Groundwater Research Report
WR04R004

OCCURRENCE OF ESTROGENIC ENDOCRINE DISRUPTORS IN GROUNDWATER

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Occurrence of Estrogenic Endocrine Disruptors in Groundwater

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July 1, 2004-June 30, 2006

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Project Summary

Background:

Concern has emerged about a group of trace organic compounds identified in the aquatic environment which might affect reproduction and development of wildlife species and humans due to endocrine disruption (Colborn et al., 1996; Roefer et al., 2000). Studies in recent years have documented a high occurrence of these endocrine disrupting compounds (EDCs) in aquatic ecosystems which may have serious implications for groundwater quality (e.g., Kolpin et al., 2002). As the hydraulic demand on groundwater supplies increases, resulting in greater groundwater draw downs, the potential for EDC contaminated surface water input to groundwater increases, especially in areas where high capacity wells are located near surface waters. An additional route for entrance of EDCs into groundwater is through the leaching of septic system effluents. Areas such as non-sewered subdivisions may have an increased susceptibility to contamination of the groundwater by EDCs.

Compounds with endocrine disrupting activity include both anthropogenic chemicals produced industrially (such as surface active agents, pesticides, polychlorinated biphenyls (PCBs), plasticizers, food additives, birth control pills, herbal supplements and cosmetics) and natural occurring compounds (such as sex steroids, plant-produced estrogens and heavy metals) (National Research Council, 1999). EDCs are used in large quantities by consumers and industry. Domestic and industrial wastewater and agricultural run-off are recognized as the major sources of EDCs. Due to their physical-chemical properties and partial resistance to biotransformation, EDCs have been detected not only in wastewater effluents, but also at low concentrations in surface and groundwaters used as a source for water supply, and at very low concentrations in tap water samples (Stumpf et al., 1996; Ternes, 1999; Baronti et al., 2000).

Objectives:

1. To test high capacity municipal water supply wells located near surface waters impacted by industrial and municipal effluents will be tested for estrogenic EDCs.
2. To sample wells constructed to monitor groundwater close to non-conventional small scale on site waste disposal systems will be tested.
3. The breast cancer cell line (MCF-7) assay (E-screen) will be used to evaluate groundwater samples and septic samples for estrogenic activity.

Methods:

High capacity wells that may be impacted by nearby surface waters were selected from five Wisconsin communities. Associated drinking water and WDNR personnel were enlisted to perform sample

collection. Each well and associated surface water were sampled four times per year to evaluate seasonal variability. A total of 21 samples were collected from traditional and non-conventional septic systems throughout Southeast Wisconsin. Soil pore water and groundwater samples were collected from lysimeters and monitoring wells installed beneath and adjacent to two of these systems.

Water samples were passed through a C_{18} solid phase extraction disk (Empore^{TM}). Compounds were eluted from the disks and concentrated to 1.5 ml. MCF-7 breast cancer cells were exposed to the extract. Cell proliferation was measured after five days of exposure using the SRB colorimetric protein assay.

Results and Discussion:

All surface waters contained some levels of estrogenic EDC activity. Water from high capacity wells did not contain any measurable estrogenic EDC activity. Estrogenic activity was detected in 20 of 21 septic effluent samples, although concentrations were markedly reduced in systems utilizing either sand filtration or aerobic pretreatment as compared to traditional systems. Although low levels of activity have been detected in soil water directly beneath one septic system, no estrogenic activity was found in groundwaters in this study.

Conclusions/Implications/Recommendations:

There appears to be no infiltration of estrogenic endocrine disrupting chemicals from the surface waters into the associated ground waters. Advanced pretreatment technologies (aerobic, sand filtration) appear to be quite effective at removing estrogenic compounds from septic effluent. Additional removal of EDCs occurs in unsaturated soils beneath septic leach fields; no EDCs were detected in groundwater beneath two systems without advanced pretreatment.

Related Publications:

Publication in progress: A manuscript based on the results from the septic effluents is in preparation in collaboration with Jeff Wilcox and will be submitted to the Journal of Environmental Quality.

Key Words:
Endocrine disruptors, high capacity wells, septic, non-sewered subdivisions, E-Screen.

Funding:
University of Wisconsin System portion of the Wisconsin Groundwater Research Program through the Water Resources Institute.

INTRODUCTION

During the past decade, concern has emerged about a group of trace organic compounds identified in the aquatic environment which might affect reproduction and development of wildlife species and humans due to endocrine disruption (Colborn et al., 1996; Roefer et al., 2000). Studies in recent years documented a high occurrence of these endocrine disrupting compounds (EDCs) in aquatic ecosystems which may have serious implications for groundwater quality (e.g., Kolpin et al., 2002). As the hydraulic demand on groundwater supplies increases, resulting in greater groundwater draw downs, the potential for EDC contaminated surface water input to groundwater increases, especially in areas where high capacity wells are located near surface waters. An additional route for entrance of EDCs into groundwater is through the leaching of septic system effluents. Areas such as non-sewered subdivisions may have an increased susceptibility to contamination of the groundwater by EDCs. The proliferation of non-conventional small scale on site waste disposal systems in rural areas may also result in the entrance of EDCs to groundwater. It is expected that such non-conventional systems will usually be installed in vulnerable groundwater settings, such as areas of shallow bedrock or high water tables, where conventional on-site septic systems would not be allowed.

Compounds with endocrine disrupting activity include both anthropogenic chemicals produced industrially (such as surface active agents, pesticides, polychlorinated biphenyls (PCBs), plasticizers, food additives, birth control pills, herbal supplements and cosmetics) and natural occurring compounds (such as sex steroids, plant-produced estrogens and heavy metals) (National Research Council, 1999). EDCs are used in large quantities by consumers and industry. Domestic and industrial wastewater and agricultural run-off are recognized as the major sources of EDCs. Due to their physical-chemical properties and partial resistance to biotransformation, EDCs have been detected not only in wastewater effluents, but also at low concentrations in surface and groundwaters used as a source for water supply, and at very low concentrations in tap water samples (Stumpf et al., 1996; Ternes, 1999; Baronti et al., 2000).

The objectives for this project were:

1. To test high capacity municipal water supply wells located near surface waters impacted by industrial and municipal effluents for estrogenic EDCs.
2. To sample wells constructed to monitor groundwater close to non-conventional small scale on site waste disposal systems as allowed by recently enacted Comm 83 rules for construction of septic systems and/or wells which already are suspected of having septic influences.
3. We used the breast cancer cell line (MCF-7) assay (E-screen) to evaluate >60 groundwater samples for estrogenic activity (breast cancer cells grow in the presence of estrogen or compounds that mimic estrogen).

High capacity wells expected either through modeling or direct empirical evidence to be impacted by nearby surface waters were chosen in consultation with DNR and USGS hydrologists. Drinking water personnel from selected communities were enlisted to perform sample collection. Each facility and associated surface water was sampled four times per year to evaluate seasonal variability. We sampled wells from five Wisconsin communities that utilize high capacity wells for their drinking water. Septic samples were collected from a housing subdivision that utilized unique septic systems. Approximately 31 samples were collected, either from monitoring wells or from cooperative homeowners.

Water samples were passed through a C_{18} solid phase extraction disk (Empore^{TM}). Compounds were then eluted from the disks and concentrated to 1.5 ml. MCF-7 breast cancer cells were then exposed to the extract. Cell proliferation was measured after five days of exposure using the SRB colorimetric protein assay. The WSLH has successfully developed the E-screen assay for use on water samples, and find it to be a highly effective and sensitive tool for detecting estrogenically active substances.

PROCEDURES AND METHODS

High capacity wells were sampled from five different Wisconsin municipalities along with their nearby surface water. All surface water samples were taken upstream from the well sites. Specific information on each sampling site can be found in Appendix B. Well water from two of the municipalities was further treated before distribution to the public. These post-treatment plant waters were also collected and tested. Each municipality was sampled four times to assess seasonal variation. A total of 80 well samples, 12 post treatment drinking waters, and 24 surface waters were collected and assayed for endocrine disruption activity. Collections of these waters occurred in November and December 2004, February and March 2005, May and June 2005, and August and September 2005.

Also, 21 septic-effluent samples were collected at 15 different residences in southeastern Wisconsin between April 2005 and February 2006. The types of septic systems included six aerobic treatment units, seven sand filters, and eight without any secondary pretreatment (Wisconsin Mound). Samples were collected before and after pretreatment from six of the systems using sand filtration. Aerobic treatment units and single-pass sand filters are used routinely in Wisconsin and elsewhere when site conditions are not considered suitable—due to shallow bedrock, high seasonal saturation, or limited infiltration capacity—for traditional systems. All samples were collected using peristaltic pumps and new Teflon tubing. In addition, soil-water and groundwater samples were collected beneath and immediately adjacent to two septic systems that utilize Wisconsin Mound distribution using previously-installed lysimeters and monitoring wells.

Water from the high capacity wells, nearby surface waters and septic systems was collected in 1 liter amber glass bottles. Samples were shipped overnight on ice to the laboratory. Samples (volumes of 1-2L) were extracted through a C_{18} disk, which was then eluted with solvents to capture the estrogenic chemicals. The extraction solvents were transferred into ethanol for a final volume of 1.5 ml. The extraction method was evaluated continually throughout the study using blanks, spikes, and duplicates. To ensure that the sampling method was not adding activity, sampling blanks were run using Type I water. Method and matrix spikes using 17β-estradiol were analyzed to ensure the extraction method was recovering the suspected EDCs. Interferences due to sample constituents, especially organic matter, were evaluated using matrix spikes. Duplicates were used to evaluate the consistency of the extraction procedures and assay.

All extracts were tested using the *in vitro* breast cancer cell proliferation assay known as the E-Screen. This assay utilizes the MCF-7 breast cancer cell line. The breast cancer cells proliferate in response to the presence of estrogen or chemicals that mimic estrogen. MCF-7 cells were grown in Dulbecco's Modified Eagle's Medium with 5% fetal calf serum at 37^{o}C and 6.5% CO_2 and were sub-cultured every 7 days. To begin the assay, MCF-7 cells were trypsinized, counted with flow cytometry and plated into 24-well tissue culture plates at approximately 25,000 cells per well. Twenty-four hours after seeding, the media was removed and experimental media was added. The experimental media was Dulbecco's Modification of Eagles Medium without phenol red. The 5% fetal bovine serum used in this media is stripped of steroids with a charcoal dextran (CD) stripping procedure (referred to as CD-media). For plates containing the standards, the MCF-7 cells were exposed to 15 concentrations of estradiol ranging from 1 x 10^{-13} to 1 x 10^{-8} M. All treatments were done in quadruplicate and three plates were used to obtain a complete standard curve. Four control wells (CD-media only) were included on each plate. For plates on which samples were assayed, 0.1 ml of the water extract (in ethanol) was added to 9.9 ml of CD-media. A 50% dilution series was then made for a total of 5 concentrations. Each concentration was applied to four wells. In one of those wells, 1 x 10^{-9} M estradiol was added as a positive control. Again, each plate contained 4 control wells. After five days, cell proliferation was measured by the SRB protein assay: Cells were fixed with 10% tricholoracetic acid, rinsed and allowed to dry. The SRB dye was added to

each well, followed by a rinse with 1% glacial acetic acid and allowed to dry. The dye was resuspended with 10mM Tris buffer at pH 10.5. The samples were read at 515 nM with a Molecular Devices microplate reader. To determine the estradiol equivalents (Eeq) of the samples, the standard curve was fit with a 4-parameter logistic equation, calculated by the Softmax PRO v. 2.6 analytical software package for the microplate reader. Estradiol equivalents for each sample was calculated by inserting the absorbance readings from the sample into the equation derived from the standard curve. Negative and positive wells were evaluated to ensure that the assay is running correctly. Low growth in the positive control wells may indicate the presence of substances that impede the normal growth of the cells. The limit of detection (LOD) was determined to be 0.02 ng/L Eeq and the limit of quantification (LOQ) was 0.04 ng/L Eeq.

RESULTS AND DISCUSSION

Two high capacity wells from five different municipalities were sampled four separate times. Well water from two of the municipalities was further treated before distribution to the public. These post-treatment plant waters were also collected and tested. Nearby surface water samples were also collected. Eighty well samples, twelve post treatment samples and twenty-four surface water samples were collected (Figures 1 through 5). Septic sampling occurred three times, with a total of 21 septic-effluent, four monitoring-well, and six soil-lysimeter samples being collected. Quality assurance samples include five matrix spikes, four travel blanks, three duplicates, two lab water blanks and two lab water spikes. In all, 121 samples have been analyzed for this study. The first round of high capacity well sampling had five of the six surface waters showing estrogenic activity which ranged from 0.05-0.09 ng/L with the sixth site having no estrogenic activity, while all of the well samples showed no estrogenic activity. The second round of sampling, for the high capacity wells had all six of the surface waters exhibiting estrogenic activity at an elevated rate from the first sampling. This activity ranged from 0.04-0.91 ng/L. The well waters from this round of sampling indicated activity levels that ranged from no detect (< 0.02 ng/L) to less than the LOQ (< 0.04 ng/L). The third round of sampling for the high capacity wells had all six surface waters with estrogenic activity ranging from 0.05-0.24 ng/L, while all of the well samples were below LOD. The fourth round of sampling for the high capacity wells had all six surface waters showing estrogenic activity which ranged from 0.07-0.66 ng/L. One of the sites showed estrogenic activity in both their well waters and post treatment waters. These activity levels ranged from <LOQ to 0.05 ng/L. The rest of the well waters did not exhibit estrogenicity. The levels of activity found in these samples are at or near the level of quantification (0.04 ng/L) and may be a result of sampling error or variation in the assay. Seasonality may have played a role in the increase in estrogenic endocrine disruptor activity in the second round of sampling. This round of sampling occurred during February and March, which follows a long period of cold temperatures. Biological activity of surface waters may lower as the temperature decreases. This phenomenon would explain the higher activity level if bacterial degradation of chemicals was happening at a slower rate.

Estrogenic activity in the 21 septic effluent samples ranged from <LOQ to 192.5 ng/L (Figure 6). The highest estrogenic activity (192.5 ng/L) was detected in a malfunctioning aerobic treatment unit. Excluding this data point, activity in samples collected following sand filtration and aerobic activity ranged from 0.058-3.81 ng/L (mean=0.76 ng/L) and 0.07-6.06 ng/L (mean=1.6 ng/L), respectively. Activity in eight samples with no advanced pretreatment—including the samples collected prior to sand filtration—ranged from 11.6-50.0 ng/L with a mean of 23.7 ng/L. These results indicate that concentrations of estrogenically-active substances can be significantly reduced in septic systems utilizing advanced pretreatment. As a comparison, a national survey of eight municipal wastewater treatment plants in which the E-screen was used to evaluate removal during secondary treatment indicated estrogenic activity ranging from 17 to 95 ng/L Eeq after primary treatment and 0.62 to 7.9 ng/L Eeq after secondary treatment (Drewes et al., 2005). Thus, removal appears fairly similar between municipal treatment and systems with advanced pretreatment.

Of the two systems that were instrumented with soil lysimeters and monitoring wells, one system showed effluent activity at 25.50 ng/L while the corresponding soil-water samples had no activity. The second system had an activity level of 19.57 ng/L in the effluent while activity in the three soil-water samples ranged from 0.28-0.38 ng/L. No estrogenic activity was detected in any of the groundwater samples.

Figure 1

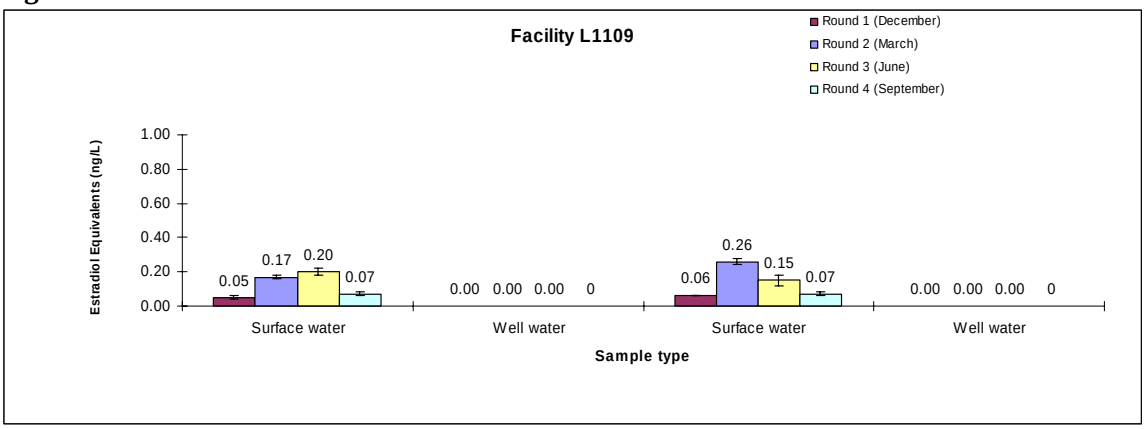


Figure 2

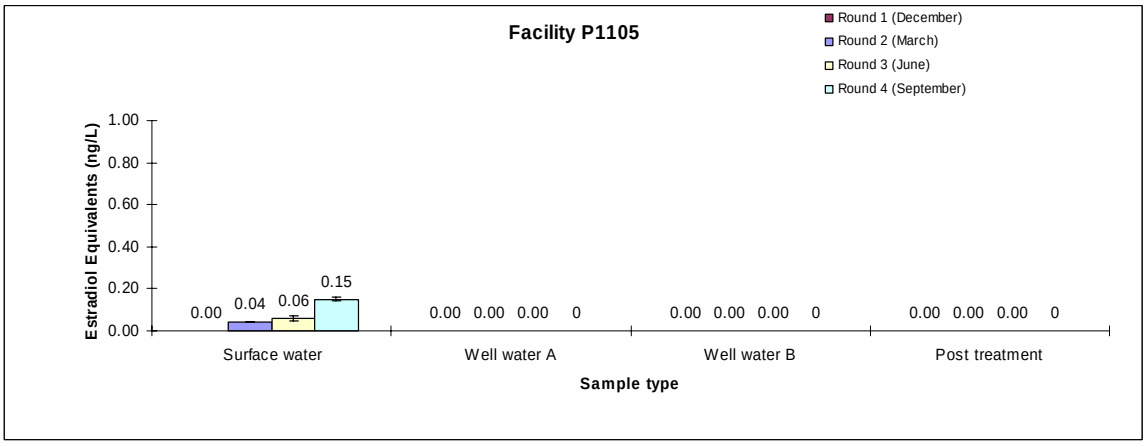


Figure 3

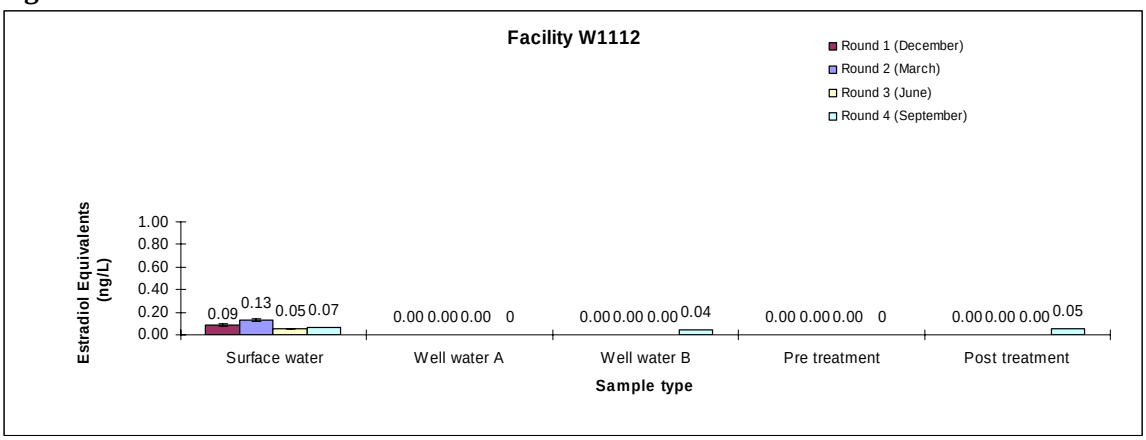


Figure 4

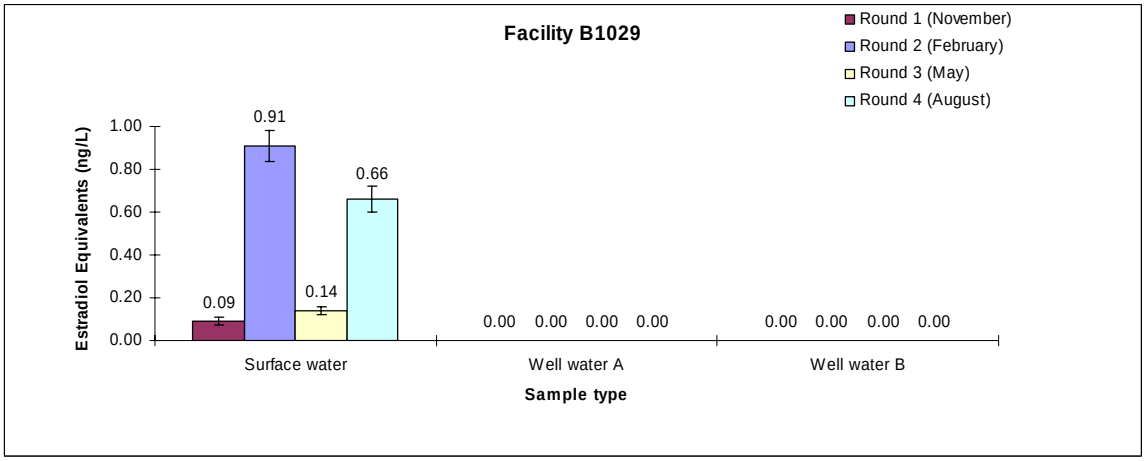


Figure 5

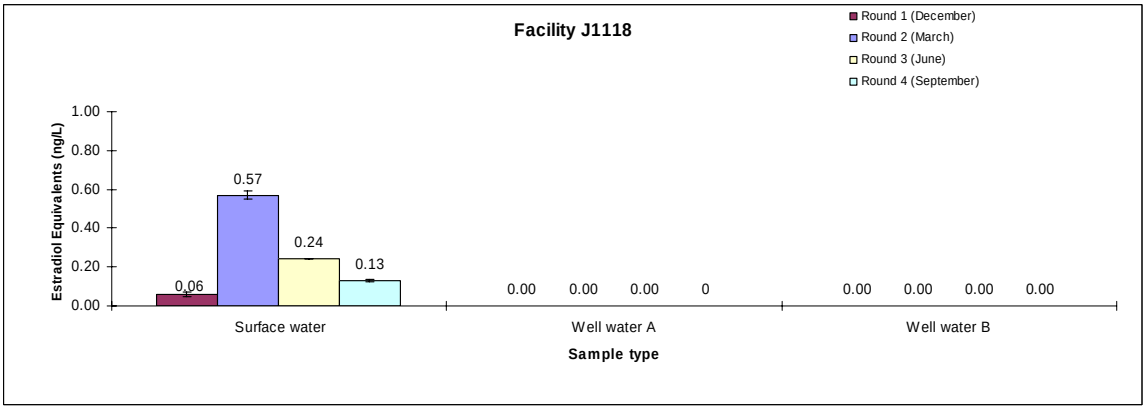
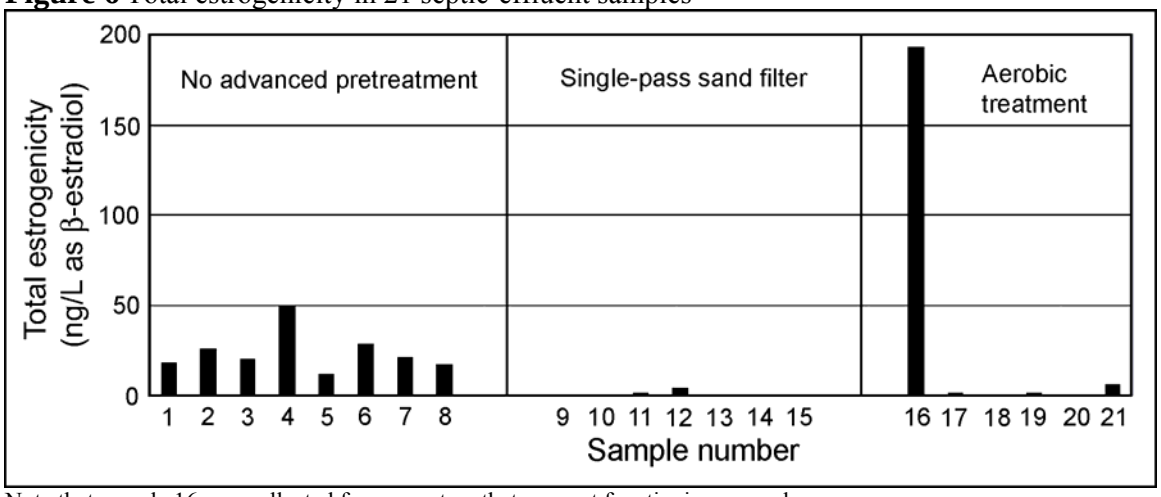


Figure 6 Total estrogenicity in 21 septic-effluent samples



Note that sample 16 was collected from a system that was not functioning properly.

Table 1 QC Sample Results

	QC sample		QC sample		QC sample		QC sample		QC sample
Sampling Round	Lab Blank	Sampling Round	Travel Blank	Sampling Round	Lab Spike % Recovery	Sampling Round	Matrix Spike % recovery	Sampling Round	Duplicate % recovery
1st	ND	1st	ND	2nd	95	2nd	85	2nd	104
1st	ND	2nd	ND	3rd	88	2nd	81	3rd	127
2nd	ND	3rd	ND			3rd	77	4th	71
3rd	ND	4th	ND			4th	85		

CONCLUSIONS AND RECOMMENDATIONS

With the exception of one sample, all the surface waters tested in this study exhibited estrogenic activity although this activity was at very low levels (all < 1 ng/L Eeq). When samples from high capacity wells that had the potential to be contaminated by the surface waters were tested, the vast majority of samples were below the detection limits of the assay. Therefore there is no evidence that the surface water is infiltrating into the groundwater. With respect to the pretreatment of septic effluent, both sand filtration and properly functioning aerobic treatment resulted in markedly reduced estrogenic activity in the samples relative to septic effluents with no pretreatment. However, estrogenic activity was reduced from untreated septic effluent in the soil column. Samples collected with a lysimeter indicated greater than 98% removal and removal below detection levels in samples collected from the monitoring wells.

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APPENDIX A: AWARDS, PUBLICATIONS, REPORTS, PATENTS AND PRESENTATIONS

Presentation:

Wilcox, J.D., J.M. Bahr, C.J. Hedman, and K.R. Bradbury, 2006, Investigation of source concentrations and transport of hormones and pharmaceuticals beneath on-site wastewater treatment systems: GSA Annual Meeting Abstracts with Programs, vol. 38.

Barman, M., Sonzogni, W., Hemming, J., Geis, S. 2005, Occurrence of Estrogenic Endocrine Disruptors in Groundwater. Midwest Regional Society of Environmental Toxicology and Chemistry. Platform presentation April 2005, Madison WI.

Publication in progress:

A manuscript based on the results from the septic effluents is in preparation in collaboration with Jeff Wilcox and will be submitted to the Journal of Environmental Quality.

APPENDIX B:

Table 2 Well Information

Facility Code										
	1029 A	**1029 B**	**1105 A**	**1105 B**	**1109 A**	**1109 B**	**1112 A**	**1112 B**	**1118 A**	**1118 B**
Well distance from surface water (ft)	NA	NA	2640	3960	400	1800	900	100	50	800
Well depth (ft)	NA	NA	110	118	100	93	100	150	100	105
Municipal discharges	NA	NA	0	0	15	12	16	16	19	19
Industrial discharges	NA	NA	0	0	2	3	14	14	3	3
NA = information not provided by municipality										