Proceedings Of The National Conferences
On Undergraduate Research (NCUR) 2014
University of Kentucky, Lexington
April 3-5, 2014

Evaluating Pharmaceutical Sorption to Soils and Ground Water from an Eastern North Carolinian Family Farm

Brittany Ford
Bio-Chemistry
The University of North Carolina at Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Jeffrey Wilcox

Abstract

The fate and transport of pharmaceuticals through soil and ground water is an important research topic because of their possible toxic environmental effects. Representative sites are often used for testing the probability of a compound to adsorb to the soil or spread within the groundwater. In this study, the Brisson Farm in North Carolina was selected as a representative site due to the recent disposal of multiple pharmaceuticals into the septic tank on the property and the lack of organic carbon in the soil. This research used batch experiments to calculate sorption coefficients for various pharmaceuticals that were disposed of on August 24, 2012. It is commonly thought that organic carbon content is the key factor in determining the fate of such chemicals. Data from this study provides evidence that soil minerals have a distinct role in the adsorption, fate, and transport of a medication. Results from the Brisson Farm were compared to other representative soils in the mountains of Western North Carolina and various soil components.

Keywords: Pharmaceutical Sportion, Ground Water, Hazards

1. Introduction

Identifying the presence of pharmaceuticals in soil is important since the potential hazards of drug reactions are greater if the water system is contaminated with pharmaceuticals. America has become increasingly medicated and these medications can have very severe consequences if mixed together. In 2004, Daughton outlined hundreds of gaps in the research on the effects of pharmaceuticals on the environment which require contributions from several fields including chemistry, biology, engineering, hydrology, soil science, toxicology, and public health.[1] Another aspect of this study's importance is the damaging environmental effects of untreated pharmaceuticals left in the ecosystem. That is why it is imperative that the environment be analyzed for pharmaceutical contamination; whether it is in soil or ground water, both could negatively affect society's quality of life in the future. If pharmaceuticals are able to be spread throughout ground water and different soils, then remediation methods need to be found and implemented.

Sorption of soil in the environment has been studied and is described by the equation $K_d = f_{oc} * K_{oc}$.[2,3,4] The amount of organic carbon within the soil, f_{oc}, is indexed as a percentage. K_{oc} is the variable for the organic carbon-water partition coefficient, whose value can be determined from property tables or determined experimentally. The K_d for soil sorption testing is the adsorption coefficient of distribution between the concentration of a chemical in soil vs in water.[5] Units of adsorption for K_d (L/kg) are found by dividing an adsorbed concentration of chemical to soil (units of mg/kg) by the concentration of chemical dissolved in solution (units of mg/L).[6] High K_d values suggest that the pharmaceutical has been adsorbed to the soil and contamination can be easily remediated by the removal of soil. However if the K_d is low then the pharmaceutical is likely spreading through the ecosystem via groundwater

requiring the area of possible contamination to be expanded. Even common substances can wreak havoc on the environment.

Studies have shown that blood pressure medications, antibiotics, steroids and antidepressants are damaging to the environment, animals and people. [7] When medications are free to interact with microbes, viruses, bacteria and pathogens a resistance to antibiotics, various defense mechanisms, and tolerances can be built towards medications. [7,8] This resistance to antibiotics can lead to mutations of infectious microbes producing "superbugs"[9], ensuring the drugs used to fight against them are less effective, in turn creating a society that is increasingly more susceptible to disease. Furthermore, the daily metabolism and functioning of an individual can be greatly disrupted by excess exposure to pharmaceuticals, which can lead to the development of cancer. [10] A study conducted by Kunde and associates found that pharmaceuticals are resistant to wastewater treatment[11]; once the drugs are present, the removal is increasingly difficult. Furthermore, plants, especially the lipids therein, are often final destinations for highly water-insoluble organics.[12] These compounds can be found in surrounding foliage even if small quantities are present within the soil.

Hazards of the chemicals tested within this study can be extreme. Bisphenol A has been linked to changes in insulin uptake, abnormal liver enzymes, reproductive system damage, and heart disease. [13] The prevalence of Bisphenol-A in the urinalysis of 93% Americans across the United States caused it to be discontinued in the making of plastics.[14] Carbamazepine can cause severe damage to skin and internal organs from allergic reactions, Stevens - Johnson Syndrome or Toxic Epidermal Necrolysis.[15] Acetaminophen has been linked to hearing loss, skin irritations, greater probabilities for asthma, and liver damage.[16, 17] Caffeine, the most common pharmacologically active of all the chemicals tested in this study, can cause changes in the calcium uptake of the body, possibly induce mutations, and increase the risk of preterm deliveries.[18] These are not including the mild agitation, increased performance, heart arrhythmias and changes in blood pressure some individuals receive after ingesting substances containing Caffeine.

The comparison soils from Western North Carolina were chosen based on their organic carbon and clay percentages. The soil components of the phyllosilicate group of minerals, montmorillonite and kaolinite, were found within and compared to sorption values from the Brisson Farm soil. This family of minerals forms microscopic crystals which turn to clay.

1.1 Motivation

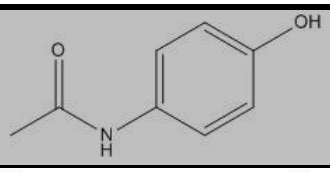
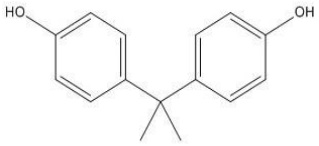
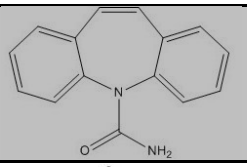
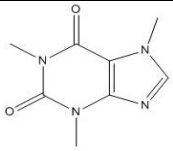
The motivation behind this study was numerous bottles of daily prescriptions that were flushed into the septic tank of Robert Brisson on August 24, 2012 following the death.[19] His land is part of a small family farm and the possible contamination created from the spread of pharmaceuticals in the soil and ground water could be harmful to the ecosystem of the Brisson Farm.

1.2 Objectives

This study sought to find whether pharmaceuticals flushed into the septic tank of the Brisson Farm are likely to have been adsorbed onto the soil or spread throughout the surrounding area via ground water. This was done by taking soil from the farm then adding known amounts of each drug to the soil. The sorption and desorption for each pharmaceutical was then annotated. The pharmaceuticals tested using the Brisson Farm soil are Caffeine, Acetaminophen, Carbamazepine, and Bisphenol A; shown in Table 1. Of the chemicals being tested, Caffeine is less likely to be adsorbed onto the soil[8] and possibly is contaminating the environment of the Brisson Farm.

It is known that high performance liquid chromatography (HPLC) must be used in the testing of pharmaceutical identification in soil and ground water because the chemical composition of pharmaceuticals is destroyed when gas chromatography (GC) is used. This research sought to determine the sorption of the medications which were flushed into the septic tank and thus allowed to interact with the ground water and soil of the Brisson Farm leading to possible contamination.

Table 1. Chemical Structures of Pharmaceuticals tested using the Brisson Farm soil

Pharmaceutical / Chemical	Uses	Structure
Acetaminophen **Log K_{ow} :** 0.46 **H_2O solubility:** 12,744 mg/L	Acetaminophen has been used for pain management.	
Bisphenol A **Log K_{ow} :** 3.32 **H_2O solubility:** 120 mg/L	BPA was synthesized in 1891 as a synthetic estrogen,[20] its resin properties were used to manufacture plastic from 1957 til 1999.	
Carbamazepine **Log K_{ow} :** 2.45 **H_2O solubility:** 30.5 mg/L	Carbamazepine was for treatment of epilepsy but is now used for depression and bi-polar disorders.	
Caffeine **Log K_{ow} :** -0.07 **H_2O solubility:** 21,600 mg/L	Coffee was used to dissolve the medications before introduction to septic tank.	

2. Methodology

The procedure of this project included the OCED (Organization for Economic Co-operation and Development) test guideline 106 for batch experiments as adopted by the EPA in 2000 and a soil processing technique. The soil used for this study was gathered in January 2013. The soil gathered from the Brisson Farm was collected at a distance of 50 feet from the septic tank, which had been sectioned off. At that location a hole was dug to a depth of two feet under the organic topsoil layer and the soil bagged. Next the soil was air dried and ground to the consistency of a fine powder by striking the soil, placed in an envelope, with a rubber mallet. Afterwards the soil was sifted through a 2 mm sieve, bagged and labeled.

The OCED batch experiment had controls without soil and samples which used soil. These samples were prepared by taking various weights of soil and placed in a vial with 10 mL of ultra-pure water. For the Brisson Farm soil 1 gram was used. Trials for montmorillonite and Kaolinite were conducted using 1 gram and 2 grams respectively. Western North Carolina soils used 2 grams of soil. The different drugs were then added by pipette into the vial at increments of 20, 40, 60, 80 and 100 µL to make concentrations of 2, 4, 6, 8, and 10 ppm. These volumes were taken from a 1000 ppm stock of pharmaceutical, with methanol as the solvent. Then the whole mixture was shaken "end over end" on a rotary shaker for 24 hours and centrifuged for 10 minutes or until the supernatant liquid was not cloudy. At this time the supernatant liquid was filtered and dispensed into respectively labeled HPLC vials. To ensure there was no cross contamination between samples, the filter on the end of the pipette was cleaned with ultra-pure water between each draw by running the water through the filter then discarding it into a waste beaker. Next a cap and membrane were put on the vial. These samples are stored in a refrigerator pending a run through the HPLC.

Standard HPLC operations were used, although the mixture of solvents was changed for better elution of the individual pharmaceutical from the column. These adapted solvent mixtures were 30% methanol with 70% ultra-pure water for Caffeine trials, 40% methanol with 60% ultra-pure water for Acetaminophen, 70% methanol with 30% ultra-pure water for Carbamazepine, and 60% methanol with 40% ultra-pure water for Bisphenol-A trials. The HPLC runs were five minutes in length and used a C-18 column. Detection peaks for the soil samples were printed out by a Shimadzu Chromatogram. These peaks were then analyzed and compared to those of the controls. Control samples were made and conducted by following standard operating procedures except there was no soil within the vial, only ultra-pure water and pharmaceutical. Corresponding HPLC peaks between the controls and the samples for each pharmaceutical show the degree of adsorption onto the soil of the Brisson Farm.

To determine the amount of organic carbon within the soil the procedure by Schulte and Hopkins was used.[21] This technique called for 5 grams of sifted and air dried soil to be weighed into a 15 mL crucible. Next the crucible of soil was oven dried at 105 ºC for one hour, placed in a desiccator, and then reweighed. The soil was then combusted in a muffle furnace for 2 hours at 360 ºC after which the oven was cooled to 105 °C. The sample baked at this new temperature for one hour. Once the hour elapsed and sample was cooled to room temperature it was placed in a desiccator and reweighed. The calculation for amount of organic carbon can then be determined by using the Loss-on-Ignition equation, ((oven dried soil weight - soil weight after combustion)/oven dry soil weight) * 100. This testing was done in triplicate for the Brisson Farm soil.

3. Results

The soil of the Brisson Farm according to Caffeine trials had high adsorption with the K_d value of 41.2 ± 1.3 L/kg. Such a high value is uncommon in western areas of the state since the Caffeine trials for the Fannin A, BRP-A, BRP-B, Dellwood, and Suncook resulted in K_d values of 10.7, 14.5, 19.4, 5.67, and 1.49 mg/kg respectively. This comparison has provided further evidence that Brisson soil, with its 28.2% clay content, has greater adsorption than high organic carbon soils of the North Carolina Mountains.

Soil testing in tandem with Dr. Moorhead found the organic carbon value to be 2.2%. The adsorption of the soil from the Brisson Farm should then be roughly zero; however, the sorption of Caffeine is much higher. A lack of organic carbon (f_{oc}) but not sorption in the soil of the Brisson Farm has dismissed the major tenant behind environmental adsorption, $f_{oc}*K_{oc} = K_d$.[2] X-ray diffraction of the Brisson Farm soil then discovered the presence of phyllosilicate mineral deposits, namely kaolinite and montmorillonite, to be within the soil. The K_d of montmorillonite for Caffeine has been measured to be 547 L/kg. It can therefore be concluded that this soil mineral component must be the cause for the elevated K_d values seen for Caffeine.

Bisphenol-A trials resulted in a 3.82± 0.43 L/kg K_d value for the soil of the Brisson Farm. With a high log K_{ow} for Bisphenol-A it was assumed that the adsorption could potentially be higher than that of Caffeine since Caffeine has a log K_{ow} of -0.07 while that of Bisphenol-A is 3.32. Nevertheless, a smaller K_d was measured because Bisphenol-A has a much weaker attraction to soil minerals and the soil lacked organics to which it could have adsorbed.

Carbamazepine, almost insoluble in water and highly attracted to organic carbon, was anticipated to sorb to the Brisson Farm soil. It however did not. The calculated K_d value was below 0.2 L/kg, the limit of quantification.

The proposed K_d for Acetaminophen was assumed to be between that of Caffeine and Bisphenol-A based on values for water solubility and log K_{ow} since there was no soil sorption data for Acetaminophen from previous studies. The Brisson Farm soil K_d value for Acetaminophen was calculated to be below the level of quantification, 0.2 L/kg. Acetaminophen, with solubility greater than Carbamazepine, was measured to have nearly the same value. The reason behind such low values must be that the pharmaceuticals are not attracted to minerals present in the soil, such as montmorillonite, strongly enough to permit sorption.

4. Conclusion

The high adsorption of Caffeine to the Brisson Farm soil along with the sorption of Bisphenol-A, Acetaminophen and Carbamazepine without organic carbon was inconsistent with the generally held assumption that organic carbon determines the amount of soil sorption. Any pharmaceutical sorption seen at the Brisson Farm has presumably been attributed to the presence of the soil minerals montmorillonite and kaolinite. The exact reason for the adsorption to minerals is not understood and awaits further research; however, testing using montmorillonite and kaolinite has provided that minerals as well as clay content can be a driving force for pharmaceutical sorption onto a soil. It is clear that the assumption *high organic carbon content leads to high adsorption* is not a sufficient analysis of all soil types, especially those with low organic carbon. Thus the current method for theoretically calculating sorption of a soil is ineffective and should be reevaluated to include possible sorption due to the presence of minerals and clay. Future avenues of research involve analysis of the soil using HPLC MS/MS to confirm which pharmaceuticals are present in the Brisson Farm soil. All compounds not found in this analysis will be assumed to be in the ground water and can then be tested for via water wells placed at the farm.

5. Acknowledgements

I would like to thank Dr. Wilcox for help with design, analysis and methodology of this project; Dr. Moorhead for analysis of soil and loss ignition methodology; the UNC-Asheville Departments of Chemistry and Environmental Studies for logistical and financial support. Additional funding for this work was provided for by the UNC-Asheville Undergraduate Research Program.

6. References

1 Daughton CG. PPCPs in the environment: Future Research— Beginning with the End Always in Mind, in Kummerer, K., ed., *Pharmaceuticals in the Environment* **2004,** *33*, 463-495.

2 Chiou, Cary; Kile, Daniel. Contaminant Sorption by Soil and Bed Sediment Is There a Difference? *USGS Fact Sheet-087-00* [online] **2000.**

3 U.S. EPA. Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites Table C-1, *Peer Review Draft* **2001,** *OSWER* 9355.4-24.

4 U.S. EPA. Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites Table C-2, *Peer Review Draft* **2001,** *OSWER* 9355.4-24.

5 U.S. EPA. Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites Table C-4, *Peer Review Draft* **2001,** *OSWER* 9355.4-24.

6 Baes, C.F. (III); R.D., Sharp. A Proposal for Estimation of Soil Leaching and Leaching Constants for use in Assessment Tools, *J. Environ. Quality* [online] **1983,** *12 (1),* 17-28.

7 Drilla Panagiota; Stamatelatou Katerina; Lyberatos Gerasimos. Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere* [online] **2005,** *60*, 1034-1044.

8 McMillen, Matt. Antibiotics No Help for Sinus Infections. *Health Magazine* [online] **2012,** *February 2014 Web Post.* Health Media Ventures, Inc. Santa Monica, CA.

9 Falco, Miriam. CDC sets threat levels for drug-resistant 'superbugs'. *CNN Health* [online] **2013**, *Cable News Network.* Atlanta, GA.

10 Salvia, Marie-Virginie; Vulliet, Emmanuelle; Wiest, Laure; Baudot, Robert; Cren-Olive, Cecile. Development of a multi-residue method using acetonitrile-based extraction followed by liquid chromatography-tandem mass spectrometry for the analysis of steroids and veterinary and human drugs at trace levels in soil. *Journal of Chromatography* [online] **2012,** *1245*, 122-133. DOI: 10.1016/j.chroma.2012.05.034.

11 Lin, Kunde; Bondarenko, Svetlana; Gan, Jay. Sorption and persistence of wastewater-borne psychoactive and antilipidemic drugs in soils. *Journal of Soils and Sediments* [online] **2011,** *11* (8), 1363-1372. DOI: 10.1007/s11368-011-0421-9.

12 Chiou, C.T.; Sheng, G.; Manes, M. A partition-limited model for plant uptake of organic contaminants from soil and water. *Environmental Science Technology* [online] **2001,** *35* (7), 1437-1444.

13 Cone, Marla; Meyers, Pete. Bisphenol A linked to diabetes, heart disease in humans. *Environmental Health News* [online] **2008,** *September 2008 ed*. Investigative News Network. New York City, NY.

14 Biello, David. Like a Guest That Won't Leave, BPA Lingers in the Human Body. *Scientific American* [online] **2009**, *January 2009 ed.*

15 U.S. Library of Medicine. Carbamazepine. *Medline Plus*. [online] **2012,** American Society of Health-Systems Pharmacists, Inc. Bethesda, MD.

16 Wohl, Y.; Goldberg, I.; Sharaz, I.; Brenner S. A case of paracetamol-induced acute generalized exanthematous pustulosis in a pregnant woman localized in the neck region. *Skinmed* [online] **2004**, *3*, 47-49.

17 FDA. Drug Safety Communications [online] **2013,** Rockville, MD.

18 Nawrot, P.; Jordan, S.; Eastwood, J.; Rotsein, J.; Hugenholtz, A.; Feeley, M. Effects of Caffeine on human health. *Food Additives and Contaminants.* [online] **2003,** *20* (1), 1-30.

19 Brisson Family Farm Records

20 Reagents of the University of Minnesota. History of Bisphenol A. *Class Notes for PUBH 6101: Environmental Health on University Website.* [online] **2008**, University of Minnesota, Minneapolis, MN.

21 Schulte, E.E.; Hopkins, B.G. Estimation of Organic Matter by Weight Loss-on-Ignition **1996**, 21-31. *In* Magdoff, F.R. Soil Organic Matter: Analysis and Interpretation. *SSSA Spec. Publ. 46*. SSSA, Madison, WI.