Fate of Personal Care Products and Pharmaceuticals and Growth Response for Reclaimed Water Irrigated Turf Grass

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Introduction:

Many pharmaceuticals and personal care products or PCPPs enter and leave wastewater treatment plants (WWTP) unaltered or incompletely removed and are subsequently released into the environment through wastewater outflows or solids application. Fish exposed to PPCPs in wastewater outflows have been shown to exhibit intersex characteristics. Instead of releasing effluent into streams, wastewater can be further treated and beneficially reused for irrigation of golf courses, farmland or forestry plantations. In addition, biosolids can be used in place of costly fertilizers. However, the persistence of PCPPs once applied to a soil system, such as a golf course, has yet to be quantified.

This study was conducted to assess the benefits and potential for exposure to PCPPs by turf grass irrigated with reclaimed water or fertilized with biosolids. Turf grass collected from the Foster Links Golf Course in Tukwila was irrigated with Class A sand filter treated water from the King County WWTP in Renton in a greenhouse study. The biosolids were obtained from the West Point Treatment Plant. The PCPPs that were monitored for mobility through the soil, presence in the soils at the end of the growing period, and uptake into leaf tissue included: the estrogens: estriol (E1), 17β-estradiol (E2) and ethinylestradiol (EE2), the pharmaceutical ibuprofen, and the antimicrobial triclosan A review of the behavior of each of these compounds in the environment follows.

Ibuprofen: a literature review of behavior in the environment

Ibuprofen or (+)-2-(4-isobutylphenyl) propionic acid is a non-steroidal anti-inflammatory drug used for relief of the symptoms of arthritis, primary dysmenorrhea, fever and as an analgesic (Flippin et al 2007). Ibuprofen requires a relatively high therapeutic dose of 600-1200 mg/d. The majority of this dose, up to 80%, is excreted as the parent compound or in the form of one of three metabolites (Hue et al 1983 cited in Buser et al 1999). The principal metabolites are hydroxy-ibuprofen, carboxy-ibuprofen and carboxy-hydratopic acid (Buser et al 1999).

Fate of Ibuprofen in the environment:

Ibuprofen is released into aquatic environments through wastewater outflows and has been detected at mean concentrations of 4 µg/L up to a maximum of 24.6 µg/L in treated effluents (Metcalfe et al 2003). In a survey of 139 streams in 30 states ibuprofen was detected in 84 streams at a median concentration of 0.20 µg/L and a maximum of 1.0 µg/L (Kolpin et al 2002). In other studies, ibuprofen was detected at lower concentrations in the ng/L range. The occurrence of ibuprofen in surface waters was monitored in Lake Greifensee. Concentrations of ibuprofen were detected in the range of 2-8 ng/L (Buser et al 1999). The concentration of ibuprofen released to the environment through wastewater outflows is highly dependent on the wastewater treatment with higher concentrations being released by secondary treatment systems than tertiary treatment systems.

Ibuprofen and its metabolites are efficiently degraded during wastewater treatment (Buser et al 1999). A variety of studies have shown that ibuprofen is present in very small concentrations in the both the effluent and solids, with no preference being shown for either the aqueous or solid phases. Removal rates were >90% for all treatments ranging from secondary treatment to an MBR system. The main mechanism attributed to the high removal is biodegradation (Kimura et al 2007). Other studies concur with these results. A submerged MBR of 21 L active volume with Kubota plate membranes operated at an HRT range of 8.8-10.0 h and SRT of 37 days exhibited 96% removal of ibuprofen. Concentrations of ibuprofen decreased from the influent (5.7 µg/L) to the effluent (0.18 µg/L) (Quintana et al 2005). The removal rates are sufficiently high to prevent release of ibuprofen at levels that cause documented wildlife effects.

The ibuprofen that is released from wastewater treatments is immediately degraded in the environment through a variety of mechanisms. Ibuprofen can be removed from surface water through photodegradation (Lin and Reinhard, 2005). Solutions were irradiated with a zenon arc lamp (765 W/m^2) and analyzed. In river water, ibuprofen exhibited a half-life of 15 hours (Lin and Reinhard, 2005). Therefore, ibuprofen is readily degraded in surface waters when exposed to sufficient levels of light. Ibuprofen is also removed by sorption to particulate matter or through biotransformation (Buser et al 1999). The compound exhibited a half-life of 20 days in aquatic systems (Buser et al 1999).

Ibuprofen is introduced into terrestrial environments through solids application or water reuse. Exposure to ibuprofen in a terrestrial system is minimal due to low introduced concentrations, limited mobility of the compound and rapid degradation (Oppel et al 2004, Scheytt et al 2005). This behavior has been seen in laboratory studies. Column experiments were run to investigate the transport behavior of ibuprofen under unsaturated conditions (Scheytt et al 2006). Ibuprofen exhibited a removal rate of 54% on column suggesting that ibuprofen is significantly retarded in a soil system and also eliminated during soil passage.

Triclosan:

5-chloro-2-(2,4-dichlorophenoxy)phenol) or triclosan is an antibacterial and antifungal agent active against gram + and gram – bacteria (Heidler and Halden, 2007). Triclosan or TCS binds to and inhibits the bacterial enoyl-acyl carrier protein reductase enzyme involved in lipid biosynthesis (Adolfsson-Erici et al 2002). TCS is found in many household products including anti-bacterial soaps, deodorants, toothpastes, mouthwashes and cleaning supplies.

The release of triclosan into the environment is of concern due to its similarity in structure to the thyroid hormone. Vedhoen et al (2006) demonstrated that exposure to actual environmental concentrations of TCS altered the rate of thyroid-induced metamorphosis and interferes with gene expression in *Rana catesbiana* or American bullfrog tadpoles. TCS has not been shown to be toxic to mammals but it is toxic to water living organisms such as fish (LC_{50} rainbow trout = 0.35 µg/L) and Daphnia magna (EC_{50} = 0.39 mg/L) (Orvos et al 2002). In addition, the degradation byproduct methyl-TCS bioaccumulates in fish. Methyl-TCS has been detected in *Rutilus rutilus* at concentrations up to 365 ng/g lipid weight (Balmer et al 2004). While triclosan has negative impacts on aquatic wildlife, algae are especially sensitive to exposure (EC_{50} = 1.5 µg/L) (Orvis et al 2002). Streams exposed to TCS in wastewater outflows exhibit changes in the structure and function of algal communities, which can result in shifts in nutrient processing capacity and food web structure (Wilson et al 2003).

A further complication of releasing triclosan into the aquatic environment is that it is likely converted to 2,8-dichlorodibenzo-*p*-dioxin or DCDD in sunlight-irradiated waters (Latch et al 2003; Sanchez-Prado et al 2006). 2,8-DCDD which is an EPA priority pollutant. Dioxins are persistent in the environment and have the potential to bioaccumulate within the fat tissues of organisms (Birkett and Lester, 2003). They also are primarily carcinogenic but have been shown to exhibit weak estrogenic activity (Birkett and Lester, 2003).

Triclosan has a low water solubility value of 10 mg/L and a log K_{ow} of 4.8 at neutral pH indicating an affinity for particle sorption. In addition, volatilization is deemed negligible due to a low vapor pressure of $4*10^{-6}$ mm HG at 20˚C. A pKa of 7.9 indicates that the pH of surface waters can have a significant influence on its speciation. K_{ow} of the protonated form is 5.4. (Singer et al 2002).

Fate in the environment:

TCS is primarily released into the environment through wastewater outflows or biosolids application. A survey of 139 streams by USGS indicated that triclosan is present in over 60% of the streams at a median concentration of 0.14 µg/L and a max of 2.3 µg/L (Kolpin et al 2002). This is a cause for concern due to research that indicates triclosan is potentially toxic and bioaccumulates in aquatic organisms.

Once released into an aquatic environment TCS is removed by photodegradation in surface waters (Lindstrom et al 2002) or sedimentation (Singer et al 2002). Photodegradation is not a preferred pathway of removal due to the formation of more toxic degradation products such as dioxin. A more environmentally benign pathway of removal is sedimentation. TCS is hydrophobic and tends to associate with suspended particles or particles that settle out of the water column. Concentrations of TCS determined in lake Greifensee sediment in Switzerland was 37 +/- 4.4 ng/g dry matter

(Singer et al 2002). A sediment core taken from the lake reflected the increased use of TCS over the last 30 years, with concentrations of TCS increasing with decreasing depth.

Triclosan can be released into terrestrial environments through solids application or reclaimed water irrigation. The US EPA reported the detection of TCS in digested sludge from a STP in WA at concentrations up to 29 mg/kg (Heidler and Halden, 2007). Other studies have reported the average concentration of TCS to occur in a range of 20-30 mg/kg in biosolids. Little to no information is present indicating the concentration o f TCS in reclaimed water. Also, the fate of TCS in a soil system is yet to be researched. It is possible that TCS may be persistent in soils due to anti-microbial activity and therefore has the potential to accumulate (Bester, 2007)

Estrogens:

Steroidal compounds are released into the environment primarily through mammalian excretion. Estrogens are released in an inactive form conjugated with sulfates or glucoronides. During sewer transport and wastewater treatment the estrogens can be cleaved into free active forms and released into the environment with the effluent of wastewater treatment plants (Joss et al 2004). Once released, these estrogens can bind to the hormone receptor of many different species and activate a hormonal response at concentrations as low as 1 ng/L (Jobling et al 2006). For example, male fish exposed to estrogens in wastewater outflows exhibit intersex characteristics such as the production of the female egg protein vitellogenin (Jobling et al 2006). Laboratory studies of *Oryzias latipes* (Japanese Medaka) showed mean lowest observed effect concentrations (LOEC) for estrone and 17β-estradiol to be 8 and 4 ng/L respectively while ethinylestradiol has a LOEC of 0.03 ng/L (Metcalfe et al 2001). Other species are similarly affected. A LOEC of 8.9 ng/L was determined for female juvenile rainbow trout exposed to17β-estradiol for 14 days (Thorpe et al 2000).

The estrogens as a group are moderately hydrophobic and thus poorly soluble in water as can be seen by their low solubility values ranging from 0.8 to 13.3 mg/L. The range of octanol water partition coefficients of 2.6 to 4.0 indicates that this group exhibits an affinity for sorption to solids. Finally, the low vapor pressure suggest that the estrogens are not easily volatilized under normal temperature and pressure conditions. Given these physicochemical characteristics it appears that the preferential behavior of the estrogens in a wastewater treatment facility or the environment is sorption onto solids. In fact, estradiol was removed in a biodegradation batch test by bio-caked membrane at two times the rate of removal of a virgin membrane (Chang et al 2006). Therefore a potential removal pathway is through sedimentation of solids and biodegradation by microbes.

Fate of estrogens in the environment:

Estrogens are ubiquitous in aquatic and terrestrial systems. Campbell et al (2006) published a review of estrogen fate and transport studies in various environmental media. The concentration of estrogens detected in different environmental compartments is listed in Table 1.

Table 1. Concentration of Natural and Synthetic Estrogens in Surface Water, Wastewater effluent and Biosolids (Campbell et al 2006)

Analyte	Surface water (ng/L)	Wastewater effluent (ng/L)	Biosolids (µg/g)
17β-estradiol	< 0.1 - 6.0	0.1 – 650	0.00057
Estrone	< 0.1 – 17	0.1 – 19	0.00143
Ethynylestradiol	< 0.1 - 5.1	0.1 – 8.9	0.00061
Estriol	1.0 – 2.5	5.0 – 7.3	-------

The concentrations of hormones in the surface water in Table 1 appear to be conservative. The various studies reviewed by Campbell et al (2006) suggest a range of concentrations from 0-17 ng/L in surface water. Much higher values were reported in a USGS survey of organic wastewater contaminants in 139 streams conducted in 2002. The natural estrogens were present in over 80 locations at a median and maximum concentration of 0.073 and 0.831 µg/L for ethinylestradiol; 0.009 and 0.2 µg/L for estradiol; and 0.027 and 0.112 µg/L for estrone (Kolpin et al 2002). In both studies, the concentrations of estrogens found in stream environments exceed the minimum effect exposure levels for aquatic wildlife reported earlier.

Estrogens are removed from the water column via three mechanisms: sorption to sediments, biodegradation or photolysis (Gray and Sedlak, 2005). Although sorption to sediments removes the estrogens from the column they can remain in an anoxic sediment environment. Only E2 can undergo anaerobic degradation at an extremely slow rate (Ying and Kookana, 2006). Subsequently, the estrogens are likely to remain within aquatic sediments. Biodegradation accounts for some removal of estrogens, however this removal is not complete. In Gray and Sedlak (2003), biotransformation of E2 and EE2 in treatment wetland test cells fed river water downstream from a treatment facility were 64% and 59% after 10 days. Finally, estrogens can also undergo photolysis in surface waters. Lin and Reinhard (2005) published half-lives of 2 to 3 hours for estriol, estrone, 17β-estradiol, and ethinylestradiol present in river water which was irradiated with a xenon arc lamp (760 W/m^2; $290 < \lambda < 700$ nm) to mimic sunny mid-summer conditions in surface waters.

Estrogens are introduced into a terrestrial environment through solids application or wastewater irrigation. Prior research has revealed that estrogens are not labile in a soil system and degrade slowly. Colucci and Topp (2001) studied the persistence of 17β-estradiol, estrone and ethinylestradiol in three agricultural soils at different temperature and moisture levels in laboratory microcosms. Initially, a rapid transformation of 17β-estradiol to estrone occurred with a TD_{50} (time to dissipate by 50%) of less than 0.5 days in all three soils. The newly formed estrone and remaining 17β-estradiol were microbially transformed into non-extractable residues resulting in low bioavailability. This observation was supported by an extremely slow mineralization rate of 17% for 17β-estradiol over a 3-month incubation period. Ethinylestradiol behaved similarly to 17β-estradiol, although dissipation was slightly slower. Ethinylestradiol exhibited a TD_{50} of

3.0 days and dissipated below the detection limit within 22 days (Colucci and Topp 2001). Stumpe and Marschner also observed an extremely slow mineralization rate of 7.4% for 17β-estradiol after a 23-day incubation period. Therefore, 17β-estradiol is likely to accumulate in soils that receive solids applications or wastewater irrigation. However, the hormone will remain static in the sediment system and is non-extractable.

Experimental Setup:

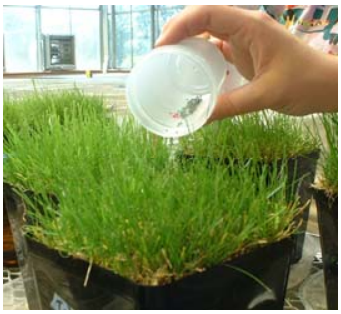
For the current study, the behavior of ibuprofen, triclosan and the three estrogens were monitored in turf grass watered using reclaimed water conventional fertilizers or biosolids. In addition to the fate of these compounds, growth response of the turf to the reclaimed water was also studied. The rate of fertilizer was varied in order to develop an appropriate rate of fertilization to account for the nutrient content of the reclaimed water.

Two distinct types of turf grass: fairway and tee were used for this study. They were collected from the Foster Links golf course in Tukwila: in July, 2007. Samples were cut by the turf manager at the site. Squares of turf including approximately 5 cm of soil and roots were collected for the study. Turf grass collected from the golf course was potted on top of sand and then assigned one of four treatments. Treatments varied by water source and fertilizer application and are listed in Table 2. The experiment was set up in the greenhouse at the University of Washington Botanic Gardens using a randomized complete block design with 4 replicates. The greenhouse is equipped with supplemental lighting and an air cooling system so that daytime temperatures during the course of the study did not exceed 29° C.

Table 2. Treatment number, water source and fertilizer rate for treatments applied to turf grass in the greenhouse study.

Treatment	Source Water	Fertilizer
T1-Control	Tap	100 % rate
T2	Reclaimed Water	None applied
T3	Reclaimed Water	50% rate
T4	Reclaimed Water	100% rate
T5	Tap	Biosolids

Figure 1. Turf grass soil systems



The grass was maintained in the greenhouse from July until mid-December for a total of 6 months. The grass was fertilized according to Curt Chandler's (the turf manager for Foster Links) recommendations. Best 23-5-10 Poly Pro fertilizer was obtained from Mr. Chandler. His standard practice is to add 6.5 lbs of fertilizer per 1000 sq ft. This was adapted to the pots to yield 0.5 and 0.25 g of fertilizer per pot. The pots were fertilized three times during the course of the study. Fertilizer was weighed out on a per pot basis. The plants were watered with aliquots of 250 or 500 mL of reclaimed or tap water depending on need. The amount of water each pot received is presented in Table 3. On a per pot basis, each pot received a total of 2.75 l of reclaimed water during the course of the trial. A discussion of the concentrations of the individual compounds in leachate water, soil and plant tissue follows.

Table 3. Water addition (acre inches) for each month of the study for all treatments

Month	Water added	Water Added
	Acre inches)	Hectare centimeters
July	4.66	4.8
August	1.69	1.74
September	2.54	2.62
October	2.12	2.18
November	2.12	2.18
December	1.69	1.74

Seventeen grams of freeze dried solids were added to each pot in treatment 5. The application rate of 38 Mt ha $^{-1}$ is calculated below. Biosolids application rate**:**

(17 g/in ^{2}pot surface)* (36 in ^{2})*(1 kg/1000 g) * (2,242,000 kg soil/ ha) = (38,114 kg/ha)*(.001 Mt/kg) = 38 Mt/ha

This application rate is above the rate that would normally be used to supply the necessary N for plant growth. However, it was necessary to exceed a N based rate to get consistent results for chemical analysis. No additional fertilizer was added to the biosolids amended turf grass.

Sample Collection:

Through the course of the study reclaimed water source samples and leachate samples from the base of the pots after watering were collected. The leachate was collected by watering the pots with 500 mLs and then collecting the water that passed through the pots. Each batch of reclaimed water and the biosolids used in the study was tested for the contaminants of concern. The leachate was collected and tested approximately every two weeks. The water samples were collected in amber jars to prevent photodegradation and were immediately transferred to King County Environmental Laboratory and refrigerated at 4° C. Plant yield was also measured at several points during the course of the study. The plants were collected by cutting the stalk of the grass approximately 1 mm above the base. Cuttings from each pot were stored in a paper bag, refrigerated and weighed within twenty-four hours to determine plant yield by treatment.

Figure 3: Deconstructing the turf grass soil pots

At the end of the study, the pots were disassembled and the soil collected to measure any changes in soil properties as a result of treatment and to test for the analytes of interest. The soil in each pot was cut so that the original soil layer and the underlying sand layer were analyzed separately. The sand layer was separated into two sections. The upper portion of the sand layer was analyzed at the same time that the soil layer was analyzed. The lower portion of the soil layer was kept for analysis in case there was evidence of compound migration into the upper sand layer. The soil was homogenized and stored in glass jars in the freezer at -20°C until analysis at the King County Environmental Laboratory.

Analytical Methods:

Currently in the scientific community, very few methods are available for extracting and correctly quantifying the estrogens, estrone, estradiol and ethinylestradiol, the anti-microbial agent triclosan and ibuprofen in the environmental samples. This is primarily due to the problems removing these compounds from their environmental matrix. For each of the compounds included in the study, analysis of the compound in water is simpler than analysis in a soil matrix. Water has much lower concentrations of organic chemicals and so analysis for a particular compound is more direct. Soil has a much larger concentration of organic compounds and so potential for contamination or

for compound adsorption is much greater. As a result of this detection limits for compounds are generally much lower in a water matrix than in the soil matrix. The extraction methods used in this study were developed by Michael Muramoto and Michael Doubrava at the King County Environmental Lab. For triclosan and ibuprofen, they are modifications of methods reported in the literature. For the estrogens, the methods developed by Michael Muramoto have a similar level of quantification to alternative methods reported in the literature. Methods for each compound are described in detail below.

Analysis of the water samples for estrogens:

The extraction and analytical methods for detection of the estrogens is extremely involved. The collected waters were combined by treatment. Following collection 500 mL samples were filtered through a zero headspace extractor (ZHE) filter pressure assembly. After filtration the samples were acidified using sulfuric acid to a pH of 3. The samples are required to sit for 10 minutes to ensure that acidification occurred. Next the filtered samples were transferred to a vacuum flask, and a magnetic stir bar and boiling chips were added. The flask was plugged and attached to the vacuum and placed on a magnetic stirrer. The stir bar was set to spin slowly and the vacuum applied for 40 minutes. The purpose of this step is to degas the samples. After degassing, 125 mL of sample was measured out for cleanup and analysis. The remaining 375 mL were stored in the refrigerator for back up. After degassing, samples can be stored for up to a year. Next, 0.625 mL of methanol and 25 µL of deuterated internal standard containing all three estrogens were added to each sample to determine recoveries. The spike blank received an additional 10 µL of native stock (MS). This is followed by an extraction using C-18 Solid Phase Extractors (SPE). The process is outlined in Figure 4.

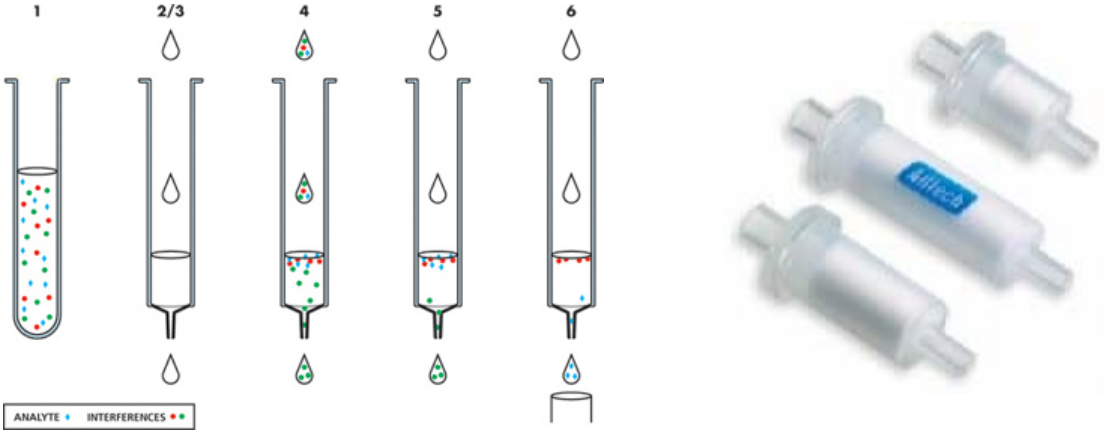


Figure 4. Solid Phase Extraction process and columns

The SPE columns were attached to a manifold vacuum apparatus which pulls all solvents and samples through the column. The manifold vacuum apparatus lid has docking bays with stop cocks into which the SPE columns are inserted. When the stop cock is open the vacuum will pull the solvent or sample through. Closing the stopcock shuts off the vacuum allowing the pull down to be stopped at certain volumes. The SPE columns are then conditioned to remove any interferences contained on the columns. This occurs by first adding 5 mL of acetone and pulling the solvent through until only 3 mLs remain. A timer is set and the columns are left for one minute. The columns are

then refilled with acetone and pulled dry. Next 6 mL of methanol is added and pulled down to 3 mLs. The columns are left for one minute and then refilled with methanol. The methanol is pulled down to approximately 3 mm above the frit. 5 mL of deionized water is added and pulled down to 3 mm above the frit. If the SPE goes dry at any point after the acetone step it must be reconditioned. Finally, the DI water is added up to 1.27 cm from the top. A Teflon tube assembly is used to connect the SPE to the sample bottle. The sample is then loaded onto the SPE at approximately 4 to 5 mL/min. After approximately 40 minutes the entire sample will have loaded onto the SPE. One mL of methanol is added to the bottle and the bottle is capped. The sample bottle is turned to coat all sides with methanol, water is added and mixed. This is a rinse to remove any of the estrogens that are attached to the sides of the sample bottle.

After the sample is loaded on the SPE the columns are conditioned again to remove any interferences introduced from the sample. Five mL of 40% methanol water mix is added to the SPE and drawn down to 3 mm. This step is repeated again. Next 5 mLs of 20% acetone/water mix is added to the SPE and drawn down to 3 mm. This step is repeated. The vacuum is set on high and the SPE is allowed to dry for 1 minute. The columns are then removed from the manifold and centrifuged for two minutes. They are then returned to the manifold and vacuum dried for 10 minutes. Finally, the samples are ready to be eluted from the SPE. The SPE columns are removed from the vacuum apparatus and placed in 40 mL volatile organic analysis (VOA) vials. Samples are eluted by adding 3 mL of acetone five times. The acetone is pushed through the columns using a steady stream of N_2. Elutions 1-4 are taken to 2 mm above the frit. The fifth elution is taken to dryness.

An artificial matrix of soy oil was added to each extract. The soy oil prevents the estrogens from evaporating off when the sample is concentrated on the turbovap. The samples were concentrated to dryness to ensure complete water removal. Once dry 1 mL of 10% hexane/methylene chloride was added and the samples were sonicated for 10 minutes. Next the samples undergo the first of three cleanup steps. The NH_2 cleanup was used to remove the bulk of remaining oils present in the sample as well as the interferences for E1. Similarly to the C-18 extraction, the NH_2 columns required conditioning prior to use. The columns are condition with 2.5 mL of methylene chloride forced through to the frit, followed by 2 x 2.5 ml of acetone forced to the frit and finally 2 x 2.5 mL of 10% hexane/methylene chloride forced through to the frit. The extract is then transferred to the column with 3 x 0.5 mL 10% hexane/methylene chloride vial washes. The extract was allowed to gravity drip through the column. Any potential contaminants were then removed by washing the SPE with 2 x 2.5 mL of 10% hexane/methylene chloride followed by 1 x 2.5 mL of methylene chloride. A collection vial was then placed under the column and the sample is eluted with 3 x 2.5 ml 10% acetone/methylene chloride. The first rinse of 3 mL was forced through using N_2 and the second two pass through the column by gravity drip. The soy oil was added again and the samples are concentrated on the turbovap. Once they have been concentrated to dryness 300 µL of acetone is added and the samples are sonicated for 1 min. Next 300 µL of hexane is added swirled and then 3.4mL of hexane is added.

Next the samples go through the second of the three clean-up steps. The silica cleanup further removes oils and any humic acids present. Silica gel columns are shown in Figure 5.

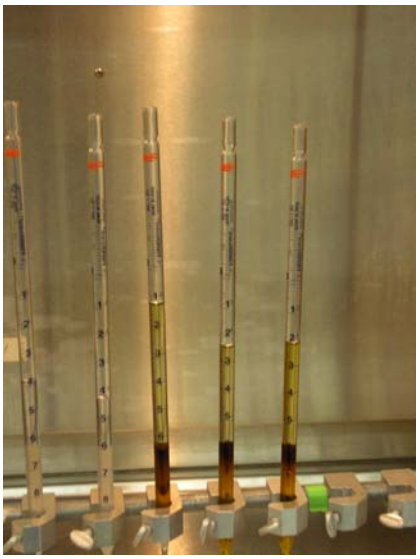


Figure 5. Silica Gel Cleanups

Silica Gel Columns are constructed by plugging a 10 mL serological pipette and then adding 2 g of deactivated silica gel. The silica gel is activated by with 7.5% acetone/hexane and the column is agitated gently to remove any air bubbles. The column is then allowed to drain. An addition 3 mls of 7.5% mix is added. Half the sample extract is added to the column (approximately 2 mLs) and allowed to sit for 5 minutes. Then the remaining two mLs are added. The 300 µL acetone/hexane addition step is then repeated. The sample vials are rinsed 1 x 2.5 mL of the acetone/hexane mix and the rinse is added to the column. Interferences are removed from the column by rinsing 2x with 4 mL of the 7.5% acetone/hexane solution. The non-polar oils and compounds such as bisphenol A, nonylphenol, and bis adipate are removed with this rinse. A sample collection vial is placed under the columns and the sample is eluted with 3 x 5 mL of 35% acetone/hexane. The collected samples are concentrated to drynesss on the turbovap. One mL of 30% methanol/acetone is added to the samples, and they are sonicated for 1 minute.

Finally, a non-retentive alumina cleanup removes an unknown contaminant that interferes with analysis of ethinylestradiol. The alumina columns are constructed similarly to the silica gel columns. A pipette is plugged with glass wool and 0.5 g of EM Science Alumina is added to the pipette. The alumina is activated by rinsing with 3 mL of 30% methanol/acetone. A sample vial is placed underneath the column. The sample is loaded onto the column with 3 x 1 mL vial rinses with the 30% methanol/acetone elution mix. The samples is then eluted with 4 x 3 mL of the elution mix. The samples are dried on the turbovap. 0.5 mL of methylene chloride are added and the samples are sonicated for 2 min. The sample is transferred to a silanized amber vial. Silanized vials prevent adhesion of the estrogens to the glass surface. The sample is then dried on the Nevap by blowdown with a slow stream of nitrogen. The Nevap setup is shown in figure 6. This occurs until it appears that crystals have formed on the bottom of the amber vial.

Figure 6. N-evap

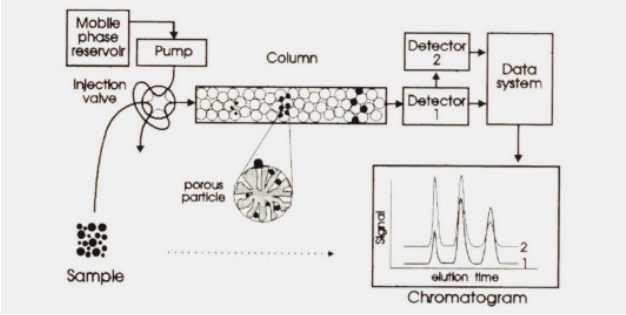
The dried extract is derivatized with 50 µL of derivitization mix. Derivitization is the process of transforming compounds into products of similar structure. To minimize exposure to air, the derivitization mix is added to the vials under a blanket of nitrogen. This occurs by placing a beaker of liquid nitrogen in a Styrofoam cooler with a lid. Then the amber vial is held towards the bottom of the cooler while the mix is added and the vial is capped. Once all the samples are capped they are placed on a heating block at 60 °C for one hour. After the hour is complete, the samples are returned to the Nevap and dried until the excess solvent is removed. Finally 490 µL of methylene chloride and 10 µL of PAH internal standard are added to the sample extracts. The samples are now ready for analysis on the Gas Chromatograph Mass Spectrophotometer (GCMS).

Analysis of the soil and solids samples for estrogens:

Soil is a more complicated matrix and therefore the method detection limit for the estrogens is much higher in the soil than in the reclaimed water. In addition, sample extraction and clean-up is similar but requires more aggressive methods. First approximately 40 g of the samples are freeze dried for 24 hours. Ten grams of dry soil is then extracted using a 10:90 acetone:95%-n-Hexane mix with sonicating the samples for 10 minutes. The solvent is collected by pouring the sample through a filter.

Next the samples go through the first of four separate clean-up steps. The extracts are run through the Gel Permeation Chromatograph (GPC)Model J2 Scientific Accuprep. This instrument functions by separating compounds by size. GPC function is outlined in Figure 7.

Fig.7 Gel Permeation Chromatography



Compounds are eluted through a column of polymer beads with different pore sizes. Larger compounds move more quickly through the column around the beads, while smaller compounds are retained and diffuse slowly through the porous beads. Subsequently, compounds of similar sizes will move through the column at similar rates. The instrument was set to collect all the compounds that eluted between 22 and 47 minutes to exclude some of the faster, larger compounds present in solids such as cholesterols and sulfurs. Collection starts at the collection time for phthalates and methoxychlor and ends at perylene.

The samples are collected and dried on the turbovap. The samples then undergo similar cleanups to the water samples but in different order. First the samples undergo NH_2 cleanup, followed by Silica and then Alumina gel cleanup and are finished up with the C-18 SPE cleanup. The samples are also derivatized and analyzed on the GC/MS.

Extraction and Analysis of TCS and Ibuprofen:

The extraction and analysis methods for ibuprofen and triclosan in water were fairly straightforward and corroborated by methods in the literature. The leachate samples were combined by treatment to achieve an initial volume of 2L. A few drops of sulfuric acid were pipetted into the 2L to reduce sample pH to 2. The samples then sat for 10 minutes to ensure acidification had occurred. Next, the 2L were added to a condenser of the continuous liquid liquid extractor (see figure 8). The water was then spiked with 50 µL of deuterated NP, a standard EDC lab mix, and 100 µL of carbon labeled TCS and native ibuprofen. Methylene chloride was then added to a 500 mL flat bottomed flask oriented on a heating element. The methylene chloride was boiled at 75-85º F where it evaporates up to the condenser. The solvent cools and condenses dropping through the water sample and settling at the bottom of the condenser. The methylene chloride then drops into the flat bottom flask and is cycled through the process again. Organic compounds, such as ibuprofen or TCS, partition from the water into the solvent. The extraction is run for 24 hours. At the end of the extraction all compounds of interest are contained in the methylene chloride solvent. The solvent is collected and run through a sodium sulfate funnel to remove any water that could be present. The samples are concentrated using the Kurderna-Danish over a steam bath. to 0.5 mL. The sample is transferred to an amber vial and then analyzed on the GC/MS.

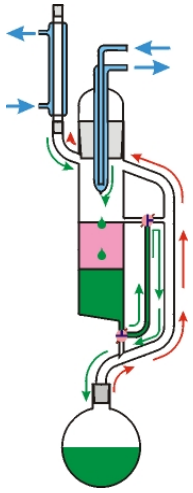


Figure 8. Continuous Liquid Liquid Extractor
(green = methylene chloride, pink = sample)

Analysis of the soil and solids samples for TCS:

In the first step, 40 g of the soil samples were freeze dried. A 10 g aliquot of the dried soil was extracted using the Accelerated Solvent Extractor with 100% methylene chloride solvent at a pressure of 13790 kPA and a temperature of 100°C. Samples were concentrated on turbovap to 1 mL. The samples were then cleaned using a silica/sodium sulfate column. The columns were constructed by plugging a 10 mL serological pipette with glass wool and adding 3 g of deactivated silica gel followed by 4 g of sodium sulfate. The sodium sulfate removes any water present in the sample that could clog the column and the silica gel retains the analytes of interest until the sample is eluted. The columns were activated with 50:50 Acetone: Methylene chloride. They were then rinsed with 5 mL of hexane. The 1 ml samples were loaded onto the column followed by a 1 mL vial rinse with hexane. Interferences were removed while the column was rinsed 3 x 5 mL of hexane. A vial was then placed underneath the column and the extract was eluted using 6 x 5 mL of 50:50 Acetone: Methylene chloride. The samples were re-concentrated to 0.5 mL on the turvbovap and transferred to 1.0 mL amber vials. The samples were analyzed on a GC/MS.

QC /QA:

The quality control and quality assurance or QC/QA methods used in this study were extensive. Four replicates of the each treatment were potted in the greenhouse experiment including the control to account for potential background exposure. The extraction and analytical methods also used extensive QC/QA by analyzing a method blank, spike blank and laboratory duplicate with each sample set. The method blank and spike blank contain a known amount of deuterated E1, E2 and EE2 carbon labeled surrogate, 13C-Triclosan or native ibuprofen that can be used to determine method recoveries. Because the labeled compounds behave similarly to the actual compounds of interest, they can be used to monitor for losses during extraction, clean-up and

analysis. The laboratory duplicate was used to test the homogeneity of the sample and reproducibility of the analysis. Recovery of individual compounds in each matrix and method dectection limits are reported in the results section.

Results and discussion:

The concentration of each compound in the reclaimed water is shown in Table 4. Results were summed to determine an approximate loading rate for each pot during the course of the experiment. Concentrations of the different estrogens in the reclaimed water varied over the course of the study with the standard error of the mean equal to approximately 50% of the mean. The concentration of triclosan in the reclaimed water was much less variable with the mean concentration 2.5 x the standard error. Concentration of ibuprofen in the reclaimed water was at or below the method detection limit for all water samples tested during the course of the study.

Table 4. Approximate addition of compounds with the source water
[Total water added (2.75 L) * (µg / L) avg concentration = approx. addition]

Compound	Concentration	Approximate Addition
	-µg/L -	µg
E1	0.068 +/- 0.049	0.187
E2	0.019 +/- 0.0065	0.052
EE2	0.021 +/- 0.011	0.058
Triclosan	1.23 +/- 0.5	3.38
Ibuprofen	0.398	1.095

The green river water which is currently used to irrigate the Tukwila golf course was also tested for the presence of these compounds. The only compound present in the water was estrone (E1) at a concentration of 0.008 µg/L. This indicates that estrogenic compounds are already being added to this soil system.

Triclosan in the greenhouse study:

Triclosan in Reclaimed Water Treatments:
The average concentration of TCS in the source water and leachate is shown in table 5.

Table 5. Concentration of TCS in source water and leachate for each of the treatments in the reclaimed water portion of the study

Compound	RW source	Control	T2- RW only	T3- 50% Fert	T4 – 100% Fert
Triclosan (µg/L)	**1.23 +/- 0.5**	< MDL	< MDL	< MDL	< MDL
Recovery (%)	119 +/- 4	102	109	120	123

- <MDL = methods detection limit is <0.025 (µg/L) sample concentration or on-column concentrations of 0.25 (µg/mL)

The concentration of triclosan was approximately 1.23 µg/L in the reclaimed water and was below detection in the leachate. This indicates that TCS is not mobile within the soil pore water and will not pass through the soil system limiting the potential for runoff. For all of the treatments that included reclaimed water, the concentration of TCS was similar to the control. It appears that the addition of TCS through irrigation with reclaimed water did not result in any movement of this compound through the soil profile. Recoveries of spikes were consistently high for all treatments across all sampling events. This confirms the accuracy of the results. The concentration of TCS in the soil system can be seen in Table 6.

Table 6. Concentration of TCS in the soil system.

Compound	Control	T2- RW only	T3- 50% Fert	T4 – 100% Fert
Triclosan (µg/L)	< MDL	< MDL	< MDL	< MDL
Recovery (%)	79	91	86	90

- The methods detection limit for the soils is 33 µg/kg

The amount of TCS added the pots by the reclaimed water totaled approximately 1.23 µg/L. The detection limit for soil is 33 µg/L. The soil analysis for this compound was conducted despite the fact that the total loading rate was below the method detection limit. The results confirm that the concentration of TCS added to soil was below detection. As the total rate of addition was so low, it is currently not possible to test for degradation of this compound in the soil matrix. However, in a companion study the concentration of TCS in biosolids amended turf was examined. As TCS partitions more to the solid phase, the concentration of TCS in the biosolids was significantly higher than the concentration in the reclaimed water. Total amount of compound added to the pots was also higher. As a result, the expected concentration of TCS (based on the concentration in the biosolids x the loading rate of the biosolids) in the pots amended with biosolids was above the method detection limit. The behavior of TCS in a soil system can be determined from the biosolids amended turf and is discussed below.

<u>Triclosan in biosolids treatments</u>

Triclosan is a hydrophobic compound and therefore, the majority of the compound that enters the wastewater treatment plant, partitions to the solids. This can be seen by the high concentrations of TCS present in Table 7.

Table 7. TCS in the biosolid samples

Compound	Biosolid 1	Biosolid 2	Biosolid 3	Average +/- st. error
Triclosan(µg/kg)	37200	32920	34800	34973 +/- 1240
Recovery (%)	117	104	111	111 +/- 3.8

The concentration of TCS present in biosolids is approximately 34973 (µg/kg) or 35 mg/kg. From this value we can determine the loading of TCS to each pot with the biosolids.

TCS = (34973 µg/kg) *(1 kg/1000g) * 17 g = 595 µg TCS/per pot.

The pots received a single initial dose of biosolids. One concern with applying biosolids are that trace contaminants such as TCS will be mobile in soil water during precipitation events. Therefore, after the pots were watered the leachate was collected and analyzed for the presence of TCS. These results are presented in Table 8.

Table 8: TCS in the leachate

Treatment	Collection	TCS (µg/kg)	Recovery
Biosolid	1	ND	139
Biosolid	2	ND	123

- ND = methods detection limit is < 0.025 (µg/L)

The leachate for the biosolids treatment replicates was combined to achieve the minimum extraction volume required of 2 L. The leachate was collected and tested on two separate occasions. These analyses reveal that TCS did not leave the system in the leachate. This behavior was suspected due to the high hydrophobic tendencies of TCS. Any removal of TCS can be attributed to degradation in the soil.

Table 9: TCS in the soil samples

Treatment	Rep	TCS (µg/kg)	Recovery
Biosolid	1	38	64
Biosolid	2	ND	81
Biosolid	3	34	56

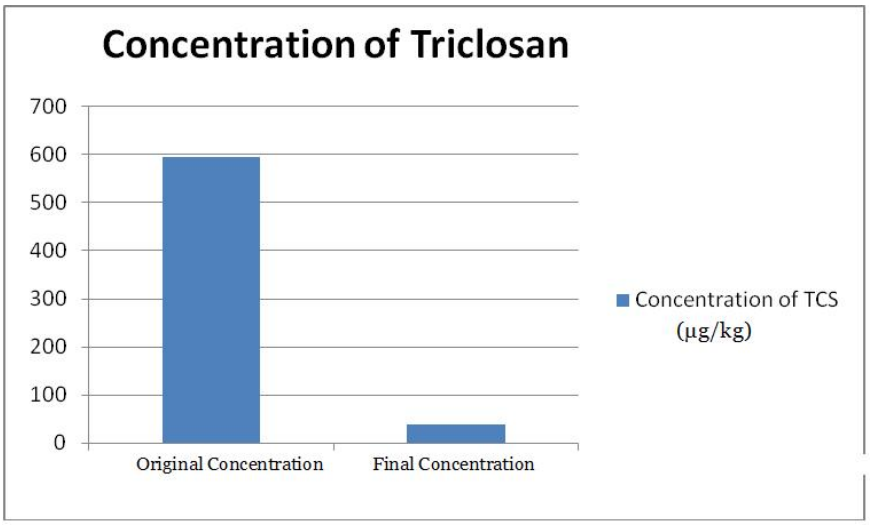
Biosolid	4	49	70
Biosolid	5	35	70
Average		39 +/- 3.44	68 +/- 5

- ND = Non-detect (the detection limit is 33 µg/kg)

No triclosan was detected below the top layer of 0-4 cm indicating that this compound does not migrate within a soil system

The triclosan added to the turf grass systems did not leach out of the soil and exhibited a change in concentration over time. The majority of the TCS added to the soils in the biosolids matrix appears to have decomposed during the course of the study (Figure 9). The biosolids were surface applied to the turf grass pots. The total concentration (595 µg TCS/per pot) apparently remained at the surface of the soils. The final soil analysis showed no movement of TCS to the lower soil horizon. In addition, the final concetration of TCS in the top 4 cm of the soil (39 ±I3 µg TCS) was significantly lower than the initial concentration indicating that the compound was degraded during the course of the study. It is highly likely that the TCS added to the soil in the reclaimed water irrigated treatments had a similar rate of degradation as in the biosolids amended soils.

Figure 9. Initial and final concentration of triclosan in biosolids amended turf grass soils.



Discussion of TCS degradation:

In this study, it appears that TCS does degrade over time and does not accumulate. Very few studies have been published to date concerning the degradation of TCS in aerobic soils. In a laboratory study conducted by Ying et al. (2007), a half-life of 18 days for TCS in aerobic soils was observed. Ying et al. (2007) spiked 1 mg/kg TCS into a vial of soil that was incubated under darkness at a temperature of 22 C. Concentration was tested at 0, 1, 7 days and weekly up to 70 days. Even though the initial TCS concentration in the biosolids treatment was much higher in this study, our results concur with previous results that under aerobic conditions TCS will degrade in a soil system.

Analysis of Ibuprofen:

One reason that ibuprofen was selected for this study was that the compound is a widely used pharmaceutical with a high level of familiarity. Also, while analyzing biosolids samples for 4-nonylphenol in a previous study, ibuprofen was occasionally detected and therefore seemed to be a logical compound to monitor. The total amount of ibuprofen added to each pot through application of reclaimed water was 1.095 µg or 0.398 µg/L. The concentration of ibuprofen fell above the detection limit of 0.125 µg/L for waters but below the practical reporting level of 0.5 µg/L. The method detection limit (MDL) is a theoretical calculation determined by running low concentration samples through the GC/MS. The reported detection level (RDL) is the lowest point based on the calibration curve and not extrapolation. In general, published work is usually based on the reporting level. Therefore, ibuprofen fell below the RDL for the reclaimed water source samples. Soil is a complex matrix that requires extensive extraction and analysis. The MDL for organic compounds in soil is approximately 33 times higher than for organic compounds in water based on lab calculations. Given the fact, that the concentration of ibuprofen was exactly at the MDL for water, the total addition of this compound to the soil was over one order of magnitude lower than the MDL. After soil analysis for TCS and confirmation that the compound could not be detected in soils, it was decided not to run a similar analysis for ibuprofen. Although this compound was generally detected in the reclaimed water samples, it was not detected in any leachate samples (data not shown). The results from this study indicate that ibuprofen in reclaimed water from the sand filter system is only present at concentrations at or below the MDL. Based on the existing scientific literature the majority of ibuprofen is removed rapidly in the environment and during wastewater treatment. Subsequently, detection of the compound after six months in a soil system was highly unlikely. This provides confirmation that ibuprofen, a widely recognized and ubiquitous pharmaceutical is not a concern when irrigating soils with reclaimed water.

Estrogens:

Estrogens in reclaimed water

The concentration of the different estrogen species varied in the reclaimed water with E1 present at the highest concentration of 68 ng/L, followed by EE2 at 21 ng/L and E2 at 19 ng/L. The concentrations are presented in table 10. EE2 response changes over time, with a decrease in sample concentration after a month of storage. Analysis of the method blank revealed twice the amount of the spiked EE2 indicating the presence of an interferent. After allowing the samples to rest for up to 30 days the concentration of EE2 in the method blank was equivalent to the spike concentration indicating that the interferent had decayed out.

Concentrations of each of the estrogens varied over time in the reclaimed water. This was most pronounced for E1 in the first collection where concentration was more than 10 x higher than in subsequent water samples. However, the concentrations of E2 and EE2 in the first collection were comparable to the concentrations in subsequent collections. It is not clear what factors were responsible for this observed high value.

Table 10. Reclaimed Water Source Concentrations:

Collections	E1 (µg/L)	Recovery %	E2 (µg/L)	Recovery %	EE2 (µg/L)	Recovery %
1	0.267	103	0.035	105	0.046	120
2	0.003	75	< MDL	79	0.035	66
3	0.038	70	0.032	72	0.0044	86
4	0.018	82	0.012	91	*	*
5	0.014	95	0.014	98	< MDL	101
Avg +/- Std. error	**0.068 +/- 0.049**		**0.019 +/- 0.0065**		**0.021 +/- 0.011**	

Each of the estrogens were monitored for mobility in soil water by measuring the concentration present in the collected leachate. These results are presented in Tables 11, 12 and 13.

Table 11. E1 in the leachate:

Treatment	E1 (µg/L)	Recovery %
Reclaimed Water-Source	**0.068 +/- 0.049**	85 +/- 6
Control	< MDL	88+/- 15
T2-RW only	.018 +/- .011	96 +/- 15

T3- 50% fert	.006 +/- .003	79+/- 9
T4 – 100 % fert	.007 +/- .002	86 +/- 16

- < MDL is less than the on-column value of 0.3 ng/mL for E1 (or final volume 0.0012 µg/L E1)
-

A small amount of E1 was detected in the leachate. There appeared to be slightly higher concentrations of E1 in the reclaimed water only treatment. The variability in this measure was also higher than for the other treatments that included reclaimed water. This may be related to fertilizer addition. The fertilizer may have resulted in some interference in the analysis or it may have altered the behavior of this compound. However, for all treatments that included reclaimed water, the concentration of E1 in the leachate was significantly reduced over the concentration in the water. This indicates that the majority of the added E1 was not mobile in the soil system.

Table 12. E2 in the leachate

Treatment	E2 (µg/L)	Recovery %
Reclaimed Water-Source	**0.019 +/- 0.0065**	89 +/- 6
Control	< MDL	98 +/- 8
T2-RW only	.004 +/- .002	105 +/- 7.5
T3- 50% fert	<MDL	94+/- 5
T4 – 100 % fert	.004 +/- .001	102 +/- 7

< MDL is less than the on-column concentration 0.5 ng/mL for E2 and EE2 (or final volume 0.002 µg/L for E2)

Similarly to E1, E2 is also present at a very low concentration in the leachate with the majority of the compounds remaining in the soil pots. For E2 there was no indication that fertilizer addition altered behavior or mobility of the compound in the pots. It should be noted that total concentration of E2 in the reclaimed water was lower than E1. As with E1, the majority of E2 added to the soils was not mobile in the soil water and partitioned to the soil fraction.

Table 13. EE2 in the leachate:

Treatment	EE2 (µg/L)	Recovery %
Reclaimed Water-Source	**0.021 +/- 0.011**	93 +/- 12

Control	<MDL	109 +/- 8
T2-RW only	< MDL	109 +/- 10
T3- 50% fert	<MDL	99 +/- 9
T4 – 100 % fert	<MDL	114 +/- 12

< MDL is less than the on-column value of 0.5 ng/mL for EE2 (or final volume 0.002 µg/L for EE2)

None of the EE2 was detected in the leachate indicating that the compound remained entirely in the soil systems. There was no indication of any interaction between fertilizer addition and movement of EE2 through the soil. For all treatments that included reclaimed water, EE2 added in the water remained in the soil and was not mobile in the soil water.

The concentration of the estrogens in the soil system showed much greater levels of removal. The first set of data has been analyzed and the final set is currently in the process of extraction. While the second set of data is required to confirm the initial results it appears that the concentration of estrogens in the soil are below detection limits in all treatments. The detection limits in the soil system for E1 was 0.03µg/kg and 0.05 µg/kg for E2 and EE2. This would indicate that the estrogens added to a soil system through the use of reclaimed water are neither mobile nor accumulating in the soil.

The amount of E1, E2 and EE2 added the pots by the reclaimed water totaled approximately 0.187, 0.052, 0.058 µg respectively. The detection limit for soil is 0.03µg/kg for E1 and 0.05 µg/kg for E2 and EE2. The total loading rate was below the method detection limit. The results confirm that the concentration of the estrogens added to soil was below detection. As the total rate of addition was so low, it is currently not possible to test for degradation of this compound in the soil matrix. However, in a companion study the concentration of the three estrogens in biosolids amended turf was examined. The concentration of the estrogens in the biosolids were significantly higher than the concentration in the reclaimed water. Total amount of compound added to the pots was also higher. As a result, the expected concentration of the estrogens (based on the concentration in the biosolids x the loading rate of the biosolids) in the pots amended with biosolids was above the method detection limit. The behavior of the three estrogens in a soil system can be ascertained from the biosolids amended turf and is discussed below.

Estrogens in biosolids amended turf

The concentration of the estrogens in the solids was much higher than the concentration in the reclaimed water. The concentrations are presented in table 14.

Table 14. Concentration of Estrogens in Biosolids

Compound	Biosolid 1 (µg/L)	Biosolid 2 (µg/L)	Average +/- st. error
E1	153.2	150.4	151.8
E2	10.8	10.4	10.6
EE2	4.6	5	4.8

From these initial concentrations the loading values of the estrogens added with the biosolids can be calculated. The initial concentration of estrone (E1) added was 2581 , estradiol (E2) was 180 , and ethinylestradiol was 82 . The final concentration of each of these compounds at the end of the study was below the detection limit.

The concentration of estrogens added to the turf system with the biosolids was much higher than that that added in the reclaimed water. The leachate from the biosolids treatment was still below the MDL as can be seen in Table 15. This indicates that once added to the system regardless of high concentration the estrogen compounds are not prone to movement out of the system during precipitation events.

Table 15. Concentration of Estrogens in the Biosolids leachate (

Compounds	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
E1	<MDL	<MDL	<MDL	<MDL	0.001364
E2	<MDL	<MDL	<MDL	<MDL	<MDL
E3	<MDL	<MDL	<MDL	*	<MDL
E1 D4 recovery	124	75	76	49	111
E2 D4 recovery	117	79	93	99	118
E3 D4 recovery	145	66	99	*	130

The estrogens did not move from the system with the leachate. The possibility of uptake by plants was also taken into consideration. The results are presented in table 16.

Table 16. Concentration of Estrogens in the Plant Samples

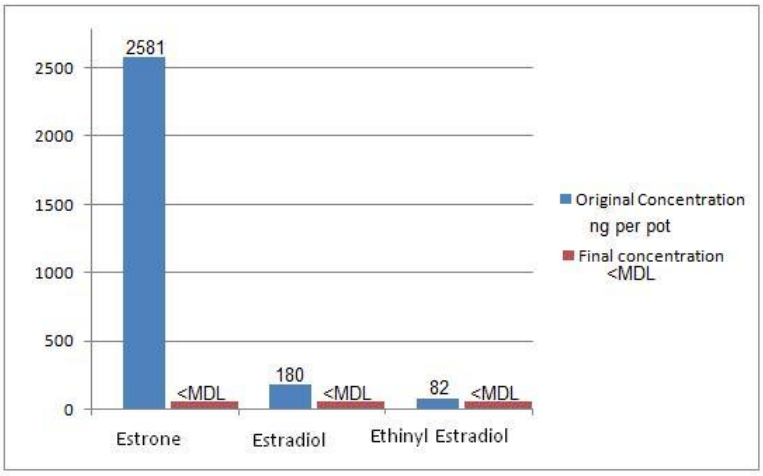
Treatment	E1 (µg/kg)	Avg. Recovery +/- st. error	E2 (µg/kg)	Avg. Recovery +/- st. error	EE2 (µg/kg)	Avg. Recovery +/- st. error
T1	< MDL	102+/-0.4	< MDL	111+/-1.5	< MDL	101+/-1.0
T2	< MDL	106+/-0.6	< MDL	151+/-4	< MDL	100+/-1.0
T3	< MDL	105+/-0.6	< MDL	107+/-0	< MDL	143+/-4
T4	< MDL	103 +/-0.5	< MDL	105+/-0.3	< MDL	94+/-0.9
Biosolids	< MDL	101 +/- 0.5	< MDL	102 +/- 0.6	< MDL	95+/-0.6

No estrogens were detected in the plant samples, indicating that the compounds did not adhere to the plant surface or were not absorbed into the plant material.

All three estrogens added to the soils in the biosolids matrix decomposed during the course of the study (Figure 10). It is highly likely that the estrogens added to the soil

in the reclaimed water irrigated treatments had a similar rate of degradation as in the biosolids amended soils.

Figure 10. Initial and final concentration of E1, E2, and EE2 in biosolids amended turf grass soils.



Note that the concentration of the estrogens in the soil at the end of the experiment all fall below the method detection limit. Therefore, no bars are present on the graph. To reiterate, estrone (E1) was applied at 2.581 µg and degraded to below 0.0003 µg/10 g extraction. Estradiol (E2) was applied at 180 ng and ethinylestradiol (EE2) initially appeared at 82 ng. Both these compounds degraded below 0.0005 µg/10 g extraction.

Discussion of Estrogen degradation:

The degradation of the three estrogens can be seen by the significant change in concentration over time in the biosolids treatments. Other studies concur with these results. Ying and Kookana (2005) saw complete degradation of all three estrogens in a lab study within 7 days. Concentration of 1 mg/kg of each compound were added to soil and incubated at 20 C. The concentration was tested art 0, 1, 3, 7 and then weekly until 70 days. The detection limit for each compound was 5 µg/L. Colucci and Topp (2001) monitored the degradation of estradiol, estrone and ethinylestradiol under varying moisture and soil temperature conditions in laboratory microcosms. An initial concentration range of 0.1 to 10 mg/kg was used for all three compounds. The authors detected degradation of ethinylestradiol (EE2) to below the detection limit within 44 days of application. Removal of ethinylestradiol occurred within 22 days in soils with sufficient moisture levels. E1 and E2 showed a much faster rate of removal with almost complete removal to an unextractable form within 3 days after application. Stumpe and Marschner found similar results to Colucci and Topp. While the rates of degradation vary the ultimate message is that the estrogens degrade or are unavailabe within aerobic soil systems.

Plant Yield:

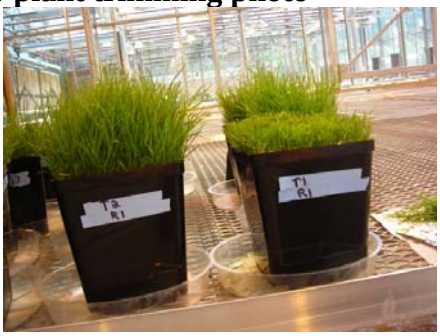
Reclaimed water contains nitrogen and phosphorous which are nutrients essential for plant growth. These nutrients are routinely added to golf courses by applying chemical fertilizers. Plants were collected and weighed periodically throughout the study to

determine the potential for reclaimed to supplement or replace fertilizer amendments. Plant yield is presented in Table 17.

Table 17. Plant yield (in grams +/- standard error)

Treatment	Harvest 1	Harvest 2	Harvest 3
Control	7.07 +/- 1.46	9.89 +/- 1.93	7.34 +/- 1.91
Reclaimed Water Only	7.25 +/- 1.48	7.5 +/- 1.9	8.4 +/- 2.6
RW + 50% fert	10.10 +/- 2.55	9.87 +/- 1.79	11.45 +/- 2.18
RW + 100% fert	14.55 +/-1.04	12.67 +/- 3.5	11.39 +/- 2.42

Figure 3. Before and after plant trimming photo



Yield for the reclaimed water only treatment were statistically similar to the control for each harvest. Yield in the two reclaimed water treatments with fertilizer addition were higher than either the control or the reclaimed water only treatments. This suggests that the reclaimed water contains sufficient N, P, and K to provide the required fertility for the turf grass. Additional fertilizer applications may not be required if the reclaimed water is used as the sole source of irrigation water.

Currently the plants are also being analyzed for the presence of the estrogens or TCS. Detection of these compounds would indicate that either plant uptake is occurring or the compounds of interest are adhering to the foliage when being added to the system through watering. Either outcome would create a means of exposure for wildlife through ingestion of grass leaves. The results from this analysis will be available by the end of May at the latest.

Nutrient Properties:

Additional analysis was run on the soil samples to measure soil properties including: pH, EC, and total carbon and nitrogen ratio. These properties are used to determine soil quality for supporting plant life. A concern exists that adding reclaimed water could alter the pH or increase the salt concentration of the soil. Another concern is the mobility of the added nitrogen. The soil property results are included in table 18.

Table 18. Soil properties for the surface (a) and underlying sand layer (b)

a. Treatment	Turf	Carbon	Nitrogen	pH	EC
Surface	0-5 cm	%	%		dS M-1
Control	Tee	1.1	0.32	4.85	0.2
Reclaimed		2.74	0.37	5.49	0.3
Reclaimed +.5 Fert		1.54	0.38	5.29	0.2
Reclaimed +Fert		1.69	0.31	5.31	0.4
Control	Fairway	1.84	0.22	5.16	0.1
Reclaimed		3.25	0.45	5.43	0.3
Reclaimed +.5 Fert		3.22	0.43	5.5	0.15
Reclaimed +Fert		4.14	0.54	5.23	0.5

b. Treatment	Turf	Carbon	Nitrogen	pH	EC
Sand	>5 cm	%	%		dS M-1
Control	Tee	> MDL	0.29	6.1	0.02
Reclaimed		0.19	0.25	6.56	0.04
Reclaimed +.5 Fert		> MDL	0.22	7.06	0.02
Reclaimed +Fert		0.19	0.22	6.14	0.03
Control	Fairway	2.73	0.43	6.03	0.03
Reclaimed		> MDL	0.3	7.03	0.03
Reclaimed +.5 Fert		> MDL	0.23	7.05	0.03
Reclaimed +Fert		0.03	0.21	6.69	0.1

Soil pH was increased in all treatments that included reclaimed water. This increased was most pronounced in the undying sand layer. This is to be expected as the buffering capacity of the sand is much lower than the soil. Total carbon also increased in the surface soil in all of the reclaimed water treatments in comparison to the control. This may be the result of dissolved carbon in the reclaimed water. Nitrogen concentrations in the reclaimed water treatments in the Fairway samples were elevated over the control. However, total N in the sand below the soil shows no evidence of excess N being added to the system. In the Tee samples, total N was similar in the soil and sand horizons for all treatments. In the surface soil of the Tee samples, soil electrical conductivity was similar in the control and reclaimed water + 50% fertilizer treatments and was increased in the other treatments. For the Fairway soils, all soils that were watered with the reclaimed water had elevated EC in comparison to the control. For all treatments, EC in the sand was very low. In addition, although elevated, EC were well below levels that would result in stress to the turf grass.

The leachate water was collected and analyzed for nitrogen and phosphorous. The data is presented in table 19.

Table 19. Concentration of Nitrogen and Phosphorous in the Leachate

Treatment	Nitrogen	Phosphorous
Source	*- mg/L -*	*- mg/L -*
Reclaimed Water	28.3	2.28
Leachate		
Control	3.47 +/-0.63	0.26 +/-0.05
Reclaimed Water	4.98+/-0.64	0.92+/-0.25
Reclaimed +.5 Fert	5.55+/-0.61	0.75+/-0.11
Reclaimed +Fert	6.72+/-1.1	0.94+/-0.22

The concentration of N and P decreased significantly through the soil profile indicating the nutrients are remaining in the soil. These nutrients are then available for plant uptake. The control exhibited the lowest concentration of nutrients in the leachate with the reclaimed water and fertilizer combination treatments having the highest concentrations. Even the highest concentrations are significantly low and runoff is not a major concern.

Data on the nutrient content of the reclaimed water was provided by the staff at the Renton Wastewater Treatment Plant. Nitrogen data consisted of organic and inorganic forms of N measured at approximately weekly intervals over a 7 month period. Data for P and K were from three samples collected over a similar period.

The concentration of the nutrients in the water was converted to provide quantities of each nutrient in pounds per acre inch as well as pounds per 1000 sq ft. Means as well as standard deviations for each of the nutrients were calculated. This information can be used to quantify the amount of fertilizer the turf is receiving based on the total water use. With this information, applications of synthetic fertilizer can be adjusted to take into account the fertilizer applied with the irrigation water. Nitrogen data is provided both as total and organic N.

Table 20. Nutrient concentrations in sand filter water from the Renton WWTP

Nutrient	Pounds per inch per acre	Pounds per inch per 1000 sq ft
Total N	15.8 ± 2.3	0.36
Inorganic N	7.5 ± 2.5	0.17
Total P	0.5 ± 0.1	0.01
Total K	3.6 ± 0.2	0.08

Conclusions

The results of this study indicate that the three chemicals of concern tested in this study, estrogens, triclosan and ibuprofen, did not persist in soils irrigated with reclaimed water or fertilized with biosolids. Minimal to no movement of any compound was detected from water collected at the bottom of the pots. For the reclaimed water irrigated pots, predicted soil concentrations were well below detection limits. For the biosolids amended soils, although initial concentrations were above detection limits, for all compounds studied, a highly significant decrease in concentration was observed at the end of the study.

Response of the turfgrass to reclaimed water irrigation suggests that the water can provide sufficient fertilization for plant growth and that no additional fertilization will be required. This would also reduce the potential for nitrogen leaching in reclaimed water irrigated turf. In order to more effectively document the value of this water as a fertilizer, it is recommended that King County routinely test the water for total P content.

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