

Estrogenic activity, selected plasticizers and potential health risks associated with bottled water in South Africa

Natalie H. Aneck-Hahn, Magdalena C. Van Zijl, Pieter Swart, Barry Truebody, Bettina Genthe, Jessica Charmier and Christiaan De Jager

ABSTRACT

Potential endocrine disrupting chemicals (EDCs) are present in bottled water from various countries. In South Africa (SA), increased bottled water consumption and concomitant increases in plastic packaging create important consequences for public health. This study aimed to screen SA bottled water for estrogenic activity, selected target chemicals and assessing potential health risks. Ten bottled water brands were exposed to 20 °C and 40 °C over 10 days. Estrogenic activity was assessed using the recombinant yeast estrogen screen (YES) and the T47D-KBluc reporter gene assay. Solid phase extracts of samples were analyzed for bis(2-ethylhexyl) adipate (DEHA), selected phthalates, bisphenol-A (BPA), 4-nonylphenol (4-NP), 17 β -estradiol (E₂), estrone (E₁), and ethynylestradiol (EE₂) using gas chromatography–mass spectrophotometry. Using a scenario-based health risk assessment, human health risks associated with bottled water consumption were evaluated. Estrogenic activity was detected at 20 °C ($n = 2$) and at 40 °C ($n = 8$). Estradiol equivalent (EEq) values ranged from 0.001 to 0.003 ng/L. BPA concentrations ranged from 0.9 ng/L to 10.06 ng/L. Although EEq and BPA concentrations were higher in bottled water stored at 40 °C compared to 20 °C, samples posed an acceptable risk for a lifetime of exposure. Irrespective of temperature, bottled water from SA contained chemicals with acceptable health risks.

Key words | bisphenol-A, bottled water, endocrine disrupting chemicals (EDCs), estrogenic activity, health risk assessment, polyethylene terephthalate (PET)

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INTRODUCTION

The bottled water industry is growing globally. Between 1999 and 2012, the South African bottled water industry grew by 315% (Ronquest-Ross *et al.* 2015). The preference for bottled water can be attributed to perceived health benefits of bottled water or concerns regarding the quality of tap water, superior taste, lack of access to other suitable water sources, convenience, and marketing influences (Ronquest-Ross *et al.* 2015). The growth of the bottled water industry and concomitant increasing use of plastic packaging may have important consequences for public health.

Ambient air, water, food, and personal care products are sources of potentially hazardous chemicals in the environment (Fox & Aoki 2010). Packaging material can contaminate food products. Some chemicals can migrate from food packaging into the food and are ingested by consumers. The type of packaging material, the chemical and physical nature of the food content, storage time and temperature can influence the migration of hazardous substances from packaging material into food (Muncke *et al.* 2014). Chemicals migrating from food packaging contribute to

the total exposure of humans to endocrine disrupting chemicals (EDCs) that are associated with chronic disease (Muncke 2011). Accurate assessment of human health risks associated with food contact materials is complicated by the presence of unexpected or unknown toxins or if toxicological data for a substance are lacking. While the effects of prolonged exposures to hazardous chemicals on human health are not yet fully known (de Fátima Poças & Hogg 2007), EDCs have been linked to adverse health effects.

EDCs mimic the occurrence of natural hormones. They can block the production of hormones by inhibiting or stimulating the endocrine system (Soares *et al.* 2008). Exposure to mixtures of similar acting EDCs and/or during sensitive windows of development are of particular concern to humans (Muncke 2011). Exposure to EDCs is associated with adverse effects on thyroid and brain function, reproductive disorders, cardiovascular diseases, diabetes, obesity, and cancer (UNEP/WHO 2012). A wide range of EDCs has been identified, and many of them are associated with the plastics industry (UNEP/WHO 2012).

Bisphenol-A (BPA), an EDC, is used for the manufacture of plastics, particularly polycarbonates and epoxy resins (Careghini *et al.* 2015). Its endocrine disrupting activity is associated with cardiovascular disease, diabetes, obesity, and liver dysfunction. Fetal and early childhood exposure to BPA may cause secondary sexual developmental changes, neurobehavioral alterations and immune disorders (Erler & Novak 2010). The EDC, 4-nonylphenol (4-NP) is used for the manufacture of surfactants and plastics (Inoue *et al.* 2001). Exposure to 4-NP is a concern due to its toxic and estrogenic activity (Toyo'oka & Oshige 2000). Even though phthalates are not used in the manufacturing of polyethylene terephthalate (PET), several reports suggest that they may leach from PET bottles into the contents. Phthalates are associated with increased adiposity and insulin resistance, decreased levels of sex hormones, and other adverse effects on the human reproductive system. Infants and children are especially vulnerable to the toxic effects of phthalates (Sax 2010). A major exposure route for humans to BPA, 4-NP, and phthalates is through the ingestion of contaminated food (Careghini *et al.* 2015).

EDCs are known to migrate from food packaging material into food (Muncke 2011). The presence of EDCs in bottled water has been reported in various countries around the world. Bioassays revealed estrogenic activity in bottled water from

Germany (Wagner & Oehlmann 2009, 2011) and Italy (Pinto & Reali 2009). BPA and 4-NP were detected in bottled water from Japan (Toyo'oka & Oshige 2000), Guangzhou, China (Li *et al.* 2010), and Spain (Guart *et al.* 2014a). Diethyl phthalate (DEP), diisobutyl phthalate (DiBP), dibutyl phthalate (DBP), and di(2-ethylhexyl) phthalate (DEHP) were detected in PET bottled water from Canada (Cao 2008). DBP, DiBP, and DEHP were also found in bottled water from Portugal (Santana *et al.* 2014). DEHP was the most abundant phthalate in bottled water from Hungary (Keresztes *et al.* 2013). Wagner & Oehlmann (2009) suggested three possible sources of estrogenic contamination of bottled water: the water source may be contaminated, contamination through the production process (e.g., disinfectants used to clean the filling system), or the migration of EDCs from the packaging material.

The growth of the bottled water industry in South Africa and the associated increase in use of plastic packaging necessitates the assessment of EDCs in locally bottled water. In this study, common South African brands of bottled water were screened for estrogenic activity and selected target chemicals. The effect of storage temperature on estrogenic activity and chemical concentrations was investigated. A health risk assessment was conducted to determine the potential health risks associated with the consumption of the bottled water.

MATERIALS AND METHODS

Bottled water samples

Ten local brands of bottled water were purchased from supermarkets in Gauteng (South Africa). For each brand, eight 500 mL bottles (4 L) from the same batch were purchased. All the brands were in PET bottles and contained water from various sources, mineral composition and areas in South Africa and some of the brands were subjected to additional purification treatments before bottling. The composition of the bottled water samples appeared on the labels (Table 1). Four bottles of each brand were incubated for 10 days in the dark at 20 °C, representing normal storage conditions or 40 °C, respectively. The incubation period of 10 days at 40 °C is in accordance with the standard European Economic Community (EEC) migration test

Table 1 | Characteristics of the analyzed bottled water samples

Brand	Source	Additional treatment	pH	Mineral content (mg/L)									
				Ca	Mg	Na	K	Cl	SO ₄	CaCO ₃	N*	F	TDS
A	Natural spring		7.3	10	10	3	1	2	4	65	1	<0.1	83
B	Natural spring		4.1	1.1	3.2	23.1	0.9	45.8	3.3	10.0	0.7	0.0	143
C	Natural spring	0.2 micron filtered UV treated	6.8	8.6	3.2	18	4.2	31	13	31	<0.3	0.14	104
D	Public/private distribution system	Reverse osmosis Ozonation	6.5	0.6	3.2	<5	3.4	5.6	14	9.8	<1	<0.1	44
E	Natural mineral water		7.7	34	4	6	1	<5	<5	100	3.4	<0.2	148
F	Natural spring		6.8	2.5	3.7	7.0	3.0	<2	<5	–	<0.1	<1	64
G	Water in quartz rock formations		6.9	7.9	9.7	8.1	1.3	7	<10	51	4.2	0.1	104
H	Underground spring		6	2	1.6	9.3	2.1	11	3	<15	1.6	<0.2	70
I	Natural spring	Filtered	4.5	<0.2	1.2	10.6	<0.2	18.5	1.0	1.0	0.2	<0.1	58
J	Mineral water from deep dolomite lakes	UV treated	7–8	43.8	25.5	5.9	0.5	7	8	203	<0.3	<0.2	225

*N = nitrate.

(82/711/EEC) (Ceretti *et al.* 2010). Samples were extracted immediately after the incubation period.

Chemicals

Recombinant yeast was obtained from Prof. J. P. Sumpter's laboratory (Department of Biology and Biochemistry, Brunel University, UK). T47D-KBluc cells were purchased from the American Type Culture Collection (Manassas, VA, USA). HPLC grade solvents, methanol, ethanol, and D(+)-glucose were purchased from Merck (Darmstadt, Germany). Methyl tertiarybutyl ether (MtBE), dichloromethane, 17 β -estradiol (E₂), bis(2-ethylhexyl) adipate (DEHA), DEHP, DBP, BPA, 4-NP, estrone (E₁), ethynylestradiol (EE₂), RPMI 1640 powder, sodium bicarbonate, glycylglycine (1 M), adenosine 5'-triphosphate (ATP), bovine serum albumin (BSA), and magnesium chloride solution (1 M) were purchased from Sigma-Aldrich (St Louis, MO, USA). Chlorophenol red- β -D-galactopyranoside (CPRG) were purchased from Roche Diagnostics (Mannheim, Germany). HEPES buffer solution (1 M), sodium pyruvate (100 mM), antibiotic/antimycotic solution (10,000 units/mL of penicillin, 10,000 μ g/mL of streptomycin, and 25 μ g/mL amphotericin B) and phosphate buffered saline (PBS) were purchased from Gibco (Life Technologies Corporation, Paisley, UK). Fetal bovine serum (FBS) and

charcoal/dextran-treated FBS were purchased from Hyclone Laboratories (Utah, USA). ICI 182,780 (henceforth, ICI) was purchased from Tocris Biosciences (Missouri, USA). Reporter lysis buffer (5 \times) and beetle luciferin were purchased from Promega (Madison, WI, USA).

Double distilled water (ddH₂O) was produced by a Millipore Milli-Q synthesis ultrapure water system from Merck Millipore (Darmstadt, Germany).

Sample extraction

The extraction of EDCs from water samples was done using the solid phase extraction (SPE) procedure outlined by Oasis (Waters 2009). The pH of the water samples was adjusted to 3 before extraction. Oasis HLB SPE glass cartridges (5 cc/200 mg) were pre-conditioned with 5 mL 10% methanol in MtBE, 3 mL methanol, and 3 mL ddH₂O. One-liter samples (2 \times 500 mL bottles) were loaded onto each cartridge. The cartridges were washed with 3 mL 5% methanol in ddH₂O and dried under vacuum. The samples were eluted from the cartridges using 6 mL 10% methanol in MtBE. Samples were extracted in duplicate. One sample was evaporated to dryness under a gentle nitrogen stream and reconstituted in 1 mL ethanol for bioassay analysis. Reconstituted samples

were stored at -20°C . The second sample was split into two 3 mL fractions and evaporated. One fraction was derivatized and the other was reconstituted in 1 mL dichloromethane for gas chromatography–mass spectrophotometry (GC-MS) analysis.

***In vitro* bioassays for estrogenic activity**

Yeast estrogen screen (YES)

The YES assay was developed in the Genetics Department at Glaxo and is described in detail in [Routledge & Sumpter \(1996\)](#). Preparation of the growth medium and the YES assay was performed according to standard assay procedures ([De Jager *et al.* 2011](#)). In short, serial dilutions (1:2) of the extracts, E_2 positive control (ranging from 1×10^{-8} M to 4.8×10^{-12} M) and vehicle control (ethanol) were prepared in ethanol. Aliquots (10 μL) were transferred to triplicate 96-well microtiter plates. The ethanol was allowed to evaporate to dryness before 200 μL assay medium containing the yeast and chromogenic substrate (CPRG) were dispensed into each sample well. The plates were sealed with autoclave tape and placed in a naturally ventilated incubator at 32°C for 3 to 5 days. On days 3, 4, and 5 the absorbance (abs) was measured on a Multiskan Spectrum 96-well plate reader, at 540 nm for color change and 620 nm for turbidity of the yeast culture. Samples were considered cytotoxic at concentrations where the absorbance of the sample at 620 nm was 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:

$$\text{Corrected value} = \text{test abs (540 nm)} - [\text{test abs (620 nm)} \\ - \text{median blank abs (620 nm)}]$$

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.

T47D-KBluc reporter gene assay

The T47D-KBluc reporter gene assay is described in detail in [Wilson *et al.* \(2004\)](#). 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 supplemented with 2.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 10% FBS, 100 units/mL penicillin, 100 units/mL streptomycin, and 0.25 $\mu\text{g}/\text{mL}$ amphotericin B. One week prior to the assay, cells were placed in media containing 10% dextran-charcoal-treated FBS without antibiotic supplements. Cells were seeded at 5×10^4 cells per well in 96-well luminometer plates 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 as well as in the presence of 0.1 nM E_2 (to test for anti-estrogenic activity) or ICI. 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), and background control (vehicle plus 10 nM ICI) were included on each plate. The exposed cells were incubated for 24 h (37°C , 5% CO_2). After 24 h exposure, cells were washed with PBS and 25 μL lysis buffer 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, programmed to add 25 μL reaction buffer (25 mM glycylglycine, 15 mM magnesium chloride, 5 mM ATP, 0.1 mg/mL BSA, pH 7.8), followed by 25 μL 1 mM D-luciferin (5 seconds later) 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.

GC-MS analysis of target chemicals

Bottled water samples were analyzed for DEHA, DEHP, DBP, BPA, 4-NP, E_2 , E_1 , and EE_2 . E_2 , E_1 , and EE_2 were included as potential contaminants of source water,

whereas the other target chemicals might be present in the source water or migrate from the packaging material into the water content. The fraction of the extract reconstituted in dichloromethane was analyzed for DEHA, DEHP, and DBP using GC-MS. The second fraction was derivatized before it was analyzed for BPA, 4-NP, E₂, E₁, and EE₂. The fraction that had to be derivatized was reconstituted in 100 µL 10% (v/v) methanol/MtBE containing α-cholestane as the internal standard at a concentration of 1 µg/mL. The reconstituted samples were then evaporated to dryness with nitrogen at 40 °C. 100 µL of 2% methoxyamine in pyridine was added and the samples were derivatized for 30 minutes in an oven at 50 °C. The vials were allowed to cool to room temperature and evaporated to dryness. The final derivatization was obtained by adding 100 µL of 0.05 M triethylamine in pyridine and 50 µL MSTFA to the vials, followed by heating for 1 hour at 80 °C. The derivatives were allowed to cool to room temperature before being transferred to a GC vial with an insert. Stock solutions of the standards were prepared in methanol and the calibration dilutions were done in 10% (v/v) methanol/MTBE. The standards were processed in the same manner as the samples. The parameters for GC-MS analyses are given in Table 2.

Human health risk assessment

The method described by the United States Environmental Protection Agency (USEPA) and the World Health Organization (WHO) (USEPA 1987, 1992; WHO 2010) was used to conduct the human health risk assessment.

Human exposure to the identified substances on a daily basis was calculated using:

$$ADD = \frac{(C_m \times IR)}{BW}$$

where *ADD* is the average daily dose (mg/kg/day), *C_m* is the contaminant concentration (mg/kg), *IR* is the ingestion rate (kg/day), and *BW* is the body weight (kg).

For non-cancer risks, a hazard quotient (*HQ*) was calculated. The expected exposure to the agent is compared to an exposure that is assumed not to be associated with toxic effects. A hazard quotient less than 1 is considered to be

Table 2 | GC-MS parameters for water sample analysis

Instrument: Agilent 6890N GC coupled with an Agilent 5975 MS
Column: Rtx®-5MS (30 m, 0.25 mm ID, 0.25 µm film thickness)
Instrument settings

Injector temperature	250 °C		
Injection volume	5 µL		
Injection mode	Splitless		
Purge flow	50 mL/min		
Purge time	5.00 min		
Total flow	33.9 mL/min		
Flow rate	1.2 mL/min		
Gas saver	On		
Saver flow	50.0 mL/min		
Saver time	10.00 min		
Carrier gas	Helium		
MS mode	EI + (SIM)		
Scanning mass range	40 to 800 m/z		
Oven temperature program			
Oven ramp	°C/min	Temp (°C)	Hold (min)
Initial		40	0
Ramp 1	10	260	3
Ramp 2	25	280	7
Ramp 3	50	330	6.2
Total run time: 40.00 min			

safe for lifetime exposure:

$$HQ = \frac{ADD}{RfD}$$

where *RfD* is the reference dose reported by the USEPA (2011).

Exposures to carcinogens that last less than a lifetime (*LADD*) is calculated as:

$$LADD = \frac{ADD \times ED}{Lft}$$

where *LADD* is lifetime average daily dose (mg/kg/day), *ED* is exposure duration (years), and *Lft* is lifetime (years).

Carcinogenic risk was calculated as a function of oral potency factor (*β*) and dose:

$$Risk = \beta \times LADD$$

The WHO acceptable risk level is 10⁻⁵ (WHO 2004).

RESULTS

In vitro bioassays for estrogenic activity

In the YES assay, no estrogenic activity or cytotoxicity was observed in any of the samples. However, in the T47D-KBluc assay, estrogenic activity was measured in two of the bottled water brands that were incubated at 20 °C and in eight brands that were incubated at 40 °C (Figure 1). EEq values ranged from 0.001 to 0.003 ng/L and no anti-estrogenic activity was observed.

GC-MS analysis of target chemicals

The limit of detection (LOD) and limit of quantification (LOQ) for each target chemical is given in Table 3. Due to the fact that the samples were concentrated a thousand times with the SPE procedure, target chemicals could be detected at concentrations a thousand times lower than the detection limits.

DEHA, DEHP, DBP, 4-NP, E₂, E₁, and EE₂ were below the detection limit in all the bottled water samples. BPA was detected at concentrations ranging from 0.9 ng/L to 10.06 ng/L (Figure 2). Except for brands A and B, BPA concentrations were slightly higher in samples stored at 40 °C

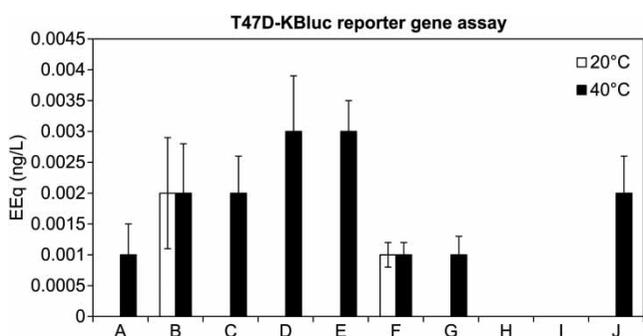


Figure 1 | Estrogenic activity found in bottled water in South Africa using the T47D-KBluc reporter gene assay.

Table 3 | LOD and LOQ of target chemicals using GC-MS

	DEHA	DEHP	DBP	BPA	4-NP	E ₂	E ₁	EE ₂
LOD (ng/L)	8.7	4.4	5.7	13	3.2	6.2	8.8	3
LOQ (ng/L)	29	15	19	42	11	21	29	10

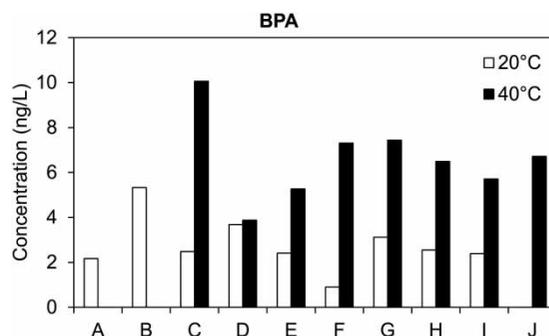


Figure 2 | BPA concentrations in bottled water from South Africa.

compared to samples stored at 20 °C. The mean BPA concentration for samples incubated at 20 °C was 2.78 ng/L and 6.61 ng/L for samples at 40 °C.

Human health risk assessment

A South African framework for guideline development for EDCs in drinking water recommended a precautionary risk-based assessment based on the WHO framework. A trigger value of 0.7 ng/L was derived for estrogenic activity in drinking water (Genthe *et al.* 2010). This trigger was based on the acceptable daily intake (ADI) of 200 pg/kg/day (as opposed to 50 ng/kg/day suggested by the WHO (2004)) to compensate for sensitive subpopulations, individual variation, percentage availability, and a safety factor of 1,000. If the trigger value is exceeded, further investigation and testing of the water is necessitated (Genthe *et al.* 2010). In this study, the estrogenic activity of the bottled water was below the trigger value of 0.7 ng/L at both storage temperatures.

The exposure parameters used for the human health risk calculations are as follows: events per year 350; body weight 70 kg; life time 70 years; ingestion rate 1 L of water per day; chronic exposure duration 30 years. DEHA, DEHP, DBP, and 4-NP concentrations were below the method detection limit. The BPA concentrations and associated health risks were higher in bottled water stored at 40 °C compared to 20 °C. The human health risk calculations can be found in Table 4.

The hazard quotient for all the target chemicals was well below 1, indicating that these chemicals are unlikely to pose a human health risk from drinking 2 L of bottled water every

Table 4 | Human health risk calculations from exposure to BPA

	BPA 20 °C average	BPA 20 °C 95th percentiles	BPA 40 °C average	BPA 40 °C 95th percentiles
Dose (mg/kg)	2.78	4.67	6.61	9.14
ADD (mg/kg/day)	7.95×10^{-8}	1.33×10^{-7}	1.89×10^{-7}	2.61×10^{-7}
RFD (mg/kg/day)	0.0125	0.0125	0.0125	0.0125
HQ	6.36×10^{-6}	1.07×10^{-5}	1.51×10^{-5}	2.09×10^{-5}

day. The carcinogenic risk was also well below the WHO acceptable risk of 10^{-5} .

DISCUSSION

Estrogenic activity and selected EDCs were found in bottled water samples from South Africa at both temperatures. Bottled water from studies in other countries had varied results. French and Italian bottled waters analyzed using a transcriptional activation assay with HepG2 cells (Bach *et al.* 2013) and the HELN cell line (Maggioni *et al.* 2013), respectively, reported no estrogenic activity. Estrogenic activity was detected with the YES assay in German bottled water (2.65 to 75.2 ng/L) (Wagner & Oehlmann 2009) and in Italian bottled water samples (0.9 ng/L to 23.1 ng/L) (Pinto & Reali 2009).

The YES assay did not detect estrogenic activity in any South African bottled water samples (this study). However, the T47D-KBluc reporter gene assay, which is more suitable for samples with lower levels of estrogenic activity (Connolly *et al.* 2011), detected activity in eight brands of bottled water (EEq 0.001 ng/L to 0.003 ng/L) (this study). Similar EEq values (0.00096 ng/L to 0.0122 ng/L) were detected in German, French, and Italian bottled water samples reanalyzed using the E-screen bioassay (Wagner & Oehlmann 2011).

BPA was detected in all the bottled water samples at concentrations ranging from 0.9 ng/L to 10.06 ng/L (this study), which may have contributed to the estrogenic activity. Similar BPA levels (2 ng/L to 10 ng/L) were found in PET bottled water from Japan (Toyo'oka & Oshige 2000). Low BPA concentrations (0.83–1.13 ng/L) were detected in PET bottled water from Italy (Maggioni *et al.* 2013). Higher concentrations of BPA (17.6–324 ng/L)

were recorded in PET and polycarbonate bottled water samples from Guangzhou, China (Li *et al.* 2010). Water stored in polycarbonate bottles in Lebanon had BPA levels ranging from 50 to 1370 ng/L (Dhaini & Nassif 2014). BPA is used in the manufacturing of polycarbonate plastic and migrates from polycarbonate bottles into the water. BPA is not used in the manufacturing of PET bottles and the detection of BPA in water stored in PET bottles is unexpected (Fan *et al.* 2014). The BPA may be as a result of contamination of the original water source (Wagner & Oehlmann 2009), for example from degrading plastic pollution.

4-NP could not be detected in South African bottled water samples (this study). 4-NP was also below the detection limit in PET bottled water from France even when exposed to increased storage temperatures (Bach *et al.* 2013). Other studies have found 4-NP in bottled water at comparably higher concentrations ((19–78 ng/L (Toyo'oka & Oshige 2000)); (108–298 ng/L (Li *et al.* 2010)); (7.9 ng/L (Amiridou & Voutsas 2011)).

No phthalates were detected in our water samples (this study). Similarly, no phthalates were detected in bottled water from Italy (Ceretti *et al.* 2010) or France (Bach *et al.* 2013). Phthalates have been reported to leach from PET bottles into water even though they are not used as substrates or precursors in the manufacturing of PET (Sax 2010). Specific phthalates, DEHP (median = 350 ng/L) and DBP (median = 44 ng/L) were found in bottled water from Greece (Amiridou & Voutsas 2011). DEHP (mean = 118 ng/L) and DBP (mean = 138 ng/L) were found in bottled water from Canada (Cao 2008). These concentrations of EDCs in bottled waters may vary geographically and temporally and could be influenced by conditions at the water source or during storage.

The use of bottled water is pronounced in hot climates. The demand for bottled water is met by widespread transport and storage facilities that are not often climate

controlled. Temperature may influence estrogenic activity. Estrogenic activity was measured in two of the bottled water samples incubated at 20 °C, and in eight samples incubated at 40 °C. This estrogenic activity may be associated with the higher BPA concentrations recorded at 40 °C (6.61 ng/L) than at 20 °C (2.78 ng/L). Larger sample sizes are needed to confirm the possible migration of BPA into water bottled in South Africa. Exposure to increased temperatures does not always affect BPA levels in water from PET bottles (Toyo'oka & Oshige 2000; Li *et al.* 2010; Amiridou & Voutsas 2011). The water samples in this study were stored in the dark, mimicking typical warehouse conditions, while Amiridou & Voutsas (2011) stored their samples in sunlight. In this study, the samples were stored at increased temperatures for 10 days, while Toyo'oka & Oshige (2000) exposed their water samples to increased temperatures for 8 hours. The release of BPA may increase as storage duration and temperature increase (Fan *et al.* 2014). Fan *et al.* (2014) controlled for water source contamination by filling PET bottles with Milli-Q water and stored the bottles for 4 weeks at a temperature of 70 °C. The source of the BPA was unclear since BPA is not used to manufacture PET (Fan *et al.* 2014). Migration tests have shown BPA can migrate from high-density polyethylene (HDPE) and low-density polyethylene (LDPE) caps and polystyrene septa used in some caps (Guart *et al.* 2011). The type of bottle cap used may also contribute to the contamination of water content.

Contamination of bottled water by EDCs needs to be expressed in terms of human health risks. Even though the EEds and BPA concentrations were higher in bottled water stored at 40 °C compared to 20 °C (this study), the samples posed a more than acceptable risk for a lifetime of exposure. The risks associated with South African bottled water were 10,000 times smaller than the acceptable WHO guideline values. The levels of EDCs in bottled water from Guangzhou, China (Li *et al.* 2010) and Greece (Amiridou & Voutsas 2011) were well below the maximum safe doses for chronic oral exposure and had very little risk to human health. Guart *et al.* (2014b) tested 77 bottled waters from 27 countries around the world for 69 organic contaminants and concluded that the very low concentrations of the detected compounds are an indication of the good quality of bottled water worldwide.

CONCLUSIONS

The estrogenic activity and BPA concentrations in PET bottled water from South Africa are similar to bottled water from other countries. Although the risk determined in the current assessment is low, other chemicals, not tested for in this study, may be present in bottled water and contribute to the total health risk. As an example, antimony is classified as a possible carcinogen and has been shown to migrate from PET (Westerhoff *et al.* 2008). Furthermore, this study only measured the contribution of bottled water to EDC exposure and other sources may increase contamination levels. The observed increased estrogenic activity and higher concentrations of BPA at 40 °C lead to the recommendation that bottled water should be stored at room temperature or below so that the potential exposure to EDCs is minimized.

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