In a time where marijuana legalization is becoming increasingly prevalent around the world, this proposal will focus on the quantification of the main metabolite of marijuana, 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) in wastewater by sewage-based drug epidemiology. With the implementation of Initiative 502 in Washington, we have a unique opportunity to analyze the changes in marijuana consumption before and after legal dispensaries open. Past research has involved sewage based drug epidemiology for analysis of other illicit drugs including cocaine, heroin, and methamphetamine, but most analyses of marijuana in wastewater have been less successful due to its specific chemical properties. This project will focus on method development for analysis of THC-COOH in wastewater, as well as quantification. I plan to perform wastewater testing on raw influent sewage samples from the Central Wastewater Treatment Plant in Tacoma, WA, to assess the changes in consumption of THC in samples taken from early 2014 through the summer.
Marijuana Usage

Marijuana (or cannabis) is currently the most abused illicit drug in the world.\textsuperscript{1-5} Since it was first banned in California 100 years ago, the last decade has seen a rise in recreational marijuana use in the United States.\textsuperscript{6} Use is becoming increasingly prevalent within the youth population of the U.S. and a huge part of the youth culture in the Netherlands, Portugal, and Uruguay. For example, cannabis usage in Amsterdam in adults ranging from ages 18-20 rose from 34\% to 44\% between 1987 and 1997.\textsuperscript{7} In 2001, Portugal decriminalized all drugs including marijuana (though it is still not legalized).\textsuperscript{8,9} Though it has been decriminalized, both the Netherlands and Portugal do not yet have the means to sell or grow cannabis legally.\textsuperscript{5} In addition, the implementation of medical marijuana in over 20 states has become a pressing issue.\textsuperscript{4} Washington and Colorado were the first two states in the U.S. to legalize adult recreational marijuana in 2012 (by Initiative-502 and the Colorado Marijuana Retail Code) with hopes that they could reap the benefits of marijuana sales from the black market, such as revenue from tax income as well as reduction in the costs of incarceration. Washington, Colorado, and Uruguay, who will begin selling cannabis at a dollar per gram starting April 2014, will be the first to implement legal growing and sales.\textsuperscript{10} However, since medical marijuana will likely not be taxed, it is possible that users will still turn to “green cards” and the black market for recreational pot instead of legal dispensaries (which will be taxed at a total of 75\% from growing, producing, and retail).\textsuperscript{5}

There is no current scientific research regarding the effects of these new laws and it is difficult to know what the implications of legalization will be. Since dispensaries opened in Colorado, they have noticed an increase in accidents involving driving under the influence of marijuana.\textsuperscript{11} Many studies have shown negative effects from marijuana in coordination, thinking, problem solving, learning, and memory.\textsuperscript{4} According to a study at Columbia University, the percentage of drivers killed in car accidents who tested positive for marijuana nearly tripled between 1999 and 2010.\textsuperscript{11} With legalization, it is likely that these numbers will continue to rise. Determining usage patterns associated with marijuana legalization may come too late for Washington, but it will help weigh the pros and cons for other states considering legalization. This crucial transition period at
the beginning of legalized recreational marijuana allows for a unique opportunity to gather data concerning trends in marijuana usage due to its legalization.

Typically, studies concerning illicit drug use come from self-report surveys, police reports, and autopsies. However, in the last 10 years a promising new type of research has evolved for the quantification of illicit drugs. Research teams in Western Europe have used a new method called sewer-based drug epidemiology to quantify several illicit compounds in wastewater. A 2005 study in Milan, Italy specifically assessed the concentration of cocaine’s metabolite, benzoylcegonine, discovering that it was present in untreated urban wastewater and a major river. The significance of this research prompted teams in Switzerland, Spain, England and other parts of Italy to perform the same experiments on several other substances (and/or their metabolites) including heroin, ecstasy, ephedrine, and tetrahydrocannabinol (THC). All of these were abundantly detected in raw sewage samples except for THC, due to its hydrophobic properties. Analysis of marijuana by sewer based drug epidemiology is much more effective if the metabolite of THC, 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) is quantified because it is more hydrophilic and thus more likely to be found in the aqueous phase. In addition, analyzing the metabolite gives information about the quantities of marijuana that were actually consumed. This proposal seeks to apply sewer-based drug epidemiology to THC-COOH in influent sewage samples in South Tacoma, WA pre- and post-legalization, as well as focus on method development for optimal quantification.

**Wastewater Testing**

Sewage-based drug epidemiology relies on two methods, solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) for isolation and quantification of drug analytes. Using this method of wastewater testing, I will test levels of THC-COOH in wastewater samples collected both before and after legalization. To aid in this comparison, a human biomarker will be used to account for wastewater dilution and excretion rates. The biomarker for this project is creatinine, a break-down product of creatine phosphate that is regularly produced by the human body at a fairly constant rate (1-3.3 g/person/day). THC-COOH concentrations can be
normalized against creatinine concentrations to better compare daily THC-COOH levels. THC-COOH concentrations in wastewater are very low, in the parts per billion (ppb) range. For this reason, raw influent sewage samples must be highly concentrated for the metabolite to be detected by LC-MS/MS. This leads us to the need for SPE.

**Solid Phase Extraction of 11-nor-9-carboxy-tetrahydrocannabinol**

Solid Phase Extraction (SPE) is a method of sample preparation used to isolate specific analytes from a solution. SPE consists of two main procedural components—first, concentration and purification of analytes from solution by sorption onto a material (in this case an SPE cartridge), and then extraction of analytes by elution with an appropriate solvent as shown in Figure 1.\(^\text{17,18}\) SPE uses the same chromatographic principles as high-performance liquid chromatography (HPLC), but in this case it is used to prepare a liquid sample before analysis by another method (i.e. MS/MS).\(^\text{19}\) The main benefits of Solid Phase Extraction include sample purification, simplification of matrix effects (the detection of non-analyte sample components), fractionation, and enrichment of low-level compounds.\(^\text{19}\)

As mentioned above, THC-COOH is found in wastewater in the ppb range, so pre-concentration is needed to analyze the metabolite by MS/MS. For example, 100 mL of sewage sample could be run through an SPE cartridge and then reconstituted in just 200 microliters of solvent, creating a 500-fold concentration step. This is an extremely powerful step for sample preparation. I will be performing ion-exchange SPE, in which the analyte will be retained due to attractions of charged functional groups (i.e. COO\(^-\)). In order to retain or elute the analyte, I will need to spend the first part of the summer testing solutions with varying pH with various brands of SPE cartridges to optimize our method. SPE is a vital step in wastewater analysis because it is efficient and allows easy fractionation of different compounds. It is also good for a wide range of volumes, which is beneficial for this project because we will be using small sample sizes.\(^\text{17}\)

**Liquid Chromatography Tandem Mass Spectrometry**

LC-MS/MS is the analytical method required to quantify THC-COOH in prepared samples. Liquid chromatography (LC) involves the separation of liquid sample components between a mobile phase and a stationary phase. Mass spectrometry can be coupled with LC by ionizing the sample components when they elute from the
chromatography column and then quantifying them based on characteristic mass-to-charge ratios. LC-MS/MS is the best method for this procedure because it combines the benefits of chemical isolation by LC and mass-to-charge separation and quantification by MS/MS. In addition, MS/MS gives the most accurate results because it induces fragmentation of selected precursor ions allowing subsequent analysis of specific product ions. Professor Burgard’s research group has access to an Agilent 6460 triple quadrupole mass spectrometer at the UW-Tacoma Center for Urban Waters.

Research Methods

My proposal seeks to quantify the amount of marijuana consumed and trends in marijuana use in Tacoma during the 6 months leading up to the legal dispensaries (which are slated to open in June or July of this year) as well as the initial month or two after legalization (my plans are to continue this research into my senior year and continue analyzing the first 8 months of legalized marijuana for my senior thesis). Professor Burgard has been collecting and freezing samples from the treatment plant since November 2013. In addition to continued sampling, the first step of this project will be significant method development of the extraction process. Since research will begin in May 2014 and dispensaries likely won’t be open until June or July, we will be able to compare amounts of THC-COOH before and after legalization. If we find significantly greater amounts of THC-COOH in the first few months after dispensaries open, we can assume that total consumption increased, instead of just a shift in source (from illegal to legal). It will also be possible to know the degree of increased use by comparing our findings to previous illegal marijuana usage levels. The nature of this project will also allow Professor Burgard’s research group to look at trends in the amount of THC-COOH found in two distinct regions of Tacoma. My project will involve analysis of samples from the Central Wastewater treatment plant (WWTP) which serves approximately 200,000 people. A second treatment plant in the North End serves approximately 50,000 people. The boundary between these two plants is shown in Figure 2. As this figure shows, there are different demographics between the populations served by the two plants. The demographics of the populations servicing the two treatment plants can be determined from U.S. census data because the treatment boundaries fall nearly on census
tract boundaries.\textsuperscript{16} These data would then be useful to other states or municipalities considering legalization.

Wastewater testing is a fast, reliable method for drug epidemiology. By testing influent samples from the Central WWTP, we will get valuable data that will directly relate to the implementation of I-502. I will be able to see trends, if they exist, in marijuana use over time because our data spans pre- and post-legalization. Since there is no current scientific data in this field, my project will provide cutting edge research that could be applied globally.\textsuperscript{16} In a time where marijuana legalization is sprouting all around the world (it was even added to the ballot in Alaska for this fall), countries are struggling to find a way to regulate and control marijuana usage. This method will satisfy many pressing issues surrounding I-502 including consumption patterns, demographics, and economics.
Appendix

Figure 1. Example of Solid Phase Extraction

Figure 2. Average Tacoma incomes for regions of the North and Central Wastewater Treatment Plants (Darker colors mean higher incomes)

Figure 3. Chemical Structure of 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH)
References


SUMMER RESEARCH GRANT
in SCIENCE AND MATHEMATICS

McCORMICK BUDGET PROPOSAL

Attach an explanation of any items which need clarification.

Supplies (Itemized) $800

Solid Phase Extraction Cartridges $600
Analytical Standards (THC-COOH,
THC-COOH-d3, THC-COOH-d9) $200

Services (Itemized) $ N/A

Not Applicable $

Other Expenses (Itemized) $ N/A

Not Applicable $

Transportation $ N/A

Fare (air, train, etc.) $
Auto miles $

Lodging $ N/A
Meals $ N/A
Total Requested $ 800
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