Steroid estrogens in ocean sediments

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Abstract

Ocean sediment samples were taken adjacent and 7km from a deep ocean outfall (average depth 80m), the discharge point of an enhanced primary sewage treatment plant. All samples contained steroid estrogens at nanogram per gram levels with higher concentrations at the 7km sampling site. The concentration of estrone ranged from (0.16 - 1.17 ng/g), 17β-estradiol (0.22 – 2.48 ng/g) and the synthetic 17α-ethinylestradiol (<0.1 – 0.5 ng/g). The values detected correspond with estimates based on the proportion of estrogens sorbed to particles in the effluent and the expected proportion of sewage origin particles in the ocean sediments. The results suggest that estrogens associated with the particulate fraction aggregate on contact with high ionic strength seawater and settle to the seafloor after discharge through deep ocean outfalls.

Keywords: estrone, estradiol, ethinylestradiol, sediment, wastewater, sewage

1. Introduction

Understanding the fate of estrogens in the environment is key to understanding the potential for human released estrogens to induce abnormal reproduction in aquatic environments (Purdom et al., 1994; Desbrow et al., 1998; Routledge et al., 1998). Estrogens have been detected in rivers (Kuch and Ballischnitter, 2001; Snyder et al. 2001; Alder et al., 2001), lakes (Matsui et al., 2000; Shen et al., 2001; Snyder et al., 2004) and marine samples (Atkinson et al., 2003; Tashiro et al., 2003) and the primary sources are considered to be from sewage treatment plants and agricultural runoff or discharge (Tashiro et al., 2003; Kołodziej et al., 2004). There is now increasing evidence that estrogens such as estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) are sorbing to riverbeds (Peck et al., 2004; Williams et al., 2003; de Alda et al., 2002; Holtñas et al., 2002), lake (Mibú et al., 2004) and estuarine (Thomas et al., 2004; Thomas et al., 2001) sediments where degradation, especially for EE2 under anaerobic conditions, is slow (Ying and Kookana., 2003; Ying et al., 2003; Jürgens et al., 2002). The release and fate of estrogens into the marine environment is little studied but of considerable interest as they appear to be relatively stable and show limited degradation even under aerobic conditions (Ying et al., 2003). Their affect on coral reefs is of particular concern (Atkinson et al., 2003). When estrone concentrations are greater than 300 pg/L, there is the potential of net uptake and potential accumulation in the reef benthos (Atkinson et al., 2003). Estrogens in the coastal marine environment may affect reproductive biology through embryonic development (Hathaway and Black, 1969), altered enzymatic activities (Ghosh and Ray, 1993a; Ghosh and Ray, 1993b) or cellular damage or apoptosis (Wiens et al., 1999; Viarengi et al., 2000). Much more information is required to characterise the presence of human-derived estrogens in marine environments and to determine their potential effects on the marine ecosystem. This paper reports on preliminary measurements of estrogens in ocean sediments.

2. Materials and Methods

2.1. Ocean sediment sampling

Ocean sediment samples were collected near the discharge point from a large coastal enhanced primary sewage treatment plant (STP). The STP is located in eastern Sydney and services domestic sewage (75%) and industrial wastewater (25%). It provides enhanced primary treatment (i.e. with FeCl₃ addition) for an average flow of 480 ML/day with ultimate disposal by deep ocean discharge (3.6km offshore, average 80m deep). A Motor Vessel (MV) Oceanographer was used as a stable platform, from which a “Smith McIntyre” grab (capacity approximately 5L) (Fig. 1) was deployed to collect sediment samples. Samples were collected from 2 locations (2 samples per location), adjacent to the Malabar deep ocean outfall and approximately 7km south of the outfall (Fig. 2). The vessel was manoeuvred to hold its position until the grab had reached the seafloor and a sediment sample was taken. In order to ensure samples are as representative as possible, the angle and speed at which the grab was lowered to the seafloor, was controlled and maintained for all the sub-sites. The grab was lowered to approximately 3 m above the seafloor and then released to collect the sample. In setting the angle and speed at which the grab is lowered, consideration was given to two things: maximising the volume of the sediment sample retrieved; and minimising the bow wave generated from the grab moving through the water column. This method of controlling the grab fall rate has been shown elsewhere to reduce the loss of the fine surface material (Blomquist, 1992). The samples were transferred to a 0.5L glass bottle and stored at –20°C prior to analysis.
2.2. Standard Preparation

Estroge (E1), estradiol (E2), ethinylestradiol (EE2), and estrone-2, 4,16,16-d4 (d4- E1) were obtained from Sigma (Aldrich, Sydney, Australia). The d4-E1 was used as internal standard. Stock solutions of individual non-deuterated standards and deuterated internal standard were prepared by dissolving known amounts of dimethanol in to obtain concentration of 0.10 mg/ml. Working standard solutions were obtained by further diluting stock solutions with water to obtain final concentrations of 0.5 pg/ml. The stock solution of internal standard was further diluted with water to obtain a final concentration of 100 pg/ml. Methanol and acetonitrile HPLC grade were obtained from Ajax Finechem, Sydney, Australia. Other solvents were of analytical grade, and they were used as supplied (Ajax Finechem, Sydney, Australia). Milli-Q water was used for all experimental procedures.

2.3. Preparation of sediment samples

Analysers were extracted from the sediment samples using liquid phase extraction (LPE). Prior to sample preparation, internal standard was added. Samples were then frozen with liquid nitrogen and then dried in a vacuum refrigerator for 24 hours at -70°C. 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 minutes followed by stirring and heating to 80°C for an hour in a hot plate. The solventsample mixture was then filtered through Whatman No. 1 glass fibre filter paper (Whatman, Sydney, Australia), the film rinsed with a mixture of acetone/hexane (50:50) followed by sonolvetation at 80°C in a water bath. The residues were dissolved in water. Analysers were then extracted from water samples by solid phase extraction (SPE) using the LC-18 SPE cartridges filled with 1.0 g of C18 (Supelco, Sydney, Australia). After fitting the SPE cartridge into a 12-Port Viscograp DL Vacuum Manifold (Supelco, Sydney, Australia), the SPE was sequentially conditioned with 2x10 ml methanol, 1x10 ml Mill-Q water. sample loading was achieved by passing standards and water samples through the LC-18 SPE cartridge. After sample loading, cartridges were dried in a vacuum desiccator for 30 to 40 minutes. Elution of the analyte 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 acetonitrile before derivatisation and analysis.

3. Results and discussion

Marine sediments taken near the ocean outfall were found to contain all three estrogens (Table 1; Fig. 3). The concentration of estrogens 7 km from the outfall were often considerably lower than immediately adjacent the outfall (Fig. 3). Previous studies monitoring total organic carbon (TOC) and particle size in the sediments indicate a slight increase in TOC and presence of finer particles at the 7 km sampling site compared to the outfall (AWT, 2000). Substantially higher TOC and a greater presence of fine particles was found close to the outfall, suggesting that higher concentrations of particles and hence estrogens may be located in this region.

Based on silver enrichment data in marine sediments approximately 1-2 km from the outfall, it is estimated that sewage particles account for approximately 2% to the surficial sediment (top 10 cm) (Mattah et al., 2002). The majority of particles comprise of natural contributions from settling marine particulate material and resuspended sediment. Using the 2% figure as a guide and assuming that there is no desorption of estrogens from the particles on contact with the seawater, the estimated concentration in the surficial sediments is close to the measured values (Table 2). It is expected that there will be a large fraction of estrogens attached to fine particles (Holthaus et al., 2002; Holbrook et al., 2004) and such particles are likely to aggregate on contact with higher ionic strength waters and show greater settleability. Indeed, after adding various concentrations of salt to the STP effluent samples to induce aggregation, the concentration of E1 in particular in the aqueous sample (i.e. without pellet) decreased markedly without increasing salt concentration (Fig. 4). EE2 was not detected in any of the centrifuged liquid samples.

These preliminary results suggest that estrogens attached to particles in the effluent and, on contact with higher ionic strength seawater, are aggregating and settling to the sea floor. As the measured concentration is similar to the estimated concentration in the marine sediments suggests that the degradation in the sediments is slow. The mean sewage flux 1-2 km from the outfall is 0.23 g/m² per hour. Cooling the solution was reduced to 100 ml, 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 trimethylsilylacetamide 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 5899B MS Engine (Agilent Technologies, Ryde, Australia). Chromatographic separations were performed using an HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The GC oven temperature was programmed at 150°C for 1.5 min and then 2°C per minute to 310°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°C, respectively. Pulse split 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 µL. The standards 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.
treatment at the STP plant (Braga et al., 2005), 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, although picomolar concentrations have been shown to affect the development of aquatic organisms (Routledge et al., 1998; Schoenmakers et al., 1981; Harries et al., 1997). Discharges into large bays or harbours where flushing is limited could prove problematic (Atkinson et al., 2003). The presence of trace levels of estrogens in marine sediments needs to be considered in the context that estrogens are one of many classes of compounds that exhibit estrogenic behaviour that are likely to be found in the sediments (e.g. PAHs and organochlorines (Leeming et al., 1991)). There are almost certainly other contaminants in the marine sediments, present at higher concentrations, but with lower estrogenic activity in the sediments. Further studies are required to determine the extent that estrogens present at nanogram per gram concentrations in the sediments affect marine invertebrates, the base of the aquatic food chain, and whether the presence of estrogens poses a risk to ecosystem function.

4. Conclusions
Steroid estrogens were found in deep ocean sediment samples at nanogram per gram concentrations. The presence of the synthetic 17α-ethinylestradiol in the sediments confirms that the estrogens (at least 17α-ethinylestradiol) are of human origin. While there are likely to be other compounds that exhibit estrogenic behaviour in the sediments, steroid estrogens have high estrogenic activity and their presence may affect the sediment ecology. Further studies are needed to determine whether estrogens, at low concentrations detected, affect the development of marine invertebrates and whether there is potential for estrogen bioaccumulation in the marine environment.

5. Acknowledgements
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6. References


**Figure captions**

Figure 1: Smith McIntyre grab sampler

Figure 2: Sediment sample collection locations

Figure 3: Concentration of estrogens in sediment samples taken 0km and 7km from the deep ocean outfall. Error bars indicate standard error of the mean.

Figure 4: Estrogen content in the separated liquid sample after the addition of NaCl to STP effluent samples and high speed centrifugation. EE2 was not detected in any liquid samples after centrifugation.

**Table caption**

Table 1. Measured and expected concentration of estrogens assuming no desorption from effluent sewage particles upon ocean disposal.

Table 1

<table>
<thead>
<tr>
<th>Estrogen</th>
<th>Mean concentration in treated effluent (ng/L)</th>
<th>Mean fraction adsorbed to particles (%)</th>
<th>Estimated concentration on particulates (ng/g)</th>
<th>Estimated concentration in surficial sediments (ng/g)</th>
<th>Measured concentration in surficial sediments (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>54</td>
<td>24</td>
<td>96</td>
<td>1.9</td>
<td>0.16 - 1.17</td>
</tr>
<tr>
<td>E2</td>
<td>14</td>
<td>43</td>
<td>45</td>
<td>0.9</td>
<td>0.22 - 2.48</td>
</tr>
<tr>
<td>EE2</td>
<td>&lt;5</td>
<td>100</td>
<td>19</td>
<td>0.4</td>
<td>&lt;0.1 - 0.5</td>
</tr>
</tbody>
</table>


- assuming suspended solids concentration is 135 mg/L (SWC, 2002)

- assuming EE2 concentration in treated effluent of 2.5 ng/L

- assuming sewage particles contribute 2% to the total surficial sediments (Matthai et al., 2002)