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Original citation & hyperlink:
Langdon, KA, Warne, MSJ, Smernik, RJ, Shareef, A & Kookana, RS 2011, 'Selected personal care products and endocrine disruptors in biosolids: An Australia-wide survey' Science of the Total Environment, vol. 409, no. 6, pp. 1075-1081. https://dx.doi.org/10.1016/j.scitotenv.2010.12.013

DOI 10.1016/j.scitotenv.2010.12.013
ISSN 0048-9697
ESSN 1879-1026

Publisher: Elsevier

Selected personal care products and endocrine disruptors in biosolids: an Australia-wide survey

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ABSTRACT

Personal care products (PCPs) and endocrine disrupting compounds (EDCs) are groups of organic contaminants that have been detected in biosolids around the world. There is a shortage of data on these types on compounds in Australian biosolids, making it difficult to gain an understanding of their potential risks in the environment following land application of biosolids. In this study, 14 biosolids samples were collected from 13 Australian wastewater treatment plants (WWTPs) to determine concentrations of eight compounds that are PCPs and/or EDCs: 4-t-octylphenol (4tOP), 4-nonylphenol (4NP), triclosan (TCS), bisphenol A (BPA), estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethinylestradiol (EE2). Concentration data were evaluated to determine if there were any differences between samples that had undergone anaerobic or aerobic treatment. The concentration data were also compared to other Australian and international data. Only 4tOP, 4NP, TCS, BPA were detected in all samples and E1 was detected in four of the 14 samples. Their concentrations ranged from 0.05 to 3.08 mg/kg, 0.35 to 513 mg/kg, < 0.01 to 11.2 mg/kg, < 0.01 to 1.47 mg/kg and < 45 to 370 µg/kg, respectively. The samples that were obtained from WWTPs that used predominantly anaerobic treatment showed significantly higher concentrations of the compounds than those obtained from WWTPs that used aerobic treatment. Overall, 4NP, TCS and BPA concentrations in Australian biosolids were lower than global averages (by 42%, 12% and 62%, respectively) and 4tOP concentrations were higher (by 25%), however, of these differences only that for BPA was statistically significant. The European Union limit value for NP in biosolids is 50 mg/kg, which 4 of the 14 samples in this study exceeded.

Keywords: biosolids; estrogens; triclosan; bisphenol A; 4-t-ocylphenol; 4-nonylphenol

1. Introduction

Personal care products (PCPs) and endocrine disrupting compounds (EDCs) are two groups of organic contaminants that have received interest recently due to their potential release into the environment following wastewater treatment and the potential for subsequent environmental risks. Environmental research into PCPs and EDCs has predominantly focussed on their removal from the aqueous phase during wastewater treatment (e.g. Zorita et al., 2009) and their potential deleterious effects to aquatic organisms when released in effluents (e.g. Batty and Lim, 1999; Castro et al., 2007). The removal of PCPs and EDCs from the aqueous phase occurs via degradation, as a result of treatment processes, or through sorption to the solid waste phase, referred to as biosolids. The levels of PCPs and EDCs that are found in biosolids may also be of environmental concern, as in many countries, including Australia, biosolids are applied to land as a supplement or replacement for inorganic fertilisers.

Numerous PCPs and EDCs have been identified in biosolids (Ternes et al., 2002; Braga et al., 2005; Kinney et al., 2006; Chu and Metcalfe, 2007), however, the potential environmental risks that these compounds pose varies. Eight PCPs and EDCs were selected for the current study because of environmental concerns, including their potential to cause adverse impacts to aquatic (Langdon et al., 2010) and/or terrestrial ecosystems (Waller and Kookana, 2009). The compounds selected were the EDCs 4-nonylphenol (4NP), 4-t-octylphenol (4tOP) and bisphenol A (BPA), the antimicrobial agent triclosan (TCS) and the natural and synthetic estrogenic compounds 17β-estradiol (E2), estrone (E1), estriol (E3) and 17α-ethinylestradiol (EE2).

The surfactant metabolites 4NP and 4tOP and the industrial chemical BPA are all compounds that have been found to mimic natural hormones and interfere with estrogen receptors in non-target

organisms (Jobling and Sumpter, 1993; Jobling et al., 1996; Fukuhori et al., 2005). The compound 4NP tends to be very prevalent in biosolids at concentrations ranging from 600 to 438 000 µg/kg (Kinney et al., 2006). This finding is consistent with the widespread use of the parent alkylphenol ethoxylate compounds in many industrial and domestic surfactant products (Ying et al., 2002). In comparison, the parent compounds that ultimately degrade to 4tOP are used to a lesser extent in surfactant products, resulting in lower biosolids concentrations of this compound, with reported concentrations in the range from 167 to 2400 µg/kg (Kinney et al., 2006). The compound BPA, which is used in the production of polycarbonate plastics, epoxy resins and flame retardants (Staples et al., 1998), has been detected in biosolids at a similar range of concentration of 100 – 4600 µg/kg (Kinney et al., 2006).

Triclosan is a commonly used antimicrobial agent found in many domestic personal care products (e.g., soaps, detergents, surface cleaners, disinfectants, cosmetics and other topical personal care products, pharmaceuticals and oral hygiene products), with published concentrations in biosolids ranging from 90 µg/kg (Ying and Kookana, 2007) to 21 740 µg/kg (Campbell-Board, 2005). As TCS is used specifically for its antibacterial properties, its subsequent release into the environment may lead to toxicity to non-target organisms, with a specific risk to micro-organisms. In the recent Targeted National Sewage Sludge Survey (TNSSS), conducted by the United States Environmental Protection Agency (USEPA), TCS was detected in 94% of the samples at concentrations ranging from 0.43 to 133 mg/kg (USEPA, 2009).

The naturally occurring estrogen compound E2, its metabolites E1 and E3, and the synthetic estrogen compound EE2 (the active compound used in the female contraceptive pill) mainly enter the environment via WWTPs, following excretion from humans. These compounds have received

considerable attention recently as they are highly potent compounds and can produce estrogenic responses in non-target organisms at trace concentrations, in the ng/L range (Mills and Chichester, 2005). In the TNSSS, the three naturally occurring estrogens, E1, E2 and E3, were detected in 71%, 13% and 21% of sludge samples, respectively, with the lowest overall concentrations being for E3 (7.6 to 232 µg/kg) and the highest being for E1 (26.7 to 965 µg/kg) (USEPA, 2009). Other published biosolids concentration values for E1 and E2 range from 12 to 150 µg/kg and 0.31 to 49 µg/kg, respectively (Ternes et al., 2002; Braga et al., 2005; Kinney et al., 2006). In comparison, EE2 has been detected in biosolids samples at considerably lower concentrations ranging from 0.42 to 17 µg/kg (Ternes et al., 2002; Braga et al., 2005), and in the TNSSS it was below the limit of detection (LOD) (i.e. < 21 µg/kg) in all samples that were analysed.

The aim of this study was to conduct a survey of Australian biosolids to obtain data on concentrations of 4tOP, 4NP, TCS, BPA, E1, E2, E3 and EE2 and to determine if concentrations varied between WWTPs that used differing treatment processes (i.e. anaerobic or aerobic treatment). In addition, the aim was to compare the concentrations of the selected compounds to previous Australian and global concentration data, as well as threshold limits where available.

2. Materials and methods

2.1. Biosolids sample collection and preparation for analysis

Fourteen different biosolids samples, each collected as four replicates, were obtained between January and March 2009 from 13 WWTPs located in all six Australian states and the Northern Territory. Personnel at each WWTP collected the four replicates in pre-cleaned 250 mL glass jars with Teflon-lined lids. At the time of sampling, the personnel filled out an information sheet providing a description of the treatment processes used on the samples. After collection, all samples

were placed in insulated containers with ice packs and sent by overnight courier to the laboratory where they were immediately placed in a freezer at -18°C. All samples were then freeze dried, homogenised using a mortar and pestle and sieved to < 2 mm.

2.2. Sample extraction and gas chromatography-mass spectrometry analysis

All replicates of the 14 different biosolids samples were extracted and prepared for analysis of the eight target compounds, 4tOP, 4NP, TCS, BPA, E1, E2, E3 and EE2. All glassware used for extraction and preparation of the samples had been pre-cleaned by solvent rinsing and baking at 350°C. One day prior to sample extraction, 1 g of each biosolids sample was weighed into a glass tube (i.e. one tube for each replicate). For quality assurance, one of the replicates from each WWTP was duplicated and a method blank was run with each batch of samples. The method blank was an empty glass tube (i.e. containing no biosolids), which was run through the entire extraction and preparation concurrently with the biosolids samples. This was done to ensure that there was no contamination in any of the solvents or sample preparation steps. Two randomly selected samples from each batch were also spiked with labelled surrogates in methanol (i.e. 4nNP-d_8, TCS-$^{13}C_{12}$, BPA-d_{16}, E1-d_4, E2-d_4, EE2-$^{12}C_2$) that were used to determine recoveries (see Table 1 for details). Following surrogate spiking, samples were left overnight in the dark for extraction the following day. Each sample was extracted three times. Each extraction involved adding 10 mL of a 1:1 mixture of methanol and acetone to the sample and placing it in an ultrasonic bath for 10 minutes. After ultrasonication, the sample was centrifuged at 630 × g for 20 minutes and the supernatant decanted into a 500 mL clean glass amber bottle. The subsequent two supernatants were added to the same amber bottle after extraction and centrifugation. The extracts were diluted to 500 mL with MilliQ (MQ) water and loaded onto Oasis HLB® solid phase extraction (SPE) cartridges which had been preconditioned with 5 mL of methanol and equilibrated with 5 mL of MQ water. Samples

were loaded onto cartridges using a vacuum manifold at a rate of approximately 2 mL/min. Each SPE cartridge was then washed with 5 mL of MQ water and then dried thoroughly under vacuum. The target compounds were eluted off each SPE cartridge using 3 × 2.5 mL methanol, followed by 3 × 2.5 mL acetone and 3 × 2.5 mL ethyl acetate. Each eluted sample was then blown to dryness under a gentle stream of N_2 gas and reconstituted in 4 mL of methanol. From the 4 mL sample, a 1 mL subsample was taken and blown to dryness using N_2 gas. The sample was then reconstituted in 400 µL of pyridine and 100 µL of the silylation agent *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) + 1% trimethyl-chlorosilane (TMCS) and placed on a dry heating block at 75°C for 1 h (based on the method of Shareef et al., 2006). This process induces a reaction that converted all target compounds to their respective trimethylsilyl derivatives to increase their suitability for analysis using gas chromatography (GC). Following the derivatization, anthracene d_{10} was added to each sample as an instrument internal standard (IS) prior to GC analysis.

For analysis, 2 µL of each sample was injected into an Agilent 6890 Series GC system, fitted with a DB-5MS (30 m × 0.25 mm internal diameter) capillary column with a 0.25 µm film thickness, that was interfaced with an Agilent 5973 Network Mass Spectrometer (MS). The oven temperature was held at 75°C for 1 minute, ramped at 10°C / minute to 150°C, then at 15°C / minute to 280°C and held at this temperature until the completion of the run time of 32 minutes. Helium was used as the carrier gas at a linear flow rate. The MS was operated in electron impact ionisation (EI) mode at 70 eV. Table 1 shows the typical retention times of each of the compounds and the target ion and qualifier ions used for quantification. The relative response factors, which were determined based on the IS, were used to determine the concentrations of each of the compounds in the samples. If the concentrations were outside of the linear range of the calibration curve for each compound, the sample was diluted until they were within the linear calibration range. All samples were adjusted for

extraction recoveries based on the concentration of the labelled surrogates in the previously spiked samples. Table 1 indicates which labelled surrogates were used for the recovery adjustment of each target compound. The limit of detection (LOD) and limit of quantification (LOQ) for each of the compounds were determined as 3-times and 10-times the signal to noise ratio, respectively, and are reported in Table 1.

2.3. Data interpretation and statistical analysis

The concentrations obtained for each replicate were used to determine the average and range of concentrations of each of the target compounds from the different biosolids samples. For samples where a compound was not above the LOD in all replicates, only the replicates with detectable concentrations were used to determine the average values.

To assess differences in concentrations of compounds based on variations in treatment processes, the biosolids samples were divided into two categories: (i) those that had undergone predominantly anaerobic treatment and; (ii) those that had undergone predominantly aerobic treatment (where aerobic treatment included aerobic digestion, aerobic bioreactors and dissolved air floatation). Statistical differences between the two groups were determined by conducting a repeated measures general linear model (GLM) using PASW® Statistics 17 on the concentration data following a logarithm to base 10 transformation. The repeated measures factor used was the target compounds (i.e. each of the compounds that were detected in the samples), with the independent variable being the treatment type (i.e. anaerobic or aerobic). The repeated measures GLM identified if there was a significant main effect ($p < 0.05$) of the treatment type on the concentrations of the compounds overall and a significant ($p < 0.05$) interaction of treatment by compound.

3. Results and discussion

Table 2 summarises selected characteristics of each of the biosolids samples collected for this study, including the duration between completion of biosolids treatment and sampling ("age"), the estimated population serviced by the WWTP where the sample was collected, the location (capital city or regional centre) of the WWTP and a brief description of the biosolids treatment processes. Nine of the samples obtained were collected immediately following completion of the biosolids treatment and this is indicated as an age of < 1 day. A further three biosolids samples were aged ≤ 30 days (samples A, B and J) and two samples had been stockpiled on site prior to collection (sample D for one year and sample E for 3-6 years). The estimated population sizes for each of the WWTPs from which samples were obtained ranged from 20 000 to 1.3 million people and there were equal numbers of samples obtained from WWTPs that were located in capital cities and regional centres. A range of aerobic and anaerobic treatment processes were used and a range of drying processes, including belt filter presses, centrifuges and solar drying (e.g. lagoon systems and drying pans). It should be noted that samples D and F were both obtained from the same WWTP and had undergone the same treatment and drying process, with the only difference being that sample D had been stockpiled for one year and sample F was collected immediately.

3.1. Data quality assurance and extraction recoveries

The method blanks run with each batch of biosolids samples were below detection for all of the compounds except for 4NP. The concentrations of 4NP in the method blank varied between each run, however ranged from approximately 50 to 200 µg/L in the final solution. These background concentrations of 4NP were subtracted from each of the samples prior to the concentrations being converted to µg/kg. The variation between each of the duplicated samples in the majority of cases

was less than 25%. The variation between duplicates was the lowest for 4NP, ranging up to 15%, whereas it was the highest for 4tOP, ranging up to 38%.

The extraction recovery values that were obtained from the spiked surrogate compounds varied considerably between the different biosolids samples, however, the variation between replicates of the same sample was low (all of the recovery data from this study are shown in Supplementary material Table S1). Overall, the recovery values ranged from 76 to 352% for 4nNP-d_8, 45 to 314% for TCS-$^{13}C_{12}$, 55 to 359% for BPA-d_{16}, 10 to 283% for E1-d_4, 27 to 295% for E1-d_4 and 120 to 382% for EE2-$^{13}C_2$, with average recoveries of 180%, 125%, 125%, 111%, 156% and 230%, respectively. In several cases, for the labelled estrogen compounds (i.e. E1-d_4 and E2-d_4 from sample G and EE2-$^{13}C_2$ from sample G and I), recovery values could not be determined as the concentrations were below the detection limit. Although some of the recovery values obtained in this study are high, a similar range of recoveries was observed in a recent survey of pharmaceuticals and PCPs in biosolids conducted in the United States (McClellan and Halden, 2010), which reported recovery values ranging from 12 to 493%. The high recoveries seen in the current study are likely to be a result of the effect of the complex biosolids matrix on the GC-MS response of the analytes and the internal standard (i.e. anthracene-d_{10}). As these differences will be the same for the labelled surrogates and the analytes, this effect is removed when the concentrations are adjusted for recovery.

3.2. Concentration in biosolids

The estrogen compounds, E2, E3 and EE2 were below the LOD (i.e. 45 µg/kg) in all replicates of all samples. In all 14 biosolids samples, concentrations of 4tOP, 4NP, TCS and BPA were above the LOD, whereas, E1 concentrations were above the LOD in only four samples (F, H, J and L). The

averages and ranges of these concentrations are summarised in Table 3. The concentrations of the compounds ranged from 0.05 to 5.35 mg/kg for 4tOP, 0.35 to 513 mg/kg for 4NP, < 0.03 (i.e., < LOD) to 11.2 mg/kg for TCS, < 0.03 (i.e., < LOD) to 1.47 mg/kg for BPA and < 0.045 (i.e. < LOD) to 0.37 mg/kg for E1 (Table 3). It should be noted, however, that the concentration data provided in Table 3 for E1, for samples H, J and L are below the LOQ for this compound and should therefore only be used as an indication of the concentrations of E1 in these samples.

The variation within the replicates from each sample was reasonably low (in 75% of cases, the relative standard deviation, RSD, was ≤ 20%), however, there was considerable variability in concentrations between the different biosolids samples. TCS showed the lowest variation between different biosolids samples with a RSD of 84%, whereas 4NP showed the highest variation with a RSD of 208%. For 4tOP, 4NP, TCS and BPA, samples F, G and I overall had the highest concentrations, whereas sample E was consistently low.

Overall, concentrations of 4NP were considerably higher than all the other compounds, with an average concentration of 58.7 mg/kg. This average 4NP concentration is approximately 16-times higher than the next most concentrated compound TCS, which had an average of 3.77 mg/kg. The high concentrations of 4NP measured in biosolids in this study are probably due to the high domestic and industrial use of the parent nonylphenol ethoxylate (NPE) surfactant compounds (Ying et al., 2002).

The concentrations of the compounds were compared between samples D and F to determine the effect of stockpiling on the final concentrations in the biosolids. The compounds 4tOP, 4NP, TCS and BPA, which were detected in both samples, showed considerably lower concentrations in the

stockpiled biosolids compared to the non-stockpiled biosolids. This difference in concentration was the greatest for the compounds 4tOP and BPA, where the average concentration in the non-stockpiled biosolids was approximately 10-times higher than in the stockpiled biosolids. The difference was not as great for the compounds 4NP and TCS, where it was approximately 6-times higher in the non-stockpiled biosolids. In addition, the estrogen metabolite compound E1 was detected in the non-stockpiled biosolids sample at an average concentration of 280 µg/kg, whereas in the stockpiled biosolids this compound was below the LOD of 45 µg/kg. These differences are likely due to an increase in degradation of the compounds over time, however, may also be due in part to different initial concentrations of these compounds, as although the WWTP and treatment processes were the same, these were different samples in terms of the timing that they entered the WWTP.

The samples were divided into two categories based on whether they had undergone predominantly anaerobic or aerobic treatment and the resulting average concentrations for the four compounds detected in all samples (i.e., 4tOP, 4NP, TCS and BPA) are shown in Figure 1. The repeated measures GLM using the four compounds produced a highly significant ($p < 0.0005$) main effect of treatment type on the concentrations of these compounds, where concentrations were higher in the anaerobically treated biosolids than the aerobically treated biosolids. This result is consistent with other research that indicates that these compounds show minimal degradation under anaerobic conditions (e.g. Brunner et al., 1988; McAvoy et al., 2002; Press-Kristensen et al., 2008). There was also a highly significant interaction ($p = 0.004$) between treatment and compound, as the magnitude of this difference varied between the compounds, indicating the aerobic treatment has a more pronounced effect on some compounds compared to others. The differences in concentration between the treatments were more evident for 4tOP and 4NP which showed concentrations that

were 36% and 27% lower, respectively, in the aerobically treated samples than the anaerobically treated samples. In contrast, for TCS and BPA, the concentrations were only 14% and 17% lower, respectively, in the aerobically treated biosolids samples.

3.3. Comparisons with Australian and global data

Apart from TCS, there is limited data on the concentrations of the compounds measured in this study in Australian biosolids. A study measuring concentrations of TCS in Australian biosolids was conducted by Ying & Kookana, 2007, where samples were collected in 2004 and 2005 from 19 WWTPs across 4 states (South Australia, Queensland, Western Australian and Victoria) and the Australian Capital Territory. Due to the WWTPs that provided the biosolids not being identified in either the current study or that of Ying & Kookana, 2007, direct comparisons were not possible between individual WWTPs, however, overall comparisons can be made. The study by Ying & Kookana, 2007 measured TCS at concentrations ranging from 0.09 to 16.8 mg/kg with average and median concentrations of 5.6 mg/kg and 2.3 mg/kg, respectively. In the current study, the median TCS concentration was similar at 2.7 mg/kg, indicating there is little difference in the range of concentrations measured for TCS. However, both the upper limit of the concentration range and the average concentration were lower in the current study at 12.2 mg/kg and 3.8 mg/kg, respectively.

The results from this study can also be compared with the range of concentrations for these compounds that have been measured in biosolids samples globally. Figure 2 shows the average and standard error of the concentrations for each of the four compounds detected in biosolids samples A to N and these are compared to the average values for each compound globally (for sources of the global data, see Langdon et al., 2010). From Figure 2 it can be seen that 4NP, TCS and BPA in this study all have lower average concentrations than the global average. TCS had the smallest

difference, being 12% lower than the global average, whereas the differences for 4NP and BPA were much larger at 42% and 62%, respectively. 4tOP was the only compound whose average was higher (by 25%) than the global average. These differences were only significant for BPA ($p = 0.04$), whereas for all other compounds, there was no significant difference between the average from this study and that from the global data (all p-values > 0.32).

The estrogen metabolite compound E1 was detected in four samples (F, H, J and L) in this study, with concentrations of the replicates ranging from 50 to 370 µg/kg, whereas all other samples were below the LOD of 45 µg/kg. The concentrations of E1 measured in sample F are higher than the concentrations measured by Kinney et al., 2006 where the maximum concentration reported was 150 µg/kg. The concentrations of E1 measured in the current study, however, are within the range of E1 concentrations in biosolids from across the USA in the more recent TNSSS study, which reported concentrations ranging from 26 to 965 µg/kg (USEPA, 2009). The other natural estrogen compounds E2 and E3 and the synthetic estrogen compound EE2 were below the LOD (45 µg/kg) in all samples analysed in the current study. The maximum concentrations of E2 and E3 that were detected in the TNSSS were 355 µg/kg and 232 µg/kg (USEPA, 2009), respectively, which are only marginally higher than the LOQ for the method used in this study. The synthetic estrogen EE2 was not detected in any samples analysed in the TNSSS, however it has been detected elsewhere up to a concentration of 17 ug/kg (Ternes et al., 2002), which is considerably lower than both the LOQ and LOD for EE2 for the method used in the current study (Table 1). These results indicate that in order to obtain significant datasets for estrogens in Australian biosolids a method with increased sensitivity is required.

Currently, the eight compounds that were analysed for as part of this study (4tOP, 4NP, TCS, BPA, E1, E2, EE2 and E3) do not require monitoring in Australian biosolids (EPA NSW, 1997; SA EPA, 1997; DPIWE, 1999; WA DEP, 2002; EPA Victoria, 2004; NRMMC, 2004), nor are there any maximum permissible concentrations in Australian soils for these compounds. This is also generally the case internationally, with the exception of 4NP in the European Union (EU). The EU Working Document on Sludge (EU, 2000) has set a limit value for nonylphenol ethoxylates (NPEs), which comprises the compounds nonylphenol and nonylphenolethoxylates (with 1 or 2 ethoxy groups), of 50 mg/kg. Four of the fourteen biosolids sampled in this study (samples B, F, G and I) exceed this EU limit value for NPE in sludge used on land. The high levels of 4NP present in samples B, F, G and I may be partly due to these samples being collected from WWTPs located in capital cities and therefore having high input of industrial chemicals.

4. Conclusion

Fourteen biosolids samples were collected from 13 WWTPs across Australia to determine levels of eight selected personal care products and endocrine disrupting chemicals (i.e., 4-t-octylphenol, 4tOP; 4-nonylphenol, 4NP; triclosan, TCS; bisphenol A, BPA; estrone, E1; 17β-estradiol, E2; estriol, E3; and 17α-ethinylestradiol, EE2). The estrogen compounds E2, EE2 and E3 were below detection in all of the samples, whereas E1 was detected in four of the 14 samples. 4tOP, 4NP, TCS and BPA were detected in all 14 biosolids samples, with 4NP detected at the highest concentrations (average of 58.7 mg/kg) and BPA at the lowest concentrations (average of 0.47 mg/kg). The samples that were obtained from WWTPs that used predominantly anaerobic treatment had significantly higher concentrations of 4tOP, 4NP, TCS and BPA than those obtained from WWTPs

that used aerobic treatment. The average concentrations of 4tOP, 4NP and BPA were lower in the current study than those that have been measured globally, whereas, the average concentration of 4tOP was higher. These differences were only significant for BPA. In four of the samples analysed in this study, the concentrations of 4NP exceeded the EU threshold limit for NPEs in sludge used in land application. The information generated in this study will assist with future hazard and risk assessments and the management of organic contaminants in biosolids.

Acknowledgements

The principal author (KL) wishes to acknowledge the financial support of Water Quality Research Australia (WQRA) (formerly the Co-operative Research Centre for Water Quality and Treatment – CRCWQT), Western Australia Water Corporation, Victorian Department of Human Services and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The authors would also like to thank Judy Blackbeard and all participating Water Authorities for providing samples for this study.

References

Batty J, Lim R. Morphological and reproductive characteristics of male mosquitofish (*Gambusia affinis holbrooki*) inhabiting sewage-contaminated waters in New South Wales, Australia. Arch. Environ. Contam. Toxicol. 1999; 36: 301-307.

Braga O, Smythe G, Schafer A, Feitz A. Fate of steroid estrogens in Australian inland and coastal wastewater treatment plants. Environ. Sci. Technol. 2005; 39: 3351-3358.

Brunner PH, Capri S, Marcomini A, Giger W. Occurrence and behavior of linear alkylbenzenesulfonates, nonylphenol, nonylphenol monophenol and nonylphenol diethoxylates in sewage and sewage-sludge treatment. Water Res. 1988; 22: 1465-1472.

Campbell-Board M. Cross-departmental research project: Removing the roadblocks to the beneficial reuse of biosolids and treated effluent, phase two: Sampling program. Water Solutions Ltd, Dunedin, New Zealand. 2005.

Castro LFC, Melo C, Guillot R, Mendes I, Queiros S, Lima D, Reis-Henriques MA, Santos MM. The estrogen receptor of the gastropod *Nucella lapillus*: Modulation following exposure to an estrogenic effluent? Aquat. Toxicol. 2007; 84: 465-468.

Chu SG, Metcalfe CD. Simultaneous determination of triclocarban and triclosan in municipal biosolids by liquid chromatography tandem mass spectrometry. J Chromatogr. A 2007; 1164: 212-218.

DPIWE (Department of Primary Industry, Water and Environment). Tasmanian biosolids reuse guidelines. DPIWE, Hobart, Australia. 1999.

EPA NSW (Environmental Protection Authority New South Wales). Environmental guidelines: Use and disposal of biosolids products, NSW EPA, Sydney, Australia. 1997.

EPA (Environmental Protection Authority) Victoria. Guidelines for environmental management. Biosolids land application. Publication 943, EPA Victoria, Melbourne, Australia. 2004.

EU (European Union). Working document of sludge, 3rd draft. European Union, Brussels. 2000.

Fukuhori N, Kitano M, Kimura H. Toxic effects of bisphenol A on sexual and asexual reproduction in *Hydra oligactis*. Arch. Environ. Contam. Toxicol. 2005; 48: 495-500.

Jobling S, Sumpter JP. Detergent components in sewage effluent are weakly estrogenic to fish – an in-vitro study using rainbow-trout (*Oncorhynchus mykiss*) hepatocytes. Aquat. Toxicol. 1993; 27: 361-372.

Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environ. Toxicol. Chem. 1996; 15: 194-202.

Kinney CA, Furlong ET, Zaugg SD, Burkhardt MR, Werner SL, Cahill JD, Jorgensen GR. Survey of organic wastewater contaminants in biosolids destined for land application. Environ. Sci. Technol. 2006; 40: 7207-7215.

Langdon KA, Warne MStJ, Kookana RS. Aquatic hazard assessment for pharmaceuticals, personal care products and endocrine disrupting compounds from biosolids-amended land. Integrated Environmental Assessment and Management. 2010; 6: 663-676.

McAvoy DC, Schatowitz B, Jacob M, Hauk A, Eckhoff WS. Measurement of triclosan in wastewater treatment systems. Environ. Toxicol. Chem. 2002; 21: 1323-1329.

McClellan K, Halden RU. Pharmaceuticals and personal care products in archived US biosolids from the 2001 EPA national sewage sludge survey. Water Res. 2010; 44: 658-668.

Mills LJ, Chichester C. Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? Sci. Total Environ. 2005; 343: 1-34.

NRMMC (National Resource Management Ministers Council). Guidelines for sewerage systems – Biosolids management. National Water Quality Management Strategy Paper No 13, NRMMC, Canberra, Australia. 2004.

Press-Kristensen K, Lindblom E, Schmidt JE, Henze M. Examining the biodegradation of endocrine disrupting bisphenol A and nonylphenol in WWTPs. Water Sci. Technol. 2008; 57: 1253-1256.

SA EPA (South Australian Environment Protection Authority). South Australian biosolids guidelines for the safe handling, reuse or disposal of biosolids. SA EPA, Adelaide. 1997.

Shareef A, Angove MJ, Wells JD. Optimization of silylation using N-methyl-N-(trimethylsilyl)-trifluoroacetamide, N,O-bis-(trimethyl.silyl)-trifluoroacetamide and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide for the determination of the estrogens estrone and 17 alpha-ethinylestradiol by gas chromatography-mass spectrometry. J. Chromatogr. A 2006; 1108: 121-128.

Staples CA, Dorn PB, Klecka GM, O'Block ST, Harris LR. A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 1998; 36: 2149-2173.

Ternes TA, Andersen H, Gilberg D, Bonerz M. Determination of estrogens in sludge and sediments by liquid extraction and GC/MS/MS. Anal. Chem. 2002; 74: 3498-3504.

USEPA (United States Environmental Protection Agency). Targeted national sewage sludge survey and analysis technical report, EPA-822-R-08-016. U.S. Environmental Protection Agency, Washington, USA. 2009.

WA DEP (Western Australia Department of Environmental Protection). Western Australian guidelines for direct application of biosolids and biosolid products. Department of Environmental Protection, Perth, WA, Australia. 2002.

Waller NJ, Kookana RS. Effect of triclosan on microbial activity in Australian soils. Environ. Toxicol. Chem. 2009; 28: 65-70.

Ying GG, Williams B, Kookana R. Environmental fate of alkylphenols and alkylphenol ethoxylates - a review. Environ. Int. 2002; 28: 215-226.

Ying GG, Kookana RS. Triclosan in wastewaters and biosolids from Australian wastewater treatment plants. Environ. Int. 2007; 33: 199-205.

Zorita S, Martensson L, Mathiasson L. Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. Sci. Total Environ. 2009; 407: 2760-2770.

List of Figures

Figure 1

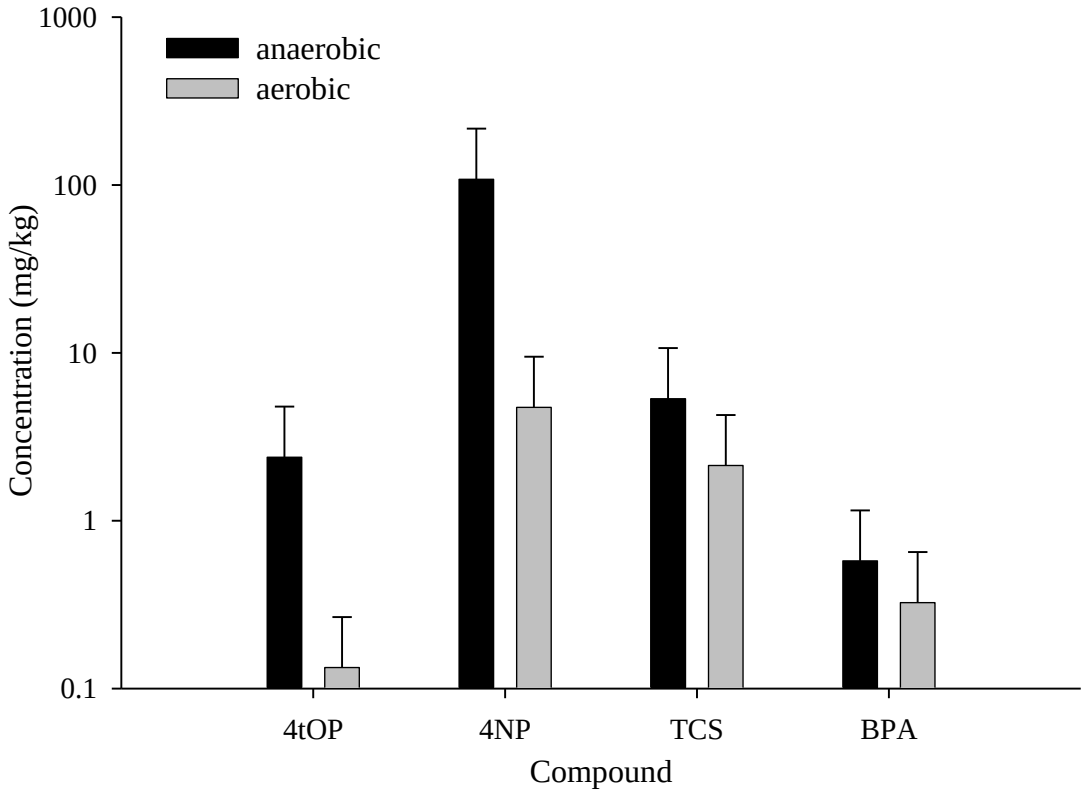
1000
100
10
1
0.1
Concentration (mg/kg)
anaerobic
aerobic
4tOP
4NP
TCS
BPA
Compound

Figure 2

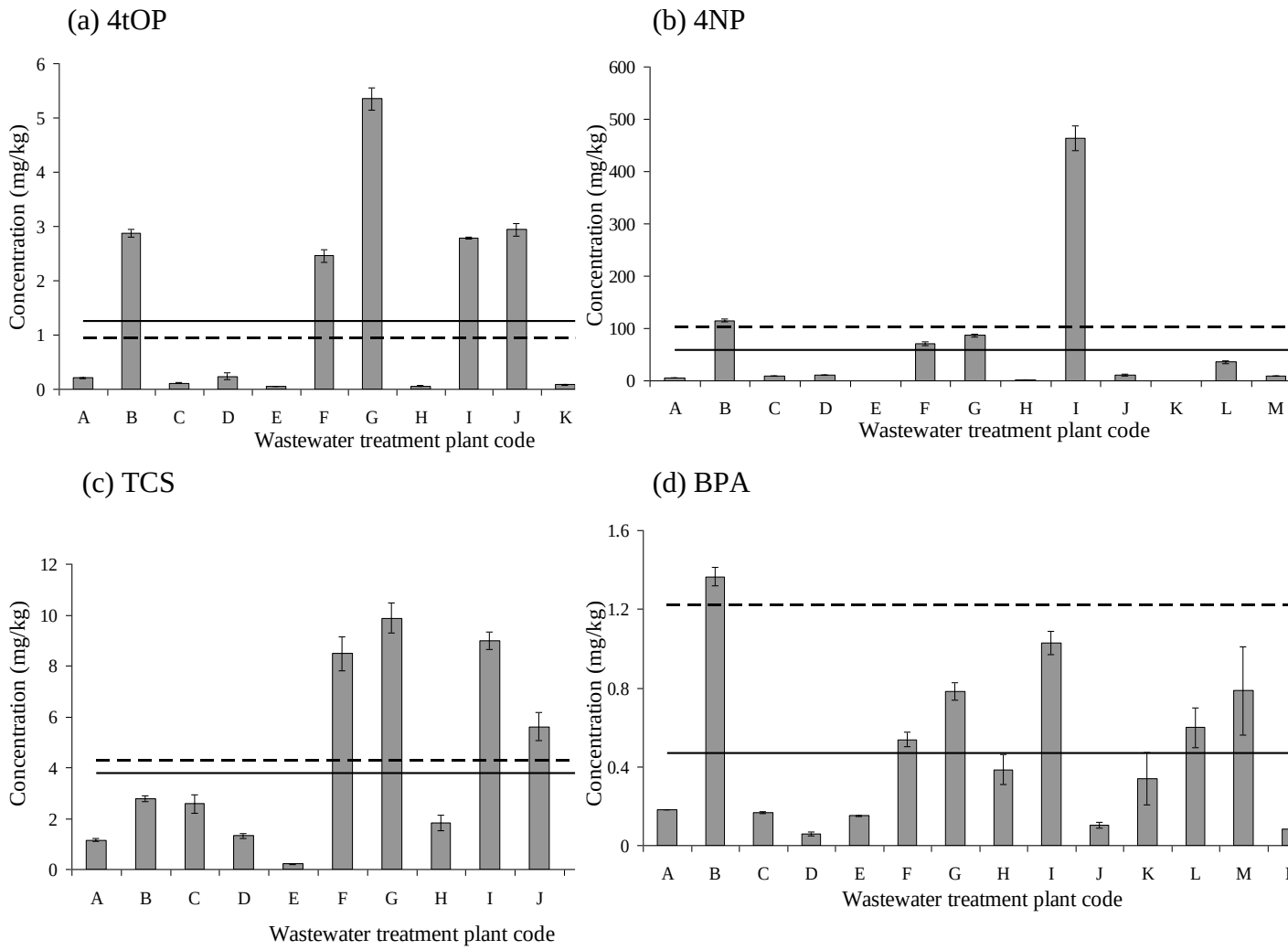
(a) 4tOP
Concentration (mg/kg)
0
1
2
3
4
5
6
A B C D E F G H I J K
Wastewater treatment plant code
(b) 4NP
Concentration (mg/kg)
0
100
200
300
400
500
600
A B C D E F G H I J K L M
Wastewater treatment plant code
(c) TCS
Concentration (mg/kg)
0
2
4
6
8
10
12
A B C D E F G H I J
Wastewater treatment plant code
(d) BPA
Concentration (mg/kg)
0
0.4
0.8
1.2
1.6
A B C D E F G H I J K L M
Wastewater treatment plant code

Table 1: Typical retention times for the internal standard, labelled surrogates and target compounds using gas chromatography mass spectrometry (GCMS) and the corresponding level of detection (LOD) and level of quantification (LOQ).

Compound type	**Compound name**	**Retention time (min)**	**Quantitation ion (*m*/*z*)**	**Qualifier ion 1 (*m*/*z*)**	**Qualifier ion 2 (*m*/*z*)**	**Qualifier ion 3 (*m*/*z*)**	**LOD (µg/kg)**	**LOQ (µg/kg)**
Internal standard	Anthracene d_{10}	12.31	188	158	94	—		
Labelled surrogates	4nNP-d_8	13.11	185	300	285	—		
	TCS-$^{13}C_{12}$	14.52	206	357	372	322		
	BPA-d_{16}	14.98	368	386	217	—		
	E1-d_4	17.43	346	220	261	246		
	E2-d_4	17.63	420	287	234	220		
	EE2-$^{13}C_2$	18.37	427	232	442	272		
Compounds	4tOP[a]	10.89	207	263	278	—	10	30
	4NP[a]	12.09	207	221	193	179	55	180
	TCS[b]	14.47	200	347	362	310	10	30
	BPA[c]	15.02	357	372	191	—	10	30
	E1[d]	17.43	342	257	244	218	45	150
	E2[e]	17.63	416	285	327	232	45	150
	EE2[f]	18.37	425	285	300	440	45	150
	E3[e]	18.97	311	345	504	386	45	150

Superscripts indicate for each analyte the labelled surrogate that was used to determine recoveries: [a] 4nNP-d_8; [b] TCS-$^{13}C_{12}$; [c] BPA-d_{16}; [d] E1-d_4; [e] E2-d_4; [f] EE2-^{13}C

Table 2: Summary of information collected about the age of the biosolids samples, the waste water treatment plants (WWTPs) that they were collected from, the population size serviced by each WWTP, the location of the WWTP and a brief description of the treatment processes used.

Sample	Age[a] (days)	Population	WWTP location[b]	Treatment description
A	30	45 000	regional	aerobic sludge digestion; dewatered by gravity drainage and then belt filter press
B	17	210 000	capital	belt filter press and thermal hydrolysis; anaerobically digested; centrifuged
C	< 1	70 000	regional	extended aeration; bioreactors (with anoxic and aerobic zones) thickening; dewatered using belt filter press
D	1 yr	1 200 000	capital	activated sludge thickened; anaerobically digested; dried in sludge drying pans (lined with clay)
E	3-6 yrs	40 000	regional	anaerobically digested sludge; dewatered primary lagoon sludge, stockpiled
F	< 1	1 200 000	capital	activated sludge thickened; anaerobically digested; dried in sludge drying pans
G	< 1	24 000	capital	anaerobically digested sludge; dewatered using belt filter press
H	< 1	20 000	regional	aerobic activated sludge treatment; dewatered using polymer and passed over primary belt, lime added
I	< 1	1 300 000	capital	activated sludge; anaerobically digested, dewatered using centrifuge
J	7	135 000	regional	activated sludge; anaerobically digested, dried using lagoon system
K	< 1	na	regional	liquid waste pumped from waste stabilisation ponds; dissolved air flotation tanks used to separate solids
L	< 1	40 000	capital	lime amended, chemically assisted settling of solids, pumped through drum filters
M	< 1	350 000	capital	thickened in dissolved air flotation tanks; mixed with raw sludge; centrifuged; lime added
N	< 1	52 000	regional	aerobically digested sludge; dewatered using belt filter press

[a] duration of time after the completion of treatment that the sample was collected

[b] location of the WWTP in a capital city or a regional centre in Australia

na information not available

Table 3: Summary of concentration data for each of the compounds that were above the limit of detection (LOD). Data shown as an average of the four replicate samples with the range in parentheses

Sample	**Concentration (mg/kg)**				
	4tOP	**4NP**	**TCS**	**BPA**	**E1**
A	0.21 (0.19-0.22)	5.28 (4.92-5.64)	1.15 (1.05-1.25)	0.18 (0.18-0.19)	< LOD
B	2.88 (2.74-3.08)	114 (109-122)	2.77 (2.44-2.93)	1.37 (1.27-1.47)	< LOD
C	0.11 (0.10-0.12)	9.69 (9.18-10.1)	2.57 (2.03-3.60)	0.17 (0.16-0.18)	< LOD
D	0.24 (0.13-0.39)	10.8 (8.83-12.3)	1.32 (1.13-1.48)	0.06 (0.04-0.09)	< LOD
E	0.06 (0.05-0.06)	0.84 (0.67-1.01)	0.22 (0.15-0.29)	0.15 (0.15-0.16)	< LOD
F	2.46 (2.18-2.71)	70.1 (60.9-87.2)	8.49 (7.02-10.2)	0.54 (0.48-0.64)	0.28 (0.17-0.37)
G	5.35 (4.78-5.73)	87.1 (79.7-91.1)	9.89 (8.64-11.2)	0.78 (0.71-0.90)	< LOD
H	0.06 (0.06-0.07)	1.88 (1.70-2.03)	1.83 (1.38-2.76)	0.39 (0.29-0.61)	0.07* (<LOD-0.08)
I	2.78 (2.75-2.83)	464 (418-513)	8.99 (8.18-9.87)	1.03 (0.91-1.13)	< LOD
J	2.94 (2.76-2.89)	10.2 (8.01-15.1)	5.62 (5.02-7.31)	0.10 (0.09-0.14)	0.10* (0.08-0.13)
K	0.09 (0.07-0.11)	0.48 (0.35-0.60)	0.29 (<LOD-0.29)	0.34 (<LOD-0.67)	< LOD
L	0.11 (0.10-0.13)	36.3 (31.9-43.4)	2.74 (2.60-3.01)	0.60 (0.33-0.76)	0.06* (0.05-0.07)
M	0.25 (0.24-0.27)	8.94 (7.57-10.1)	4.74 (3.10-5.47)	0.79 (0.03-1.11)	< LOD
N	0.08 (0.07-0.11)	2.12 (2.05-2.22)	2.19 (2.07-2.34)	0.08 (0.07-0.10)	< LOD
average	1.26	58.7	3.77	0.47	0.13

* sample below limit of quantification (LOQ)

Supplementary Material

Table S1: Recovery values for the labelled surrogate compounds, 4-n-nonylphenol-d_8 (4nNP-d_8), triclosan-$^{13}C_{12}$ (TCS-$^{13}C_{12}$), bisphenol A-d_{16} (BPA-d_{16}), estrone-d_4 (E1-d_4), 17β-estradiol-d_4 (E2-d_4) and 17α-ethinylestradiol-$^{13}C_2$ (EE2-$^{13}C_2$) in the 14 biosolids samples (A to N). All recoveries are shown as percentages and the values from two spiked replicates are shown. Missing values indicate samples where recoveries could not be determined as concentrations were below detection limits.

Sample	**4nNP-d_8**		**TCS-$^{13}C_{12}$**		**BPA-d_{16}**		**E1-d_4**		**E2-d_4**		**EE2-$^{13}C_2$**	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
A	84	76	314	218	125	113	106	112	128	121	347	336
B	133	132	199	131	113	105	120	101	134	161	382	358
C	124	109	126	140	119	127	109	101	156	154	360	352
D	124	128	59	57	61	56	72	74	120	154	120	163
E	112	120	89	89	102	114	245	283	136	146	136	146
F	174	173	45	55	55	60	68	58	173	147	202	154
G	182	169	193	226	159	140	-	-	-	-	-	-
H	197	211	163	154	159	177	100	93	154	142	154	142
I	198	182	63	68	106	140	9.6	10	27	27	-	-
J	127	109	94	79	78	72	154	135	295	253	189	157
K	158	172	68	91	98	89	129	137	181	189	495	214
L	352	320	199	177	323	359	200	199	173	185	152	149
M	299	298	91	102	141	149	32	42	148	148	158	148
N	245	351	98	102	87	81	96	108	187	207	251	258