Removal mechanisms of endocrine disrupting compounds (steroids) during soil aquifer treatment

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Abstract The objective of this study was to determine the primary removal mechanisms of endocrine disruptors such as steroidal hormones present in reclaimed water, specifically 17β-estradiol, estriol, and testosterone, during groundwater recharge via soil aquifer treatment (SAT). Steroidal hormones were quantified using enzyme-linked immunosorbent assays. Bench-scale studies and laboratory-scale soil column experiments were employed to determine what mechanisms (i.e., adsorption, biodegradation, photolytic degradation) dominate the removal of the three compounds of interest during SAT. Findings of these studies revealed that the dominating removal mechanism for the compounds of interest during SAT is adsorption to the porous media matrix and additional attenuation to below the detection limit occurred in the presence of bioactivity. This additional removal occurred regardless of dominating redox conditions (aerobic vs. anoxic) or the type of organic carbon matrix present (hydrophobic acids, hydrophilic carbon vs. colloidal carbon).

Keywords Endocrine disrupting compounds; groundwater recharge; soil-aquifer treatment; steroidal hormones; water reuse

Introduction

With an increasing water demand and lack of alternative sources, utilities are attracted to reuse treated domestic wastewater for groundwater recharge to augment drinking water supplies. With the potable reuse of this water, there is a concern that organic pollutants known as endocrine disrupting compounds (EDCs) might survive water treatment and transport through soil-aquifer treatment (SAT) systems resulting in residual concentrations in drinking water, which might pose potential adverse human health effects (Drewes and Shore, 2001). The presence of EDCs in drinking water sources is of concern because EDCs can either mimic natural hormones or inhibit the effects of a hormone causing an increase or decrease in the production of hormones. Multiple studies have shown that EDCs can survive wastewater treatment processes and are present in wastewater effluents in concentrations that can cause adverse effects on wildlife, both in nature and in a laboratory setting (Routledge *et al*., 1998; Iguchi *et al*., 2001; Silva *et al*., 2002). Understanding the mechanisms of removal of these compounds in natural and engineered systems may provide insight into types of treatment processes that are needed to ensure that drinking water supplies are not contaminated.

Steroidal hormones, such as 17β-estradiol, estriol, and testosterone (Table 1), are commonly detected in wastewater effluents as well as surface waters that receive wastewater effluents (Belfroid *et al*., 1999; Huang and Sedlak, 2001). Certain wastewater treatment processes have been shown to better remove these compounds (Lee *et al*., 2003; Strenn *et al*., 2003). Ternes *et al*. (1999) reported removal efficiencies for 17β-estradiol of up to 99.9 percent in plants that employ activated sludge treatment. Takigami *et al*. (2000) reported significantly higher levels of 17β-estradiol in sewage sludge as compared to the concentrations in the final effluent. These results indicate that sorption to biosolids is occurring. Holbrook *et al*. (2002) found significantly higher estrogenic activity in digester

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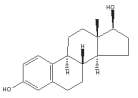
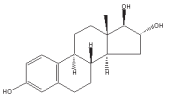
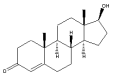
Table 1 Physical and chemical properties of 17β-estradiol, estriol, and testosterone

Compound	Water solubility (mg/L)	Log Kow*	Chemical Structure
17β-Estradiol	3.6	4.01	
Estriol	441	2.45	
Testosterone	23.4	3.32	

* SciFinder Scholar 2002, calculated using Advanced Chemistry Development (ACD) Solaris V4.67

feeds comprised of sewage sludge than in the raw influent to a specific wastewater treatment facility. These results would also indicate that steroids responsible for the estrogenicity are sorbing to the sludge.

Understanding the fate of steroidal hormones during wastewater treatment processes can provide indications about their fate and transport during SAT. Compounds that are being removed primarily via sorption have the potential to remobilize. If the compounds are being degraded, then they may be transformed to a non-estrogenic form and no longer capable of causing endocrine disruption. Studies regarding the fate and transport of EDCs especially hormones during SAT have so far provided some indications to removal but not comprehensively addressed the removal mechanisms during travel through the subsurface. Cordy *et al*. (2003) studied removal of 34 organic wastewater compounds, and did not detect any hormones after 3 m of infiltration through desert soils and a retention time of 21 days. Lai *et al*. (2000) investigated the sorption potential of estriol and estradiol onto sediments. These studies showed an initial high rate of sorption followed by a lower rate of sorption and subsequent desorption, all within a 5-hour time period. Tanaka *et al*. (2000) reported that hormones were enzymatically degraded on the surface of sea sand. Layton *et al*. (2000) showed that 17β-estradiol and testosterone are capable of being mineralized by biosolids from four different municipal wastewater treatment plants. In addition to biological degradation, photolytic degradation of the compounds might have occurred. Gray and Sedlak (2003) reported a 95 percent removal of 17β-estradiol after a period of 100 hours when hormone amended wetland water was exposed to sunlight.

The objective of this study was to fundamentally explore mechanisms leading to removal of 17β-estradiol, estriol, and testosterone during transport through porous media. During this study, 3 sets of soil columns under different redox conditions and flow regimes as well as batch tests were employed to simulate processes occurring during SAT and to study steroid removal during water percolation through porous media.

Methods

Laboratory-scale studies

Two sets of biotic soil column tests (i.d. 6.5 cm, length 30 cm) filled with silica sand (d_{10} = 0.19 mm, d_{60} = 0.75 mm, f_{OC} <0.1 percent) and a pore volume equal to 200 mL were used in this study. Each test consisted of four individual columns fed with bulk secondary treated effluent and three different organic matter matrices fractionated from the bulk secondary effluent. The isolated fractions included hydrophobic acids (HPO-A), hydrophilic organic matter (HPI), and colloidal organic matter. HPO-A, HPI, and colloids were fractionated from the bulk secondary effluent using methods (such as XAD-8 resin and dialysis bags with a cut-off of 6,000 Dalton) as decribed by Rauch and Drewes (in press). The first test

consisted of four columns ("recycle columns") that were operated under unsaturated aerobic conditions and run in recycle mode where the column effluent was recycled to the feed container. All column influents contained 5 mg/L dissolved organic carbon of the respective carbon fraction and were spiked with nominal concentrations of approximately 200 ng/L of each of the three hormones of interest. A phosphate buffer at pH 7 was added to adjust the pH as well as the ionic strength of the solutions. Water was recycled on these columns until organic carbon concentrations remained steady which usually occurred after 5 days of operation (corresponding to 5.2–7.4 hrs. effective contact time of the samples in the porous media). In addition to the four recycle columns a fifth recycle column of the same set-up was operated under abiotic conditions in order to quantify removal by physical adsorption. The abiotic column was fed with milli-Q water spiked with 480 ng/L of each target compound, adjusted to 5 mg/L effluent DOC and a pH of 7. Sodium azide (2 mM) was added to inhibit aerobic microbial activity in this column. Samples were taken on day 0 and day 5 and analyzed for initial and residual hormone concentrations. The second column system consisted of four columns operated in flow-through mode simulating the initial infiltration (0–30 cm) of SAT ("flow-through columns"). These columns were operated under saturated, aerobic flow-through conditions simulating a total detention time of 18 hours in the subsurface. These columns were also fed with the four different bulk organic carbon fractions (adjusted to a DOC of 5 mg/L and a pH of 7) and spiked with nominal concentration of approximately 200 ng/L of each of the target compounds.

Bench-scale studies

Batch studies were used to determine the sorptive potential of the three hormones onto soil. Glass jars were filled with 200 grams of a soil as dry weight with an f_{OC} of 1 percent and a relative sand/silt/clay distribution of 59/17/24 percent. 1 L solutions containing 2mM sodium azide (to assure abiotic conditions), phosphate buffer at pH 7, and target concentrations of 0, 10, 30, 50, and 500 ng/L of the three compounds, respectively, were added to each of the jars. The jars were shaken on a shaker table for 24 hours in the dark and then analyzed for liquid phase concentrations of the three hormones. Additional batch studies were conducted to determine adsorption kinetics of the three hormones onto the silica sand used in the soil column studies (f_{oc}<0.1%). 200 g of silica sand were placed in 3 glass jars, and solutions containing 2 mg/L of each of the target compounds, 2mM sodium azide, and a phosphate buffer at pH 7 were added to each jar. The jars were shaken in the dark and samples taken every 30 minutes over a period of 5 hours, and after 24 hours.

Photodegradation studies were conducted in open glass beakers using secondary effluent spiked with 17β-estradiol and testosterone. The beakers were exposed to sunlight for 1 day. In parallel, a control was kept in the dark during the experiment.

Analytical methods

Soil samples were collected from the top 1–3 cm of the columns for soil biomass activity measurements quantified as dehydrogenase enzyme activity. (Previous analysis of soil cores over complete column depth had identified that the major biomass accumulation occurred in the top part of the columns.) Analysis followed the procedure described in detail in Rauch and Drewes (submitted). In short, soil was incubated with the color reagent 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) and subsequently the formation of INT formazan was quantified by photo-spectroscopy and directly related to soil biomass activity. Soil analysis was performed in triplicates.

The analytical methods for steroid isolation and quantification in aqueous samples were modified from Huang and Sedlak (2001). Sample concentration was achieved by filtering a 1 litre sample through a 0.45 μm cellulose acetate membrane filter (Advantec MFS, Inc.)

followed by a high performance extraction disk (C18, Empore). Subsequently, the disk was dried for 10 minutes. Once the C18 disc was dry, the disk was first eluted with 10 ml of a 40/60-methanol/water solution. This fraction was discarded. 2 × 10 ml of a 90/10-methanol/water solution was used to rinse the original sample container, then filtered through the disk. The entire 20 ml of 90/10 solution was then dried to complete dryness under a gentle stream of nitrogen. Once dry, the dried samples were resuspended in 1 ml of a 15/85-acetonitrile/water solution.

A Hewlett Packard 1050 HPLC with an ultra-violet (UV) detector (at 220 nm) was used to separate target compounds from remaining organic matter and to isolate fractions of samples that contain the compounds of interest. Separation was achieved through two chromatographic columns. A size exclusion column (Alltech) with a packing material size of 300Å and a mobile phase 15/85-acetonitrile/water (flowrate of 1 ml/min) was used to separate steroids from interfering organic matter and other impurities in the sample that were both smaller and larger in size. A standard mixture of hormones (estriol, Aldrich; 17β-estradiol and testosterone, Sigma) was injected into the column to determine the detention time of the compounds. 200 μL volumes of standards and samples were used. A sample was injected under the same conditions as the standards, and the fraction of the sample that corresponded to the detention times of the compounds of interest was collected in a glass test tube, dried under a gentle stream of nitrogen, and reconstituted in a 50/50-acetonitrile/water solution. A reverse phase column with 5 μm particles, 9% carbon load, was used next. A standard mixture of 1 mg/L of each hormone in a 50/50-acetonitrile/water solution was injected into the column in a mobile phase of 55/45-acetonitrile/water. The detention time and peak width of each hormone in the reverse phase column was determined. Each sample was then injected into the column. A one minute fraction prior to the estriol peak, the estriol peak, and a one minute fraction after the estriol peak were collected. The same procedure was applied for the 17β-estradiol and testosterone peak. 17β-estradiol, estriol, and testosterone enzyme linked immunosorbent assay (ELISA) kits were obtained from Cayman Chemical Company (Lansing, MI). These kits are based on the competition between free hormone and a hormone-acetylcholinesterase (AChE) tracer for a set number of hormone specific rabbit antiserum binding sites. The developed color intensity is inversely proportional to the concentration of free hormone and was quantified using an Opsys plate reader (Thermo Labsystems).

The recoveries for all four target compounds through the extraction procedure and the HPLC clean-up were 95 ± 3 percent when the initial spiking concentration was 10 μg/L quantified on the HPLC. Recoveries of all compounds were verified using a standard addition of 30 ng/L prior to solid-phase extraction. For the ELISA total recoveries ranged from 45–95%. The limit of detection for 17β-estradiol was 0.4 ng/L, estriol was 0.6 ng/L, and testosterone was 0.5 ng/L, respectively.

Results and discussion

Adsorption studies

The adsorption isotherms for each of the three steroids on soil collected from a field site are shown in Figure 1. The results indicate that the compounds have a high tendency to adsorb onto porous media.

A distribution coefficient (K_d) was calculated for each of the compounds using the solid and liquid phase equilibrium concentrations. Using the ratio between the mass of soil to volume of liquid (c_s), a relative distribution coefficient ($K_{d,p}$) was also determined. This relative distribution coefficient can be used to calculate a retardation factor (R_d) for each of the compounds. The K_d, $K_{d,p}$, and R_d for each compound are listed in Table 2, along with the log K_{ow} values of the target compounds. The calculation of the retardation factor assumed a bulk density of 1.5 g/cm^3 and a porosity of 0.3, respectively.

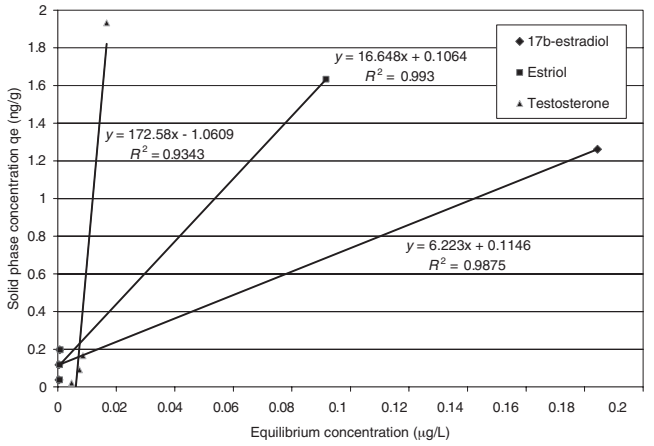


Figure 1 Adsorption isotherms of 17β-estradiol, estriol, and testosterone on soil (f_{oc} = 1%)

Table 2 Coefficients and factors of compounds of interest

	17β-estradiol	Estriol	Testosterone
K_d (ml/g)	6.22	16.65	172.6
K_d,p	60.9	84	97.7
R_d	32.1	84	864
Log K_{ow}	4.01	2.45	3.32

Based on the log K_{ow} values, 17β-estradiol should exhibit the highest affinity to adsorb to soil followed by testosterone and estriol. However, a comparison between the log K_{ow} values and the retardation factors calculated from the experimental data indicated that testosterone is the most retarded in the soil, followed by estriol and 17β-estradiol. This adsorption behavior would suggest that 17β-estradiol is the most mobile compound in subsurface systems.Solid phase concentrations of each steroid after 0.5 and 5.2 hours effective bed contact time when being recycled through the abiotic column are presented in Figure 2. The results from the abiotic column indicate that 79.3 ± 2.4% of 17β-estradiol, 84.3 ± 14.6% of estriol, and 97.5 ± 1.7% of testosterone, respectively, were removed via adsorption to silica sand. Estriol and testosterone reached an adsorption equilibrium with the sand in less than 30 minutes, while 17β-estradiol appears to follow slower adsorption kinetics. A

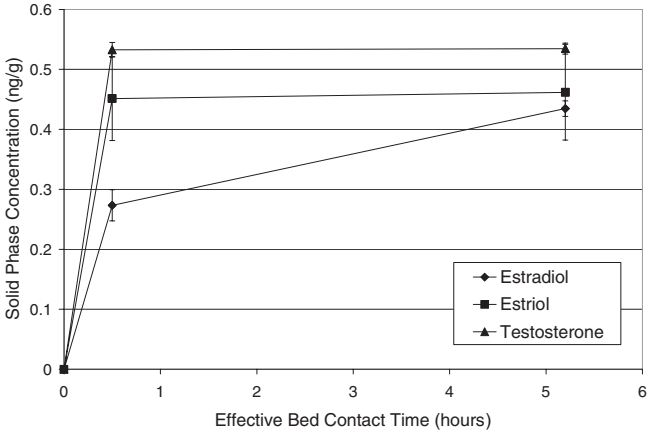


Figure 2 Adsorption kinetics of hormones on silica sand observed during soil-column studies

batch study with the three hormones and silica sand was performed in order to examine adsorption kinetics (Figure 3). These results verified the adsorption behavior of estriol and testosterone. However, 17β-estradiol exhibited similar rapid sorption kinetics in the batch experiment. All three compounds reached equilibrium within the first 0.5 hours of the study, which is consistent with findings reported by Lai *et al*. (2000) regarding estriol. Lai *et al*. (2000) also reported a slight desorption of estriol after 5 hours, which has also been observed in this experiment.

Degradation studies

Results from the biotic soil columns indicate that the three hormones were not only adsorbed but also subject to degradation, regardless of the type of organic carbon present in the sample. Figure 4 shows a comparison of the final concentration of 17β-estradiol in each of the 4 biotic columns and abiotic column after 5.2 hours effective contact time.

This study proved the potential of hormones to be degraded in an aerobic biologically active environment. Very similar results were achieved with soil columns operated under anoxic conditions (data not shown). Biomass activity measurements were taken for each of the columns in an attempt to correlate soil biomass activity with 17β-estradiol removal. Figure 5 shows 17β-estradiol concentration after completed column studies versus soil bioactivity as determined by INT formazan formation.

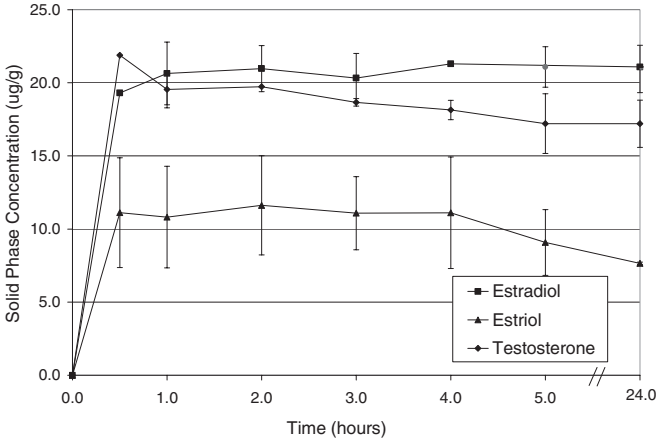


Figure 3 Adsorption kinetics of steroids on silica sand

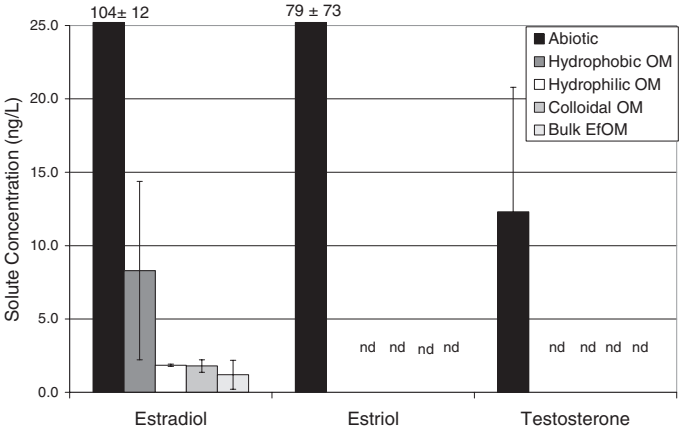


Figure 4 Effluent quality of abiotic and biotic soil columns

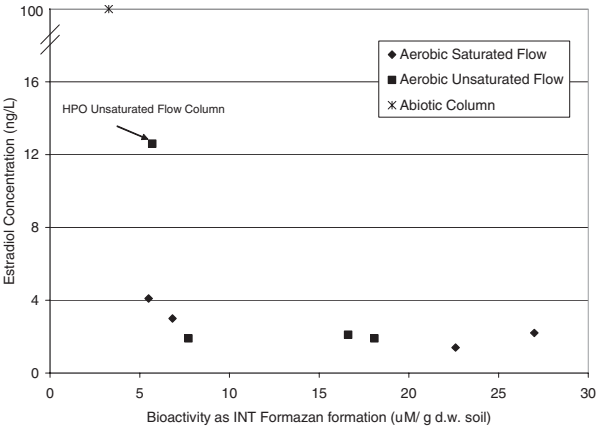


Figure 5 17β-estradiol concentration versus biomass activity in soil columns experiments

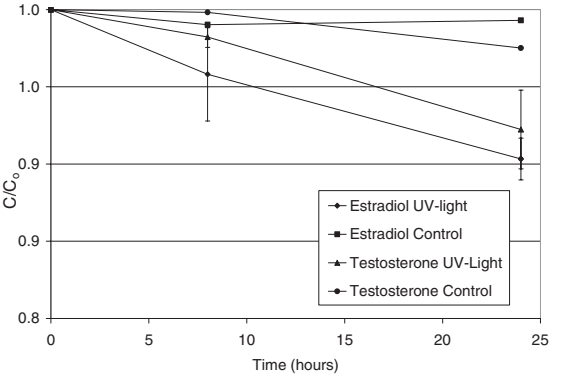


Figure 6 Photolytic degradation of 17β-estradiol and testosterone in secondary effluent (spiked samples exposed to UV light and controls kept in the dark)

Estriol and testosterone were not detected in the biotic column effluents. The average removal of 17β-estradiol in all eight columns was 99.1 ± 0.9%. It was expected that steroid removal would be stimulated in the presence of higher soil microbial activities. Removal of 17β-estradiol, however, appeared independent of biomass activity as long as a minimal soil microbial activity was present. The square symbols in Figure 5 represent recycle column effluent concentrations with a total contact time of approximately 5.2 hours, while the dime-shaped symbols represent biomass results from flow-through columns with an average contact time of 18 hours. The recycle column fed with hydrophobic organic matter with a contact time of 5.2 hours had the highest final concentration of 17β-estradiol, which could be attributed to both the short contact time and the minimal amount of bioactivity present. This particular column showed a bioactivity only slightly higher than the abiotic column. The flow through column fed with HPO-A and with an 18 hour contact time also showed low bioactivity measurements, but had a higher percent removal of 17β-estradiol. This could be attributed to the longer contact time of the sample in the column.

Results from photodegradation experiments showed that the three hormones are only minimally degraded during ultraviolet light exposure over a 24 hour period. 17β-estradiol and testosterone were removed by approximately 10% after 24 hours. Controls kept in the dark showed no degradation of the two compounds (Figure 6). These results are confirming studies by Gray and Sedlak (2003), who reported little degradation of 17β-estradiol in less than 50 hours.

Conclusions

By employing laboratory experiments simulating SAT systems, this study proved that SAT is efficient in removing hormones present in reclaimed water used to augment groundwater supplies. Our study showed that adsorption is the primary mechanism of removal for the three hormones studied. Removals by physical adsorption of 17β-estradiol, estriol, and testosterone on silica sand were 79.3 ± 2.4%, 84.3 ± 14.6%, and 97.5 ± 1.7%, respectively. Removal efficiencies were higher on soil containing a higher content of silt, clay and organic content. When compared to abiotic systems, the three steroids were further removed in the presence of low soil microbial biological activity, indicating that degradation of hormones is occurring during soil infiltration under field site conditions contributing to a sustainable removal of these compounds in the environment. Photolytic decay accounted for a removal of the target compounds of only less than 10% in 24 hours.

Acknowledgements

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