

Report for 2004KS37B: Pharmaceuticals in Surface Water

- Articles in Refereed Scientific Journals:
 - 1. Koch, D.E.; Bhandari, A.; Close, L.; Hunter, R.P. "Azithromycin extraction from municipal wastewater and quantitation using liquid chromatography / mass spectrometry." *Journal of Chromatography, A*. 1074:17-22. 2005.
 - 2. Xia, K.; Bhandari, A.; Das, K.; Pillar, G. "Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in biosolids." *Journal of Environmental Quality*. 34:91-104, 2005
- Conference Proceedings:
 - 3. Bhandari, A.; Close, L.; Koch, D.; Hunter, R. "The occurrence and fate of antimicrobials in municipal wastewater treatment plants - a northeast Kansas perspective." Paper presented at the 39th Midwest Regional Meeting, American Chemical Society, Manhattan, KS, Oct 19-22, 2004.

Report Follows

PROGRESS REPORT

Pharmaceutical Agents in Surface Waters: The Occurrence and Fate of Pharmaceuticals in Northeast Kansas Wastewater Treatment Facilities

PROJECT TITLE: Pharmaceutical Agents in Surface Waters: The Occurrence and Fate of
Pharmaceuticals in Northeast Kansas Wastewater Treatment Facilities

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INVESTIGATORS & AFFILIATIONS: Alok Bhandari, Ph.D., P.E., Associate Professor of Environmental
Engineering, Department of Civil Engineering, Kansas State University

Robert Hunter, M.S., Ph.D., Assistant Professor of Veterinary
Pharmacology, Department of Anatomy and Physiology, Kansas State
University

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PROGRESS REPORT

Pharmaceutical Agents in Surface Waters: The Occurrence and Fate of Pharmaceuticals in Northeast Kansas Wastewater Treatment Facilities

Principal Investigators

Alok Bhandari, Ph.D., P.E.
Associate Professor of Environmental Engineering
Department of Civil Engineering, Kansas State University

Robert Hunter, M.S., Ph.D.
Assistant Professor of Veterinary Pharmacology
Department of Anatomy and Physiology, Kansas State University

Problem and Research Objectives

Discharges from municipal wastewater treatment plants (WWTPs) are among the major sources of surface water and groundwater contamination by antibiotics and other pharmaceutical drugs. The presence of antibiotics in surface waters and groundwater is of concern because these chemicals have the potential to perturb microbial ecology, increase the proliferation of antibiotic-resistant pathogens, and pose serious threat to human health. Pharmaceutical chemicals are introduced into municipal wastewater streams from human excreta, which contain large quantities of non-metabolized or partially metabolized medicinal compounds. In order to develop solutions that control the release of antibiotics and other pharmaceutical agents into the environment, it is important to estimate the amounts of these chemicals discharged into surface waters and on land. Recent studies have detected more than 40 different pharmaceutical drugs in environmentally significant quantities in discharges from wastewater treatment facilities in Europe and across the eastern United States. Very few studies, however, have been conducted in the Midwestern United States, and these studies have not correlated the occurrence of target pharmaceuticals to community types or removal in WWTPs to treatment processes and seasonal changes.

The overall objective of proposed project is to evaluate the occurrence and fate of three widely prescribed antibiotics – azithromycin (AZI), sulfamethoxazole (SUL), and ciprofloxacin (CIP) – in raw and treated wastewater, and biosolids at four northeast Kansas wastewater treatment facilities. Information generated from this research will provide critical and timely information about the mass input of these drugs at northeast Kansas WWTPs and extent of environmental release through effluent discharges and biosolids. The proposed work will consist of measuring concentrations of target pharmaceutical drugs in raw and treated wastewater, and biosolids at four northeast Kansas WWTPs. The target antibiotics were selected because they are among the most widely used pharmaceutical drugs in the United States and are commonly detected in municipal wastewaters. The four treatment plants to be evaluated encompass a wide range in the type and size of populations served and the treatment processes employed.

The specific objectives of this research include:

- (i) determining the occurrence of the target antimicrobials in the influent, effluent, and biosolids collected from the selected WWTPs;
- (ii) determining fate of the antimicrobials as the water is processed in the WWTPs;
- (iii) monitoring seasonal changes in antimicrobial concentrations;
- (iv) correlating antimicrobial concentrations with the types of treatment processes and raw wastewater characteristics;
- (v) conducting a screening evaluation of the water and biosolids samples for a wider variety of pharmaceuticals including methylxanthines (caffeine, theobromine, and theophylline), opioids (morphine, fentanyl, butorphanol, etc.), and acetaminophen;
- (vi) conducting hourly and 24-hour composite samples at selected WWTPs to evaluate temporal trends in the mass input and output of pharmaceutical agents at these facilities;

Description of Methods

The four WWTPs selected for this study are located along the Kansas River. The effluent from these treatment facilities is discharged directly into the Kansas River. The four WWTPs were selected because of their proximity to Manhattan, the wide range in the size and type of communities they serve, the wide range of treatment processes employed at these facilities, and our established relationships with the personnel at these municipal plants. Raw wastewater, primary effluent, secondary effluent and sludge samples will be collected at each WWTP and transported to the environmental engineering research laboratory under ice. Sampling at each plant was performed at least once in each of the four seasons: spring (Mar-May), summer (Jun-Aug), fall (Sep-Nov), and winter (Dec-Feb). All water samples will be collected in 1-L pre-washed amber glass bottles and transported to the laboratory under ice. In the laboratory, the wastewater samples were stored at -70°C until extraction. Sludge samples were collected from the aerators, digesters and dewatering equipment at the four WWTPs. Biosolids were separated from water by centrifugation and freeze-dried before extraction. Samples not extracted immediately were stored at -70°C .

Extraction and analytical methods were based on the most recent literature as detailed in the original proposal. AZI was extracted by liquid-liquid extraction using MTBE and quantified using LC/MS. SUL and CIP were extracted using mix-mode solid-phase extraction cartridges, eluted with methanol and quantified using HPLC/UV/fluorescence. All samples were subjected to rigid QA/QC protocols during collection, transport, storage, preparation and analysis. Each collected sample was divided into 3 sub-samples. Appropriate surrogate and internal standards were used during extraction and HPLC or LC/MS analyses. External standards and solvent blanks were analyzed at frequent intervals to assure equipment stability. Appropriate statistical methods were used to analyze data and differentiate treatment effects.

Work Accomplished

The accomplishments thus far for this project include sampling at the four different wastewater treatment plants in the Northeast region of Kansas and method development for the analysis of and for the sample preparation of AZI, SUL and CIP in wastewater samples.

A summary of the samples collected at the four WWTPs is presented in Table 1. At least four replicate liquid samples were collected from each plant and at least two replicate solids or slurry samples were collected from clarifiers, digesters, aerators and belt filter presses.

Table 1. Description of samples collected from the WWTPs in May and August 2003.

PLANT I.D.	NUMBERS, TYPES, AND LOCATIONS OF SAMPLES		
	Solid	Slurry	Liquid
Plant 1	0	4 return line, digester	9 plant influent, plant effluent
Plant 2	2 belt filter press (BFP)	5 nitrification tank, clarifier	19 plant influent, nitrification tank, clarifier effluent, plant effluent, BFP effluent
Plant 3	2 BFP	4 primary clarifier, secondary clarifier	14 plant influent, secondary clarifier effluent, plant effluent
Plant 4	2 BFP	4 primary clarifier, secondary clarifier	17 plant influent, secondary clarifier, BFP effluent, plant effluent

Analytical method development has been completed for sulfamethoxazole, ciprofloxacin and azithromycin. A single isocratic high pressure liquid chromatography (HPLC) method was developed for sulfamethoxazole and ciprofloxacin based on a binary gradient method reported by Adams *et al.* (2002). This method utilizes a HPLC with UV/VIS and fluorescence detectors positioned in series. In prior studies, sulfamethoxazole was detected by a UV/VIS detector, (Adams *et al.*, 2002), and ciprofloxacin by fluorescence detection (Golet *et al.* 2001). Solid-phase extraction methods for sulfamethoxazole and ciprofloxacin are currently being developed. These methods are based on those reported by Adams *et al.* (2002), Kolpin *et al.* (2002), and Golet *et al.* (2001) and utilize cation exchange/reverse phase cartridges, MPC (Waters[®]) or MCX (3M[®]). A method based on liquid-liquid extraction followed by LC-MS analysis has been developed to quantify azithromycin in water to concentrations as low as 50 ppb. The samples shown below were all extracted from 1 mL of water. The extraction method is as follows: to 1 mL of water, add 100 μ L of 0.5 M K₂CO₃, vortex and add 10 mL of methyl-*t*-butyl ether (MTBE). Vortex for 1 minute and centrifuge at \sim 1,000 $\times g$ for 10 minutes. Transfer supernatant to fresh centrifuge tube and dry using N₂ at 40 °C in a H₂O bath. Reconstitute using 100 μ L of mobile phase. Chromatography is performed using a Luna C18(2) (30 \times 2 mm) reversed phase

column. The mobile phase used is a 24:24:2:50 methanol:H₂O:tetrahydrofuran:acetonitrile with 10 mM ammonium hydroxide.

Analytical results obtained so far indicate that all four treatment plants received raw sewage containing AZI at concentrations ranging from 0.4 to 15 µg/L. No significant change in aqueous AZI concentrations was seen as the wastewater moved through the treatment plants; AZI concentrations in the effluent ranged from 0.8 to 3.8 mg/L. The data showed no discernible seasonal trend for aqueous AZI concentrations. Mass loading of AZI into the Kansas River ranged from 1.5 g/day to 81 g/day.

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- Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. *Environ. Sci. Technol.* 2002, 36, 1202-1211.

Publications and Presentations

1. Close, L.; Koch, D.; Hunter, R; Bhandari, A. "Occurrence and fate of antibiotics in KS wastewater treatment facilities." Invited paper at the 21st Annual Water and the Future of Kansas Conference, Lawrence, KS, Mar 11, 2004.

Information Transfer

1. "K-State Researchers Track Antibiotics in Kansas River Waters" Press release by KSU Research & Extension. Apr 5, 2004
2. "Researchers Track Antibiotics in Kansas River Waters" News article published by About.com (www.about.com), Apr 6, 2004.
3. "Antibiotics in Rivers Raise Concerns" News article published by Harris News Service, Apr 12, 2004.
4. "Water Samples Reveal Presence of Antibiotics" News article published by Topeka Capitol Journal Online, May 4, 2004.

Students Supported

1. Larry Close, MS Candidate, Department of Civil Engineering, KSU
2. Zachary Cook, MS Candidate, Department of Civil Engineering, KSU