Steroid estrogens in primary and tertiary wastewater treatment plants

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Abstract: The concentrations of two natural estrogens (Estrone (E1) and Estradiol (E2)) and one synthetic progestin (Ethinylestradiol (EE2)) were measured for different unit operations in an advanced sewage treatment plant and in a large coastal enhanced primary sewage treatment plant. The average influent concentration to both plants was similar – 55 and 53 ng/L for E1 and 22 and 12 ng/L for E2 for the advanced and enhanced primary STPs, respectively. The activated sludge process at the advanced STP removed up to 85% and 96% of E1 and E2, respectively. The enhanced primary sewage treatment plant was mostly ineffective at removing the steroids with only 14% of E1 and 5% of E2 being removed during the treatment process. EE2 was not been detected during the study period in the influent or effluent of either STP. The difference in the observed removal between the two plants is primarily linked to plant performance but the extent to which removal of steroid estrogens is due to bacterial metabolism (i.e. the advanced STP) rather than adsorption to the bacterial biomass remains unclear. The poor removal observed for the coastal enhanced primary STP may have implications for the receiving environment in terms of a greater potential for abnormal reproductive systems in marine animals, particularly if discharges are into large bays or harbours where flushing is limited.

Keywords: Estrone, estradiol, ethinylestradiol, estrogen, wastewater, sewage.

Introduction

The release of steroid estrogens into the environment has been linked to abnormal reproductive systems in freshwater and marine dwelling animals (Purdom *et al.*, 1994; Desbrow *et al.*, 1998; Routledge *et al.*, 1998). Human excretion is considered the principal source of estrogens and progestins in the environment, predominantly via discharge from sewage treatment plants (Sumper, 1998; Sole *et al.*, 2000; Ariese *et al.*, 2001). While there is increasing data on the concentration of estrogens in rivers (Kuch and Ballschmitter, 2001; Snyder *et al.*, 2001; Alder *et al.,* 2001) and wastewater effluent (Ternes *et al.*, 1999; Baronti *et al.*, 2000; Huang and Sedlak, 2001), there are comparatively few studies that have determined the concentration of estrogens in the raw sewage (prior to sample pre-treatment with filtration) due to the difficulties encountered when processing a high organic matter, fats and suspended solids matrix. This paper reports on measurements of estrogens in raw sewage for two different Australian sewage treatment plants (STP), the concentration of the estrogens for different unit operations during the wastewater treatment process, and reports on the concentration of estrogens in the effluent from enhanced primary and advanced STPs.

Materials and Methods

Sampling Sites

A small inland advanced sewage treatment plant and large coastal enhanced primary sewage treatment plant were studied in order to assess steroid estrogen removal rates for different unit operations. The advanced sewage treatment plant is located in western Sydney and services only domestic sewage. This plant consisted of activated sludge treatment (2 sequential batch bioreactors). The secondary effluent taken to the tertiary treatment unit that consisted of continuous microfiltration (CMF), reverse osmosis (RO) and chlorination/de-chlorination. The activated sludge treatment consists of two basins with anoxic and aerobic zones. The solids retention time (SRT) is approximately 16 days and the hydraulic retention time (HRT) is 4 hours (2 hours each in the anoxic zone and aerobic zones). The coastal enhanced primary sewage treatment plant is located in eastern Sydney and services domestic sewage (75%) and industrial wastewater (25%). It provides enhanced primary treatment (i.e. with FeCl$_3$ addition) for an average flow of 480 ML.d^{-1} with ultimate disposal by deep ocean discharge.

Standard and SPE Preparation

Estrone (E$_1$), estradiol (E$_2$), ethinylestradiol (EE$_2$), and deuterated estrone-2, 4,16,16-*d4* (d4- E$_1$) were obtained from Sigma Aldrich (Sydney, Australia). The d4- E$_1$ 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. L^{-1} to 500 pg. L^{-1}. The stock solution of internal standard was further diluted with water to obtain a final concentration of 100 pg. L^{-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 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) and after dechlorination. 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 within a week of collection).

Sample Preparation and Solid Phase Extraction

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.

Sample Derivatization for GC-MS Analysis

The derivatization was carried out using a modified version of the method used by Nakamura *et al*. (2001) 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 µL 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   m 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 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   L. The instrumental limit of detection (LOD) for E2 and EE2 was 0.1pg. L^{-1} of injection and for E1 was 0.5 pg. L^{-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 and 0.1 ng.L^{-1} for secondary and tertiary effluents. Note that this is the LOQ of the raw sample prior to any pre-treatment (e.g. filtering).

Results

The average concentrations of E1, E2 and EE2 in the raw sewage of the advanced STP were 55, 22 and <1 ng/L respectively. The average concentrations of E1, E2 and EE2 in the raw sewage of the enhanced primary STP were 53, 12 and <1 ng/L respectively. The E1 and E2 figures are generally in good agreement with reported raw sewage concentrations in other studies but the EE2 figures were consistently lower (Table 1). The fact that there is little difference between the pre-filtered results obtained in this study and filtered samples from other studies suggests that estrogens are mostly present in the colloidal or dissolved fraction in the raw sewage. The extent of estrogen removal for each unit process in the advanced sewage treatment plant is summarised in Figure 1. The sequential batch reactor (SBR) removes on average 85% of the incoming E1 and 96% of the E2. These figures do not consider the potential transformation of E2 to E1 (Ternes *et al*., 1999b; Lee and Liu, 2002). The biologically treated effluent is then stored in a holding tank before passing through the microfiltration plant. During microfiltration the concentration of E1 and E2 are further reduced from 4.1 and 0.75 ng/L to 1.2 and 0.1 ng/L, respectively. No E1 or E2 was detected after reverse osmosis and later dechlorination.

Table 1. Reported individual estrogen concentrations in the influent to sewage treatment plants and an indication of which analytical methods pre-filter before total estrogen analysis of water samples

E1 (ng/L)	E2 (ng/L)	EE2 (ng/L)	Pre-filter	Reference
4 - 47	13 - 70	2 - 28	Y*	Lagana *et al.* (2000)
9 - 48	18 - 140	<0.2 - 8.8	Y	Johnson et al (2000)
27 - 40	15 - 21	-	Y	Ternes *et al* (1999a)
7.6 - 8.6	4.9 - 7.2	2.0 - 5.2	Y	Mouatassim-Souali *et al.* (2003)
4 - 25	25 - 132	0.40 - 13	Y	Baronti *et al.* (2000)
<1 - 25	11	-	Y	DAscenzo *et al.* (2003)
-	20 - 94	-	?	Nasu *et al.* (2001)
39	19	<5	?	Chapman (2003)
54.9 - 76.6	12.2 - 19.5	6.2-10.1	Y	Anderson *et al.* (2003)
36 – 81	6.3 – 29	<1	N	This study (Enhanced Primary STP)
29 - 93	2.2 – 72	<1	N	This study (Advanced STP)

* sample residual on filter paper washed with methanol

Unlike the advanced STP where some 85-96% of estrogens are removed in the initial biological treatment stage, there is effectively no removal of the estrogens during treatment at the enhanced primary STP as the solids concentration and hydraulic residence time is too low (i.e. 45min). The ocean discharge concentration for E1 is 46 ng/L and 12 ng/L for E2, equating to only 14% removal for E1 and 5% for E2 (Figure 2).
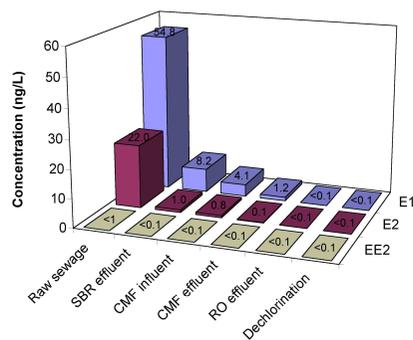

Figure 1. Average estrogen removal at the advanced STP for different unit operations from 16 samples (Estrone (E$_1$), estradiol (E$_2$) and ethinylestradiol (EE$_2$)). SBR = sequential batch reactor; CMF = cross-flow microfiltration; RO = reverse osmosis.
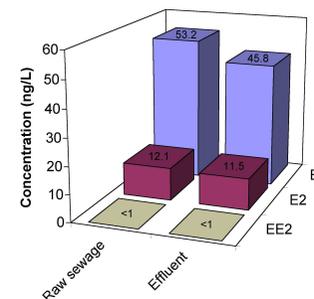

Figure 2. Average estrogen removal at enhanced primary STP for raw sewage influent and treated effluent from 16 samples (Estrone (E$_1$), estradiol (E$_2$) and ethinylestradiol (EE$_2$)).

Discussion

The percentage removal for estrogens obtained during biological treatment at the advanced sewage treatment plants is consistent with other studies, which typically range from 60 – 85% (Ternes *et al.*, 1999b; Baronti *et al.*, 2000; Johnson *et al.*, 2000). The SBR is therefore generally effective for the removal of the estrogens but the extent that removal is due to adsorption onto bacterial biomass or biological degradation remains unclear. Further studies are required to accurately determine the degree of partitioning during treatment together with the removal mechanism within biological treatment. Several studies have performed mass balances around the biological treatment stage at STPs and shown that most of the estrogens removal can be accounted for by biological oxidation (Holbrook *et al.*, 2002; Anderson *et al*., 2003). Radiolabelled estrogen studies have confirmed that E2 is susceptible to mineralization but EE2 is resistant to mineralization (Layton *et al.*, 2000). Other authors have also confirmed that E1 and E2 are readily degraded by sewage bacteria during small batch experiments (Ternes *et al.*, 1999b; Lee and Liu, 2002).

Poor removal of estrogens is observed at the enhanced primary STP in contrast to the advanced STP. This result is consistent with the reported performance of primary clarifiers in secondary or tertiary treatment plants (Holbrook *et al.*, 2002; Anderson *et al*., 2003). With only 14% of E1 and 5% of E2 being removed at the plant, it is important to consider the potential impacts of such a large load of estrogens on the receiving environment. The high levels of dilution obtained during ocean disposal may prove sufficient for the prevention of endocrine effects in marine animals but could prove problematic for discharges into large bays or harbours where flushing is limited (Atkinson *et al.,* 2003). The life-time for estrogens in fully aerobic seawater is in the order of weeks (Ying and Kookana, 2003). Enhanced primary STPs are considered to be one of the most suitable technologies for coping with the vast quantities of wastewater from mega cities such as Mexico City, Los Angles, Hong Kong, Sao Paulo, Rio de Janeiro and Istanbul (Harleman and Murcott, 1999; Eroglu *et al.*, 2001). This study suggests that most of the estrogens will not be removed from such plants and the STP discharges could result in a high load of estrogens to the receiving environment.

Conclusions

The concentrations of estrone (E$_1$), estradiol (E$_2$) and ethinylestradiol (EE$_2$) in Australian sewage is similar to that reported for other countries. High removal efficiencies are observed during biological treatment but poor removal is observed for enhanced primary treatment plants. This poor removal has implications for the receiving environment in terms of a greater potential for abnormal reproductive systems in marine animals, particularly if discharges are into large bays or harbours where flushing is limited.

Acknowledgements

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