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Use of Passive Samplers to Evaluate Pharmaceutical Fate in Surface Waters

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USE OF PASSIVE SAMPLERS TO EVALUATE PHARMACEUTICAL FATE IN SURFACE WATERS

by

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Emerging contaminants have been of importance in recent water research. Wastewater treatment plants (WWTPs) have proved ineffective at handling present-day antibiotic loads from hospital and municipal sources. Kolpin et al. (2002) performed a study that identified pharmaceuticals in numerous waters downstream from effluent discharge. Though present in trace levels, concern has been raised regarding pharmaceutical persistence in natural environments. In the present study, uptake rates were quantified in the laboratory for 25 pharmaceutical compounds using Polar Organic Chemical Integrative Samplers (POCIS). Twenty new uptake rates were determined for compounds that have no previously reported literature values. POCIS was also used to evaluate the fate of polar organic contaminants in Nebraska surface waters impacted by WWTP effluent. Select pharmaceuticals were observed to persist for at least 1300 m downstream. Carbamazepine and DEET showed persistence and the highest average concentrations of 110 and 60 ng/L, respectively. Decay rates were determined for 25 pharmaceuticals in receiving waters. Pharmaceutical loading rates were calculated for each compound using the average in-stream concentration and volumetric flow rate.

Acknowledgements

I would like to thank my adviser Dr. Shannon Bartelt-Hunt for doing an excellent job at supervising and directing this project. During times of frustration and misdirection, she provided advice that allowed me to circumvent problems along the path to completion. At the beginning of the paper I was a bit overwhelmed at the task before me, but as I finalized corrections suggested by my committee, I have acquired a new found appreciation for option I MS students and research as a whole. Preparation and defense of my research has been time-consuming, but a very satisfying experience, nonetheless.

Dr. Daniel Snow also played a pivotal role in the completion of this work. He accompanied me to field, rubber waders and all, to deploy passive samplers in the effluent streams at both sites. The Hastings drainage canal was memorable due to stream turbidity, smell, and “sinking” sediment substance located at bottom of the ditch. Dr. Snow and Teyona Damon at the Water Science Laboratory were responsible for carrying out the analytical methods and recovery of pharmaceuticals from samplers. The data recovered was an essential part of accomplishing objectives set forth during research. Dr. Tian Zhang provided suggestions that made this thesis a lot more concise. After his editorial comments, I believe the average student with little to no prior knowledge

of emerging contaminants could read this paper and understand passive sampling technology and its ability to provide water quality data.

My family has been instrumental in providing support and encouragement for as long as I can remember. Looking back, I am actually thankful for the times when I thought they were just being hard for no reason. My late grandmother always had high expectations for me, and as I developed and matured her expectations became my own. I am firm believer in the ideology that preparation and ambition are fundamental precursors to success.

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Chapter 1

Introduction

Recently there has been increased interest in the occurrence and behavior of organic wastewater microconstituents, including pharmaceuticals, personal care products, and steroid hormones, in waters across the country. One of the primary sources of these compounds to natural waters is effluent from wastewater treatment plants (WWTPs) (Glassmeyer et al. 2005; Lee and Rasmussen 2006; Miao et al. 2004). One of the potential concerns about the presence of these compounds is that they may be biologically active, with negative consequences for aquatic species. There are few published studies of the ecotoxicological impacts of chronic low-level exposures of therapeutic or illicit pharmaceuticals in aquatic systems (Fent et al. 2006; Pounds et al. 2008), though these chemicals may have effects at environmentally-relevant concentrations (Raldua et al. 2008; Schreiber and Szewzyk 2008). Because these compounds are biologically-active, both ecotoxicological and human health impacts are of potential concern.

Although the occurrence and concentration of illicit and therapeutic pharmaceuticals in natural waters have been documented based on discrete sampling events, there are fewer data available regarding the time-weighted average concentrations of these compounds in receiving waters downstream of

WWTP outfalls. Traditional water sampling approaches, such as grab and composite sampling, are effective for documenting the occurrence of pharmaceuticals, but these sampling techniques only capture information at the time of sample collection, and may miss events such as changes in the flow regime, chemical inputs and/or the influence of precipitation (MacLeod et al. 2007). Monitoring temporal changes in pharmaceutical concentrations via continuous on-line sampling methods may be prohibitively expensive. One device that has been developed for use in sampling trace organic compounds is the Polar Organic Chemical Integrative Sampler (POCIS). This sampling device is designed to trap polar organic compounds from water. Its ease of use and apparent resistance to biofouling make it particularly attractive for determining time-weighted average (TWA) concentrations of organic compounds in water (Alvarez et al. 2004). POCIS samplers have been used previously for both qualitative and quantitative evaluation of pharmaceuticals, pesticides and hormones in surface waters (Alvarez et al. 2004, 2007; Arditsoglou and Voutsa 2008; Harman et al. 2008; Jones-Lepp et al. 2004; MacLeod et al. 2007; Zhang et al. 2008).

The hypothesis of this study is that passive samplers can be used to evaluate the occurrence and behavior of pharmaceuticals in surface waters impacted by wastewater treatment plant effluent. The objectives of this research project were: (1) to quantify POCIS uptake rates for 25 pharmaceutical

compounds by conducting a laboratory uptake study, (2) to deploy passive samplers to evaluate the fate of polar organic contaminants in surface waters in Nebraska impacted by wastewater treatment plant (WWTP) effluent, and (3) to determine decay rates for pharmaceuticals in receiving waters.

Chapter 2

Literature Review

Traditional sampling technologies

Accurate assessments of contaminant concentrations based on traditional grab sampling methods are not always possible. Very large sample volumes are required to accurately sample contaminants at low levels and there is often low recovery of polar compounds in liquid to liquid extraction techniques. Volatilization, adsorption to container walls, and chemical degradation are also of concern when using grab sampling techniques. Due to the short sample collection period along with transport and storage implications, discrete sampling only provides information on the instantaneous concentration, in contrast to data regarding time weighted average (TWA) concentrations provided by integrative passive samplers (Greenwood et al. 2009).

Automated sampling methods give a better indication of average water constituents than grab sampling. Automated samplers are designed to take samples at specified intervals, which can provide a clearer indication of variation in pollutant concentration over time. However, this process is also subject to high levels of contamination via sampling tubes, valves, and pumps (Greenwood et al. 2009). Contamination by trace level compounds plays an important part in the integrity of the sample. Modeling and assessments drawn from sampling studies may be potentially skewed due to excess contamination.

Aquatic organisms have also been used to determine the biological relevance associated with the presence of organic microcontaminants. Often, fish have been deployed downstream from wastewater treatment plant (WWTP) effluent to assess changes in pollutant concentration via bioaccumulation. Variation in body tissue concentration over a given time period is comparable to fluctuation in aqueous pollutant concentration. This approach of measuring pollutant concentrations in water also has limitations. The aquatic organisms cannot be exposed to environments where concentrations may exceed toxic levels. Information on background levels of contaminant present in the organism prior to deployment presents a problem as well as cost associated with tissue sample recovery (Greenwood et al. 2009). Biological indicators also do not typically specifically identify the compound present, but rather, identify the biological effect resulting from exposure to a compound or other environmental stressors. Passive samplers have been recommended as an approach to circumvent potential problems associated with grab sampling, automated sampling, and use of biological indicators.

Passive sampling technologies

Passive diffusion is a transport mechanism in which molecules move from an area of high concentration to an area of lower concentration until equilibrium conditions are reached. Unlike active transport, passive diffusion does not

require any additional energy or forcing against concentration gradients, which provides unbiased results regarding contaminant transport. Diffusion-based passive samplers rely on this method to monitor chemical uptake. These samplers consist of a porous hydrophilic membrane that allow for accumulation of certain organic contaminants, while rejecting others. Fick's first law of diffusion describes the flow of contaminant during passive sampling.

$$N_A = (DS/L)C \qquad (1)$$

where N_A is the mass flow rate, C is the analyte concentration, S and L are surface area and diffusive length, respectively and D is the analyte diffusive coefficient in air (Ballesta et al. 1993).

Passive samplers have been used in environmental monitoring for over 30 years. Original passive sampling technologies were developed for air monitoring applications. Ballesta et al. (1993) developed a diffusion-based passive sampler to detect toluene present in air. The sampler contained a Teflon base and cover, porous membrane, stainless steel rings and locks for adsorbent media compaction. The adsorbent used for toluene collection was pre-activated coconut charcoal. In addition to quality toluene sampling rates near 50 cm^3/min, the sampler is reusable and adsorbent exchangeable (Ballesta et al. 1993).

The Radiello passive sampler was established by Maugeri of Italy, in order to sample inorganic and organic pollutants. These include but are not limited to benzene-toluene-xylenes (BTX), VOC, nitrogen dioxide (NO_2), sulfur dioxide

(SO_2), ozone (O_3), and other airborne pollutants (Namiesnik et al. 2005). The sampler is named after its radial geometric shape. It has a cylindrical, diffusive membrane with micropores and an adsorbing cartridge for compound accumulation (Namiesnik et al. 2005).

More recently, passive samplers have been developed to monitor pollutant concentrations in water and soil (Greenwood et al. 2009). The need for using passive samplers for contaminant identification has been discussed in the literature, primarily due to low levels of contamination present in environmental media and the high analyte recovery in passive samplers at low concentrations (Alvarez et al. 2004).

For example, the Ceramic Toximeter was designed to combine an integrative passive sampling technique compatible with bioassay analysis for groundwater applications (Bopp et al. 2007). Biosilon, a high surface area polystyrene microsphere typically used as a growth support for bacteria, was used as the sorbent media to sequester polycyclic aromatic hydrocarbons. The results from the experiment confirmed the ability for passive samplers to remain in the linear uptake phase for an extended period of time. The Ceramic Toximeter displayed linear uptake through the 42-day deployment without fouling, (i.e. membrane deterioration or hydrodynamic flow). Discrete samples were also taken in duplicates bi-weekly for comparison to validate results from the Ceramic Toximeter. The only limitation was the sampler's inability to uptake

smaller aromatic compounds compared to larger compounds over the same period of time (Bopp et al. 2007).

The passive in-situ concentration-extraction sampler (PISCES) was introduced in 1993 (Namiesnik et al. 2005). The sampler is comprised of a metallic t-shaped pipe with hexane as the solvent. Once sealed, the PISCES is suspended in a water column with the membranes facing downward for contaminant accumulation. PISCES was successful for identifying organic microconstituents, such as poly-chlorinated biphenyls (PCBs), and has since been considered for field studies evaluating the occurrence of other contaminants (Namiesnik et al. 2005).

Semi-permeable membrane devices or SPMDs, were among the first passive sampler devices being designed. From 1990 until the present, over 200 studies have been completed using SPMDs for environmental monitoring (Vrana et al. 2005). SPMDs are comprised of a flat laying, low-density polyethylene (LDPE) tubing filled with a high-molecular weight lipid (Namiesnik et al.2005; Vrana et al. 2005). Synthetic triolein (Glycerine trioleate) is often the filling of choice. LDPE is a non-porous material, so it is selective and only allows fully dissolved and unbound molecules to diffuse through the membrane (Vrana et al. 2005). Along with the ease of use, their ability to quantify pollutant-aqueous phase concentrations, their flexible field deployment periods and their ability to

determine TWA concentrations, SPMDs are one the most effective passive sampling technologies available (Namiesnik et al. 2005; Zhang et al. 2008).

Solid-phase microextraction (SPME) is a passive sampling method, which does not require the use of any solvent. This method is accomplished in two distinct ways: Direct, where the extraction fiber of SPME is immersed in media and Indirect, where the extraction fiber of SPME is placed in the headspace layer at equilibrium with media. The SPME fiber extracts analytes without collecting a sample. This is accomplished by compound sorption onto the thin film of a stationary phase coated on SPME fibers (Namiesnik et al. 2005). One drawback to this technique is that SPME cannot be used for long-term monitoring. SPME data obtained over longer periods of time was only comparable to grab sample quality, which excludes changes over time (Namiesnik et al. 2005).

Polar Organic Integrative Samplers (POCIS)

Polar Organic Chemical Integrative Samplers (POCIS) were designed by scientists at United States Geological Survey (USGS) to sequester hydrophilic compounds from water. The POCIS is comprised of a solid sequestration media inside a polyethersulfone (PES) membrane, which is held together by stainless steel compression rings (Figure 1).

Figure 1. POCIS and Deployment Canister

The stainless steel screws securing the rings are passively resistant to oxidation allowing optimal performance in water. Two designs of POCIS media exist for distinct target analytes. A copolymer, Oasis HLB (polydivinylbenzene-co-N-vinylpyrrolidone) is the sorbent media aimed at pharmaceuticals, while Triphasic admixture is designed for pesticides (Alvarez et al. 2004). The sampler has three components: the water boundary layer, the diffusive membrane, and the receiving phase (Greenwood et al. 2009). The water boundary layer comprises the zone of aqueous solution immediately adjacent to the bulk water environment. The diffusive membrane allows specific contaminants from the water boundary layer to reach the receiving phase. The diffusive membrane is derived of PES, with micropores that allow polar compounds to enter, while rejecting particulates, colloids, and other microbes. The receiving phase,

comprised of sorbent media, acts an infinite sink for the contaminants by maintaining a concentration close to zero. This results in optimal mass transfer by diffusion. The only limitation for mass transfer is the actual surface area available for contaminant transfer (Greenwood et al. 2009).

The process of compound accumulation on the sorbent media is a first order reaction (Alvarez et al. 2004). First-order kinetic models include an integrative phase, curvilinear phase, and equilibrium partitioning phase. During the integrative phase, the sampler acts as an infinite sink for contaminants with log-linear uptake, as shown in Figure 2. (Alvarez et al. 2004).

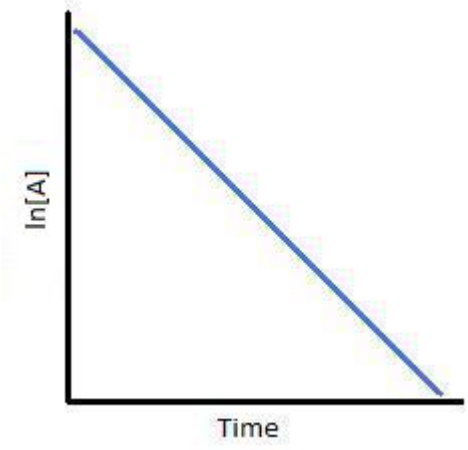


Figure 2. Time series concentration change illustrating First Order (log linear) uptake rate

In order to use the POCIS quantitatively, an uptake rate (R_s) must be determined experimentally for the compounds of interest (Alvarez et al. 2004). The uptake rate can be determined as:

$$R_S = (D_w/L_w)A \qquad (2)$$

where the uptake rate R_s is in units of (L/d), D_w is the compound-specific aqueous diffusive coefficient (m^2/s), L_w is the aqueous film layer thickness (m), and A is the available surface area (m^2). Once an uptake rate has been calculated, the time-weighted average water concentration of the contaminant of interest can be calculated as:

$$C_w = C_s M_s / R_s t \qquad (3)$$

where C_w (ng/L) and C_s (ng/g) are the analyte concentration in water and sorbent, respectively; M_s (g) is the mass of the sorbent, R_s (L/d) is the uptake rate determined from equation above; and t (d) is the exposure time.

POCIS have the advantage of being able to retain contaminants from the initial integrative phase, while still being able to acquire additional contaminants. They have the ability to handle large volumes of water over time with the addition of evaluating variations in contaminant concentration and flow rates (Alvarez et al. 2004). Though the POCIS has been used in numerous studies investigating the occurrence of organic wastewater contaminants (Alvarez et al. 2004; Jones-

Lepp et al. 2004; MacLeod et al. 2007; Togola and Budzinski 2007), its use for estimating concentrations have been limited. Uptake rates have to be calculated for compounds of interest before POCIS can be used quantitatively (Soderstrom et al. 2009). Published sampling rates are available for only a relatively small number of pharmaceuticals (Bartelt-Hunt et al. 2009). In addition, calculated uptake rates have been demonstrated to be sensitive to a number of environmental factors including salinity, temperature, and pH (Togola and Budzinski 2007; Soderstrom et al. 2009). Variability of uptake rates within a factor of 2 to 3 is consistent with variability in contaminant concentrations observed in the field based on continuous monitoring over an extended period (Togola and Budzinski 2007). POCIS samplers have been used previously for both qualitative and semi-quantitative evaluation of pharmaceuticals, pesticides and hormones in surface waters (Alvarez et al. 2004, Jones-Lepp et al. 2004; Alvarez et al. 2007; MacLeod et al. 2007; Arditsoglou and Voutsa 2008; Harman et al. 2008; Zhang et al. 2008; Sellin et al. 2009).

Occurrence of Pharmaceuticals in WWTP effluents

More than 70% percent of antibiotics are excreted in their active state (Kummerer 2009). Some antibiotic and pharmaceutical compounds are reduced or eliminated in biological wastewater treatment plant processes, while others are converted to their biologically active form. If not eliminated during sewage

treatment or purification processes, these pharmaceuticals persist and can be discharged in surface water. These de-conjugated or metabolized byproducts have toxicity equal to or exceeding the original compound (Nikolaou et al. 2007).

Clofibric acid ($C_{10}H_{11}ClO_3$) in the range of 0.80 to 2.0 µg/L was the first reported pharmaceutical evidence in wastewater effluent by Garrison in the late 1970s (Jones-Lepp et al. 2004). Since that time, numerous studies have investigated the occurrence of pharmaceuticals in WWTP effluents. The occurrence of emerging contaminants was investigated by Kolpin et al. (2002) study that examined surface waters downstream from areas of urbanization and livestock production. Over a one-year period, samples were collected from over 100 streams in 30 separate states. Approximately half of the streams contained at least 7 contaminants, one-third had 10 or more different compounds, and a maximum of 38 contaminants were identified in one stream (Kolpin et al. 2002). Steroids, nonprescription drugs, and detergent metabolites were the most frequently detected compounds within the streams analyzed. Steroids, nonprescription drugs, and antibiotics occurred at maximum concentrations of 18.3, 17.4 and 3.6 µg/L, respectively.

Nikolaou et al. (2007) conducted a study that compiled effluent pharmaceutical occurrence from previously published sources. Antibiotics, anti-inflammatory drugs, lipid regulators, steroids, and hormones were identified as common pharmaceuticals discharged from hospital and municipal environments.

German WWTP effluents and river waters were found to contain 32 different pharmaceutical compounds in a moderate ng/L range. The Hoje River, a Swedish river, was found to contain ibuprofen, ketoprofen, naproxen, diclofenac, atenolol, metoprolol, propanolol, trimetoprim, sulfamethoxazole, carbamazepine, and gemfibrozil in the range of 0.12 to 2.2 µg/L. Carbamazepine has been detected at over 40 American rivers at an average concentration of 60 ng/L. Sewage treatment effluent studies in the United Kingdom (UK) identified ibuprofen in 86% of all streams surveyed at an average concentration of 3086 ng/L. Norwegian WWTP effluents displayed mean concentrations of caffeine, triclosan, and ibuprofen of 151, 1.3 and 10 µg/L, respectively. A study of antibiotics in New Mexico hospital effluents revealed sulfamethoxazole, trimethoprim, ciprofloxacin, ofloxacin, lincomycin, and penicillin G. Ibuprofen and sulfamethoxazole showed persistence in surface waters assessed in mean concentrations of 4.2 and 0.6 µg/L, respectively (Carballa et al. 2004).

Spongberg and Witter (2008) performed a study on 3 WWTPs located in northwestern Ohio. Influent and effluent concentrations from urban, surburban, and rural locations were measured to analyze degradation and persistence. Influent concentrations were identified for caffeine, carbamazepine, cotinine, sulfadimethoxine, sulfamethazine, and sulfamethoxazole of 2.5, 0.04, 0.20, 0.003, 0.03 and 0.26, respectively. The effluent concentrations varied by specific compound. Caffeine was readily degraded over the time period and had minimal

residue in the effluent. Carbamazepine and sulfamethoxazole, however, both had effluent concentrations that exceeded influent by a factor of 2. The remaining 3 compounds failed to be detected due to limits of quantification.

Wu et al. (2009) performed a study around the agricultural area of Lake Erie basin. A total of 18 pharmaceuticals were investigated for occurrence and fate in aqueous and soil locations. Surface waters in the Lake Erie basin do not receive wastewater effluent, but are susceptible to agricultural and septic tank runoff. Yearly application of biosolids to sampling area also plays a part in microcontaminant transport. The sampling area was separated into 3 watersheds, where caffeine was by far the most frequently detected compound and found in the largest quantity of 4275 ng L^{-1}. Erythromycin, lincomycin, sulfamethazine, and sulfamethoxazole were the pharmaceuticals with veterinary applications, and had lower detection frequencies ranging from 6 to 24 percent. These compounds had maximum detected concentrations in ng L^{-1} of 438, 5, 10, and 112, respectively. Of all sediment samples collected, no pharmaceutical compound was present above method detection limits (MDL). Wu et al. (2009) described the pharmaceuticals as polar and hydrophilic, favoring to aqueous phase over partitioning into sediment.

Xu et al. (2009) published a study on agricultural soils, where wastewater effluent is used for irrigation purposes. The following pharmaceutical and personal care products (PPCPs): clofibric acid, ibuprofen, naproxen, triclosan,

diclofenac, and bisphenol A were observed for degradation and adsorption properties. These particular compounds were selected due to prevalence in agricultural runoff studies. Degradation and adsorption were assessed as function of 4 agricultural soil types. Handford loamy sand (HLS), Arlington sandy loam (ASL), Imperial silty clay (ISC), and Palouse silt loam (PSL) were the soils tested in experimental portion of study. 1^{st} order exponential decay model and Freundlich isotherm were used to determine degradation rate constant and adsorption coefficient. All PPCPs in the four soils exhibited persistence. The half lives of the compounds ranged from 0.8 to 20.4 d for bisphenol A and diclofenac, respectively.

Due to widespread use of antibiotics for disease control and disinfection, waters have suffered an unusual loading of contaminants. Tong et al. (2009) discussed available methods in which to rapidly detect these contaminants that may harm the environment. The 13 antibiotics studied are often used in veterinary medicines that belong to sulfonamide, fluoroquinolone, tetracycline, and chloramphenicol categories. Grab samples were collected at two pig farm waste streams (P1 & P2) during the summer and winter in Hubei, China. Eight distinct samples were collected from the sites, which include groundwater summer, groundwater winter, lake water summer, lake water winter, P1 summer, P1 winter, P2 summer, and P2 winter. Fluoroquinolone and tetracycline concentrations were considerably higher in the winter months, which may be

attributed to decreased microbial activity. Ciprofloxacin had the highest winter lake water concentration spike of 12 ng L^{-1}. Tetracycline displayed the largest summer lake water concentration of 12 ng L^{-1}. Groundwater samples were not as large lake water and were in the range of 1.6 to 8.5 ng L^{-1}. Large quantities of antibiotics were present in P1 and P2 wastewater effluents. Sulfamerazine had the largest P1 summer, P2 summer, and P2 winter concentrations, all exceeding 10,000 ng L^{-1}. Ciprofloxacin had the highest treatment system elimination rate of approximately 96%, while doxycycline had the lowest of 65%. Sulfathiazole and chlortetracycline were barely detected in the wastewater effluents, which may be attributed to adequate treatment removal, biodegradation, sorption, or photolysis processes.

Kuchta et al. (2009) conducted a study, which identified lincomycin in snowmelt runoff water after land application of liquid swine manure. Land application of biosolids is a method that provides nutrients to soil, and reduces the necessity of land filling or incineration. A couple of closed basins, ephemeral wetlands, and dugouts were sampled in Saskatchewan, Canada during the study. The amount of liquid manure applied to closed basin section of field 1 and field 2 were 88,000 and 110,000 L ha^{-1}, respectively. Lincomycin was present in all runoff samples acquired from each site location. There was a mean concentration of 0.27 µg L^{-1} and 0.39 ug L^{-1} for field 1 and field 2, respectively. Manure was not applied to wetlands, so as expected the mean

concentration was a bit lower approximately 0.16 μg L^{-1}. Lincomycin had a mean concentration in the dugout portion of field 1 of 0.12 ug L^{-1} and field 2 was 0.21 μg L^{-1}. Water present in the dugouts may have contributed to antibiotic dilution and decrease in concentration.

Andreu et al. (2009) produced a study that acknowledged a superior method for extracting compounds from soil, which was a combination of ethylenediamine tetraacetic acid-treated sand, water at a temperature of 70^{o}C, followed by SPE cleanup. CTC, DC, OTC, and TC were the –tetracycline compounds evaluated and had recovery rates from soil ranging from 71 to 96%. Recovery rates were calculated at 1.2 and 12.5 μg L^{-1}. Though the magnitude of the concentrations varied, the overall trend of each distinct compound was comparable.

Fate of Pharmaceuticals in receiving waters

There have been a limited number of studies investigating the fate of pharmaceuticals in receiving waters. Pharmaceuticals can be removed from environmental systems by a number of processes. These processes include but are not limited to photolysis, sorption, biodegradation, and hydrolysis. These elimination pathways remove some fraction of pharmaceuticals in natural systems, but certain pharmaceuticals can likely persist in aquatic systems with potentially adverse effects. One study determined an influent concentration for

caffeine of 63.2 µg/L (Miao et al. 2005). Another study operated under similar conditions found a caffeine effluent concentration of 4.5 µg/L that verified degradation during treatment (Batt et al. 2006). Batt et al. (2006) evaluated the persistence of additional antibiotics in receiving waters at distances of 10 m, 20 m, and 100 m from the effluent source, and found that many of these compounds persisted for at least 100 m from the source. Pharmaceutical concentrations observed in surface waters were lower than those measured at the WWTP outfall, indicating that dilution and/or degradation processes must be occurring (Batt et al. 2006).

Photolysis. Photolysis is a degradation process, which effects light-sensitive compounds and serves as a removal method of pharmaceuticals in shallow surface waters downstream from WWTP effluent (Kummerer 2009). Some antibiotics are sensitive to light, but not all are photodegradable. Tetracyclines, sulfa-drugs, and tylosin have all show high photodegradation in previous surface waster studies. Photolysis is directly related to light intensity and frequency, so it is not a dominant mode of degradation in heavily turbid waters (Kummerer 2009).

Few studies exist, which address pharmaceutically active compounds and their interaction with UV light. A study conducted by Pereira et al. (2007) determined photodecomposition of different pharmaceuticals. Carbamazepine,

an anti-convulsant, was found to be minimally affected by light sources. After exposure to light, less 5% of the compounds underwent degradation. Carbamazepine and other compounds with similar structures tend to absorb light within the lower wavelength range of 200-240 nm, so as expected degradative properties without additives, i.e. hydrogen peroxide, barely exist (Periera et al. 2007).

Biodegradation. Biodegradation is a biological process of breaking down organic contaminants. It is a natural occurring pathway in which contaminants can be eliminated from water. Biodegradation affects compounds of various structures in different ways. When compounds have low adsorptive properties, biodegradation is the primary means of removal (Carballa et al. 2004). Many treatment facilities utilize hydraulic retention times (HRT), which are lower than the half-lives of common pharmaceuticals, so adequate degradation does not occur (Kinney et al. 2006).

Loffler et al. (2005) conducted a study on biodegradation, in which an experimental set-up with 100 ng/g of spiked pharmaceuticals, which was analyzed for a 100-day period. Samples were taken a 0, 0.25, 1, 2, 7, 14, 28, 56 and 100 days. Quality control measures were implemented to maintain a fairly constant pH and dissolved oxygen concentration over the specified time period. After the 100-day period, 83 percent of the carbamazepine present in the initial

spike was recovered. Carbamazepine displayed resistant behavior to various biodegradation processes during soil interactions (Loffler et al. 2005; Kinney et al. 2006).

Sorption. Sorption is a combination of adsorption and absorption processes, and varies given the physio-chemical properties of each compound. With particle binding, pH, and partitioning coefficients, assessing the sorption behavior of antibiotics is difficult (Kummerer, 2009). Clofibric acid exhibited high persistence and was negligible to sorption under normal conditions in the Loffler study that followed the environmental persistence of microcontaminants. Tetracylines form complexes and bond with alkaline earth metals, like calcium and magnesium (Kummerer, 2009). Sorption to solid materials (i.e. clay, soil, coagulants) is a key factor in the microcontaminant removal. This process does not play a large role in the removal of contaminants with low adsorption coefficients. The study by Carballa et al. (2004) highlighted pharmaceuticals resistant to degradation. Ibuprofen and naproxen have low solid-liquid partitioning coefficients, in addition to their acidic structures. Of the initial concentrations observed carbamazepine, ibuprofen, and sulfamethoxazole had recoveries of 67, 90, and 75 percent of initial, respectively. These microcontaminants remained in the aqueous phase and were resistant to settling, flocculation, and other removal processes (Carballa et al. 2004). Tolls (2001) performed a review on the sorption of veterinary pharmaceuticals in soil. Various

compounds were analyzed and their interaction with soil was quantified. Sulfadiazine, fluoroquinolones, and other sulphonamides had prevalent elimination by sorption processes to soil.

Hydrolysis. Hydrolysis is the decomposition of organic compounds when reacted with water. The compound is separated into two or more distinct parts with addition of a hydrogen ion. As hydrolysis occurs, compounds are broken down into smaller compounds, which often produce degradation byproducts with unknown toxicity (Nikolaou et al. 2007). Hydrolysis is a primary elimination method for pesticides. The composition of the pesticide compounds allow for the reaction with water enabling removal (EPA 1990).

Sulphanomides and quinolones are two classes of compounds, which are resistant to hydrolysis (Kummerer, 2009). The stable structure is these organic microcontaminants allow are resistant to the breakdown initiated by the protonated ion. Other wastewater contaminants, like some tetracylines, have instability and are hydrolyzed when submerged in aqueous environments.

The four removal processes: biodegradation, photolysis, sorption, and hydrolysis all have limitations. Though not originally designed for current municipal and hospital pharmaceutical loads, adequate WWTP removal is necessary due to the drawbacks of each process. Biodegradation is not a factor when a compound has high adsorptive properties, photolysis is a minimal factor

in turbid waters, sorption is not a factor when compounds fail to form complexes with sediment, and hydrolysis is not a factor when hydrophobic behavior persists.

Chapter 3

Materials and Methods

POCIS samplers.

POCIS, holders and deployment canisters were obtained from Environmental Sampling Technologies (EST Inc, St. Joseph, MO). For the field deployment, each stainless steel canister was fitted with three pharmaceutical POCIS filled with Oasis HLB sorbent (Waters Corporation, Milford, MA). Each POCIS had a surface area of 41 cm^2 and contained 200 mg of sorbent medium.

Laboratory Uptake Study

Uptake rates were measured in the laboratory by submerging a single POCIS sampler in a 2L beaker of ultrapure water spiked with the pharmaceuticals of interest (Table 1) at an initial concentration of 500 ng/L under flowing conditions. The experiment was performed with four replicates. The water temperature was measured to be approximately 25^0 C throughout the duration of the experiment. A negative control experiment was performed in duplicate, which consisted of a beaker containing water and POCIS, but excluded any spiked compounds to assess the potential for contamination during the experiment. A positive control experiment was performed, consisting of a 2L beaker containing ultrapure water spiked with the same concentration of contaminants, but contained no POCIS.

The purpose of the positive control was to monitor natural degradation of the pharmaceuticals unrelated to POCIS uptake. The beakers were covered with foil and 100 mL water samples were removed from each beaker at 0, 3, 7, 14, and 30 days. At the end of the 30-day exposure period, the POCIS was removed. All aqueous samples and the POCIS were stored at -20^{0}C until analysis. The water flow rate in the beakers was determined to be approximately 4.5 m/s based on travel time around the circumference of the container.

Table 1. Pharmaceuticals evaluated and LC-MS parameters.

Compound	Use	CAS No.	Mol. Weight (g/mol)	Retention Time (min)	MRM	Collision Energy (eV)	Cone Voltage (V)	IDL (ng)
Non-prescription Drugs								
Acetaminophen	Analgesic/ Anti-pyretic pain reliever	103-90-2	151.16	10.73	152>110	14	30	1.58
Caffeine	Stimulant	58-08-2	194.19	11.94	195>138	18	32	0.33
1,7-dimethylxanthine	Caffeine metabolite	611-59-6	180.16	11.25	181>124	20	32	0.61
Cotinine	Nicotine metabolite	486-56-6	176.22	10.30	177>78	20	35	0.28
d-amphetamine	Stimulant	51-64-9	135.21	10.90	136>91	16	18	0.70
DEET	Insect repellent	134-62-3	191.27	16.70	192>119	15	25	0.66
Diphenhydramine	Anti-histamine	58-73-1	255.35	12.89	256>167	14	25	0.35
Ibuprofen	Anti-inflammatory	15687-27-1	206.28	18.51	207>161			
Methamphetamine	Stimulant	537-46-2	149.23	10.99	150>91	20	20	0.43
Ractopamine	Beta agonist	90274-24-1	301.38	11.09	302>164	18	16	0.18
Prescription Drugs								
Carbamazepine	Anti-convulsive	298-46-4	236.27	15.66	237>194	22	32	0.71
Veterinary and Human								

Antibiotics								
Azithromycin	Antibiotic	83905-01-5	748.98	12.63	750>592	25	40	2.79
Erythromycin	Antibiotic	114-07-8	733.93	14.8	734>576	32	22	0.57
Lincomycin	Antibiotic	154-21-2	406.54	10.73	407>359	38	20	0.28
Monensin	Antibiotic	17090-79-2	406.54	20.54	688>635	22	17	0.025
Sulfachloropyridazine	Antibiotic	280-32-0	284.72	12.20	285>156	15	24	0.60
Sulfamethazine	Antibiotic	57-68-1	278.33	12.03	279>156	30	18	0.19
Sulfamethazole	Antibiotic	144-82-1	270.33	11.33	271>156	24	13	0.29
Sulfadimethoxine	Antibiotic	122-11-2	310.33	13.24	311>156	20	28	0.76
Sulfamethiazole	Antibiotic	144-82-1	270.33	11.68	271>156	13	24	0.17
Sulfamethoxazole	Antibiotic	723-46-6	253.28	12.20	254>156	15	23	0.34
Sulfamerazine	Antibiotic	127-79-7	264.30	11.51	265>156	16	28	0.24
Sulfathiazole	Antibiotic	72-14-0	255.32	10.99	256>156	14	25	0.46
Thiabendazole	Anthelmintic	148-79-8	201.25	12.38	202>175	24	35	0.17

Tiamulin	Antibiotic	55297-95-5	493.74	14.56	494>192	32	24	0.44
Tylosin	Antibiotic	1401-69-0	916.10	14.44	916>772	55	32	0.041
Virginiamycin	Antibiotic	21411-53-0	525.59	16.35	526>355	16	25	0.78
Internal Standards								
phenyl-$^{13}C_6$ – sulfamethazine		57-68-1	284.1	11.95	285>124	25	30	
d_9-methamphetamine		537-46-2	158.1	10.99	159>93	18	20	
$^{13}C_3$-caffeine		58-08-2	197.1	11.87	198>140	18	32	

Sampling Rate Calculations

Values of R_s were determined by fitting experimental uptake data to equation 3. Data were fit for each set of sequential sampling events, and an average R_s was calculated for the overall experiment. Some of the compounds had significant decreases in aqueous concentration over time in the positive control experiments. Evaporation of all solutions produced increases in concentrations for some compounds. Dissipation data from the positive control experiments was used to correct aqueous concentration data observed in the experimental reactors by subtracting mass losses due to dissipation and not POCIS uptake. Varying degrees of uptake were observed for carbamazepine, DEET, diphenylhydramine, erythromycin, ibuprofen, ractopamine, sulfadimethoxine, sulfamerazine, thiabendazole, tylosin, azithromycin, and sulfacholorpyridazine. Lincomycin and tiamulin experienced rapid uptake, so experimental uptake rates could only be determined from data present between 0 and 3 days. The R_s values for these two compounds are significantly higher than others and include no standard error because data was only obtained from one time period.

Field Deployment

Two field sites were chosen to investigate pharmaceutical fate in receiving water: Salt Creek, downstream from the Theresa St. WWTP in Lincoln, Nebraska and the west fork of the Big Blue River, which receives discharge from the WWTP at Hastings, Nebraska. Additional information about the two field sites may be found in Table 2. At each location, POCIS were placed in the effluent prior to discharge, in stream within the

effluent mixing zone, at a location approximately 500 m downstream from the effluent discharge, and at a location approximately 1500 m downstream from the effluent discharge. POCIS were deployed in triplicate in a steel canister and secured in place using a metal stake to avoid displacement of the samplers. POCIS were deployed on May 5, 2009 and retrieved on May 28, 2009, for a 23-day exposure period. At the end of the exposure period, POCIS were retrieved, rinsed gently with DI water, and stored at -20˚C until analysis. Data on the ng of each compound recovered from the POCIS samplers were converted to aqueous concentrations using the laboratory uptake rates determined in this study.

Table 2. Wastewater Treatment Facilities sampled in Nebraska.

Facility Location	**Receiving Water Body**	**Community Population (2008)**	**Secondary Treatment Technique**	**Average Daily Flow (MGD)**
Hastings, NE	West Fork of the Big Blue River	25,394	Trickling Filter	4.0
Lincoln, NE	Salt Creek	251,624	Activated Sludge and Trickling Filter	18

Determination of pharmaceutical decay rates

To determine decay rates for pharmaceuticals in receiving waters, the experimental data was fit to the following equation:

$$C_t/C_o = \exp(-kt) \quad (4)$$

where C_t (ng/L) is the concentration at a specific time t, C_o (ng/L) is the initial concentration at time zero, k (d^{-1}) is the decay rate coefficient, and t (d) is duration of time.

The sampling locations (m) were converted to a time dividing by the velocity in each channel. Channel velocities were determined by dividing the volumetric flow rate by the cross-sectional area of the channel. At Hastings, flow in the channel is entirely effluent, so volumetric flow rates were determined from plant discharge data. At Lincoln, flow in Salt Creek was determined from the Salt Creek at Lincoln, NE gauging station located at 40 50 48N, 96 40 54W. The depth and width of the channel at Hastings were measured at the time of deployment. The depth in Salt Creek was measured at the time of deployment, and the width at the sampling point was estimated based on the size of channel.

Decay rate coefficients (k) were determined by minimizing the root mean square error (RMSE) of the model using the Solver function in Excel. The equation for the root mean square error is defined in equation 5.

$$RMSE = [(y_1 - y_0)^2 / n]^{1/2} \qquad (5)$$

Where n is the number of points analyzed, y_1 (ng/L) is the value of the measured concentration, and y_0 (ng/L) is value of the modeled concentration.

Analytical Methods

Solvents and Internal Standards. Reference materials, metabolites and labeled standards, including $^{13}C_3$-caffeine and d_9-methamphetamine, were obtained from Sigma-Aldrich (St. Louis, MO). Phenyl-$^{13}C_8$-sulfamethazine was purchased from Cambridge Isotopes (Andover, MA). Solvents used in sample preparation were high purity grade (OPTIMA, Fisher Scientific, St. Louis, MO).

Extraction Methodology. Handling and elution of POCIS followed procedures described previously (Alvarez 2004; Jones-Lepp et al. 2004). After the exposure period, each individual POCIS device was removed from its deployment canister, briefly rinsed with water if needed to remove debris and opened. The contents of the POCIS were transferred using approximately 20 mL of high-purity methanol directly into silane-treated vials. Vials containing the methanol and sorbent were held at -20^{o}C until they could be processed for analysis.

Target compounds were eluted by passing 50 mL of high-purity methanol through silane-treated glass gravity flow chromatography columns into 120 mL evaporation tubes (RapidVAP, Labconco, Kansas City, MO). Approximately 1 ng of d_9-methamphetamine, $^{13}C_3$-caffeine, and phenyl-$^{13}C_8$-sulfamethazine internal standards were added to the eluate and used for quantification. Extracts were evaporated under nitrogen to approximately 1 mL, and quantitatively transferred to autosampler vials for analysis by liquid chromatography tandem mass spectrometry (LC/MS/MS). Standards and spiking solutions were prepared from stock solutions (5 µg/µL) in methanol.

Calibration solutions (2, 5, 12.5, 25 and 50 pg/µL) were prepared in 50:50 methanol and water. All standards and extracts were stored in amber vials at -20˚C.

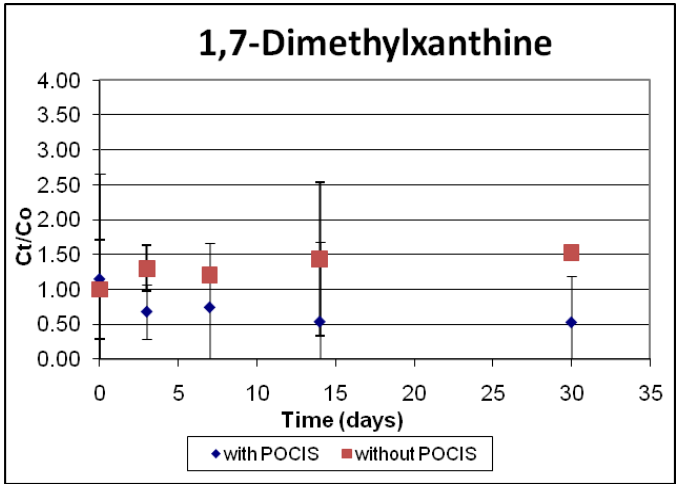
Liquid Chromatography-Tandem Mass Spectrometry. POCIS extracts were analyzed for twenty-five pharmaceuticals and metabolites, as listed in Table 1. Standards and extracts were analyzed on a Quattro Micro triple quadrupole with a Waters 2695 high pressure liquid chromatography (HPLC) and autosampler. Electrospray ionization in positive ion mode was used for detection of target compounds by multiple reaction monitoring (MRM) with argon collision gas. A Thermo (Bellefonte, PA) Betabasic-18 column (250x2.1 mm, 5 um, 50°C) was used for separation at a flow rate of 0.2 ml/min with a gradient of methanol with 0.1% formic acid in water. Mass spectrometer operational parameters were optimized by infusing each compound separately (Table 2). The source conditions were: capillary 2.5 kV, extractor 2 V, RF lens 0.8 V, source temp 90°C, desolvation temp 400°C, cone gas flow at 30 L/hr, and desolvation gas flow at 700 L/hr. Compound retention times, ionization modes and MRM transitions are listed in Table 2. A five point internal standard calibration curve was used for quantification of each analyte. Methamphetamine-d_3 was used as the internal standard for methamphetamine and D(extro)-amphetamine, phenyl-$^{13}C_8$-sulfamethazine was used for sulfa antibiotics and $^{13}C_3$-caffeine was used as the internal standard for all other target compounds. Based on the variability of the lowest standard (2 pg/µL), the estimated detection limits for most compounds are less than 1 pg/µL, corresponding to 1 ng recovered from the POCIS. Recovery of target compounds was checked by

analysis of fortified blanks spiked with known amounts of each compound and averaged 123 ± 30%. Two laboratory reagent blanks were processed with the POCIS samples, with all compounds below instrument detection limits listed in Table 1. Additional information on the analytical methods is included in Appendix C and further referenced in Bartelt-Hunt et al. (2009).

Chapter 4

Results

Results from Laboratory uptake experiments for 25 pharmaceuticals are presented in Figure 3 through 8. In each figure, the average concentration observed in beakers containing POCIS samplers and the average concentration observed in the positive controls (beakers with pharmaceuticals but no POCIS). The error bars represent the standard error of the mean. Calculated uptake rates are presented in Table 3.



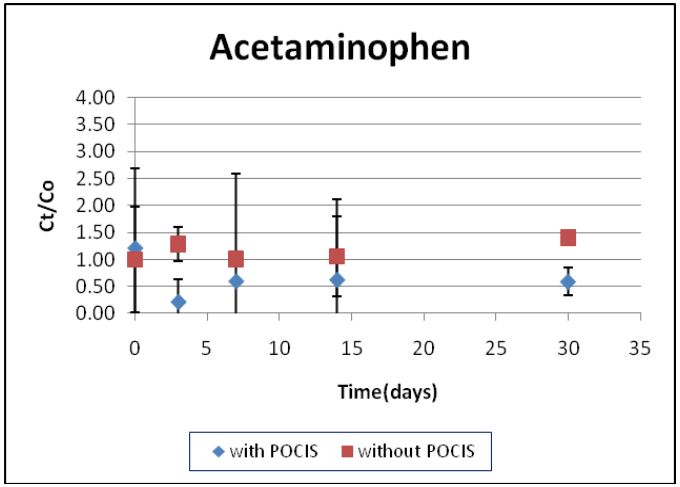
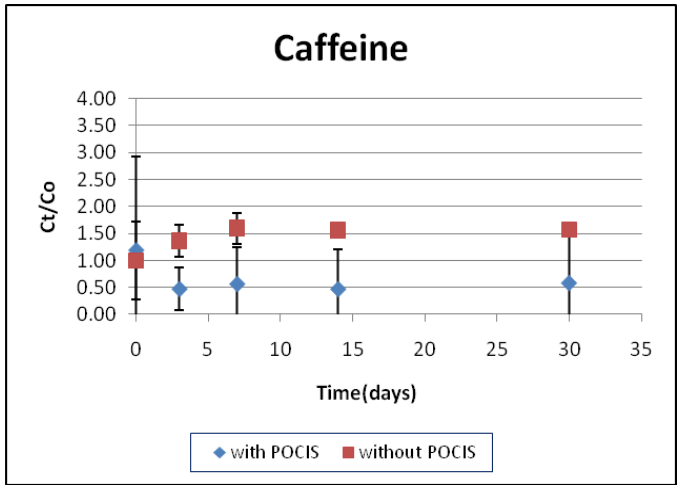
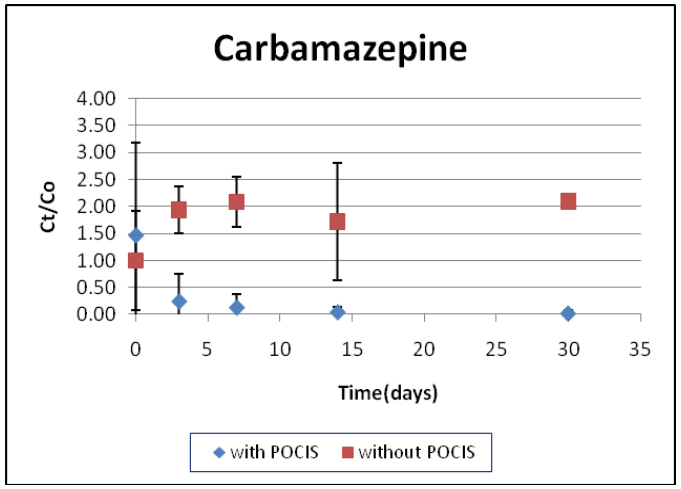
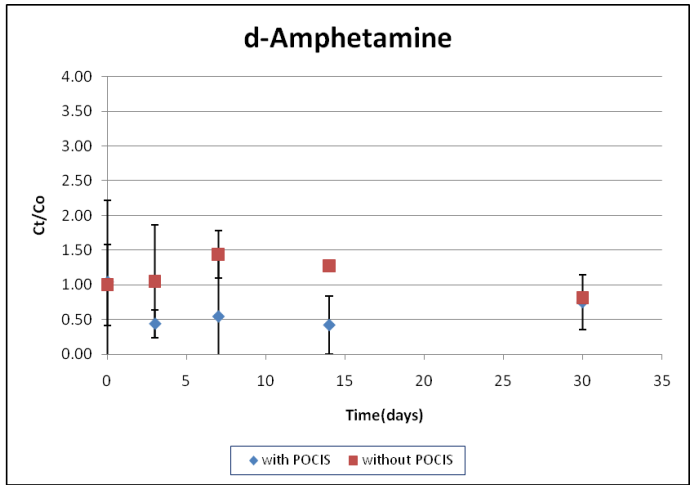


Figure 3. Laboratory Uptake Data







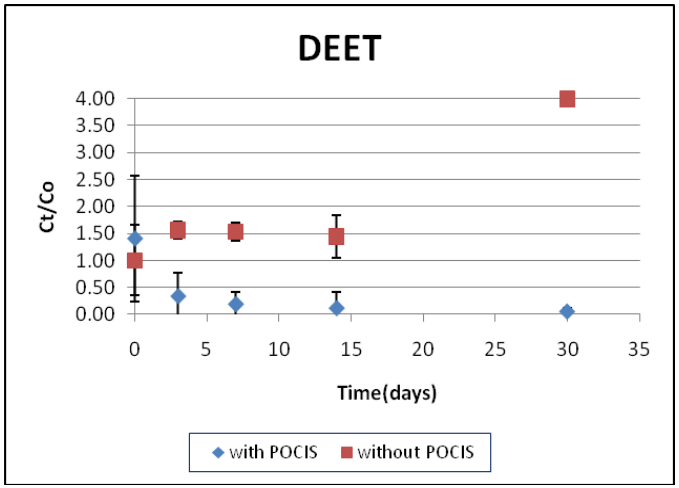
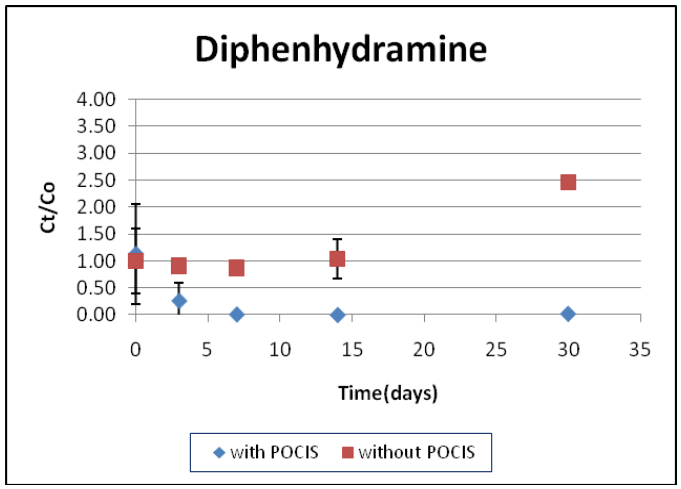
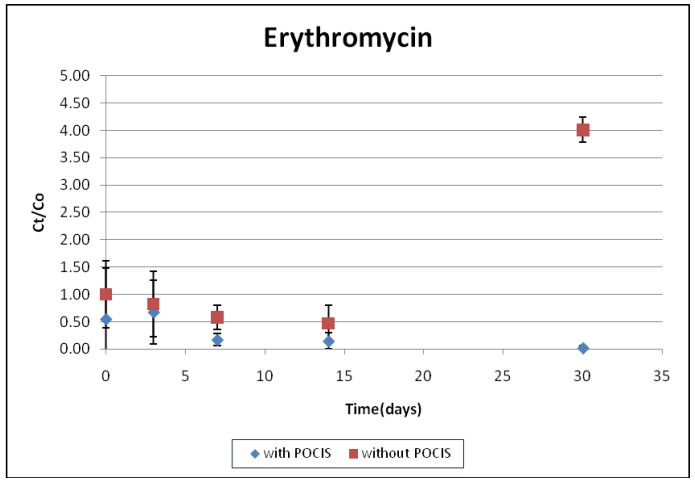
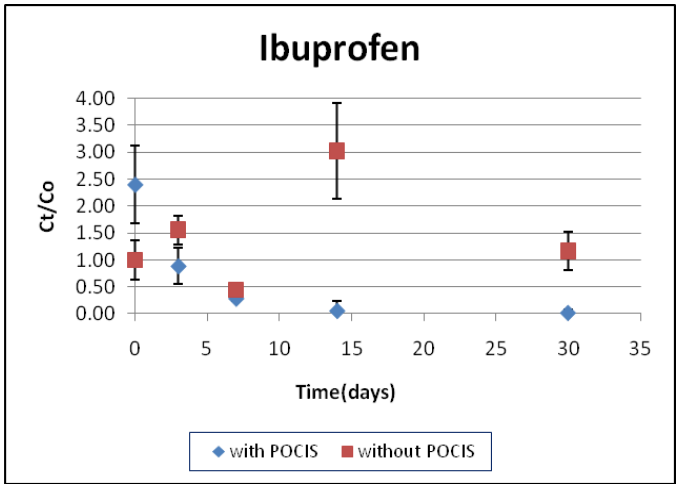


Figure 4. Laboratory Uptake Data







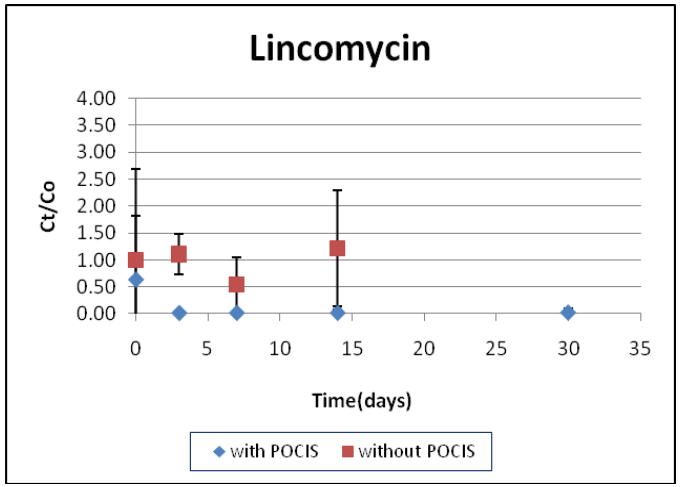
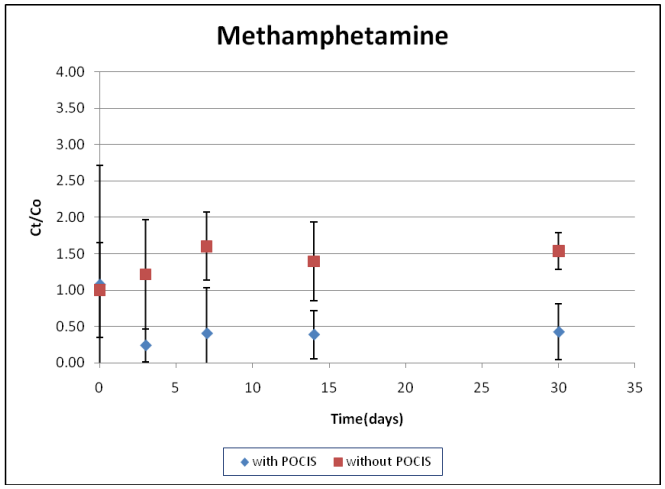
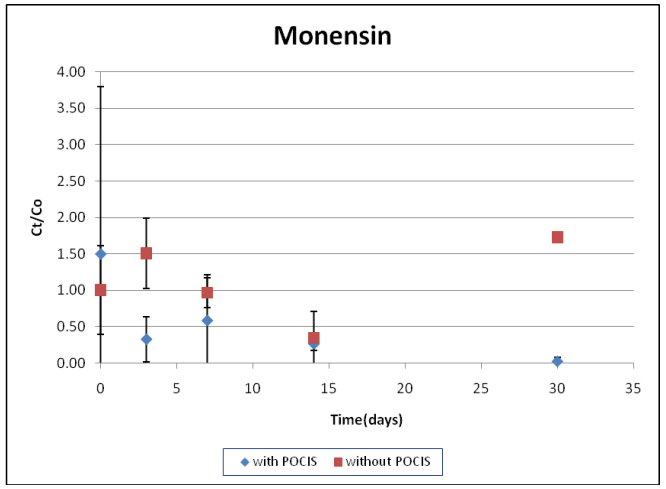
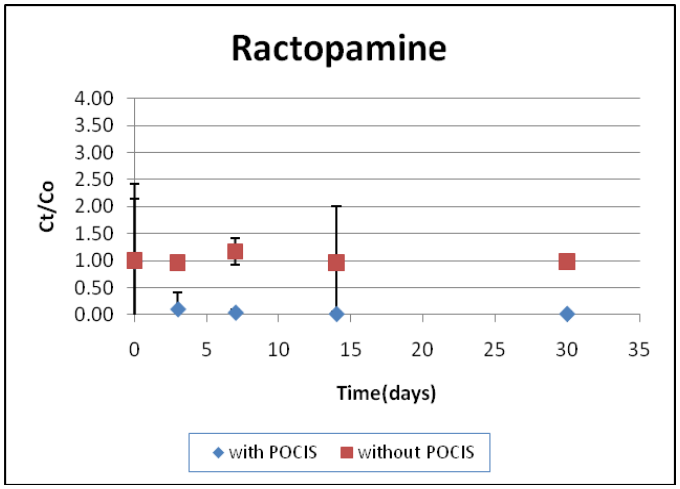


Figure 5. Laboratory Uptake Data







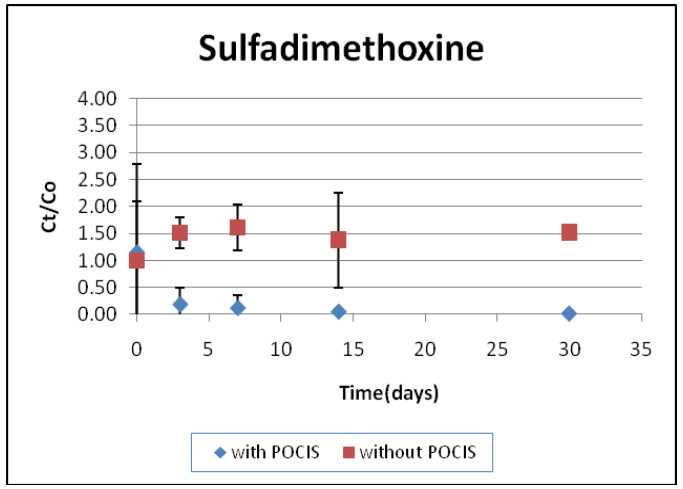
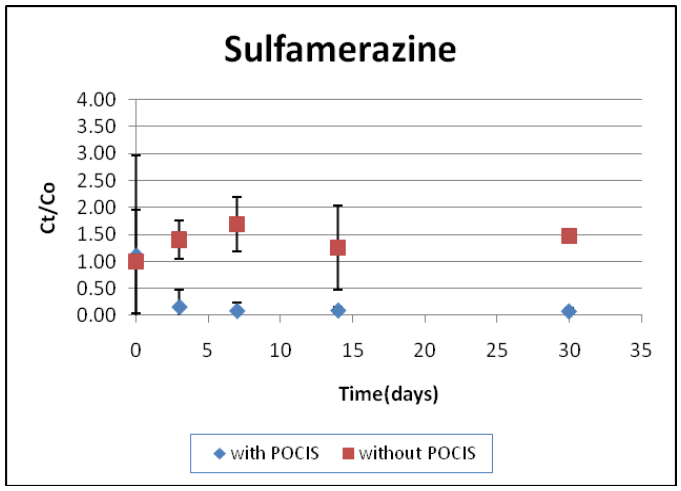
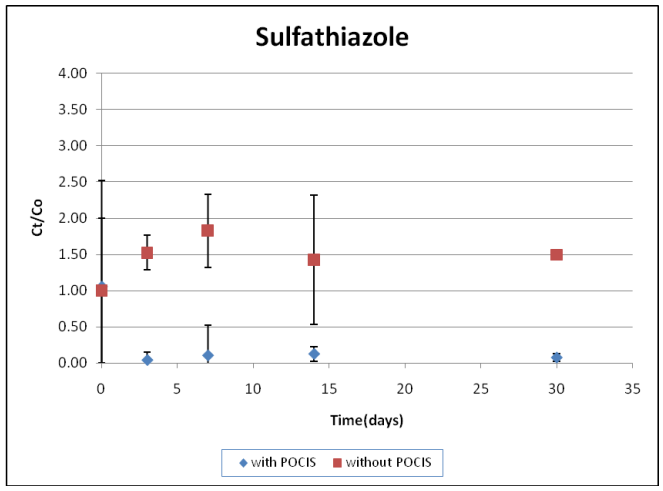
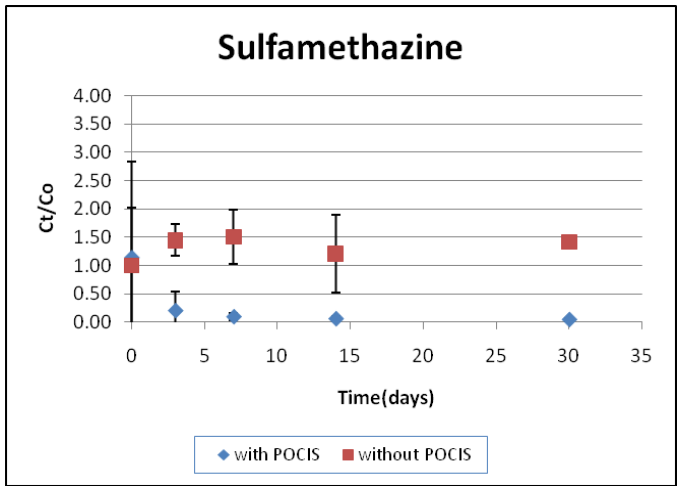


Figure 6. Laboratory Uptake Data







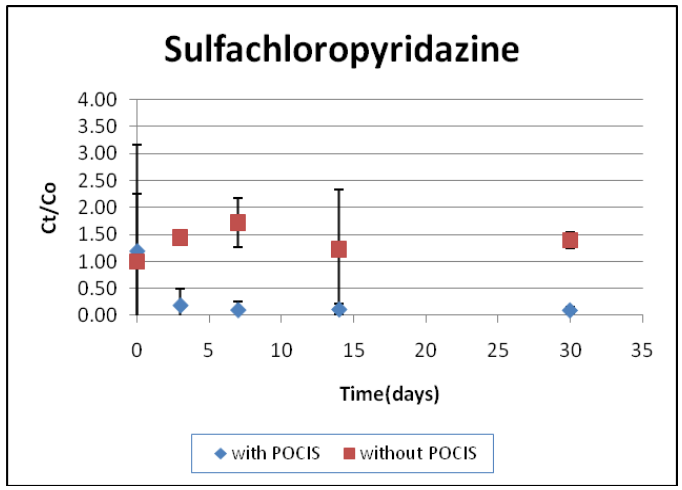
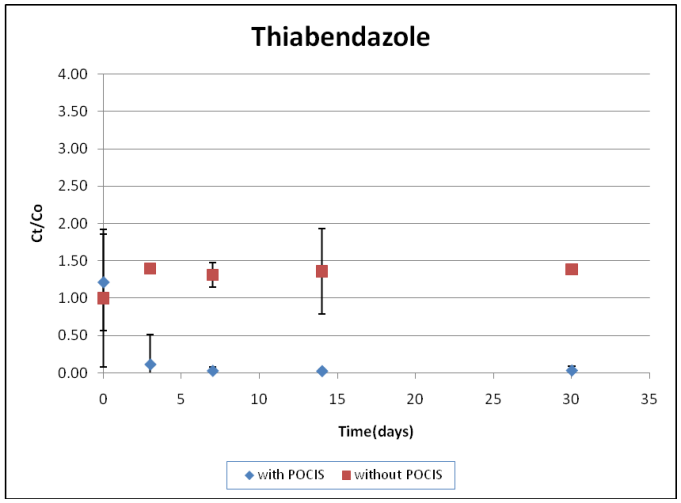
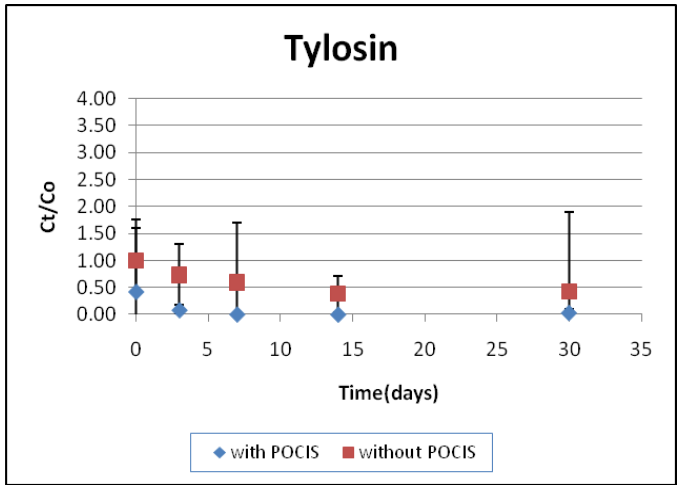
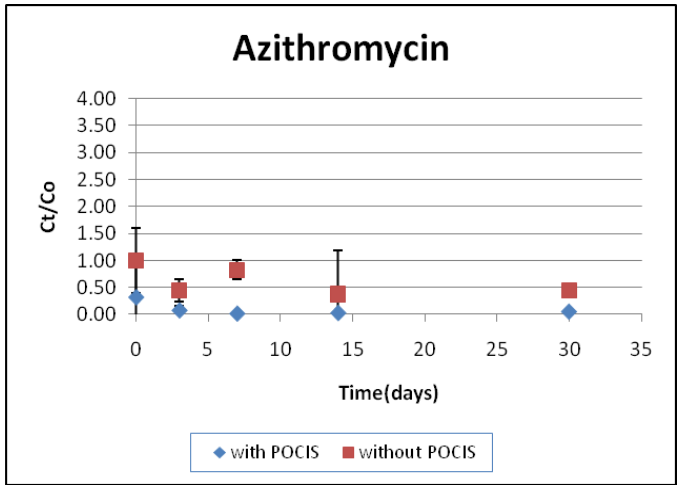


Figure 7. Laboratory Uptake Data







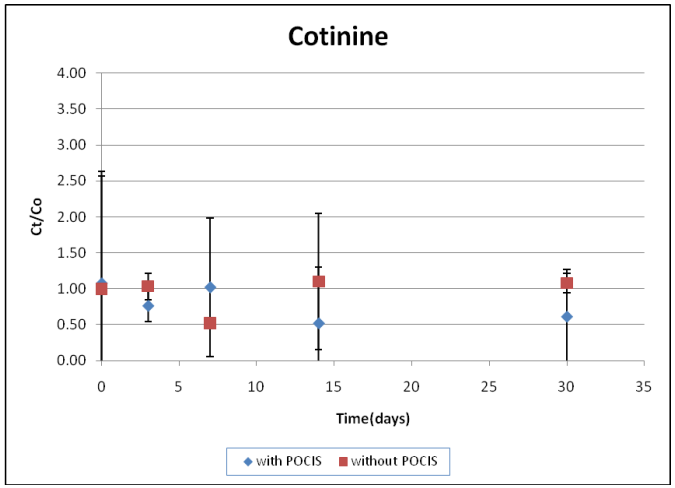


Figure 8. Laboratory Uptake Data

Table 3. Calculated R_s values and comparisons to literature data.

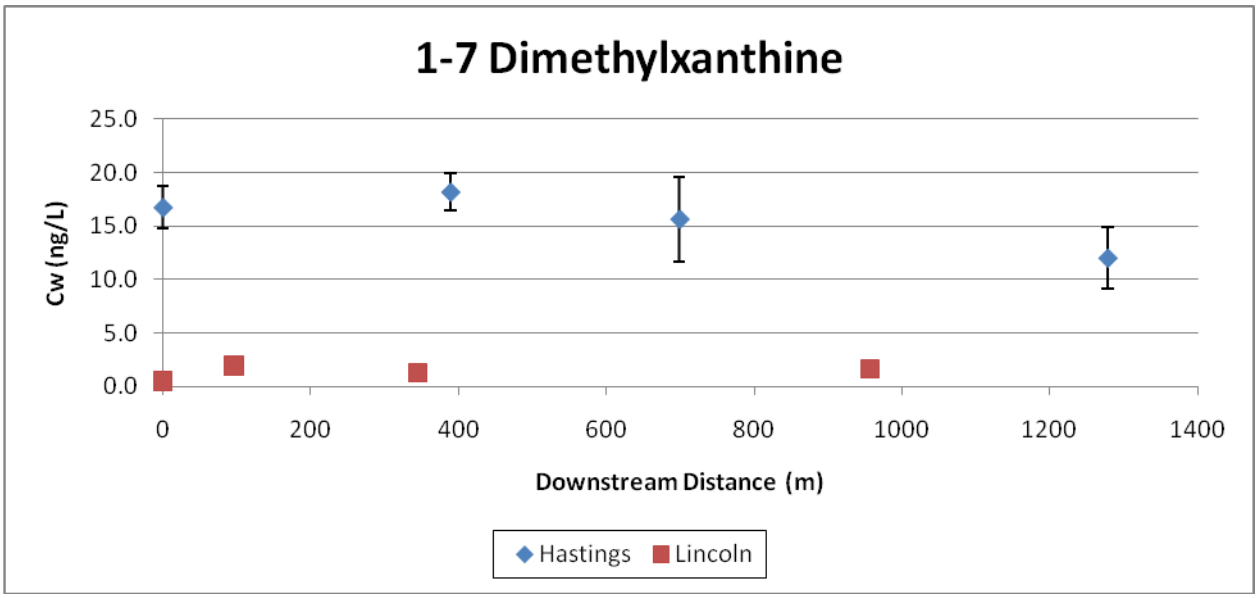
Target Compounds	Experimental Flowing, Rs (Lday-1)	Standard Error (Unitless)	Reported Flowing, Rs (Lday-1)	Source
1,7 dimethylxanthine	0.078	0.022		
Caffeine	0.2	0.097	0.1	(Togola and Budzinski 2007)
Acetaminophen	0.268	0.185		
Carbamazepine	0.227	0.045	0.31, 0.3	(MacLeod et al. 2007; Togola and Budzinski 2007)
d-Amphetamine	0.154	0.067		
DEET	0.21	0.0043		
Diphenylhydramine	0.376	0.066		
Erythromycin	0.146	0.039		
Ibuprofen	0.27	0.05		
Lincomycin	0.666	-		
Methamphetamine	0.283	0.158	0.089	(Alvarez et al. 2007)
Monensin	0.24	0.07		
Ractopamine	0.261	0.063		
Sulfadimethoxine	0.227	0.05		
Sulfamerazine	0.201	0.06		
Sulfamethazole	0.16	0.075		
Sulfamethoxazole	0.146	0.056		
Sulfathiazole	0.237	0.113		
Thiabendazole	0.33	0.091		
Tiamulin	0.664	-		
Tylosin	0.379	0.074		
Azithromycin	0.157	0.041	0.27	(Alvarez et al. 2007)
Cotinine	0.105	0.029		
Sulfachloropyridazine	0.203	0.058		
Sulfamethazine	0.228	0.054	0.1	(MacLeod et al. 2007)

[1] Values represent experimental data reported for 41 cm^2 POCIS under flowing conditions.

(-) indicated no standard error due to rapid uptake over one sampling period

The data obtained from the field deployment is presented in Figures 9 through 18. In each plot, the average in-stream concentration for each of the two sampling locations, Hastings, NE and Lincoln, NE is presented for each pharmaceutical compound.

D-amphetamine, erythromycin, sulfachloropyridazine, sulfathiazole, tiamulin, and tylosin were not detected in the field POCIS at either location.



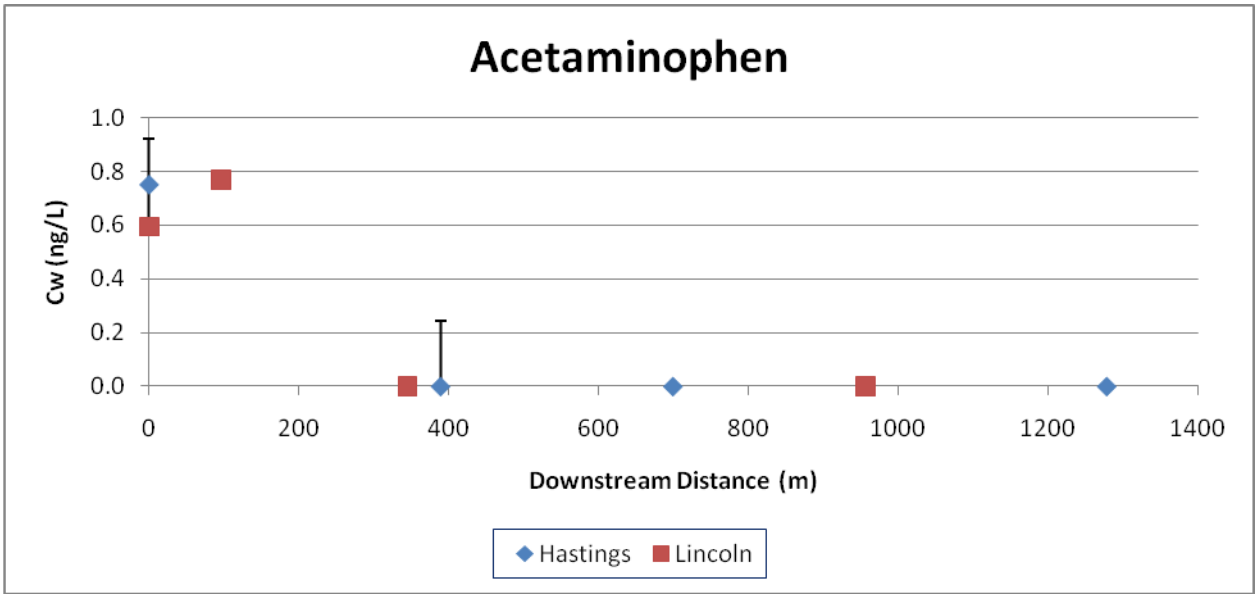
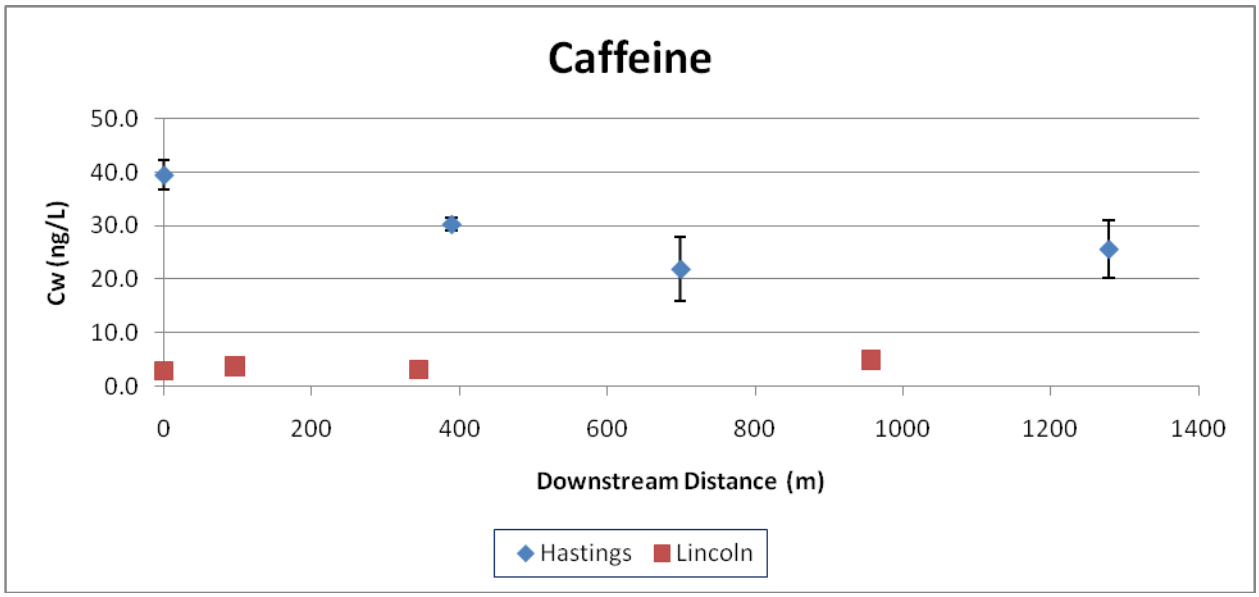


Figure 9. Field Deployment Data



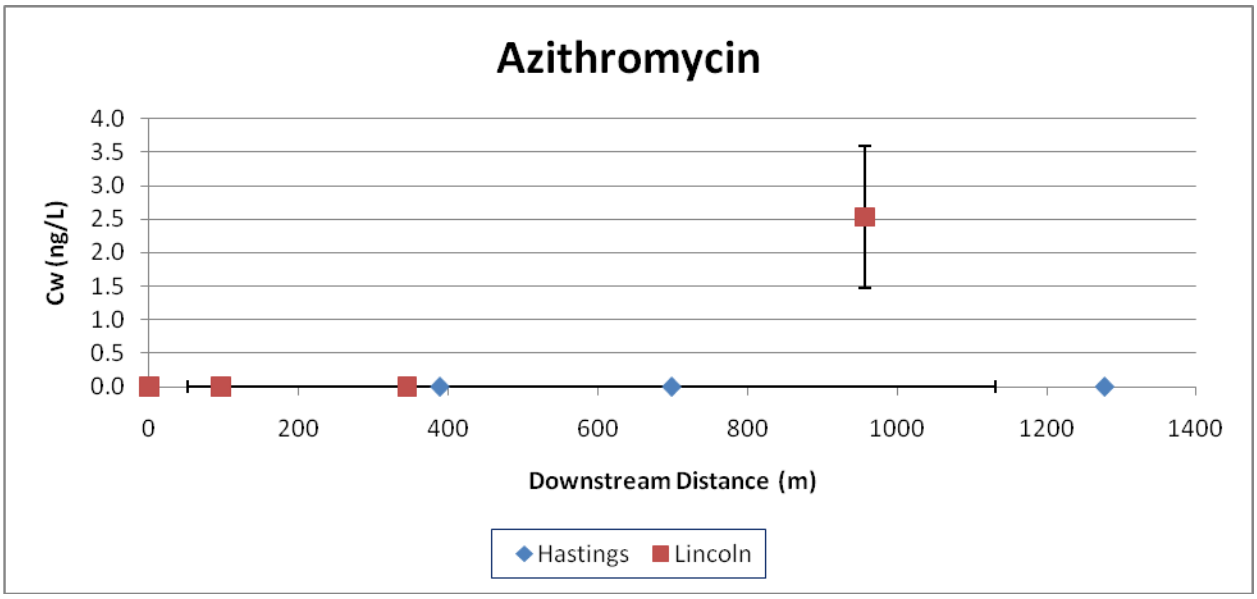
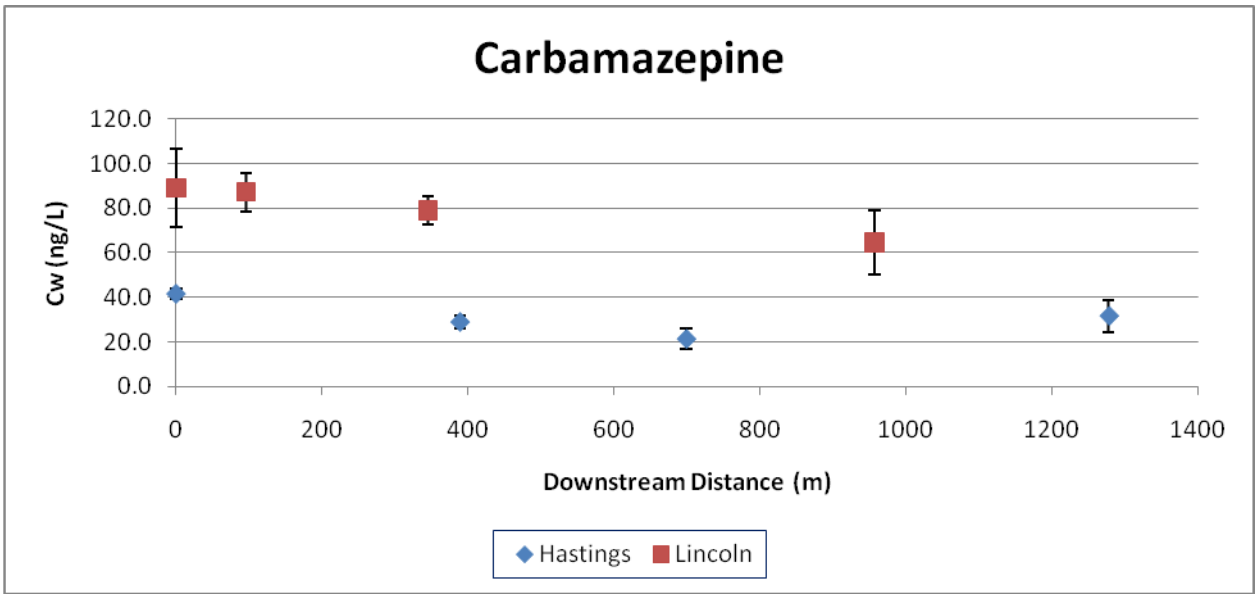
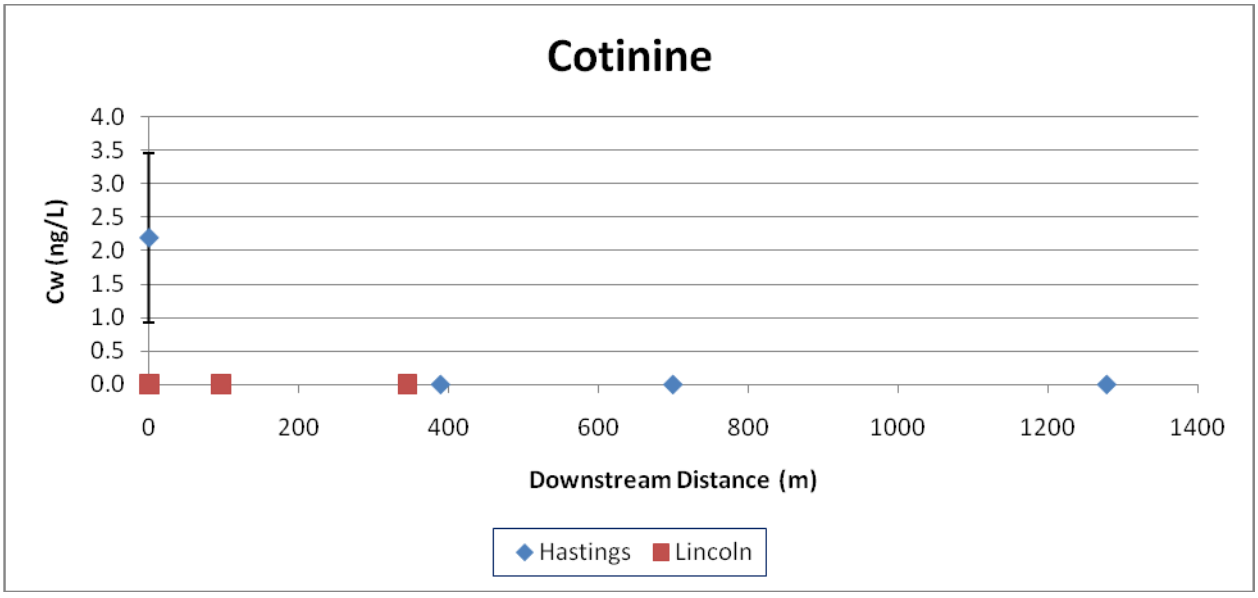


Figure 10. Field Deployment Data





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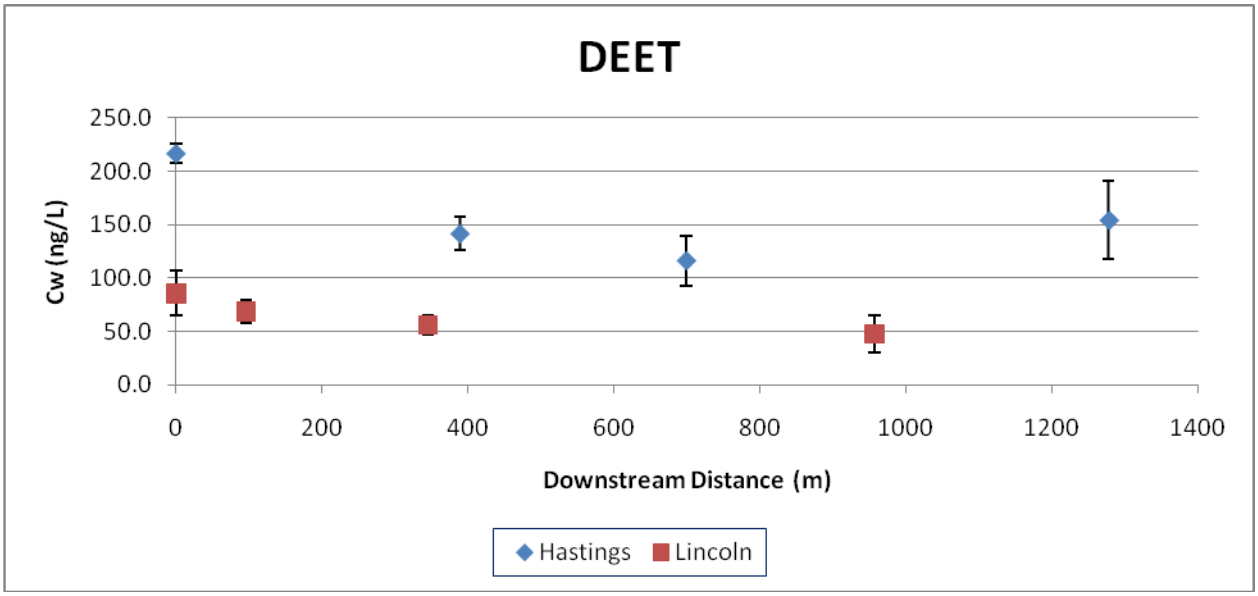

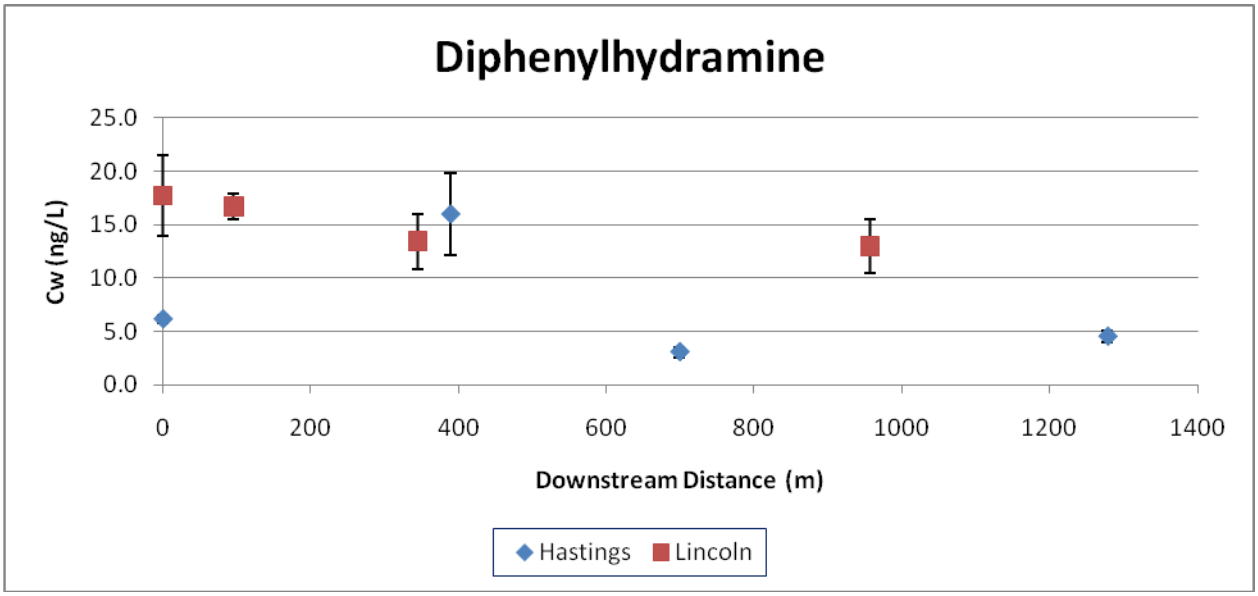

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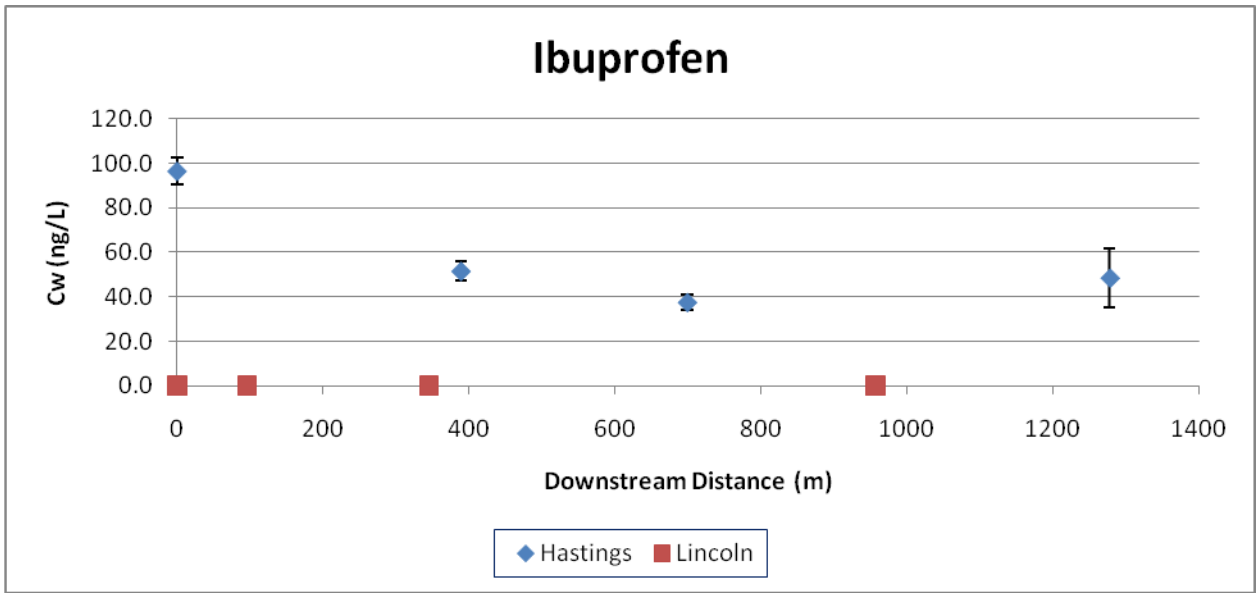

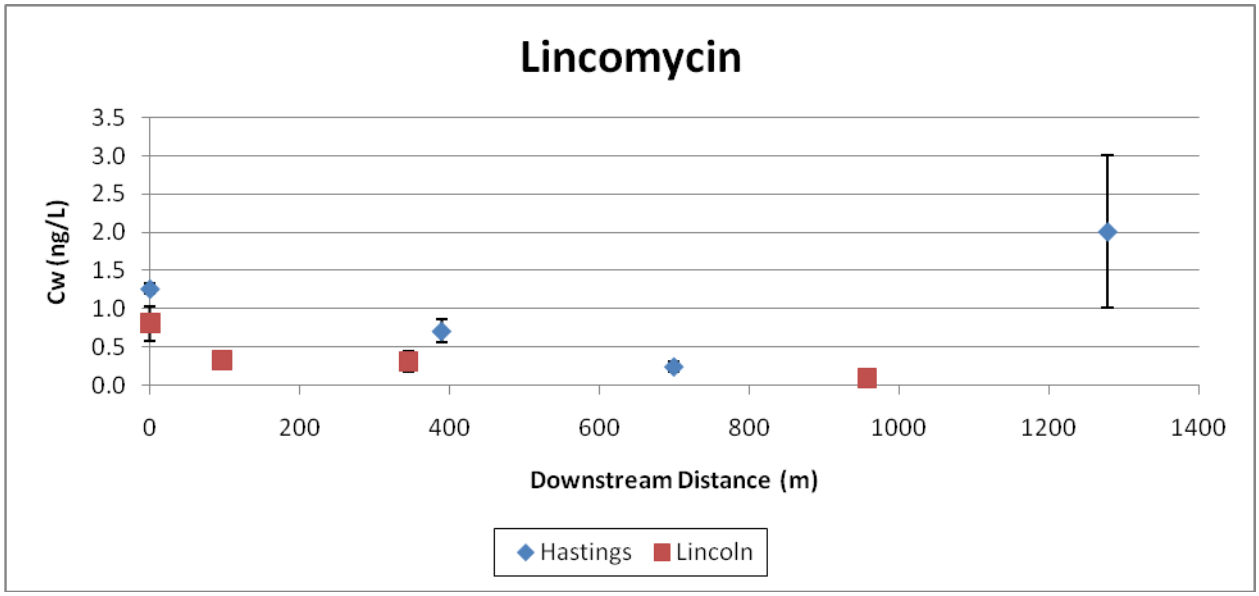

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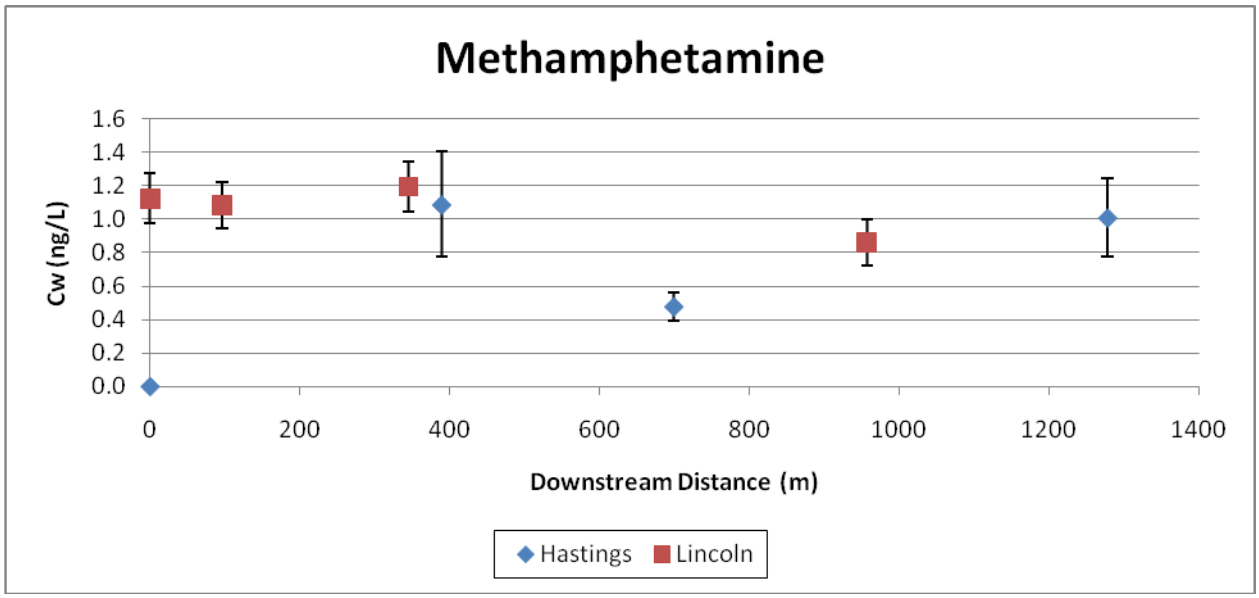

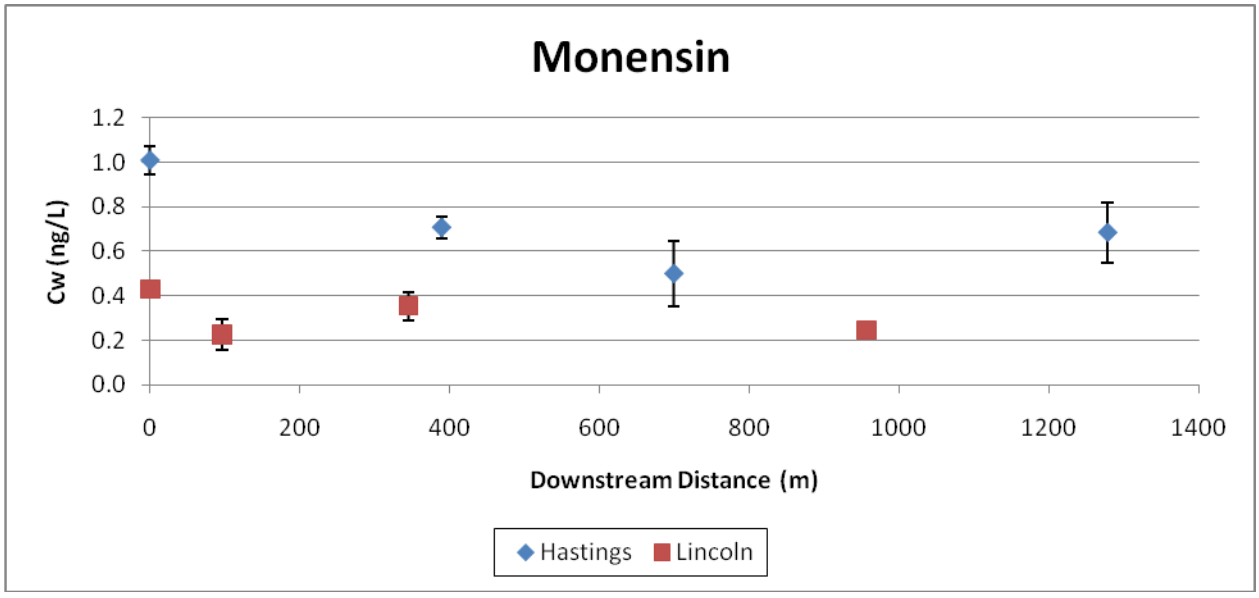

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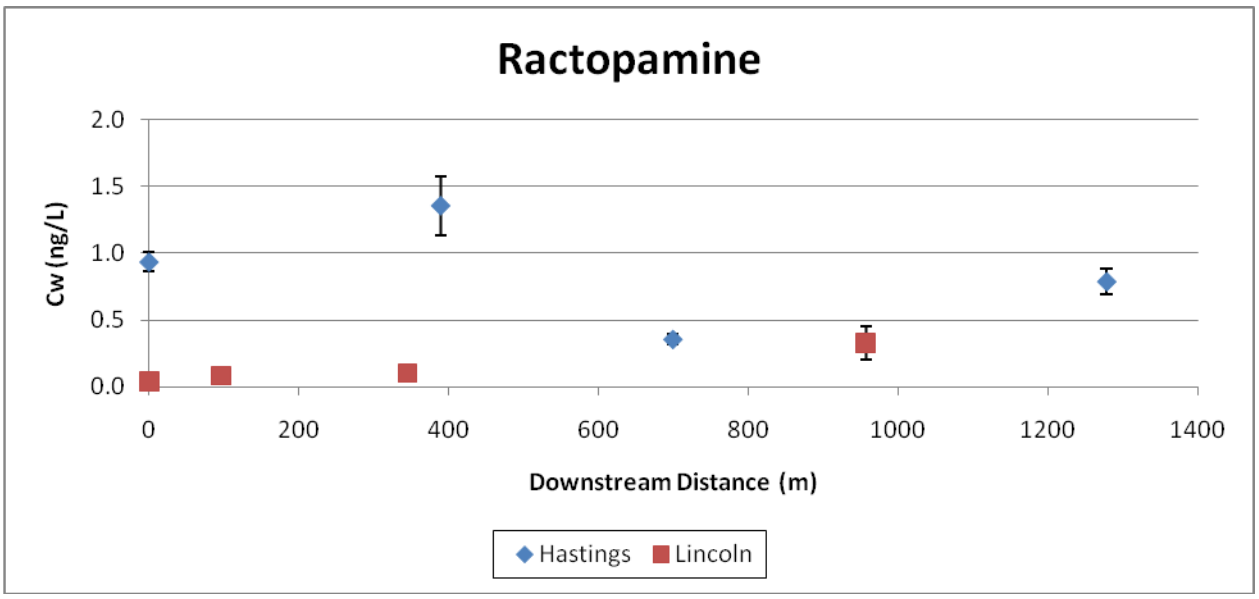

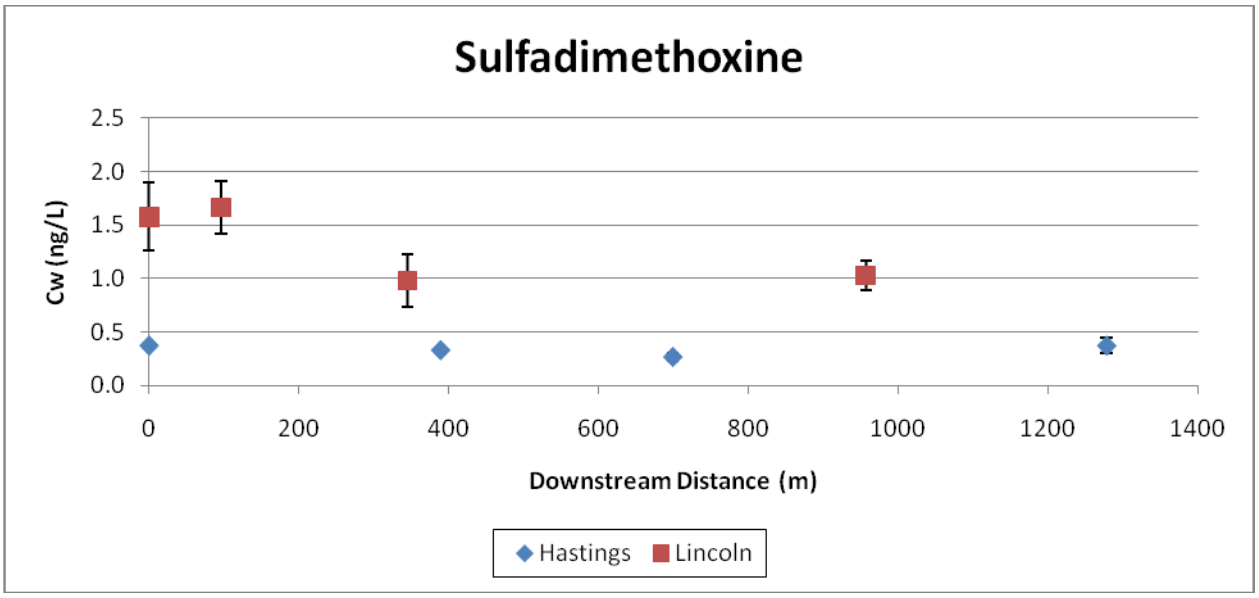

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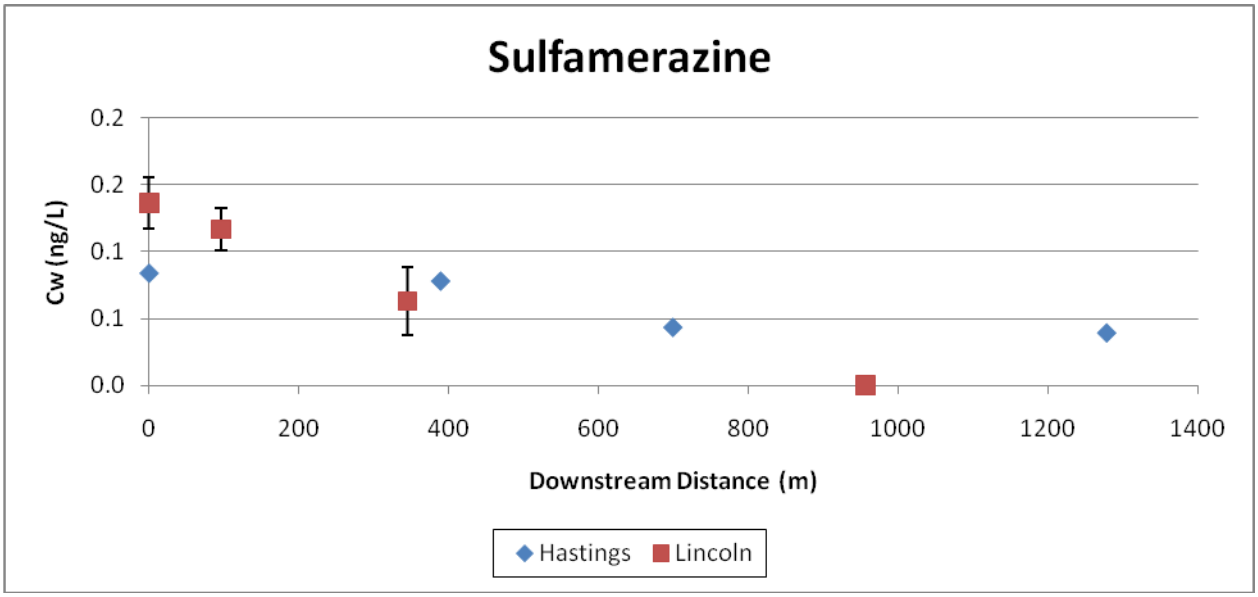

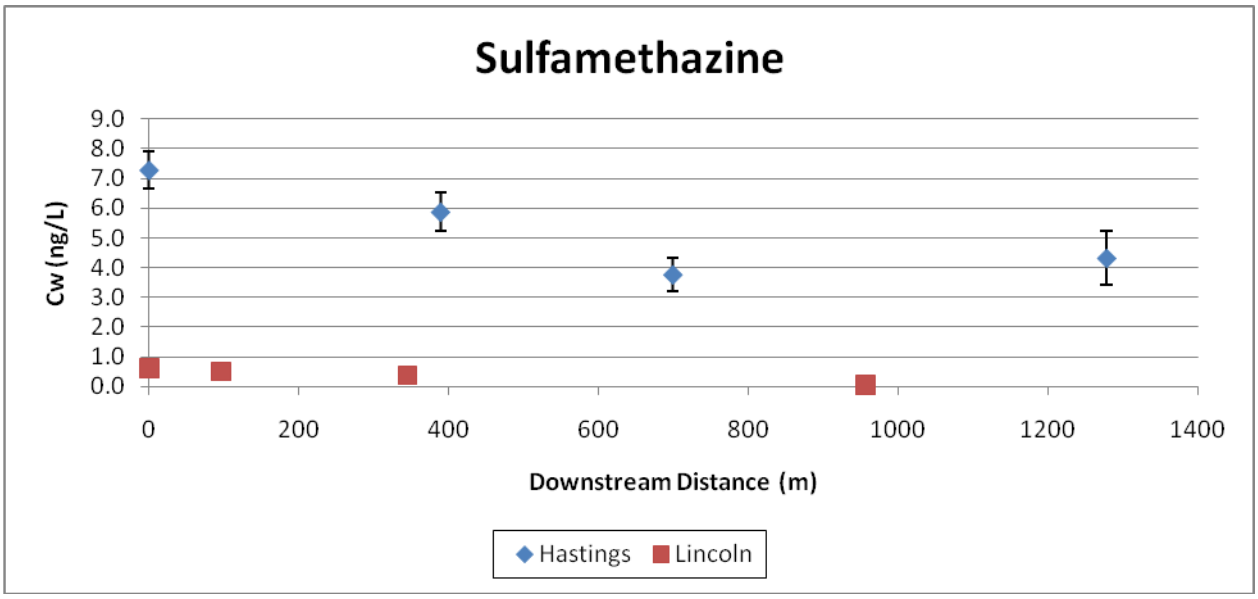

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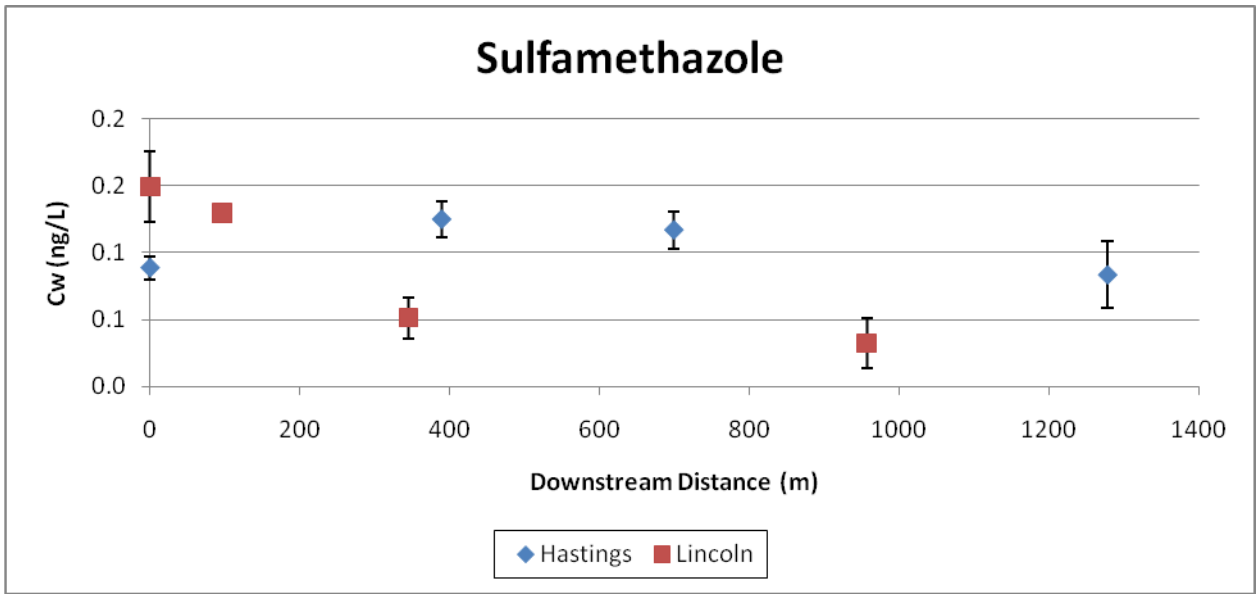

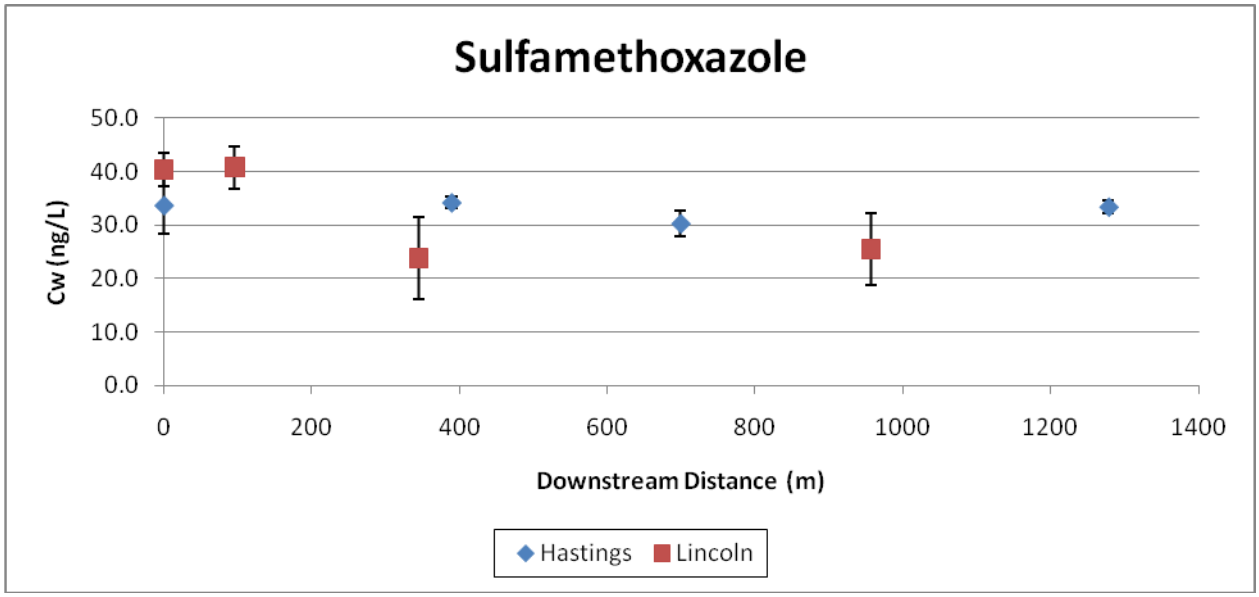

Figure 17. Field Deployment Data

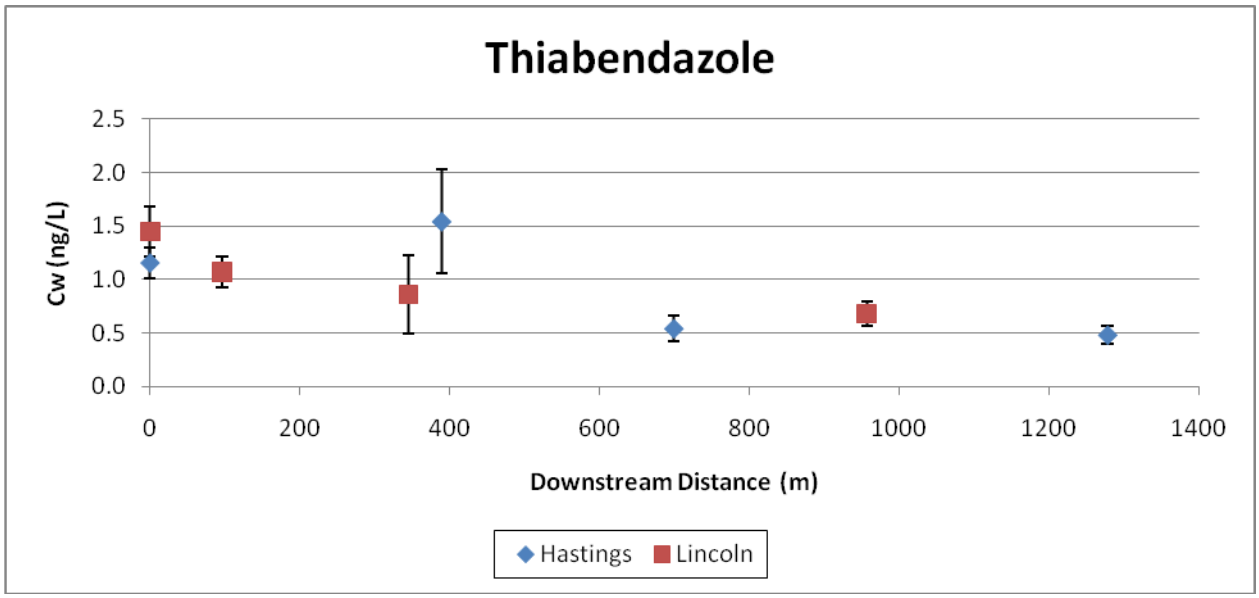

Figure 18. Field Deployment Data

Table 4. Calculated Decay Coefficients and RMSE values.

	Hastings		Lincoln	
Target Compounds	**Decay Coefficient, k (day-1)**	**RMSE**	**Decay Coefficient, k (day-1)**	**RMSE**
1,7 dimethylxanthine	0.260	1.757	0	1.169
Caffeine	0.773	4.607	0	1.249
Acetaminophen	53687091	0.701	19.8	0.261
Carbamazepine	0.695	7.731	2.63	1.437
d-Amphetamine	Not Determined	-	Not Determined	-
DEET	0.791	37.897	6.236	7.317
Diphenhydramine	0.027	6.043	3.211	1.143
Erythromycin	Not Determined	-	Not Determined	-
Ibuprofen	1.479	16.01	Not Determined	-
Lincomycin	0.072	0.827	31.2	0.12
Methamphetamine	0	0.906	1.052	0.099
Monensin	0.807	0.169	5.408	0.108
Ractopamine	0.222	0.398	0	0.191
Sulfadimethoxine	0.208	0.069	4.805	0.214
Sulfamerazine	2.407	0.034	9.175	0.0298
Sulfamethazole	0	0	9.175	0.0298
Sulfamethoxazole	0.051	1.68	5.11	5.46
Sulfathiazole	Not Determined	-	Not Determined	-
Thiabendazole	0.901	0.36	7.18	0.107
Tiamulin	Not Determined	-	Not Determined	-
Tylosin	Not Determined	-	Not Determined	-
Azithromycin	Not Determined	-	0	1.443
Cotinine	53687091	0	Not Determined	-
Sulfachloropyridazine	Not Determined	-	Not Determined	-
Sulfamethazine	0.867	0.778	11.41	0.045

Chapter 5

Discussion

Laboratory Uptake Experiments

The experimental R_s values determined for the pharmaceutical compounds evaluated in this study are comparable to published uptake rates under similar flowing conditions (Table 3). For example, previously-published R_s values for carbamazepine were 0.31 and 0.3 L/day, respectively (MacLeod et al. 2007; Togola and Budzinski 2007). This compares well with the value for carbamazepine of 0.227 L/day calculated in this study. Similarly, the experimental R_s value determined for caffeine in this study is 0.2 L/day, which is comparable to a value of 0.1 determined by Togola and Budzinski (2007). Alvarez et al. (2007) reported an uptake rate for azithromycin of 0.27 L/day, while the experimentally-determined uptake rate for azithromycin is 0.157 L/day. MacLeod et al. (2007) reported an R_s value of 0.1 L/day for sulfamethazine, while the current study calculated an uptake rate 0.228 L/day. Methamphetamine had experimental R_s of 0.283 L/day, while Alvarez et al. (2007) reported an R_s of 0.089 L/day under comparable flowing conditions. In addition to these five compounds with previously-published uptake rates, experimental uptake rates were determined for 20 additional compounds with no previously reported values. Lincomycin and tiamulin had the highest rates of uptake during the laboratory study. Similar to other compounds analyzed, between days 0 and 3 there was sharp decrease in contaminant concentration. This was originally assumed to be extensive uptake, but was later determined to be dry POCIS saturation in water

and evaporation. The negative control confirmed that contamination was limited during the experiment. The raw data for this control and others is provided in Appendix C. The positive controls also exhibited inconsistencies throughout the 30-day observation period. Often pharmaceutical concentration values exceeded the initial spike amount at day 0. This was attributed to evaporation and/or contamination during methods of recovery. A correction factor was utilized to correct all pharmaceutical concentrations larger than initial sample concentration taken at time 0.

During this study, all pharmaceuticals were analyzed at relatively low concentrations, so some degree of analytical error may be present. Standard error provided further validity to the data reported. The agreement between the experimental uptake rates and those reported in other studies provides good evidence that organic compound quantification using POCIS is reproducible, at least over the range of environmental conditions employed in our study and previously-published studies.

Field Deployment Data

Pharmaceuticals were detected receiving waters downstream of the wastewater treatment plant outfall at both sampling locations. At Hastings 1,7-dimethylxanthine, caffeine, carbamazepine, DEET, diphenylhydramine, ibuprofen, lincomycin, methamphetamine, monensin, ractopamine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethazole, sulfamethoxazole, and thiabendazole were detected at every point downstream

from the WWTP. In Lincoln 1,7-dimethylxanthine, caffeine, carbamazepine, deet, diphenylhydramine, lincomycin, methamphetamine, monensin, ractopamine, and sulfadimethoxine sulfamerazine, sulfamethazine, sulfamethazole, sulfamethoxazole, and thiabendazole were all detected in the field study. All pharmaceuticals detected in the Lincoln WWTP effluent were also present in the Hastings effluent with the exception of ibuprofen. It appears that antibiotics, specifically the sulfa –based compounds, showed high levels of persistence in wastewater effluents. DEET and carbamazepine had the highest average effluent concentrations of 110 and 60 ng/L, respectively. Both also showed persistence at both sites surveyed. At Lincoln, azithromycin originally showed no occurrence in the wastewater effluent, but a spike of 2.2 ng/L appeared approximately 1000m downstream effluent discharge point. This may be due to desorption from sediment or additional runoff from surroundings non-point sources. Although tiamulin was observed to undergo rapid uptake in the laboratory uptake study, we observed persistence of this compound at both at the Hastings and Lincoln locations. This may be due to enhanced persistence in the natural environment due to association with aquatic sediment.

Some of the compounds appeared in the wastewater effluent, but dissipated after discharge to the receiving water. At Hastings, acetaminophen, cotinine, and sulfathiazole were degraded in-stream. At Lincoln, acetaminophen, azithromycin, and sulfathiazole were degraded in stream. Acetaminophen and cotinine are non-prescription drugs that appear to be easily eliminated at trace levels. Sulfathiazole, a prescription antibiotic, showed substantial degradation

over deployment period. This may be attributed to a smaller molecular weight than other sulfa compounds studied. Compound stability may also be of concern, as well.

Decay coefficients were determined from the collected field data as described previously, and are presented in Table 4. Sulfamethazole was not degraded at the Lincoln location in the study, therefore the decay coefficient and RMSE (Table 4) for this compound was 0. Due to no effluent occurrence, decay coefficients were not determined for sulfachloropyridazine, azithromycin, tylosin, tiamulin, sulfathiazole, erythromycin, and d-amphetamine at Hastings. Decay coefficients were not determined for sulfachloropyridazine, sulfathiazole, tylosin, tiamulin, ibuprofen, erythromycin, and d-amphetamine at the Lincoln field site. Acetaminophen and cotinine had very high decay coefficients, due to high initial spikes and immediate dissipation. The very high dissipation rates made it difficult to determine RMSE, due to only one non-zero data point. The opposite was apparent for a few compounds in Lincoln with continuous occurrence. Decay coefficients of 0 were calculated for 1,7-dimethylxanthine, caffeine, and ractopamine at Lincoln. This was due to persistence along each POCIS sampling site and minimal to no degradation. First-order decay models using the calculated decay coefficients were fit to experimental data as presented in Appendix A.

Various pharmaceutical compounds investigated in this study were present in effluents and appeared to be resistant to WWTP removal and degradation processes. Bartelt-Hunt et al. (2009) performed a field study at the

same Nebraska sampling locations, with the exception of Columbus, Grand Island, and Omaha, where similar compounds were analyzed. A laboratory uptake experiment was not conducted, so R_s values were estimated using Equation 2. Though estimations were used, various data exists in this study that is comparable to previous work by published Bartelt-Hunt et al. (2009). The experimental sampling rates for the sulfa-based drugs in this study were approximately 0 to 0.5 units from values theoretically derived a few years prior. Bartelt-Hunt et al. (2009) reported calculated flowing rates for sulfachloropyridazine, sulfamethazine, sulfadimethoxine, sulfamethazole, sulfamethoxazole, sulfamerazine, and sulfathiazole of 0.20, 0.18, 0.17, 0.21, 0.21, 0.20, and 0.22 L/day, respectively. In this study experimental flowing rates of 0.203, 0.228, 0.227, 0.16, 0.146, 0.201, and 0.237 L/day were determined for the same compounds, respectively. Acetaminophen, carbamazepine, DEET, and methamphetamine were comparable as well with R_s values of 0.3 and 0.268, 0.20 and 0.227, 0.19 and 0.21, 0.22 and 0.283, L/day respectively. Both studies failed to detect d-amphetamine and sulfathiazole at any point downstream from either effluent discharge. Though previously detected, virginiamycin was not analyzed in our current study.

In addition to sampling rates, Bartelt-Hunt et al. (2009) reported field occurrence data that was comparable to data generated in this study. Sulfamethoxazole had reported values downstream from the Lincoln and Hastings effluent of 343 and 173 ng/L. This was approximately one order larger than the mean concentration detected in this study of 35 ng/L. Ibuprofen

displayed persistence at one location in this study, in contrast to the prior study. A mean concentration of 60 ng/L was identified in the Hastings effluent. Bartelt-Hunt et al. (2009) detected DEET concentrations of 181 and 1616 ng/L downstream from Lincoln and Hastings, respectively. This study had DEET Lincoln and Hastings concentration values of 55 and 155 ng/L, respectively. Though the amount compound detected varied, a strong correlation is apparent in the persistence and degradation of specific polar organic microcontaminants.

Chapter 6

Conclusions and Future Directions

The objectives set forth in this study were accomplished. Uptake rates were quantified for 25 pharmaceutical compounds by conducting a laboratory uptake study, using POCIS. 20 additional uptake rates were calculated for specified pharmaceuticals no previously documented values. Acetaminophen, carbamazepine, methamphetamine, azithromycin, and sulfamethazine all displayed uptake rates that compared to reported literature values. POCIS was also used to evaluate the fate of polar organic contaminants in Nebraska surface waters impacted by WWTP effluent. The field deployment data provided a distinction between compounds that persist and those that degrade. We concluded that select pharmaceuticals can persist for at least 1300 m downstream. Batt et al. (2006) conducted a similar study but only at maximum distance of 100m downstream from effluent. The first documented decay rates were determined for 25 pharmaceuticals in receiving waters. Decay rates were determined by minimizing RMSE and a combination of all loses which include: biodegradation, hydrolysis, photolysis, and sorption.

The hypothesis that POCIS can evaluate occurrence and behavior of pharmaceuticals in WWTP effluent was confirmed by data collected throughout this study. This passive sampling technology performed efficiently at replicating continuous exposure conditions, while be resistant to fouling. The data collected here is reproducible and comparable to other studies conducted under similar conditions and methods. Pharmaceutical loading rates, included in Appendix B,

were calculated for each compound that displayed persistence using the average in-stream concentration and volumetric flow rate. This gave a quantitative description of daily microcontaminant mass discharged downstream

Future work may be conducted on both the laboratory uptake and field deployment studies. Lincomycin and tiamulin had similar behavior under controlled conditions, but differed in the natural environment. Lincomycin was observed to persist in the field setting; however, it underwent rapid uptake in the laboratory experiments. Tiamulin displayed rapid uptake in the laboratory, but was not detected in the field study. Strategies to eliminate evaporation during uptake study should also be explored. Evaporation played a large role in the fluctuation of contaminant concentration during the laboratory uptake study. There were frequent spikes where analyte concentration exceeded the original spiked amount at time 0. Because the experiment was operated under controlled conditions, it was concluded that evaporation was the reason for increase in concentration. Though polar compounds have low volatility, measures should be implemented to seal beakers. The sulfa-based compounds, DEET, and carbamazepine showed the highest level of persistence, so their behavior and potential effects on the environment should be further investigated.

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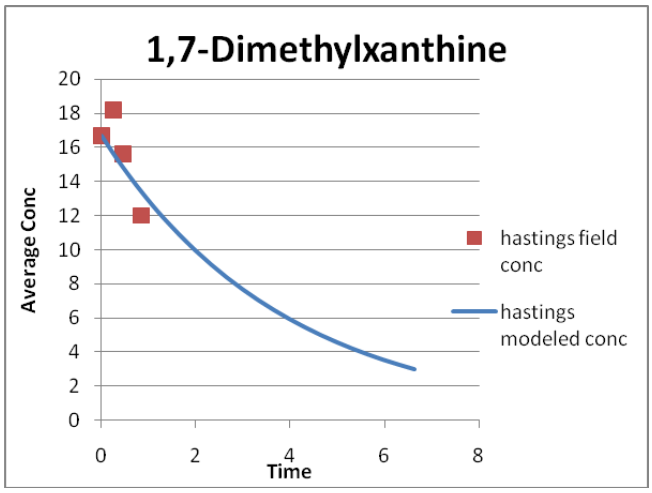
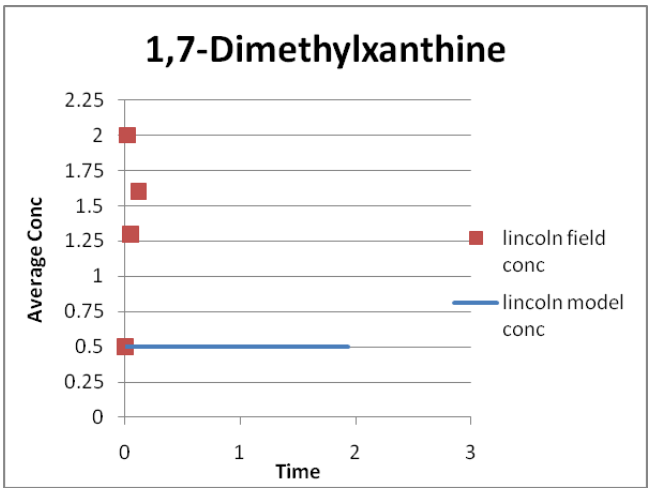
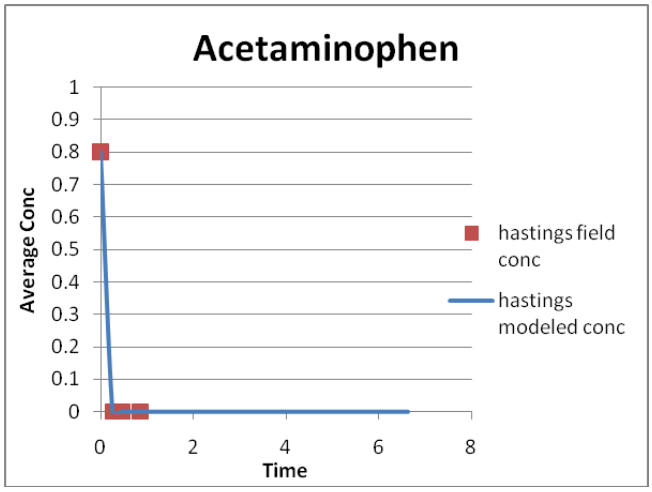
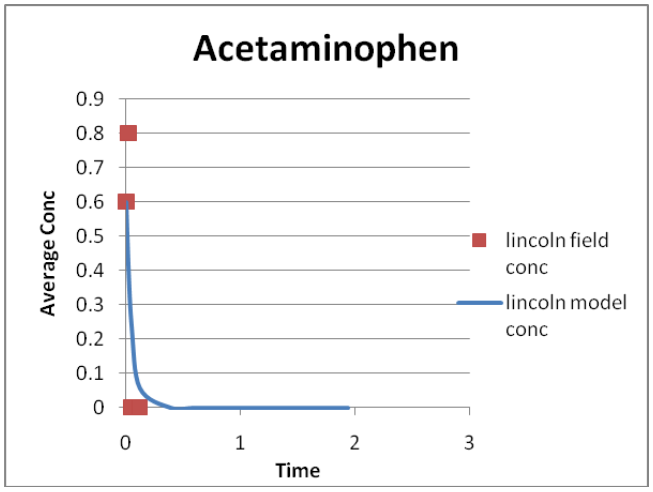
Appendix A

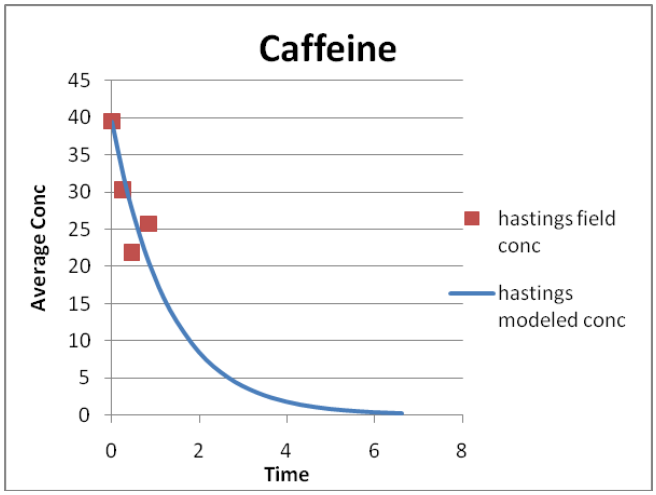
Model Fits for all 25 compounds analyzed at Hastings and Lincoln locations.

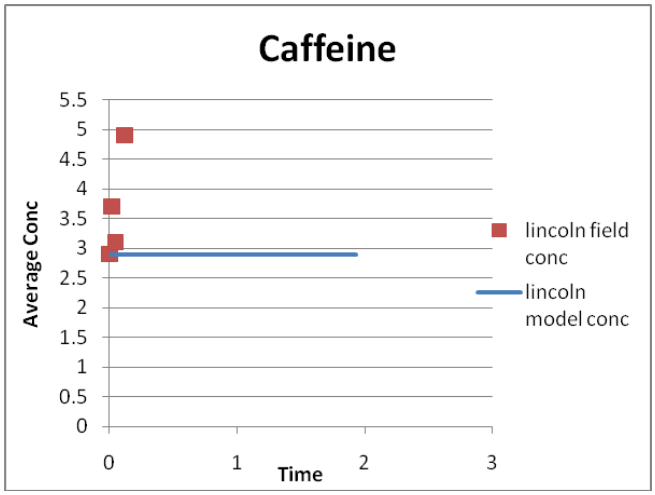


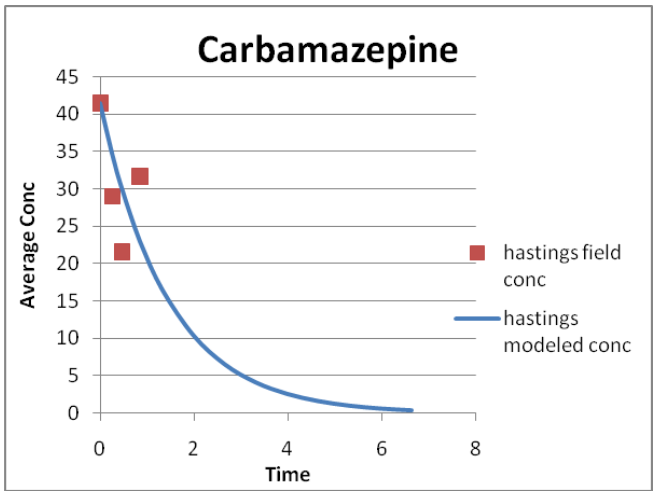


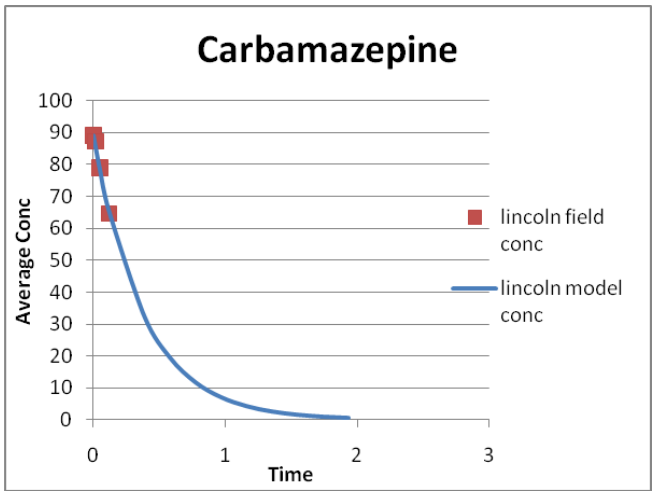


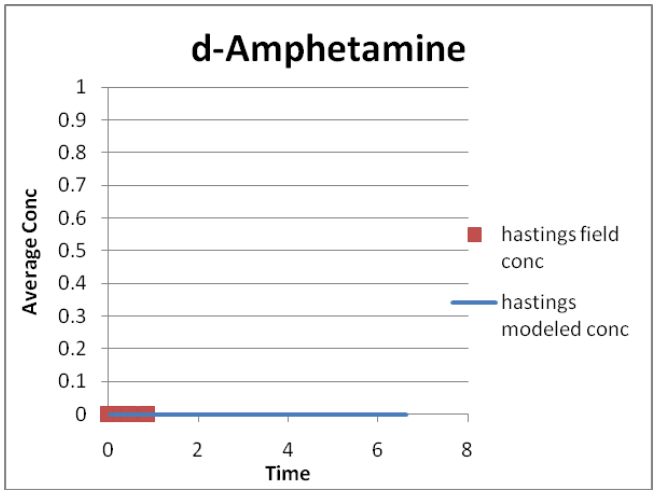


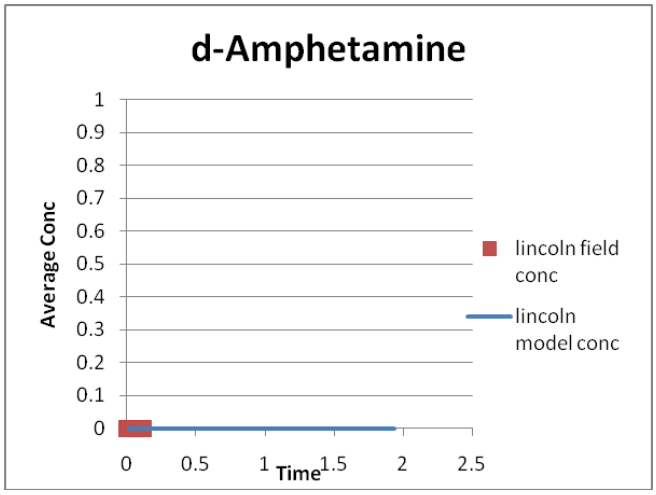

Caffeine
Average Conc
Time
hastings field conc
hastings modeled conc

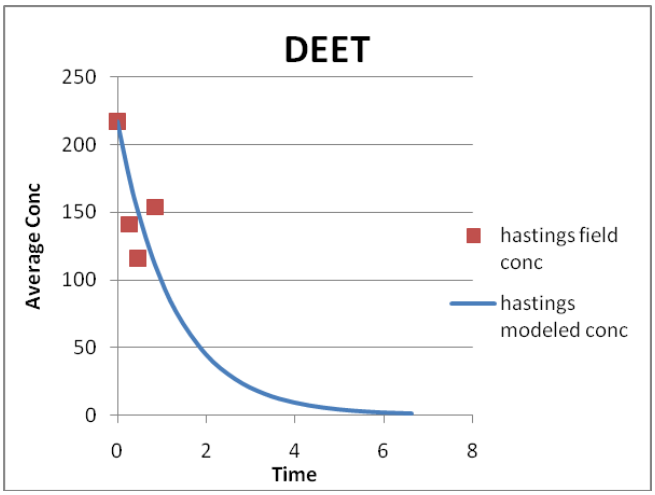

Caffeine
Average Conc
Time
lincoln field conc
lincoln model conc

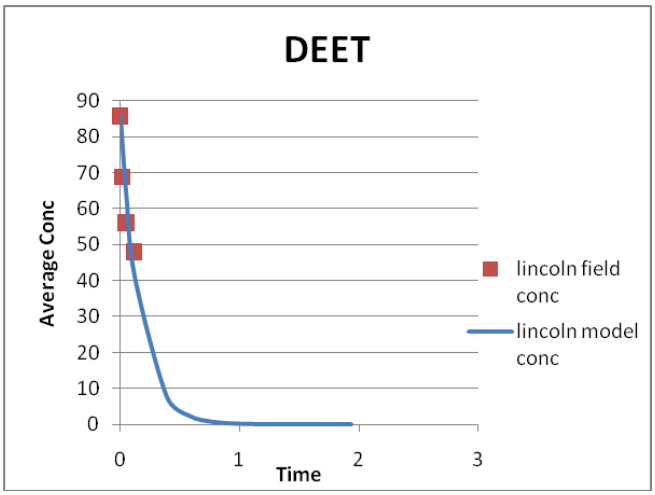

Carbamazepine
Average Conc
Time
hastings field conc
hastings modeled conc

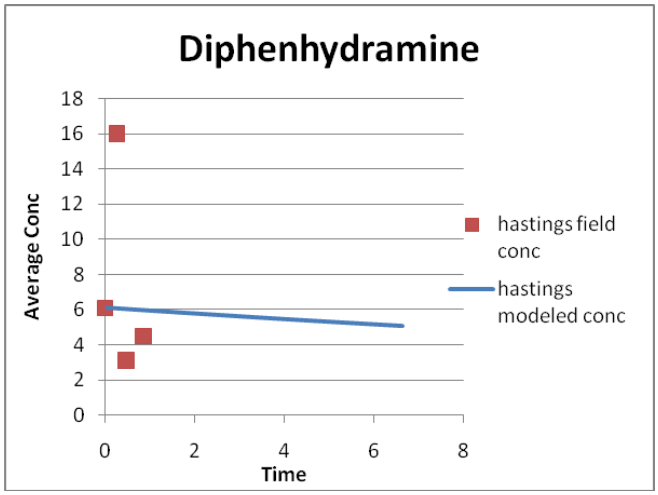

Carbamazepine
Average Conc
Time
lincoln field conc
lincoln model conc

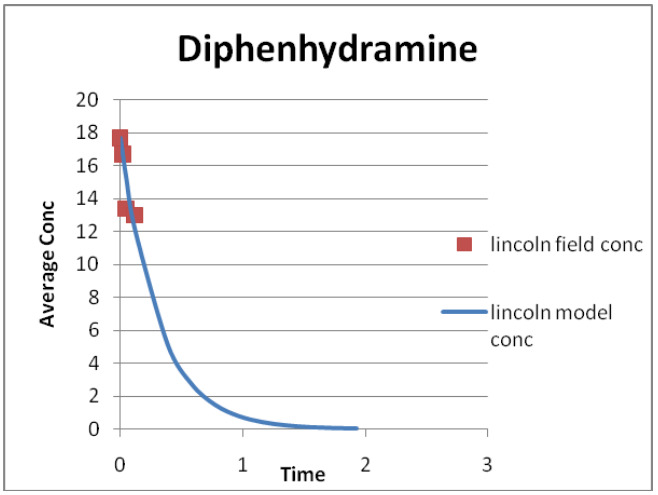

d-Amphetamine
Average Conc
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
Time
hastings field conc
hastings modeled conc

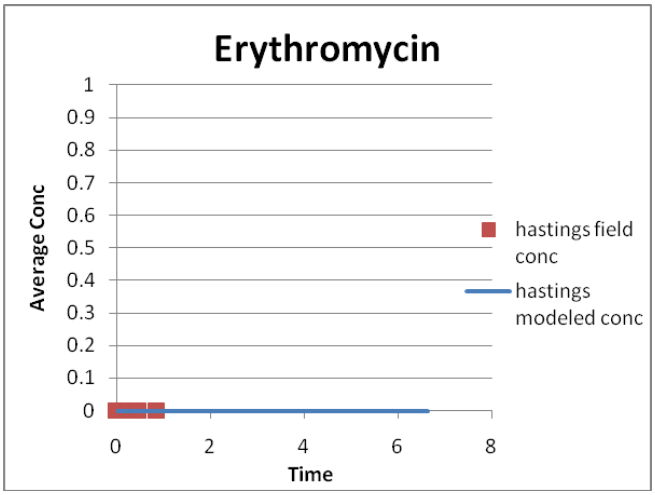

d-Amphetamine
Average Conc
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
2
2.5
Time
lincoln field conc
lincoln model conc

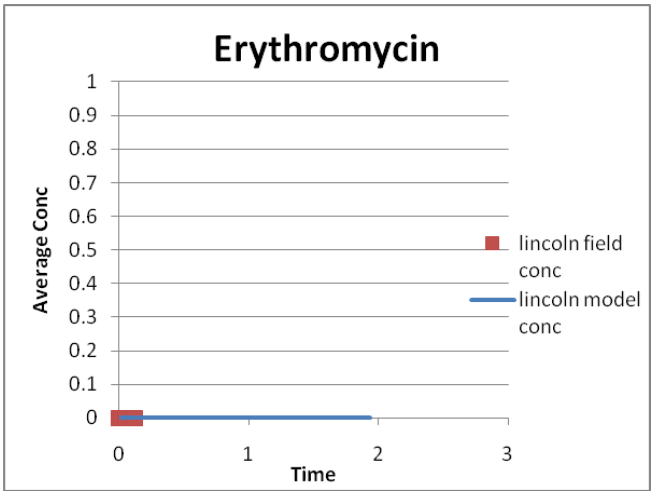

DEET
Average Conc
250
200
150
100
50
0
0
2
4
6
8
Time
hastings field conc
hastings modeled conc

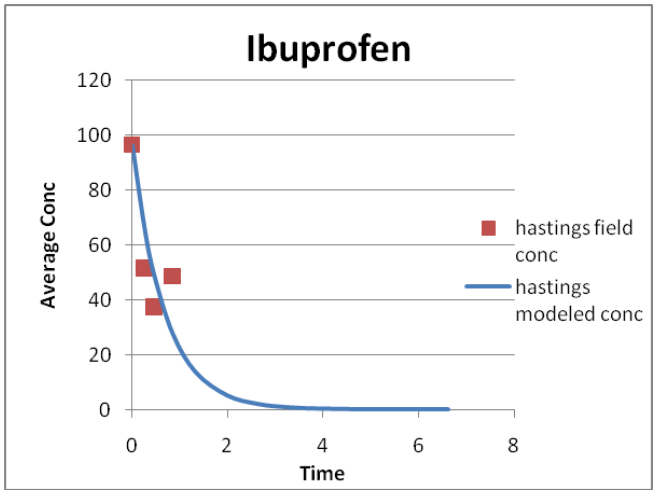

DEET
Average Conc
90
80
70
60
50
40
30
20
10
0
0
1
2
3
Time
lincoln field conc
lincoln model conc

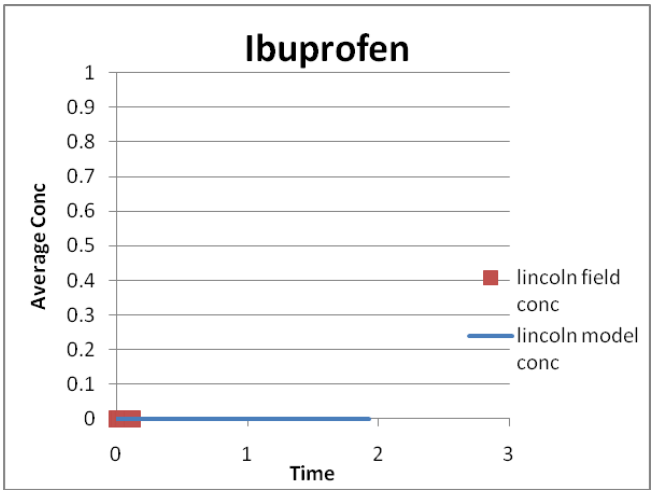

Diphenhydramine
Average Conc
Time
hastings field conc
hastings modeled conc

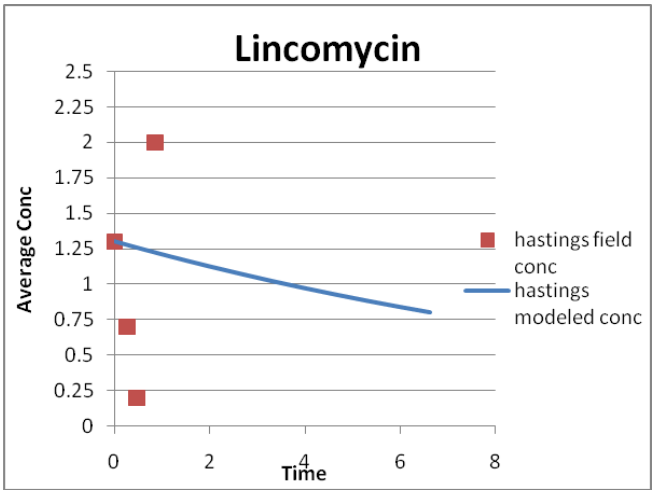

Diphenhydramine
Average Conc
Time
lincoln field conc
lincoln model conc

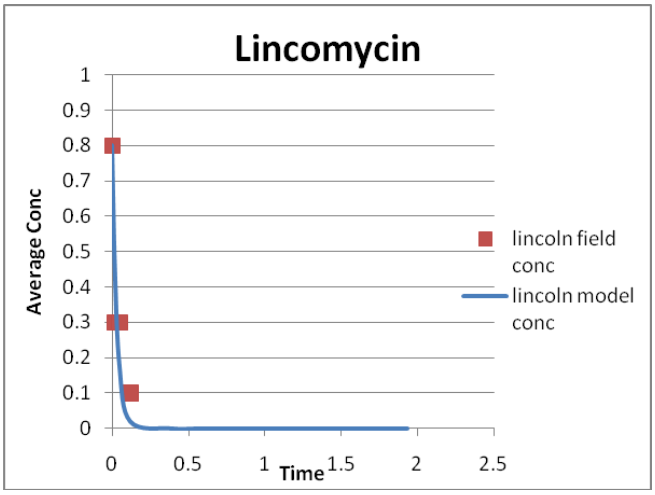

Erythromycin
Average Conc
Time
hastings field conc
hastings modeled conc

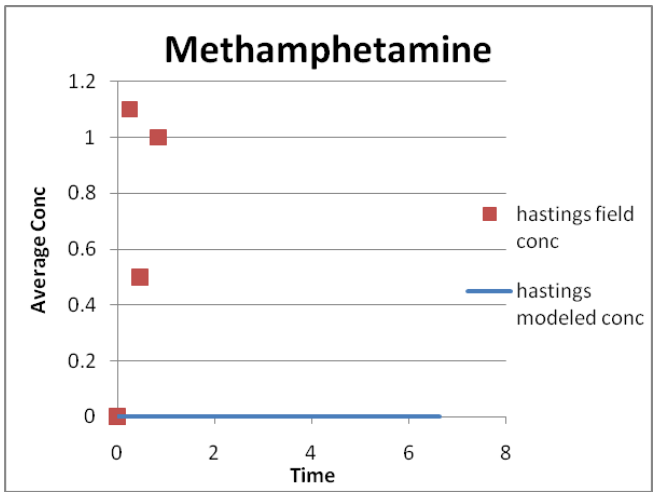

Erythromycin
Average Conc
Time
lincoln field conc
lincoln model conc

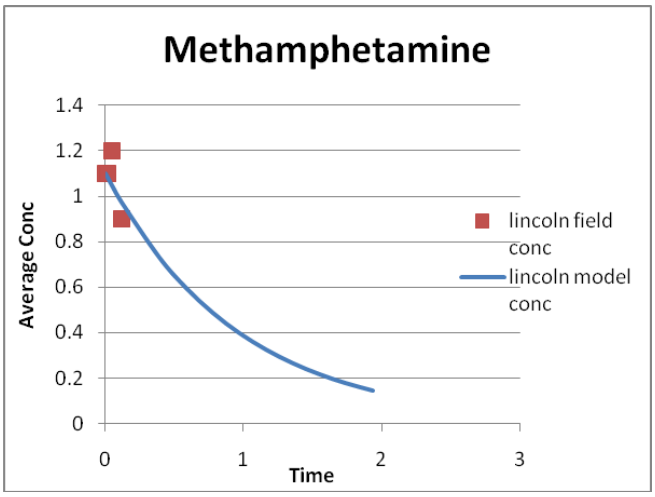

Ibuprofen
Average Conc
Time
hastings field conc
hastings modeled conc

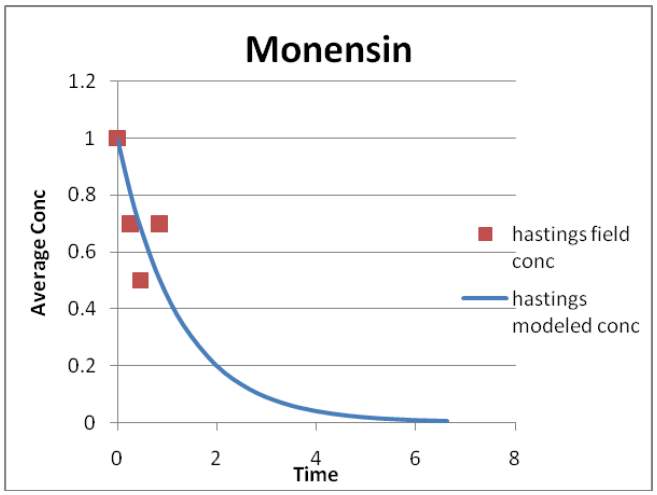

Ibuprofen
Average Conc
Time
lincoln field conc
lincoln model conc

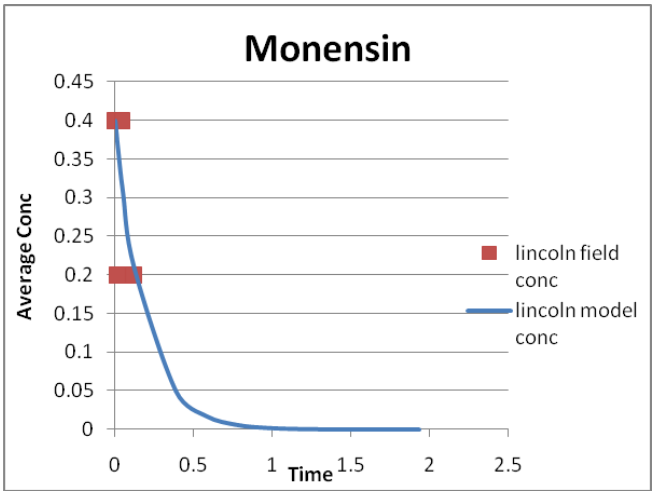

Lincomycin
Average Conc
Time
hastings field conc
hastings modeled conc

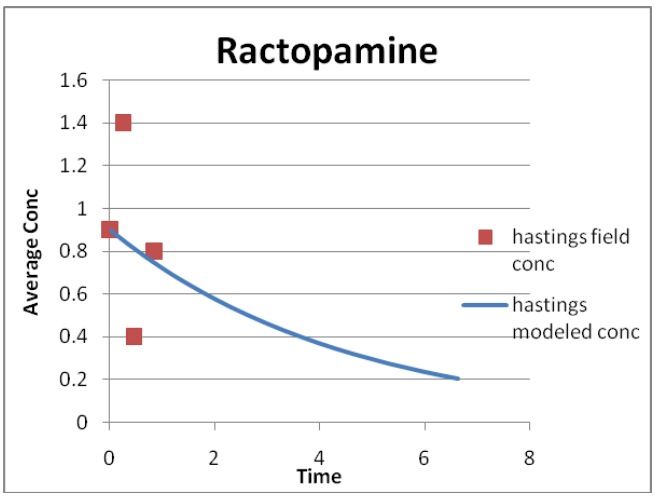

Lincomycin
Average Conc
Time
lincoln field conc
lincoln model conc

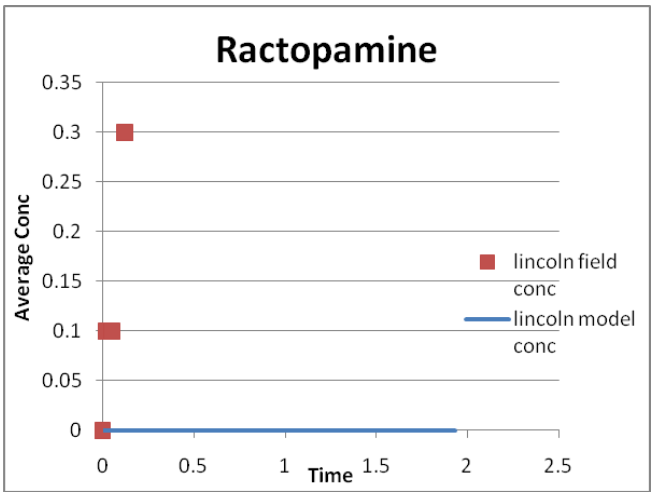

Methamphetamine
Average Conc
Time
hastings field conc
hastings modeled conc
0
0.2
0.4
0.6
0.8
1
1.2
0
2
4
6
8

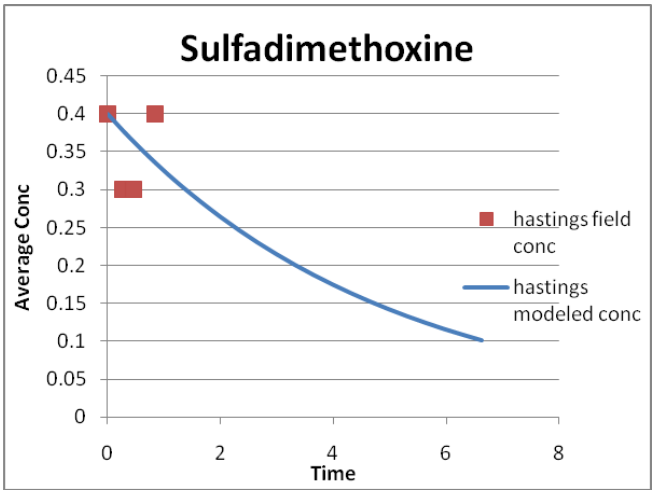

Methamphetamine
Average Conc
Time
lincoln field conc
lincoln model conc
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
1
2
3

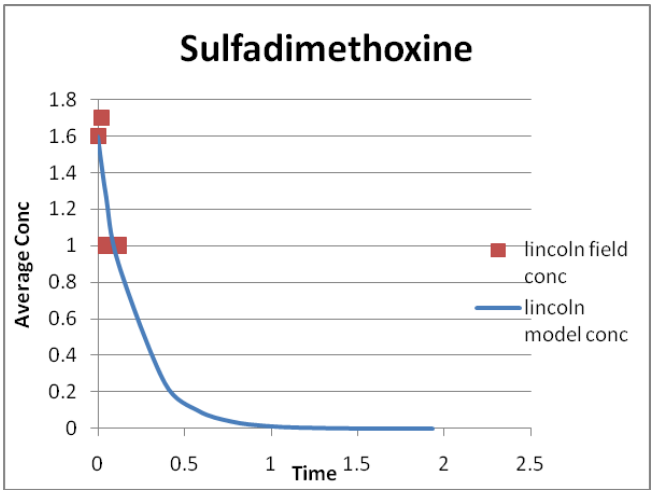

Monensin
Average Conc
Time
hastings field conc
hastings modeled conc
0
0.2
0.4
0.6
0.8
1
1.2
0
2
4
6
8

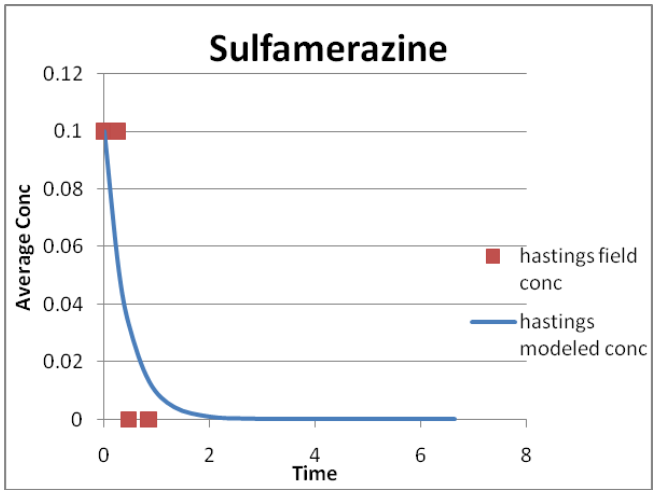

Monensin
Average Conc
Time
lincoln field conc
lincoln model conc
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0
0.5
1
1.5
2
2.5

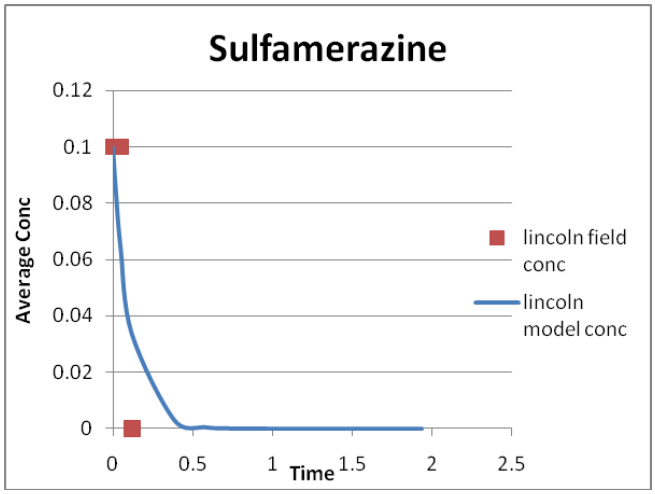

Ractopamine
Average Conc
Time
hastings field conc
hastings modeled conc

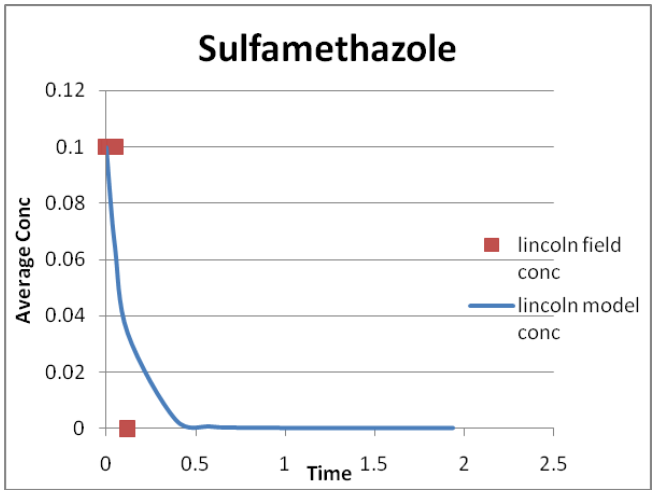

Ractopamine
Average Conc
Time
lincoln field conc
lincoln model conc

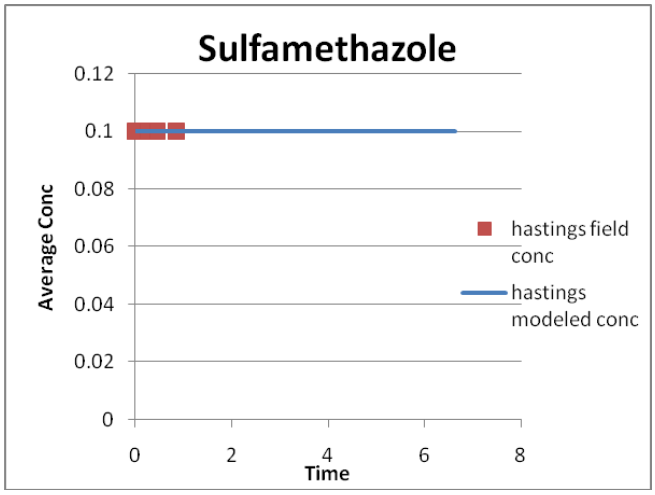

Sulfadimethoxine
Average Conc
Time
hastings field conc
hastings modeled conc

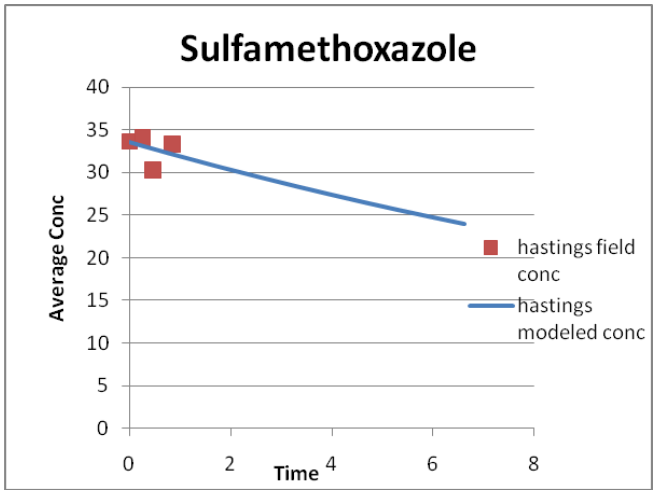

Sulfadimethoxine
Average Conc
Time
lincoln field conc
lincoln model conc

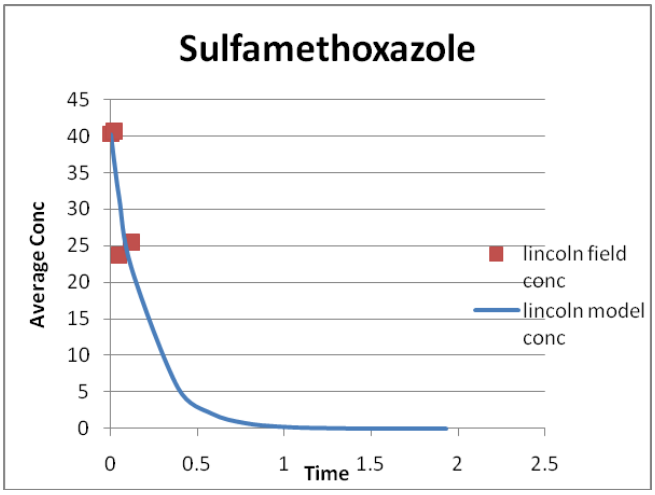

Sulfamerazine
0.12
0.1
0.08
0.06
0.04
0.02
0
Average Conc
0
2
4
6
8
Time
hastings field conc
hastings modeled conc

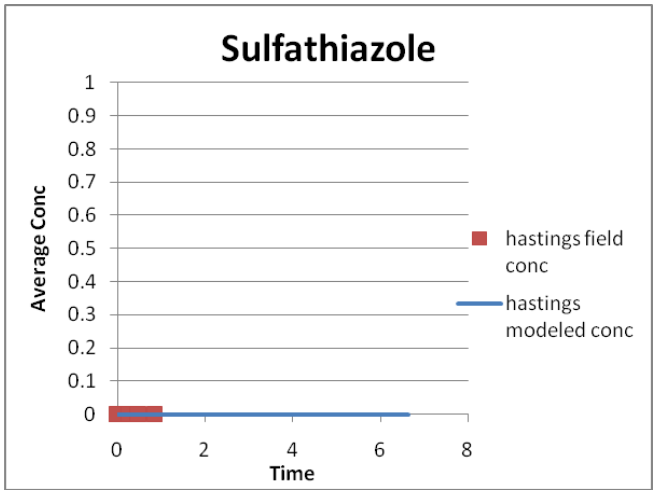

Sulfamerazine
0.12
0.1
0.08
0.06
0.04
0.02
0
Average Conc
0
0.5
1
1.5
2
2.5
Time
lincoln field conc
lincoln model conc

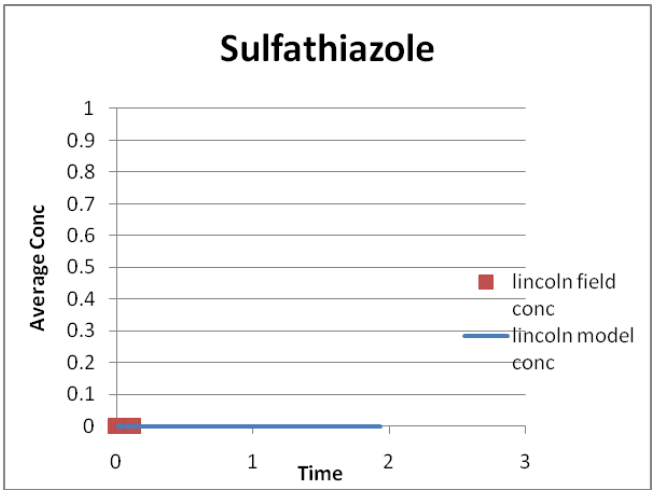

Sulfamethazole
0.12
0.1
0.08
0.06
0.04
0.02
0
Average Conc
0
0.5
1
1.5
2
2.5
Time
lincoln field conc
lincoln model conc

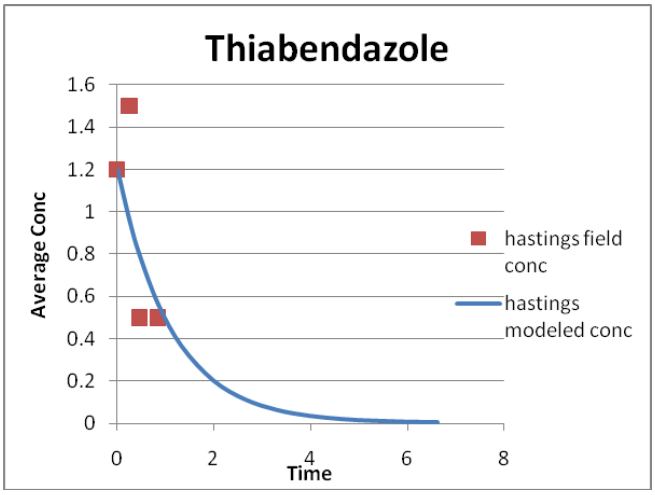

Sulfamethazole
0.12
0.1
0.08
0.06
0.04
0.02
0
Average Conc
0
2
4
6
8
Time
hastings field conc
hastings modeled conc

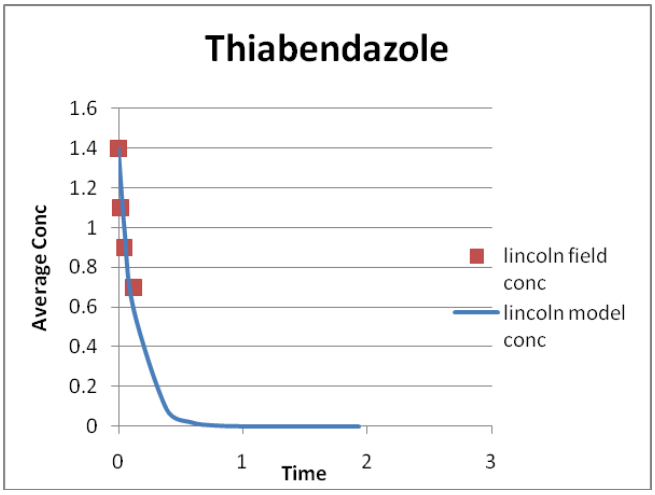

Sulfamethoxazole
Average Conc
Time
hastings field conc
hastings modeled conc

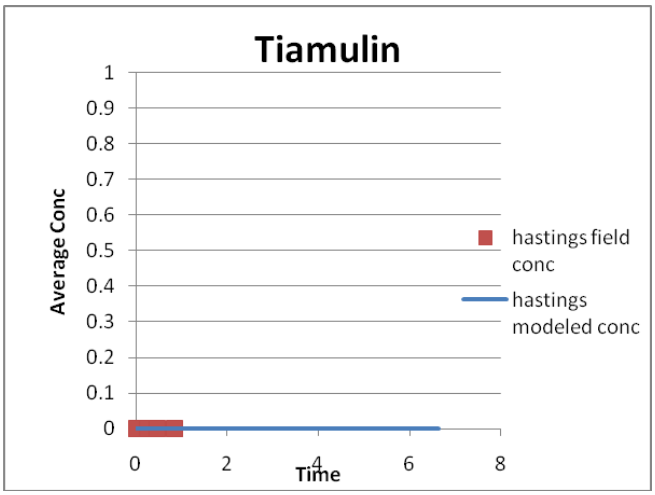

Sulfamethoxazole
Average Conc
Time
lincoln field conc
lincoln model conc

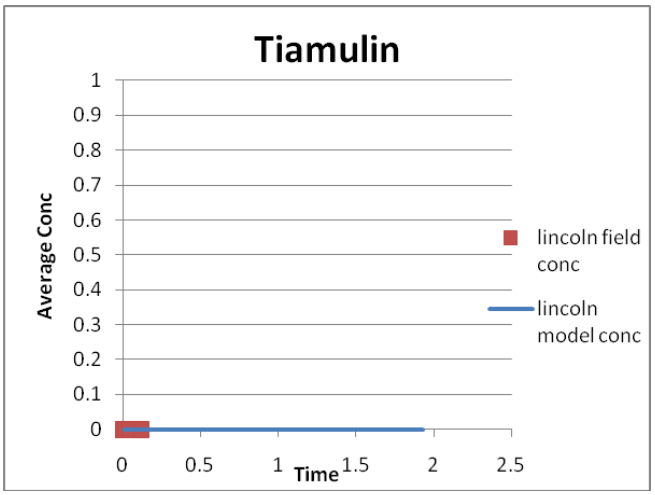

Sulfathiazole
Average Conc
Time
hastings field conc
hastings modeled conc

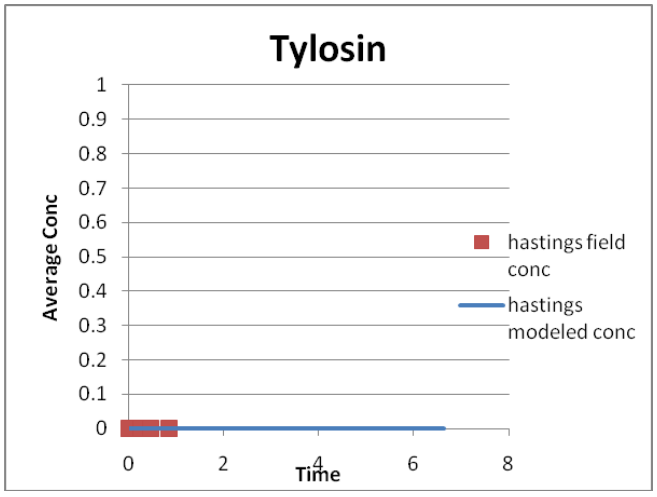

Sulfathiazole
Average Conc
Time
lincoln field conc
lincoln model conc

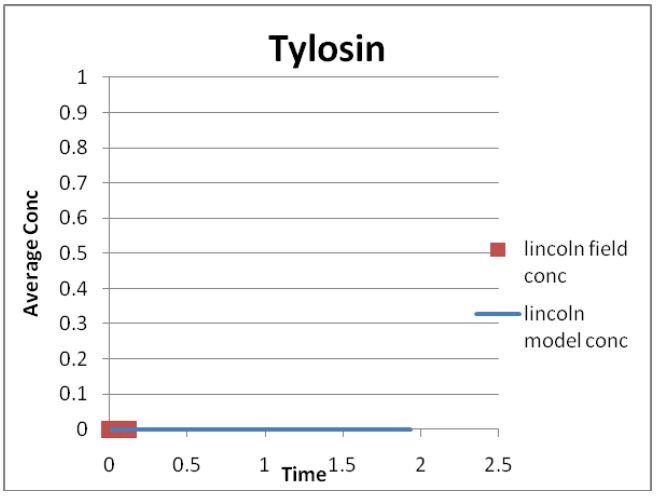

Thiabendazole
Average Conc
Time
hastings field conc
hastings modeled conc

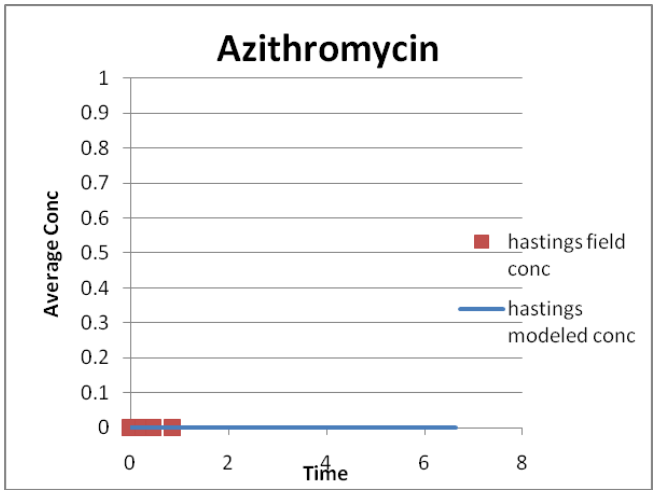

Thiabendazole
Average Conc
Time
lincoln field conc
lincoln model conc

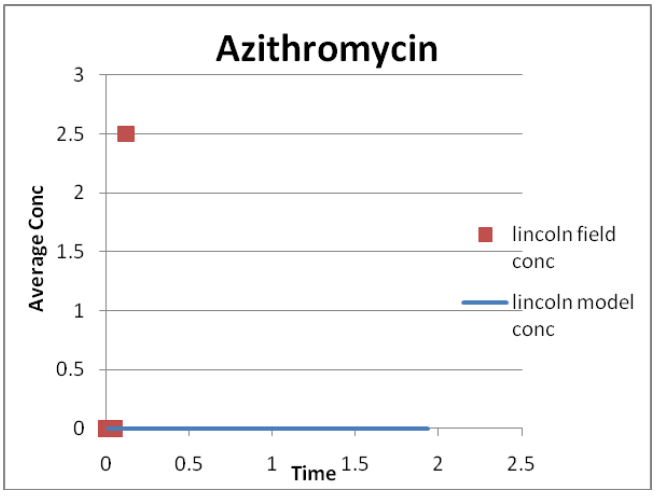

Tiamulin
Average Conc
Time
hastings field conc
hastings modeled conc

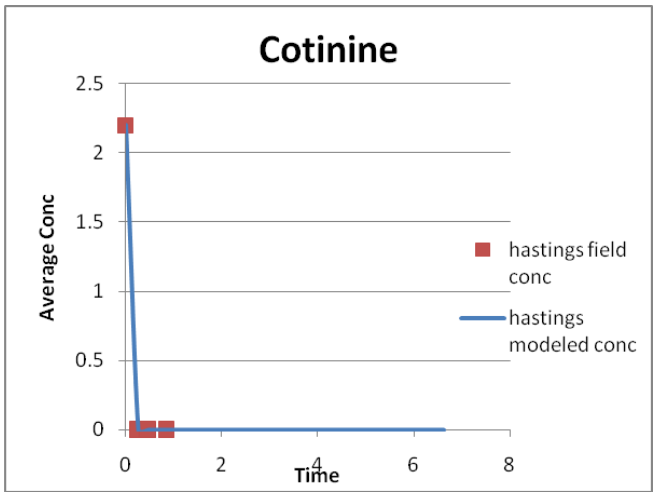

Tiamulin
Average Conc
Time
lincoln field conc
lincoln model conc

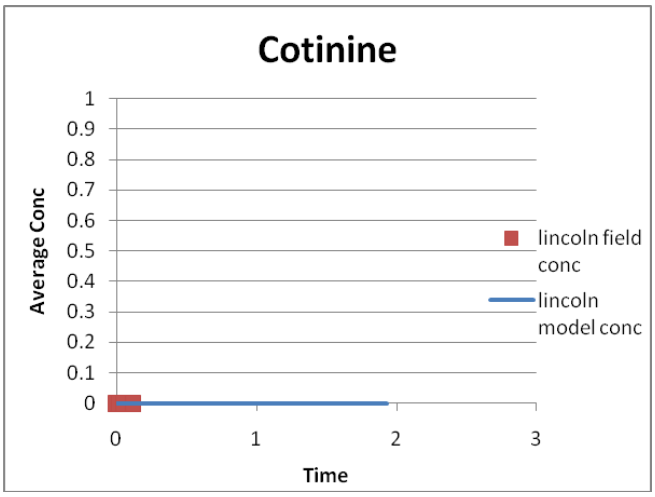

Tylosin
Average Conc
Time
hastings field conc
hastings modeled conc


Tylosin
Average Conc
Time
lincoln field conc
lincoln model conc


Azithromycin
Average Conc
Time
hastings field conc
hastings modeled conc


Azithromycin
Average Conc
Time
lincoln field conc
lincoln model conc


Cotinine
Average Conc
Time
hastings field conc
hastings modeled conc
0
0.5
1
1.5
2
2.5
0
2
4
6
8


Cotinine
Average Conc
Time
lincoln field conc
lincoln model conc
0
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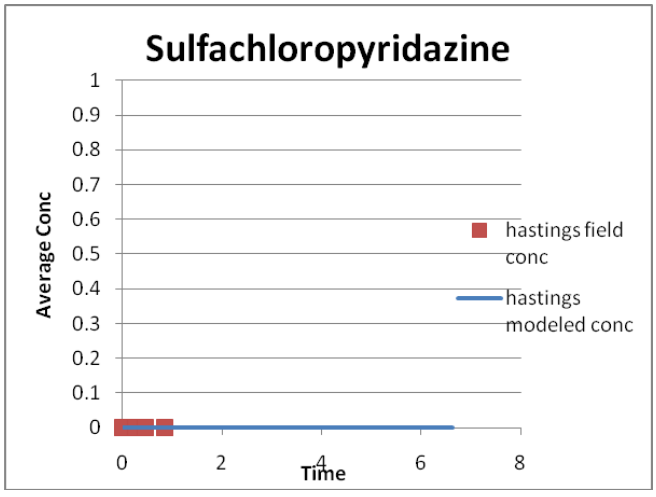
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Average Conc
Time
hastings field conc
hastings modeled conc
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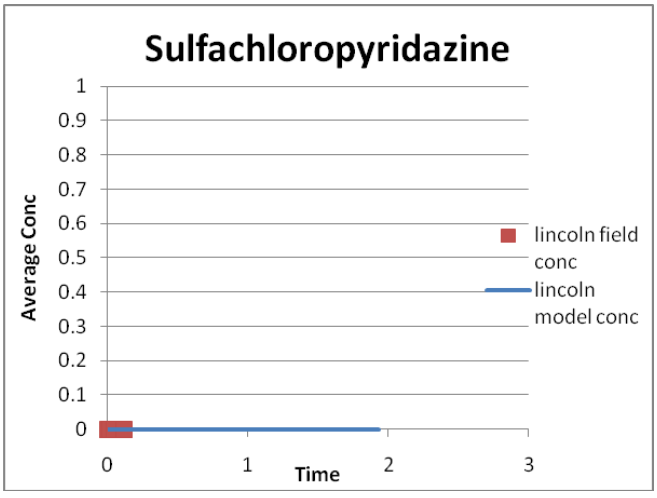
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Time
lincoln field conc
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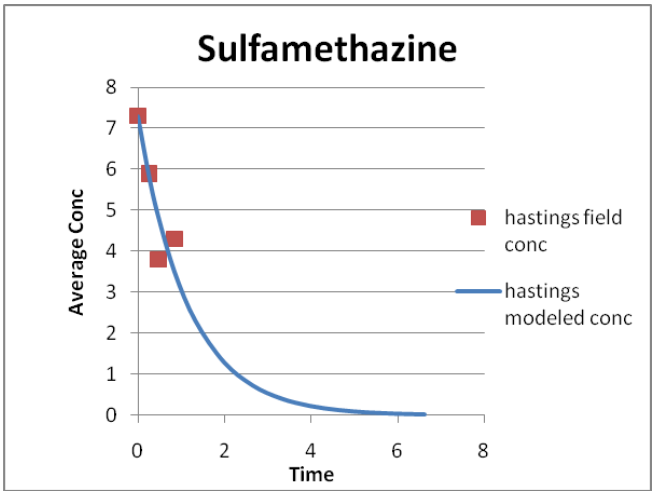
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Average Conc
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5
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7
8
0
2
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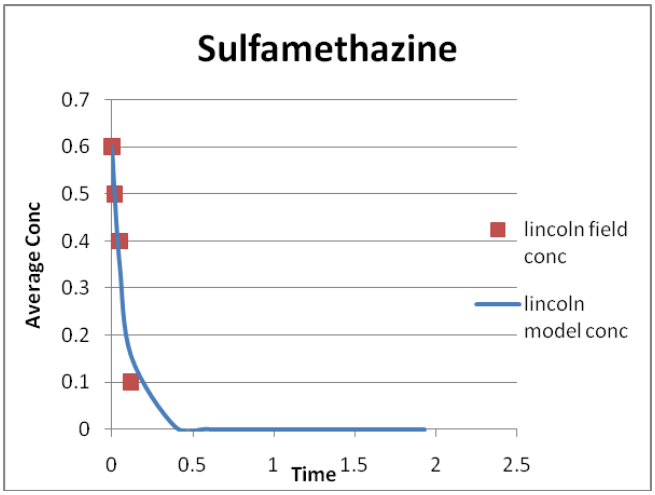
6
8
hastings field conc
hastings modeled conc


Sulfamethazine
Average Conc
Time
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0.2
0.3
0.4
0.5
0.6
0.7
0
0.5
1
1.5
2
2.5
lincoln field conc
lincoln model conc

Appendix B – Table of Loading Rates

Target Compounds	Hastings loading rates (kg*day-1)	Lincoln loading rates (kg*day-1)
1,7-dimethylxanthine	1.75E-04	1.44E-04
Caffeine	nd	nd
Acetaminophen	3.75E-04	4.40E-04
Carbamazepine	4.62E-04	5.81E-03
d-Amphetamine	nd	nd
DEET	2.24E-03	4.31E-03
Diphenhydramine	6.56E-05	1.17E-03
Erythromycin	nd	nd
Ibuprofen	7.08E-04	nd
Lincomycin	2.91E-05	8.99E-06
Methamphetamine	1.46E-05	8.09E-05
Monensin	1.02E-05	1.80E-05
Ractopamine	1.17E-05	2.70E-05
Sulfadimethoxine	5.83E-06	8.99E-05
Sulfamerazine	nd	nd
Sulfamethazole	1.46E-06	nd
Sulfamethoxazole	4.85E-04	2.29E-03
Sulfathiazole	nd	nd
Thiabendazole	7.29E-06	6.29E-05
Tiamulin	nd	nd
Tylosin	nd	nd
Azithromycin	nd	2.25E-04
Cotinine	nd	nd
Sulfachloropyridazine	nd	nd
Sulfamethazine	6.27E-05	8.99E-06

nd – not detected throughout field deployment period

Appendix C – Analytical Methods QA/QC

Pharmaceutical Analysis of Water Samples

Extraction and analysis of pharmaceuticals in the aqueous samples was based on previous methods (Snow et al. 2003; Batt et al. 2006) and modified to permit the use of automated solid phase extraction (SPE) with detection by liquid chromatography-tandem mass spectrometry. A Spark Holland Symbiosys Environ (Spark Holland, Emmen, The Netherlands) on-line solid phase extraction system was used with detection by electrospray ionization liquid chromatography tandem mass spectrometry (LC/MS/MS. Up to twenty-five milliliter subsamples water were weighed into 40-mL amber glass vials along with 10µL of reagent grade formic acid. Each sample was spiked at 0.500 ng/mL (ppb) of sulfamethazine-phenyl-13C6 (internal standard), 13C3 -caffeine and demeclocyline (surrogate). Samples and standards were automatically extracted using Prospekt 2/Symbiosis 2.0 x 10mm Oasis HLB solid phase extraction cartridges and immediately eluted for LC/MS/MS analysis using a Quattro Micro triple quadrupole mass spectrometer. A stepwise gradient separation was performed using a Waters 2695 high pressure liquid chromatograph (HPLC). A Thermo HyPurity C18 5um, 2x250mm column was used with a mobile phase comprised of 97:3 water/methanol (A) and 3:97 methanol/water (B) each containing 0.1% (v/v) formic acid. Elution and separation began with 95:5 A to B for 2 min, changing to a linear gradient of 50:50 A to B to 25:75 A to B at 8 minutes, to 100% B for 18 minutes. Mobile phase composition was returned to starting conditions until the end of the run.

LC/MS/MS conditions and transitions were determined and optimized in positive electrospray (ESI +) by infusing with concentrated standards and similar to those shown in Table 1. A capillary voltage of 4.0 kV, an extractor of 3 V and an RF lens of 0.1 V was used. The source temperature was 120°C and the desolvation temperature was 500°C. The nebulizer flow rate was 700 L/hr in the desolvator and 30 L/hr in the cone. Resolutions were set at 14 across the board and ion energy 1 was 0.8 and ion energy 2 was 1.5. Instrument calibration was performed using a five-point calibration curve over a concentration range from 10 to 1000 ng/L.

Lab_ID_String	Sample_ID	1,7-Dimethylxanthine	Acetaminophen	Azithromycin	Caffeine	Carbamazepine	Chlorotetracycline	Cotinine	d-Amphetamine	DEET	Diphenylhydramine	Erythromycin	Ibuprofen	Lincomycin	Methamphetamine	Monensin	Oxytetracycline	Ractopamine	Sulfachloropyridazine	Sulfadimethoxine	Sulfamerazine	Sulfamethazine	Sulfamethazole	Sulfamethoxazole	Sulfathiazole	Tetracycline	Thiabendazole	Tiamulin	Tylosin	Batch	Analysis Date
09-863	PHARM1	94	257	598	833	2330	10	1284	494	896	2018	1668	482	4818	511	293	16	3323	1045	2117	859	1999	651	1289	2222	15	810	2124	1939	W09046	4/3/2009
09-864	PHARM2	237	375	319	724	2301	6	1241	223	688	1116	1522	830	4541	881	216	8	4832	819	1958	418	1715	549	1241	1643	9	459	787	795	W09046	4/3/2009
09-865	PHARM3	165	332	779	669	2252	6	1197	557	957	2095	3602	743	6348	1111	261	8	3282	1071	2125	586	2027	848	1388	1955	11	704	1519	1962	W09046	4/3/2009
09-866	PHARM4	143	345	1031	783	2408	13	1425	812	971	1830	3414	341	7928	1068	268	13	2964	1514	1988	804	2456	1414	1251	1616	26	528	1948	2455	W09046	4/3/2009
09-867	N1				6	1	1		3	10	10				3		1	1	3	12	3	5	2	3	2	2	2	4	1	W09046	4/3/2009
09-1599	H-1-1	98			746	1053		80		4694	290		2857	85		22		31		10	1	200	1	405		96	38			W09140	6/19/2009
09-1600	H-1-2	148	43		1137	1281				5992	299		3629	113		30		33		11	2	236	2	578		114	62			W09140	6/19/2009
09-1601	H-1-3	204	26		898	913				5025	208		2500	91		32		21		9	2	137	2	710		114	31			W09140	6/19/2009
09-1602	H-2-1	197			701	876				4034	674		1725	28		21		30		10	2	121	3	762		65	26			W09140	6/19/2009
09-1603	H-2-2	109			632	484				2103	1199		1158	40	52	15		64		6	1	212	1	368		87	27			W09140	6/19/2009
09-1604	H-2-3	182			800	914				4095	200		1924	94	55	23		29		9	3	129	3	589		82	122			W09140	6/19/2009
09-1605	H-3-1	200			757	738				3579	104		1327	16	20	23		13		8	1	126	2	723		32	24			W09140	6/19/2009
09-1606	H-3-2	18			31	157				845	204		799	6	6			7		3		48	3	66		11	5			W09140	6/19/2009
09-1607	H-3-3	203			755	794				3969	90		1376	34	21	19		12		9	2	122	1	735		41	32			W09140	6/19/2009
09-1608	H-4-1	19			211	230				1071	151		427	6	13	6		14		4	0	41	0	168		7	7			W09140	6/19/2009
09-1609	H-4-2	138			623	1343				6373	164		2869	418	58	25		27		15	1	182	3	778		119	23			W09140	6/19/2009
09-1610	H-4-3	166			972	912				3705	271		1227	37	27	26		30		10	2	117	2	732		50	25			W09140	6/19/2009
09-1611	L-1-1		26		71	3572				3535	994			100	48	13				62	3	21	4	960		151	73			W09140	6/19/2009
09-1612	L-1-2		29		59	2574				2172	1093			84	41	8				48	4	23	3	633		141	68			W09140	6/19/2009
09-1613	L-1-3	15			71	833				504	209			2	20	14		4		14	2	4	1	437		8	25			W09140	6/19/2009
09-1614	L-2-1	20	27		102	2494				2015	900			30	42					64	4	17	2	745		54	59			W09140	6/19/2009
09-1615	L-2-2	18			69	1521				783	615			7	20	11		7		25	2	5	2	669		25	28			W09140	6/19/2009
09-1616	L-2-3	16	44		91	2816				2184	652			38	44	8				41	3	18	3	638		57	35			W09140	6/19/2009
09-1617	L-3-1	14			78	1868				1085	283			5	26	5		4		12	0	4	1	303		50	2			W09140	6/19/2009
09-1618	L-3-2	12			91	1673				902	514			6	35	15		5		16	1	4		357		15	16			W09140	6/19/2009
09-1619	L-3-3	9			50	2643				2068	945			59	55	9				48	3	22	2	536		74	79			W09140	6/19/2009
09-1620	L-4-1	18		27	61	1173				923	294			2	16	4		8		18			2	361		7	11			W09140	6/19/2009
09-1621	L-4-2	26		110	185	2981				2490	921			9	43	10		22		39				442		11	35			W09140	6/19/2009
09-1622	L-4-3				97	907				57	473			10	25	7				24		4		483		9	31			W09140	6/19/2009