Estrogens, triclosan and derivatives in sediments of Barker Inlet, South Australia

A report prepared for the
Adelaide and Mount Lofty Ranges Natural Resources Management Board and the South Australian Environment Protection Authority

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EXECUTIVE SUMMARY

Many chemicals included in pharmaceutical and personal care products are pollutants that escape wastewater treatment and are discharged with effluents into receiving water bodies. Some of these chemicals persist in the environment, posing the risk of causing adverse effects to human health and aquatic ecosystems. Exposure has been associated with several toxicological effects in both flora and fauna, including disruption of the normal functioning of the endocrine system. Adelaide metropolitan waters receive approximately 72 gigalitres (GL) of wastewater effluents annually, discharged from three wastewater treatment plants spread over 50 km of coastline. The largest input comes from the Bolivar wastewater treatment plant, which discharges 46 GL into Barker Inlet. In this work, we investigated the occurrence of the persistent organic pollutants triclosan, methyl-triclosan and the steroid hormones estrone, 17β-estradiol and ethinylestradiol, in sediments of the inlet. Our goals were not only to expand a previous survey conducted in the area in 2008 for the Adelaide and Mount Lofty Ranges Natural Resources Management Board, but to provide a detailed assessment of local concentrations and potential exposure to marine organisms, and an indication of the likely mechanisms promoting accumulation in the sediments and bioavailability to marine life.

Triclosan is a chlorinated phenoxyphenol used widely as an anti-microbial agent, and was detected in all samples collected in this study, suggesting its persistence in Barker Inlet. The concentrations recorded here (4-27 $\mu g\ kg^{-1}$) were comparable to values found in other marine sediments under the influence of wastewater outfalls, typically <100 $\mu g\ kg^{-1}$. Triclosan transport and burial appeared to be closely associated with the fine and organic-rich fractions of the sediments. The concentration of this compound in sediment porewaters was estimated to be of the order of 1 $\mu g\ L^{-1}$. This value is lower than the threshold reported for harmful effects to occur in the couple of species of marine phytoplankton investigated to date (3 $\mu g\ L^{-1}$). However, toxicological information for marine organisms is very limited, and studies on freshwater algae suggest that harmful effects can occur at concentrations as low as 0.015 $\mu g\ L^{-1}$.

Methyl-triclosan is the main degradation product of triclosan, and was detected in several samples at concentrations <11 $\mu g\ kg^{-1}$. The accumulation of both triclosan and methyl-triclosan was more pronounced in close proximity to the Bolivar wastewater outfall and in deep sediments towards the inner part of the inlet. Currently, no other data is available for methyl-triclosan in marine sediments worldwide. This compound is more lipophilic than the parent compound and thus has a greater potential for accumulation in animal tissues. The implications of its

occurrence to ecosystem health in Barker Inlet are difficult to predict given the lack of ecotoxicological data in the current literature.

The presence of steroid hormones in aquatic systems has been linked to the disruption of hormonal systems and poor reproductive success in both humans and wildlife. These compounds are typically present in wastewater effluents at concentrations similar to triclosan, but only estrone was detected in the sediments analysed here. Estrone was found at a concentration of 5.4 $\mu g\ kg^{-1}$ in one sample from Section Bank, suggesting a sediment porewater concentration of 2.1 $\mu g\ L^{-1}$. As for triclosan, the paucity of toxicological data for marine species makes an assessment of the risk of exposure to estrone difficult. However, freshwater fish species are deleteriously affected when exposed to estrone concentrations as low as 0.06 $\mu g\ L^{-1}$. The absence of other hormones in this survey might have been dictated by the method of analysis, and further studies are recommended to accurately define their risk to the local environment.

The results from this work highlight the need to better understand the toxicological response of South Australian flora and fauna to persistent organic pollutants present in wastewater effluents. In particular, the effects of triclosan on plankton health need to be clearly defined in nursery habitats receiving wastewater discharges. Given the toxic response of several freshwater photosynthetic organisms to triclosan exposure, it is also important to investigate its effects on protected mangrove and mudflat habitats. The potential for bioaccumulation, and hormonal disruption, are other topics deserving further research, particularly considering that Barker Inlet is the only sanctuary established in the state of South Australia to protect Indo-Pacific bottlenose dolphins (*Tursiops aduncus*).

1. INTRODUCTION

A large number of chemicals present in pharmaceuticals and personal care products are released into the environment through wastewater discharges (Hester and Harrison 1999; Andresen et al. 2007). A common example is triclosan, a preservative or antiseptic chlorinated phenoxyphenol included in the formulation of several products used daily by people across the globe, such as soaps and toothpastes (Singer et al. 2002; Sabaliunas et al. 2003). As a consequence, triclosan is one of the most common anthropogenic compounds found in surface waters in the United States (Kolpin et al. 2002). This aromatic compound is moderately persistent in the environment and toxic to bacteria, algae and other aquatic biota, potentially interfering with processes supporting the base of the food chain, such as the remineralisation of nutrients and primary productivity (Tatarazako et al. 2004; Dobretsov et al. 2007; Farre et al. 2008; NICNAS 2009; Waller and Kookana 2009). Triclosan also has low solubility in water, and therefore can accumulate in sediments (Reiss et al. 2002). One of its degradation products, methyl-triclosan, has a greater ability to dissolve in fats than the parent compound and thus can accumulate in animal tissues (Lindstrom et al. 2002).

Other compounds discharged with wastewater effluents, such as natural steroid hormones released by humans, or synthetic hormones present in contraceptive formulations, are endocrine disrupters and have been linked to the disruption of hormonal systems and poor reproductive success in both humans and wildlife (Hester and Harrison 1999; Campbell et al. 2006). These estrogens can be persistent in the environment (Ying and Kookana 2003), with the estrogenic activity of contaminated waters starting at trace quantities (Routledge et al. 1998; Kidd et al. 2007) and spreading for large distances from the point source (Harries et al. 1997).

Adelaide metropolitan waters receive approximately 72 GL of wastewater effluents annually, discharged from three wastewater treatment plants (WWTPs) spread over 50 km of coastline (Wilkinson et al. 2003). The largest input comes from the Bolivar WWTP, which discharges 46 GL into Barker Inlet, followed by the Glenelg and Christies Beach WWTPs (18 and 8 GL, respectively), discharging directly into Gulf St Vincent. These effluents carry both triclosan and endocrine disrupting chemicals into coastal waters (Ying et al. 2004; Ying and Kookana 2007), but the magnitude of inputs and associated roll-over effects to the marine ecosystem are currently unknown. A preliminary study suggested the accumulation of these chemicals in

parts of Barker Inlet, potentially promoted by anaerobic conditions (Fernandes et al. 2008).

In this work, we investigated the occurrence of triclosan, methyl-triclosan and the steroid hormones estrone, 17β-estradiol and ethinylestradiol, in sediments of Barker Inlet. The region receives not only effluents from the Bolivar WWTP, but also industrial discharges from facilities bordering the adjacent Port River, and stormwater from the surrounding catchments. It encompasses two aquatic reserves protecting mudflat and mangrove habitat (Barker Inlet-St Kilda and St Kilda-Chapman Creek), and two Conservation Parks (Port Gawler and Torrens Island). The inlet is also part of the Adelaide Dolphin Sanctuary.

The initial survey of concentrations of triclosan and endocrine disrupting chemicals in sediments of Barker Inlet included only a few samples with a limited spatial coverage (Fernandes et al. 2008). In this work, we produced a more detailed survey of the area, and concentrated efforts on the south-western shores between the Section Bank and Torrens Island. The two main reasons for this choice of effort are (i) the area showed the highest concentrations in the initial survey, and (ii) it is predicted to accumulate wastewater inputs during winter when predominant northerly winds force particles to drift along the shores (Pattiaratchi et al. 2007). Our goals were not only to provide a more detailed and confirmatory assessment of local concentrations and potential exposure to marine organisms, but also to have an indication of the likely mechanisms promoting accumulation in the sediments and bioavailability to marine life.

2. METHODS

2.1. Sampling

Three digested sludge samples were collected in glass jars from an activated sludge tank inside the Bolivar WWTP in July 2009. One empty glass jar was taken to the field and kept as a procedural blank. To follow recovery during transport, storage and analysis, one sample was spiked with 100 μL of a 1 mg L^{-1} mixture of surrogate standards (deuterated estrone, and ^{13}C-enriched estradiol, ethynylestradiol, triclosan and methyl-triclosan). The sludge samples were transported on ice to the laboratory and stored frozen (-20 ^{o}C).

Twenty shallow (<2 m) and 10 deep sites (>3 m) were sampled in Barker Inlet either by snorkelling or SCUBA diving in July/August 2009 (Figure 1). Sampling equipment was washed with acetone, MilliQ water and seawater between sampling sites. Five replicate cores were collected at each site using 74 mm (i.d.) stainless steel tubes capped with PTFE stoppers. Surface oxidation-reduction potential (redox) was obtained by inserting a 10 mm platinum electrode with a calomel reference (ORP, Phoenix) into the top 1 cm of each core. The overlying water in the tube was then carefully discarded, the sediment extruded and the top 1 cm sliced, and any fragments of roots and other plant material removed. The five replicates collected at each location were combined and homogenized in a stainless steel bowl before sub-samples were transferred into pre-combusted glass jars. Three sub-samples were collected at each site, one for particle size analysis, one for organic carbon analysis and one for estrogen/triclosan analysis. Three empty glass jars were taken to the field and kept as procedural blanks. Sediment samples were transported on ice and stored frozen (-20 ^{o}C). Of the sediments collected for estrogen/triclosan analysis, 3 shallow samples and 2 deep samples were spiked with the mixture of surrogate standards described above.

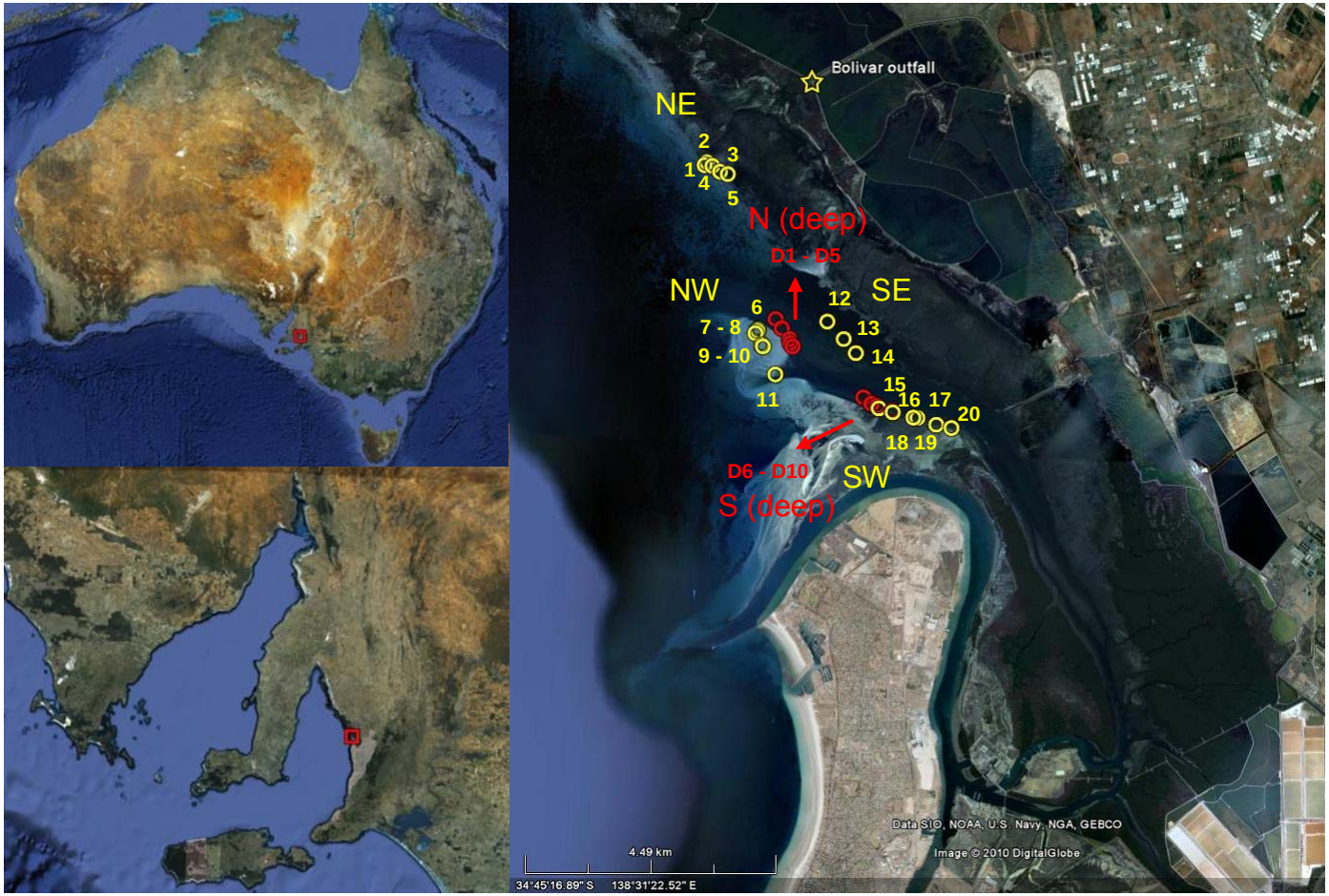

Figure 1. Map of the study area showing the location of both shallow (in yellow) and deep (in red) sampling sites.

2.2. Analysis

Samples for particle size analysis were left to thaw at room temperature overnight and wet sieved to 1 mm just before analysis in a Malvern Mastersizer 2000 laser diffraction analyser. Particle size distributions were analysed with the software package GRADISTAT (Blott and Pye 2001).

For all other analyses, sediment and sludge samples were freeze-dried, sieved to 2 mm and homogenized. Sediment sub-samples for organic carbon analysis were pre-treated with 1 N hydrochloric acid to remove carbonates, rinsed with MilliQ water to remove hygroscopic salts and oven-dried at 50 ºC using a method modified from Fernandes and Krull (2008). Organic carbon content was calculated from the carbon content of pre-treated and untreated sub-samples, determined in a LECO TruSpec elemental analyser. Inorganic carbon content was calculated from the difference between total carbon and organic carbon, and later translated into carbonate content using molecular weights.

For the analysis of organic compounds, 5 g of freeze-dried sediments or 0.5 g of the sludge samples, and procedural blanks, were spiked 48 h before extraction with 100 μL of the same mixture of surrogate standards as used in the field (see the sampling section). The samples were extracted three times by ultrasonication for 10 minutes with a mixture of methanol and acetone (1:1 v/v). For the first extraction, 20 mL of this solvent mixture was used, followed by two additional extractions with 10 mL. After centrifugation at 600 *g*, the extracts were combined and diluted to 500 mL with MilliQ water. The combined extracts were then purified using Oasis HLB® solid phase extraction (SPE) cartridges (Waters, Sydney, Australia). The cartridges were pre-conditioned with 2 x 4 mL of methanol and 2 x 4 mL of MilliQ water. The extracts were loaded onto the cartridges at a rate of approximately 15 mL min^{-1}. The cartridges were then washed with 3 mL of MilliQ water and dried under vacuum. The dried cartridges were eluted with 3 x 3 mL methanol, 3 x 3 mL acetone and 3 x 3 mL ethyl acetate. After each elution, fractions were concentrated to dryness under a gentle stream of nitrogen. The combined dried fractions were reconstituted in methanol and derivatised (Shareef et al. 2006) before analysis by gas chromatography-mass spectrometry (GC-MS).

Organic compounds were analysed in an Agilent 6890 GC, coupled with a 5973 MS, equipped with a HP-5MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm), with helium as the carrier gas (flow rate of 1 mL min^{-1}). A 2 μL aliquot of each sample was injected in splitless injection mode. The oven temperature was

programmed from 75 °C (1 min) to 150 °C (10 °C min^{-1}) and then to 280 °C (15 °C min^{-1}) and held for 10 min. The injector and interface temperatures were set at 280 °C, with the MS quadrupole set at 150 °C and the MS source at 230 °C. The mass spectrometer was operated in the positive ion electron impact mode with an ionisation voltage of 70 eV using selected ion monitoring (SIM).

The method limit of quantitation (LOQ) was estimated at 2 μg kg^{-1} for triclosan and methyl-triclosan, and 1 μg kg^{-1} for the steroid hormones estrone, 17β-estradiol and ethinylestradiol. The LOQ was determined based on 10x the signal-to-noise ratio of the response of the isotopically labelled standards spiked into each sample prior to extraction. For quantitation, relative response factors were calculated for the internal standard deuterated anthracene, which was added to each sample extract immediately before injection into the GC-MS. Seven point calibration curves in the range 25 to 1000 μg L^{-1} were used and resulted in good linearity ($r > 0.99$) and repeatability.

Results were analysed with the software package STATISTICA (StatSoft, Tulsa, OK). Principal component analysis was used as an exploratory technique to extract relationships between variables and sample groupings. Data were standardized using the mean and standard deviation of each variable. Analysis of variance (ANOVA) was used to identify statistical differences in recovery between field and laboratory spiked samples.

3. RESULTS

Sediments typically consisted of fine to medium sands with a mean particle size between 100 and 450 μm and low silt and clay content (Figures 2 and 3). Lower mean particle sizes towards the south of the inlet, and on western shores, were generally driven by the accumulation of silts and clays. The exception was Section Bank (sites 6-11), where lower mean grain sizes were explained by a higher content of fine sands (125-250 μm) but negligible amounts of silt and clay.

Surficial sediments were aerobic, with organic carbon contents below 1% (Figures 4 and 5). Redox potential declined with organic carbon accumulation, with both variables being well correlated to silt and clay content ($r^2 > 0.5$). One deep site in the south of the study area (D8) had comparatively little organic matter burial despite the clear dominance of silt and clay fractions.

Sediments on the eastern shores of the inlet were calcareous (carbonate content 70-95%), whereas sediments on the western shores were sandy (carbonate content <20%) (Figure 5). The composition of deep sediments in the north of the study area indicated a mixture of sandy and calcareous particles, as carbonate contents reached values close to 50%. Further south, carbonate contents in deep sediments were below 20%.

The principal component analysis of sediment physicochemical characteristics gives evidence for the geographical differences discussed above (Figure 6). The analysis produced two factors with eigenvalues >1. The first factor accounts for 56% of the total variance in the dataset, and is driven chiefly by silt and clay contents, organic carbon contents and redox potential. This factor clearly separates the southernmost shallow (18-20) and deep sites (D6-D10), as these sediments are organic rich and finer, with lower redox potential. The second principal component, in contrast, relies heavily on carbonate contents and accounts for 22% of the variance in the dataset. This factor clearly separates calcareous sediments on the eastern shores (sites 1-5 and 12-14) from those with low carbonate content on the western shores.

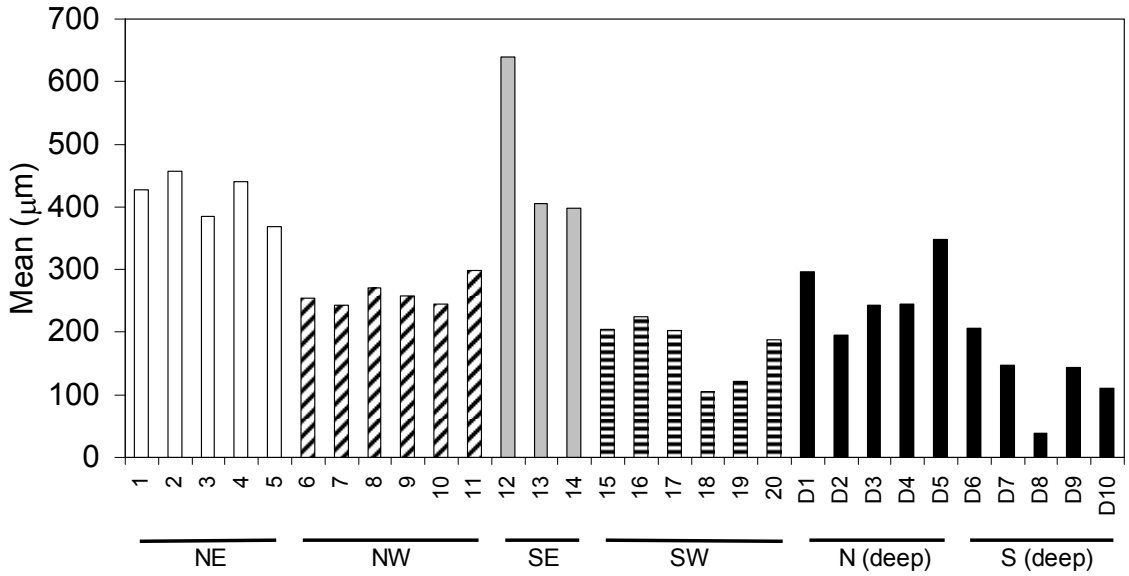

Figure 2. Mean particle size of shallow and deep sediments.

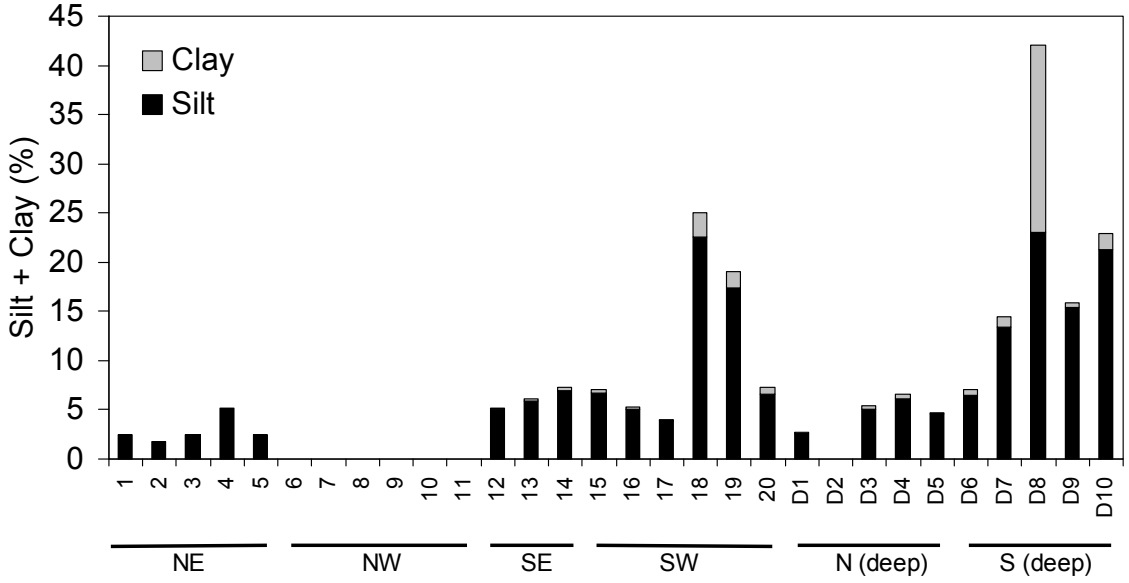

Figure 3. Clay (0-4 μm) and silt (4-63 μm) content of shallow and deep sediments.

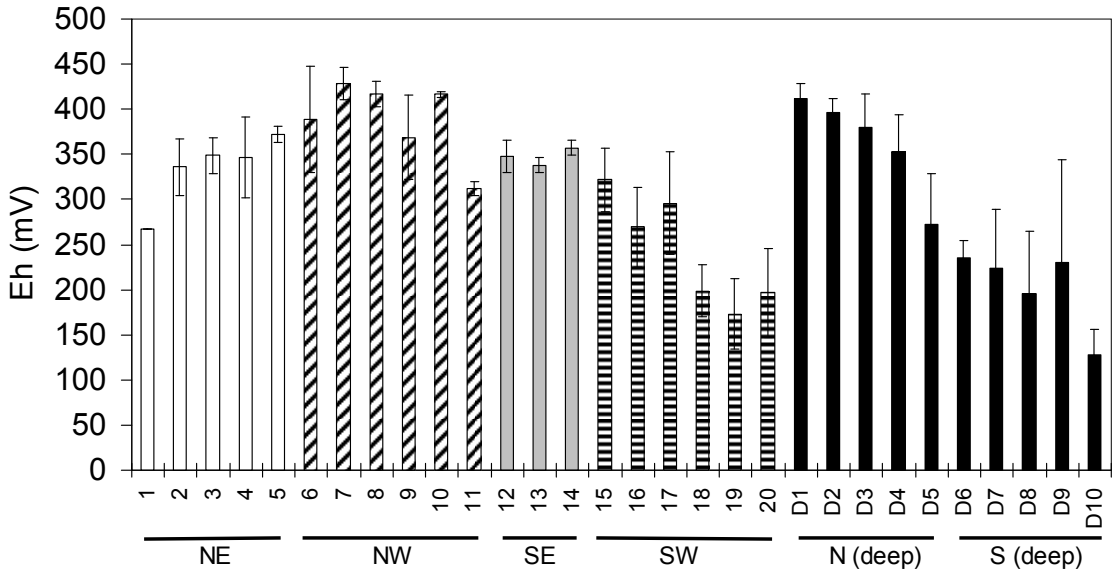

Figure 4. Redox potential at the surface of shallow and deep sediments.

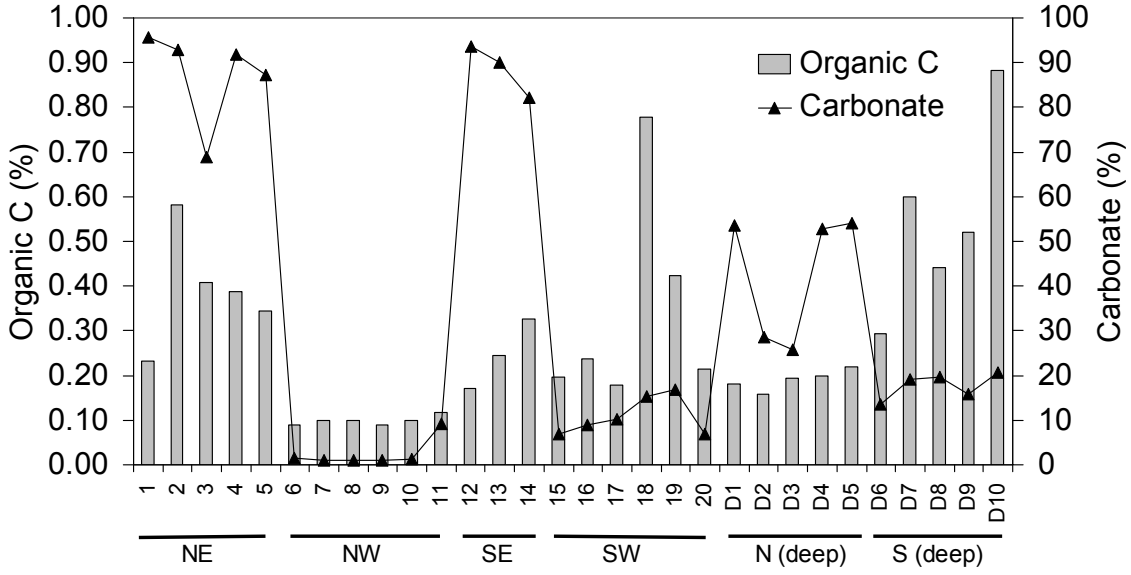

Figure 5. Organic carbon and carbonate content of shallow and deep sediments.

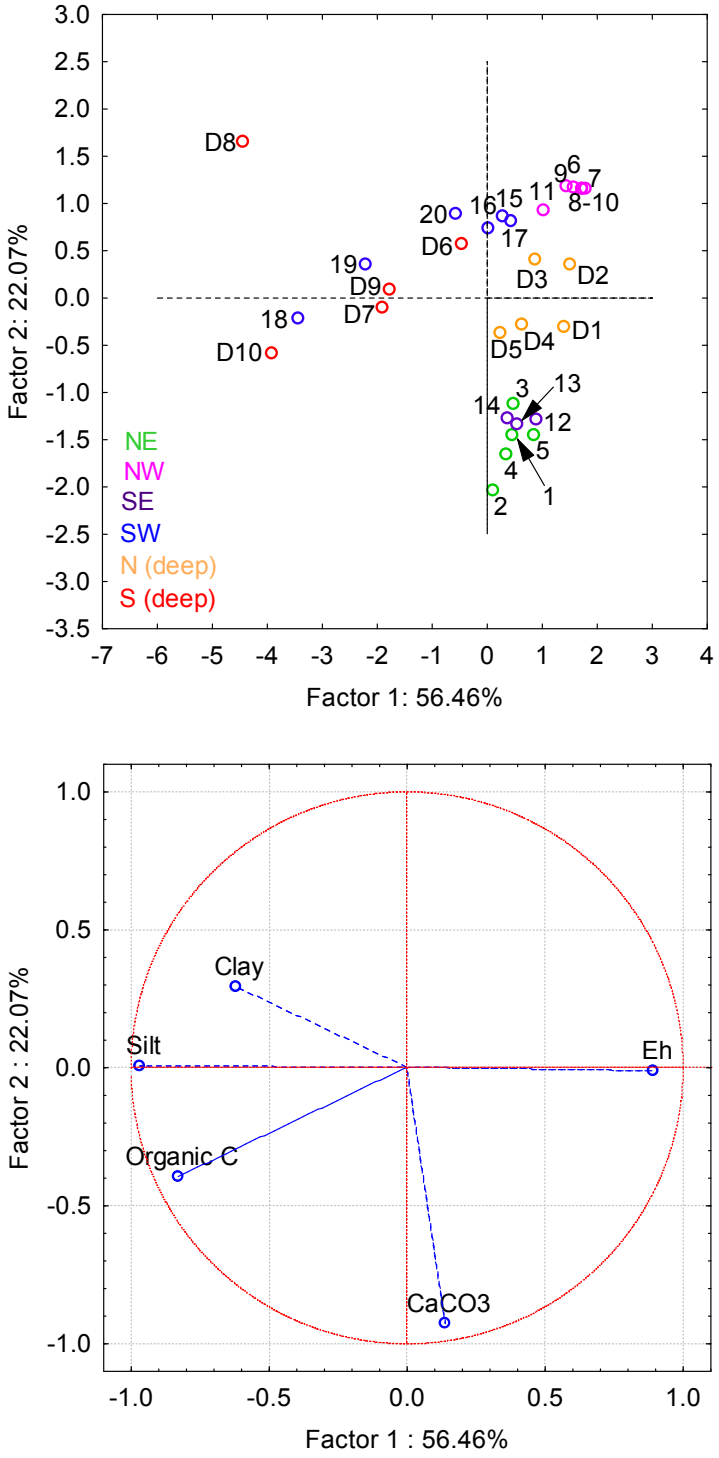

Figure 6. Results for the principal component analysis of physicochemical characteristics of sediments according to (a) sites and (b) variables.

The recovery of spiked surrogate standards in the procedural blanks was almost complete, typically >80%. When standards were added to the samples in the laboratory, recovery was lower, on average approximately 50%. Marginally higher recoveries (~60%) were recorded for the estrogens, particularly estrone and ethinylestradiol. The lowest recoveries (< 20%) were associated with the organic-rich and fine sediments of sites D7-D9. There was no significant difference in recovery between laboratory and field spiked samples for triclosan, methyl-triclosan, or ethinylestradiol. The recovery of estrone and 17β-estradiol, however, was significantly lower when samples were spiked in the field.

The sludge samples had higher concentrations of triclosan (1366 ± 40 μg kg^{-1}) and methyl-triclosan (89 ± 2 μg kg^{-1}), than of steroid hormones, with only estrone detected at a concentration of 8 ± 1 μg kg^{-1}. None of the organic pollutants investigated here were detected in the procedural blanks, and 17β-estradiol and ethinylestradiol were also absent from the sediment samples. The only estrogen detected in the sediments was estrone, present at a concentration of 5.4 μg kg^{-1} at site 11. In contrast, triclosan was detected in all samples, with concentrations ranging from 4.7 to 27.0 μg kg^{-1} (Figure 7), and a mean value of 8.4 ± 5.0 μg kg^{-1} (SD). Methyl-triclosan was detected in several samples at lower concentrations (<11.4 μg kg^{-1}), with the mean value being approximately half of the mean concentration recorded for triclosan (4.1 ± 2.8 μg kg^{-1}). This compound was mostly found in close proximity to the Bolivar outfall (sites 1-5), and in deep sediments (sites D3-D5, and D7-D10) (Figure 7).

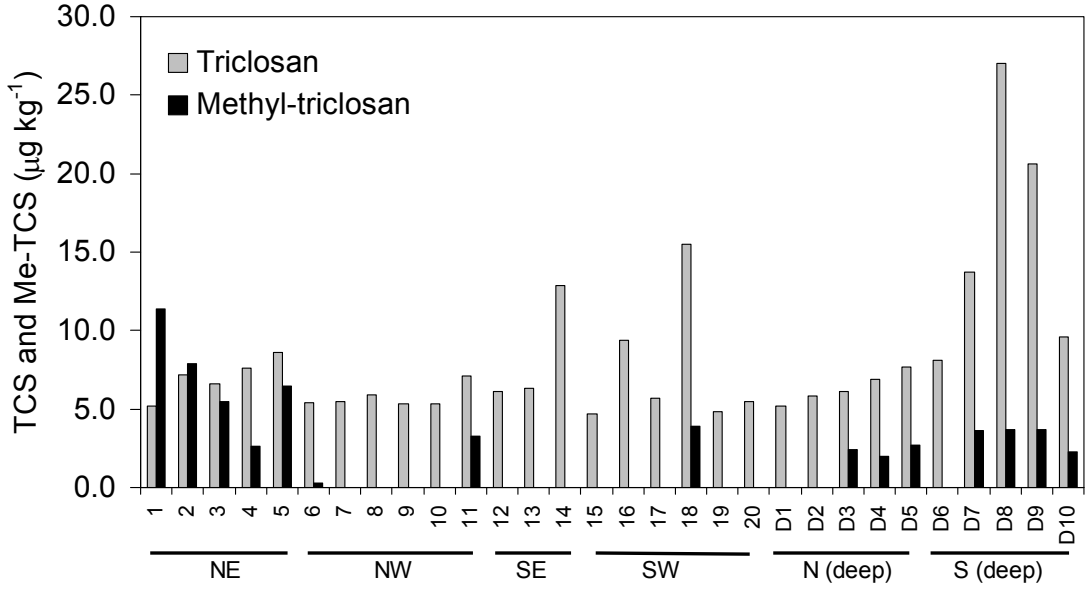

Figure 7. Triclosan and methyl-triclosan content of shallow and deep sediments.

The best physicochemical predictor for triclosan accumulation in the sediments was silt content (Figure 8a). Triclosan also showed some correlation with organic carbon (r^2 = 0.29) and redox potential (r^2 = 0.23). Methyl-triclosan did not co-vary with any of the physicochemical parameters measured, except for carbonate content (Figure 8b).

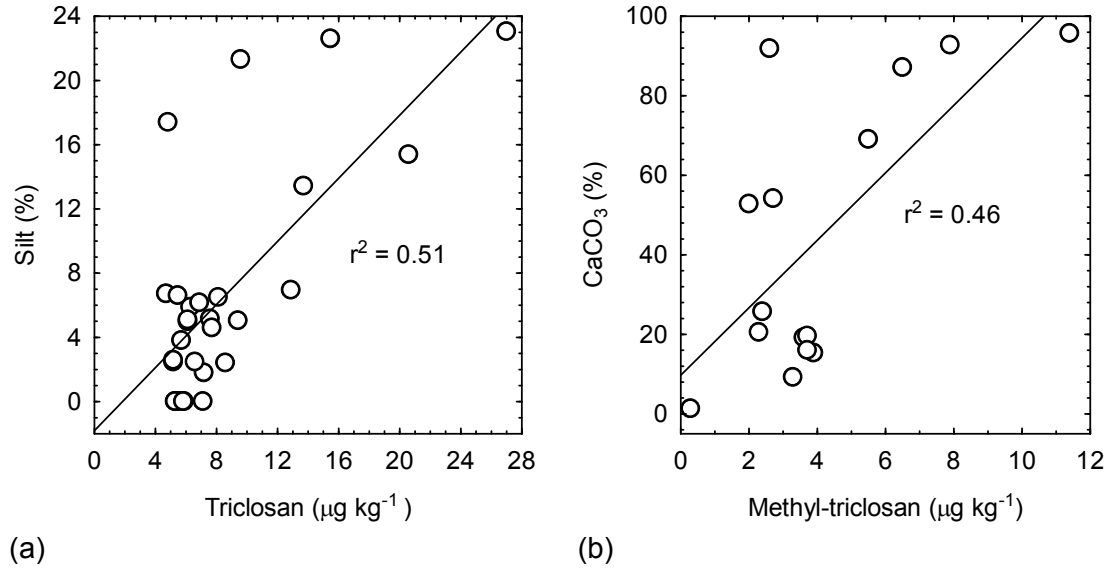

Figure 8. Correlation of triclosan with silt content (a), and of methyl-triclosan with carbonate content (b).

4. DISCUSSION

The separation of sediment types based on physicochemical data was consistent with hydrodynamic patterns for this coastal region (Peter Christy, SA EPA, personal communication). The eastern shores are more exposed to current and wave energy incursion from Gulf St Vincent, and were characterized by coarser and calcareous sediments indicative of a marine origin, with increasing organic carbon accumulation near the Bolivar outfall. The area adjacent to the outfall also showed the highest concentrations of triclosan and methyl-triclosan amongst shallow sediments.

The western shores, in contrast, had finer sediments with lower contents of carbonate, suggesting the preferential deposition of land-derived sediments, particularly towards the south. Depositional centres were evident around the shallow sites 11 and 18, and in deep sites (mostly D7-D10), where fine and organic-rich sediments carry both triclosan and methyl-triclosan.

Site 11 in Section Bank was unique in that it was the only location where a steroid estrogen was detected, with estrone levels equivalent to those measured in an earlier

survey of the area (Fernandes et al. 2008), but higher than detected near a deep ocean outfall near Sydney (Braga et al. 2005b), or in other marine sediments worldwide (generally <1 μg kg^{-1}) (Schlenk et al. 2005; Isobe et al. 2006). Estrone usually dominates the mixture of steroid hormones found in wastewater, and is further formed in the receiving environment as a degradation product of 17β-estradiol (Desbrow et al. 1998; Johnson and Williams 2004; Braga et al. 2005a). The reason why this steroid hormone was found only at this one site in Section Bank is unclear, as is the total area affected.

No other estrogen was found throughout the study area, despite the fact that 17β-estradiol was detected at a concentration of approximately 3 μg kg^{-1} in 6 out of 7 samples surveyed in 2008. There are two inter-related explanations for this discrepancy between the two surveys: (i) lower recoveries associated with the introduction of the SPE clean-up procedure in the present study, (ii) insufficient quantities of sediment extracted. The SPE clean-up procedure was introduced in this study to minimize interference and co-elution with other organic compounds during GC-MS analysis. The concentrations recorded here should therefore be seen as a conservative estimation of contamination, as the low recovery of spiked standards suggests strong matrix sequestration for all of the compounds surveyed, particularly in fine and organic-rich sediments.

The sludge samples had a mean concentration of methyl-triclosan that was less than 10% of the mean value for triclosan (89 versus 1366 μg kg^{-1}), with only traces of estrone. These values are similar to those reported in the literature for sludge samples from WWTPs in Australia, the US and Europe (Ying and Kookana 2007; Combalbert and Hernandez-Raquet 2010). Although we did not analyse any effluent samples from the Bolivar WWTP, available data indicates it carries similar concentrations of triclosan and methyl-triclosan (20-45 ng L^{-1}; Shareef and Kookana, unpublished results), to estrone (20-55 ng L^{-1}), but much lower levels of 17β-estradiol (5-8 ng L^{-1}) and no detectable concentrations of ethinylestradiol (<1 ng L^{-1}) (Holmes et al. in press).

In the sediments, triclosan was present in all samples, at levels (4-27 μg kg^{-1}) comparable to those found in other marine sediments affected by wastewater effluents worldwide, typically <100 μg kg^{-1} (Aguera et al. 2003; Morales-Munoz et al. 2005a; Morales-Munoz et al. 2005b; Miller et al. 2008; Wilson et al. 2008). Methyl-triclosan levels in coastal environments are not available in the current literature, but values for river sediment samples can be substantially higher (up to 450 μg kg^{-1}) than found here (up to 11 μg kg^{-1}) (Heim et al. 2004; Kronimus et al. 2004). The absence

of estrogens in the sediments supports the theory that these compounds are quickly broken down under aerobic conditions (Ying and Kookana 2003).

The fact that methyl-triclosan concentrations in close proximity to the outfall were similar to triclosan, but declined to about 30% or less in sediments further afield, suggests some of the pathways associated with environmental dispersal. The precipitation of organic compounds around the outfall through the change in salinity, the so-called salting out effect (Turner 2003), would explain the higher proportion of methyl-triclosan found here. The comparative decline in other parts of the estuary, however, potentially suggests larger-scale dispersal of small quantities of sludge, accompanying bedload transport of effluent-derived organic compounds deposited near the outfall. The composition observed in deep depositional sites might also reflect *in situ* conditions favourable to the microbial methylation of triclosan (Lindstrom et al. 2002). These dispersal mechanisms would explain the close association of triclosan with silt particles. In contrast, the correlation of methyl-triclosan with carbonate is likely to be spurious, driven by the high levels recorded in the calcareous sediments adjacent to the outfall.

There is a lack of information on the effects of sedimentary concentrations of triclosan and methyl-triclosan on estuarine fauna and flora. Most studies have looked at the toxicological responses to concentrations in water rather than sediment, and for this reason we decided to assess the risk of the compounds investigated here based on dissolved concentrations in porewaters. To estimate dissolved concentrations, we used sedimentary concentrations and organic carbon-water partition coefficients (K_{oc}) (Lindstrom et al. 2002; Campbell et al. 2006; Fernandes et al. 2008; Miller et al. 2008).

The maximum dissolved concentrations for triclosan were calculated to be in the order of 1 $\mu g\ L^{-1}$, a value lower than the threshold for harmful effects to occur in the few marine organisms so far investigated, including some species of phytoplankton (3 $\mu g\ L^{-1}$), bacteria (53 $\mu g\ L^{-1}$) and shrimp larvae (154 $\mu g\ L^{-1}$) (DeLorenzo and Fleming 2008; DeLorenzo et al. 2008; Chalew and Halden 2009). The toxicity data currently available for marine organisms, however, is very limited. Several studies have shown that algae are particularly susceptible to triclosan exposure, and negative effects have been recorded in freshwater species at concentrations as low as 0.015 $\mu g\ L^{-1}$ (Orvos et al. 2002; Wilson et al. 2003). Further studies are therefore necessary to establish if the triclosan levels found in Barker Inlet might be detrimental to benthic and pelagic primary productivity in this important nursery habitat.

There is also limited toxicological information on methyl-triclosan to this date, and the dissolved concentrations calculated here (<0.15 μg L^{-1}) were well below the lowest observed effect concentration for bacteria (75 μg L^{-1})(Farre et al. 2008). Although estrone only occurred at one site, the calculated porewater concentration of 2.1 μg L^{-1} is higher than the threshold for freshwater fish, in the order of 0.06-0.3 μg L^{-1} (Thorpe et al. 2003; Mills and Chichester 2005). This compound is the least estrogenic steroid hormone of the suite investigated (Thorpe et al. 2003).

Based on current toxicological information and the levels recorded here, no clear biological effects can be predicted at the scale of the estuary. However, two important questions remain, (i) are the triclosan levels found in this study detrimental to estuarine primary productivity, and (ii) can localized hotspots of estrone accumulation induce problems in fish living in close association with the sediments. This assessment is based on estimated porewater concentrations, which may not reflect true bioavailability, and the current state of knowledge of toxicological responses. Exposure to sources other than those dissolved in the water column, and the combined effects of exposure to multiple chemicals, are not included. Neither of these factors are well understood, but they should be included in future assessments.

5. CONCLUSIONS AND RECOMMENDATIONS

This limited spatial survey indicated that triclosan, and to a lesser extent, methyl-triclosan, are persistent organic pollutants in sediments of Barker Inlet. In contrast, steroid estrogens were only detected at one site, leading to the assumption that these compounds undergo rapid environmental breakdown, with restricted accumulation in a limited number of hotspots.

Very little information is available on the toxicological response of marine organisms to these compounds. Triclosan has been identified as toxic to freshwater algae at trace levels (ng L^{-1}). Further research is thus necessary to ascertain the risks associated with its accumulation in Barker Inlet, particularly to photosynthetic organisms at the base of the food chain, from phytoplankton to mangroves. The compounds investigated here also have the potential for bioaccumulation, and to cause hormonal disruption, and the effects to the local fauna, including its resident population of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) and seasonal populations of migratory shorebirds, requires further research.

The question remains as to whether 17β-estradiol is widely distributed or not in Barker Inlet. The previous survey, conducted in 2008, indicated widespread

accumulation, whereas in the present study this compound was below the detection limit imposed by the small quantities extracted and the new laboratory method of analysis. We suggest that any further surveys should extract larger quantities of sample and evaluate analytical steps to search and accurately quantify steroid hormones in the area.

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