Title: Biotransformation of pharmaceuticals and personal care products (PPCPs) at an effluent land application site.
Focus Category: Treatment, Waste Water, Water Quality
Keywords: effluent**,** biotransformation, 17β-estradiol, estrone, estriol, and 17α-ethinylestradiol, ciprofloxacin, triclosan, ibuprofen
Duration: March 1, 2009 through February 28, 2010.
Federal Funds Requested: $4967
Non-Federal (matching Funds Pledged: $10,025
Principal Investigator: Deborah Carr, Texas Tech Univ., Dept. of Environmental Toxicology, Ph.D. Candidate, Deborah.Carr@ttu.edu, (806) 885-4567, Box 41163, Lubbock TX 79409-1163
Co-Principal Advisor: Todd Anderson, Texas Tech Univ., Dept. of Environmental Toxicology, Professor, Todd.Anderson@ttu.edu, (806) 885-4567, Box 41163, Lubbock TX 79409-1163
Congressional District where Project will Occur: Congressional District 19

Abstract

Pharmaceuticals and personal care products are emerging contaminants of concern to drinking water supplies and aquatic ecosystems. This proposal addresses the ability of the soil microbial community to remove commonly occurring compounds, 17β-estradiol, estrone, estriol, and 17α-ethinylestradiol, ciprofloxacin, triclosan and ibuprofen from wastewater effluent prior to entering the groundwater or surface waters.

Statement of Critical Regional Water Problem

As water supplies become more limiting (especially in the arid Southwest) and water re-use practices change, pharmaceuticals and personal care products in municipal water supplies and the level of effluent treatment become an important human and environmental health issue. Information generated from the proposed research will broadly impact our ability to evaluate the effectiveness of wastewater treatment systems to minimize micropollutants and their transport to downstream drinking water supplies.

Proposed Research

We propose to investigate the long-term fate of pharmaceuticals and personal care products (PPCPs) in the soil from a large municipal waste water treatment plant (WWTP) effluent land application site. The Lubbock Waste Water Reclamation Plant 3 employs simple primary and secondary processing with no advanced treatment processes. Effluent from this plant is applied at the Lubbock Land Application Site (LLAS) for non-edible crop production and nutrient removal before entering the underlying groundwater aquifer. Because the LLAS has received wastewater effluent for over 70 years it is an ideal field site to study the persistence and fate of micropollutants in the soil. <u>The goal of this research is to determine the role of soil bacteria and fungi in transforming a variety of common PPCPs before they are transported to the groundwater.</u> As the City of Lubbock (and other areas of the southwest U. S.) prepares to change their water reuse practices, the fate of these micropollutants should be evaluated. Information generated from the proposed research will broadly impact our ability to evaluate the effectiveness of wastewater treatment systems to minimize micropollutants and their transport to groundwater and downstream drinking water supplies. The proposed research outlined here is part of a larger project (funded by the U.S. EPA) on the long-term fate (occurrence, sorption, biodegradation, plant uptake) of pharmaceuticals and law and policy questions related to water re-use. The proposed research will only focus on a portion of those objectives consistent with the amount of funds requested.

Background

Several recent media accounts have focused attention on prescription, over the counter drugs, and personal care products showing up in the nation's drinking water sources. While PPCPs have been identified in the environment for several decades (Kummerer, 2001), the fates and persistence of these compounds are not well known. These compounds are considered micropollutants and can be found in wastewater and surface and groundwater near wastewater discharge areas. They can include birth control hormones, antibiotics, antimicrobials, pain relievers, insect repellants and caffeine to name only a few. In general these are compounds with known pharmacological actions in humans and animals and, although quantities of these contaminants in wastewater may be low, they are continuously present and constitute a constant exposure. In most cases, no regulatory limits have been set for these compounds and their discharge from WWTPs; their fate and persistence in the environment are largely unknown.

Natural and synthetic estrogens (4-nonylphenol, DDT and Bisphenol-A) have become emerging contaminants of concern because of their ability to disrupt the endocrine system (EDC) and cause long-term impacts to wildlife and human health (Lee and Liu, 2002; Hermanowicz and Wozei, 2002; Norris and Carr, 2006). Investigations into the removal of EDCs from water in WWTPs have shown that bacteria in activated sludge systems can reduce concentrations of natural and synthetic estrogens and their breakdown products (Hermanowicz and Wozei, 2002; Anderson et al. 2003). Sorption to sludge particulate matter and biodegradation appear to be the most important removal processes for EDCs in wastewater treatment systems, though microbial populations vary in their ability to degrade estrogens (Layton et al. 2000). While studies have confirmed the ability of EDCs to partition to solids in WWTPs (Gomes et al. 2004), less than 3 percent of the estrogenic activity was found in the sludge (Körner et al. 2000) and only 5 percent of the estrogens were absorbed onto digested wastewater sludge (Anderson et al. 2003). This implies a significant fraction may be susceptible to microbial degradation.

Several studies have detected antibiotics such as ciproflaxacin (Alder et al. 2000), erythromycin (Hirsch et al. 1999), and penicillin types (Hallig-Sorensen et al. 1998) in WWTP effluents at ng/L to µg/L concentrations. According to a study by the U.S. Geological Survey (Kolpin et al. 2002) out of 31 antibiotics and antibiotic metabolites measured only 17 were detected, indicating a strong need for information regarding the fate and transport of antibiotics in the environment.

Many PPCPs other than antibiotics and EDCs have been detected in surface water and wastewater effluents. In the above mentioned USGS study, the most frequently detected compounds were coprostanol (fecal steroid), cholesterol (plant and animal steroid), insect repellant (N,N-diethyltoluamide), caffeine, triclosan (antimicrobial disinfectant), fire retardant ((tri(2-chloroethyl)phosphate) (Kolpin et al. 2002). Caffeine and Ibuprofen have been shown to persist up to 6 months in groundwater aquifers (Drews et al. 2003). The concentrations of these chemicals leaving water treatment facilities and their environmental persistence have profound implications for water quality and health standards.

Hypothesis

Biotransformation and sorption limit the accumulation of micropollutants at the Lubbock Land Application Site (LLAS) and prevent their contamination of the groundwater and downstream drinking water supply.

Specific Aim

To assess the role of biotransformation and sorption of micropollutants applied to the LLAS under aerobic and anaerobic conditions in soils of long-term exposure to PPCPs and soils not exposed to PPCPs. The micropollutant compounds to be evaluated in this study are often found in drinking water sources and have been determined to have a negative environmental effect in surface water ecosystems. They include:

- Endocrine disruptors: 17β-estradiol (E2), estrone (E1), estriol (E3), and 17α-ethinylestradiol
- Antibiotics: Ciprofloxacin
- Personal Care Products: Ibuprofen and triclosan

Research Plan

We propose to assess the role of biotic and abiotic processes on the micropollutants applied at the LLAS using aerobic and anaerobic bottle studies. Differing chemical structures lend themselves to either aerobic or anaerobic transformation processing by indigenous bacteria (Maier et al. 2000). Upper soil layers will have higher oxygen levels while saturated or lower soil layers are potential low oxygen environments resulting in differing microbial populations. To examine all potential fates for the applied micropollutants both anaerobic and aerobic bottle studies will be conducted. Killed (autoclaved) controls will be used to determine abiotic sequestering potential in these soils. Soils from an effluent application site and from a control site with no exposure to WWTP effluent will be compared for their ability to biotransform commonly occurring PPCP compounds.

The aerobic bottle study will be conducted at 50% field capacity conditions. Ten grams of soil will be added to a 120 ml clear glass bottle and spiked with known concentrations of the micropollutants. The headspace will be sparged with oxygen, the bottles sealed. No additional carbon or nutrients will be added. The bottle studies will be conducted in triplicate over 14 d. and soil samples will be collected at 0, 3, 7, and 14 d. and analyzed for micropollutant concentrations. The data collected will indicate the ability of these pollutants to be degraded aerobically by indigenous microbes as well as the rate of this biological degradation. Autoclaved soils from the exposed and non-exposed sites will be subjected to the same treatment described above, and will be used to determine the ability and potential rate of abiotic degradation as well as the partitioning of the micropollutant to the soil matrix at the study site. The anaerobic bottle study will be conducted in the same fashion as the aerobic bottle studies; however the headspace will be sparged with nitrogen rather than oxygen.

At the conclusion of each incubation period (0, 3, 7 and 14 d.), micropollutants will be extracted from the soil in the bottles with acetonitrile using published methods (Golet et al. 2003; Christian et al. 2003). Micropollutant concentrations will be determined by conventional HPLC (UV, Fluorescence) analytical techniques (Wibawa et al. 2002; Golet et al. 2001; Kolpin et al. 2002). The above analytical equipment is available within the facilities of the investigator. Decreases in micropollutant concentration will be compared among treatments by ANOVA and Tukey- Kramer multiple comparison test.

A comparison of the microbial populations between the control and effluent-exposed sites will be conducted by pyrosequencing extracted DNA in cooperation with Scott Dowd at USDA.

Expected Results and Limitations

The expected results of this project include both short-term and long-term outcomes. The outcomes of this work include identifying the fate and transport of micropollutants in a natural system and assessing the role of biotic and abiotic mechanisms on the persistence of micropollutants in the environment. While it is expected that results obtained in this study will be applicable to many arid soils, complex environmental interactions may limit the ability to transfer these results to generalized soil environments.

Planned Dissemination of Results and Future Support

Results of this study will be presented orally to the City of Lubbock and initially at regional meetings of the Society of Environmental Toxicology and Chemistry (SETAC). A final report including manuscripts resulting from this research will be presented at the completion of the project. Results will be submitted as a manuscript(s) to a peer-reviewed journal.

Literature Cited

Alder, A.C., McArdell, C.S., Giger, W., Golet, E.m., Ibric, S., Molnar, E. and Nipales, N.S. (2001). Occurance and fate of flouroquinolone, macrolide, and sulfonamide antibiotics during wastewater treatment and in ambient waters in Switzerland. In: Daughton C.G., Jones-Lepp , T. (eds.) Pharmaceuticals and personal care products in the environment: scientific and regulatory issues. American Chemical Society, Symposium Series 791, 56-69.

Anderson H., Siegrist, H., Hallig-Sørensen, B., and Ternes, T.A. (2003). Fate of Estrogens in a Municipal Sewage Treatment Plant. *Environmental Science and Technology*, Vol. 37, No. 18:4021-4026.

Christian, T., Schneider, R.J., Farber, H.A., Skutlarek, D., Goldbach, H.E. (2003). Determination of antibiotic residues in manures, soil and surface waters. *Acta Hydrochimica et Hydrobiologica*. 1:36-44.

Drews, J.E., Herberer, T., Rauch, T., and Reddersen, K. (2003). Fate of Pharmaceuticals During Ground Water Recharge. *Ground Water Modeling and Remediation*, 23(3), 64-72.

Golet, E.M., Alder, A.C., Hartmann, A., Ternes, T.A., and Giger, W. (2001). Trace determination of flouroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. *Analytical Chemistry*. 73:3632-3638.

Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C., and Giger, W. (2003). Environmental exposure assessment of flouroquinolone antibacterial agents from sewage to soil. *Environmental Science and Technology*, 37:3243-3249.

Gomes, R.L., Avcioglu, E., Scrimshaw, M.D., and Lester, J.N. (2004). Steroid estrogen determination in sediment and sewage sludge: a critique of sample preparation and chromatographic/mass spectrometry considerations, incorporating a case study in method development. *Trends in Analytical Chemistr*y, Vol. 23, No. 10-11:737-744.

Hallig-Sørensen, B., Nielsen, S.N., Lanzky, P.F., Ingerslev, F., Holten Lutzhoft, H.C., and Jorgensen, S.E. (1998). Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment –A Review. *Chemosphere*, 36(2), 357-393.

Hermanowicz, S.W., and Wozei, E. (2002). Biodegredation of estrogenic compounds and its enhancement in a membrane bioreactor. http://repositories.cdlib.org/wrc/tcr/hermanowicz

Hirsch, R., Ternes, T. Haberer, K., and Kratz, K.L. (1999). Occurrence of antibiotics in the aquatic environment. *Sci Total Environ*, 225, 109-118.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., and Buxton, H.T. (2002) Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. *Environmental Science and Technology*, 36, 1202-1211.

Körner,W., Bolz, U., Süβmuth, W., Hiller, G., Schuller, W., Hanf, V., and Hagenmaier, H. (2000). Input/output balance of estrogenic active compounds in a major municipal sewage plant in Germany. *Chemosphere* 40: 1131-1142.

Kummerer, K. (ed). (2001). Pharmaceuticals in the Environmant: Sources, fate, effects and risks. Berlin, Germany: Springer-Verlag.

Layton, A.C., Gregory, B.W., Seward, J.R., Shultz, T.W., and Sayler, G.S. (2000). Mineralization of Steroidal Hormones by Biosolids in Wastewater Treatment Systems in Tennessee U.S.A. *Environ. Sci. Technol.* 34:3925-3931.

Lee, H.B. and Liu, D. (2002). Degradation of 17β-Estradiol and its Metabolites by Sewage Bacteria. *Water, Air, and Soil Pollution* 134: 353-368.

Maier, R.M., Pepper, I.L., and Gerba, C.P. (eds.) (2000). *Environmental Microbiology*. Academic Press, San Diego, CA.

Norris, D.O. and Carr, J.A. (eds.) (2006). *Endocrine Disruption: Biological Bases for Health Effects in Wildlife and Humans.* Oxford University Press, New York, NY.

Wibawa, J.I., Fowkes, D., Shaw, P.N., and Barrett, D.A. (2002). Measurement of amoxicillin in plasma and gastric samples using high-performance liquid chromatography with fluorimetric detection. *Journal of Chromatography* B. 774-141-148.