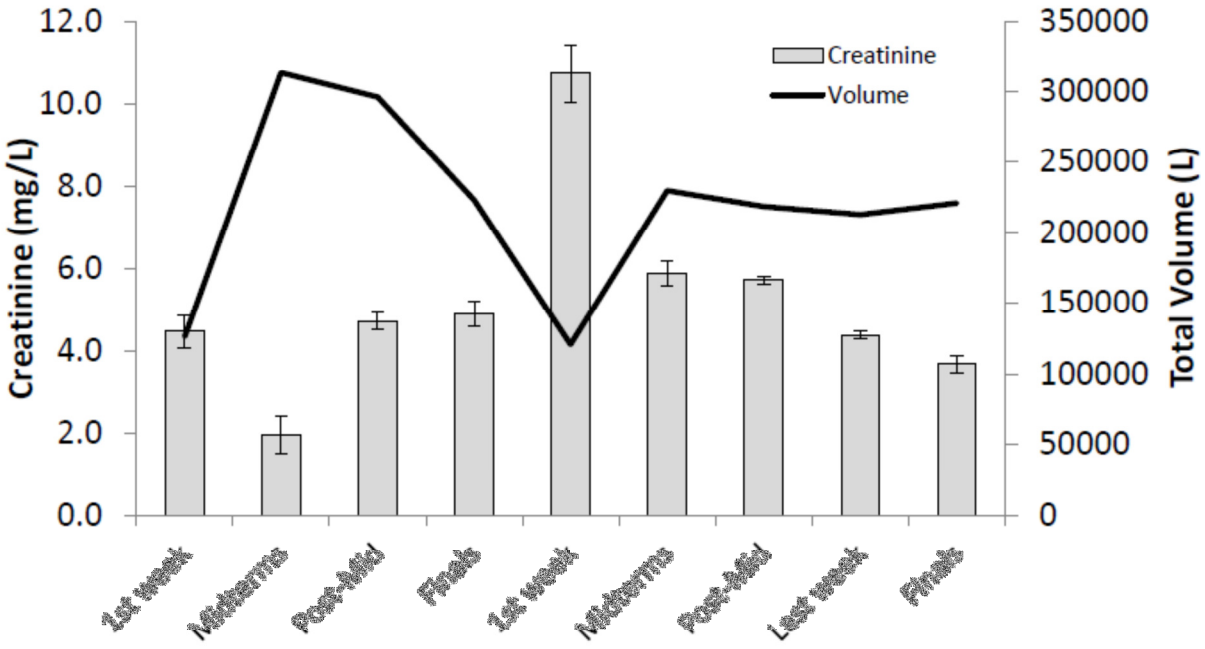

Figure 1. Average creatinine concentrations for each of the 72 h composite samples. The line shows the total volume that passed the sampler. The uncertainties are the standard deviations.

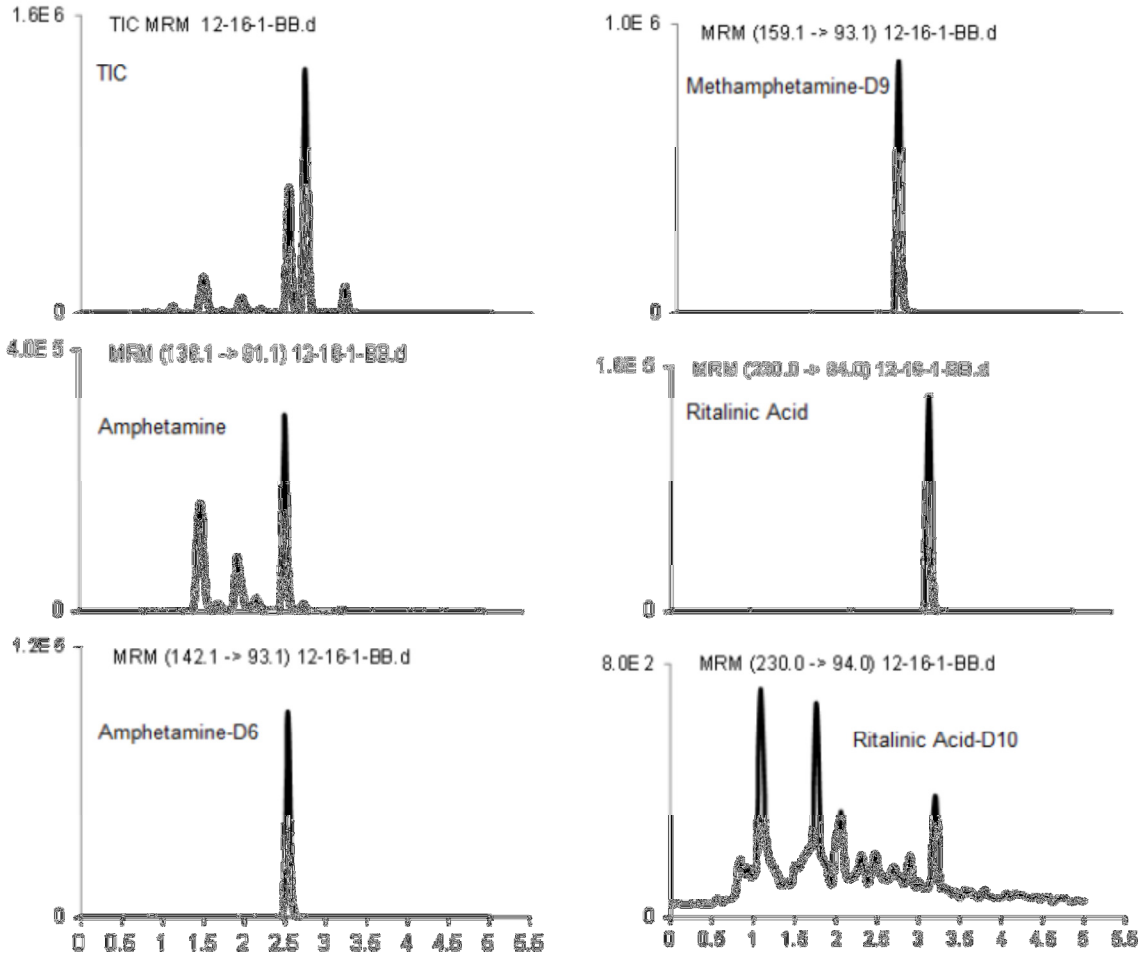

Figure 2. Total ion count and chromatograms for the two metabolites, their deuterated internal standards and the surrogate in a typical wastewater sample (finals week first semester) The retention times from amphetamine, amphetamine-D6, methamphetamine-D9, ritalinic acid and ritalinic acid-D10 are 2.55 min, 2.53 min, 2.74 min, 3.22 min, and 3.20 respectively.

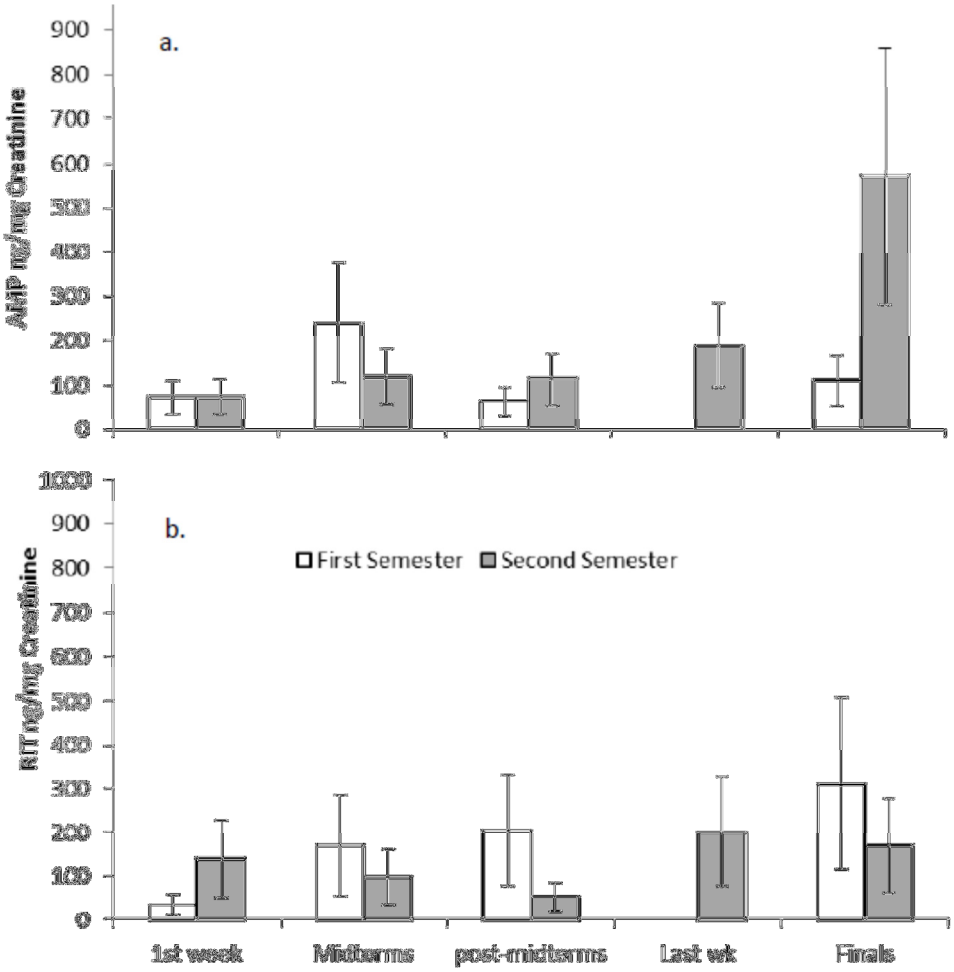

Figure 3. Average mass of a) amphetamine and b) ritalinic acid normalized to the human marker creatinine. The last week of classes was only sampled during the second semester. The uncertainties are the standard deviations as determined from flow, analytical and sampling uncertainty.