Estrogenic activity, chemical levels and health risk assessment of municipal distribution point water from Pretoria and Cape Town, South Africa

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Highlights

• Estrogenic activity was detected in Pretoria and Cape Town drinking water.

• Estrogens, bisphenol-A and phthalates were detected in distribution point water.

• Distribution point water is associated with acceptable health and carcinogenic risks.

Abstract:

Endocrine disrupting chemicals (EDCs) are ubiquitous in the environment and have been detected in drinking water from various countries. Although various water treatment processes can remove EDCs, chemicals can also migrate from pipes that transport water and contaminate drinking water. This study investigated the estrogenic activity in drinking water from various distribution points in Pretoria (City of Tshwane) (n=40) and Cape Town (n=40), South Africa, using the recombinant yeast estrogen screen (YES) and the T47D-KBluc reporter gene assay. The samples were collected seasonally over four sampling periods. The samples were also analysed for bisphenol A (BPA), nonylphenol (NP), di(2-ethylhexyl) adipate (DEHA), dibutyl phthalate (DBP), di(2-ethylhexyl) phthalate (DEHP), diisononylphthalate (DINP), 17β-estradiol (E_2), estrone (E_1) and ethynylestradiol (EE_2) using ultra-performance liquid chromatography-tandem mass spectrophotometry (UPLC-MS/MS). This was followed by a scenario based health risk assessment to assess the carcinogenic and toxic human health risks associated with the consumption of distribution point water. None of the water extracts from the distribution points were above the detection limit in the YES bioassay, but the EEq values ranged from 0.002 to 0.114 ng/L using the T47D-KBluc bioassay. BPA, DEHA, DBP, DEHP, DINP E_1, E_2, and EE_2 were detected in distribution point water samples. NP was below the

detection limit for all the samples. The estrogenic activity and levels of target chemicals were comparable to the levels found in other countries. Overall the health risk assessment revealed acceptable health and carcinogenic risks associated with the consumption of distribution point water.

Keywords:

Endocrine disrupting chemicals (EDCs); estrogenic activity; bisphenol-A; estrogens; phthalates; health risk assessment

Abbreviations:

ADD	Average daily dose
β	Oral potency factor
BPA	Bisphenol A
BW	Body weight
C_{medium}	Concentration of substance in water
CPRG	Chlorophenol red-β-D-galactopyranoside
DBP	Dibutyl phthalate
dBPA	Deuterated BPA
DCM	Dichloromethane
DEHA	Di(2-ethylhexyl) adipate
DEHP	Di(2-ethylhexyl) phthalate
DINP	Diisononyl phthalate
dl	Detection limit
E_1	Estrone
E_2	17β-Estradiol

ED	Exposure duration
EDCs	Endocrine disrupting chemicals
EE_2	Ethynylestradiol
EEq	Estradiol equivalents
FBS	Fetal bovine serum
HQ	Hazard quotient
IR	Daily intake rate
LADD	Lifetime average daily dose
Lft	Lifetime
loq	Level of quantification
MtBE	Methyl tertiarybutyl ether
NP	Nonylphenol
PBS	Phosphate buffered saline
PVC	Polyvinyl chloride
RfD	Reference dose
SPE	Solid phase extraction
UPLC-MS/MS	Ultra-performance liquid chromatography-tandem mass spectrophotometry
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
YES	Yeast estrogen screen

1. Introduction

The demand for the supply of clean water is increasing due to the continuing human population growth (Loos et al., 2007). However, population growth and urbanization is associated with a reduction in water quality with industrial and agricultural activities contributing to the contamination of water sources (Guillette and Crain, 2000; Sumpter, 2005; Falconer et al., 2006). Some of these contaminants are endocrine disruptors that have the potential to cause adverse health effects in humans.

Endocrine disrupting chemicals (EDCs) can enter the aquatic environment via direct discharge into water, effluents from sewage treatment plants, leaching (e.g. leakage from septic tanks and landfill sites), storm water runoff and accidental spills (Slabbert et al., 2008; Burkhardt-Holm, 2010). Many environmental EDCs are estrogenic and estrogenic activity was found at varying concentrations in raw and treated water in various countries, including South Africa (Slabbert et al., 2008; Burkhardt-Holm, 2010; Genthe et al., 2010; Manickum and John, 2014). Natural and synthetic estrogens, bisphenol-A (BPA), nonylphenol (NP) and short chain phthalates are some of the substances that contribute to the estrogenic load in water bodies and may cause adverse effects in aquatic organisms (Kunz et al., 2015).

Natural hormones, including estrogens, can be released into the environment via sewage effluent and from such sources such as agricultural and pharmaceutical activities (Falconer et al., 2006; Slabbert et al., 2008; Burkhardt-Holm, 2010). Estrogenic potencies of natural and synthetic estrogens are three to seven orders of magnitude greater than the potencies of other EDCs, making them the major contributor to e strogenic activity in environmental water (Desbrow et al., 1998;

Tanaka et al., 2001; Nakada et al., 2004; Racz and Goel, 2010). Although natural estrogens are essential for normal development and reproduction, natural and synthetic estrogens are also known human carcinogens (Metzler et al., 1998).

BPA may be present in some plastics, polyvinyl chloride (PVC) products and thermal receipts (Biederman et al., 2010; Rochester, 2013). These products are often disposed of in landfill sites. Compounds, like BPA, can enter waterways through leachate from landfill sites (Kawagoshi et al., 2003). BPA exposure is associated with adverse reproductive and developmental effects, metabolic disease, disruption of thyroid function and immune disorders (Rochester, 2013). NP is used in the manufacturing of industrial and household surfactants and plastics. It is introduced to the environment mainly through industrial effluents and wastewater discharges (Loyo-Rosales et al., 2004; Burkhardt-Holm, 2010). In addition to the steroid hormones, NP may contribute substantially to the estrogenic activity in aquatic environments (Ternes et al., 1999b; Johnson et al., 2005; Galli and Braun, 2008). The endocrine disruptive effects of NP include feminization of aquatic organisms and decreased male fertility (Soares et al., 2008).

Phthalates are used as plasticisers in PVC plastics and are found in numerous consumer products. Phthalates are not covalently bonded to the plastics in which they are used, and are therefore continuously being released from the products (Heudorf et al., 2007). Contamination of waterways may therefore be through leachate from landfill sites (Burkhardt-Holm, 2010). Phthalates are associated with increased adiposity and insulin resistance (Grun and Blumberg, 2009), decreased levels of sex hormones (Pan et al., 2006) and other adverse effects on the human

reproductive system (Hauser and Calafat, 2005; Sax, 2010) as well as attention deficit hyperactivity disorder and reduced IQ scores in children (Cho et al., 2010). Di(2-ethylhexyl) adipate (DEHA) is used as an alternative to phthalates in flexible PVC products (Ghisari and Bonefeld-Jorgensen, 2009) and has been classified by the United States Environmental Protection Agency (USEPA) as a possible human carcinogen (USEPA, 1992).

EDCs can potentially be found in drinking water if drinking water treatment processes are not effectively removing these environmental water contaminants from the source water. Water treatment process technology differs at different water treatment plants and various steps in the water treatment process can remove estrogenic activity to some degree (Slabbert et al., 2008; Burkhardt-Holm, 2010). Treatment options to remove EDCs include separation processes, adsorption and biological and chemical conversion (Chang et al., 2009). Each treatment method has its own limitations and benefits to remove EDCs. Although water treatment processes can be effective in removing EDCs from drinking water, chemicals might also migrate from the water lines/pipes that transport water to distribution points and to the home, thereby adding to the contamination of the drinking water . NP, phthalate esters and BPA can migrate from reservoirs and pipes containing polyethylene plastic, epoxy resins or paints (Romero et al., 2002; Casajuana and Lacorte, 2003).

Limited information is available on estrogenic activity and levels of EDCs in drinking water from South Africa. This study investigated the estrogenic activity in drinking water from various distribution points in Pretoria (City of Tshwane) and Cape Town,

South Africa. The drinking water samples were also analysed for selected target chemicals that included natural and synthetic estrogens, BPA, NP, selected phthalates and DEHA, followed by a scenario based human health risk assessment.

2. Materials and Methods

2.1 Sample collection and extraction

Treated drinking water samples were collected seasonally over four sampling periods from two study areas, namely Pretoria (City of Tshwane) and Cape Town. Both are larger, well-functioning municipalities, but in different geographical areas of South Africa. The main water distribution points in Pretoria and Cape Town were identified and ten representative sampling sites per city were selected. Samples were taken from selected distribution points and not from individual homes (i.e. not point of use). These points rather than points in private homes were selected in order to prevent the possibility of confounding factors in the form of the different types of piping used in private homes and to provide an indication of the quality of water supplied by the water service provider. Table 1 summarizes the treatment processes that were applied to the water from each distribution point.

Table 1: Treatment processes applied to water samples from distribution points

Site	Treatment processes
PTA01	Dose flocculant, sedimentation, dissolved air flotation, filtration, chlorination
PTA02	Pre-oxidation with $KMnO_4$ or ozone, dose flocculant, dissolved air flotation, sedimentation, filtration, granular activated carbon, chlorination
PTA03	Fountain water from 2 fountains, chlorination only
PTA04	Fountain water from PTA03, chlorination only
PTA05	Mix of PTA06 and PTA07 water
PTA06	Dose flocculant, dissolved air flotation combined with filtration, granular activated carbon, chlorination
PTA07	Dose flocculant, sedimentation, filtration, granular activated carbon, chlorination
PTA08	Fountain water, chlorination only
PTA09	Dose flocculant, sedimentation, filtration, chlorination
PTA10	Dose flocculant, dissolved air flotation, filtration, chlorination
CPT01	Water from a combination of treatment plants, mostly CPT10
CPT02	Coagulation and pH adjustment, powder activated carbon, flocculation, settlement, filtration, stabilisation, chlorination
CPT03	Coagulation and pH adjustment, powder activated carbon, flocculation, settlement, filtration, stabilisation, chlorination
CPT04	Coagulation and pH adjustment, flocculation, settlement, filtration, stabilisation, chlorination
CPT05	Water from a combination of treatment plants, mostly CPT02
CPT06	Coagulation and pH adjustment, flocculation, settlement, filtration, stabilisation, chlorination
CPT07	Coagulation and pH adjustment, flocculation, settlement, filtration, stabilisation, chlorination
CPT08	Water from a combination of treatment plants, including CPT03
CPT09	pH adjustment, ion-exchange softening, chlorination
CPT10	Coagulation and pH adjustment, powder activated carbon, flocculation, settlement, filtration, stabilisation, chlorination

Water samples were collected in triplicate in 1 L glass bottles, prepared by rinsing the bottles with HPLC grade methanol (Merck, Darmstadt, Germany). The lids of the bottles were lined with tin foil to prevent the samples from coming into contact with the plastic lids, which can be a possible source of EDC contamination. The pH of the water samples were adjusted to 3 using 32% hydrochloric acid (Merck, Darmstadt, Germany). The water was stored at 4 °C in the dark until it was extracted. To account for seasonal variations in levels of EDCs, samples were collected from the

identified sites in October 2013 (spring), January 2014 (summer), April 2014 (autumn) and July 2014 (winter).

Water samples were extracted by solid phase extraction (SPE) using Oasis HLB 200 mg cartridges (Waters, Milford, Massachusetts, USA) according to the method recommended by the supplier for EDCs in water samples (Waters, 2009). SPE cartridges were pre-conditioned with 5 mL 10% methanol in methyl tertiarybutyl ether (MtBE) (Sigma-Aldrich, St. Louis, MO, USA), followed by 3 mL methanol. The cartridges were equilibrated with 3 mL double-distilled water, before the samples were loaded (1 L/cartridge). The cartridges were washed with 3 mL 5% methanol in double-distilled water and dried. The samples were eluted from the cartridges using 6 mL 10% methanol in MtBE. Throughout the extraction procedure the flow rate was never allowed to exceed 10 mL/minute and care was taken not to let the cartridge run dry until the whole sample volume passed through the cartridge. Glass serological pipettes were used for all solvents and disposable serological pipettes were used for the samples. After elution, the solvent was evaporated to dryness at 37 °C under a gentle nitrogen stream. The sample residue was reconstituted in 1 mL HPLC grade ethanol (Merck, Darmstadt, Germany) for analysis in the bioassays (concentration factor of 1 000 x). A separate extract of each sample was derivatized for ultra-performance liquid chromatography-tandem mass spectrophotometry (UPLC-MS/MS) analysis. Reconstituted samples were stored at -20 °C.

2.2 Bioassays for estrogenic activity

2.2.1 Recombinant yeast estrogen screen (YES)

The YES bioassay was developed in the Genetics Department at Glaxo and is described in detail in Routledge and Sumpter (1996). The genetically modified *Sacchromyces cerevisiae* yeast strain was obtained from Prof JP Sumpter's laboratory, in the Department of Biology and Biochemistry, Brunel University, Uxbridge, Middlesex in the United Kingdom. Preparation of the growth medium and the YES bioassay was performed according to standard assay procedures (Routledge and Sumpter, 1996; De Jager et al., 2011). In short, yeast cells were incubated overnight in a rotating water bath (28 °C, 155 upm) until turbid. Serial dilutions (1:2) of the extracts, 17β-estradiol (E_2) positive control (Sigma-Aldrich, St. Louis, MO, USA) ranging from 1 x 10^{-8} M to 4.8 x 10^{-12} M and vehicle control (ethanol) were prepared in ethanol. Aliquots (10 µL) were transferred to triplicate 96 well micro-titre plates (Thermo Fisher Scientific, Denmark). The ethanol was allowed to evaporate to dryness before 200 µL assay medium containing yeast and the chromogenic substrate chlorophenol red-β-d-galactopyranoside (CPRG) (Roche Diagnostics, Mannheim, Germany) were dispensed into each sample well. The plates were incubated in a naturally ventilated incubator at 32 °C for 3 to 5 days. On day 3, 4 and 5 the absorbance was measured on a Multiskan Spectrum 96-well plate reader (Thermo Fisher Scientific, Vantaa, Finland), at 540 nm (for colour) and 620 nm (for turbidity, as an indicator of yeast cell growth). Samples were considered cytotoxic at concentrations where the absorbance of the sample at 620 nm were less than the average absorbance elicited by the solvent control (blank) minus three times the standard deviation. The following equation was applied to correct for turbidity:

Corrected value = test abs (540 nm) - [test abs (620 nm) - median blank abs (620 nm)]

The detection limit of the yeast assay was calculated as the absorbance elicited by the solvent control plus three times the standard deviation. The E_2 standard curve was fitted (sigmoidal function, variable slope) using Graphpad Prism (version 4), and the estradiol equivalents (EEq) of water samples with three or more points above the detection limit were interpolated from the standard curve and corrected with the appropriate dilution factor for each sample.

2.2.2 T47D-KBluc reporter gene bioassay

The T47D-KBluc reporter gene bioassay was developed by the USEPA to screen environmental samples and chemicals for estrogenic and anti-estrogenic activities and is described in detail in Wilson et al. (2004). The T47D-KBluc cells were purchased from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). The assay was performed according to standard assay procedures (Wilson et al., 2004; De Jager et al., 2011). T47D-KBluc cells were maintained in RPMI growth media (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 2.5 g/L glucose (Merck, Darmstadt, Germany), 10 mM HEPES, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate (Gibco, Life Technologies Corporation, Paisley, UK), 10% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, Utah, USA), 100 units/mL penicillin, 100 units/mL streptomycin and 0.25 µg/mL amphotericin B (Gibco, Life Technologies Corporation, Paisley, UK). One week prior to the assay, cells were placed in media containing 10% dextran-charcoal treated FBS without antibiotic

supplements. Cells were seeded at 5 x 10^4 cells per well in 96-well luminometer plates (Corning Incorporated, New York, USA) and allowed to attach overnight. The dosing medium contained 5% dextran-charcoal treated FBS and the vehicle (ethanol) did not exceed 0.2%. Each sample was tested alone in a four dilution series as well as in the presence of 0.1 nM E_2 (to test for anti-estrogenic activity) or 10 nM ICI 182,780 (Tocris biosciences, Ellisville, MO, USA). An E_2 standard curve (ranging from 100 pM to 0.1 pM), vehicle control (ethanol), antagonist control (100 pM E_2 plus 10 nM ICI 182,780) and background control (vehicle plus 10 nM ICI 182,780) were included on each plate. The exposed cells were incubated for 24h (37 °C, 5% CO_2). After 24 h exposure, cells were washed with phosphate buffered saline (PBS) (Gibco, Life Technologies Corporation, Paisley, UK) and 25 µL lysis buffer (Promega, Madison, Wisconsin, USA) was added to each well. The lysis buffer was activated by one freeze-thaw cycle. The assay plate was placed in a luminometer with two injectors (LUMIstar OPTIMA, BMG Labtech, Offenburg, Germany), programmed to add 25 µL reaction buffer, followed by 25 µL 1 mM D-luciferin (Promega, Madison, Wisconsin, USA) to each well. Relative light unit readings were converted to a fold induction above the vehicle control. The E_2 standard curve was fitted (sigmoidal function, variable slope) using Graphpad Prism (version 4). EEq values of extracts with a greater than two-fold induction above the vehicle control were interpolated from the E_2 standard curve and corrected with the appropriate dilution factor for each sample.

2.3 Target chemical analyses

Extracts were analysed for the following target chemicals using UPLC-MS/MS: BPA, NP, DEHA, dibutyl phthalate (DBP), di(2-ethylhexyl) phthalate (DEHP), diisononylphthalate (DINP), E_2, estrone (E_1) and ethynylestradiol (EE_2).

Stock solutions of 10 mg/mL were prepared for all the standards. All standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), except for the deuterated BPA (dBPA) internal standard which was from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA). NP, BPA, dBPA (internal standard), E_2 and EE_2 were prepared in methanol (Romil, Cambridge, UK), E_1 in acetone (Sigma-Aldrich, St. Louis, MO, USA) and DEHA, DEHP and DBP in dichloromethane (DCM) (Sigma-Aldrich, St. Louis, MO, USA). A concentration range of stock solution was prepared in crimp vials ranging from 250 µg/L to 5 ng/L for NP, BPA, E_1, E_2 and EE_2 and from 1 mg/L to 100 µg/L for DEHA, DEHP and DBP. For each concentration, 1 mL was transferred to a new vial and evaporated under a stream of nitrogen at 40 °C. The dried extract samples and standards (except for the DEHA and phthalate standards) were resuspended in 100 µL 1 M sodium carbonate (Na_2CO_3) (Merck, Darmstadt, Germany). A 2 mg/L solution of dansyl chloride (Sigma-Aldrich, St. Louis, MO, USA) was prepared in acetone and 100 µL was added to each vial. The vials were capped and vortexed and derivatized for 2 minutes at 60 °C and analysed using UPLC-MS/MS. The DEHA and phthalate standards were resuspended in 200 µL acetonitrile (Romil, Cambridge, UK) and were injected without further preparation. The detection limit (dl) and level of quantification (loq) obtained for the target chemicals were 0.5 ng/L (dl) and 5 ng/L (loq) for BPA, NP, E_2 and EE_2, 800 ng/L (dl) and 1 000 ng/L (loq) for DEHA and DINP, 10 000 ng/L (dl) and 40 000 ng/L (loq) for

DBP and DEHP and 5 ng/L (dl) and 10 ng/L (loq) for E_1. In order to determine the recoveries of the target chemicals after the extraction process, triplicate 1 L ddH_2O samples were spiked with a standard cocktail containing all the target chemicals. The final concentration of each target chemical was 200 ng/L. The spiked and unspiked (control) samples were extracted and analysed for the target chemicals. Recoveries obtained for this method were 104% for BPA; 64% for NP; 163% for DEHA; 167% for DBP; 102% for DEHP; 88% for E_2; 84% for E_1 and 89% for EE_2. The instrument and method details for UPLC-MS/MS analyses are available in the supplementary information (see A.1).

2.4 Human health risk assessment

A Human Health Risk Assessment was conducted to assess the potential human health impacts of the chemicals found in the tested water samples. The assessment followed the methodology described by Genthe et al. (2010). The health risk assessment was based on adult exposure excluding children and vulnerable populations. The following exposure parameter values were used for the risk calculations: 350 events per year; body weight of 70 kg; lifetime of 70 years; consumption of 2 L water per day and chronic exposure duration of 30 years.

Bioassay results were compared to the trigger value of 0.7 ng/L for estrogenic activity in drinking water suggested by Genthe et al. (2010). If the trigger value is exceeded, possible adverse health effects are implicated and necessitate further investigation and testing of the water.

The concentrations of the identified target chemicals were used to calculate the total dose a person may be exposed to. The computer programme Risk*Assistant was used for the calculations of potential exposure concentrations. The following calculation was used to determine human exposure to the identified substances on a daily basis:

$ADD = (C_{medium} \times IR) / BW$

where:

ADD is the average daily dose (mg/kg/day)

C_{medium} is the concentration of the substance in the water (mg/kg)

IR is the ingestion rate (L/day)

BW is the body weight (kg)

For toxic chemicals a hazard quotient (HQ) was calculated using the following formula:

$HQ = ADD/RfD$

where:

RfD is the reference dose reported by the USEPA (USEPA, 2011)

A HQ less than 1 is considered to be safe for a lifetime exposure

For carcinogenic chemicals for exposures that last less than a lifetime, the lifetime average daily dose (LADD) was calculated as:

$$LADD = ADD \times ED/Lft$$

where:

ED is the exposure duration (years)

Lft is lifetime (years)

The excess cancer risk was calculated as a function of oral potency factor (β) as reported by the USEPA (USEPA, 2011) and dose:

$$Risk = \beta \times LADD$$

The WHO acceptable risk level is 10^{-5}, meaning that the excess risk of developing cancer is deemed acceptable if ingestion of the substance results in one additional cancer case per hundred thousand of the population or less.

3. Results and discussion

3.1 Bioassays for estrogenic activity

None of the water extracts from the distribution points were above the detection limit in the YES bioassay. Although the YES bioassay has several advantages, including its robustness, lack of endogenous receptors that can interfere with the response of the cells, lower cost and uncomplicated bioassay procedure, yeast-based bioassays are less sensitive compared to human cell lines (Connolly et al., 2011; Kunz et al., 2015). Estrogenic activity was also below the detection limit in drinking water from Brazil (Bergamasco et al., 2011) and Finland (Omoruyi and Pohjanvirta, 2015) using

yeast based assays. The YES bioassay is therefore not as suitable for detection of low levels of estrogenic activity normally found in drinking water.

Estrogenic activity was detected in distribution point water using the T47D-KBluc bioassay, with EEq values ranging from 0.002 to 0.114 ng/L. The EEq values for each distribution point is summarized in Table 2. None of the samples showed anti-estrogenic activity. Estrogenic activity was also detected in drinking water from Italy (Maggioni et al., 2013), Taiwan (Gou et al., 2016), The Netherlands (Brand et al., 2013) and the US (Stanford et al., 2010) using mammalian cell line assays, with EEq values ranging from below the level of quantification to 1.3 ng/L (Table 4).

3.2 Target chemical analyses

BPA, DEHA, DBP, DEHP, DINP, E_2 and E_1 were detected in distribution point water extracts from Pretoria and Cape Town. EE_2 was only detected at one distribution point in Pretoria (PTA08) in all four sampling periods. None of the Cape Town samples had EE_2 concentrations above the dl. NP was below the detection limit in all the extracts. The results of the target chemical analysis are summarized in Table 3 (the individual results for each sampling period is available in the supplementary information, see A.2). The presence of EDCs in drinking water could possibly be ascribed to either, the contamination of the source water and ineffective water treatment methodologies or, in the case of the plasticizers, migration from reservoir linings or plastic pipes at water distribution systems.

Table 2: Estradiol equivalent (EEq) concentrations (ng/L) in distribution point water from Pretoria (PTA) and Cape Town (CPT), South Africa, using the T47D-KBluc bioassay

Site	Oct 2013	Jan 2014	Apr 2014	Jul 2014
PTA01	0.089 ± 0.006	0.015 ± 0.003	0.074 ± 0.003	0.058 ± 0.006
PTA02	<dl	<dl	<dl	0.023 ± 0.005
PTA03	0.061 ± 0.009	<dl	<dl	0.077 ± 0.031
PTA04	<dl	<dl	<dl	<dl
PTA05	<dl	<dl	<dl	0.024 ± 0.006
PTA06	0.032 ± 0.004	0.015 ± 0.005	<dl	0.077 ± 0.025
PTA07	<dl	<dl	<dl	<dl
PTA08	<dl	<dl	<dl	<dl
PTA09	0.013 ± 0.001	<dl	<dl	<dl
PTA10	<dl	<dl	<dl	<dl
Site	**Oct 2013**	**Jan 2014**	**Apr 2014**	**Jul 2014**
CPT01	<dl	<dl	<dl	<dl
CPT02	<dl	<dl	<dl	<dl
CPT03	<dl	<dl	<dl	<dl
CPT04	0.004 ± 0.0003	0.004 ± 0.001	0.003 ± 0.0003	0.005 ± 0.001
CPT05	0.002 ± 0.001	<dl	<dl	0.003 ± 0.001
CPT06	0.004 ± 0.001	0.003 ± 0.0003	0.004 ± 0.0004	0.002 ± 0.0004
CPT07	0.004 ± 0.001	<dl	0.003 ± 0.001	0.041 ± 0.014
CPT08	0.006 ± 0.001	0.005 ± 0.002	0.004 ± 0.001	0.026 ± 0.004
CPT09	<dl	<dl	0.044 ± 0.015	0.020 ± 0.001
CPT10	0.005 ± 0.001	<dl	<dl	0.114 ± 0.044

<dl: below detection limit of the assay

Table 3: Target chemical concentrations (ng/L) in distribution point water from Pretoria (PTA) and Cape Town (CPT), South Africa, using UPLC-MS/MS

Site[a]	BPA	NP	DEHA	DBP	DEHP	DINP	E_2	E_1	EE_2
PTA01	0.44	<dl	2.66	214.11	78.17	38.49	<dl	<dl	<dl
PTA02	0.67	<dl	2.67	272.14	155.71	106.31	0.03	2.32	<dl
PTA03	3.79	<dl	3.19	319.36	81.99	26.05	<dl	3.41	<dl
PTA04	2.63	<dl	2.62	240.51	119.65	28.56	<dl	<dl	<dl
PTA05	2.66	<dl	1.97	260.45	99.04	8.34	<dl	<dl	<dl
PTA06	0.85	<dl	3.71	300.97	93.27	59.84	<dl	3.61	<dl
PTA07	0.42	<dl	3.20	176.15	147.98	105.38	<dl	<dl	<dl
PTA08	1.08	<dl	2.27	248.01	60.71	30.51	<dl	<dl	0.02
PTA09	0.35	<dl	2.68	283.28	124.94	15.96	<dl	<dl	<dl
PTA10	0.25	<dl	2.64	336.31	238.07	54.74	<dl	<dl	<dl
Median	**0.39**	**<dl**	**2.85**	**260.18**	**78.09**	**37.50**	**0.03**	**2.87**	**0.02**
CPT01	0.17	<dl	3.93	629.09	165.99	350.09	<dl	<dl	<dl
CPT02	0.17	<dl	2.65	418.73	60.78	61.86	0.02	0.36	<dl
CPT03	1.99	<dl	2.54	366.40	104.63	194.91	0.04	<dl	<dl
CPT04	1.03	<dl	2.84	383.46	3415.19	50.75	<dl	<dl	<dl
CPT05	7.43	<dl	3.46	618.66	256.44	330.81	<dl	<dl	<dl
CPT06	0.26	<dl	3.43	287.90	186.13	65.75	0.05	<dl	<dl
CPT07	0.58	<dl	3.26	231.02	74.24	79.52	0.04	<dl	<dl
CPT08	0.41	<dl	3.77	318.71	95.69	52.59	<dl	<dl	<dl
CPT09	1.76	<dl	3.40	480.23	360.36	44.75	<dl	1.14	<dl
CPT10	0.61	<dl	4.07	536.16	127.10	250.26	<dl	<dl	<dl
Median	**0.26**	**<dl**	**3.38**	**342.62**	**103.25**	**54.81**	**0.04**	**0.75**	**<dl**

[a] The table present average concentrations over four sampling periods; <dl: below detection limit. Values were adjusted for the 1 000 x concentration of the samples during SPE

BPA concentrations ranged from 0.01 to 28.83 ng/L and were detected in 90% of the Pretoria and 93% of the Cape Town samples. NP was below the detection limit in this study. Drinking water might contain residual amounts of chlorine from the disinfection procedure that reacts with NP to form diverse chlorinated byproducts (Hu et al., 2002). This could explain why NP was not detected in the samples from this project. DEHA was detected in all the samples, with concentrations ranging from 1.07 to 4.97 ng/L. DBP was detected in 88% of the Pretoria samples and in all Cape Town samples. DBP concentrations ranged from 109.48 to 1065.14 ng/L, but the median concentration was 260.18 ng/L in Pretoria and 342.62 ng/L in Cape Town samples. DEHP was detected in 65% Pretoria and 83% Cape Town samples and ranged from 40.20 to 5150.76 ng/L, with median concentrations of 78.09 ng/L for Pretoria and 103.25 ng/L for Cape Town samples. DINP were detected in 70% samples from Pretoria and 85% samples from Cape Town. The concentrations ranged from 3.02 to 1250.75 ng/L, with a median concentration of 37.50 ng/L for Pretoria and 54.81 ng/L for Cape Town samples.

E_2 was detected in 2.5% Pretoria and 10% Cape Town samples, with concentrations ranging from 0.02 to 0.04 ng/L. E_1 was detected in 10% Pretoria and 5% Cape Town samples and concentrations ranged from 0.36 to 4.89 ng/L. EE_2 was detected in 10% of the Pretoria samples and the concentrations ranged from 0.003 to 0.06 ng/L. In this study, E_1 concentrations were at least twenty times higher than the E_2 concentrations. Compared to other estrogens, E_1 is excreted by humans and livestock at relatively high concentrations (Wise et al., 2011). Furthermore, E_2 is biodegradable to E_1 in surface waters (Jurgens et al., 2002) and during sewage

treatment plant processes (Ternes et al., 1999a), rendering E_1 one of the most frequently detected estrogens (Burkhardt-Holm, 2010).

More advanced treatment systems might be more effective at removing EDCs from drinking water, however, it is not always a viable option due to much higher operating costs (Racz and Goel, 2010). Strategies to limit source contamination and effectively remove EDCs from source water are also recommended and may include the development of more effective water treatment technologies and public awareness campaigns (e.g. to reduce the use of and recycle plastic products and to dispose of unused pharmaceuticals in a responsible manner).

When comparing the chemical and bioassay results, EEq values were much lower than expected. For example, for PTA03 in October 2013 the E_1 concentration was 3.41 ng/L. Taking into account the relative potency of E_1 in the T47D-KBluc assay (0.46), an EEq of at least 1.57 ng/L was expected. However, the EEq for this sample was only 0.016 ng/L. The results indicate that antagonists might be present in the water samples. On the other hand, estrogenic activity was detected in some of the samples that were below the detection limit for the estrogens E_1, E_2 and EE_2. As the concentrations of BPA were below the concentrations expected to react in the bioassays, other chemicals or pharmaceuticals with estrogenic activity might be present in those samples. A broad chemical screening is therefore recommended to identify other chemicals/pharmaceuticals that may be present in the water samples.

Table 4 presents a comparison of the estrogenic activity and levels of the target chemicals in this study to the activity and concentrations found in drinking water from

other countries. From the table, it is clear that BPA, NP, DEHA, phthalates and natural and synthetic estrogens are present in drinking water worldwide at a wide range of concentrations. Very few studies assessed drinking water for DINP, but this phthalate was detected in drinking water from this study as well as in drinking water from eastern China (Shi et al., 2012). It is therefore recommended to include DINP when screening environmental or drinking water samples for phthalates.

3.3 Human health risk assessment

When analysing water samples using bioassays, trigger values are useful to judge whether further investigation is needed. Exceeding the trigger value does not necessarily mean that a health effect is expected, but further investigation is needed to identify substances responsible for the activity and could ultimately lead to a full risk assessment (Brand et al., 2013). In this study, none of the samples were above the 0.7 ng/L trigger value for estrogenic activity in drinking water proposed by Genthe et al. (2010).

For the health risk assessment 95th percentiles were used as 'reasonable maximum' values to determine the risks associated with the consumption of distribution point water. The health risk assessment is summarised in Table 5. Using reasonable maximum values, the HQ for E_1 in Pretoria distribution point samples were above 1, indicating potential health risk. In contrast, the HQ based on EEq values from the T47D-KBluc assay were below 1 for the Pretoria distribution point samples, indicating acceptable health risks. This might indicate the presence of substances with antagonistic effects in the water samples. Chemical screening assays are

Table 4: Comparison of target chemicals in drinking water from various countries

Country	BPA (ng/L)	NP (ng/L)	DEHA (ng/L)	DBP (ng/L)	DEHP (ng/L)	DINP (ng/L)	E_2 (ng/L)	E_1 (ng/L)	EE_2 (ng/L)	EEq (ng/L)	Reference
Pretoria and Cape Town, South Africa	0.01-28.83	<dl	0.91-4.97	109-1 065	39-5 150	3-1 251	0.02-0.05	0.36-4.89	0.003-0.06	0.002-0.144[d]	This study
Brazil (Campinas)	160[b]	<dl	-	-	-	-	<loq	<loq	<dl	-	(Sodré et al., 2010)
Brazil (São Paulo)	<dl	-	-	-	-	-	<dl	<dl	<dl	<dl[e]	(Bergamasco et al., 2011)
Brazil (Piracicaba city)	-	-	-	-	-	-	<dl	<dl	<dl	-	(Torres et al., 2015)
Canada (Ontario)	-	-	-	-	-	-	-	0.03-1.5	-	-	(Metcalfe et al., 2014)
China (Chongqing)	-	100-2700	-	-	-	-	-	-	-	-	(Shao et al., 2005)
China	-	-	<dl-25[b]	1.1 – 930[b]	6.2 – 280[b]	<dl – 29[b]	-	-	-	-	(Shi et al., 2012)
China	10.8[a]; 128[c]	27[a]; 558[c]	-	-	-	-	0.04[a]; 0.1[c]	0.3[a]; 1.7[c]	-	-	(Fan et al., 2013)
China	-	-	-	180[a]; 350[b]	180[a]; 770[b]	-	-	-	-	-	(Liu et al., 2015)
China (Jiangsu province)	0.17-1.22	<dl	-	-	-	-	-	-	-	0.02-0.20[e]	(Lv et al., 2016)
Finland	-	-	-	-	-	-	-	-	-	<dl[e]	(Omoruyi and Pohjanvirta, 2015)
France (Paris)	-	-	-	-	-	-	-	-	-	<dl[f]	(Jugan et al., 2009)
France	<9-50	<35-505	-	-	-	-	-	-	-	-	(Colin et al., 2014)
Germany	0.50-2.0	2.50-16	-	-	-	-	0.20-2.1	0.20-0.60	0.15-0.50	-	(Kuch and Ballschmiter, 2001)
Germany (Leipzig)	-	-	-	380	50	-	-	-	-	-	(Luks-Betlej et al., 2001)
Italy	<dl	<dl	-	-	-	-	<dl	<dl	<dl	-	(Loos et al., 2007)
Italy	0.82-102	10.30-84.00	-	-	-	-	<dl	<dl	<dl	0.0136[c,g]	(Maggioni et al., 2013)

Poland	-	-	-	640	60	-	-	-	-	-	(Luks-Betlej et al., 2001)
Portugal (Lisbon)	-	-	90	520	60	-	-	-	-	-	(Serodio and Nogueira, 2006)
Portugal	-	-	-	<dl	130-190	-	-	-	-	-	(Santana et al., 2014)
Portugal	<dl	<loq	-	-	-	-	<dl	<dl	<dl	-	(Carvalho et al., 2015)
Spain (Catalonia)	6-25	24	-	16-32	331	-	-	-	-	-	(Casajuana and Lacorte, 2003)
Spain (Madrid)	-	-	-	633 ± 255	<dl	-	-	-	-	-	(Dominguez-Morueco et al., 2014)
Spain (Madrid)	3.7-50.3	2.5-20.5	-	-	-	-	<dl	<dl	<dl	-	(Esteban et al., 2014)
Taiwan	<dl	<dl	-	163-210[b]	773-1350[b]	<dl	-	-	-	<dl-1.3[d]	(Gou et al., 2016)
The Netherlands	-	-	-	-	-	-	-	-	-	0.022-0.032[h]	(Brand et al., 2013)
US (Cape Cod, Massachusetts)	20-44	<dl	-	-	-	-	-	-	-	-	(Rudel et al., 1998)
US	420[c]	<dl	-	-	-	-	-	-	-	-	(Stackelberg et al., 2004)
US	25	97[a]	-	-	<dl	-	<dl	<dl	<dl	-	(Benotti et al., 2009)
US	-	-	-	-	-	-	-	-	-	0.19-0.77[i]	(Stanford et al., 2010)
US (Southeastern)	0-44.3	12.4-60.6	-	-	-	-	-	-	-	-	(Padhye et al., 2014)
US	-	-	-	-	-	-	<dl	<dl	<dl	-	(Conley et al., 2016)

[a]: Median concentration; [b]: Mean concentration; [c]: Maximum concentration; [d]: T47D-KBluc bioassay; [e]: yeast-based bioassay; f: luciferase reporter gene assay using MELN cells; [g]: HELN-ERα luciferase reporter assay; [h]: ERα CALUC; [i]: E-screen; -: not analysed; <dl: below detection limit; <loq: below level of quantification

therefore recommended to try and identify possible chemicals or pharmaceuticals in the water with antagonistic activity.

Although the HQ for E_1 was above 1 in the Pretoria distribution point samples, E_1 was only detected in four samples and in three different sampling points, indicating that consumers are not continuously exposed to E_1 in their drinking water. It is therefore a more realistic scenario to calculate the HQ using the average concentration. If the HQ is recalculated using the average concentration, the HQ is 0.21, indicating acceptable health risks associated with E_1 in Pretoria distribution point water. A monitoring strategy is however advised, to provide a more accurate assessment of the frequency of E_1 detections in drinking water, for a more accurate health risk assessment. For all other distribution points the HQ was below one for all the target chemicals and is therefore considered safe for a lifetime exposure. The carcinogenic risks for DEHP and DEHA were also below 10^5, and therefore deemed acceptable.

Overall, this study indicated acceptable human health and carcinogenic risks associated with exposure to BPA, phthalates and estrogenic compounds through the consumption of distribution point water. EDCs in drinking water were also below the levels expected to have adverse health effects in Spain (Dominguez-Morueco et al., 2014), China (Liu et al., 2015) and Portugal (Santana et al., 2014). Although this study indicated an acceptable human health and carcinogenic risk associated with the consumption of the distribution point water, it should be kept in mind that this study only focussed on selected target chemicals. Other hazardous chemicals, not

Table 5: Health risk assessment of distribution point water in Pretoria and Cape Town

Target Chemical	Water source	Concentration (95th percentile in ng/L)	ADD (mg/kg/d)	LADD (mg/kg/d)	RfD (mg/kg/d)	Trigger value (ng/L)[a]	Hazard Quotient	Slope	Risk
BPA	PTA	5.84	1.67E-07	7.15E-08	0.05[b]		0.000003		
	CPT	2.42	6.91E-08	2.96E-08	0.05		0.000001		
DEHA	PTA	3.95	1.13E-07	4.84E-08	0.6[c]		2E-07	0.0012	5.81E-11
	CPT	4.71	1.35E-07	5.77E-08	0.6		2E-07	0.0012	6.92E-11
DBP	PTA	391.29	1.12E-05	4.79E-06	0.1[d]		0.00011		
	CPT	954.88	2.73E-05	1.17E-05	0.1		0.00027		
DEHP	PTA	251.42	7.18E-06	3.08E-06	0.02[e]		0.00036	0.014	4.31E-08
	CPT	4127.59	1.18E-04	5.05E-05	0.02		0.0059	0.014	7.08E-07
DINP	PTA	118.99	3.40E-06	1.46E-06	0.115[f]		0.00003		
	CPT	770.95	2.20E-05	9.44E-06	0.115		0.00019		
E_2	PTA[g]	0.03	8.57E-10	3.67E-10		0.7	0.04		
	CPT	0.04	1.14E-09	4.90E-10		0.7	0.06		
E_1	PTA	2.38	6.80E-08	2.91E-08		1.5	1.56		
	CPT	0.02	5.71E-10	2.45E-10		1.5	0.01		
EE_2	PTA	0.01	2.86E-10	1.22E-10		0.6	0.02		
	CPT	<dl				0.6	-		
EEq	PTA	0.08				0.7	0.11		
	CPT	0.04				0.7	0.06		

[a] Trigger value suggested by Genthe et al. (2010) and adjusted for relative potencies of E_1 (0.46) and EE_2 (1.26) in the T47D-KBluc bioassay; [b] USEPA (2002); [c] USEPA (1992); [d] USEPA (2000b); [e] USEPA (2000a); [f] NTP-CERHR (2003); [g] only one sample above detection limit; <dl: below detection limit

tested for in this study, might also be present in the water samples and would add to the potential health risk. Furthermore, this study only focussed on estrogenic activity and selected target chemicals and did not account for other EDC activities, e.g. androgenic activity, anti-androgenic activity, thyroid activity, etc. This study was conducted in two of the larger, well-functioning municipalities in South Africa, but should also be extended to other municipalities in the country, where the EDC status of drinking water might be different. This is especially the case in municipalities where the water quality might not be of as high a quality as indicated by Blue Drop status (a certification programme indicating compliance of municipalities to drinking water quality requirements, available from http://www.dwa.gov.za/dir_ws/DWQR/default.asp).

5. Conclusions

Due to their ubiquity in the environment and endocrine disruptive activity, the potential impact of EDCs on public health is a reason for concern. This study revealed the presence of BPA, phthalates, DEHA and estrogenic hormones in distribution point water from Pretoria and Cape Town, South Africa. The estrogenic activity and levels of target chemicals were comparable to the levels found in other countries.

The presence of EDCs in drinking water can be ascribed to the contamination of the source water and ineffective water treatment methodologies or migration from reservoir linings or plastic pipes at water distribution systems. E_1 in Pretoria distribution point samples posed the highest potential health risk, but overall this

study indicated acceptable human health and carcinogenic risks associated with the consumption of distribution point water from Pretoria and Cape Town.

Due to the fact that EDCs were frequently detected in Pretoria and Cape Town distribution point water, a monitoring strategy is recommended that can act as an early warning system. Other municipalities should also be assessed and assays for androgenic and thyroid activity should be included. Strategies to limit source contamination and effectively remove EDCs from source water are also recommended.

Conflict of interest:

The authors declare no conflict of interest.

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