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Fate of steroid estrogens in Australian inland and coastal wastewater treatment plants

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Abstract

A comparison of estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) removal at a coastal enhanced primary and inland advanced sewage treatment plant (STP) is reported. The average concentration of estrogens in the raw sewage is similar to reports in other studies. The sequential batch reactor at the advanced STP removed on average 85% of the incoming E1 and 96% of the E2. Further removal was observed during later microfiltration with the estrogen concentration below detection (<0.1 $ng.L^{-1}$) after reverse osmosis. Some 6% of the influent E1+E2 was removed in the waste activated sludge. The detection of EE2 in the waste activated sludge (0.42 $ng.g^{-1}$ solids dry weight), undetectable in the raw sewage, suggests that EE2 is resistant to biological treatment in the sequential batch reactor and is primarily removed due to sorption. Little estrogen removal was observed at the enhanced primary with only 7% of E1 and 0% of E2 removed. Low removal is expected based on the degree of estrogens partitioning in the organic fraction given the relatively low solids concentration, but surprisingly, some 43% of E2, 24% of E1 and 100% of EE2 remains associated with the solids fraction in the treated effluent. Further research is necessary to determine whether the low level of estrogen removal for the coastal treatment plant will adversely affect the receiving marine environment.

Introduction

There are increasing concerns that the release of steroid estrogens post wastewater treatment is leading to abnormal reproductive systems in freshwater and marine dwelling animals (*1-3*). Human excretion is considered to be the primary source of steroid estrogens from the urban environment and are is released by individuals into sewerage system in both conjugated and unconjugated forms at µg levels per day (*4-6*). Johnson and Williams (*6*) recently developed a model to estimate the quantities of estrogens by the human population, taking in consideration conjugation and metabolism of natural and synthetic hormones in the body and the different quantities released by different population groups. The average human excretion of estrogens per head is reported to be 10.5 $µg.d^{-1}$ for estrone (E1), 6.6 $µg.d^{-1}$ for 17β-estradiol (E2), with an additional 3.3 $µg.d^{-1}$ transformation of the E1 to E2 in the sewer (*6*). The population normalized concentration of the synthetic steroid 17α-ethinylestradiol, an active agent in the contraceptive pill, is 1 $µg.d^{-1}$ per head (*6*).

While there are increasing data on the presence of estrogens in rivers (*7-9*), coastal samples (*10,11*) and in the sediments of river beds (*12-15*), lakes (*16*) and estuaries (*17,18*), relatively little is known about the movement or degradation characteristics of individual human estrogens within wastewater treatment systems. Sorption to sludge is likely to play an important role in the initial removal of these compounds during wastewater treatment (*4*) as the octanol-water partition coefficients (K_{ow}) for E2 ($logK_{ow}$ = 3.9), E1 ($logK_{ow}$ = 3.4) and EE2 ($logK_{ow}$ = 4.1) (*5*) are moderately high, although lower K_{ow}s have been obtained by Holthaus et al (*19*) for E2 and E1. The concentration of estrogens in the raw wastewater (*20-22*) and in treated effluent (*23-26*) is well characterized; however, few studies have monitored the fate of individual estrogens through the solids cycle during wastewater treatment (*27,28*). The recent study by Holbrook et al. (*27*), using an estradiol-equivalent screening assay, suggests that the treated effluent contains the greatest estrogenic activity with the sludge only accounting for approximately 10% of the influent estrogenic activity. This paper attempts to provide greater detail on the movement of E1, E2 and EE2 through two different Australian sewage treatment plants (STP) and focuses on the fate of these compounds in the solids cycle.

Materials and Methods

Sampling Sites

A small inland advanced sewage treatment plant (STP) and large coastal enhanced primary STP were selected for the study in order to assess steroid estrogen removal rates for different unit operations. The advanced STP is located in western Sydney and services only domestic sewage from a nearby suburb (population approximately 3000) and major sporting venues. The plant consists of activated sludge treatment (2 sequential batch reactors (SBRs)) with an average flow of 1.9 $ML.d^{-1}$. The secondary effluent is taken to the tertiary treatment unit that consists of continuous microfiltration (CMF) (exclusion size 0.2µm), reverse osmosis (RO) and chlorination/de-chlorination. It provides 852 ML of recycled water each year from sewage and stormwater. The activated sludge treatment consists of two basins, each 987 kL in volume, with anoxic and aerobic zones. The volume of the aerobic zone, where sludge is monitored and wasted from, is 775 kL and the sludge in the basin is kept at 30% of the total volume. Approximately 37 kL of the activated sludge is wasted each day and sent for dewatering. The solids retention time (SRT) is approximately 16 days and the hydraulic retention time in the SBR is 4 hours (2 hours each in the anoxic zone and aerobic zones). The coastal enhanced primary STP is located in eastern Sydney and services domestic sewage (75%) and industrial wastewater (25%) with an equivalent population of 1,700,000. It provides enhanced primary treatment (i.e. with $FeCl_3$ addition) for an average flow of 480 $ML.d^{-1}$ and hydraulic retention time of 45min. Ultimate disposal is by deep ocean discharge 3.6 km offshore at an average of 80 m depth.

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Standard and SPE Preparation

Estrone (E1), estradiol (E2), ethinylestradiol (EE2), and deuterated E1, 4,16,16-*d4* (d4- E1) were obtained from Sigma Aldrich (Sydney, Australia). The d4- E1 was used as the internal standard. Stock solutions of individual non-deuterated standards and deuterated internal standard were prepared by dissolving known amounts of in methanol to obtain a concentration of 0.10 mg.mL^{-1}. Working standard solutions were obtained by further diluting stock solutions with water to obtain final concentrations of 0.5 pg.µL^{-1} to 500 pg.µL^{-1}. The stock solution of internal standard was further diluted with water to obtain a final concentration of 100 pg.µL^{-1}. HPLC grade methanol and acetonitrile were obtained from Ajax Finechem (Sydney, Australia). Other solvents were of analytical grade and they were used as supplied. Milli-Q water was used for all experimental procedures. Analytes were extracted from aqueous samples by solid phase extraction (SPE) using the LC-18 SPE cartridges filled with 1.0 g of C_{18} (Supelco, Sydney, Australia). After fitting the SPE cartridge into a 12-Port Visiprep DL Vacuum Manifold (Supelco, Sydney, Australia), the SPE was sequentially conditioned with 2x10 mL methanol, 1x10 mL Milli-Q water.

Sample Collection

Duplicate grab samples were collected in 1L Pyrex glass bottles from each sampling point within the two STPs. In the advanced STP, samples were collected from the raw sewage, outlet from sequential batch reactor (SBR), inlet/outlet from cross flow microfiltration (CMF), outlet from reverse osmosis (RO), after chlorination, from the wasted mixed liquor suspended solids (MLSS) and from the dewatered sludge. For the enhanced primary STP, samples were collected from the raw sewage and treated effluent. All samples were passed though SPE, dried and stored in a 10-mL tube on the collection day. The stored samples were analysed together once sampling was finished (normally a week after collection). All samples at each of the plants were taken at the same time of day, 8 - 8.30 am, during weekdays, periodically over a period of 4 months for the advanced STP and between 10 – 10.30 am, during weekdays, periodically over a period of 1 year for the enhanced primary STP.

Sample Preparation and Solid Phase Extraction

Water: Analytes were extracted from 0.5 L (raw sewage) to 1 L for all other samples. Before samples were processed, internal standard (see above) was added to each sample, followed by the removal of suspended particle by a prefiltration step with an AP-15 filter (Millipore, Sydney, Australia). This step was performed to avoid SPE cartridge plugging. Sample loading was achieved by passing standards and environmental water samples through the LC-18 SPE cartridge. After sample loading, cartridges were dried in a vacuum desiccator for 30 to 40 min. Elution of the analytes was achieved by passing 2x5 mL methanol that was collected in a 10 mL culture tube with screw cap. The collected solution was dried down under vacuum and reconstituted to 1 mL with acetone before derivatisation and analysis.

Sludge: Before samples were processed, internal standard (see above) was added to a 50 mL sludge sample, followed by autoclaving and freeze-drying. The dried pellets were weighed and then dissolved in a mixture of 100 mL acetone/hexane (50:50). The solvent-sample slurry was then sonicated for 30 min followed by heating at 80^{0}C for an hour in a water bath. The solvent-sample mixture was then filtered through Whatman No. 1 glass fibre filter paper (Whatman, Sydney, Australia), the pellet rinsed with a mixture of acetone/hexane (50:50), followed by solvent evaporation in a rotatory evaporator. The residues were dissolved in water and an extraction was carried out with LC-18 SPE (see above) before sample derivatization and analysis.

Sample Derivatization for GC-MS Analysis

The derivatization was carried out using a modified version of the method used by Nakamura et al. (*30*) for the pentafluorobenzyl-trimethylsilyl derivative. To the acetone extract, 100 µL of 10% aqueous potassium carbonate and 10 µL of pentafluorobenzylbromide reagent were added, and were kept at 70^{0}C for 1 hour. After cooling, the solvent was reduced to 100 uL under vacuum. 1 mL of toluene was added, and the organic phase was washed with 0.5 mL of Milli-Q water. The water layer was discarded and the toluene layer completely removed under vacuum. 100 µL of trimethylsililacetamide was then added to the vial and kept at room temperature for 30 min. Toluene was added to 1 mL before analyses.

Gas Chromatography Mass Spectrometry Conditions

All GC-MS analyses were carried out using an Agilent 5890 gas chromatograph interfaced to an Agilent 5989B MS Engine (Agilent Technologies, Ryde, Australia). Chromatographic separations were performed with an HP-5MS capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The GC oven temperature was programmed at 150^{0}C for 1.5 min and then 36^{0}C per minute to 310^{0}C, final hold 7.0 min. The GC-MS interface heater, the ion source, quadrupole, and injection port temperatures were maintained at 260, 240, 100 and 260^{0}C, respectively. Pulse splitless injection was used with a pulse

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pressure of 241 kPa (1.1 min) and purge time delay of 8 min. The MS analyses were performed with an electron-capture negative-ion (ECNI) source, using methane as reagent gas (Ultrapure grade, Matheson Gas Products Inc.) and selected ion monitoring mode. The [M^-] ion and [M-TMS^-] ions were monitored for all compounds with a dwell time of 100 ms per single ion. The injection volume was 1.0 µL

Recoveries and blanks

For the determination of recoveries, the raw sewage and secondary and tertiary effluents were spiked with stock solution containing the individual non-deuterated and deuterated steroid estrogens. The resulting concentration were varied and ranged between 1ng.L^{-1} to 100 ng.L^{-1}. The blank samples of each matrix were only spiked with the internal standard.

Calibration and Quantification

The working solutions containing all the estrogens (non-deuterated and deuterated) at accurate defined concentrations were derivatized as described above. Quantification was carried out by calculation of the response factors (RF) based on the area of the non-deuterated and deuterated estrogens standard. These ratios were converted to concentrations using a linear regression equation, which was used to assign the unknown concentrations. Signals for method limit of detection (LOD) and limit of quantification (LOQ) was set at 3- and 6-fold height of noise, respectively

Results and Discussion

Detection limits and recoveries of the SPE-GC-MS method

The instrumental limit of detection (LOD) for E2 and EE2 was 0.1pg.µL^{-1} of injection and for E1 was 0.5 pg.µL^{-1} injection, which was estimated at a signal-to-noise ratio of 3. The method limit of quantification (LOQ) was determined to be 1 ng.L^{-1} for raw sewage (5 ng.L^{-1} for EE2 in raw sewage) and 0.1 ng.L^{-1} for secondary and tertiary effluents. Mean accuracies of all the analytes generally range from 90% to 103 % in tertiary effluent, 80% to 123 % in secondary effluent, and 91% to 113% in raw sewage, with the exception of ethinylestradiol (between 40% and 55%) (Table1). A sample chromatograph is shown in Figure 1. Note that accuracies are based on the raw sample before any sample preparation or filtration. The relative standard deviation (RSD) varied from 1% to 13% for all matrices studied, with the exception of ethinylestradiol in raw sewage (RSD between 14% and 48%). These values indicate a satisfactory reproducibility and precision of the whole analytical procedure for E1 and E2 for each studied matrix but lower confidence for EE2 in the raw sewage. The poorer analytical sensitivity of EE2 in the raw sewage is believed to be due to the higher solids content in the raw sewage and greater adsorption of EE2 compared to E1 and E2 on the sludge particles, which are removed during filtration prior to SPE. In contrast, high accuracies of EE2 are obtained from secondary effluent, tertiary effluent and the sludge validation results, which also pass through the SPE clean-up procedure. Mean accuracies of each analyte from dewatered waste activated sludge were 75% for E1, 87% for E2, and 95% for EE2. The RSD varies from 5% for E1, 19% for E2, and 8% for EE2 (Table 1). These values indicate a satisfactory reproducibility and precision of the whole analytical procedure for the sludge matrix.

Steroid estrogen concentration and behaviour within the Advanced STP

The average concentrations of E1, E2 and EE2 in the raw sewage were 55, 22 and <5.0 ng.L^{-1} respectively. The E1 and E2 figures are in good agreement with reported raw sewage concentrations in other studies (*20-22,24,27*) and the extent of estrogen removal for each unit process in the advanced sewage treatment plant is summarised in Table 2. The sequential batch reactor (SBR) removed on average 85% of the incoming E1 and 96% of the E2, similar to reported percentages removed by activated sludge in other studies (*20-22,27,31*). Studies indicate that E1 could be formed by the

oxidation of E2 under aerobic conditions (*31-33*). In this work, there is no clear evidence of this reaction in the water samples analysed; however, when non-autoclaved dewatered waste activated sludge (WAS) from this plant is spiked with a mixture containing the same concentration of E1, E2, and EE2, analysis 40 min later gives almost double the amount of E1, similar to the spiked concentration for EE2, and very low concentrations of E2 (data not shown) indicating almost 100% conversion of E2 to E1. The significantly lower proportion of E2 found in the mixed liquor suspended solids (MLSS; wastewater and activated sludge) (2% of influent) compared to E1 (34% of influent) (see mass balance in Figure 2) also suggests that E2 is being oxidized to E1 during the biological oxidation process. The mass balance around the SBR indicates that 25% of the E1+E2 total mass load accumulates in MLSS, whereas 9% of E1, 1% of the E2 and 6% of the E1+E2 total mass load is removed from the activated sludge process in the form of WAS. This is similar to the 10% of the influent estrogenic activity found by Holbrook et al. (*27*) using an estradiol-equivalent screening assay but much lower than Takigami et al. (*34*) who found extensive accumulation in activated sludge with some 30% of E2 being removed in the waste sludge. The dewatered sludge contains similar although slightly lower estrogen levels, possibly due to estrogen losses in the filtrate. The extensive concentration factor of solids in the sludge enables the detection of EE2 (0.42 ng.g^{-1} of solids), previously undetectable in the raw sewage. EE2 persistence has been documented by Vader et al (*35*) and Layton et al. (*36*) who found only 40% mineralization of ^{14}C-EE2 to $^{14}CO_2$ over a 24 h period.

The biologically treated effluent is then stored in a holding tank before passing through the microfiltration plant. During microfiltration the concentrations of E1 and E2 were further reduced from 4.1 and 0.75 ng.L^{-1} to 1.2 and 0.1 ng.L^{-1}, respectively. No E1 or E2 was detected after reverse osmosis and later chlorination. The high level of estrogen removal during microfiltration is most likely due to a combination of adsorption onto the hollow-fiber membranes (*37*) and onto the dynamic membrane filter (*38*). Chang et al. (*37*) recently demonstrated almost 100% removal of radiolabelled E1 by adsorption on 0.2 µm hydrophobic polypropylene hollow fibre membranes (the same as microfiltration membranes used at the advanced STP). Huang and Sedlak (*12*) also detected the presence of E2 (1.36 ng.L^{-1}) and EE2 (0.14 ng.L^{-1}) after microfiltration and even trace levels of E2 (0.24 ng.L^{-1}) after reverse osmosis. The transport mechanisms underpinning estrogen removal during nanofiltration and reverse osmosis membranes are discussed further in Khan et al (*39*) and Nghiem and Schäfer (*40*).

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Estrogens in the enhanced primary STP

The concentration of estrogens in the raw sewage of the enhanced primary STP is similar those found in the advanced STP, i.e. the average concentration are 58 ng.L^{-1} for E1, 14 ng.L^{-1} for E2 and <5.0 ng.L^{-1} for EE2 (Table 3). EE2 concentration for both plants is comparatively low, however, below the 8.8 ng.L^{-1} identified in Johnson et al. (*41*), 8.2 ng.L^{-1} in Andersen et al. (*28*) and 6 ng.L^{-1} in Ternes et al. (20). The results are consistent with the lower levels reported by Baronti et al (21). The lower concentration of EE2 is primarily due to the low abundance expected in sewage. Prescriptions records in Australia (*42*) indicate that the total amount of EE2 dispensed in its various forms is approximately 9.77 kg.yr^{-1} during 2000 (Table 4). The total proportion of women aged between 18-50 during 2000 was 46.9% (*43*) and of this approximately 26.7% used oral contraceptives (*44*). The equivalent annualized daily EE2 dose for women using oral contraceptives with EE2 is therefore 22.2 µg.p^{-1}.d^{-1}. The majority of Australian women that use oral contraceptives use the low EE2 dose 30 µg - levonorgestrel formulations (*42*,*45*). When taking into account 21/28 effective days, this equates to an expected annualized daily dose of 22.5 µg.p^{-1}.d^{-1}. The coastal enhanced primary sewage treatment plant treats wastewater for some 1.7 million people, of which approximately 100,000 people would be women taking oral contraceptives based on the above statistics. Assuming that all EE2 is excreted and detectable in the raw sewage, and neglecting possible losses and non-active forms, the maximum concentration of EE2 expected would be 4.9 ng.L^{-1}. Johnson and Williams (6) estimate that 40% of EE2 ingested, following deconjugation, will be available as free EE2 in the sewer which would lower the maximum (without considering losses in the system) to 2.0 ng.L^{-1}. This is considerably less than detected in other studies and is a reflection of the proportion of women aged between 18-50 of the total population and the degree that oral contraceptives are used.

Unlike the advanced STP where some 85-96% of estrogens are removed in the initial biological treatment stage, there is essentially no removal of the estrogens during treatment at the enhanced primary STP. The ocean discharge concentration for E1 is 54 ng.L^{-1} and 14 ng.L^{-1} for E2, equating to only 7% removal for E1 and 0% for E2 (Table 3). Similar poor estrogen removal rates during primary treatment has been observed by Andersen et al. (*28*), Ternes et al. (*20*), Holbrook et al. (*27*), Svenson et al. (*24*) and Desbrow et al. (*2*). Despite a 50% reduction in influent non-filterable residue at the enhanced primary STP, from an average of 258 to 130 mg.L^{-1}, and 60% reduction in oil and grease concentration, there is no corresponding drop in E1 or E2 concentration. The percentage removal results suggest that the compounds are not partitioning onto the nonpolar fat and lipid material in the raw sewage. This low removal is not surprising given the relatively low solids concentration in the raw wastewater (i.e. TSS = 258 mg.L^{-1}). For example, if considering estrone, the generalized expression for relating K_{oc} and K_{ow} values for nonpolar compounds (*46*) can be used to estimate the logK_{oc} (logK_{ow} = 3.4):

$$\log K_{oc} = \log K_{ow} - 0.317$$
$$\log K_{oc} = 3.4 - 0.317 = 3.1$$

The logK_{oc} value is similar to that determined by Lee et al. (47) for estrone partitioning onto different soils (i.e. logK_{oc} = 3.1 cf. 3.2). It is then possible to estimate the weight fraction of reduced organic carbon (f_{oc}) given that the volatile suspended solids of the raw wastewater is 85% and using the convention that organic carbon is approximately 50% of the natural organic matter (*48*), i.e.

$$f_{oc} \sim 0.85 \times 0.5 \sim 0.43$$

By only considering sorption due to partitioning onto the organic matter and neglecting any possible electrostatic effects or complexation adsorption reactions, the sorption constant, K_d, can be estimated by (*48):*

$$K_d = K_{oc} \times f_{oc} = 1258 \times 0.43 = 540$$

The fraction of estrone in the water, f_w, can then be related to K_d and the solids concentration in the wastewater, r_{sw}, using the following expression (*48*):

$$f_w = \frac{1}{1 + r_{sw} \cdot K_d}$$

$$f_w = \frac{1}{1 + 0.000258 \cdot 540} = \frac{1}{1 + 0.14} = 0.88$$

Hence, only 12% of the E1 - and using the same approach – 30% of E2 would be expected to be associated with the organic fraction. Given that approximately 50% of the suspended solids are removed, only a small fraction of E1 and E2 (6% and 15%) are likely to be removed via this mechanism, which is similar to the low observed removal rates, i.e. 7% for 0% for E1 and E2, respectively. There is likely to be little conversion of E2 to E1 under the anaerobic conditions encountered during primary clarification. In contrast, the r_{sw} for the activated sludge processes in the ASTP plant is almost ten times greater and hence a much greater degree of initial sorption is expected.

The extent to which E1 and E2 are sorbed onto fine particles that are not removed at the treatment plant is not clear. Treated effluent samples were collected and centrifuged at high-speed (10,000 rpm, 2 x 20 min, 10°C) to separate solids from the aqueous layer and both layers were analysed for estrogens. Results clearly showed the presence of E2 and EE2 in the solid phase in each of the analysed samples. A precise quantification was not possible at such low concentrations using d4-E1 is used as internal standard; however, by comparing the estrogens concentration in water samples that were not centrifuged to the aqueous phase from centrifuged samples it was possible to estimate a percentage of estrogen attached to solid particles. EE2 was only identified in the solids fraction (i.e. 100% adsorption), whereas the extent of adsorption for E1 in the effluent was 24% and 43% for E2 (n=6).

We are obliged to be somewhat speculative with regards to the processes determining this high level of association with the solids fraction given that it cannot be accounted for by bulk partitioning onto organic matter. Lai et al. (*5*) observed increasing sorption onto river sediments with 0.3-3.3% organic carbon but also some 40% removal of estrogens sorbed to pure iron oxide, presumably through ion exchange between the surface hydroxyl group on the oxide and the polar phenolic steroids. Similar, although lower, sorption was observed by Schäfer et al. (*38*) for E1 onto hematite and clays in the presence of natural organic matter. The influent to the enhanced primary STP, unlike the advanced STP, is pre-dosed with $FeCl_2$ upstream in the sewerage system to control sulfide odours before reaching the plant. This results in the formation of fine black FeS particles that have been observed to leave in the treated effluent. The equivalent dose is 2 mg Fe.L^{-1} wastewater and an additional 10 mg $FeCl_3$.L^{-1} is added during the chemical assisted sedimentation (CAS) process. Iron is the dominant metal in the treated effluent (Table 5) and is presumably present as a mixture of surface hydrolyzed iron sulfides (*49*) and Fe(II) and Fe(III) hydroxides. Iron and aluminum are also the dominant metals in the sludge. During a period of no CAS addition, the Fe content of the raw sludge was 1.2 g.$100g^{-1}$ and the Al content 0.7 g.$100g^{-1}$ on a dry weight basis. It is proposed that estrogens may be sorbed onto fine inorganic-organic aggregates leaving the effluent, enhanced by sorbed natural organic matter, and pass

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through the treatment system unaffected by the flocculation and sedimentation process. In addition, the K_{oc} for sorption onto organic colloidal particles may be considerably greater than that determined from bulk partitioning (*50,51*) as the nature of the organic constituents strongly affects the K_{oc} (*52*). Recently Holbrook et al (*53*) have shown that the sorption coefficients for E2 and EE2 on organic colloids are higher than expected based on the octanol-water partition coefficient. Further, recent analyses of marine sediment samples adjacent and 7km from the deep ocean outfall were found to contain all three estrogens at nanogram per gram concentrations (*54*). Studies monitoring total organic carbon (TOC) and particle size in the ocean sediments indicate that there is a slight increase in TOC and presence of finer particles at the 7 km sampling site compared to at the outfall (*55*). Substantially higher TOC and a greater presence of fine particles was found 3 to 5 km from the outfall, suggesting that higher concentrations of particles and hence estrogens may be located in this region.

A clear implication of this work is that enhanced primary treatment does not to remove estrogens and any large scale treatment using this technology may result in a considerable estrogen load to the surrounding marine environment. Their effect on sensitive environments, such as coral reefs, is of particular concern due to the potential for estrone accumulation in the reef benthos (*10*). In addition, as a proportion of the estrogens remain adsorbed to particles, it appears that they are likely to aggregate in higher ionic strength seawater and settle to the sea floor (*54*). The effect that estrogens have on marine invertebrates in the sediments and the wider marine ecosystem is unknown. Since enhanced primary STPs are considered one of the most suitable technologies for coping with the vast quantities of wastewater from mega cities such as Mexico City, Los Angeles, Hong Kong, Sao Paulo, Rio de Janeiro and Istanbul *(56,57*), it is important to determine whether the release of estrogens into the environment will affect the marine ecosystem. The high levels of dilution may prove sufficient for the prevention of endocrine effects in marine animals from deep ocean coastal outfalls but could prove problematic for discharges into large bays or harbours where flushing is limited (*10*). Under such conditions, microbial mediated degradation of estrogens may take several weeks (*58*). The presence of estrogens in anoxic marine sediments, where degradation is slower (*58*), presents a greater chance for estrogenic activity and accumulation in the environment. Further studies are needed to determine whether estrogens present at nanogram per gram concentrations in the sediments affect marine invertebrates and whether this poses a risk to the marine ecosystem.

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Table captions

Table 1. Precision and accuracy of the ECNI GC-MS assays for E1, E2 and EE2 in each studied water matrix and in dewatered sludge

Table 2. Concentration of estrogens in the advanced STP

Table 3. Concentration of estrogen at the enhanced primary STP

Table 4. Ethinyloestradiol use in Australia during 2000 (*42*)

Table 5. Major ions in EP STP treated effluent (*59)*

Figure captions

Figure 1. Sample ECNI GC-MS chromatogram for analysis of E1, E2 and EE2 in raw sewage spiked with 10 ng.L^{-1}. The [M^-] ion and [M-TMS^-] ions were monitored for all compounds. The traces show the monitored ions 271, 272, 273, 269, 343, 367 m/z for d4-E1 (internal standard), E1, E2, and EE2 respectively.

Figure 2. Mass balance for E1, E2 and E1+E2 for the advanced STP sequential batch reactor (MLSS = Mixed liquor suspended solids)

Table 1

Tertiary Effluent (ng/L)	E1	Accuracy (%)	E2	Accuracy (%)	EE2	Accuracy (%)
1						
Mean	0.92	92	0.90	90	0.99	99
%RSD	5.4		2.8		3.7	
10						
Mean	10.2	102	10.3	102	10.3	103
%RSD	4.3		1.7		3.7	
100						
Mean	104	103	102	102	103	103
%RSD	1.2		1.5		1.3	
Secondary Effluent (ng/L)	E1	Accuracy (%)	E2	Accuracy (%)	EE2	Accuracy (%)
10						
Mean	8.4	84	9.5	95	9.1	91
%RSD	2.5		5.5		3.9	
50						
Mean	47.7	95	50.1	100	39.9	80
%RSD	2.0		1.3		0.8	
100						
Mean	101	101	123.2	123	99.6	100
%RSD	3.7		2.7		2.4	
Raw Sewage (ng/L)	E1	Accuracy (%)	E2	Accuracy (%)	EE2	Accuracy (%)
10						
Mean	11.3	113	9.4	94	5.3	55
%RSD	5.7		3.9		13.6	
50						
Mean	53.8	108	53.5	107	24.0	48
%RSD	2.8		3.2		10.0	
100						
Mean	96.5	96	91.0	91	40.2	40
%RSD	2.4		9.4		47.6	
Dewatered Sludge (pg/ul)	E1	Accuracy (%)	E2	Accuracy (%)	EE2	Accuracy (%)
100						
Mean	74.6	75	87.4	87	94.5	95
%RSD	4.9		18.9		7.6	

* RSD is relative standard deviation

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Table 2

Water samples		E1 (ng/L)		E2 (ng/L)		EE2 (ng/L)	
	n	Average	Std dev.	Average	Std dev.	Average	Std dev.
Raw sewage	16	54.8	14.3	22.0	15.9	< 5.0	
SBR Effluent	15	8.1	4.2	0.95	0.55	< 0.1	
% removal		85		96		-	
CMF influent	10	4.1	3.6	0.75	0.99	< 0.1	
CMF effluent	16	1.2	2.6	0.10	0.24	< 0.1	
% removal		70		87		-	
RO effluent	9	< 0.1		< 0.1		< 0.1	
Chlorination	10	< 0.1		< 0.1		< 0.1	
% removal		-		-		-	
Sludge samples		**E1 (ng/g)**		**E2 (ng/g)**		**EE2 (ng/g)**	
	n	Average	Std dev.	Average	Std dev.	Average	Std dev.
Activated sludge	4	11.8	4.7	0.31	8.11	0.42	0.32
Dewatered sludge	4	14.3	3.8	0.57	2.87	0.61	0.67

Table 3

Sample		E1 ng/L		E2 ng/L		EE2 ng/L	
	n	Average	Std dev.	Average	Std dev.	Average	Std dev.
Raw sewage	19	58.0	15.0	14.0	11.0	< 5.0	-
Effluent	19	54.0	13.0	14.0	10	< 5.0	-
% removal		45		61			
Centrifuged effluent	6	41.0	18.0	8.0	5.0	<1.0	

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Table 4

		Code	Number EE2 tablets/ pkg	Quantity of EE2/ tablet ug	Number scripts	No. pkg per script	total EE2 kg
Fixed combinations	Desogestrel with ethinyloestradiol	14249	28	30	123283	4	0.41
	Ethinyloestradiol with gestodene	15082	21	30	35320	2	0.04
		15084	21	30	36066	2	0.05
	Levonoergestrel with ethinyloestradiol	1393	21	30	35308	4	0.09
		1394	21	30	121547	0 4	3.06
		1455	21	50	11275	4	0.05
		1456	21	50	125346	4	0.53
		3186	21	50	15945	4	0.07
		3188	21	50	32621	4	0.14
		16212	28	50	77411	4	0.43
		16217	28	20	156242	4	0.35
		16970	21	20	2454	4	0.00
	Norethisterone with ethinyloestradiol	2772	21	35	8885	4	0.03
		2773	21	35	4959	4	0.01
		2774	21	35	186511	4	0.55
		2775	21	35	91006	4	0.27
Seq. preparations	Ethinyloestradiol with gestodene	15087	6	30	9069	2	0.00
			5	40	9069	2	0.00
			10	30	9069	2	0.01
		15088	6	30	5305	2	0.00
			5	40	5305	2	0.00
			10	30	5305	2	0.00
	Levonoergestrel with ethinyloestradiol	1391	6	30	19348	4	0.01
			5	40	19348	4	0.02
			10	30	19348	4	0.02
		1392	6	30	123654	3 4	0.89
			5	40	123654	3 4	0.99
			10	30	123654	3 4	1.48
		1458	11	50	41115	4	0.09
			10	50	41115	4	0.08
	Norethisterone with ethinyloestradiol	2776	21	35	27231	4	0.08
	Total						9.77

Table 5

Analyte	Average concentration (mg/L)
Al	0.76
Ammonia	26.6
Cl	194
Fe	5.6
Oil and grease	26
Phosphate	6.6
Sulfate	57
Total suspended solids	130
Total N	39.1
Zn	0.13

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Figure 1

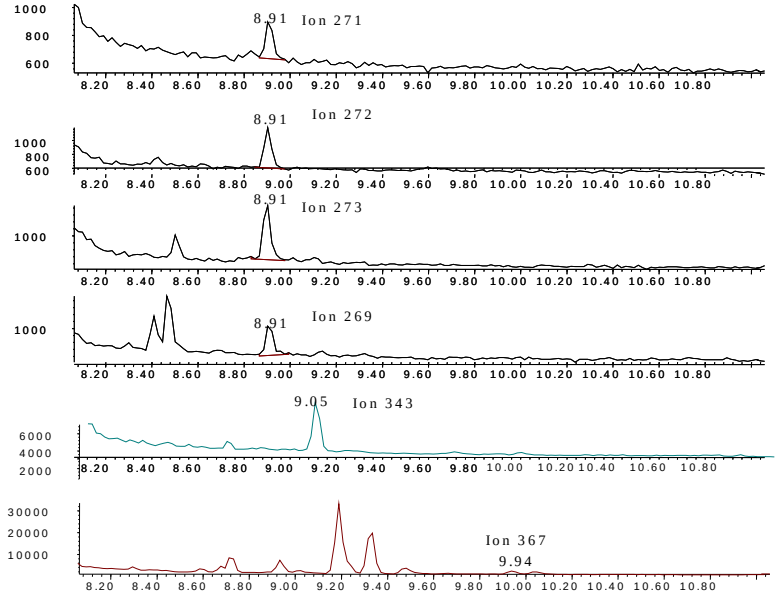

Figure 2